

Legume cover crops under no-tillage favor organomineral association in microaggregates and soil C accumulation

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ARTICLE INFO

Keywords:

Organic matter
Soil aggregates
Carbon stabilization
Tillage systems
Cropping systems

ABSTRACT

Both no-tillage and legume cover crops have been shown to increase soil organic carbon (SOC) in subtropical soils. However, the mechanisms underpinning management system effects on SOC accumulation are still not well understood. We used a combination of aggregate size and density fractionation to elucidate these mechanisms at a 30-year old experiment on an Acrisol in southern Brazil. The effects of two tillage systems [conventional system (CT) and no-tillage (NT)] combined with three cropping systems [oat/maize (O/M), vetch/maize (V/M) and oat + vetch/maize + cowpea (OV/MC)] were evaluated in the top 20 cm soil layer. Overall, macroaggregation (> 0.25 mm) was significantly influenced by tillage with NT showing values 14% greater than CT in the 0–5 cm soil depth. On average, the occluded light fraction-C content in macroaggregates was more than twice as high under NT compared to CT (4.4 vs. 1.8 g kg⁻¹). This effect was more pronounced when legume cover crops were grown. However, the most significant effect of cover crops was observed in the organomineral fraction of microaggregates, especially under NT (12.1 under O/M and 19.8 g kg⁻¹ under OV/MC). Our results suggest that, although NT increased the occluded light fraction-C compared to CT, this effect was smaller than the gains that legume cover crops offered in organomineral association.

1. Introduction

Soil organic carbon is essential for more sustainable and resilient agricultural systems, especially in tropical and subtropical soils, owing to its central role in determining soil properties that strongly affect crop yield and the quality of the wider environment (Lal, 2009; Powlson et al., 2016).

Conservation management systems can make agricultural soils potential sinks of atmospheric CO₂. An accumulation of SOC in agricultural systems of subtropical Brazil can be achieved through management strategies such as reduced tillage or NT that decrease the decomposition rate of soil organic matter (Bayer et al., 2006), and high C-input intensive cropping systems (Diekow et al., 2005a,b; Zanatta et al., 2007). Switching from CT to NT in Brazilian subtropical regions can result in C sequestration rates ranging from 0.11 to 0.68 Mg ha⁻¹ yr⁻¹ depending on the intensity of cropping systems

adopted (Bayer et al., 2006). Intensifying cropping systems in Southern Brazil usually involves the insertion of cover-crop species, especially legumes, for which a positive effect on C input is usually related to soil N supply (Amado et al., 2006; Diekow et al., 2005b).

Physical fractionation (density or size) has been used to study the effect of management systems on SOC stabilization (Diekow et al., 2005a; Alvaro-Fuentes et al., 2009; Andruschkewitsch et al., 2013; Conceição et al., 2013). Despite the wide range of fractionation schemes used (Cambardella and Elliott, 1992; Diekow et al., 2005a; Conceição et al., 2008), three main fractions related to mechanisms of C stabilization are usually assessed. The free light fraction (free-LF) which is considered a non-protected C-pool, the occluded light fraction (occluded-LF) that is fraction located inside of soil aggregates and assumed to be physically-protected against the attack of soil microorganisms and their enzymes, and the mineral-associated fraction (min-OM) that is a colloidal fraction interacting with mineral particles (Six et al., 1999; Rumpel et al., 2010; Conceição et al.,

Abbreviations: SOC, soil organic carbon; C, carbon; SOM, soil organic matter; CT, conventional tillage; NT, no-tillage; O/M, oat/maize; V/M, vetch/maize; OV/MC, oat + vetch/maize + cowpea; MWD, mean weight diameter; free-LF, free light fraction; occluded-LF, occluded light fraction; min-OM, mineral-associated fraction; SPT, polytungstate; CaCl₂, calcium chloride.

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<https://doi.org/10.1016/j.still.2019.03.003>

Received 31 October 2017; Received in revised form 23 February 2019; Accepted 9 March 2019

Available online xxx

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Table 1

Geographic coordinates, regional climate parameters, characteristics and classification of the soil, and C input by cover crops and maize across the whole experimental period (30 years) in Southern Brazil. CT = conventional tillage, NT = no-tillage, O/M = oat/maize, V/M = vetch/maize, and OV/MC = oat + vetch/maize + cowpea.

Characteristic			
County	Eldorado do Sul, RS		
Geographic coordinates	30°51'S, 51°38'W		
Altitude (m)	96		
Mean annual temperature (°C)	19.4		
Mean annual rainfall precipitation (mm)	1440		
Köppen Climate Classification	Cfa		
Soil group (WRB) ^a	Acrisol		
Soil type (US taxonomy)	Typic Paleudult		
Soil texture in 0–20 cm layer			
Particle size distribution			
Clay	220		
Silt	240		
Sand	540		
Mineralogy ^b			
Feo ^c	0.9 g kg ⁻¹ soil		
Fed ^d	11.8 g kg ⁻¹ soil		
Feo/Fed	0.1		
Gt/(Gt + Hm) ^e	0.1		
Soil bulk density (Mg m ⁻³) ^f	CT	NT	
0–5 cm	1.5	1.3	
5–10 cm	1.7	1.6	
10–20 cm	1.7	1.7	
Annual C input (Mg ha ⁻¹) ^g	Maize	Cover crops	Total
O/M	2.3	2.7	5.0
V/M	2.6	3.8	6.4
OV/MC	5.2	3.7	8.9

^a FAO, 2015.

^b Original data presented by Inda et al. (2007).

^c Ammonium oxalate soluble Fe.

^d Dithionite-citrate-bicarbonate soluble Fe.

^e Gt: goethite; Hm: hematite.

^f NT, no-tillage with glyphosate desiccation prior to maize planting; CT, conventional tillage with plowing and two disking prior to maize planting.

^g Original data presented by Veloso et al. (2018), on average of two tillage systems; mean values across the whole experimental period (30 years).

2013). Carbon occlusion and organomineral association are considered the main mechanisms of C stabilization in tropical and subtropical soils with a predominance of Kaolinite (1:1 clay) and sesquioxides of Al and Fe (Conceição et al., 2013; Deneff et al., 2007; Zotarelli et al., 2007).

It has been suggested that SOC stabilization in NT soils is closely linked to soil macroaggregation (Six et al., 1999; Conceição et al., 2013). The slower turnover of macroaggregates would favor the formation and stabilization of microaggregates within macroaggregates (Six et al., 1999; Deneff et al., 2004; Du et al., 2015), and provide an environment for C to be chemically stabilized by soil minerals (Conceição et al., 2013). However, the mechanisms by which different cropping systems affect SOC stabilization is still unclear, especially in tropical and subtropical soils.

The hypothesis of this work was that soil management systems involving no soil disturbance (NT) and associated with large and diversified crop residue input, increase the C occlusion in macroaggregates and, consequently, favour the formation of organomineral associations and ultimately SOC stabilization. Our objective was to test this hypothesis using a combination of aggregate size and organic matter density fractionation, on a 30 year-old experiment of southern Brazil combining tillage and cropping systems with legume cover crops.

2. Materials and methods

2.1. Field experiment

This study was based on a long-term field experiment (30 years) initiated in 1985 in an area previously cultivated for approximately 16 years under conventional tillage, with plowing and disking, at the Experimental Station of the Federal University of Rio Grande do Sul, Eldorado do Sul, Rio Grande do Sul State, Brazil. Soil and climate characteristics are presented in Table 1.

The ongoing experiment consists of the combination of two tillage [conventional tillage (CT) and no tillage (NT)] systems with three cropping systems [oats (*Avena strigosa* Schreb)/maize (*Zea mays* L.) (O/M), vetch (*Vicia sativa* L.)/maize (V/M) and the consortium of oats + vetch/maize + cowpea (*Vigna unguiculata* (L.) Walp) (OV/MC)] distributed in a randomized block experimental design with split plots and three replicates. Tillage systems were assigned to the main plots (15 × 20 m) and cropping systems to the subplots (5 × 20 m). These tillage systems combined with cropping systems were managed at two levels of fertilization: 0 and 180 kg ha⁻¹ of N-urea (0 N and 180 N), applied manually in strips in the maize crop, characterized the sub-subplots (5 × 10 m). Only the sub-subplots with no nitrogen fertilization were used in this study.

Winter crops, managed as cover crops, were established in April–May of each year using direct drilling in both CT and NT treatments. Oats, when grown alone, were seeded at a rate of 80 kg ha⁻¹. When cultivated with vetch, oats were seeded at 30 kg ha⁻¹ and vetch at 50 kg ha⁻¹. For vetch cultivated on its own, 80 kg ha⁻¹ was used. In the OV/MC system, cowpea (summer cover crop) was manually sown 15–20 days after maize, between the lines of this crop, in pits 40 cm apart, with 3–4 seeds per pit. After the maize was harvested, cowpea was allowed to grow to its full development, remaining until its mechanical management by knife roller before the winter crops were planted (oats and vetch).

The CT plots were ploughed to a furrow-depth of 17 cm once a year in spring before maize sowing using a three-disk plough and harrowed twice to a depth of 10 cm using a disk harrow mixing the crops residues in this layer. At the same time, glyphosate-based herbicide (Roundup, Monsanto) was applied in the NT plots at 1.4 kg ha⁻¹ with respect to final glyphosate concentration, and two or three days later the winter cover crops were managed with a knife-roller and the aboveground residues left on the soil surface. In NT, soil disturbance occurred only in the sowing line, and the residues of the cover crop were left on the soil surface.

Maize was planted with a NT planter in both tillage treatments in September–October, with 90 cm spacing between rows and using a sowing rate adjusted to obtain 50–70 thousand plants per hectare. The fertilizers were applied at 8 cm soil depth at maize sowing, at rates of 21.5 and 41.5 kg ha⁻¹ of P and K, respectively (50 kg ha⁻¹ of P₂O₅ and K₂O each). The mean historic annual C input for both cover crops and maize are provided in Table 1. The mean annual C input (aboveground and root, with roots being assumed to account for 30% of the aboveground portion) was calculated from cover crop data and the dry-matter maize yields compiled by Zanatta et al. (2007) for the period 1985–2006, which were subsequently updated to 2014 (Fig. 1) (Veloso et al., 2018).

2.2. Soil bulk density and total organic carbon determinations

Soil sampling was carried out in September 2014 in the 0–5, 5–10 and 10–20 cm soil layers, prior to soil tillage for maize crop establishment. Trenches were opened, perpendicular to the sowing lines, for sampling for bulk density which was measured in duplicate using

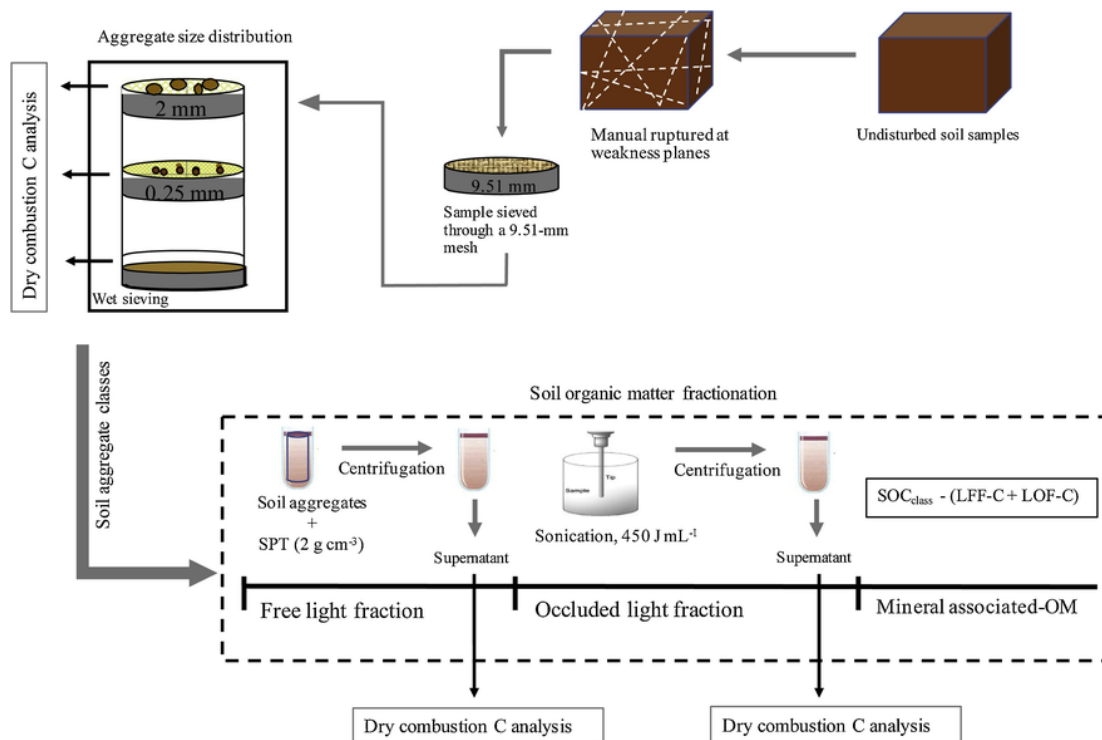


Fig. 1. Schematic map of density fractionation of soil organic matter in soil aggregate classes.

the volumetric ring method (volume of 101.5 cm³) (Blake and Hartge, 1986). Soil samples were collected from the different layers for SOC analysis. The samples were air-dried, ground and passed through a 2-mm-mesh sieve, and then ground in an agate mortar. The determination of SOC concentrations by dry combustion was performed using an elemental analyzer (FlashEA 1112, Thermo, Electron Corporation, Milan, Italy).

The SOC stocks up to a depth of 20 cm were calculated using the equivalent mass approach (Ellert and Bettany, 1995), taking the soil mass of CT O/M management system as the reference. The annual SOC accumulation rate was calculated as the difference between the SOC stocks of the treatments and the reference system, divided by the time elapsed since the implementation of the experiment, *i.e.* 30 years.

2.3. Aggregate size distribution and physical fractionation of SOM

At the same period, additional trenches were excavated with a cutting blade in order to sample undisturbed soil in the 0–5, 5–10 and 10–20 cm layers for aggregate fractionation. These undisturbed samples were manually ruptured at their weakness points until the entire sample could be passed through a 9.51-mm mesh and dried in the shade. Aggregates larger and smaller than 2 mm were weighed to determine the proportion of aggregates making up the soil mass required to analyze the aggregate size distribution in water and the physical density fractionation of SOM.

To determine the size distribution of aggregates in water, 50 g samples of soil were weighed and moistened by capillarity for 12 h on filter paper. Separations were run in duplicate. Afterwards, they were transferred to 1000 ml plastic tubes containing 500 ml of water and placed on a rotary shaker for 2 min at 16 rpm. The samples were then transferred to the vertical oscillation shaker, following the procedure of Carpenedo and Mielniczuk (1990). The samples were placed on a set of sieves with mesh sizes 4.76, 2.00, 0.50, 0.25, and 0.053 mm, and shaken in water at 42 oscillations per minute for

15 min, so that water at the lowest level reached the top of the aggregates in the 4.76 mm sieve. The material that passed through the last sieve and was retained in the bucket was flocculated with potassium aluminum (5 g L⁻¹).

The contents of the five sieves and the bottom were transferred to plastic containers of known mass, dried for 48 h at 60 °C and then weighed. The mean weight diameter (MWD) was calculated from the aggregates retained in the five sieves and the bottom pan (Eq. (1)):

$$\text{MWD} = \frac{\sum m_{AGRi} \times C_i}{\sum m_{AGR}} \quad (1)$$

Where: MWD = mean weight diameter (mm); m_{AGRi} = mass of aggregates in class *i*; C_i = mean value of the class of aggregate class [(upper mesh diameter + lower mesh diameter)/2]; m_{AGR} = total mass of aggregates of the six classes. The soil aggregate classes were pooled in large macroaggregates (> 2 mm), small macroaggregates (2–0.250 mm) and microaggregates (< 0.250 mm). The SOC concentration of these aggregate size fractions was determined as previously described.

For the physical fractionation of SOM from large macroaggregates, small macroaggregates and microaggregates, 10-g samples from each soil aggregate class were placed in centrifuge tubes containing 80 ml of sodium polytungstate solution (SPT) (2.0 g cm⁻³) (Fig. 1). After closure of the stopper tube and slow and manual stirring, the free-LF of the OM was released (Conceição et al., 2008). The suspension was centrifuged at 2000g for 90 min. The supernatant, which contained the free-LF, was filtered under vacuum. To remove excess SPT, the free-LF was washed with distilled water and calcium chloride solution (CaCl₂) (0.01 mol L⁻¹). The SPT solution (contained in the free-LF supernatant) was returned to the tube and the suspension was subjected to ultrasonic vibration dispersion at the 240 J mL⁻¹ level in order to disperse the soil aggregates. This level of energy was previously defined as sufficient to disperse organomineral complexes > 2 μm in order to obtain 99% of the total clay fraction (Inda et al., 2007).

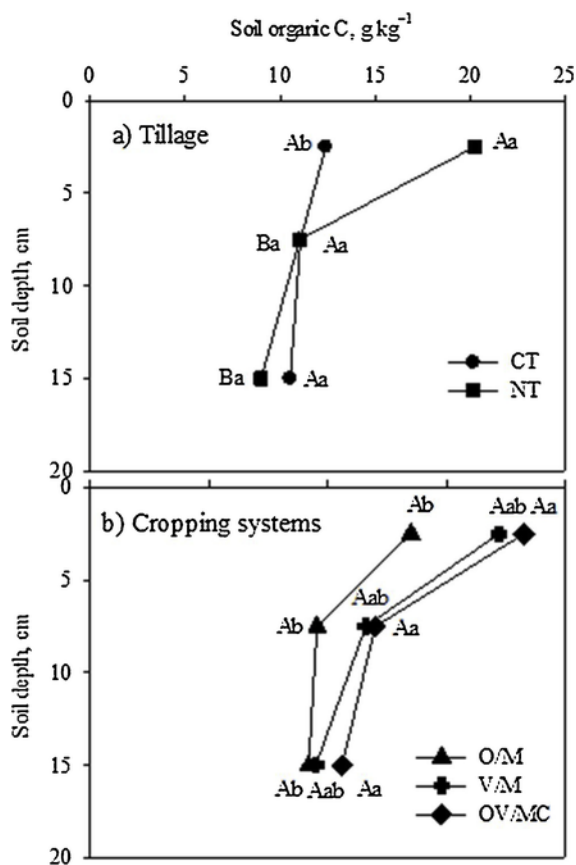


Fig. 2. Soil organic carbon concentration in 0–5, 5–10 and 10–20 cm of sandy clay loam Acrisol subjected for long-term to conventional tillage (CT) and no-tillage (NT) (a) in combination with three cropping systems [oat/maize (O/M), vetch/maize (V/M) and oat + vetch/maize + cowpea (OV/MC)] (b). The results were showed in average of cropping systems (a) and of tillage systems (b) because the triple interaction was not significant. Uppercase letters compare soil layers within the tillage systems (a) or the cropping systems (b). Lowercase letters compare tillage systems (a) or cropping systems (b) within soil layer according to the Tukey test ($p < 0.05$).

Table 2

Soil organic carbon stock in 0–20 cm layer of sandy clay loam Acrisol under conventional tillage (CT) and no-tillage (NT) in combination with three cropping systems [oat/maize (O/M), vetch/maize (V/M) and oat + vetch/maize + cowpea (OV/MC)].

Tillage	Cropping system	SOC stock (Mg ha ⁻¹)	SOC accumulation rate (Mg ha ⁻¹ year ⁻¹)
CT	O/M	32.4	–
	V/M	37.1	0.2
	OV/MC	39.8	0.3
NT	O/M	33.7	0.1
	V/M	38.0	0.2
	OV/MC	41.7	0.3
Mean CT		36.4	0.1
Mean NT		37.8	0.2
Mean O/M		33.1 b	0.0 b
Mean V/M		37.6 a	0.2 a
Mean OV/MC		40.8 a	0.3 a
F value	Tillage (T)	0.69 ns	1.00 ns
	Crop (CS)	19.51 **	15.00 **
	T*CS	0.08 ns	0.56 ns

No significant differences ($p > 0.05$) between treatments are designated with (ns); Significant differences between treatments at 1% probability of error by test F are designated with (**); Lowercase letters compare averages of crop systems; SOC = soil organic carbon; SOC accumulation rate was calculated in relation to CT O/M management system.

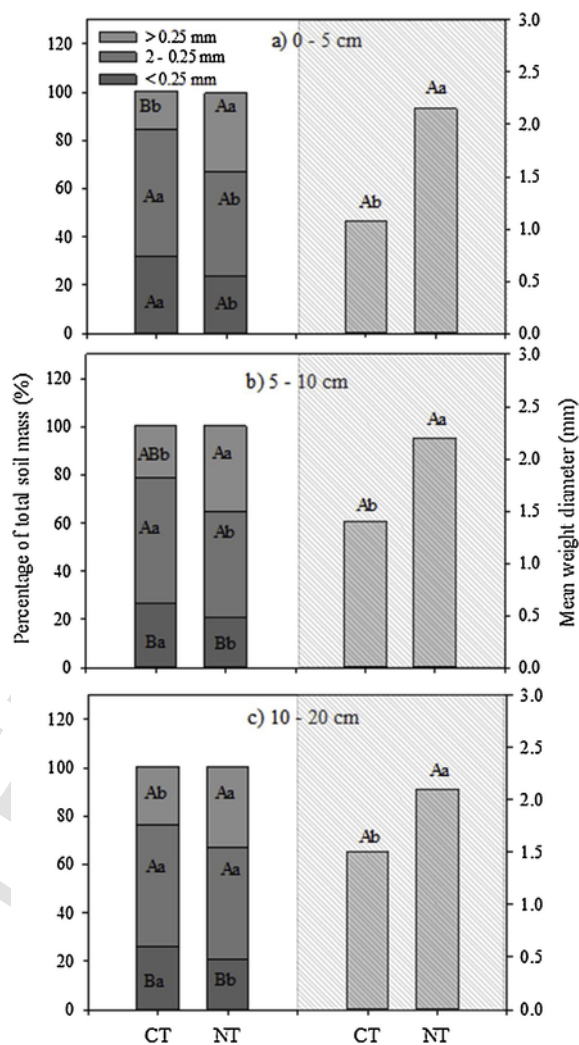


Fig. 3. Aggregate size-class distribution and mean weight diameter (MWD) in the (a) 0–5, (b) 5–10 and (c) 10–20 cm layers of a sandy clay loam Acrisol subjected to conventional tillage (CT) and no-tillage (NT). The results were showed in average of cropping systems because the triple interaction was not significant. Uppercase letters compare soil layers within the tillage systems in each soil aggregate class. Lowercase letters compare tillage systems within soil layer in each aggregate class according to Tukey test ($p < 0.05$).

After sample dispersion, the suspension was centrifuged again and the occluded-LF fraction was separated by following the same procedure as for free-LF. The free-LF and occluded-LF fractions were oven dried at 60 °C, ground in an agate mortar, and analyzed for C as previously described. The C-min-OM was obtained by calculating the difference between SOC and C of the C-free-LF + C-occluded-LF. The C recovery in each soil aggregate class ranged from 85 to 96% of the SOC.

2.4. Statistical analysis

Data were subjected to analysis of variance (ANOVA) and, when significant ($p < 0.05$), the differences between means of treatments were evaluated by the Tukey test at the 5% level. To compare the effects of the tillage (T) and cropping (CC) systems on the SOC variables, the MIXED procedure was performed, which considered the main factors and their interactions as fixed factors, and the block and the experimental errors as random variables. All analyses were performed using SAS® v.9.4 (Statistical Analysis System Institute Cary, North Carolina).

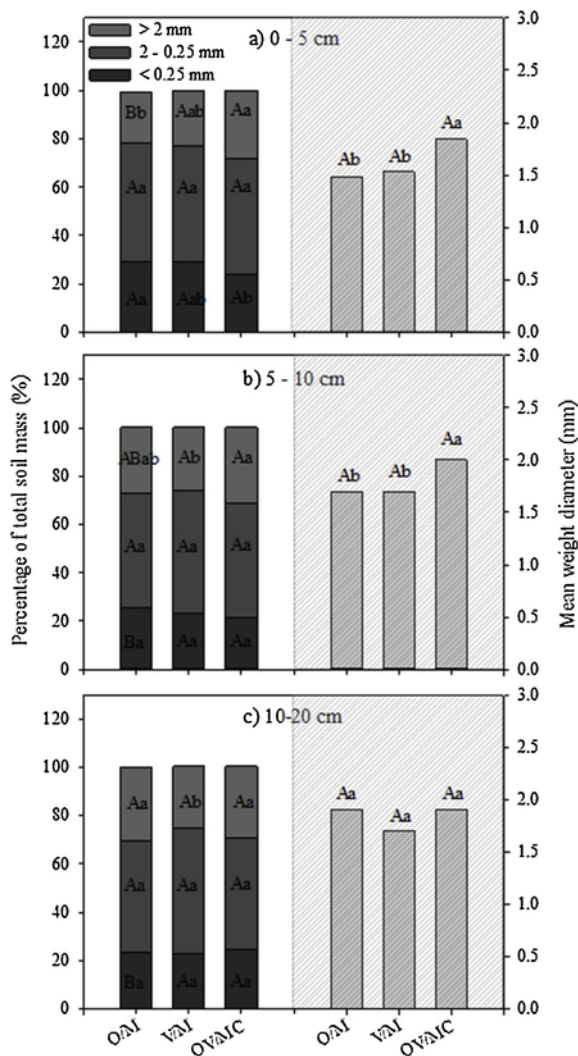


Fig. 4. Aggregate size-class distribution and mean weight diameter (MWD) in the (a) 0–5, (b) 5–10 and (c) 10–20 cm layers of a sandy clay loam Acrisol subjected to cropping systems oat/maize (O/M), vetch/maize (V/M) and oat + vetch/maize + cowpea (OV/MC) in average of tillage systems [conventional tillage (CT) and no-tillage (NT)]. The results were showed in average of tillage systems because only of cropping systems and soil layers effects were significant. Uppercase letters compare soil layers within the cropping system in each soil aggregate layer. Lowercase letters compare cropping systems within soil layer in each class of aggregate according to Tukey test ($p < 0.05$).

The statistical model used in the analysis of variance to analyze SOC or C in the classes of aggregates was as follows: $Y_{ijkl} = \mu + B_i + T_j + \text{Error}_{(ij)} + C_k + T_j C_k + \text{Error}_{(ijk)} + L_l + \text{Error}_{(jl)} + T_j L_l + C_k L_l + T_j C_k L_l + \text{Error}_{(ijkl)}$, where μ = general mean of experiment; B = block ($i = 1, 2, 3$); T = tillage systems ($j = 1, 2$); CC = cropping systems ($k = 1, 2, 3$); L = soil layer ($l = 1, 2, 3$) e Error = experimental error.

To compare the C concentration in the fractions of SOC within each soil aggregate class, the statistical model used was follows: $Y_{ijkl} = \mu + B_i + T_j + \text{Error}_{(ij)} + C_k + T_j C_k + \text{Error}_{(ijk)}$, onde μ = general mean of experiment; B = block ($i = 1, 2, 3$); T = tillage system ($j = 1, 2$); C = cropping systems ($k = 1, 2$) e Error = error experimental.

3. Results

3.1. Soil organic carbon

The SOC concentration was higher in the superficial soil layer (0–5 cm) under NT compared to CT, but this difference disappeared in the underlying layers (Fig. 2a). The SOC stocks down to a depth of 20 cm depth were therefore similar for the two tillage systems (Table 2). However, cropping systems had a strong effect on SOC stocks, which varied from 33.1 to 40.8 Mg C ha⁻¹, with a gradual increase due to legume cover crop insertion in the cropping systems (Table 2). Consequently, on average, legume-based cropping systems had the SOC accumulation rate 0.25 Mg C ha⁻¹ yr⁻¹ higher than traditional cropping system involving non legume crops (O/M) (Table 2).

3.2. Soil aggregation

The proportion of soil aggregates was strongly affected by tillage systems down to a depth of 20 cm (Fig. 3a–c). In the three soil layers, NT (average of 34.6%) resulted in a greater proportion of large macroaggregates compared to CT (average of 20.1%), yielding a MWD of 1.3 mm for CT and 2.2 mm for NT. The cropping systems influenced the proportion of soil aggregates only in the 0–5 cm depth. In this soil layer, the proportion of large macroaggregates was 40% greater and the MWD 24% greater, under OV/MC compared to O/M (Fig. 4a).

3.3. SOC concentration in soil aggregates

Tillage and cropping systems differed in their effects on SOC concentrations in the three aggregate size classes, and these effects were mostly visible in the 0–5 cm soil layer (Fig. 4). The SOC concentration in both macroaggregate fractions ranged from 10.3 to 19.5 g kg⁻¹, and was affected mainly by tillage systems (Fig. 5a and b). The SOC concentration in the large and small macroaggregates in the superficial soil layer was 5.1 and 4.0 g C kg⁻¹ higher under NT than under CT, respectively (Fig. 5a, b). On the other hand, cropping systems had a greater effect on the small macroaggregates (Fig. 5e) and an especially noticeable effect on the microaggregates in the superficial soil layer (Fig. 4f), with SOC concentrations in microaggregates varying between 15.3 and 21.3 g kg⁻¹. More noticeably, the system with two legume cover crops (OV/MC) showed a strong C enrichment in the microaggregates of the superficial soil layer compared to system with no legume cover crop (O/M) (Fig. 5f).

3.4. Distribution of C in physical fractions of soil aggregates

On average, the free-LF-C fraction of both macroaggregate fractions represented 9% of the total SOC, and was affected by both tillage and cropping systems (Table 3). The proportion of free-LF was much smaller in the soil microaggregates. On average, the C concentration in the macroaggregates was 2.4 times greater under NT compared to CT (Table 3). A less pronounced but still noticeable effect was observed in the system with two legume cover crops: the free-LF-C in small macroaggregates was 76% higher with the legume crops compared to O/M (Table 3).

The occluded-LF-C concentration varied between 1.1 and 4.6 g kg⁻¹ among soil management systems, accounting from 9 to 27% of SOC, and was strongly influenced by soil tillage systems. The occluded-LF-C concentration was 2.2-, 2.8- and 2.6-fold larger, in the large and small macroaggregates and the soil microaggregates, respectively, under NT system compared to CT (Table 3). The highest C concentration was found in the large macroaggregates under NT

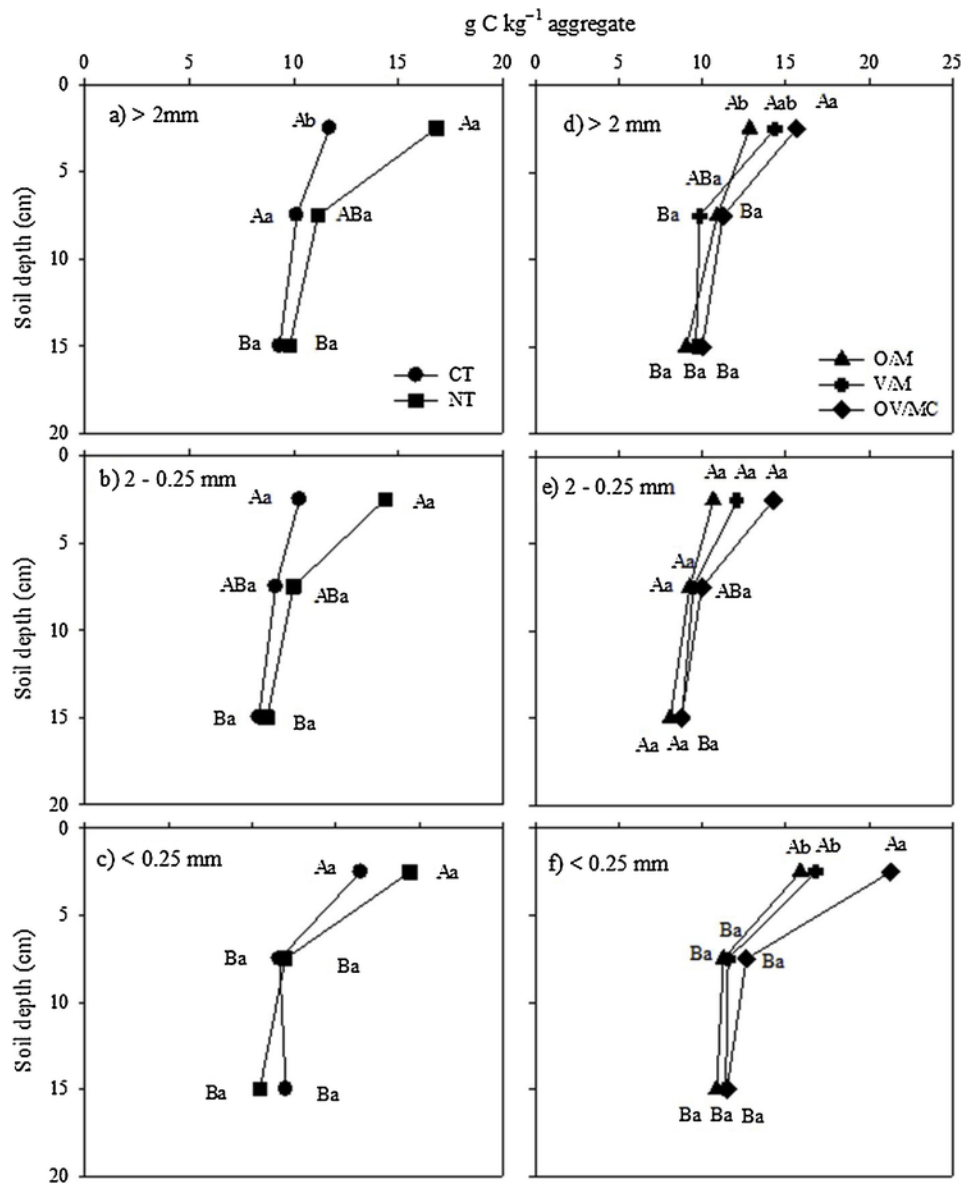


Fig. 5. Concentration of organic carbon in aggregates > 2 mm (a, d), $2 - 0.25$ mm (b, e) and < 0.25 mm (c, f) in 0–5, 5–10 and 10–20 cm soil layers of a sandy clay loam Acrisol subjected to conventional tillage (CT) and no-tillage (NT) or to three cropping systems [oat/maize (O/M), vetch/maize (V/M) and oat + vetch/maize + cowpea (OV/MC)]. As there was not triple interaction, the results were showed in average of cropping system and soil layer (a–c) and in average of tillage system because the significant interaction between tillage system and soil layer (d–f). In (a–c), uppercase letters compare soil layers within the tillage system in each aggregate class; lowercase letters compare tillage systems within soil layer in each class of soil aggregate according to Tukey test ($p < 0.05$). In (d–f), uppercase letters compare soil layers within the cropping system in each soil aggregate class; lowercase letters compare cropping systems within soil layer in each class of soil aggregate according to Tukey test ($p < 0.05$).

(4.9 g kg^{-1}) (Table 3). The occluded-LF-C concentration was 70, 69 and 84% greater in large and small macroaggregates and microaggregates, respectively, under OV/MC compared to O/M cropping system (Table 3).

The min-OM fraction accounted for the majority of the C contained in all aggregate fractions (Table 3). The cropping systems had a strong effect on min-OM of microaggregates, especially under NT (Table 3) where it was 64% greater under OV/MC compared to O/M (Table 3). Although the effect was less pronounced, the main effect of tillage system was also significant in large macroaggregates, where the min-OM-C concentration was 28% greater in NT compared to CT (Table 3).

In all three aggregate fractions, in the 0–5 cm layer only, there was a significant positive correlation between the concentration of occluded-LF-C and min-OM-C (Fig. 6).

4. Discussion

Overall, and as usually observed (Plaza-Bonilla et al., 2010; Conceição et al., 2013), soil under NT presented a greater proportion of macroaggregates than CT. These macroaggregates under NT were also enriched in C compared to CT, which is also consistent with previous studies (Conceição et al., 2013; Plaza-Bonilla et al., 2010, 2013). Macroaggregates are complex structures, enriched in labile organic material recently added to the soil (Angers and Giroux, 1996; Plaza-Bonilla et al., 2013; Puget et al., 1999; Six et al., 1999). The implementation of NT on degraded (or C depleted) soils results in the formation and stabilization of these macroaggregates and the consequent preservation of LF-C in these aggregate structures (Andruschkewitsch et al., 2013; Plaza et al., 2013). The higher pro-

Table 3

Carbon concentration in free light fraction (free-LF), occluded light fraction (occluded-LF) and mineral-associated organic matter (min-OM) in aggregates classes > 2 mm, 2–0.25 mm and < 0.25 mm of 0–5 cm layer of a sandy clay loam Acrisol under two tillage systems (CT, conventional tillage and NT, no-tillage) and two cropping systems [oat/maize (O/M) and oat + vetch/maize + cowpea (OV/MC)].

Tillage	Cropping system	> 2 mm			2–0.25 mm			< 0.25 mm		
		Free-LF	Occluded-LF	Min-OM	Free-LF	Occluded-LF	Min-OM	Free-LF	Occluded-LF	Min-OM
----- g kg ⁻¹ aggregate -----										
CT	O/M	1.2	1.1	7.6	0.7	1.0 Ba	7.3	0.5	0.8	14.87 Ba
	OV/MC	1.0	3.3	8.61	1.2	1.7 Ba	8.0	0.4	2.0	14.45 Ba
NT	O/M	1.8	4.1	9.8	2.1	2.8 Ab	7.5	0.7	2.8	12.09 Ab
	OV/MC	1.7	5.7	10.9	3.7	4.7 Aa	9.2	1.2	4.6	19.83 Aa
Mean CT		1.1	2.2	8.1 B	0.9 B	1.3	7.6	0.5	1.4	14.66
Mean NT		1.8	4.9	10.4 A	2.9 A	3.8	8.4	1.0	3.7	15.96
Mean O/M		1.5	2.6	8.7	1.4 b	1.9	7.4	0.6	1.8	13.48
Mean OV/MC		1.4	4.5	9.8	2.5 a	3.2	8.6	0.8	3.3	17.14
F value	Tillage (T)	4.58 ns	9.05 ns	21.64 *	26.80 *	223.35 **	0.96 ns	2.60 ns	3.95 ns	0.61 ns
	Crops (CS)	0.94 ns	3.11 ns	0.01 ns	7.86 *	175.66 **	3.96 ns	1.44 ns	5.57 ns	23.60 **
	T*CS	0.22 ns	0.23 ns	1.74 ns	2.45 ns	66.76 **	0.70 ns	2.14 ns	0.76 ns	29.34 **

No significant differences ($p > 0.05$) between treatments are designated with (ns); Significant differences between treatments at 5% and 1% probability of error by test F are designed with (*) and (**), respectively; Uppercase letters compare tillage systems within cropping systems and lowercase letters compare cropping systems within tillage systems.

portion of soil macroaggregates associated with higher occluded-LF-C concentration in superficial soil layer led to greater C stocks under NT compared to CT in that layer (2.4 vs. 0.8 Mg ha⁻¹, on average, data not shown).

The effect of tillage on C accumulation in macroaggregates was not apparent in the 5–10 and 10–20 cm soil layers; hence, C stocks in the 0–20 cm layer were statistically similar (Table 2; Fig. 2). Plaza-Bonilla et al. (2010) and Andruschkewitsch et al. (2014) likewise found that the higher SOC content within macroaggregates under the NT treatment was restricted to the 0–5 cm soil depth.

As opposed to the effect of tillage, which was mostly observed in the macroaggregate fractions, the effects of cover crops were essentially visible in the organomineral associations (Min-OM-C) of soil microaggregates, particularly under the NT system. Two factors probably contributed to this effect. First, there was more C input in the OV/MC than O/M (8.9 Mg ha⁻¹ vs. 5.0 Mg ha⁻¹, Table 1). Second, the greater lability of the legume cover crop residues (vetch and cowpea) likely contributed to more efficient use of this material by microorganisms, and enhanced C stabilization in soil organic matter by chemical bonding with the mineral soil matrix (Cotrufo et al., 2013, 2015).

Overall, in all aggregate fractions, the min-OM-C fraction accounted for up to 75% of the SOC and was also the fraction most influenced by cropping systems (Table 3). The cropping system, including legume cover crops, was therefore the main factor that affected SOC accumulation up to 20 cm depth. Although NT increased the occluded-LF-C compared to CT, this effect was smaller than the benefits that legume cover crops can offer through gains in organomineral association.

The significant relationship between occluded-LF-C and Min-OM-C (Fig. 6a–c) suggests that the greater C occlusion favored the formation of organomineral associations when NT and legume cover crops are combined. This may be because aggregates “provide time” and

environment for occluded LF to become associated with the mineral soil matrix forming an organomineral association (Conceição et al., 2013; Garcia-Franco et al., 2015). These results suggest that the reduced aggregate turnover under NT relative to CT (Six et al., 1999) favoring the physical protection of particulate OM as well as interactions between soil mineral particles and OM in the superficial soil layer (Courier-Murias et al., 2013) is especially efficient in stabilizing C when coupled with legume cover crops.

5. Conclusions

The mechanisms by which no-tillage and legume cover crops favor soil C accumulation in a subtropical Acrisol differ. No-tillage has a positive impact on C stabilizing in soil macroaggregates, increasing the LF-C occlusion and leading to C accumulation mainly in the top 5 cm soil layer. On the other hand, legume cover crops are associated with an increase in the amount and lability of residue inputs, which favor organomineral associations in fine particles of soil, especially when combined with NT.

Acknowledgments

The authors are grateful to Dr. Denis Angers, who have great contribution to data interpretation. Also, to the staff of the Experimental Station and Department of Soil Science of the Federal University of Rio Grande do Sul for their help with field and laboratory activities, respectively. They also thank The National Council for Scientific and Technological Development (CNPq) and Foundation for Research Support of Rio Grande do Sul State (Fapergs) for funding this work and the Coordination for the Improvement of Personnel with Graduate Education (CAPES) for award of a sandwich doctorate scholarship to M. G. Veloso in Agriculture and Agri-Food Canada.

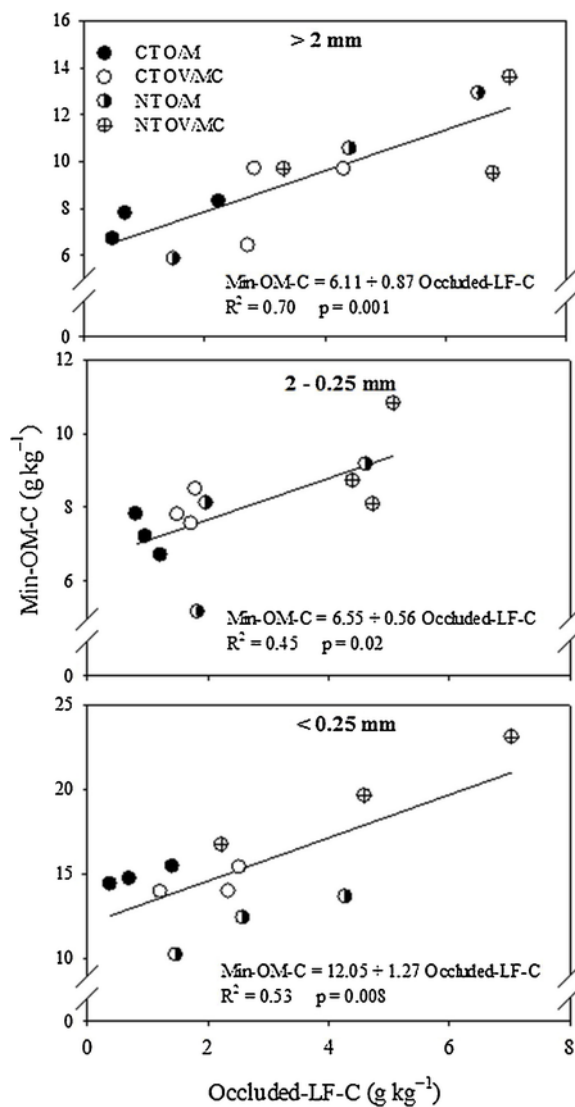


Fig. 6. Regression of C concentration in the mineral associated organic matter (Min-OM-C) as a function of the concentration of C in the occluded light fraction (Occluded-LF-C) in aggregates > 2 (a), 2–0.25 (b) and < 0.25 mm (c) in 0–5 cm layer of sandy clay loam Acrisol under conventional tillage (CT) and no-tillage (NT) in combination with oat/corn (O/M) and oat + vetch /maize + cowpea (OV/MC).

References

- Alvaro-Fuentes, J., Cantero-Martínez, C., Lopez, M.V., Paustian, K., Deneff, K., Stewart, C.E., Arrue, J.L., 2009. Soil aggregation and soil organic carbon stabilization: effects of management in semiarid mediterranean agroecosystems. *Soil Sci. Soc. Am. J.* 73, 1519–1529.
- Amado, T.J.C., Bayer, C., Conceição, P.C., Spagnollo, E., de Campos, B.H.C., da Veiga, M., 2006. Potential of carbon accumulation in no-till soils with intensive use and cover crops in southern Brazil. *J. Environ. Qual.* 35, 1599–1607.
- Andruschewitsch, R., Geisseler, D., Koch, H.J., Ludwig, B., 2013. Effects of tillage on contents of organic carbon, nitrogen, water-stable aggregates and light fraction for four different long-term trials. *Geoderma* 192, 368–377.
- Andruschewitsch, R., Koch, H.J., Ludwig, B., 2014. Effect of long-term tillage treatments on the temporal dynamics of water-stable aggregates and on macro-aggregate turnover at three German sites. *Geoderma* 217, 57–64.
- Angers, D.A., Giroux, M., 1996. Recently deposited organic matter in soil water-stable aggregates. *Soil Sci. Soc. Am. J.* 60, 1547–1551.
- Bayer, C., Martin-Neto, L., Mielniczuk, J., Pavinato, A., Dieckow, J., 2006. Carbon sequestration in two Brazilian Cerrado soils under no-till. *Soil Tillage Res.* 86, 237–245.
- Bayer, C., Dieckow, J., Amado, T.J.C., Eltz, F.L.F., Vieira, F.C.B., 2009. Cover crop effects increasing carbon storage in a subtropical no-till sandy Acrisol. *Commun. Soil Sci. Plant Anal.* 40, 1499–1511.

- Blake, G.R., Hartge, K.H., 1986. Bulk density. In: Klute, A. (Ed.), *Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods*. ASA/SSSA, Madison, pp. 363–382.
- Cambardella, C.A., Elliott, E.T., 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. *Soil Sci. Soc. Am. J.* 56, 777–783.
- Carpeneo, V., Mielniczuk, J., 1990. Estado de agregação e qualidade de agregados de latossolos roxos, submetidos a diferentes sistemas de manejo (aggregate stability and quality of latosol submitted to different management practices). *Rev. Bras. Ciên. Solo* 14, 99–105.
- Conceição, P.C., Boeni, M., Dieckow, J., Bayer, C., Mielniczuk, J., 2008. Densimetric fractionation with sodium polytungstate to investigate physical protection of soil organic matter. *Rev. Bras. Ciên. Solo* 32, 541–549.
- Conceição, P.C., Dieckow, J., Bayer, C., 2013. Combined role of no-tillage and cropping systems in soil carbon stocks and stabilization. *Soil Tillage Res.* 129, 40–47.
- Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Deneff, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Glob. Change Biol. Bioenergy* 19, 988–995.
- Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nat. Geosci.* 1–6. <https://doi.org/10.1038/NGEO2520>.
- Deneff, K., Six, J., Merckx, R., Paustian, K., 2004. Carbon sequestration in microaggregates of no-tillage soils with different clay mineralogy. *Soil Sci. Soc. Am. J.* 68, 1935–1944.
- Deneff, K., Zotarelli, L., Boddey, R.M., Six, J., 2007. Microaggregate-associated carbon as a diagnostic fraction for management-induced changes in soil organic carbon in two Oxisols. *Soil Biol. Biochem.* 39, 1165–1172.
- Diekow, J., Mielniczuk, J., Knicker, H., Bayer, C., Dick, D.P., Kögel-Knabner, I., 2005. Carbon and nitrogen stocks in physical fractions of a subtropical Acrisol as influenced by long-term no-till cropping systems and N fertilization. *Plant Soil* 268, 319–328.
- Diekow, J., Mielniczuk, J., Knicker, H., Bayer, C., Dick, D.P., Kögel-Knabner, I., 2005. Soil C and N stocks as affected by cropping systems and nitrogen fertilization in a southern Brazil Acrisol managed under no-tillage for 17 years. *Soil Tillage Res.* 81, 87–95.
- Du, Z.L., Ren, T.S., Hu, C.S., Zhang, Q.Z., 2015. Transition from intensive tillage to no-till enhances carbon sequestration in microaggregates of surface soil in the North China Plain. *Soil Tillage Res.* 146, 26–31.
- Ellert, B.H., Bettany, J.R., 1995. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. *Can. J. Soil Sci.* 75, 529–538.
- Fabrizzi, K.P., Rice, C.W., Amado, T.J.C., Fiorin, J., Barbagelata, P., Melchiori, R., 2009. Protection of soil organic C and N in temperate and tropical soils: effect of native and agroecosystems. *Biogeochemistry* 92, 129–143.
- García-Franco, N., Albaladejo, J., Almagro, M., Martínez-Mena, M., 2015. Beneficial effects of reduced tillage and green manure on soil aggregation and stabilization of organic carbon in a Mediterranean agroecosystem. *Soil Tillage Res.* 153, 66–75.
- Grandy, A.S., Robertson, G.P., 2007. Land-use intensity effects on soil organic carbon accumulation rates and mechanisms. *Ecosystems* 10, 58–73.
- Inda Junior, A.V., Bayer, C., Conceição, P.C., Boeni, M., Salton, J.C., Tonin, A.T., 2007. Variáveis relacionadas à estabilidade de complexos organo-minerais em solos tropicais e subtropicais brasileiros. *Ciência Rural* 37, 1301–1307.
- Martins, M.D., Angers, D.A., 2015. Different plant types for different soil ecosystem services. *Geoderma* 237, 266–269.
- Mazzilli, S.R., Kemanian, A.R., Ernst, O.R., Jackson, R.B., Piñeiro, G., 2015. Greater humification of belowground than aboveground biomass carbon into particulate soil organic matter in no-till corn and soybean crops. *Soil Biol. Biochem.* 85, 22–30.
- Plaza, C., Courtier-Murias, D., Fernandez, J.M., Polo, A., Simpson, A.J., 2013. Physical, chemical, and biochemical mechanisms of soil organic matter stabilization under conservation tillage systems: a central role for microbes and microbial by-products in C sequestration. *Soil Biol. Biochem.* 57, 124–134.
- Plaza-Bonilla, D., Cantero-Martínez, C., Alvaro-Fuentes, J., 2010. Tillage effects on soil aggregation and soil organic carbon profile distribution under Mediterranean semi-arid conditions. *Soil Use Manage.* 26, 465–474.
- Plaza-Bonilla, D., Cantero-Martínez, C., Viñas, P., Alvaro-Fuentes, J., 2013. Soil aggregation and organic carbon protection in a no-tillage chronosequence under Mediterranean conditions. *Geoderma* 193–194, 76–82.
- Puget, P., Angers, D.A., Chenu, C., 1999. Nature of carbohydrate associated with water-stable aggregates of two cultivated soils. *Soil Biol. Biochem.* 31, 55–63.
- Rasse, D.P., Rumpel, C., Dignac, M.F., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilization. *Plant Soil* 269, 341–356.
- Razafimbelo, T.M., Albrecht, A., Oliver, R., Chevallier, T., Chapuis-Lardy, L., Feller, C., 2008. Aggregate associated-C and physical protection in a tropical clayey soil under Malagasy conventional and no-tillage systems. *Soil Tillage Res.* 98, 140–149.
- Rumpel, C., Eusterhues, K., Kögel-Knabner, I., 2010. Non-cellulosic neutral sugar contribution to mineral associated organic matter in top-and subsoil horizons of two acid forest soils. *Soil Biol. Biochem.* 42, 379–382.
- Six, J., Elliott, E.T., Paustian, K., 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. *Soil Sci. Soc. Am. J.* 63, 1350–1358.
- Veloso, M.G., Angers, D.A., Tiecher, T., Giacomini, S., Dieckow, J., Bayer, C., 2018. High carbon storage in a previously degraded subtropical soil under no-tillage with legume cover crops. *Agric. Ecosys. Environ.* 268, 115–123.
- Zotarelli, L., Alves, B.J.R., Urquiaga, S., Torres, E., dos Santos, H.P., Paustian, K., Boddey, R.M., Six, J., 2007. Impact of tillage and crop rotation on aggregate-associated carbon in two oxisols. *Soil Sci. Soc. Am. J.* 69, 482–491.