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# Effect of 26-years of soil tillage systems and winter cover crops on C and N stocks in a Southern Brazilian Oxisol

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**ABSTRACT:** Soil management and crop rotation are key factors in controlling the accumulation of C and N in the soil profile, but their long-term effect remains poorly understood for deep soil layers, especially in subtropical conditions. Using a long-term experiment (26-years), this study aimed to evaluate the effect of different soil management systems associated with different winter cover crops on C and N accumulation in a very clayey (72 % clay) soil up to 1 m deep. Two tillage systems [conventional tillage (CT) and no-tillage (NT)] were cultivated with eight winter cover crops (black oat, rye, common vetch, hairy vetch, oilseed radish, wheat, blue lupine, and fallow) in a subtropical Oxisol from Southern Brazil. Soil samples were taken in eight soil layers up to 1.00 m soil depth after 26 years of experiment and, also from an adjacent native forest. After forest clearing, the C stock in the 0.00-0.20 m soil layer was reduced by 45 % in only 10 years (from 1976 to 1986) of soil tillage. Twenty-six years after the beginning of the experiment, C and N stock in 0.00-0.20 m soil layer were 13 and 20 % higher in NT compared to CT, with the greatest differences in C and N content observed in the 0.00-0.05 m layer. When associated with winter cover crops, NT accumulated 0.6 and 0.06 Mg ha$^{-1}$ yr$^{-1}$ more C and N than CT with winter fallow in the 0.00-0.20 m soil layer. No-tillage and CT recovered 95 and 83 %, respectively, of the C stock found in the 0.00-0.20 m layer from the native forest. However, in the 0.00-1.00 m soil layer, the positive effect of NT on soil C accumulation compared to CT was diluted, and no clear effect of NT was verified. Moreover, no difference in winter cover crops on soil C and N stocks were observed in all soil layers, possibly due to their similar residues input (3.3-4.9 Mg ha$^{-1}$ yr$^{-1}$). No-tillage associated with high biomass input through winter cover crops promoted a faster recovery of soil C and N stock than in CT and, therefore, is an efficient tool to improve soil C and N accumulation even in Oxisols with high clay content.

**Keywords:** conservation agriculture, C accumulation rate, N accumulation rate, long-term no-tillage.

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## INTRODUCTION

The key role of soils on the carbon (C) budget has been recognized and demonstrated by numerous studies (Li et al., 2020; Xiao et al., 2020). The intensification of agriculture, with land-use change from natural ecosystems (forests, grasslands, meadows) to cultivated areas induced large depletion of soil organic matter (SOM) content due to excessive tillage, that decreased aggregate stability and enhanced soil erosion from plowed soils, and favored organic matter decomposition (Paustian et al., 2016; Lal, 2018). Cultivation with conventional tillage with plowing or with no-tillage with reduced residue inputs to the soil accelerates the decrease of SOM content (Huang et al., 2020). This is especially the case in central and south Brazil where large areas of natural vegetation were converted to cultivation of grain crops, such as soybean, corn, and wheat. Moreover, in tropical and subtropical regions with intensely weathered soils (e.g., Oxisols), SOM plays a key role in several soil properties, such as cation exchange capacity, nutrient reservoir and availability, aggregate stability, and water holding capacity (Mendonça and Rowell, 1996; Oorts et al., 2003). Soil management that reduces soil tillage such as conservation agriculture or no-tillage (or direct seeding) and with permanent soil cover (crops, crop residue, cover crops) allows maintaining or increasing soil organic carbon (SOC) stocks and soil fertility/productivity (Oldfield et al., 2019). Therefore, increasing soil organic C content in tropical or subtropical soil is a sustainable way to recover soil quality and fertility while decreasing atmospheric $CO_2$.

Studies carried out under tropical and subtropical climate conditions showed that growing cover crops with high residues input in soil under no-tillage (NT) increases the C and N stocks faster than conventional tillage (CT) systems with plowing (Bayer et al., 2004; Fageria et al., 2005; Calegari et al., 2008; Conceição et al., 2013; Nascente et al., 2013; Carvalho et al., 2014; Yadav et al., 2019). The absence of soil disturbance in NT maintain high amounts of crop residues covering the soil surface, increasing the C and N sequestration in soil surface layers (0.00-0.20 m) over the years (Bona et al., 2006; Zanatta et al., 2007; Sombrero and de Benito, 2010; Conceição et al., 2013). However, these changes are slow and cumulative, which needs temporal evaluation with long-term experiments. Most of these studies demonstrated that this is effective in the topsoil (0.00-0.20 m) and has limited effect in deeper soil layers (>0.30 m depth). Moreover, these changes may not be detected in short trial periods, especially in deeper soil layers, although it represents a potential for sequestration of C in agricultural soils (Dieckow et al., 2005; Boddey et al., 2010; Reis et al., 2014). In this sense, Veloso et al. (2018) have recently demonstrated that about half of the soil organic C storage in a 30-year-old NT system in an Acrisol from Southern Brazil was due to the C increase in the subsurface layer (0.30-1.00 m).

Growing cover crops is also essential to improve soil physical and chemical properties, soil quality (Calegari et al., 2013a), and increase soil C stocks (Poeplau and Don, 2015). Three main plant families are commonly used in crop rotation/succession in Southern Brazil. The first group is the legumes, such as common (*Vica sativa* L.) and hairy vetch (*Vicia villosa* Roth), and blue lupine (*Lupinus angustifolius* L.), which biologically fix atmospheric $N_2$, have low C:N ratio and nutrient-rich biomass, that can enhance soil microbial activity and can transfer N to successor crop (Frageria and Nascente, 2014). Legume cover crops have a high potential for dry matter production, reaching C accumulation rates of 1.42 Mg $ha^{-1}$ $yr^{-1}$ in the 0.00-1.07 m in Southern Brazil (Diekow et al., 2005). Besides, N fixation by legume cover crops can be as twice as efficient in accumulating C compared to N fertilization (Veloso et al., 2018).

The second group is the grasses, such as black oat (*Avena strigosa Schreb*), rye (*Secale cereale* L.), and wheat (*Triti-cum aestivum* L.), which have a high C:N ratio and, therefore, remain long time on the soil surface, favoring N microbial immobilization during decomposition process (Thomas and Asakawa, 1993). The slow decomposition of cereals straw due to high C:N ratio participates also to the increase of SOC content of topsoil in NT system (Chen et al., 2017). Grasses have an abundant and voluminous root system, and a reticular root network that penetrates deep into the soil (Fitter and Stickland, 1991). Due to their particular architecture, the growth, death, and regeneration of the grass root system usually increase the C input, resulting in soil C accumulation rate up to 0.50 Mg ha$^{-1}$ yr$^{-1}$ in the 0.00-1.00 m soil layer (Alburquerque et al., 2015).

The third group is the Cruciferae, among which the most used species in Southern Brazil is oilseed radish (*Raphanus sativus* L.). Radish has high dry matter production and high capacity to recycle nutrients, and has a tap root system, fairly deep and aggressive, reaching over two meters deep, which contributes to soil decompaction (Doneda et al., 2012). Although some studies have found soil C accumulation rates ranging from 0.04 to 0.85 Mg ha$^{-1}$ yr$^{-1}$ in rotation cropping systems including radish (Boddey et al., 2010), the isolated effect of radish as a cover crop on the soil accumulation of C and N is still little studied.

It is well known that the literature on the effect of soil management and cover crops on the accumulation of C and N in the soil is abundant. However, there is still a consensus that it is extremely important to carry out studies of this nature especially investigating the effects of long-term monitoring (longer than 10-20 years – Poeplau and Don, 2015; Du et al., 2017; Jian et al., 2020), and in deeper layers (deeper than 0.20-0.30 m – Li et al., 2020; Xiao et al., 2020) than those usually used to assess the accumulation of C and N in the soil. Therefore, the present study was carried out aiming to evaluate the long-term (26-years) effect of soil management systems associated with different winter cover crops to accumulate C and N in a very clayey (72 % clay) soil up to 1.00 m deep.

## MATERIALS AND METHODS

The present study was carried out using one of the oldest Brazilian experiments comparing soil tillage systems to assess the effect of winter cover crops and NT on organic C and N sequestration in a very clayey (72 % clay fraction) soil after 26 years of experiment. In a previous study at 19 years after the establishment of this experiment, Calegari et al. (2008) evaluated the content and stock of C up to 0.60 m depth. Knowing the importance of the temporal evaluation of long-term experiments, we revisited this experimental area seven years later, with a new approach involving; (i) two more species of winter cover crops (*Secale cereale* L. and *Vica sativa* L.); (ii) the evaluation of soil N contents and stocks; and (iii) a more detailed assessment of C and N stocks up to 1.00 m deep. This new approach also allowed studying the temporal variation of the C stock in the 0.00-0.20 m layer, from the assessment of C stock of a native forest area adjacent to the experimental area (time 0), and the assessments made at the beginning (after 10 years of cultivation with soil tillage - 1976-1986) and 19 years after the beginning of the experiment (2005 – Calegari et al., 2008). Moreover, this new approach allowed us to investigate the possible contribution of deeper soil layers (up to 1.00 me) to accumulate N and C in the soil.

### Study site description

The experiment was carried out at the Experimental Station of the Agronomic Institute of Paraná (IAPAR), in Pato Branco, Southwest region of Paraná State (52° 41’ W and 26° 07’ S), Brazil, at an elevation of about 760 m a.s.l. The climate of the region is subtropical humid, with cool summer, with an average temperature varying between 14 and 22 °C, with a mean annual rainfall of 2,000 mm per year and without a defined dry season. The monthly average temperature and precipitation of the experimental station in the period 1979–2014 are presented in figure 1.

The landscape is gently undulating with slopes between 4 and 7 %. The soil of the experimental site is an Oxisol (Rhodic Hapludox) or *Latossolo Vermelho Aluminoférrico* (Costa, 1996), very acid (natural soil pH in water about 4.5, 1:1 ratio, v/v) with a high clay content, formed from basaltic rocks. Soil texture is very homogeneous in the 0.00-1.00 m soil layer, presenting 720 g $kg^{-1}$ of clay, 140 g $kg^{-1}$ of silt, and 140 g $kg^{-1}$ of sand. Mineralogical composition estimated by using a combination of magnetic susceptibility measurements, chemical dissolution procedures, and X-ray diffraction analysis indicates that clay fraction is composed of kaolinite (680 g $kg^{-1}$), aluminum-hydroxyl-interlayered vermiculite (HIV) (130 g $kg^{-1}$), iron oxides (140 g $kg^{-1}$), and gibbsite (50 g $kg^{-1}$) (Costa, 1996).

## Experimental design

The experimental area was covered by Atlantic Forest (*Mata Atlântica*) until 1976, when the forest was cut down and burned to cultivate corn (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L.) in the summer with intensive soil tillage with plowing and disc harrowing before each crop. From 1976 to 1986, the area was managed as commercial farming. The intense soil disturbing induced strong erosion and soil loss. In 1986 the experiment was established to compare the impact of tillage systems such as no-tillage (NT) and conventional tillage (CT, one plowing, and two disks harrowing) cultivated with different winter cover crops on soil erosion and soil properties. Winter cover crops were: black oat (*Avena strigosa Schreb*), rye (*Secale cereale* L.), common vetch (*Vica sativa* L.), hairy vetch (*Vicia villosa* Roth), oilseed radish (*Raphanus sativus* L.), wheat (*Triti-cum aestivum* L.), blue lupine (*Lupinus angustifolius* L.), and fallow. The winter cover crops plot (240 $m^2$) were randomized in each block and were conducted in triplicates. After that, each block was split into two strips of 120 $m^2$ (20 × 6 m): one strip managed in NT and the other in CT, and both of them underwent the same winter cover crops.

## History of the experimental area

The winter cover crops were grown in 13 of the 26 seasons (1986, 1987, 1988, 1989, 1990, 1992, 1994, 1999, 2000, 2001, 2005, and 2008). In the years 1991, 1995, 1996,

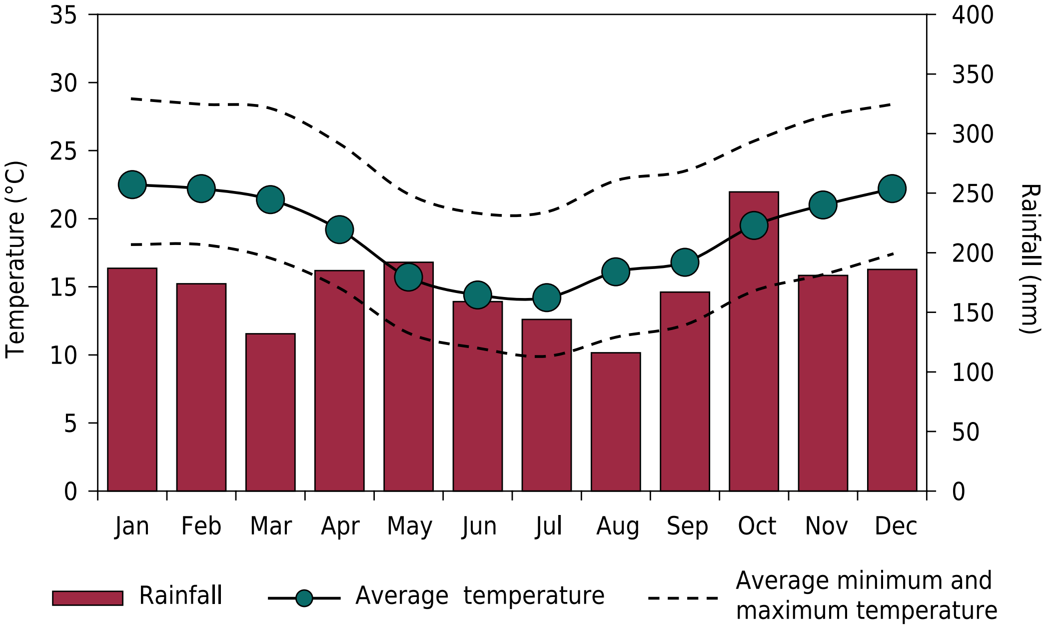

**Figure 1.** Average temperature and precipitation in the experimental area in the period of 1979–2014. Source: Meteorological Station of Experimental Station of IAPAR, Pato Branco, state of Paraná.

1998, 2006, and 2009, black oat was grown, and in 1997, 2002, 2003, 2004, and 2007, black oat intercropped with forage oilseed radish was cultivated in all plots, except in fallow treatment. In 2010, black oat was cultivated in consortium with hairy vetch in all plots, except in fallow treatment. In 2011, all cover crops were cultivated, except black oat, which was replaced by white oat in all treatments, except in fallow. In 1993, all plots remained fallow. Cover crops were controlled at the flowering stage by cutting with a knife roller or by applying herbicides (fallow), and occasionally, after the cutting-roller, control was complemented with herbicide. The plots with wheat were harvested until 1995 (seven crops), and the plant residues left on the soil surface before soil preparation for the summer crop. In the remaining years, wheat was handled like other cover crops.

In summer, the whole area was cultivated with soybean (*Glycine max* L. Merr) or corn. During the experimental period, soybean was grown 14 times (1989, 1990, 1991, 1993, 1995, 1997, 1998, 2000, 2001, 2002, 2004, 2005, 2007, and 2009) and corn was grown 11 times (1986, 1987, 1988, 1992, 1994, 1996, 1999, 2003, 2008, 2010, and 2011). In 2006, the soil remained fallow in summer.

The same amounts of fertilizer were added each year to all treatments of summer crops according to local recommendations (CQFS-RS/SC, 2016). Phosphorus (P), potassium (K), and 1/3 N were applied in the row of the direct seeding, and the remaining N, after 45 days. For the 26 years of cultivation, 771 kg ha$^{-1}$ P, 750 kg ha$^{-1}$ K, and 580 kg ha$^{-1}$ N were added to the soil. During the experimental period, eight applications of dolomitic limestone powder on the soil surface at doses of: 1.0, 2.0, 3.0, 1.5, 2.0, 2.0, 2.0, and 2.0 Mg ha$^{-1}$ in all plots in 1989, 1992, 1995, 1999, 2001, 2006, 2009, and 2011, respectively, totaling 15.5 Mg ha$^{-1}$. The production of dry matter of shoots of summer and winter crops are found in table 1.

## Soil sampling

Soil was sampled in November 2012. In each plot, a trench 0.5 m wide by 0.5 m long and 1 m deep was open with a cutting shovel. Soil samples were taken at 0.00-0.05, 0.05-0.10, 0.10-0.20, 0.20-0.30, 0.30-0.40, 0.40-0.60, 0.60-0.80, and 0.80-1.00 m. Soil samples under native forest were also collected in a contiguous area to the experimental area, which presents the same soil type and clay content of the experimental area, located approximately 500 m of experiment. Soil was dried in oven with forced air circulation at ±50 °C, sieved in 2 mm mesh, and stored. In the same trench, undisturbed soil samples were collected in each soil layer with cylinders of 4.0 cm height and 5.6 cm of diameter (100 cm$^3$) to determine soil bulk density. Three replicates were made for each soil depth.

## Soil analyses

Carbon and N soil contents were determined by dry process through an elemental analyzer. Soil samples were ground and homogenized before weighing into tin capsules, which were put in a combustion chamber at a temperature of approximately 975 °C to oxidize the material. Gases were detected by a thermal conductivity sensor and converted into a percentage of C and N. The C and N stocks (Mg ha$^{-1}$) were calculated for each soil layer based on the equivalent mass correction between the treatments using changes in bulk density with time (Ellert and Bettany, 1995), which considers the mass of a reference soil (in this case, CT fallow) to be equal to the remaining treatments. The annual rate of SOC accumulation to depths of 0.20 and 1.00 m soil of each treatment was calculated according to equation 1:

$$\textit{SOC accumulation (Mg C ha}^{-1}\textit{ yr}^{-1}\textit{)} = [(\textit{SOC stock in treatments, Mg ha}^{-1}) - (\textit{SOC stock in reference, Mg ha}^{-1})]/t \qquad \text{Eq. 1}$$

in which reference is CT fallow system and t is the time elapsed since the implementation of the experiment, i.e., 26 years.

**Table 1.** Total aboveground dry biomass yields over 26 years in different winter treatments and soil tillage systems

| Winter treatments | Aboveground dry biomass yield | | | | | |
|---|---|---|---|---|---|---|
| | Winter cover crops residues[1] | | Summer crops residues[2] | | Total | |
| | NT | CT | NT | CT | NT | CT |
| | Mg ha$^{-1}$ yr$^{-1}$ | | | | | |
| Oat | 4.87 | 4.09 | 4.49 | 4.40 | 9.36 | 8.49 |
| Rye | 4.28 | 3.87 | 4.46 | 4.27 | 8.82 | 8.14 |
| Common vetch | 4.24 | 3.83 | 4.67 | 4.31 | 8.91 | 8.14 |
| Hairy vetch | 3.95 | 3.32 | 4.41 | 4.18 | 8.36 | 7.49 |
| Radish | 4.05 | 3.34 | 4.64 | 4.52 | 8.70 | 7.86 |
| Lupin | 4.23 | 3.68 | 4.63 | 4.32 | 8.86 | 8.00 |
| Wheat | 3.83 | 3.40 | 4.23 | 4.03 | 8.06 | 7.43 |
| Fallow[3] | 1.80 | 1.39 | 4.34 | 4.22 | 6.14 | 5.61 |
| Average | 3.91 a | 3.37 b | 4.48 a | 4.28 b | 8.40 a | 7.65 b |

[1] Values are the sum of biomass yield of the winter cover crop used as treatments in 1986, 1987, 1988, 1989, 1990, 1992, 1993, 1994, 1999, 2000, 2001, 2005,2008 and 2011; plus black oat biomass yield in1991, 1995, 1996, 1998, 2006 and 2009; plus black oat + radish biomass yield in 1997, 2002, 2003, 2004, 2007 and 2010. [2] Values are the sum of crops residues produced by maize cultivated in 1986, 1987, 1988, 1992, 1994, 1996, 1999, 2003, 2008, 2009 and 2011) and by soybean cultivated in 1989, 1990, 1991, 1993, 1995, 1997, 1998, 2000, 2001, 2002, 2004, 2005, 2007 and 2010. [3] The fallow biomass yield consisted of weed biomass. CT: conventional tillage; NT: no-tillage. Means followed by the same letter in the row, comparing soil tillage systems for winter, summer, and total aboveground dry biomass yield, are not significantly different at p<0.05, according to the Tukey test.

### Statistical analyses

Before analysis, the data were analyzed for normality by the Kolmogorov-Smirnov test and homogeneity of variance by the Levene test. The data were then submitted to analysis of variance (ANOVA) and when significant ($p<0.05$), the mean of each winter cover crop and soil tillage systems were compared by Tukey's honestly significant difference (HSD) ($p<0.05$). The MIXED procedure was performed to compare the effects of winter cover crops (*C*) and soil tillage systems (*T*), which consider the main factors and their interactions as fixed factors and the variable block and experimental errors as random effects. All analyses were performed using SAS® v.9.4 (Statistical Analysis System Institute Cary, Carolina do Norte). The statistical model used in the analysis of variance of C and N content and stocks in each soil layer was:

$$Y_{ijkl} = \mu + B_i + C_j + error\ (ij) + T_k + error\ (ik) + CT_{jk} + error\ (ijk) \qquad \text{Eq. 2}$$

in which $\mu$: overall experimental average; *B*: block ($i$ = 1, 2, 3); *C*: cropping system ($k$ = 1, 2, 3, 4, 5, 6, 7, 8); *T*: tillage system ($j$ = 1, 2); and *error*: experimental error.

Furthermore, in each soil layer, we compared the data from reference soil under forest with the cultivated soil using the Mann–Whitney *U*-test (non-parametric test).

## RESULTS

### Effects of conversion from native forest into cultivated areas

The large amount of organic residue added to the soil surface by native forest and its undisturbed soil condition led to higher C and N contents (up to 0.20 m) compared to cultivated soil (NT and CT) (Figure 2). Combining our data from native forest with C stock in the 0.00-0.20 m layer at the beginning of the experiment (data from Calegari et al., 2008), a reduction of approximately 45 % in C stock could be observed in only ten years of conventional cultivation with intensive soil plowing (from 1976 to 1986) (Figure 3a).

## Effects of soil tillage system

After 26 years of experiment establishment, the content of C and N was affected by soil tillage systems only in the topsoil layer (0.00-0.05 m) (Table 2 and Figure 2). On average, the aboveground dry biomass was 10±1 % higher in NT than in CT (Table 1). In the same experimental area, a decrease in soil acidity and an increase in the availability of P, K, Ca, and Mg were observed under NT, especially in the most superficial soil layer (up to 0.20 m) (Tiecher et al., 2017). Therefore, the higher soil fertility combined with low soil disturbance and higher residues input (Table 1) in NT system provided higher content of C and N (0.00-0.05 m soil layer) compared to CT (Figure 2). As a result, C and N stocks up to 0.20 m depth were higher in NT compared to CT (Tables 3 and 4).

Since the beginning of the experiment, a recovery of C stocks in the 0.00-0.20 m soil layer was observed in both NT and CT (Figure 3a). The C stocks continued to increase

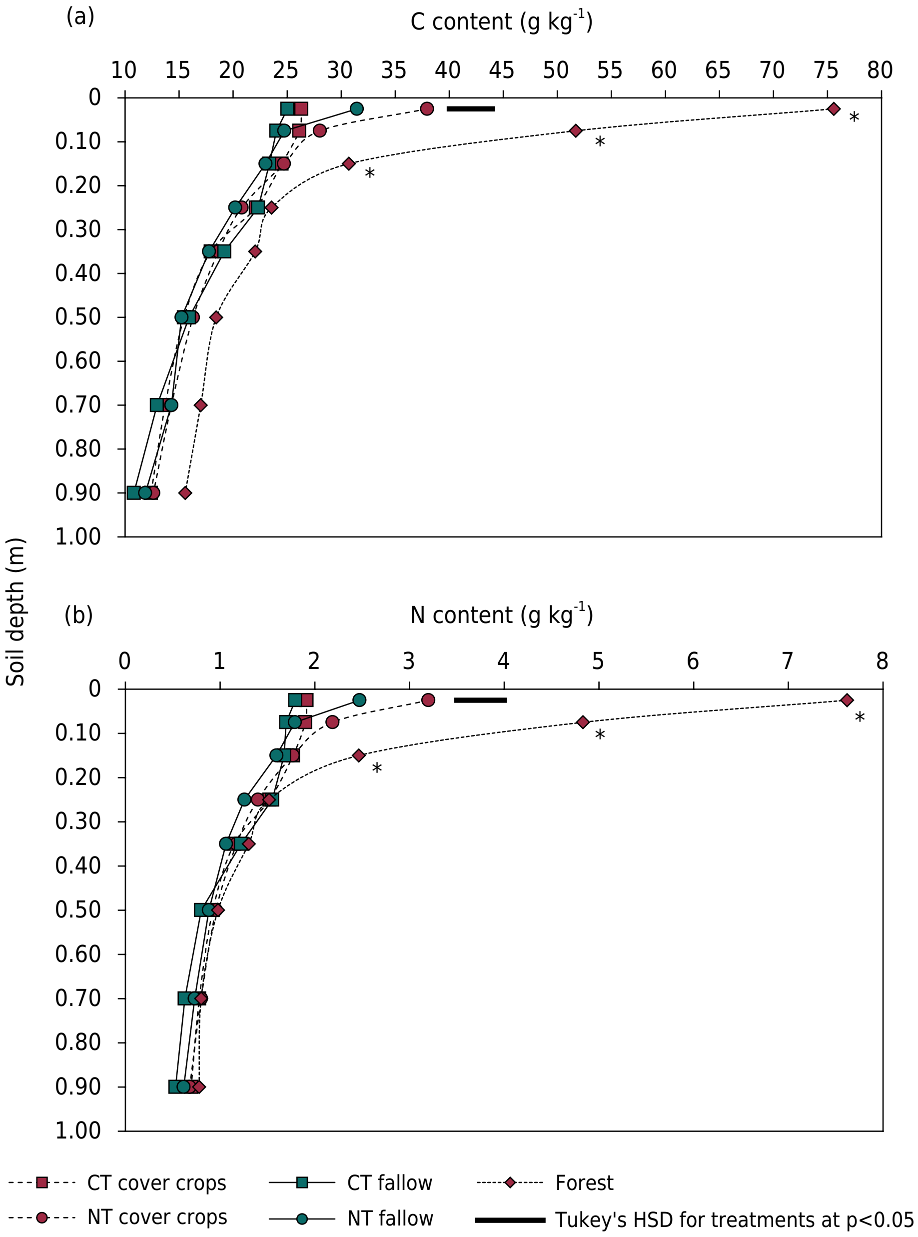

**Figure 2.** Carbon (a) and nitrogen (b) content in the 0.00-1.00 m soil layer as affected by soil tillage (CT: conventional tillage; NT: no-tillage) and winter cover crops. Forest soil was not compared statistically with the experimental treatments. Asterisks indicate a significant difference in the C or N content in each soil layer between the forest soil ($n$ = 3) and the cultivated soil (NT plus CT treatments, $n$ = 48) by the non-parametric Mann-Whitney $U$-test.

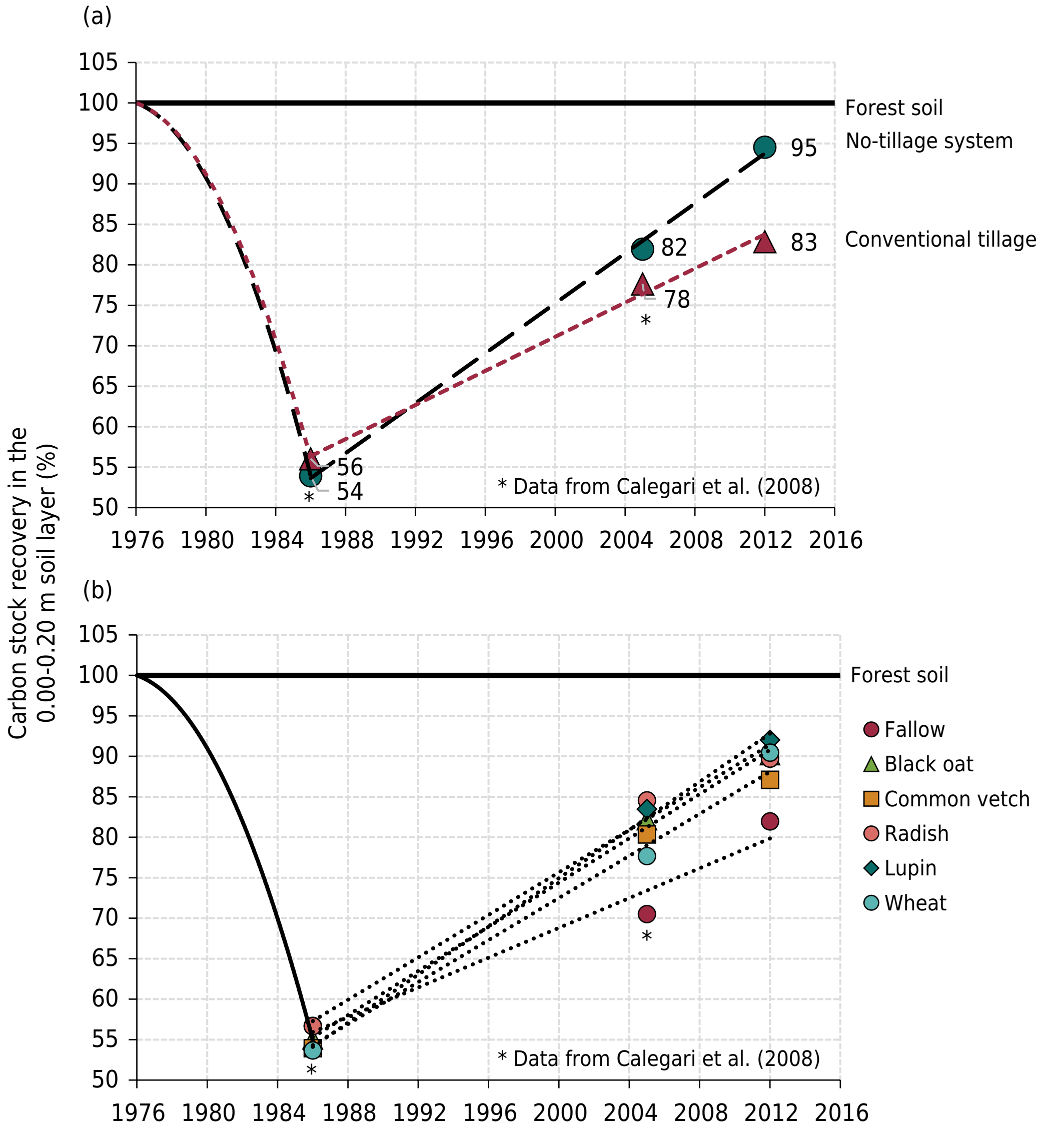

**Figure 3.** Carbon stock recovery in the 0.00-0.20 m soil layer after forest clearing (1976) for intensive cultivation with soil plowing (from 1976-1986), at the beginning of the experiment (1986), and at 19 and 26-years after as affected by soil management system (a) and winter cover crops treatments (b).

**Table 2.** Significance of the effects of experimental factors and their interactions as resulting from analysis of variance (ANOVA) for C and N content

| **Variable** | **Soil layer** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **0–0.05 m** | **0.05–0.10 m** | **0.10–0.20 m** | **0.20–0.30 m** | **0.30–0.40 m** | **0.40–0.60 m** | **0.60–0.80 m** | **0.80–0.10 m** |
| *Soil organic carbon content* | | | | | | | | |
| Winter treatment (W) | * | NS | NS | NS | NS | NS | NS | NS |
| Soil tillage (T) | ** | NS | NS | NS | NS | NS | NS | NS |
| W × T | * | NS | NS | NS | NS | NS | NS | NS |
| *Soil nitrogen content* | | | | | | | | |
| Winter treatment (W) | * | NS | NS | NS | NS | NS | NS | NS |
| Soil tillage (T) | ** | NS | NS | NS | NS | NS | NS | NS |
| W × T | * | NS | NS | NS | NS | NS | NS | NS |

NS: not significant. *Significant at $p<0.05$. **Significant at $p<0.01$.

linearly as shown by soil sampling performed at 19 (Calegari et al., 2008) and 26 years (this study) in both soil tillage systems.

However, NT soil clearly recovered the C stock faster (about 1.6 % $yr^{-1}$) than in CT (about 1.0 % $yr^{-1}$). Accordingly, following the linear trend observed, 4 and 17 years would take to recover the initial C stocks found in native forest for NT and CT, respectively. Moreover, 26-years of continuous NT recovered about 95 (Figure 3a) and 91 % of the original C stocks in the 0–0.20 and 0–1.00 m soil layer, while original N stocks were recovered by 79 and 85 % for the same soil layers (Table 4). In addition, accumulation rates of C and N were 0.38 and 0.06 Mg $ha^{-1}$ $yr^{-1}$, respectively (Figure 4) on average for all winter cover crops.

**Table 3.** Significance of the effects of experimental factors and their interactions as resulting from analysis of variance (ANOVA) for C and N stock and accumulation rates

| Variable | Soil layer | | |
|---|---|---|---|
| | 0-0.20 m | 0.20-1.00 m | 0-1.00 m |
| *Carbon stock* | | | |
| Winter treatment (W) | NS | NS | NS |
| Soil tillage (T) | * | NS | NS |
| W × T | NS | NS | NS |
| *Nitrogen stock* | | | |
| Winter treatment (W) | NS | NS | NS |
| Soil tillage (T) | * | NS | NS |
| W × T | NS | NS | NS |
| *Carbon accumulation rate* | | | |
| Winter treatment (W) | NS | NS | NS |
| Soil tillage (T) | * | NS | NS |
| W × T | NS | NS | NS |
| *Nitrogen accumulation rate* | | | |
| Winter treatment (W) | NS | NS | NS |
| Soil tillage (T) | * | NS | NS |
| W × T | NS | NS | NS |

NS: not significant. * Significant at $p<0.05$.

**Table 4.** Soil C and N stocks affected by soil tillage system after 26 years and comparison with the adjacent native forest

| Soil layer | Conventional tillage | No-tillage | Forest | P-value(1) | |
|---|---|---|---|---|---|
| | | | | Conventional tillage | No-tillage |
| Carbon stock (Mg $ha^{-1}$) | | | | | |
| 0–0.20 m | 69.6 (83) b | 79.4 (95) a | 84.0 | 0.643 | 0.165 |
| 0.20–1.00 m | 127.5 (91) a | 124.7 (89) a | 140.5 | 0.316 | 0.537 |
| 0–1.00 m | 197.1 (88) a | 204.1 (91) a | 224.5 | 0.487 | 0.487 |
| Nitrogen stock (Mg $ha^{-1}$) | | | | | |
| 0–0.20 m | 5.0 (65) b | 6.1 (79) a | 7.7 | 0.054 | 0.934 |
| 0.20–1.00 m | 7.7 (95) a | 7.3 (90) a | 8.1 | 0.247 | 0.440 |
| 0–1.00 m | 12.7 (80) a | 13.4 (85) a | 15.8 | 0.589 | 0.537 |

Values for each soil tillage system at each depth are average of eight winter treatments ($n$ = 24). Values in parenthesis are the relative C and N stock compared to native forest soil. Means followed by the same letter in the row, comparing soil tillage systems in each soil layer, are not significantly different at $p<0.05$ according to the Tukey test. (1) Probability of difference between the native forest soil ($n$ = 3) and the cultivated soil under no-tillage ($n$ = 24) or conventional tillage ($n$ = 24) by Mann–Whitney $U$-test.

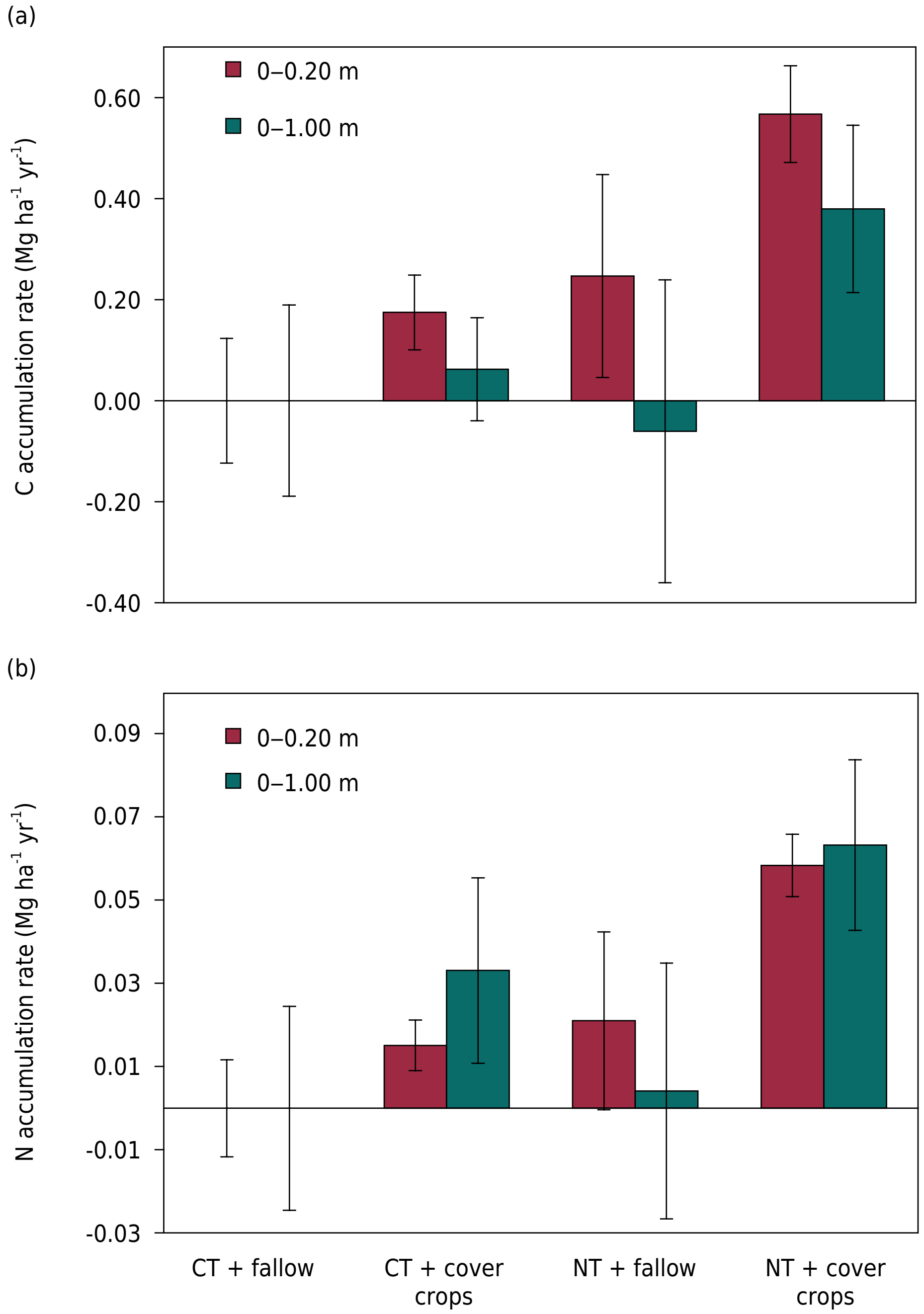

**Figure 4.** Carbon (a) and nitrogen (b) accumulation rates in the 0–0.20 and 0–1.00 m soil layer as affected by soil tillage and winter treatments. NT, no-tillage; CT, conventional tillage.

By contrast, in the 0.20–1.00 m soil layer, there was no significant difference in the stock of C and N between soil tillage systems (Tables 3 and 4) because of lower C content between 0.20 m and 1.00 m and dilution effect of organic inputs in surface. As a result, no significant difference on C and N stocks between soil tillage systems were observed when considering the whole soil profile up to one-meter depth (0–1.00 m) (Tables 3 and 4).

## Effects of winter treatments

The fallow in the winter resulted in lower C and N content in 0–0.05 m soil layer compared to cover crops in both soil tillage systems (Figure 2), which was associated to 40 % lower biomass inputted in the fallow compared to cover crops (Table 1). In CT, C and N content in 0–0.05 m soil layer with cover crops was only 5.1 and 6.7 % higher, respectively, compared to fallow, while in NT this difference was 52 and 78 % higher (Figure 2).

## DISCUSSION

Soil C content generally decreases after conversion of natural environments into agricultural systems. In a global meta-analysis with 385 studies on land-use change in the tropics, Don et al. (2011) found that conversion of primary forest into cropland resulted in an average decrease of 25 % in SOC stock. The decline in C stock observed in our study was faster than results found in the literature. Sá et al. (2014) observed a decrease in C stock around 38 % in 0–0.20 m soil layer after 29 years of cultivation. Sharma et al. (2014) observed a reduction of 25 % in C stock in 0–0.50 m soil layer after 100 years of cultivation. Cook et al. (2014) found a reduction of 33 % in C stock in the 0–0.45 m soil layer after 20 years of cultivation. The rapid decline in soil C stock in our study (45 % in SOC stock in 10 years) is possibly related to the low input of crops residues to the soil from 1976 to 1986 (data not presented) with intensive soil disturbing by plowing and disc harrowing and also due to the higher soil C mineralization rate in tropical and subtropical soils compared to temperate soils (Bayer et al., 2006) that was further promoted by tillage, aggregate disruption and subsequent erosion.

Accordingly, higher SOC losses by conversion of primary forest into cropland are observed in regions with higher precipitation (Don et al., 2011). Moreover, some authors have demonstrated that in Oxisols, the loss of SOC with deforestation may be greater than in other soil classes due to the rapid depletion of organic P and the sorption on the inorganic constituents, thus reducing its availability and consequently the biomass production and C inputs to the soil (Hartemink, 1997). This is particularly important in acidic soils and with high contents in Fe oxides as the present study.

The higher residues input and lower soil disturbance in NT increases the physical protection of C and N in aggregates and favors organo-mineral interaction, which ends up reducing the oxidative potential of the soil, increasing the stock of C and N compared to CT (Veloso et al., 2019). The higher residues input in NT observed for both summer and winter crops (Table 1) was possibly associated to the improvement in physical, chemical, and biological properties of soil. Several published studies derived from the experimental site support this affirmation. Although the amount of fertilizers and lime applied is the same in the CT and NT treatments, evaluations made at the 19 and 23 years of installation of this experiment have shown that the NT has lower soil acidity and greater base saturation in the 0-0.20 m layer, and also presents greater availability of P in the 0-0.10 m layer, compared to CT (Calegari et al., 2013a; Tiecher et al., 2012a, 2017). Additionally, it was also verified in this experiment that the NT provides greater microbial biomass, polysaccharide, glomalin-related soil protein, and soil enzyme activity (Balota et al., 2014), higher content of organic P and P stored in microbial biomass (Tiecher et al., 2012b; Rheinheimer et al., 2019), and improved soil aggregation (Calegari et al., 2013b).

The C accumulation in superficial soil layers under NT has been well documented in other studies in Brazil (Bayer et al., 2004; Fageria et al., 2005; Calegari et al., 2008; Nascente et al., 2013; Carvalho et al., 2014) and worldwide (Xiao et al., 2020). This is due to high inputs of crop residues with a permanent cover of the soil surface. However, our results show that even after 26 years of experiment, the levels of C and N in the 0.05-1.00 m soil layer did not change either by soil management system or cover crops. It is important to note that an evaluation of the C and N stock of 0-1.00 m resulted in similar values between NT and CT (Table 4). As observed in the global meta-analysis of Xiao et al. (2020), these results illustrate that using the overall SOC stock change of the whole sampling soil profile deeper than 0.30 m masks the beneficial change in SOC stock in the 0–0.05 m soil over long-term adoption of NT.

No-tillage and cover crops work synergistically to increase SOC, mainly via slowing down soil C decomposition rates and increasing cumulative C inputs (Huang et al., 2020). In the treatments with winter cover crops, the soil C accumulation rate reached 0.57 Mg ha$^{-1}$ yr$^{-1}$ in NT (Figure 4). This result is very similar to the rate of carbon sequestration of 0.56 Mg ha$^{-1}$ yr$^{-1}$

observed in a global meta-analysis of cropland soil C changes due to cover cropping (Jian et al., 2020). However, these results of soil C accumulation rates are slightly below those found in other studies in Southern Brazil (Diekow et al., 2005; Alburquerque et al., 2015), which is possibly due to the higher resilience of the studied soil with the clayey texture of the soil (720 g kg$^{-1}$ of clay) therefore dominated by kaolinite and iron oxides that bind less organic matter than smectitic soils (Lal, 2004b; Kleber et al., 2007).

Results clearly demonstrated that NT proved to be efficient in accumulating C in the 0–0.20 m soil layer, resulting in a C accumulation rate 41 % higher than CT (Figure 4). However, it is important to note that winter cover plants were more important than the soil management system used to accumulate C and N in the soil. Comparing the treatments without cover crops, the use of NT resulted in an accumulation rate of C and N about 0.25 and 0.02 Mg ha$^{-1}$ yr$^{-1}$ higher than the CT (Figure 4). By contrast, comparing the NT treatments, the use of winter cover crops resulted in soil C and N accumulation rates about 0.32 and 0.04 Mg ha$^{-1}$ yr$^{-1}$ higher than the fallow (Figure 4). The importance of cover crops is also evident by the very similar soil C and N accumulation rates between treatments NT+fallow and CT+cover crops (Figure 4). This means that even with soil plowing, but using winter crop rotations, it is possible to obtain an accumulation of C and N similar to NT without cover plants. These results corroborate the meta-analysis by Li et al. (2020), showing that the residue retention is the main factor controlling soil C accumulation. This is due to the great potential of biomass production by cover crops in these areas of subtropical climate highlighting the efficiency of conservation management systems with growing crops during the winter combined with no soil disturbance to recover the C and N stocks as reported in other studies in subtropical climate conditions (Sisti et al., 2004; Bayer et al., 2006).

Although a linear trend of increasing the C content in the soil of the 0-0.20 m layer was observed since the beginning of the experiment in all treatments, it is evident that this increase is faster in NT compared to CT, and faster with winter cover crops than winter fallow (Figure 3). Poeplau and Don (2015) also found that the time since introduction of cover crops in crop rotations was linearly correlated with SOC stock change with an annual change rate of 0.32±0.08 Mg ha$^{-1}$ yr$^{-1}$ in a mean soil depth of 0.22 m and during the observed period of up to 54 years. We would expect differences in content and stock of C and N in the soil cultivated 26 years under different cover crops. However, C and N content were not affected by the cover crops (Figure 2) because shoot biomass yield in the winter was very similar between cover crops (102±11 Mg ha$^{-1}$ – Table 1). Accordingly, Poeplau and Don (2015) also found no influence of plant functional type (legume vs. non-legume) on the annual SOC stock change. Nevertheless, growing winter cover crops has recovered around 1.4 % yr$^{-1}$ of C stock in the 0–0.20 m layer, while winter fallow increases only 1.0 % yr$^{-1}$ (Figure 3b), which is possibly associated to a lower efficiency of spontaneous plants to add C in surface soil layers compared to cover crops. In addition, over the years, there is rotation in the summer between soybean and corn in all treatments, which may have diluted the isolated effect of the grass and legume cover crops evaluated.

## CONCLUSIONS

We evaluated the effect of a quarter of a century of different soil tillage systems and cover crops on C and N stocks and accumulation rates in a very clayey Oxisol. The results obtained in the present study demonstrate the importance of long-term evaluation in studies of C sequestration, since even after 26 years, the soil organic C stock in the topsoil (0 to 0.20 m) continues to increase linearly.

The use of cover crops in winter has a greater effect on the rates of accumulation of C and N in the soil, compared to tillage effect. Despite the fact that the different groups of cover crops (legumes, grasses, and crucifers) had no effect on soil C and N contents and stocks, possibly due to the similar supply of residues in all crop rotations, the winter fallow should always be avoided, as it results in a lower rate of C and N accumulation in the soil regardless of the soil management system.

There is a synergism when using cover crops associated with NT system that promotes a faster recovery of the soil C and N stock originally contained in the soil under native forest. Both the content and the stock of C and N were influenced especially by the most superficial soil layer (0-0.05 m). Care should be taken when comparing the effect of different soil management strategies when considering deeper layers of soil below 0.20 m due to the dilution effect.

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