Journal of Soil Science and Plant Nutrition
https://doi.org/10.1007/s42729-024-01967-8

ORIGINAL PAPER

# Relationship of Microbial and Fertility Attributes to Organic Carbon Accumulation in a Subtropical Weathered Soil Impacted by a Long-Term Tillage Chronosequence

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Received: 26 October 2023 / Accepted: 29 July 2024


## Abstract

The management of crop residues by tillage is a key determinant that affects microbial processes, leading to changes in soil organic carbon (SOC) pools and fertility status. However, studies evaluating the long-term temporal changes caused by no-tillage (NT) system are still incipient. Therefore, the objective of this study was to evaluate the effects of a 22-year tillage chronosequence on microbial activity and soil fertility attributes in a subtropical ecosystem in Brazil. Field experiments were conducted at two research sites. One site was located near Tibagi (Santa Branca Farm), and the other site was located near Ponta Grossa City (Frankanna Farm), Paraná State, Brazil. The soils are classified as Typic Hapludoxes and the tillage chronosequence comprised five treatments: (i) native vegetation (NV); (ii) NT for 10 years (NT-10); (iii) NT for 20 years (NT-20); (iv) NT for 22 years (NT-22); and (v) conventional tillage for 22 years (CT-22). We determined soil microbial biomass carbon (MB-C) and nitrogen (MB-N), basal respiration (BR), microbial quotient (MB-C and SOC ratio), metabolic quotient ($qCO_2$), total polysaccharides (TP), and fertility attributes (pH, N, S, P and effective CEC). A strong interaction between soil management and microbial activity soil fertility attributes was observed. NV exhibited higher MB-C and MB-N and lower BR and $qCO_2$, suggesting that NV could support a more stable ecosystem. CT-22 depleted soil C, N, S and P concentrations, but increasing NT time restored their concentrations. There were 34%, 42% and 48% less SOC (-14.9 g C $kg^{-1}$), N (-1.4 g N $kg^{-1}$) and S (-0.13 g S $kg^{-1}$), respectively, in CT-22 in relation to the NT-22 soil at 0 to 5 cm depth. In addition, CT-22 showed significantly ($p < 0.05$) lower MB-C, MB-N and a high $qCO_2$, indicating intense competition among soil microbes for available C. The study emphasized NT system potential to sequester C in soils. Although further studies are necessary to confirm this hypothesis, it can indicate that microbial communities with high C use efficiency drive SOC sequestration and this occurred more in NT than in CT.

**Keywords** No-tillage system · Microbial biomass · C Sequestration · Microbial activity · Labile pools

**Acronyms**

| | |
|---|---|
| SOC | Soil Organic Carbon |
| NT | No-Tillage |
| NV | Native vegetation |
| CT | Conventional Tillage |
| MB-C | Microbial-biomass carbon |
| MB-N | Microbial biomass nitrogen |
| TP | Total Polyssaccharides |
| C | Carbon |
| N | Nitrogen |
| S | Sulfate |
| P | Phosphor |
| CEC | Cation Exchange Capacity |
| BR | Basal Respiration |
| MBC and SOC ratio | Microbial Quotient |
| $_qCO_2$ | Metabolic Quotient |

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Published online: 08 October 2024

## 1 Introduction

Soil organic matter (SOM) is considered imperative to maintain soil quality as it interacts significantly with chemical, physical, and biological parameters that affect plant development (Sá et al. 2020; Gupta et al., 2022). In natural environments, soil organic carbon (SOC) occurs in a stratified pathway, with greater accumulation in the surface layer due to continuous contribution of litter, driving soil health, increasing aggregation, and facilitating soil aeration (Sá and Lal 2009). This distribution pattern across the soil profile can be used as a proxy for organic matter quality and can be used to assess the sustainability of different cropping systems (Jha et al. 2022; Sá et al. 2022).

It is known that in no-tillage (NT) system, the permanence of crop residues on soil surface promotes SOM stratification and increases carbon (C) storage in the upper layers (Sá et al. 2014, 2020; Mamedov et al., 2022; Viana et al. 2023). According to Sá et al. (2020), the presence of mulch in NT often increases microbial biomass; however, the decomposition rate of crop residues is reduced, suggesting a lower activity of microorganisms. A possible explanation is C protection increase in large aggregates. Conversely, conventional soil tillage practices promote the breakdown of soil aggregates reducing the SOM into the profile, impacting the nutrient turnover, C cycling, and the availability of nutrients for crops (Gupta et al. 2022; Broring et al. 2023).

**Table 1** General description of the tillage chronosequence in regards to location, climate and soil

| Description | Parameters | Sites | |
|---|---|---|---|
| | | Tibagi | Ponta Grossa |
| Location | Latitude | 24° 36' S | 25° 20' S |
| | Longitude | 50° 23' W | 50° 20' W |
| | Altitude | 880 m | 910 m |
| Climate | Type[§] | mesothermic, wet subtropical, type cfb | mesothermic, wet subtropical, type cfb |
| | MAT[‡] | 20.7 °C | 18.7 °C |
| | MAR[‡‡] | 1532 mm | 1545 mm |
| Soil | Type | Dark Red Latosol, Typic Hapludox | Dark Red Latosol, Typic Hapludox |
| | Texture | Clayey | Clayey |
| | Parent Material | Shale + Sandstone (Reworked material) | Shale + Sandstone (Reworked material) |
| | Clay type[†] | Kao; Gib; Hem; Goe | Kao; Gib; Hem; Goe |

[§] Climate type classification according to Köppen

[‡] MAT = mean annual temperature

[‡‡] MAR = Mean Annual Rainfall

[†] Kao = kaolinite; Gib = gibbsite; Hem = hematite; and Goe = Goethite

Consequently, there is a change in microbial activity caused by subsurface aeration, contributing to C depletion, and increasing $CO_2$ emissions (Bordonal et al. 2018).

Although the soil microbial biomass represents only 1–5% of SOC (Doran et al., 1998), it can be considered a sensitive indicator of changes in SOC stocks and soil quality in agricultural systems (Stott et al. 2013). Changes in soil management and cropping systems significantly affect the diversity and composition of the microbial community over time (Silva et al. 2022). Some factors such as the absence of soil disturbance and the retention of crop residues on the soil surface, when combined with continued rhizodeposition, strengthen soil aggregation in the surface layers and lead to a slower rate of labile C degradation, protecting SOM within aggregates (De Oliveira Ferreira et al. 2018b; Briedis et al. 2023; Tivet et al. 2013).

Soil fertility attributes are also of high importance for maintenance and increase of SOC stock levels. Various studies have emphasized the significance of integrating low C/N ratio crops with high C/N ratio crops (Cotrufo et al. 2019), attributing this importance to nitrogen's (N) role in facilitating the incorporation of SOC into more stable fractions such as fulvic and humic acids (Cotrufo et al. 2013; Six et al. 2000). Furthermore, it has been reported that the stoichiometry of SOC fine fractions adheres to the ratio C: N:P: S = 10,000:833:200:143 (Kirkby et al. 2013), underscoring the criticality of N supplementation and effective soil fertility management practices for enhancing SOC sequestration.

Our hypothesis is that long-term NT promotes a continuous flow of C and N in the labile pools, promoting improvements in soil fertility attributes and greater microbial activity, boosting SOC sequestration. However, studies evaluating long-term temporal changes caused by NT are still rare. Therefore, the objective of this study was to evaluate the effects of a 22-year tillage chronosequence on microbial activity and soil fertility attributes in a subtropical ecosystem in Brazil.

## 2 Materials and methods

### 2.1 Site Description

Two study areas were selected, being one in the city of Tibagi and the other in the city of Ponta Grossa (50º 23' W and 24º 36' S; 50º 20' W and 25º 20' S, respectively) in the State of Paraná – Brazil (Table 1). The choice of these locations was based on the existence of a well-defined age tillage chronosequence, based on the original undisturbed conditions (natural vegetation and soil properties). This chronosequence facilitated an assessment of the long-term impacts of conventional tillage systems with continuous

tillage (CT) and NT on various parameters affected by SOC. The sites were developed on the same parent material, have the same soil type and landscape position, and were managed using similar soil/crop rotation and management practices except for soil plow.

The soil in the chronosequence is classified as a Typical Hapludox Oxisol (IUSS Working Group WRB 2015) and has a deep, well-structured profile, with high porosity and adequate internal drainage. The characteristics of climate, temperature and parent material are described in Table 1. The natural vegetation, prior to agricultural practices, are subtropical prairies composed mainly of C4 species, such as Andropogon sp., Aristida sp., Paspalum sp. and Panicum sp., and subtropical gallery forests, located in natural drainage channels (Maack 1981).

**Table 2** Description of the land use chronosequence in regards to management and cultivation

| Land use chronosequence | Description |
|---|---|
| Native vegetation (NV) | Was natural vegetation (native field) comprising a climax vegetation of the region. These natural vegetation, included a subtropical "prairie" composed mainly of C4 species, such as *Andropogon sp.*, *Aristida sp.*, *Paspalum sp. and Panicum sp.* |
| No-till for 10 years (NT-10) | In this area, lime was applied to the soil surface four times at the rate of 1.5 Mg $ha^{-1}$. The summer harvest consisted of 7 soybean crops [*Glycine max.* (L.) *Merrill*] and 3 of corn (*Zea mays* L.), and the winter crop was composed of 4 crops of wheat (*Triticum aestivum*) and 6 of black oats (*Avena strigosa*, Schreb). |
| No-till for 20 years (NT-20) | In this area, soil acidity was initially corrected with the application of 3.5 Mg $ha^{-1}$ of limestone, and in the following years limestone was applied to the soil surface eight times at the rate of 1.5 Mg $ha^{-1}$. Summer cultivation involved 15 harvests of soybean and 5 corn (Zea mays L.). In winter, wheat was cultivated seven times, black oats (Avena strigosa, Schreb) 11 times and lupine (Lupinusangustifollios) twice. Both black oats and lupine were grown as cover crops. |
| No-till for 22 years (NT-22) | Soil management was similar to that adopted in NT-20. Cultivation during the summer comprised 15 harvests of soybeans and 6 of corn, and this in winter composed of 10 crops of wheat, 4 of black oats and 1 of lupine and 4 of winter rye. |
| Conventional tillage for 22 years (CT-22) | This treatment involved preparing the soil with a 70 cm disc after the summer harvest and one after the winter harvest to a depth of 20 cm plus two 60 cm wide discs to break up clods). Initially, 3.5 Mg $ha^{-1}$ of limestone was incorporated into the soil to correct acidity and every 3 years 2 Mg $ha^{-1}$ of limestone was incorporated. Cultivation during the summer comprised 15 soybean and 6 corn crops, and during the winter it comprised 10 wheat crops, 4 black oats and 1 lupine crops, and 4 winter rye (*Lollium multiflorum*). |

## 2.2 Experimental Design and Sampling

The experimental design comprised of a tillage chronosequence in which the duration of CT and NT was assigned as whole plots, and the sampling depth was assigned as a subplot. The following chronosequence treatments were included in the experimental design: (i) native vegetation (NV) treatment, comprising the undisturbed vegetation; (ii) NT for 10 years (NT-10); (iii) NT for 20 years (NT-20); (iv) NT for 22 years (NT-22); and (v) CT for 22 years (CT-22). Soil preparation and crops for chronosequence treatments are briefly described in Table 2. The dimensions of each chronosequence area were 200 × 50 m, with five sub-plots representing a 40 × 50 m area. Soil samples for each replicate were obtained after digging nine profiles of 20 cm wide x 50 cm long x 50 cm deep. The samples were collected from five depths (0-2.5, 2.5-5, 5–10, 10–20 and 20–40 cm), and a composite sample from all replicates was obtained for each depth.

## 2.3 Analysis of soil Fertility Attributes

Soil pH was determined from a soil suspension in $CaCl_2$ at 0.01 mol $L^{-1}$ (1:2.5 soil/solution, v/v). Exchangeable $Al^{3+}$, $Ca^{2+}$ and $Mg^{2+}$ were extracted with 1 M neutral KCl and determined by titration, and $K^+$ was extracted with Mehlich$^{-1}$ and determined by flame photometry. The available P were extracted using a cation- and anion-exchange resin (Raij and Quaggio 1983). The effective cation exchange capacity (CEC) was obtained by the sum of exchangeable Ca, Mg and K (Table 3).

## 2.4 Soil Organic C and Total N Analyses

A portion of each sample was ground and sieved at 100 mesh to determine the SOC, N and sulfate (S) concentrations. The C, N and S concentrations were determined through the loss on ignition method (Nelson and Sommers 1982) using a CNS analyser (LECO, St. Joseph, MO, USA).

## 2.5 Measurement of soil Microbial Biomass C and N and Basal Respiration

The soil samples were stored at 5 °C to 7 °C pending analyses for soil microbial biomass C (MB-C) and N (MB-N). Prior to analysis, the samples were sieved at 2-mm, adjusted to a 40% water-holding capacity, and maintained at room

**Table 3** Changes of pH, Cation Exchange Capacity (CEC), P content, C:N, and C: S ratios in an Oxisol (Typic Hapludox) under a long-term land use chronosequence (means of five replicates)

| Soil | Depth | Land use chronosequence | | | | |
|---|---|---|---|---|---|---|
| property | (cm) | NV | NT-10 | NT-20 | NT-22 | CT-22 |
| **pH**, 0.01 M | 0-2.5 | 4.0a[§] | 6.2c | 6.3c | 6.0c | 5.3b |
| $CaCl_2$ | 2.5-5 | 3.9a | 5.8c | 5.8c | 5.5bc | 5.4bc |
| | 5–10 | 3.9a | 5.2b | 5.3b | 5.2b | 5.3b |
| | 10–20 | 4.0a | 4.9b | 4.6b | 4.6b | 5.2bc |
| | 20–40 | 4.0a | 4.6a | 4.3a | 4.2a | 4.5a |
| *P value*[‡] | *(Depth)* | > 0.001 | | | | 0.01 |
| **CEC** | 0-2.5 | 2.0c | 17.7a | 19.2a | 11.6b | 9.3b |
| **(cmol $kg^{-1}$)** | 2.5-5 | 1.2d | 11.1ab | 13.2a | 9.1c | 9.2c |
| | 5–10 | 0.8c | 7.9ab | 8.7ab | 7.3b | 8.9a |
| | 10–20 | 0.7b | 6.4a | 4.9a | 5.0a | 5.3a |
| | 20–40 | 0.4c | 4.4a | 3.0ab | 2.7ab | 2.6b |
| *P value* | *(Depth)* | > 0.001 | | | | |
| **P** | 0-2.5 | 10.6e | 82.2c | 114.6b | 165.1a | 55.6d |
| **(mg $kg^{-1}$)** | 2.5-5 | 9.4d | 47.4bc | 58.2b | 120.4a | 43.6c |
| | 5–10 | 6.6a | 14.8c | 29.4b | 36.2a | 34.8ab |
| | 10–20 | 4.2c | 9.0b | 12.2ab | 14.4a | 12.6ab |
| | 20–40 | 3.0b | 4.0ab | 4.2ab | 4.4a | 3.4ab |
| *P value* | *(Depth)* | > 0.001 | | | | |
| **C: N ratio** | 0-2.5 | 15.2a | 12.1b | 12.3b | 13.2b | 14.6a |
| | 2.5-5 | 15.3a | 12.2c | 12.6bc | 13.4b | 15.5a |
| | 5–10 | 15.9a | 13.1c | 13.8bc | 14.8ab | 16.3a |
| | 10–20 | 16.0bc | 15.4c | 15.8bc | 17.2ab | 18.0a |
| | 20–40 | 16.6b | 16.3b | 16.6b | 17.3ab | 18.9a |
| *P value* | *(Depth)* | 0.03 | > 0.001 | | | |
| **C: S ratio** | 0-2.5 | 158b | 279a | 147b | 158b | 195b |
| | 2.5-5 | 168ns | 246 | 146 | 163 | 220 |
| | 5–10 | 179b | 161b | 175b | 200ab | 244a |
| | 10–20 | 173b | 168b | 186b | 272a | 295a |
| | 20–40 | 176a | 155c | 156c | 271b | 328a |
| *P value* | *(Depth)* | 0.21 | 0.04 | 0.28 | > 0.001 | > 0.001 |

[§] Means followed by the same lowercase letter in the rows (comparison among treatments within each depth) do not differ by Tukey test at $P < 0.05$. [‡] *P* values for comparison of means among depths within each treatment

temperature for 2 days. The microbial C and N concentrations were estimated as the difference in the C and N released between fumigated and non-fumigated soils according to the fumigation-extraction method of Vance et al. (1987) and Brookes et al. (1985). Microbial activity was assessed through basal respiration (BR), where the C-$CO_2$ emission of soils incubated with sodium hydroxide was determined according to Mendonça and Matos (2017). The microbial quotient is represented by MB-C and SOC ratio, and the metabolic quotient ($qCO_2$) by BSR and MB-C ratio (Insam and Haselwandter 1989).

## 2.6 Soil Polysaccharides

The measurement of total polysaccharides (TP) was based on the release of saccharide monomers through hydrolysis with sulfuric acid, followed by colorimetric estimation using phenol-sulfuric acid following the methodology proposed by Lowe (1993).

## 2.7 Statistical Analyses

Differences among treatments were examined using analysis of variance (ANOVA) and when significance of *P* values lower than 0.05 was met we applied Tukey test at 0.05 significance. A principal component analysis (PCA) was performed using a matrix of 100 samples (i.e., 5 treatments, 5 replicates and four depths) and 11 variables (i.e., C, N and S stocks, MB-C, MB-N, BR, $qCO_2$, TP, CEC, pH, and P). Statistical calculations were performed using R Development Core Team (R Core Team 2012).

## 3 Results

### 3.1 Changes in SOC Pools and Total N, S and Polysaccharide Concentrations

The concentrations of SOC, N and S decreased significantly with increasing soil depth and exhibited different distribution patterns in the surface layer among tillage chronosequences (Fig. 1). Higher ($p < 0.05$) concentrations of SOC and N were recorded in soils NT-20 and NT-22 compared to soils NV, CT-22 and NT-10 at 0-2.5 and 2.5 -5 cm depth. Total S concentrations were significantly ($p < 0.05$) increased in NT-20 and NT-22. A decrease of 34%, 42% and 48% was observed in SOC (-14.9 g C $kg^{-1}$), N (-1.4 g N $kg^{-1}$) and S (-0.13 g S $kg^{-1}$), respectively in CT-22 compared to NT-22 at the depth of 0–5 cm. Higher C: N ratio was observed in the CT-22 soil at the depth of 0–5 cm compared to the NV and NT systems (Table 3).

For all treatments, TP concentrations decreased slightly with increasing soil depth (Fig. 1), while the proportion of TP in SOC increased with depth. TP fractions in SOC varied from 20 to 30% at depths of 0–2.5 cm and from 33 to 44% at depths of 20–40 cm. Conversion from NV to cultivated fields affected TP concentration, and higher concentrations ($p < 0.05$) were observed under NV at depths of 2.5–20 cm compared to other treatments. Furthermore, the highest TP concentrations at 0–2.5 cm depth ($p < 0.05$) were observed for treatments NT-20 and NT-22, and these values were significantly higher than those for treatments NT-10 and CT-22.

### 3.2 Changes in Microbial Biomass C and N, Basal Respiration and $qCO_2$ in Different land uses

At medium soil depths, microbial C and N concentrations represented between 1.3 and 2.8% of SOC and 3.2 and 7.1% of total N, respectively. The contents of MB-C and MB-N under NV were significantly higher ($p < 0.05$) compared to CT at 0-2.5, 2.5-5, 5–10 and 10–20 cm depth (Fig. 2a and b). Between the tillage chronosequence, the concentrations of MB-C and MB-N in the soil under NT-22 (610.5 and 121.3 µg C $g^{-1}$) were higher ($p < 0.05$) than those under CT-22 (400.8 and 55.1 µg C $g^{-1}$) at 0–10 cm depth.

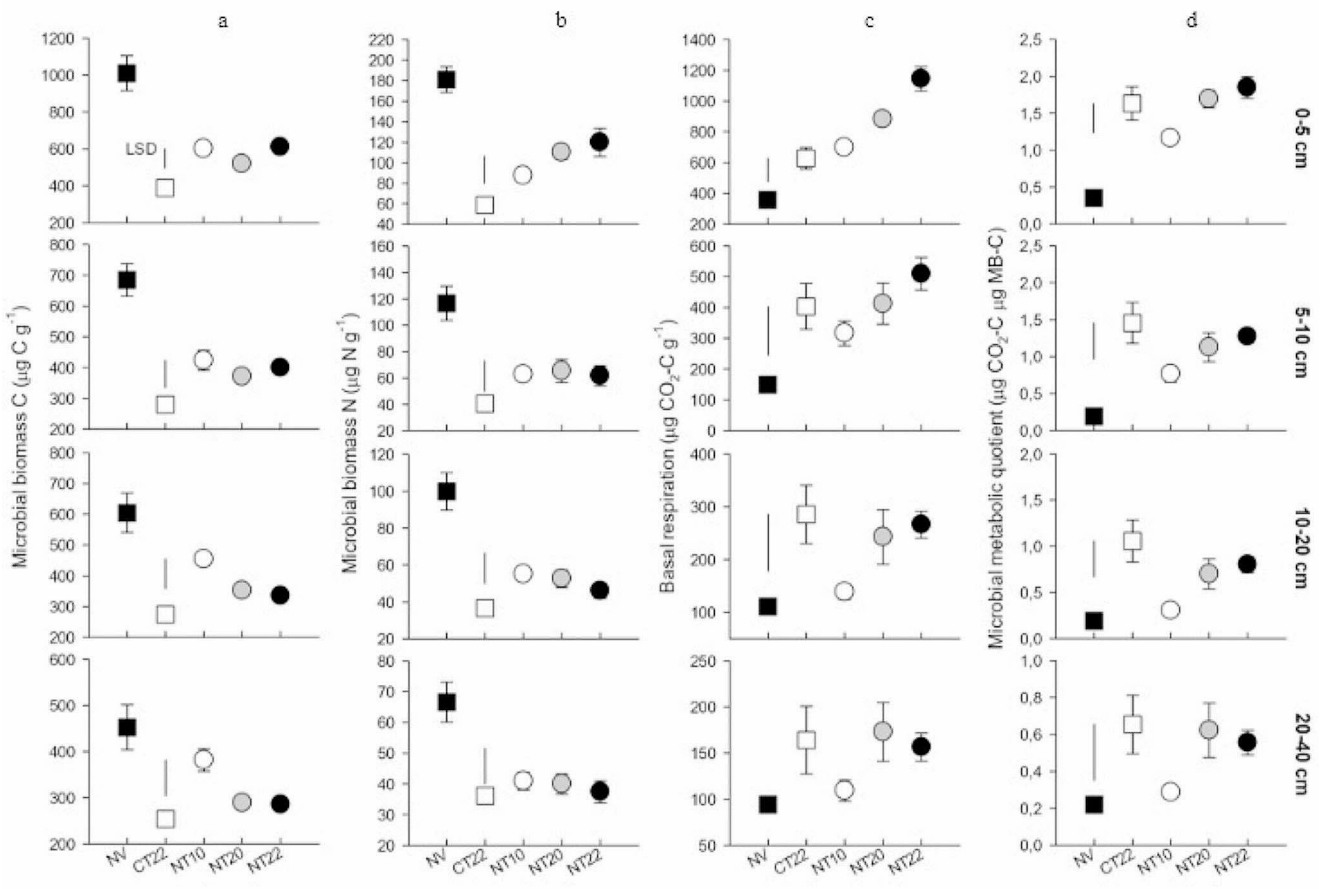


**Fig. 1** Soil carbon, nitrogen, sulfur, and polysaccharides concentrations managed under 22 years of conventional tillage (CT-22), no-tillage of different durations (NT-10 years, NT-20 years, and NT-22 years), and under the neighboring native field (NF) of a Typic Hapludox. Bars indicate the least significant difference (LSD – Student, $P < 0.05$) between means. Values are means of five replicates

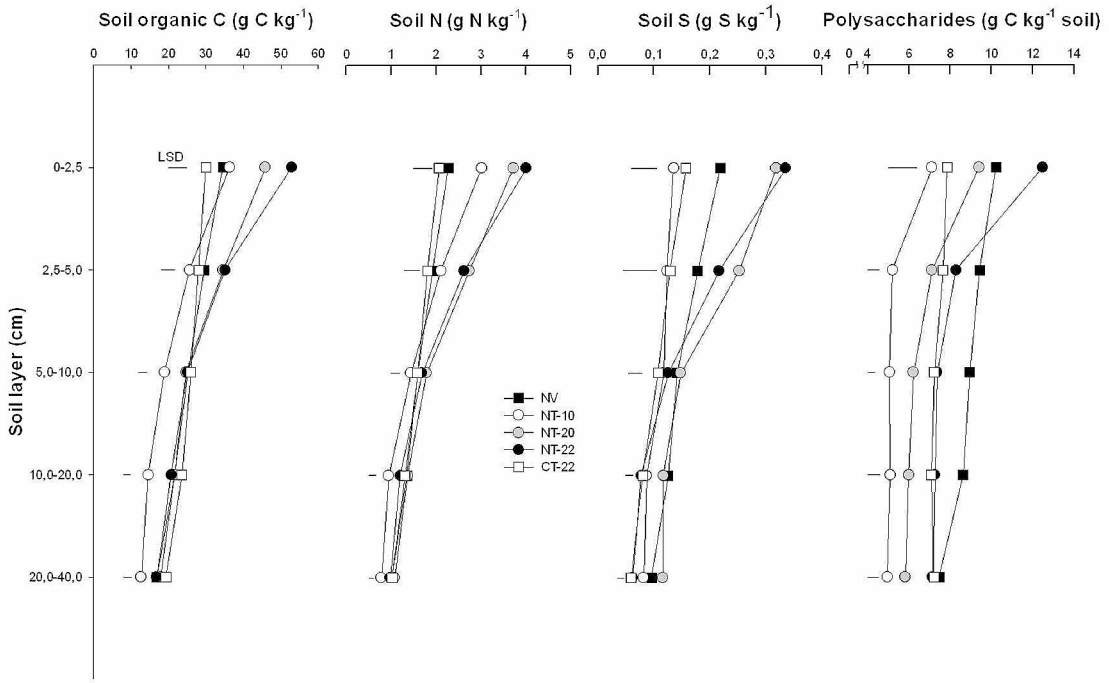


**Fig. 2** Microbial biomass C and nitrogen (**a** and **b**), basal respiration and metabolic quotient (qCO2) (**c** and **d**) managed under 22 years of conventional tillage (CT-22), no-tillage of different durations (NT-10 years, NT-20 years, and NT-22 years), and under the neighboring native field (NF) of a Typic Hapludox. Bars indicate the least significant difference (LSD – Student, $P < 0.05$) between means. Values are means of five replicates. No significant difference was observed for the basal respiration in 20–40 cm depth only

A significant difference was also observed among CT-22, NT-10 and NT-20 at 2.5-5 cm and 5–10 cm depth. The difference in microbial N concentration under NT-20 and NT-22 compared to CT-22 was even greater than that of microbial C in the surface soil layer (120% and 109%, respectively). Furthermore, significant differences were observed in the MB-C: MB-N ratio at 0–2.5 cm depth (Table 4), representing higher ratios in CT-22 (6.6) and NT-10 (6. 9) compared to NT20 (5.5), NT-22 (5.1) and NV (5.6).

Although both MB-C and SOC were reduced under conventional plow-based systems, no consistent reduction in microbial quotient was observed among CT-22, NT-20 and NT-22 at 0–5 cm depth (Table 4). However, significant changes in the MB-C: SOC ratio were observed in soil NT-10 compared to CT-22, NT-20 and NT-22 in the 0–40 cm depth and in soils NT-20 and NT-22 compared to CT-22 at 5–40 cm depth. Furthermore, significant changes at 0–40 cm depth were observed in response to land use management, with values varying among 27.7, 12.5, 28.1, 15.6 and 16.2 mg MB-C $g^{-1}$ SOC under NV, CT-22, NT- 10, NT-20 and NT-22, respectively (Table 4). Although whitout statistical differences, the microbial quotient decreased with increasing depth in NV soil and increased slightly in NT soils. Long-term tillage with plow decreased the microbial quotient and concentrations of MB-C and MB-N.

The average BR ranged from 467 to 1536 mg C-$CO_2$ $kg^{-1}$ soil 10 $day^{-1}$ at 2.5 cm depth and from 94 to 173 mg C-$CO_2$ $kg^{-1}$ soil 10 $day^{-1}$ at 20–40 cm depths. The microbial BR rate in the NV soil was lower ($p < 0.05$) than that the different tillage treatments at 0-2.5, 2.5-5, and 5–10 cm depths (Fig. 2c). In deeper soil layers, lower BR rates in NV soil were observed, although these differences were not significant. The same trend was observed for the $qCO_2$, with lower values observed in NV soil (Fig. 2d). A higher $qCO_2$ ($p < 0.05$) was observed in NT-22 soil at 0–2.5 cm depths, and no significant differences were observed between CT-22, NT-20 and NT-22 at 2.5–40 cm depths. The average $qCO_2$ in soils under NT-10, NT-20 and NT-22 was 53%, 14% and 11% lower, respectively, then that under CT-22. Using NV as the base-line, steady-state equilibrium was characterized as a higher MB-C (590 mg $kg^{-1}$ soil) and microbial quotient (27.7 mg MB-C $g^{-1}$ SOC) and a lower $qCO_2$ (0.23 mg C-$CO_2$ $mg^{-1}$ MB-C) compared with NT-22 (350 mg MB-C $kg^{-1}$ soil, 16.2 mg MB-C $g^{-1}$ SOC, 0.87 mg C-$CO_2$ $mg^{-1}$ MB-C) and CT-22 (280 mg $kg^{-1}$ soil, 12.5 mg MB-C $g^{-1}$ SOC, 0.98 mg C-$CO_2$ $mg^{-1}$ MB-C) soils.

**Table 4** Changes in MB-C: MB-N, MB-C: SOC, and MB-N: TN in an Oxisol (Typic Hapludox) under a land use chronosequence (means of five replicates)

| Soil microbial parameters[§] | Depth | Land use chronosequence | | | | |
|---|---|---|---|---|---|---|
| | (cm) | NV | NT-10 | NT-20 | NT-22 | CT-22 |
| **MB-C: MB-N** | 0-2.5 | 5.70ab | 6.65a | 4.66b | 4.97b | 6.69ab |
| | 2.5-5 | 5.43ab | 7.16a | 4.77b | 5.27ab | 6.54ab |
| | 5–10 | 5.87ns | 6.76 | 5.68 | 6.48 | 6.91 |
| | 10–20 | 6.05ns | 8.25 | 6.69 | 7.27 | 7.49 |
| | 20–40 | 6.79b | 9.33a | 7.23ab | 7.63ab | 7.02b |
| *P value*[‡‡] | *(Depth)* | 0.72 | > 0.05 | > 0.05 | 0.33 | 0.29 |
| Mean | | 6.35 | 8.44 | 6.59 | 7.08 | 7.07 |
| **MB-C: SOC** | 0-2.5 | 34.23a | 18.35b | 12.79c | 13.20c | 14.14c |
| mg SMBC $g^{-1}$ SOC | 2.5-5 | 28.42a | 20.85b | 13.04c | 14.93c | 12.55c |
| | 5–10 | 27.10a | 22.38a | 15.04b | 15.94b | 10.79c |
| | 10–20 | 28.01a | 31.24a | 16.27b | 16.17b | 11.65c |
| | 20–40 | 26.79a | 30.02a | 16.16b | 16.90b | 13.16c |
| *P value*[‡‡] | *(Depth)* | 0,576 | > 0.001 | 0,196 | 0,180 | > 0,01 |
| Mean | | 27.7 | 28.1 | 15.6 | 16.2 | 12.5 |
| **MB-N: TN** | 0-2.5 | 91.36a | 33.22b | 33.88b | 35.01b | 30.76b |
| mg SMBN $g^{-1}$ TN | 2.5-5 | 80.35a | 35.45b | 34.47b | 37.84b | 29.74b |
| | 5–10 | 73.33a | 43.53b | 36.61bc | 36.48bc | 25.44c |
| | 10–20 | 74.12a | 58.21b | 38.51c | 38.25c | 27.98c |
| | 20–40 | 65.43a | 52.35b | 37.16c | 38.21c | 35.47c |
| *P value* | *(Depth)* | 0,128 | > 0.001 | 0,898 | 0,968 | > 0,01 |
| Mean | | 71.1 | 50.5 | 37.1 | 37.8 | 31.7 |

[§] MB-C: soil microbial biomass carbon; MB-N: soil microbial biomass nitrogen; SOC: soil organic carbon; and TN: total nitrogen. [‡] Means followed by the same lowercase letter in the rows (comparison among treatments within each depth) do not differ by Tukey test at $P < 0.05$. [‡‡]*P* value for comparison of means among depths within each treatment. Mean across soil depths

### 3.3 Relationships among soil Fertility Attributes, SOC Pools and Microbial Activities

The data in Fig. 3a show the projections of land use according to the two principal components of the PCA, which respectively explained 45% and 32% of the total variance. The first axis separates NV and CT-22 from NT-22, while the second axis separates NV from CT-22 and NT-10. The variables separating NV from CT-22 are associated with soil MB-C and MB-N and lability, represented by soil TP. The main variables that separate NT-22 from CT-22 are associated with the SOC, N S and P concentrations and the BR rate and soil fertility status. Significant correlations between SOC, N, soil fertility parameters and microbial activities were consistent with the relationship revealed by the PCA (Fig. 3b).

Highly significant correlation coefficients among TP, and MB-C or MB-N emphasize the dependence of microbial biomass on easily decomposable biomass C. Although the correlation coefficients were highly significant ($p < 0.001$) among the data ($n = 150$), contrasting trends were observed within treatments ($n = 25$): (i) the correlation coefficient for the CT-22 treatment was significant ($p < 0.01$), indicating that the interrelation between the SOC and microbial biomass under long-term CT (Table 5) is also affected by residue and fertilizer incorporation; and (ii) the MB-C and MB-N concentrations rose with increasing SOC concentration under long-term NT use.

The data on the soil pH revealed different responses of MB-C and MB-N, $qCO_2$ and BR in the chronosequence. The correlation coefficient within each land use treatment ($n = 25$) revealed contrasting responses (Table 5). A non-significant correlation was observed between the pH, MB-C and MB-N for NV ($r = 0.07$ and $-0.13$) and CT-22 treatments ($r = 0.37$ and $r = 0.38$). In contrast, a significant relationship ($p < 0.001$) was observed among pH, MB-C and MB-N in NT soils. The trends in the correlation coefficients showed that long-term NT treatment changed the soil fertility, thereby increasing the pH, MB-C and MB-N.

## 4 Discussion

The results of this study indicated that after 22 years of the clearance of native fields and the setting up of NT and CT systems, the soil organic C, N, and S concentrations under CT-22 were 66%, 58%, and 52%, respectively, of those in NT-22 soils at 0–5 cm depths. The absence of soil plow allied with high C input trough crop residues increased SOC stocks. In consequence, N and S stocks that, for being

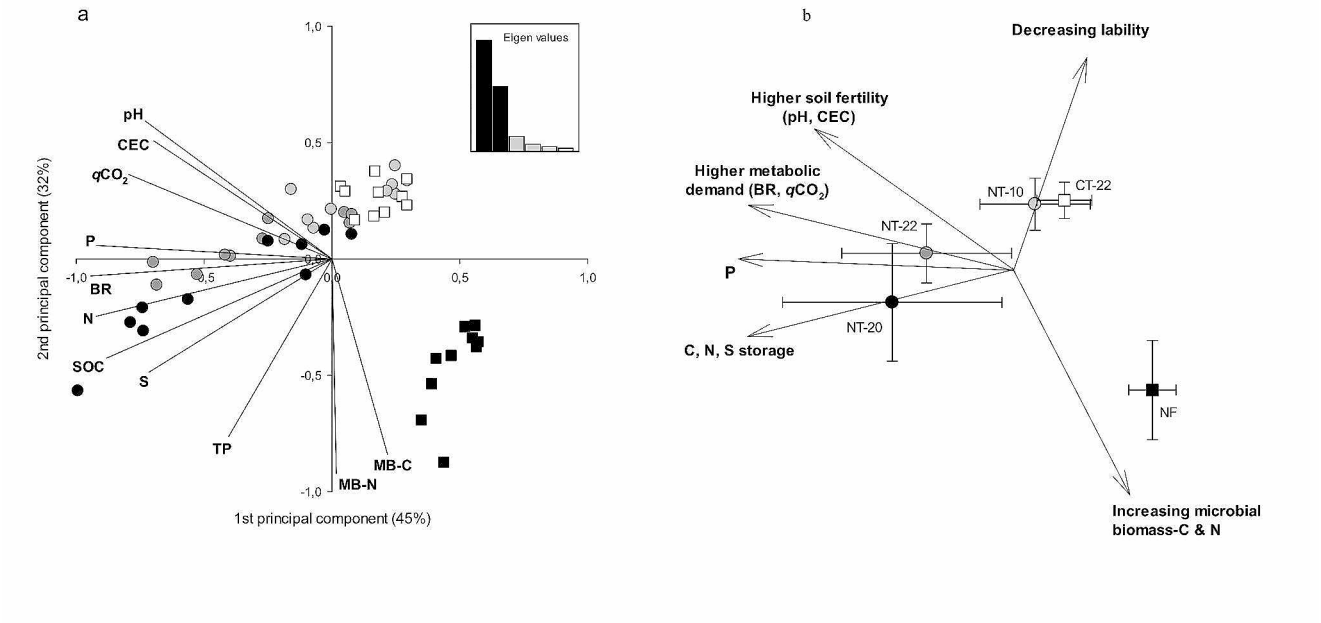


**Fig. 3** Principal component analysis (**a**) for the different land uses and management practices (black square: NF; open square: CT-22, gray circle: NT-10, dark grey circle: NT-20, and black circle: NT-22). Insert: graph of PCA eigenvalues, retained eigenvalues (> 2) in black. (**b**) Schematic diagram illustrating the main trends observed for each land use and management practices

mainly in anion forms, are highly dependent on organic matter immobilization avoiding leaching also increased. Previous studies revealed increasing the NT adoption time increased the soil C and N stocks (De Oliveira Ferreira et al. 2016; Oliveira Ferreira et al. 2018a, 2024), the inorganic-N concentration, and the net mineralization in the soil (De Oliveira Ferreira et al. 2009; Cardoso et al. 2011; Sá et al. 2011). The SOC, N, and S concentrations were highly stratified with increasing depth under NV and NT chronosequences. The stratification ratio has been proposed as an indicator of the soil quality under diverse tillages (Sá and Lal 2009), and was significantly higher under NT than under CT. An express decline in the SOC concentration upon conversion of the natural vegetation to an agricultural ecosystem is typically followed by the establishment of a new equilibrium within 30 to 50 years (Wagner 1981). Sá et al. (2022) estimate that the C losses during the first phase of cropping occurred at a mean rate of 0.88 Mg $ha^{-1}$ $yr^{-1}$ at 0–40 cm depth. In comparison, the increase in SOC under NT-22 occurred at a rate of 0.99 Mg $ha^{-1}$ $yr^{-1}$, restoring the SOC to even a higher concentration than that under NV.

Lower S concentrations were observed in NT-10 soil (Fig. 1) In addition, the C: S ratio was ∼1.5 higher in NT-10 (238) soil than in NT-22 (141) soil at a 0–5 cm depth. Most of the S in soils is bound to organic molecules, representing more than 90% of the S concentration in soils (De Oliveira Ferreira et al. 2018b). The nature of the biomass-C input was similar between the tillage chronosequences and cannot explain the difference in soil S concentration (Fig. 1). Differences in the microbial community structure between NT-10 to NT-22 might influence responses to enzyme-mediated substrate catalysis, particularly the activity of arylsulphatase, involved in the mineralization of ester sulphate in soils. Balota et al. (2014) observed higher arylsulphatase activity (+219%) in NT soil compared with CT in Oxisol. In addition, the microbial nutrient demand is driven through the relatively constrained stoichiometry of the microbial biomass (C: N:P: S ratios) (Cleveland and Liptzin 2007). Thus, the enzymatic activity might enhance the availability of the most limiting nutrients to meet microbial nutrient demands (Pereira et al. 2021). Additional analyses of plant tissues, the ratios between enzymatic activities, or the soil microbial diversity are needed to confirm this hypothesis.

The SOC stock in the 0–40 cm layer varied at rates of -4.83, +17.4, +18.9 and −0.13 Mg C $ha^{-1}$ in soils NT-10, NT-20, NT -22 and CT-22, respectively, compared to the NV soil, suggesting that the inadequate supply of available inorganic N, P and S may reflect the low level of SOC accumulation in the NT-10 soil, while the input of biomass-C was higher among land use treatments (Sá et al. 2020). Studies have shown that NT practices can limit the supply of nutrients essential for the stabilization of SOM, which largely depends on N, P and S (Briedis et al. 2021; De Oliveira Ferreira et al. 2021). It has been reported that the stoichiometry of soil organic carbon fine fractions adheres to the ratio C: N:P: S = 10,000:833:200:143 (Kirkby et al. 2013). Kirkby et al. (2014) also showed that optimal C sequestration requires more additional nutrient inputs than

**Table 5** Bivariate Pearson correlation for soil variables from the land use chronosequence comprising all data (NF, CT-22, NT-10, NT-20, and NT-22, $n = 150$), and for native field (NF), conventional plow-based tillage (CT-22), and no-tillage (NT-22) ($n = 25$)

| | pH | CEC | P | SOC | TN | TS | C:N | TP | MBC | MBN | MBC:MBN | MBC:SOC | BR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **All land use** | | | | | | | | | | | | | |
| CEC | **,922** | | | | | | | | | | | | |
| P | **,757** | **,748** | | | | | | | | | | | |
| SOC | **,581** | **,616** | **,854** | | | | | | | | | | |
| TN | **,670** | **,720** | **,886** | **,974** | | | | | | | | | |
| TS | **,450** | **,507** | **,757** | **,872** | **,871** | | | | | | | | |
| C:N | **-,639** | **-,709** | **-,633** | **-,564** | **-,718** | **-,592** | | | | | | | |
| TP | -,004 | ,029 | **,490** | **,714** | **,598** | **,635** | -,085 | | | | | | |
| MBC | -,110 | -,005 | **,194** | **,459** | **,436** | **,475** | **-,332** | **,531** | | | | | |
| MBN | -,018 | ,080 | **,335** | **,625** | **,597** | **,653** | **-,410** | **,654** | **,869** | | | | |
| MBC:MBN | -,171 | **-,188** | **-,318** | **-,486** | **-,475** | **-,487** | **,327** | **-,408** | -,165 | **-,574** | | | |
| MBC:SOC | **-,517** | **-,432** | **-,404** | **-,329** | **-,299** | -,149 | ,030 | -,063 | **,624** | **,359** | **,285** | | |
| BR | **,719** | **,733** | **,916** | **,884** | **,897** | **,763** | **-,601** | **,537** | **,272** | **,408** | **-,365** | **-,382** | |
| $qCO_2$ | **,717** | **,700** | **,749** | **,664** | **,661** | **,515** | **-,404** | **,288** | -,131 | ,047 | **-,328** | **-,655** | **,858** |
| **NF** | | | | | | | | | | | | | |
| CEC | -,170 | | | | | | | | | | | | |
| P | **-,546** | **,829** | | | | | | | | | | | |
| SOC | **-,475** | **,892** | **,948** | | | | | | | | | | |
| TN | **-,456** | **,910** | **,946** | **,990** | | | | | | | | | |
| TS | **-,469** | **,864** | **,942** | **,951** | **,949** | | | | | | | | |
| C:N | ,305 | **-,654** | **-,610** | **-,589** | **-,691** | **-,590** | | | | | | | |
| TP | **-,539** | **,728** | **,852** | **,905** | **,873** | **,873** | **-,434** | | | | | | |
| MBC | -,193 | **,786** | **,661** | **,765** | **,753** | **,751** | **-,466** | **,602** | | | | | |
| MBN | -,226 | **,904** | **,811** | **,914** | **,918** | **,839** | **-,595** | **,764** | **,843** | | | | |
| MBC:MBN | ,098 | -,257 | -,353 | -,373 | -,368 | -,239 | ,128 | **-,418** | ,059 | **-,429** | | | |
| MBC:SOC | ,117 | **,417** | ,168 | ,248 | ,256 | ,282 | -,276 | ,077 | **,792** | ,442 | **,451** | | |
| BR | -,159 | **,881** | **,806** | **,881** | **,865** | **,884** | **-,460** | **,788** | **,859** | **,850** | -,103 | **,466** | |
| $qCO_2$ | -,164 | **,670** | **,728** | **,728** | **,713** | **,736** | -,328 | **,737** | ,387 | **,542** | -,241 | -,109 | **,794** |

| | pH | CEC | P | SOC | TN | TS | C:N | TP | MBC | MBN | MBC:MBN | MBC:SOC | BR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CT-22** | | | | | | | | | | | | | |
| CEC | **,652** | | | | | | | | | | | | |
| P | **,539** | **,925** | | | | | | | | | | | |
| SOC | **,596** | **,913** | **,941** | | | | | | | | | | |
| TN | **,512** | **,885** | **,963** | **,945** | | | | | | | | | |
| TS | **,437** | **,828** | **,932** | **,878** | **,986** | | | | | | | | |
| C:N | **-,449** | **-,793** | **-,868** | **-,771** | **-,926** | **-,959** | | | | | | | |
| TP | ,352 | **,538** | **,742** | **,690** | **,721** | **,713** | **-,600** | | | | | | |
| MBC | ,370 | **,663** | **,827** | **,792** | **,869** | **,881** | **-,773** | **,780** | | | | | |
| MBN | ,382 | **,675** | **,832** | **,747** | **,833** | **,847** | **-,776** | **,742** | **,927** | | | | |
| MBC:MBN | -,210 | -,300 | -,338 | -,204 | -,246 | -,248 | ,306 | -,209 | -,169 | **-,517** | | | |
| MBC:SOC | -,121 | -,023 | ,217 | ,087 | ,273 | ,369 | -,317 | **,455** | **,675** | **,601** | ,001 | | |
| BR | ,260 | **,673** | **,812** | **,761** | **,723** | **,677** | **-,572** | **,604** | **,667** | **,704** | -,365 | ,174 | |
| $qCO_2$ | ,161 | **,576** | **,625** | **,594** | **,502** | **,433** | -,359 | ,343 | ,341 | **,420** | -,372 | -,157 | **,915** |
| **NT-22** | | | | | | | | | | | | | |
| CEC | **,939** | | | | | | | | | | | | |
| P | **,966** | **,925** | | | | | | | | | | | |
| TOC | **,932** | **,919** | **,962** | | | | | | | | | | |
| TN | **,941** | **,924** | **,974** | **,997** | | | | | | | | | |
| TS | **,944** | **,923** | **,978** | **,992** | **,999** | | | | | | | | |
| C:N | **-,868** | **-,889** | **-,883** | **-,820** | **-,856** | **-,871** | | | | | | | |
| TP | **,758** | **,728** | **,788** | **,877** | **,863** | **,854** | **-,611** | | | | | | |
| MBC | **,916** | **,917** | **,926** | **,926** | **,927** | **,926** | **-,798** | **,833** | | | | | |
| MBN | **,835** | **,813** | **,881** | **,892** | **,905** | **,911** | **-,797** | **,768** | **,823** | | | | |
| MBCMBN | **-,399** | -,363 | **-,440** | **-,442** | **-,467** | **-,485** | **,468** | -,353 | -,308 | **-,748** | | | |
| MBCTOC | **-,492** | **-,448** | **-,512** | **-,584** | **-,578** | **-,571** | **,476** | **-,451** | -,265 | **-,491** | **,404** | | |
| BR | **,905** | **,905** | **,923** | **,970** | **,967** | **,962** | **-,788** | **,899** | **,920** | **,893** | **-,481** | **-,506** | |
| qCO2 | **,869** | **,878** | **,864** | **,893** | **,897** | **,895** | **-,803** | **,761** | **,776** | **,861** | **-,580** | **-,620** | **,937** |

§ CEC: Cation Exchange Capacity; P: phosphorus; SOC: soil organic carbon; TN: total nitrogen; TS: total sulfate; C:N ratio; TP: total polysaccharides; MB-C: microbial biomass carbon; MB-N: microbial biomass nitrogen; BR: microbial basal respiration; and qCO2: metabolic quotient (respiration-to-microbial biomass C). ‡ For all data ($n = 150$), bold numbers are significant $P > 0.05$; values > 0.41 are significant according to the t-test at $P < 0.001$. ‡‡ Within treatment ($n = 25$), values < 0.41 are not significant; values from 0.41 to 0.55 are significant at $P < 0.05$; values from 0.56 to 0.65 are significant at $P < 0.01$; and values > 0.66 are significant at $P < 0.001$ according to the t-test

those required for agricultural production alone to optimize the use of the C-rich biomass input by microbial communities, thereby limiting energy loss from enzyme production. This reduces the mineralization of the most stable SOM reservoir, yet the low S concentration observed in the NT-10 soil may negatively impact SOC sequestration and should be considered.

As an active component of SOC, microbial biomass is an important attribute of soil quality as a sensitive indicator of early changes in SOM in response to land use management (Powlson et al. 1987). The high MB-C and MB-N observed at site NV and soil NT-22 could reasonably reflect the absence of soil disturbance and the availability of above and below-ground C sources, supporting increased microbial biomass and activity (Belhadj et al. 2023). Furthermore, higher concentrations of soil TP were observed in NT soils compared to CT-22, which typically reflects improvements in soil structural stability, soil aggregation, and soil biological activity level (Debska et al. 2020; Liu et al. 2005). The present study shows that conventional tillage with plowing deteriorates soil microbial properties, and NT systems are successful in recovering some of these lost properties.

NT-22 significantly increased the microbial C (26%) and microbial N (34%) across soil depths compared with CT-22. CT affects soil conditions, including the disruption of fungal hyphae, the decreased protection of the microbial habitat, increased extreme temperature conditions, and decreased soil-moisture content, resulting in a faster turnover of aggregates, increased losses in SOC, and a reduction in C availability to support microbial growth (De Oliveira Ferreira et al. 2018b; Hok et al. 2021). The results of the present study are consistent with those of previous studies, including Kaschuk et al. (2010), for a similar Oxisol in Paraná, Brazil. These authors showed that CT soils had less organic C and MB-C, compared with NT systems, resulting in a decline in other soil properties (e.g., aggregation and microbial activities).

A comparison of the NT and CT systems clearly showed that NT improved MB-C and MBN, and these differences increased with time, such that after 22 years these two parameters were approximately 57% and 104% higher, respectively, under NT-22 than CT-22 in the 0–5 cm soil depth. Although the MB-C was relatively stable after the 10th year of NT, the MB-N continued to increase with time under NT. In addition, a markedly higher MB-C/MB-N ratio was observed under CT-22 (6.6) compared with NT-20 (4.7) and NT-22 (5.1), and similar values were reported with NT-10 (6.9) at 0–5 cm depths. Several studies, including Silva et al. (2022), have shown that the increased MB-N can contribute to increased grain yields and SOC sequestration in NT soils. Furthermore, Broring et al. (2023) showed that part of the microbial biomass-derived C is retained in the soil microbial food web and contributes significantly to the formation of a stabilized SOM pool. Thus, the increase in microbial biomass is crucial for SOC sequestration.

The $qCO_2$ indicates a higher metabolic demand in the soil surface layers under CT-22, NT-20 and NT-22. NV exhibited the highest MB-C and MB-N and the lowest BR rate and $qCO_2$, suggesting that native vegetation can support a more stable ecosystem with high levels of biological activity. In contrast, higher qCO2 values were observed in CT-22, NT-20, and NT-22 soils, suggesting the presence of microbial communities with high respiration rates and the predominance of microbes with higher C resource utilization. Furthermore, increased $CO_2$ release from the NT soils might indicate a reduced potential for C sequestration.

Although additional research is warranted, the contrasting patterns in the BR, $qCO_2$ and MB-C observed in CT and NT soils seem to reflect differences in the microbial communities and the efficiency of C use by soil microbes (Gupta et al. 2022). Considering that MB-C: SOC is an index of microbial activity, the lack of relationship indicates the presence of opportunistic microbial communities in CT-22 soils, while a negative correlation in NT soils suggests the presence of microbial communities with high C use efficiency, contributing to SOC sequestration.

Several studies, including Wardle (1992) and Silva et al. (2022), have shown that the soil pH is as important as the soil C and N concentrations in influencing the size, community structure and activity of the microbial biomass. The NT soils yielded a close relationship among pH, SOC concentrations and microbial biomass activity. However, a low correlation was observed between pH and MB-C in the CT-22 soil and suggests intense soil disturbance and SOC depletion may not be strongly tied to soil pH.

## 5 Conclusions

The application of no-till (NT) systems over many years induces major changes in soil organic carbon (SOC), nitrogen (N), sulfate (S) stocks and microbial activity. The increase in SOC under NT for 22 years (NT-22) occurred at a rate of 0.99 Mg $ha^{-1}$ $yr^{-1}$, restoring SOC to a higher concentration than under native vegetation (NV), emphasizing no till potential to sequester carbon in soils. On the other hand, contrasting trends were observed in the conventional tillage for 22 years (CT-22) and NT systems. High basal respiration (BR), levels of microbial biomass (MB-C) and metabolic quotient ($qCO_2$) were observed in NT soils, compared to CT-22 suggesting that soil microbes compete intensely for the small amount of available C in CT soils. Our results can also indicate a change in microbial communities in NT compared to CT soils since NT soils

produced a close relationship between pH, SOC concentrations and microbial biomass activity. Although further studies are necessary to confirm this hypothesis, it can indicate that microbial communities with high C use efficiency drive SOC sequestration and this occurred more in NT than in CT.

**Acknowledgements** The authors would like to thank Lucio Miranda ("*in memoriam*") (Santa Branca Farm) and Franke Dijkstra (Frankanna Farm).

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