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ORIGINAL ARTICLE

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Diversified crop rotation with no-till changes microbial distribution with depth and enhances activity in a subtropical Oxisol

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Abstract

Soil microbial community and enzyme activity regulate carbon and nutrient cycling in soils. No-till has elevated levels of carbon at the soil surface compared to intensively tilled soils. The introduction of crop diversification in agricultural systems increases soil carbon protection and aggregate stability. Thus, the association of no-till with crop diversification may change the soil microbial distribution and enhance activity in comparison with tilled soils with no crop diversification. We tested this hypothesis assessing the soil microbial community and enzyme activity in a long-term (32 yr) experiment with contrasting soil management (no-tillage, (NT) and conventional tillage (CT)) and cropping systems in southern Brazil. Long-term NT increased microbial biomass at 0–5 cm. In contrast, soil microbial biomass was higher in deeper soil layers (10–30 cm) of CT. No-tillage and crop diversification increased the activity of β-glucosidase, acid phosphatase and *N*-acetyl-glucosaminidase. The benefits of an intensified/diversified crop rotation were offset by soil tillage. The higher abundance of PLFA groups at 0–5 cm and 10–30 cm of respective NT and CT soils were correlated with increased C and nutrient levels (N, Ca^{2+}, Mg^{2+}) and decreased Al^{3+}.

Highlights

- Crop diversification increased the soil microbial biomass and individual PLFA groups.
- No-till without crop diversification did not change microbial biomass.
- No-till with crop diversification increased enzyme activity.
- Subsoil fertility restricted microbial responses in a no-till Oxisol.

KEYWORDS

cropping system, hydrolase activity, phospholipid fatty acid, soil microbial biomass, soil tillage

1 | INTRODUCTION

Increasing global demand for food during the past 50 years has induced several changes in the cultivated areas of tropical and subtropical regions (Wingeyer et al., 2015). Agricultural intensification has caused noteworthy soil erosion, biodiversity depletion, soil organic matter decline and nutrient loss, making the current model of agriculture unsustainable (Adewopo et al., 2014; Amundson et al., 2015; Karlen & Rice, 2015). Therefore, the development and selection of agricultural practices capable of promoting soil health is one of the main challenges for the sustainability of agroecosystems in response to the increased demand for food (Foley et al., 2011; Pittelkow, Liang et al., 2015; Chi et al., 2016). The improvement of agricultural practices is a crucial factor for promoting food security because they mediate food production levels (Karlen & Rice, 2015; Tilman, Balzer, Hill, & Befort, 2011; Tilman, Cassman, Matson, Naylor, & Polasky, 2002).

Soil degradation is a recurring concern in tropical regions due to inadequate soil management practices, primarily by intensive soil tillage and the lack of crop diversification (Fu et al., 2015; Pittelkow, Linquist et al., 2015; Torres-Sallan et al., 2017). This improper management results in a decline of the microbial diversity and activity and crop yield (Khaledian, Kiani, Ebrahimi, Brevik, & Aitkenhead-Peterson, 2017). Mediating agricultural practices based on conservation agriculture, characterized by minimal soil disturbance (e.g., no-till (NT)), permanent soil cover and crop rotation (Derpsch & Friedrich, 2009; Hobbs, Sayre, & Gupta, 2008; Pittelkow, Linquist et al., 2015; TerAvest, Carpenter-Boggs, Thierfelder, & Reganold, 2015), can enhance microbial diversity and activity that regulate biogeochemical cycling (Ashworth, DeBruyn, Allen, Radosevich, & Owens, 2017; White & Rice, 2009).

Soil fungal and bacterial communities perform important services in the soil, including aggregate formation, organic carbon protection and decomposition of recalcitrant materials (Fabrizzi et al., 2009; de Moraes Sá et al., 2015; X. Zhang et al., 2018). β-glucosidase, *N*-acetyl-glucosaminidase and acid phosphatase are hydrolytic enzymes responsible for mediating C, N and P cycling in the soil, respectively (Bowles, Acosta-Martínez, Calderón, & Jackson, 2014; Cusack, Silver, Torn, Burton, & Firestone, 2011; Zhao et al., 2016). The potential activities of these enzymes are frequently linked to microbial biomass and used as an indicator of microbial C, N and P demand (Sinsabaugh, Hill, & Follstad Shah, 2009).

No-till can improve soil chemical, physical and microbiological properties in comparison with intensively tilled soils (Blanco-Canquiet al., 2009; De Oliveira Ferreira et al., 2016; Hansel, Diaz, Amado, & Rosso, 2017; Reichert et al., 2016; Reichert, Amado, Reinert, Rodrigues, & Suzuki, 2016; Six & Paustian, 2014). Increasing crop frequency and species diversity in agricultural systems promotes nutrient cycling, microbial biomass and activity, aggregate stability and soil protection against raindrops (Ai et al., 2018; Aschi et al., 2017; Briedis et al., 2018; Chavarría et al., 2016; Fabrizzi et al., 2009; Ferrari et al., 2015; Zuber, Behnke, Nafziger, & Villamil, 2017). However, due to soybean monoculture, crop rotation and cover crops are still uncommon practices in South American agroecosystems, especially in Brazil and Argentina (Wingeyer et al., 2015). Nonetheless, studies on how no-till and different crop rotations affect the soil microbial community distribution and enzyme activity in long-term experiments in subtropical regions are still poorly documented.

To address these issues, we investigated the impact of no-till with crop diversification on the microbial community composition and activity. We tested this by assessing the soil microbial community composition and enzyme activity in a long-term experiment (32 yr) with contrasting tillage systems and crop rotations in southern Brazil.

2 | MATERIALS AND METHODS

2.1 | Study site and field characteristics

The long-term experiment was established in April 1985 at the CCGL-TEC in Cruz Alta, State of Rio Grande do Sul, southern Brazil (28°36′ S, 53°40′ W, elevation: 409 m, slope: 4%) (Figure S1). The climate was classified as humid subtropical (Cfa) according to Peel, Finlayson, and McMahon (2007), with an average annual precipitation of 1,774 mm and an average temperature of 25°C. The rainfall was evenly distributed during the year, with droughts occurring in some years (Figure 1). During the experiment period, the site experienced udic and ustic conditions. The soil at the site was a Typic Hapludox (Soil Survey Staff, 2014), hereafter referred to as an Oxisol, predominantly composed of kaolinite and iron oxides. Table 1 documents the soil physicochemical properties for each treatment.

The experiment was a split-plot arrangement without replications. Thus, three pseudo-replications were allocated within the subplots. The research consisted of two tillage systems, no-till(NT) and conventional tillage (CT), with each plot measuring 40 m wide and 60 m long (2,400m^2), allowing all field operations to be similar to a commercial farm. The NT was performed by sowing crops with minimal soil disturbance, and the CT

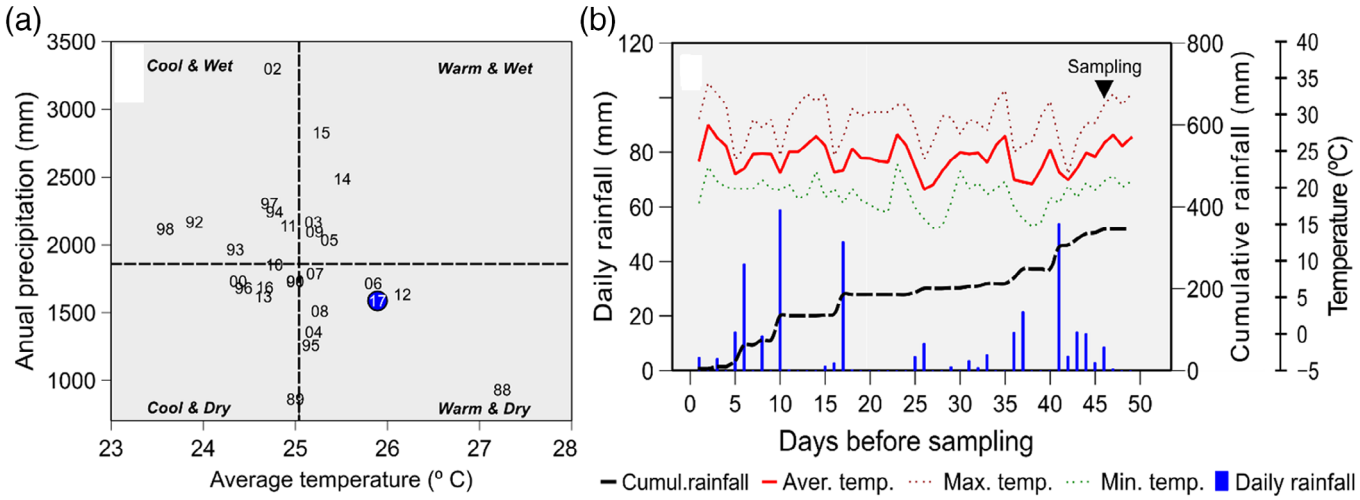

FIGURE 1 (a) Annual temperature and precipitation summaries for the experimental location using the historical weather years (1988–2017) (blue is the year of sampling). Vertical and horizontal lines show the average annual temperature and precipitation, respectively. (b) Daily rainfall, cumulative rainfall and maximum, average and minimum temperature close to sampling

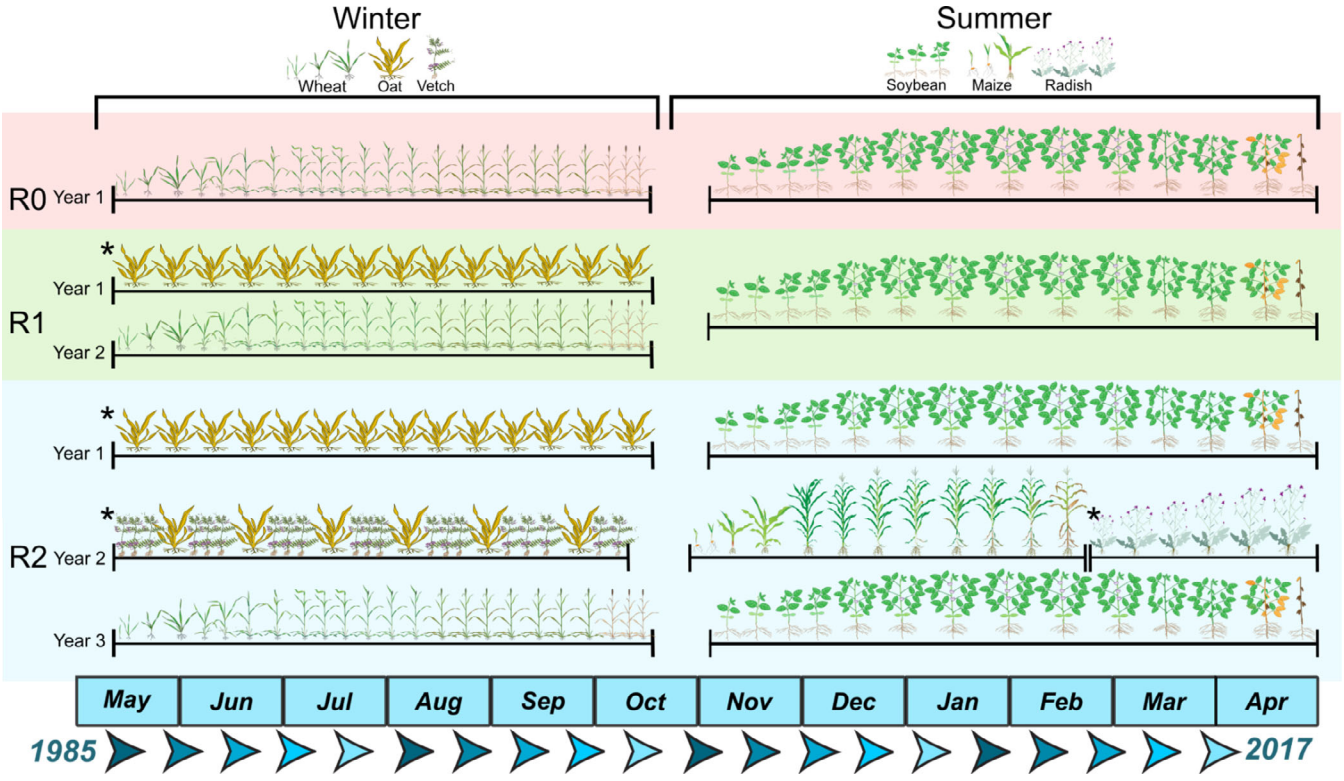

FIGURE 2 Crop rotation description. All crop rotations were tested under no-tillage and conventional tillage. Cover crops were used without the purpose of harvesting. R0: 1 year to complete the rotation system. R1: 2 years to complete the rotation system. R2: 3 years to complete the rotation system. * Cover crops

consisted of disk plough (20 cm) followed by twice disk tandem (10 cm) prior to sowing the summer and winter crops. Only before radish (rotation R2), was there no soil tillage. Three crop rotations with increasing plant diversification based on winter/summer crops were tested (Figure 2): wheat (*Triticum aestivum* L.)/soybean (*Glycine max* L.) (R0); black oat (*Avena strigose* S.)/soybean/wheat/soybean (R1); and black oat/soybean/black oat

TABLE 1 Soil physicochemical properties by tillage, crop rotation and depth ($n = 3$)

Tillage* Crop rotation	% Clay	g kg^{-1} Nitrogen	Carbon	mg dm^{-3} K^+	P	pH	cmol$_c$ dm^{-3} Al^{3+}	Ca^{2+}	Mg^{2+}	CEC_{eff}	µS cm^{-1} EC
		0–5 cm									
CT-R0	58.0 ± 1.5	0.12 ± 0.00	1.67 ± 0.04	160 ± 29.8	15.5 ± 67.0	5.3 ± 0.0	0.3 ± 0.02	4.5 ± 0.3	3.2 ± 0.2	10.5 ± 1.2	101 ± 4
CT-R1	55.0 ± 1.0	0.13 ± 0.01	1.73 ± 0.06	89 ± 22.6	15 ± 4.1	5.2 ± 0.1	0.4 ± 0.06	4.5 ± 0.6	3.2 ± 0.3	8.3 ± 0.9	68 ± 8
CT-R2	52.8 ± 1.2	0.14 ± 0.00	1.76 ± 0.03	139 ± 14.9	14.2 ± 1.3	5.1 ± 0.1	0.6 ± 0.07	4.5 ± 0.4	3.4 ± 0.3	8.5 ± 0.2	88 ± 16
NT-R0	47.7 ± 2.8	0.22 ± 0.00	2.68 ± 0.04	106 ± 28.7	28 ± 1.0	6.1 ± 0.3	0 ± 0.00	5.6 ± 0.6	3.9 ± 0.3	9.8 ± 0.9	146 ± 10
NT-R1	47.3 ± 1.8	0.20 ± 0.01	2.39 ± 0.13	88 ± 19.3	35.2 ± 9.3	5.7 ± 0.2	0 ± 0.00	6 ± 0.6	3.7 ± 0.3	8.8 ± 0.6	165 ± 3.2
NT-R2	45.7 ± 2.6	0.23 ± 0.03	3.05 ± 0.23	110 ± 33.7	23.2 ± 9.2	5.8 ± 0.1	0 ± 0.00	6.1 ± 0.9	4.1 ± 0.2	9.9 ± 1.0	229 ± 12
		5–10 cm									
CT-R0	58.3 ± 0.3	0.17 ± 0.02	1.73 ± 0.07	105 ± 23.7	20.8 ± 6.2	5.3 ± 0.2	0.3 ± 0.07	4.6 ± 0.2	3.3 ± 0.2	8.5 ± 0.2	77 ± 3.7
CT-R1	55 ± 0.68	0.15 ± 0.03	1.82 ± 0.09	98 ± 11.1	17.5 ± 4.4	5.8 ± 0.1	0.3 ± 0.04	4 ± 0.42	3.0 ± 0.2	7.6 ± 0.5	73 ± 3.2
CT-R2	52.3 ± 0.3	0.18 ± 0.01	1.71 ± 0.13	68 ± 6.3	19.9 ± 9.1	5.2 ± 0.1	0.4 ± 0.02	4.4 ± 0.6	3.2 ± 0.2	8.5 ± 0.2	74 ± 4.7
NT-R0	56.3 ± 1.4	0.15 ± 0.02	1.78 ± 0.08	59 ± 17.4	18.2 ± 3.7	5.4 ± 0.2	0.7 ± 0.24	3.7 ± 0.6	3.0 ± 0.3	7.6 ± 0.7	72 ± 5.8
NT-R1	56.3 ± 0.3	0.17 ± 0.02	1.77 ± 0.03	52 ± 8.8	17.9 ± 5.2	5 ± 0.3	1.4 ± 0.99	3.5 ± 0.5	2.7 ± 0.2	8.1 ± 0.8	102 ± 6.0
NT-R2	53.3 ± 0.9	0.18 ± 0.03	1.85 ± 0.10	55 ± 17.4	17.5 ± 1.9	5 ± 0.1	1.1 ± 0.21	4.0 ± 0.1	3.2 ± 0.1	7.7 ± 0.4	102 ± 11
		10–30 cm									
CT-R0	58.3 ± 1.2	0.14 ± 0.00	1.72 ± 0.03	88 ± 14.1	13.5 ± 1.5	5.6 ± 0.1	0.2 ± 0.06	4.8 ± 1.0	3.6 ± 0.5	7.6 ± 0.5	75 ± 8.3
CT-R1	56.3 ± 0.9	0.15 ± 0.01	1.85 ± 0.03	91 ± 31.5	17.0 ± 4.5	5.3 ± 0.1	0.3 ± 0.09	4.2 ± 0.2	3.2 ± 0.2	7.8 ± 0.4	84 ± 6.7
CT-R2	50 ± 0.6	0.18 ± 0.01	1.92 ± 0.06	72 ± 36.7	15.9 ± 1.7	5.4 ± 0.1	0.4 ± 0.1	4.5 ± 0.6	3.2 ± 0.2	8.8 ± 1.4	65 ± 18
NT-R0	58.3 ± 0.7	0.15 ± 0.03	1.57 ± 0.04	42 ± 6.5	4 ± 1.7	4.9 ± 0.2	1.5 ± 0.6	3.2 ± 1.0	2.5 ± 0.2	7.3 ± 0.8	67 ± 4
NT-R1	55.3 ± 0.9	0.13 ± 0.02	1.62 ± 0.13	36 ± 7.0	5.3 ± 2.1	5.1 ± 0.1	1.4 ± 0.8	2.8 ± 0.5	2.5 ± 0.3	8.29 ± 0.8	52 ± 17
NT-R2	55 ± 1.7	0.15 ± 0.02	1.56 ± 0.02	37 ± 17	6 ± 1.2	5.3 ± 0.2	0.7 ± 0.4	4.1 ± 0.7	2.8 ± 0.3	6.83 ± 0.3	60 ± 8

Note: Means for physicochemical properties with the standard error of the mean. CT, conventional tillage; EC, electrical conductivity; NT, no-till; R, rotation.

+ common vetch (*Vicia sativa* L.)/maize (*Zea mays* L.)/radish (*Raphanus sativus* L.)/wheat/soybean (R2). Prior to the establishment of the experiment in 1985, the area was managed with conventional tillage with wheat (monoculture) and wheat/soybean (succession winter/summer), where the wheat straw was burned. The plots were amended with lime and fertilized with N, P and K following soil analysis (Comissão de Química e Fertilidade do Solo -RS/SC, 2016).

2.2 | Soil sampling

Soil sampling occurred in February 2017. Samples were taken at 0 to 5, 5 to 10, and 10 to 30-cm soil depth for all variables analysed. Soil samples were collected with a spatula from an open pit (90 x 90 cm) with 90 cm depth. Soil samples for microbial properties were kept in a cooler (4°C) and frozen (−20 °C) within 2 hr after sampling and stored until analysis. Samples for particle size analysis and chemical properties were cleaned of roots, air-dried, ground and sieved (2 mm).

2.3 | Soil chemical properties analysis

Subsamples were finely ground in a ball mill and analysed for soil organic carbon (C) and total nitrogen (N) by dry combustion using a C/N Elemental Analyzer (Flash EA 1112 Series ThermoFinnigan Italia S.p.A./Milan, Italy). Soil water pH and saturated soil-paste electrical conductivity (EC) were measured in a saturation extract (1:1 soil:water) (Embrapa, 2011). Potassium (K^+) and phosphorus (P) were extracted with a Mehlich-I solution. K content was determined by flame photometry and the P content was measured colorimetrically using molybdenum blue (Embrapa, 2011). Calcium (Ca^{2+}), magnesium (Mg^{2+}) and aluminium (Al^{3+}) were extracted using 1.0 mol L^{-1} KCl. Ca^{2+} and Mg^{2+} were determined by atomic absorption spectrophotometry. Al was titrated with NaOH 0.025 mol L^{-1} (Embrapa, 2011). The effective cation exchange capacity (CEC) of the soil was determined by the sum of the exchangeable bases (K^+, Ca^{2+}, and Mg^{2+}) plus Al^{3+}.

2.4 | Soil microbiological properties analysis

Phospholipid fatty acid (PLFA) analysis was performed with modifications to the original procedure (White & Ringelberg, 1998). Total lipids were extracted by 10 mL of methanol, 5 mL of chloroform and 4 mL of phosphate buffer (pH 7.4) on 5 g freeze-dried soil. Water and chloroform were added after 3 hr to separate the mixture into polar and non-polar fractions, whereas total lipids remained in the non-polar phase. Phospholipids were isolated from neutral lipids and glycolipids by using silicic acid chromatography columns (Disposable BAKERBOND® SPE Columns, J.T. Baker®, Avantor, Radnor, Pennsylvania, USA) and eluted with methanol. The phospholipids were then saponified by KOH, methylated to fatty acid methyl esters (FAME) and analysed by Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) equipped with a DB5-MS column (30 m x 250 μm in diameter x 0.25 μm film thickness; Agilent Technologies, Santa Clara, California, USA). Helium was used as carrier gas. FAME peaks were recognized by different retention times in comparison with the bacterial acid methyl esters mix (BAME; Matreya 1,114; Matreya LLC, Pleasant Gap, Pennsylvania, USA). Internal standards 19:0 FAME were used to determine concentrations.

A total of 30 biomarkers were identified for all samples. Microbial groups were assigned based on characteristics of the biomarkers: *iso* and *ante-iso* branches often belong to Gram-positive bacteria; monosaturated and cyclopropyl lipids often belong to Gram-negative bacteria; actinobacteria have more methyl-branched fatty acids; and methyl linoleate typically belong to fungi (Table 2). Phospholipid fatty acid abundance was reported as nmol per gram of dry soil (nmol PLFA g^{-1} soil). Total bacteria were the sum of Gram-positive bacteria, Gram-negative bacteria and actinomycetes. Microbial biomass (MB) was the sum of all PLFA biomarkers.

Hydrolytic enzyme assays include the C-requiring enzyme (β-glucosidase, βG, EC 3.2.1.21), P-requiring enzyme (acid phosphatase, AcidP, EC 3 .1.3.2) and N-requiring enzyme (*N*-acetyl-glucosaminidase, NAG, EC 3.2.1.52). The potential activities of hydrolases were measured following a modified fluorometric method using fluorometric substrate 4-methylumbelliferone (Zeglin et al., 2013). One gram of soil was homogenized in 100 mL of 50 mM pH 5 acetate buffer. Soil slurries were added into 96-well microplates with 200 μM fluorometric substrate proxy specific to each enzyme. Each sample had six analytical replicates. Additionally, buffer blank, soil blank, negative control, 4-methylumbelliferone reference standard and quench control were measured with each sample to adjust enzyme activity values. The specific incubation time was measured for each enzyme. The times that 0.5 N NaOH solution was added in hydrolase activity assays were recorded as stop times. Fluorescent absorbance was determined by a Multi-Mode Microplate Reader (FilterMax F5, Molecular Devices, LLC, San Jose,

TABLE 2 Phospholipid fatty acid (PLFA) signatures chosen to characterize microbial community structure

Microbial group	Abbreviation	Fatty acids	References
Gram-positive bacteria	Gram+	*i*15:0; *a*15:0; *i*16:0; *i*17:0; *a*17:0	Vestal and White (1989); Velasco, Probanza, Mañero, Solano, and Lucas (2010); Tavi et al. (2013); Fichtner, von Oheimb, Härdtle, Wilken, and Gutknecht (2014)
Gram-negative bacteria	Gram-	cy19:0; cy17:0; 2-OH 10:0; 2-OH 12:0; 3-OH 12:0; 2-OH 14:0; 3-OH 14:0; 2-OH 16:0; 16:1ω7	Parker, Smith, Fredrickson, Vestal, and White (1982); Zelles (1997); Tavi et al. (2013); Zhang et al. (2014); Fichtner et al. (2014);
Actinomycetes	Actino	10-methyl 18:0; 10-methyl 19:0	Vestal and White (1989); Högberg, Högbom, and Kleja (2013); Fichtner et al. (2014)
Saprophytic fungi	Fungi	18:2ω6,9c	Stahl and Klug (1996); Velasco et al. (2010); Zhang et al. (2014)

California, USA) with 365/450 nm excitation/emission for hydrolase fluorometric plates. Potential enzyme activities were reported as nanomoles activity per gram of dry soil per hour ($nmol^{-1}\ hr^{-1}\ g^{-1}$ soil).

2.5 | Statistical analysis

The effect of tillage, crop rotation, depth and their respective interactions (fixed effects) on the response variables was analysed by ANOVA with a mixed model. Random effects corresponded to (i) crop rotation within tillage (crop rotation (tillage)) and (ii) depth within tillage × crop rotation (depth (tillage × crop rotation)). The normality of the residuals was investigated using the Shapiro–Wilk test, and square-root transformation was applied when necessary. To account for potential spatial correlation of the plotted errors, exponential, Gaussian and spherical correlation functions without nugget effect were evaluated using the *nlme* package (Pinheiro, Bates, DebRoy, Sarkar,, & R Core Team, 2017) of the R statistical software (R Core Team, 2018). This step was added to overcome the lack of randomization for soil sampling inherent to this long-term experiment. Moreover, all the models were adjusted with homogenous and heterogeneous variances for the different depths using the *weights* function from the *nlme* package. Model selection for correlation structure was carried out following the Akaike information criterion (AIC) and Bayesian information criterion (BIC). When comparing homoscedastic and heteroscedastic models, the likelihood ratio test (LRT) was used (West et al., 2007). A post-hoc comparison was used to determine significant differences among treatments for all the fixed effects presenting a significance equal to or lower than 0.05 using Tukey's honest significant difference test (*multicomp* R package) (Hothorn, Bretz, & Westfall, 2008).

Distance-based redundancy analysis (dbRDA) (Legendre & Andersson, 1999) was used to explore linear relationships between key soil physicochemical properties and axes of the ordination of sites based upon Bray–Curtis dissimilarities utilizing the function capscale of the *vegan* package (Oksanen et al., 2017) in R software. Their significance was tested by a Monte Carlo permutation test using 999 permutations. Prior to analysis, biological data were log-transformed. In the present study, dbRDA was performed separately for each of the three soil depths. The direction and magnitude of the relationship between environmental and biological variables were presented visually in the dbRDA triplot (sites as points and biological and environmental variables as vectors). Moreover, a Pearson correlation analysis was performed for all variables studied to accompany the dbRDA correlations.

3 | RESULTS

3.1 | Changes in soil microbial community

The soil microbial community was assessed by the abundance of PLFA biomarkers according to tillage, crop rotation and sampling depth (Table 3). A significant one-way and two-way interaction ($p < .05$) occurred for the crop rotation and for tillage*depth (Table S1). Crop rotation affected all PLFA groups and soil microbial biomass. Soil microbial biomass was significantly higher in the crop rotations with cover crops when compared with those with no cover crops (R1: 25.3%; R2: 21.6%). However, no differences were found between R1 and R2.

The tillage*depth interaction significantly affected all PLFA biomarkers. Higher microbial biomass was found in the soil surface (0–5 cm) under NT (40.2 nmol PLFA

TABLE 3 Changes in soil microbial community by tillage, crop rotation and depth. Results are presented in nmol PLFA g^{-1} soil

Source of variation	Microbial biomass	Total bacteria	Gram+	Gram-	Actino**	Saprophytic fungi
Crop rotation	$p = .0126$*	$p = .0048$	$p = .0036$	$p = .0058$	$p = .0105$	$p = .0316$
R0	20.0 b	8.4 b	4.3 b	3.2 b	0.9 b	1.1 b
R1	26.8 a	10.9 a	5.7 a	4.0 a	1.2 a	1.6 a
R2	25.4 a	10.6 a	5.4 a	4.1 a	1.1 a	1.7 a
Tillage*Depth	$p = \leq.0001$	$p = \leq.0001$	$p = \leq.0001$	$p = .0001$	$p = \leq.0001$	$p = \leq.0001$
CT-0_5 cm	25.4 b	10.8 b	5.7 b	4.0 b	1.1 b	1.7 b
CT-5_10 cm	24.7 b	10.5 b	5.5 bc	3.9 b	1.1 b	1.6 b
CT-10_30 cm	21.4 b	8.9 b	4.6 bc	3.3 b	1.0 bc	1.2 b
NT-0_5 cm	40.2 a	15.1 a	8.3 a	5.3 a	1.5 a	2.7 a
NT-5_10 cm	20.1 bc	8.7 b	4.0 c	3.6 b	1.1 b	1.2 b
NT-10_30 cm	12.6 c	5.8 c	2.6 d	2.5 c	0.8 c	0.5 c

Note: Means followed by the same letter are not different according to the Tukey test at the 5% level. CT, conventional tillage; NT, no-till; PLFA, phospholipid fatty acid; R, rotation.
*Type III tests of fixed effects *p* value.
**Actinomycetes.

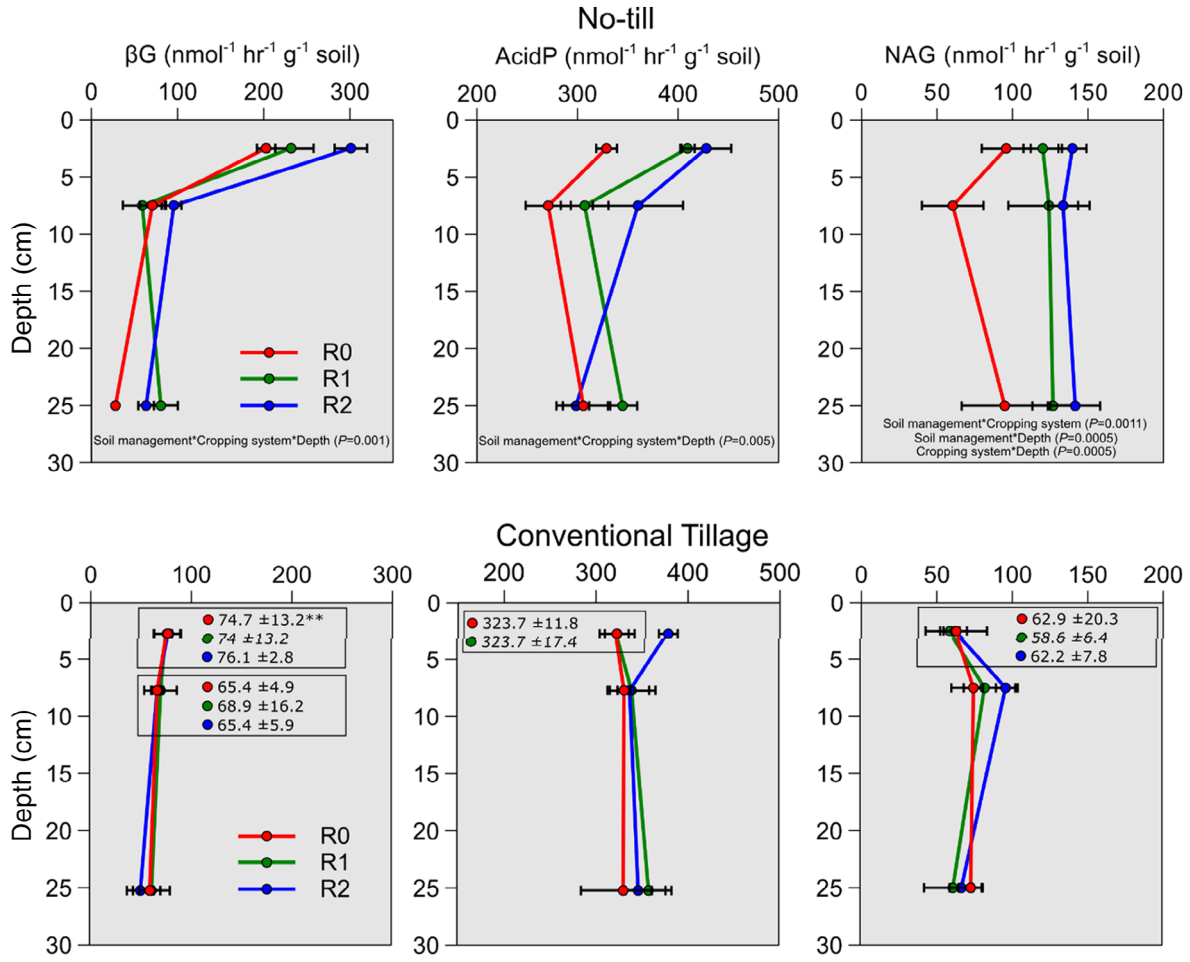

FIGURE 3 Soil extracellular enzyme activity by tillage, crop rotation and depth ($n = 3$). βG, β-glucosidase; AcidP, acid phosphatase; NAG, *N*-acetyl-glucosaminidase. Error bars represent 95% confidence intervals. **Mean and confidence interval (±)

g^{-1} soil), decreasing by 50% in the 5–10-cm layer (20.1 nmol PLFA g^{-1} soil) and 69% in the 10-30-cm layer (12.6 nmol PLFA g^{-1} soil). The different microbial groups followed the same trend as microbial biomass. No differences in soil microbial biomass or specific groups were found between soil depths with CT. Overall, NT concentrated microbial parameters at the surface and CT had a uniform distribution.

3.2 | Changes in extracellular enzyme activity

A significant three-way interaction ($P < 0.05$) among tillage, crop rotation and soil depth was found for βG activity (Table S1). Soil βG activity decreased with depth, regardless of the tillage system, excluding NTR1 treatment (Figure 3). The βG activity was 69% higher in the 0–5-cm soil layer of NT than CT. At the same depth, βG activity was enhanced by increasing crop rotation diversity in NT with no effect in CT. The differences between NTR2 to NTR0 and NTR1 were 23% and 33%, respectively.

Likewise, a three-way interaction ($p < .05$) among tillage, crop rotation and soil depth occurred for AcidP (Table S1). For NAG activity, significant two-way interactions ($p < 0.05$) occurred for tillage*crop rotation, tillage*depth and crop rotation*depth (Table S1). Both Acid P and NAG activity (Figure 3) were sensitive to the crop rotation diversity under NT, primarily in the soil surface. In this situation, crop rotation diversity increased the activity of these enzymes, regardless of the tillage system. The high-diversity crop rotation (R2) increased the

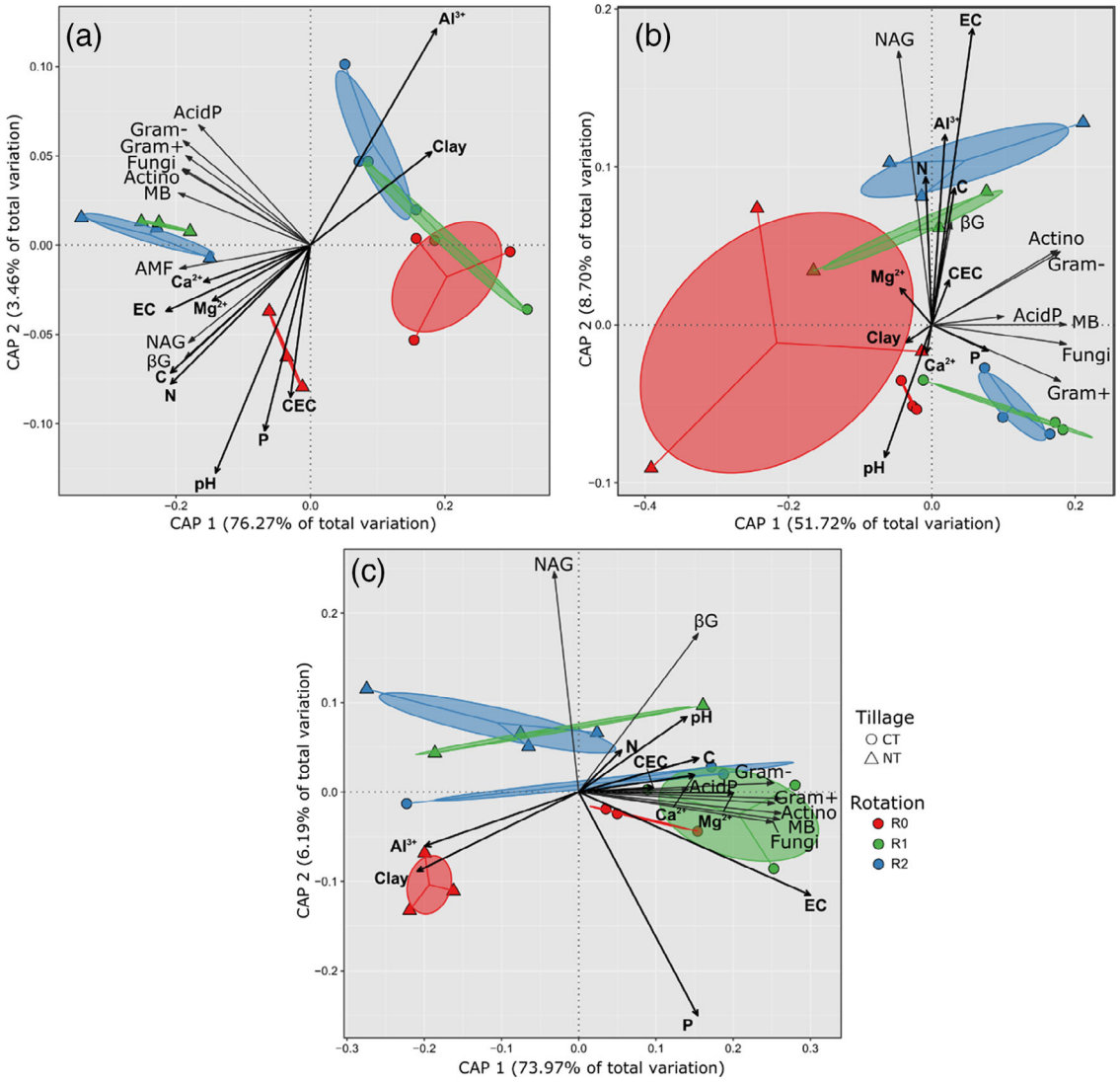

FIGURE 4 Distance-based redundancy analysis (dbRDA) of microbial community and enzyme activities constrained by soil physicochemical properties at 0–5 cm (a), 5–10 cm (b) and 10–30 cm (c). MB, microbial biomass; Gram+, Gram-positive bacteria; Gram-, Gram-negative bacteria; Actino, actinomycetes; βG, β-glucosidase; AcidP, acid phosphatase; NAG, *N*-acetyl-glucosaminidase

activity of AcidP at the surface of CT. The same did not occur for NAG, which had an increase at 5 to 10 cm, excluding NTR0 and NTR2.

3.3 | Relative influence of soil physicochemical attributes on the microbial community and enzyme activity

A distance-based redundancy analysis (dbRDA) was performed to examine the relative influence of soil physicochemical properties on the microbial community composition and enzyme activity.

For the 0–5-cm depth, dbRDA of the PLFA groups and enzymes was constrained by all soil physicochemical properties, which explained 79.7% of the total variance by the first two axes (with 999 permutations, $p = .002$) as influenced by soil tillage and crop rotation (Figure 4a). PLFA biomarkers and enzymes were strongly related to pH ($p = 0.002$), Al^{3+} ($p = .002$), Ca^{2+} ($p = .013$) and EC ($p = .012$) (Figure 1) as a result of the Monte Carlo permutation test ($p \leq .05$). Only the first axis (CAP 1, horizontal) was significant ($p < 0.003$), which was related to the aforementioned variables explaining 76% of the total variance. Microbial community and enzyme activities were positively correlated with C, N, Ca^{2+}, Mg^{2+}, EC, CEC, P and pH, and negatively correlated with clay and Al^{3+} (Figure 4a). Ordination of treatments was primarily related to CAP 1, separating the CT system from the NT (Figure 4a). The permutation test for the dbRDA model demonstrated no significant effect (with 999 permutations, $p = .679$) for 5–10-cm soil depth (Figure 4b).

For the 10–30-cm depth, dbRDA analysis revealed that the first and second axis combined accounted for 80% of the total variation (with 999 permutations, $p = .01$). Ordination was mainly related to CAP 1 as this axis was the only significant axis ($p = .006$), explaining 74% of the total variance (Figure 4c). PLFA biomarkers and enzymes were significantly related to pH ($p = .027$), Al^{3+} ($p = .021$), P ($p = 0.009$), CEC ($P = .020$) and EC ($P = .016$) from the Monte Carlo permutation test ($p \leq .05$). Microbial community and enzyme activities (excluding NAG) were positively correlated with C, N, Ca^{2+}, Mg^{2+}, EC, CEC, P and pH, and negatively correlated with clay and Al^{3+} (Figure 4c).

4 | DISCUSSION

4.1 | Changes in soil microbial community

This study contributes to the understanding of the soil microbial community distribution in a subtropical Oxisol under contrasting tillage systems and crop rotation. Crop rotation significantly affected the microbial biomass in the soil profile. Different cover crops and cash crops (that is, plant species) used in the crop rotation can release specific compounds in the soil and may lead to changes in the distribution of microbial communities (Chavarría et al., 2016). However, there were no differences between winter (R1) and winter/summer (R2) cover crops. Nonetheless, our findings demonstrate the relevance of cover crops and different plant species in the cropping system because the monocropping treatment (R0) had the lowest microbial biomass, regardless of tillage. This behaviour was similar for all PLFA groups. De Campos, Amado, Bayer, Nicoloso, and Fiorin (2011), working on the same experiment, reported that the soil C input increased with the diversification of the crop rotation, where the intensive crop rotation (R2) had the highest C input and monoculture (R0) the lowest. The difference in C and N input may have been the driver for these differences because we also found a higher C and N content in the R2 (C: 3.05 g kg^{-1}; N: 0.23 g kg^{-1}) compared to the R0 treatment (C: 2.68 g kg^{-1}; N: 0.22 g kg^{-1}).

Moreover, the presence of roots of cover crops during the intercrop period can release exudates in the soil and contribute to the greater microbial biomass (Loveland & Webb, 2003). For instance, exudates and compounds of high molecular weight such as polysaccharides (mucilage) and proteins (Kamilova et al., 2006; Haichar, El, Santaella, Heulin, Achouak, 2014) stimulate microbial biomass. Therefore, the continuous and diversified (R2) presence of live roots in the system is an essential step for greater soil health.

In subtropical regions, favourable soil moisture and temperature during the intercrop periods are a vital factor for the microbial community (Brockett, Prescott, & Grayston, 2012). Straw from the previous crop and the shoot biomass of the crops that are growing under NT is indispensable for the preservation of the soil microbial community. The residue maintains soil moisture and reduces soil temperature during the summer (De Quadros et al., 2012; Wang et al., 2011). Studying in the same field, Pes, Amado, La Scala, Bayer, and Fiorin (2011) reported lower soil temperature and higher water-filled porosity in the NT than in the CT.

Long-term NT was the dominant component for greater microbial biomass at 0 to 5 cm. The highest microbial biomass and abundance of total bacteria and fungi in this layer was due to the concentration of C and N (Amado et al., 2006; Mikha & Rice, 2004). Moreover, tillage exposes the soil to more frequent wetting and drying cycles, thus decreasing the microbial biomass (Tivet et al., 2013; Vezzani & Mielniczuk, 2009). Moreover, soil tillage inhibits fungal growth and proliferation, which

contributes to macroaggregate formation and preservation. Thus, it is crucial to adopt practices such as NT to promote fungi growth. Six years after establishment of the experiment, it was possible to verify an increase in soil macroaggregates under NT in relation to the CT (De Campos et al., 2011). Furthermore, De Campos et al. (2011) reported that crop rotation increased soil aggregation. In the same experiment, Fabrizzi et al. (2009) documented that NT had 75% more macroaggregates (>2000 μm) than the tilled treatment.

Overall, NT contributed to the maintenance of the fungal hyphal network. The higher fungal and bacterial biomass at 0–5 cm in NT was associated with three factors: minimal soil disturbance, higher soil moisture and lower soil temperature. Lower disturbance in the soil promotes growth and activity of fungi due to the establishment of extensive hyphal networks. It has been reported that soil cover allows fungi to establish bridges at the soil-litter interface facilitating residue decomposition and soil organic matter formation. Furthermore, agricultural environments that favour fungal growth promote soil carbon preservation through physical protection in macroaggregates (Fabrizzi et al., 2009; Six, Frey, Thiet, & Batten, 2006).

The abundance of Gram-negative bacteria was 53% higher at 0–5 cm than at 10–30 cm in the NT soil. No-till systems provide straw (i.e., plant material) at the soil surface and Gram-negative bacteria are more adapted to use of plant material as a C resource (Paul, 2016; Wan et al., 2015). However, Gram-positive bacteria abundance was relatively higher with depth and in the surface in relation to the Gram-negative bacteria. Gram-positive bacteria have a thick wall of peptidoglycan and the ability to produce resistance spores. This characteristic provides more resistance to environmental stresses such as wetting and drying cycles (Paul, 2016; Wan et al., 2015), supporting its greater abundance at 0 to 5 cm. Actinomycete is a Gram-negative bacteria that behaves similarly to fungi in soil. Thus, actinomycetes were similar to Gram-negative bacteria distribution, decreasing 48% from 0–5 cm to 10–30 cm. Actinomycetes contribute significantly to the degradation of recalcitrant carbohydrates.

4.2 | Changes in extracellular enzyme activity

The majority of the variation in the βG, NAG and AcidP activity was explained by tillage, crop rotation and depth (Figure 3). βG has been one of the most studied hydrolytic enzymes due to its link to the soil C cycle (Bowles et al., 2014). The βG catalyses the decomposition of cellulose (Grandy, Neff, & Weintraub, 2007; Stone, Deforest, & Plante, 2014). Because cellobiose is a disaccharide with rapid decomposition (Liu et al., 2016), its presence may explain the strong positive correlation between the activity of βG and the C content (Table S2) (Matsuoka, Mendes, & Loureiro, 2003). Thus, βG is a good indicator of soil activity due to its rapid response to the changes in soil management (Peixoto et al., 2010).

Under NT, the increase in crop rotation diversity increased βG activity. Crop residues from cover crops and maize (NTR2) and the proportion of readily decomposable organic components returned to the soil from these crops is likely to have enhanced βG activity. In this case, the βG activity was 48.49% and 29.9% higher in relation to minimal crop diversity (NTR0) and winter rotation (NTR1), respectively. Soil disturbance by CT decreased βG activity and offsets the positive effect of crop diversity.

βG, NAG and AcidP activities were significantly higher in NT than CT, excluding CTR2 for AcidP. The effect of tillage for βG and AcidP was restricted to 0 to 5 cm. No difference was found at greater depths. Similar results have been reported by Zhang, Li et al. (2014) and Zuber and Villamil (2016). Synthesis of extracellular enzymes is induced by the presence of their substrates (Nannipieri et al., 2012). Thus, the higher activity of the hydrolytic enzymes in NT indicates greater availability of cellulose and nutrients such as C, N and Ca^{2+} (Blanco-Canquiet al., 2009; Six & Paustian, 2014). The presence of substrates from multiple crops in the diversified rotation promoted the greater hydrolytic activity.

AcidP activity facilitates organic P mineralization into phosphate by hydrolysing phosphoric ester bonds under acid conditions (German et al., 2011; Grandy et al., 2007). In most cases, P deficiency in the soil can stimulate AcidP activity. However, this theory could not be supported by our results. Furthermore, we did not find a correlation between AcidP activity and P content (Table S2). Even though our results were not related to the P content, diversified crop rotation systems with cash crops and cover crops such as R2 treatment may increase P demand, inducing high AcidP activity. This finding is in agreement with Bell, McIntyre, Cox, Tissue, and Zak (2008) because the highest AcidP activities were reported in the NTR2, NTR1 and CTR2 treatments, respectively. Moreover, the C content can explain the higher AcidP activity in the NTR1 and NTR2 treatments. Soil with higher soil organic carbon content will have higher organic P (Table 1; Table S2) (Margalef et al., 2017).

Conversely, NAG activity was not correlated with either C or N content. The NAG activity was only weakly negatively correlated with clay (Table S2). NAG had higher activities under NT in the R1 and R2 crop rotations. Nonetheless, over crop successions (soybeans/

wheat monoculture (R0)), there was a decrease of NAG activity at 5 to 10 cm, presumably due to the lack of crop diversity and lower microbial biomass. Stone et al. (2014), reported that in deep soils, such as Oxisols, bacterially derived NAG enzymes play an indispensable role in recycling organic N from microbial biomass.

4.3 | Relative influence of soil physicochemical attributes on microbial community and enzyme activity

Based on the dbRDA, the variation in the microbial community and enzyme activity was explained by tillage and then by crop rotation. Clustering clearly separated microbial and physicochemical properties correlated with NT or CT at 0–5 and 10–30 cm, respectively. Results from the Monte Carlo permutation indicated that pH and Ca^{2+} were crucial abiotic factors regulating microbial communities. Soils from tropical and subtropical regions are acidic and rich in Al^{+3}. In subtropical environments, the correction for acidity (liming) must be considered an essential tool to improve the chemical quality and increase the microbial biomass, as well as the crop yield, of these soils (Mühlbachová & Tlustoš, 2006; Xue, Huang, Yao, & Huang, 2010). Our findings strengthen the importance of soil acidity correction, because NT creates a nutrient gradient impairing root development and decreasing exudates in the subsurface (Dalla Nora & Amado, 2013). Furthermore, the residue incorporation under CT increased all microbial groups in deeper layers, which reinforces that Oxisols managed under NT results in a strong nutrient stratification. Also, the microbial communities and enzyme activity were moderated by abiotic factors.

5 | CONCLUSIONS

We investigated the effect of tillage and crop rotation on the soil microbial community distribution and extracellular enzyme activity known to regulate carbon and nutrient cycling in agricultural soils. As predicted, tillage had a profound effect on soil microbial biomass distribution and enzyme activity. Long-term NT increased microbial biomass at the soil surface. In contrast, soil microbial biomass was increased in deeper soil layers of CT.

Nonetheless, the use of crop rotation with either summer or winter diversified crops increased all tested microbial groups in comparison with the wheat/soybean rotation. The association of NT and increased crop rotation increased the activity of all three enzymes in the soil surface. Differences between crop rotations decreased with soil depth and were small under CT.

Distance-based redundancy analysis revealed that β-glucosidase and *N*-acetyl-glucosaminidase were closely related to the concentration of their specific substrates (C and N) at 0–5, 5–10 and 10–30 cm. In contrast, there was no correlation between acid phosphatase and soil test P levels. The higher abundances of microbial groups at 0–5 cm and 10–30 cm of respective NT and CT soils were correlated with increased nutrient levels (C, N, Ca^{2+}, Mg^{2+}) and decreased Al^{3+}, as a result of residue and nutrient retention on the surface of NT soils and incorporation within the plough layer in CT soils. Our results suggest that amelioration of subsoil acidity and fertility is a pathway for enhancing microbial biomass in deeper soil layers of NT soils. Our analysis also demonstrated that an increase in crop rotation diversity favoured the abundance of both microbial community and extracellular enzyme activity in the surface of NT soils. It supports that agricultural intensification increases soil microbial biomass and activity in subtropical agroecosystems.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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SUPPORTING INFORMATION

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