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Article

Soil organic matter fractions in an Oxisol under tillage systems and winter cover crops for 26 years in the Brazilian subtropics

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ABSTRACT: The improvement of carbon (C) accumulation in soils has been one of the main purposes of the conservation systems in agricultural production. This study aimed to assess the long-term effect of conventional tillage (CT) and no-tillage (NT) combined with winter cover crops, black oat and oilseed radish, and fallow on C accumulation and stabilization in a very clayey Oxisol in Southern Brazil. Soil samples were collected in the 0-0.05, 0.05-0.10 and 0.10-0.20 m layers of a 26-year-old experiment. Distribution of size-class aggregates, C stock in aggregates, total C stock, and C stocks in the physical fractions, free particulate organic matter (free-POM), occluded particulate organic matter (occluded-POM) and mineral-associated organic matter (min-OM) were assessed. NT had a higher percentage of macroaggregates and C stock in this size-class, and also higher C stock in bulk soil, free-POM and occluded-POM fractions than CT in 0-0.05 m (Tukey's test $p < 0.05$), due to higher input of biomass and minimum soil mobilization in NT. Oat and radish had higher C stock in macroaggregates than fallow in 0.05-0.10 m (Tukey's test $p < 0.05$). Radish had the highest C stock in the free-POM (0-0.05 m). Fallow decreased the stabilization of macroaggregates and C accumulation in free-POM, due to the lower C input from aboveground biomass over the years. In conclusion, NT after 26 years improved C accumulation and stabilization, mainly in the superficial layer and in POM fractions, and winter cover crops favored the formation and stability of macroaggregates.

Key words: carbon stabilization, conservation agriculture, soil macroaggregates, density physical fractionation.

INTRODUCTION

Carbon (C) sequestration is one of the central strategies to climate change mitigation, which is one of the main global problems of the 21st century, directly and indirectly affecting the entire world population (Lal 2020). The soil, as the greatest global C pool, has three times higher C pools than in terrestrial vegetation and atmosphere (Lal 2004, Lehmann and Kleber 2015), with a great capability to act as a sink of atmospheric CO_2-C.

The improvement of C stock in soils has been one of the main objectives of the conservation systems in agricultural production, such as no-tillage (NT), globally recognized and consolidated as the basis for sustainable agriculture, and recommended by the Food and Agricultural Organization (Kassam et al. 2019). In 2022, NT in Brazil completes 50 years, and data from long-term experiments contributes to the promotion of this system, especially due to the improvements of soil properties. The association among NT and cover crops, used as soil cover and green manure, is an important strategy to increase the C accumulation and stabilization in tropical and subtropical soils (Bayer et al. 2006, Veloso et al. 2018, Veloso et al. 2019, Xavier et al. 2019), and also to improve the physical, chemical and biological properties of the soil (Balota et al. 2014, Moraes et al. 2016, Tiecher et al. 2017).

In addition, the adoption of NT with cover crops can alter the distribution of C soil among soil organic matter (SOM) physical fractions as reported by Briedis et al. (2018), and Conceição et al. (2013) who found that more C accumulated in the particulate organic matter (POM) fractions in NT than in conventional tillage (CT). Besides that, the C protected inside the macroaggregates, as occluded-POM, is also affected by management systems (Briedis et al. 2021) and constitutes the most important pool to increase the stabilizing and accumulating C in NT (Six et al. 2000; Veloso et al. 2019). The plant residue fragments (litter) protected within macroaggregates have a longer residence and interaction time with microaggregates and mineral particles in the soil (Tivet et al. 2013, Briedis et al. 2018, Ferreira et al. 2018), which could lead to organic matter stabilization as mineral-associated organic matter, which is really important for weathered soils.

However, the effects of long-term adoption of tillage systems and cover crops could be further explored, with a focus on C stabilization and its mechanisms. The hypothesis were:

- Conventional tillage decreases C stabilization, mainly in the particulate organic matter pools;
- Fallow decreases C stabilization in all C pools continuously over the years.

This study aimed to evaluate the effect of tillage systems and winter cover crops, in the long-term (26 years), on the SOM fractions and C stabilization in a very clayey Oxisol in the Brazilian subtropics.

MATERIALS AND METHODS

The study was carried out as part of a long-term experiment at the Experimental Station of the Agronomic Institute of Paraná (IAPAR) (currently Rural Development Institute of Paraná – IDR-Paraná), in Pato Branco, Southwest region of Paraná State, Brazil (52°41’ W, 26°07’ S; 600 m altitude). The climate is subtropical, humid mesotermic (Cfb, Köppen) (Alvares et al. 2013), with monthly average temperatures varying between 14 and 22°C, and mean annual precipitation of 2,000 mm distributed throughout the year. The monthly average temperature and precipitation in the experimental station over 30-years are presented in Fig. 1.

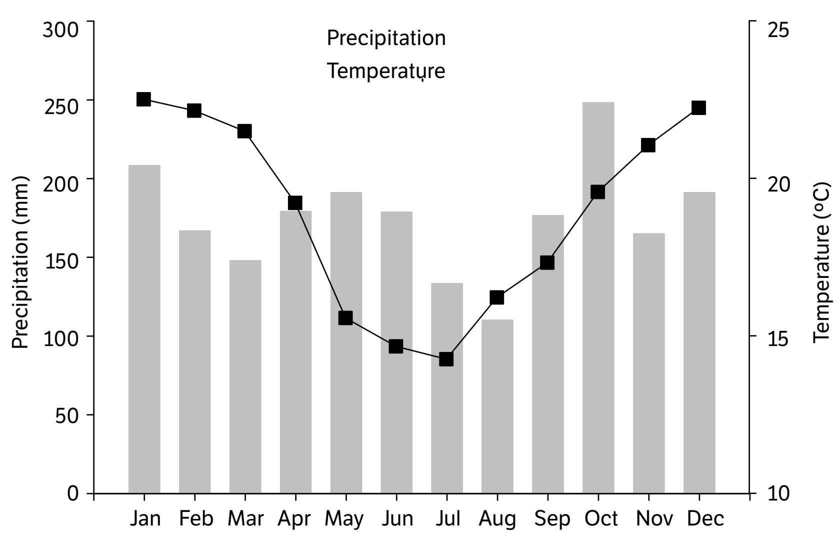


Figure 1. Thirty-years average precipitation and average temperature in the experimental area.

Source: Meteorological Station of Experimental Station of Agronomic Institute of Paraná (IAPAR) (currently Rural Development Institute of Paraná – IDR-Paraná), Pato Branco, Paraná State, Brazil.

The soil was classified as Latossolo Vermelho aluminoférrico – Brazilian Soil Classification System (Embrapa 2018) –, or Rhodic Hapludox – Soil Taxonomy (Soil Survey Staff 1999) –, with very clayey texture in the top 0.10 m (720 $g \cdot kg^{-1}$ clay, 140 $g \cdot kg^{-1}$ silt and 140 $g \cdot kg^{-1}$ sand). The mineralogical composition of the clay fraction is 68% kaolinite (phyllosilicate type 1:1), 13% vermiculite and/or montmorillonite (silicate type 2:1), 14% iron oxides and 5% gibbsite.

The local native vegetation was subtropical forest (*floresta ombrófila mista*), which was replaced by annual crops of corn (*Zea mays* L.), soybean (*Glycine max* Merrill.) and common bean (*Phaseolus vulgaris* L.) in conventional tillage for 10 years (1976-1986).

The experiment was installed in 1986 as a factorial combination of soil tillage systems and winter cover crops, arranged in a completely randomized block design with three replicates. The cover-crop plots (12 × 20 m) were randomly distributed in each block, and each block was subdivided into two strips comprising two soil tillage systems (6 × 20 m). Winter cover crops (main plots) were: black oat (*Avena strigosa* Schreb.) and oilseed radish (*Raphanus sativus* L.). The other plot was fallow, in which there was volunteer growth mainly of ryegrass (*Lolium multiflorum* Lam.) and/or spergula (*Spergula arvensis* L.). Tillage systems (subplots) were conventional tillage (CT), with one disc-plowing and two disc-harrowings, both for the establishment of winter and summer crops (twice a year); and no-tillage (NT). An adjacent area of native forest was used as soil reference, located approximately 500 m from the experiment, with similar characteristics of soil type and texture, and without anthropic effects.

The treatment cover crops were grown in winter 1986–1990, 1992, 1994, 1999–2001, 2005, 2008, 2011, and 2012 (14 years) (Fig. 2). However, all the plots (except fallow) were cultivated in winter 1991, 1995, 1996, 1998, 2006, and 2009 with black oat; in winter 1993 it was left fallow; in winter 1997, 2002, 2003, 2004, and 2007 it was under black oat plus radish intercropped; and in winter 2010 under black oat plus hairy vetch. This protocol was adopted to minimize phytosanitary problems associated with the continuous growth of the same species in the same plot. Cover crops were managed at the flowering stage with a knife roller. When necessary for weed control, herbicide was applied before summer sowing, and for the fallow plots, herbicide was applied to kill the volunteers plants.

Plots											
Plots	Oats	O M	O M	O M	O S	O S	O S	O M	F S	O M	O S
	Radish	R M	R M	R M	R S	R S	O S	R M	F S	R M	O S
	Fallow	F M	F M	F M	F S	F S	F S	F M	F S	F M	F S
	Year	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995

Plots	Oats	O M	O+R S	O S	O M	O S	O S	O+R S	O+R M	O+R S	O S
	Radish	O M	O+R S	O S	R M	R S	R S	O+R S	O+R M	O+R S	R S
	Fallow	F M	F S	F S	F M	F S	F S	F S	F M	F S	F S
	Year	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005

Plots	Oats	O -	O+R S	O M	O S	O+V M	O M	O M
	Radish	O -	O+R S	R M	O S	O+V M	R M	R M
	Fallow	F -	F S	F M	F S	F M	F M	F M
	Year	2006	2007	2008	2009	2010	2011	2012

O Black oats
R Radish
F Fallow
O+R Oats and radish intercropped
O+V Oats and vetch intercropped
M Maize
S Soybean

Figure 2. History of crops succession in winter and summer (maize and soybean) in the treatment plots (oats, radish and fallow) under no-tillage and conventional tillage, over the 26 years of the long-term experiment.

As summer crops, maize was cultivated in 12 years and soybean in the other 14 years, in a crop rotation scheme. The aboveground biomass of winter cover crops and aboveground residue of summer crops (maize and soybean) over 26 years are shown in Table 1.

Table 1. Aboveground biomass of winter cover crops and aboveground residue of summer crops (maize and soybean) in three winter cover-crop treatments (oat, radish, and fallow) and two soil tillage systems (CT and NT) for 26 years.

Treatments	Winter cover crops[A]		Summer crops[B]		Total		Mean annual	
	CT	NT	CT	NT	CT	NT	CT	NT
	(Mg DM ha^{-1})							
Oat	106.4	126.5	114.3	116.8	220.7	243.3	8.4	9.3
Radish	86.9	105.4	117.4	120.7	204.3	226.1	7.8	8.7
Fallow[C]	36.1	46.8	109.8	112.9	145.9	159.7	5.6	6.1

[A]Values are the sum of biomass yield of the winter cover crop used as treatments; [B]values are the sum of crops residues produced by maize and soybean; [C]the fallow biomass yield consisted of weed biomass; DM: dry matter; CT: conventional tillage; NT: no-tillage.

Fertilizations occurred in the summer crops according to soil analysis and each crop recommendations, with the same amount of fertilizer to each plot. For both, direct and conventional seeding, N (1/3 rate), P and K were applied in the row, and the remaining N was applied after 45 days to maize. The total soil fertilization (26 years) was 580 kg N·ha^{-1}, 771 kg P·ha^{-1}, and 750 kg K·ha^{-1}, over time. Dolomitic limestone powder was applied eight times during the experimental period, applied on the soil surface for NT and incorporated by plowing and harrowing for CT, totaling 15.5 Mg·ha^{-1}.

Soil samples from the 0-0.05, 0.05-0.10, and 0.10-0.20 m layers were collected in November/2012, when the experiment was 26 years old. Samples were collected as undisturbed blocks with lateral dimensions of 0.10 × 0.20 m, and then manually and gently disaggregated in their natural weakness planes, until the entire sample passed through a 19 mm mesh sieve, before being air-dried. Soil samples were also collected in the native forest stand located near the experiment. Air-dried soil samples were properly stored in polystyrene packaging until the soil analysis.

Aggregate stability was evaluated by a wet sieving method. Briefly, 50 g subsamples, in duplicates, were capillary-wetted for 10 min in filter paper, and then shaken vertically for 15 min (30 oscillations/min) in a nest of six sieves (8, 4, 2, 1, 0.50, 0.25 mm mesh). Stable aggregates retained in each sieve were transferred to aluminum pans and dried at 60°C for 24 h and weighed. The remaining suspension (< 0.25 mm) was flocculated with 50 mL of aluminum sulfate 5% and also dried, but its mass was obtained by difference. The initial soil moisture was measured drying 50 g of soil at 60°C for 48 h, and then used for correction in the calculations. The mean weight diameter (MWD) of aggregates was calculated according to Kemper and Rosenau (1986). A subsample of each size-class of aggregates was ground to < 2 mm and used to determine the C concentration by wet combustion (Yeomans and Bremner 1988).

For discussion purposes, the results obtained separately in the seven aggregate size-classes were grouped into three classes, as macroaggregates (> 8, 8-4, and 4-2), mesoaggregates (2-1, 1-0.50, and 0.50-0.25), and microaggregates (< 0.25).

Soil samples were physically fractionated based on particle density to obtain the free particulate organic matter (free-POM) (density < 2 kg·dm^{-3}), occluded particulate organic matter (occluded-POM) (density < 2 kg·dm^{-3}) and mineral-associated organic matter (min-OM) (density > 2 kg·dm^{-3}), using the method described by Conceição et al. (2008), with adaptation to the aggregates-size in this study.

Ten grams of air-dried soil (aggregates < 19 mm) were added to a 100 mL centrifuge tube containing 80 mL sodium polytungstate (SPT) solution of density 2 kg·dm^{-3}. The tube was closed with a rubber stopper and inverted slowly and manually for five times, to release the free-POM, without breaking the aggregates. The suspension was centrifuged at 1,591 g during 45 min, and the supernatant was filtered (Whatman GF/C) under vacuum. Then, the filter + free-POM were washed with distilled water to remove excess SPT, and dried at 50 °C for 24 h. To obtain the occluded-POM, the SPT was returned to the tube containing the aggregates pellet, and it was suspended again and subjected to ultrasound dispersion, using an energy level of 1,212 J·mL^{-1}, which was the energy level required to disperse this soil (Inda Junior et al. 2007). After dispersion, the suspension was centrifuged at 1,414 g for 60 min and then filtered under vacuum. The filter + occluded-POM were washed with distilled water and dried at 50°C for 24 h.

The free-POM and occluded-POM fractions were weighted and analyzed by dry combustion for C concentration in a CNHS elemental analyzer (EuroVector EA3000). A soil sample was also analyzed to quantify the total C concentration from soil, so the C concentration in min-POM was obtained from the difference between total C and C POM fractions (free + occluded). C stocks in aggregate size-classes and in physical fractions were calculated based on C concentration, soil bulk density and thickness of the analyzed soil layer. Soil bulk density was determined in undisturbed soil samples collected with volumetric cylinders (4 cm height × 5.6 cm diameter) in each soil layer (Blake and Hartge 1986).

The experimental design used was a split-plot factorial scheme, with the winter cover crops as the main plots and the tillage systems as the subplots. Data was tested for normality by the Shapiro-Wilk test. Then, data was submitted to analysis of variance (ANOVA) by the F-test ($p < 0.05$), and the means were compared using Tukey's test ($p < 0.05$), using the Sisvar statistical software.

RESULTS

Soil aggregation

Regarding soil aggregates, there was no significant interaction between tillage system and cover crop. Therefore, results from those two factors are presented separately. Macroaggregates (> 2 mm) represented a proportion of the soil mass that was 2-3 times greater in NT than in CT (40.5 *vs.* 12.8-14.2% to 0.10 m depth and 29.1 *vs.* 14.3% in 0.10-0.20 m layer) (Table 2). Meanwhile, mesoaggregates (2-0.25 mm) and microaggregates (< 0.25 mm) of all layers were more abundant in CT than NT (e.g., 60.9 vs. 41.5% for mesoaggregates in 0-0.05 m). Accordingly, the MWD of aggregates was 2-3 times larger in NT than in CT (e.g., 3 *vs.* 1.1 mm in 0-0.05 m) (Table 2).

Table 2. Distribution of aggregates size-classes, mean weight diameter (MWD) of aggregates, and C stock in aggregate size-classes (macroaggregates > 2; mesoaggregates 2-0.25; and microaggregates < 0.25 mm) in a very clayey Oxisol under conventional tillage (CT) or no-tillage (NT) combined with winter cover crops oat, radish, and fallow; and forest (reference).

Management systems	Aggregate distribution (% of soil mass)			MWD (mm)	C stock ($Mg \cdot ha^{-1}$)		
	Macro	Meso	Micro		Macro	Meso	Micro
0-0.05 m							
Tillage system							
CT	12.8 b	60.9 a	26.3 a	1.1 b	1.6 b	7.5 ns	3.6 ns
NT	40.5 a	41.5 b	18.0 b	3.0 a	7.1 a	6.5	3.1
Winter cover crop							
Oat	29.1 ns	50.6 ns	20.3 ns	2.1 ns	4.9 ns	6.8 ns	3.1 ns
Radish	31.8	47.9	20.3	2.5	5.0	6.5	3.0
Fallow	19.0	55.2	25.8	1.5	3.2	7.7	4.0
Forest	36.6	43.5	19.9	2.3	6.8	7.2	4.0
0.05-0.10 m							
Tillage system							
CT	14.2 b	62.7 a	23.1 a	1.2 b	1.9 b	8.0 a	3.1 a
NT	40.5 a	45.9 b	13.6 b	2.3 a	6.3 a	6.4 b	2.0 b
Winter cover crop							
Oat	33.1 a	50.7 b	16.2 b	1.9 a	5.2 a	7.2 ns	2.4 ns
Radish	31.2 a	51.3 b	17.5 ab	2.0 a	4.6 a	6.9	2.4
Fallow	17.8 b	60.9 a	21.3 a	1.3 b	2.5 b	7.4	2.8
Forest	48.4	35.8	15.8	3.0	7.8	5.2	2.4
0.10-0.20 m							
Tillage system							
CT	14.3 b	64.7 a	21.0 a	1.2 b	4.3 b	18.0 a	6.1 a
NT	29.1 a	55.9 b	15.0 b	1.8 a	8.2 a	14.5 b	4.0 b
Winter cover crop							
Oat	25.2 ns	58.3 ns	16.5 ns	1.7 ns	7.5 ns	16.1 ns	5.0 ns
Radish	21.6	60.2	18.1	1.5	6.4	16.3	4.8
Fallow	18.3	62.3	19.4	1.3	4.9	16.3	5.3
Forest	49.9	36.3	13.9	2.6	11.1	7.5	3.0

Means followed by the same letter within a column are not significantly different according to Tukey´s test ($p < 0.05$); ns: not significant (F-test $p < 0.05$).

The effects of cover crops occurred only in the 0.05-0.10 m layer, in which the highest proportions of macroaggregates occurred under oat (33.1%), and radish (31.2%), and the lowest under fallow (17.8%) (Table 2). On the contrary, the highest amount of mesoaggregates occurred in fallow (60.9%), and the lowest in oat and radish (50.7-51.3%). Therefore, fallow had the lowest MWD (1.3 mm) compared to oat and radish (1.9-2 mm) (Table 2).

The forest soil had a proportion of macroaggregates higher than the treatments, except in the top layer, in which NT had the highest proportion. This trend also occurred for the MWD (Table 2).

Carbon stock in aggregates

The C stocks in macroaggregates were larger in NT than in CT, four times larger in 0-0.05 m layer (7.1 vs. 1.6 Mg·ha^{-1}) and three times larger in 0.05-0.10 m layer (6.3 vs. 1.9 Mg·ha^{-1}) (Table 2). For mesoaggregates and microaggregates, the highest C stock occurred in CT, in layers 0.05-0.10 and 0.10-0.20 m.

The effects of cover crops on C stocks in aggregates were also restricted to the 0.05-0.10 m layer, in which C stock in macroaggregates in radish and oat (4.6-5.2 Mg·ha^{-1}) was almost twice that in fallow (2.5 Mg·ha^{-1}) (Table 2). C stocks in mesoaggregates and microaggregates were not significantly different among cover crops.

The forest soil showed high C stocks in macroaggregates in all layers (6.8 to 11.1 Mg·ha^{-1}), but its values were similar to NT in the superficial layer. Forest C stocks decreased as the diameter of aggregates decreased, but in managed systems the highest C stock was in mesoaggregates.

Carbon stock and physical fractions

In the top 0-0.05 m layer, a higher total C stock was observed in NT than in CT (25.7 *vs*. 20.8 Mg·ha^{-1}), but in deeper layers there were no significant differences between the two tillage systems (Table 3). Among cover crops, total C stock did not differ significantly, in any of the soil layers (Table 3).

Regarding SOM fractions, the C stock of the free-POM in the 0-0.05 m layer was twice as large in NT that in CT (1.6 *vs*. 0.8 Mg·ha^{-1}), and represented 6.3% of the total C stock in this layer under NT and 4.1% under CT (Table 3). However, in the 0.05-0.10 m layer, CT had a higher C stock in free-POM than NT (0.7 vs. 0.4 Mg C·ha^{-1}). Among winter cover crops, free-POM was affected only in the top layer, in which radish had higher free-POM C (1.5 Mg·ha^{-1}) than oat and fallow (1 and 1.1 Mg·ha^{-1}, respectively) (Table 3). In radish, the free-POM represented 7.2% of the C stock in the top layer, while in fallow it represented 4.5% (Table 3). Forest had the highest stock of free-POM C, mainly in the 0-0.05 m layer (4.1 Mg·ha^{-1}), in which it represented 19.3% of total C stock (Table 3). This proportion decreased to 5.2% in the 0.05-0.10 m layer.

For the occluded-POM, NT had more C in this fraction than CT in the top 0.05-m layer (2.3 *vs*. 1.4 Mg·ha^{-1}), but in the 0.10-0.20 m layer CT had more C (3 *vs*. 2.3 Mg·ha^{-1}) (Table 3). The occluded-POM stored 4.6 to 9.7% of the total C stock in soil tillage systems, more than was stored by the free-POM (Table 3). Among cover crops, there was no difference in occluded-POM carbon. The forest soil had 3 Mg C ha^{-1} of occluded-POM in the 0-0.05 m layer, which was numerically higher than in managed systems and corresponded to 14.4% of total C stock in the layer.

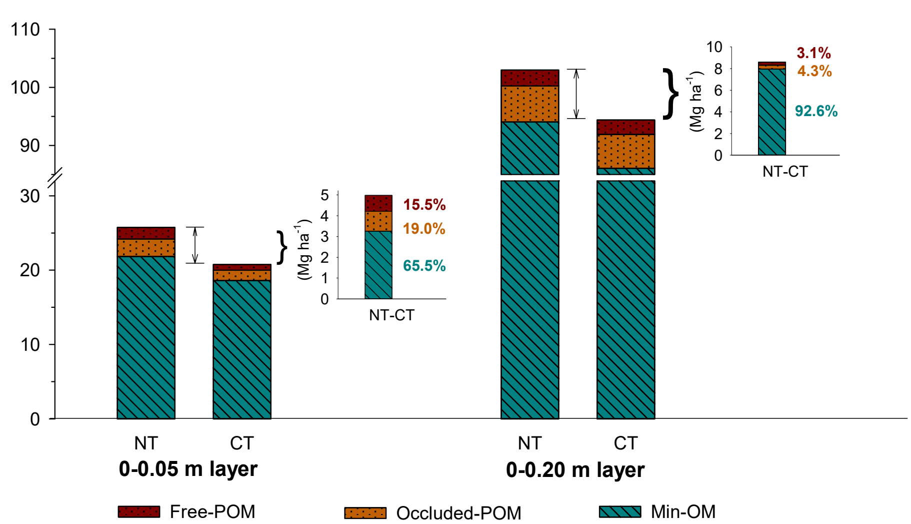
In the min-OM fraction, C stocks differed neither between tillage systems nor among winter cover crops for all soil layers, but stocks in this fraction were 5-17 times greater compared to the stocks in free- or occluded-POM of managed systems (Table 3). The proportion of C stock in min-OM relative to total C ranged from 84.4 to 93.9% for NT for all layers, but in the top layer of the forest soil it was only 66.6% (Table 3).

Considering the whole 0-0.20 m layer, there were no differences in the total C stock between the two tillage systems nor among the three winter cover crops. However, NT increased the total C stock by 8.6 Mg·ha^{-1} (0-0.20 m layer), from which 92.6% was in the min-OM (7.9 Mg·ha^{-1}) and only 7.4% as POM fractions (Fig. 3). In the 0-0.05 m layer, the difference between NT and CT was 5 Mg·ha^{-1}, of which 3.3 Mg·ha^{-1} was in min-OM, representing 65.5%, while the POM fractions represented the other 34.5%.

Table 3. Carbon stock and distribution of total carbon in free particulate organic matter (free-POM), occluded particulate organic matter (occluded-POM), and mineral-associated organic matter (min-OM) fractions, in a very clayey Oxisol under conventional tillage (CT) or no-tillage (NT) combined with winter cover crops oat, radish and fallow; and forest (reference).

Management systems	C stock ($Mg \cdot ha^{-1}$)			Total	Distribution of total C (%)		
	Free-POM	Occluded-POM	Min-OM		Free-POM	Occluded-POM	Min-OM
0-0.05 m							
Tillage system							
CT	0.8 b	1.4 b	18.6 ns	20.8 b	4.1	7.2	88.7
NT	1.6 a	2.3 a	21.8	25.7 a	6.3	9.3	84.4
Winter cover crop							
Oat	1.0 b	1.8 ns	21.7 ns	24.5 ns	4.0	7.6	88.4
Radish	1.5 a	1.9	16.9	20.3	7.2	9.7	83.1
Fallow	1.1 b	1.9	22.1	25.1	4.5	7.5	88.0
Forest	4.1	3.0	14.9	22.0	19.3	14.1	66.6
0.05-0.10 m							
Tillage system							
CT	0.7 a	1.4 ns	20.1 ns	22.3 ns	3.7	7.0	89.3
NT	0.4 b	1.5	22.5	24.4	1.7	6.3	92.0
Winter cover crop							
Oat	0.5 ns	1.5 ns	23.2 ns	25.1 ns	1.9	6.0	92.0
Radish	0.7	1.6	19.1	21.4	3.7	7.7	88.7
Fallow	0.5	1.4	21.6	23.5	2.6	6.3	91.2
Forest	1.0	1.7	16.9	19.6	5.2	8.9	86.0
0.10-0.20 m							
Tillage system							
CT	1.0 ns	3.0 a	47.4 ns	51.4 ns	2.0	6.4	91.6
NT	0.8	2.3 b	49.7	52.8	1.5	4.6	93.9
Winter cover crop							
Oat	0.7 ns	2.5 ns	54.5 ns	57.6 ns	1.4	4.7	94.0
Radish	1.1	2.8	45.3	49.2	2.2	6.1	91.7
Fallow	0.9	2.6	45.9	49.4	1.7	5.8	92.5
Forest	1.1	1.5	33.0	35.6	3.2	4.5	92.3

Means followed by the same letter within a column are not significantly different according to Tukey's test ($p < 0.05$); ns: not significant (F-test $p < 0.05$).



NT: no-tillage; CT: conventional tillage; POM: particulate organic matter; OM: organic matter.

Figure 3. Carbon stock in physical fractions free-POM, occluded-POM and min-OM, in a very clayey Oxisol under CT or no NT and, difference between them, in 0-0.05-m and 0-0.20-m soil layers.

The distribution pattern of the total C stock within the 0.20 m depth was similar in both tillage systems, with the first 0.05 m depth storing 22% of the total stock in CT and 25% in NT (Fig. 3). With respect to distribution of the fractions to 0.20 m depth, the 0.05 m topsoil stored 21.6% of min-OM C in CT and 23.2% in NT, 31.9% of free-POM C in CT and 56.8% in NT, and 23.8% of occluded-POM C in CT and 37.7% in NT.

DISCUSSION

Effect of tillage systems on C accumulation

The effect of tillage systems on C accumulation occurred mainly in the superficial soil layer (Tables 2 and 3). Regarding to total C stock, the difference of 5 $Mg{\cdot}ha^{-1}$ after 26 years between NT and CT (Table 3) was possibly related to the NT fundamental principles, such as deposition of plant residues (straw) on the soil surface and minimum soil mobilization (Kassam et al. 2019). NT decreases C losses by reducing microbial decomposition of the organic matter, soil erosion, and leaching of soluble organic compounds (Bayer et al. 2006) and increases the C input via crop residues (Briedis et al. 2018). That results in a greater C accumulation in soil, as reported in the literature by Calegari et al. (2008), Veloso et al. (2018), and Briedis et al. (2021), with studies in different soils, climatic conditions, and crop systems in Brazil.

No-tillage changed the distribution of C among physical fractions, especially by improving C accumulation in POM fractions, which shows the key role of labile fractions on C accumulation in conservation management systems (Briedis et al. 2018) and the importance of SOM fractionation evaluations. The POM fractions are important to the supply of C and nitrogen to microorganisms, the availability of nutrients to plants, and for formation and stabilization of aggregates (Tivet et al. 2013, Balota et al. 2014, Ferreira et al. 2018).

The C accumulation in free-POM in NT could be related to the higher input of aboveground dry biomass in this system, which was 0.8 $Mg{\cdot}ha^{-1}$ $year^{-1}$ greater than in CT (Table 1). Besides that, these crop residues were deposited and maintained on the soil surface, which favors the interaction among organisms, POM, and soil particles, leading to further C stabilization by the recalcitrance of organic compounds (Sollins et al. 1996, von Lützow et al. 2006). That was also verified by Conceição et al. (2013) in an 18-year experiment over an Acrisol in Southern Brazil, where twice more C was accumulated in the free-POM of NT soil than in CT soil (0-0.05 m layer).

Moreover, NT also improved the C accumulation in the occluded-POM of the top layer as a result of the minimal soil mobilization (Briedis et al. 2018, Ferreira et al. 2018, Veloso et al. 2019). The occluded-POM is the SOM pool physically protected inside aggregates, which limits the action of microorganisms and their enzymes on organic substrates because of less accessibility and lower oxygen diffusion for decomposition processes (Sollins et al. 1996, von Lützow et al. 2006).

On the other hand, the soil mobilization in CT, with plowing and disc-harrowing operations before each crop, breaks the macroaggregates (> 2 mm) and exposes the SOM to decomposition processes (Six et al. 2002). This could be verified in the proportion of aggregates size-classes in CT soil, which had the highest amounts of mesoaggregates and microaggregates, and the highest C stock in these aggregates (Table 2). Besides that, with soil mobilization, the crop residues were incorporated into deeper layers, leading to an increase of C, as seen for the free-POM in 0.05-0.10-m layer and occluded-POM in 0.10-0.20 m layer (Table 3).

So, in NT soil there is a mutual relationship between organic matter and aggregates which is important for soil structuring and soil C stabilization. That was also observed in the greater amount of macroaggregates and C stock for NT (Table 2), agreeing with the studies of Tivet et al. (2013), Ferreira et al. (2018), Wuaden et al. (2020) and Cooper et al. (2021) in Brazilian soils.

Although C accumulated in the POM fractions in NT soil, it was not enough to overcome the greatest amount of C stock in the POM fractions of forest soil (Table 3). The min-OM fraction was the one that stored the largest proportion of the organic C (Table 3) and represents the organic compounds associated with soil fine mineral particles (Kleber et al. 2015), stable to microbial degradation in the soil through organo-mineral interactions (Sollins et al. 1996, von Lützow et al. 2006). This very clayey Oxisol has a predominance of clay minerals and Fe and Al oxides, which improves the organo-mineral interaction and the C stabilization. Higher C stock in organic fractions associated to minerals was also verified by Conceição et al. (2013) in an Acrisol and Briedis et al. (2021) in an Cambissol and Ferralsol, both in Southern Brazil.

The effect of NT on C increments occurred mainly in the topsoil layer, and when observing the whole 0-0.20 m soil layer, this effect was weakened and not significant (Fig. 3), which was also checked by Tiecher et al. (2020) considering the 0-1.0 m soil layer. Besides that, the effect of NT could be seen in the proportion of POM fractions between the soil layers, in which 56.8% of C of free-POM and 37.7% of occluded-POM was in the superficial layer (Fig. 3). This fact highlights the importance of maintaining and preserving the topsoil, the most active soil layer and susceptible to degradation due to poor soil management. This shows the importance of NT in Brazil since its introduction in the 1970s, reducing and controlling erosion and the loss of soil, nutrients, and water (Telles et al. 2013).

The SOM fraction, which plays a role in nutrient retention and availability, water retention, soil porosity and structure, and microbial activity are related to soil quality and agronomic yields. So, the C increase of 8.6 $Mg{\cdot}ha^{-1}$ in the 0-0.20 m layer of NT relative to CT after 26 years of cultivation is important for soil improvement, as well as physical, chemical and soil biological properties, as shown by Calegari et al. (2008), Balota et al. (2014), and Tiecher et al. (2017) in addition to increasing the soil productive capacity.

Effect of winter cover crops on C accumulation

The effect of winter cover crops was observed in the free-POM fraction, in which the difference between the C stock of radish, and oat or fallow could be related to the characteristics of each species, belonging to different botanical families. The free-POM fraction is related to the organic matter stabilization mechanism of recalcitrance, which protects and stabilizes C in the soil through its characteristics of chemical/molecular composition of litter, in which the most labile compounds are decomposed initially by soil microorganisms (Sollins et al. 1996, von Lützow et al. 2006).

The C input by radish was not as high as that of oat, as seen by the mean annual input of aboveground biomass, 8.9, 8.3 and 5.9 $Mg{\cdot}ha^{-1}{\cdot}year^{-1}$, for oat, radish, and fallow, respectively (Table 1). On the other hand, differences in root biomass between oat and radish could be affecting the C stock over the years, as seen in the study of Santos et al. (2011), in which there was a good relation between C stock and belowground C additions. Redin et al. (2014) reported that roots of plant species of the Brassicaceae family (radish) had higher levels of cellulose and lignin compared to species of the Poaceae family (black oat), in addition to the higher amount of coarse roots, which may decrease the mineralization rate of crop residues in soil (Cotrufo et al. 2015, Poirier et al. 2018).

The effect of fallow on soil aggregation was observed only in the 0.05-0.10 m layer, in which the proportion of macroaggregates, the MWD, and C stock was reduced by fallow compared to radish or oat (Table 2). The lower aboveground biomass obtained under fallow likely decreased the formation of organic cores in the soil, which represents the interaction between crop residues (litter), soil fine particles and microbial organic compounds, and is the main variable responsible for the accumulation of C in macroaggregates and for the stabilization of these aggregates (Tivet et al. 2013, Ferreira et al. 2018).

Besides the low C input via crop biomass, the poor soil conditions under fallow are associated with a smaller plant canopy and less mulch cover, high susceptibility to erosion, high temperatures, low microbial activity and other factors, which favor the decomposition of organic compounds and C loss (Calegari et al. 2008, Dieckow et al. 2009, Balota et al. 2014).

A much greater difference in soil aggregation between fallow and cover crops was expected in the whole 0-0.20 m layer, but possibly in this Oxisol the clay minerals and Fe and Al oxides, which strongly contribute to soil aggregation (Six et al. 2000, Maltoni et al. 2017), partially mitigated the negative effects of fallow. This corroborates the great resilience and recovery capability of clayey Oxisols (Bonetti et al. 2017). In addition, in the winter fallow under NT, there was the predominance of ryegrass among the volunteer plants, which is considered a plant with excellent root characteristics, contributing to aggregates formation and stabilization.

CONCLUSION

The adoption of NT for 26 years promoted C accumulation in soil, mainly in the 0-0.05 m layer, verified by the highest total C stock, highest C stock in macroaggregates and highest C stock in POM fractions (free-POM and occluded-POM), changing the distribution of SOM fractions.

The use of cover crops in winter increased the formation and stability of macroaggregates in comparison to fallow, but it occurred only in the 0.05-0.10 m layer. Radish increased C accumulation in free POM, mainly in the 0-0.05 m layer. Future studies should be conducted in order to evaluate the potential of the intercropping of different botanical families, such as oat and radish, under NT, on the C accumulation in soils.

Regardless of the tillage system and cover crop species, the mineral-associated organic matter fraction is the one that stores the largest stock of C in the soil, due to the high interaction of organic compounds with the fine soil particles as clay and Fe and Al oxides, predominant in this very clayey Oxisol. This shows the potential of soil to stabilize C and to act as a sink of C from the atmosphere.

Using cover crops and the NT system has an important role in tropical and subtropical soils, regarding the potential of C accumulation and stabilization, mitigation of greenhouse gases emission, as well as on soil quality and maintenance of crop productivity.

AUTHORS' CONTRIBUTION

Conceptualization: Amadori, C. and Conceição, P. C.; **Methodology:** Amadori, C., Conceição, P. C., and Casali, C. A.; **Investigation:** Amadori, C.; **Writing – Original Draft:** Amadori, C. and Conceição, P. C.; **Writing – Review and Editing:** Amadori, C., Conceição, P. C., Dieckow, J., Casali, C. A. and Canalli, L. B. S.; **Project Administration:** Calegari, A. and Canalli, L. B. S.; **Supervision:** Amadori, C. and Conceição, P. C.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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