Impact of tillage and crop rotation on light fraction and intra-aggregate soil organic matter in two Oxisols

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Received 25 February 2006; received in revised form 23 December 2006; accepted 5 January 2007

Abstract

It is well known that no-tillage (NT) practices can promote greater stocks of soil organic matter (SOM) in the soil surface layer compared to conventional tillage (CT) by enhancing the physical protection of aggregate-associated C in temperate soils. However, this link between tillage, aggregation and SOM is less well established for tropical soils, such as Oxisols. The objective of this study was to investigate the underlying mechanisms of SOM stabilization in Oxisols as affected by different crop rotations and tillage regimes at two sites in southern Brazil. Soils were sampled from two agricultural experiment sites (Passo Fundo and Londrina) in southern Brazil, with treatments comparing different crop rotations under NT and CT management, and a reference soil under native vegetation (NV). Free light fraction (LF) and intra-aggregate particulate organic matter (iPOM) were isolated from slaking-resistant aggregates. Of the total C associated with aggregates, 79–90% was found in the mineral fraction, but there were no differences between NT and CT. In contrast, tillage drastically decreased LF-C concentrations in the 0–5 cm depth layer at both sites. In the same depth layer of NT systems at Londrina, the concentrations of iPOM-C were greater when a legume cover crop was included in the rotation. At Londrina, the order of total iPOM-C levels was generally NV > NT > CT in the 0–5 cm depth interval, but the difference between NT and CT was much less than in Passo Fundo. At Passo Fundo, the greatest concentrations and differences in concentrations across tillage treatments were found in the fine (53–250 μm) iPOM fractions occluded within microaggregates. In conclusion, even though no aggregate hierarchy exists in these Oxisols, our results corroborate the concept of a stabilization of POM-C within microaggregates in no-tillage systems, especially when green manures are included in the rotation.

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Keywords: No-tillage; Crop rotation; Soil aggregates; Soil organic matter; Oxisols

1. Introduction

Soil organic matter (SOM) tends to decline when native ecosystems are converted to cropping systems for several reasons. The top soil may be subject to erosion and tillage, which leads to breakdown of soil aggregates and oxidation of organic matter. This loss of SOM reduces the availability of plant nutrients and also detrimentally affects soil physical properties. In the Cerrado (savanna) region of western Bahia in Brazil, the
conversion of native vegetation to conventional soybean [\textit{Glycine max} (L.) Merr.] cropping decreased the SOM in the 0–15 cm depth by 41–80% across different soil textures after only 5 years of conversion (Da Silva et al., 1994). Conventional tillage (CT) practices result in the mixing of crop residues into the soil profile and disruption of soil aggregates (especially macroaggregates (>250 \textmu m)), which enhance residue decomposition rates and SOM transformations (Beare et al., 1994; Six et al., 2002). However, the adoption of no-tillage (NT), may enhance SOM stabilization in comparison to CT (Havlin et al., 1990; Beare et al., 1994; Bayer et al., 2000a; Sisti et al., 2004; Diekow et al., 2005). A greater C accumulation in NT than CT in temperate soils has been related to the preservation of aggregates due to the lack of soil disturbance in NT (Beare et al., 1994; Six et al., 2002).

In temperate soils, it has been found that the formation rate of new macro- and microaggregates is directly related to the dynamics of particulate organic matter (POM). According to Golchin et al. (1994, 1998), macroaggregates are formed around fresh coarse residues (POM > 250 \textmu m), which become colonized by microorganisms and encrusted with mineral materials. The continuous decomposition of coarse intra-aggregate POM (iPOM) into smaller fragments promotes the formation of microaggregates (<250 \textmu m) within macroaggregates (Six et al., 2000b). Under CT, with frequent disruption of aggregates and input of new residues in the plough layer, macroaggregate turnover is enhanced, resulting in decreased time for microaggregate formation and consequently a diminished stabilization of C within microaggregates (Six et al., 2000b). In other words, under NT, the amount of water-stable macroaggregates is generally greater and the rate of turnover of macroaggregates is estimated to be half the rate of that under CT (Six et al., 1999, 2000b), resulting in greater mean residence times and accumulation of soil C in the surface layer of NT compared to CT systems (Six et al., 2002).

Several factors may contribute to soil aggregation, SOM accumulation and/or C stabilization in Oxisols. Since Oxisols are very weathered soils, there is a predominance of 1:1 clay minerals and oxides, while temperate soils are dominated by 2:1 clay minerals. These different characteristics may play a dominant role in the stabilization mechanisms of C (Denef et al., 2004; Oades and Waters, 1991; Six et al., 2000a). Fe and Al oxy-hydroxides associated with 1:1 clay minerals are strong binding agents and Oxisols dominated by this mineralogy form a very strong fine granular structure after long periods of time (Resende et al., 1997). In this process, free POM can become incorporated into this granular structure and be transformed into occluded POM (Roscoe et al., 2004), but SOM is generally less important as an aggregation agent (Six et al., 2000a; Zotarelli et al., 2005). Therefore, it has been suggested that the formation and stabilization of microaggregates within macroaggregates in soils with greater amounts of 1:1 clay mineral and oxides is less dependent on SOM (Denef et al., 2004; Six et al., 1999).

Unprotected SOM pools, such as the light fraction (LF) and free POM, are strongly influenced by the quality and quantity of crop residues and by soil management practices, such as NT versus CT, and crop rotation (Alvarez et al., 1998; Janzen et al., 1992). Even though the total C inputs within the same cropping sequence are usually quite similar for NT and CT (Paul et al., 1997; Sisti et al., 2004; Zotarelli et al., 2005), a greater C stabilization is observed in most long-term NT systems, in part due to greater aggregation which promotes physical protection of soil C (e.g. Six et al., 2000a,b). Soil texture also has an important influence on C accumulation in aggregates; i.e. with increasing clay content, the amount of occluded organic C stored in aggregates increases (Köbl and Kögel-Knabner, 2004). The lower disturbance in NT systems can promote the interaction between clays and slower decomposing C inputs to form soil aggregates. However, faunal populations and microbial biomass (especially fungal biomass) are also greater under NT (Cattelan et al., 1997; Frey et al., 1999) and these organisms play an important role in soil aggregation (Doran, 1987; Tisdall, 1991; Jungerius et al., 1999; Rillig and Mummey, 2006). Furthermore, the root system has been considered an effective agent for stabilization of macroaggregates in NT systems, whereas new C inputs from surface residues seem to not contribute as much to macroaggregate-associated C pools (Gale et al., 2000).

In our previous study (Zotarelli et al., 2005) on Oxisols, it was shown that aggregate size was greater under NT than under CT but in contrast to studies on high activity (2:1) clay soils there was no increase in C concentration with aggregate size class, or according to Tisdall and Oades (1982) there was no clear “aggregate hierarchy”. The objective of this study was to deepen our understanding of the underlying mechanisms of SOM stabilization at the same sites studied by Zotarelli et al. (2005) by (1) determining the C distribution in different mineral-associated C, occluded POM-C, and free POM-C fractions and (2) testing in Oxisols the model of aggregate formation and accumulation of soil organic matter proposed by Six et al. (2000b) for temperate soils. Our overall hypotheses were that the conceptual model of macroaggregate turnover (sensu
Six et al., 2000b) would be valid for Oxisols and that NT management and the inclusion of green manures in the crop rotation would be synergistic in increasing SOC accumulation.

2. Materials and methods

2.1. Site descriptions

2.1.1. Experiment 1: Passo Fundo

The first study was performed at the Experimental Station of the Embrapa Wheat Research Center in Passo Fundo, located 28°15′S and 52°24′W, 687 m altitude. The experiment was installed in 1986 in an area of native vegetation (tropical semi-deciduous forest open Araucaria woodland. In November of 1985, the soil was ploughed and limed with 7 Mg ha$^{-1}$ of dolomitic lime, but no further lime additions were made thereafter. The tillage treatments were: no-tillage (NT) and conventional tillage (CT). The CT plots were disk ploughed (20 cm) followed by a disk harrow twice a year, before every sowing. Two crop rotations were tested: PFR$_1$ was characterized by presence of two-grain crops per year, a summer soybean (*Glycine max* L.) and a winter wheat (*Triticum aestivum* L.). PFR$_2$ was characterized by two summer grain crops, soybean and maize (*Zea mays* L.), which were planted in alternate years, two winter crops, oat (*Avena sativa* L.) and wheat also planted in alternate years. In PFR$_2$, hairy vetch (*Vicia villosa* L.) was included as green manure that was cut down at the flowering stage and the residues were maintained on the soil surface. In PFR$_2$, the crop sequence wheat-soybean, hairy vetch-maize, and oats-soybean was repeated every 3 years.

2.1.2. Experiment 2: Londrina

The second site was at the Experimental Station of the Embrapa Soybean Research Center in Londrina, located 23°23′S and 51°11′W, 566 m altitude. The native vegetation was tropical semi-deciduous forest, and previous to the installation of the experiment, the area was under conventional agriculture since the 1980s, with a rotation of soybean and wheat. Between 1995 and 1997, the whole area was converted to NT with a sequence of soybean/lupin (*Lupinus* sp.)–maize/oat–soybean/oat. In November 1997, the experiment was installed with two soil management systems: NT and CT, and three crop rotations. The crop sequence between 1997 and 2001 for LAR$_1$ was maize, oat, soybean, wheat, soybean, lupin, maize, oat, for the LAR$_2$ was soybean, lupin, maize, oat, soybean, wheat, soybean, lupin, and LAR$_3$ was soybean, wheat, soybean, lupin, oat, maize. The management used for hairy vetch in Passo Fundo was adopted for lupin in Londrina.

The CT plots were disk ploughed (20 cm deep) followed by disk harrow before each of the two cropping seasons per year. The general soil characteristics of the agricultural sites are shown in Table 1.

2.2. Soil sampling and physical fractionation

At both sites, the soils were kaolinitic Oxisols with a clayey texture (Haplorthox–Ferralsol). Both sites had

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Tillage</th>
<th>Passo Fundo 0–5 cm</th>
<th>5–20 cm</th>
<th>Londrina 0–5 cm</th>
<th>5–20 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total organic C (g m$^{-2}$)</td>
<td>NV</td>
<td>1613 ± 36$^a$</td>
<td>3108 ± 111</td>
<td>1954 ± 96</td>
<td>2875 ± 132</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>1146 ± 82</td>
<td>3457 ± 172</td>
<td>1228 ± 159</td>
<td>3305 ± 360</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>820 ± 44</td>
<td>3384 ± 107</td>
<td>1180 ± 66</td>
<td>3437 ± 204</td>
</tr>
<tr>
<td>Total N (g m$^{-2}$)</td>
<td>NV</td>
<td>159 ± 11</td>
<td>317 ± 15</td>
<td>230 ± 16</td>
<td>297 ± 8</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>109 ± 4</td>
<td>330 ± 11</td>
<td>106 ± 9</td>
<td>311 ± 11</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>80 ± 3</td>
<td>311 ± 8</td>
<td>89 ± 5</td>
<td>245 ± 9</td>
</tr>
<tr>
<td>Bulk density (g cm$^{-3}$)</td>
<td>NV</td>
<td>0.97 ± 0.35</td>
<td>1.07 ± 0.15</td>
<td>0.94 ± 0.41</td>
<td>1.01 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>1.11 ± 0.09</td>
<td>1.10 ± 0.08</td>
<td>1.03 ± 0.13</td>
<td>1.23 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>1.37 ± 0.15</td>
<td>1.37 ± 0.14</td>
<td>1.23 ± 0.09</td>
<td>1.25 ± 0.11</td>
</tr>
<tr>
<td>Year of establishment</td>
<td></td>
<td>1985</td>
<td></td>
<td>1997</td>
<td></td>
</tr>
<tr>
<td>Texture (S, Si, C)$^b$</td>
<td></td>
<td>(240, 130, 630)</td>
<td></td>
<td>(200, 80, 720)</td>
<td></td>
</tr>
</tbody>
</table>

0–5 and 5–20 cm denote the soil depth.

$^a$ Standard devition.

$^b$ (S, Si, C) = g kg$^{-1}$ of sand, silt, and clay.
native vegetation (NV), CT, and NT treatments. However, the NV plots were located in adjacent areas near the plots of the field experiment and not included within the experimental area.

In July 2001, soils from each treatment were sampled at two depths (0–5 cm and 5–20 cm). Before the samples were taken, the litter layer was removed. Once in the laboratory, the moist soil was passed through an 8 mm sieve by gently breaking apart the soil and the samples were then air dried and stored at room temperature. The method of aggregate separation was adapted from Elliott (1986). Briefly, aggregates were separated by wet sieving air-dried soil through a series of three sieves (2000, 250 and 53 μm). The air-dried soil was quickly submerged in deionized water on top of the 2000-μm sieve, resulting in slaking of the soil. The isolated aggregates were oven dried (50°C). The method of separation of free LF and intra-aggregate POM was described by Six et al. (1998). A 5 g subsample was suspended in 35 mL of 1.85 g cm⁻³ sodium polytungstate in a 50 mL graduated conical centrifuge tube. The suspended subsample was mixed without breaking the aggregates by slowly reciprocal shaking by hand (10 strokes). The sample was put under vacuum (138 kPa) for 10 min to evacuate air entrapped within the aggregates. After 20 min equilibration, the sample was centrifuged (1250 g) at 20 °C for 60 min. The floating material (LF) was aspirated onto a 20-μm nylon filter, rinsed thoroughly with deionized water to remove sodium polytungstate, transferred to a small aluminum pan, and dried at 50 °C. The heavy fraction was rinsed twice with 50 mL of deionized water and dispersed by shaking in 15 ml of 0.5% of hexametaphosphate with 12 glass beads (5 mm diameter each) per sample for 18 h on a reciprocal shaker. The dispersed heavy fraction was passed through 250 and 53 μm sieves and different POM size classes were distinguished: fine iPOM (<250 μm) and coarse iPOM (>250 μm). The material remaining on the sieve, iPOM + sand was dried (50 °C) and weighed.

Aggregate-associated C and mineral-associated C were calculated by difference:

aggregate-associated C

\[ \text{aggregate-associated C} = \text{total aggregate C} - \text{free LFC} \]  

(1)

mineral-associated C

\[ \text{mineral-associated C} = \text{aggregate-associated C} - \text{iPOM-C} \]  

(2)

where total aggregate C was the total C measured in aggregates prior to the LF flotation.

2.3. Carbon analysis

Carbon concentrations were measured with a LECO CHN-100 analyser (Leco Corp., St. Joseph, MI) for the aggregate, LF, and iPOM size fractions. For appropriate comparisons between treatments and sites it was necessary to correct for the sand content in each aggregate size class (Elliott et al., 1991).

2.4. Statistical analysis

In Londrina, the field experiment (two tillage treatments × three rotations) was arranged in a randomized complete block design with four replicates. The Passo Fundo field experiment (two tillage treatments × four rotations) was arranged in a randomized split plot design with three replicates and the tillage treatments in the main plots and the rotations in the subplots. At both sites, the areas of NV were outside of the experimental design and therefore the NV was not included in the statistical analyses. The data were initially analyzed using the MSTAT-C software (Michigan State University, USA) for analysis of variance to determine the effects of the main variables (tillage and rotation) on the measured parameters at a P value of 0.05.

For the experiment at Londrina, which was a factorial design with all treatments in main plots, it was necessary to examine the effect of the different crop rotation on any parameter within the same tillage treatment (or vice versa), the variance associated with the difference between the individual means was compared to that of the residual and the F test was applied.

For the split plot design of the experiment at Passo Fundo, this was achieved using the procedure described by Little and Jackson-Hills (1978): The calculation of the least significant difference between means (LSD, Student) was applied where

\[ \text{LSD}_{0.05} = t_{ab} \sqrt{2 \times \frac{(b-1)E_b + E_a}{rb}} \]  

(3)

where \( b \) is the number of subplot treatments, \( r \) the number of replicates, \( E_a \) and \( E_b \) are the mean squares of the sub-plot and main plot errors, respectively, and \( t_{ab} \) is the weighted \( t \) value for main plots and subplots calculated as described by Little and Jackson-Hills (1978).

These procedures were performed in the software SISVAR, produced by the Federal University of Lavras, Minas Gerais.
3. Results and discussion

3.1. Mineral associated carbon

When comparing the tillage treatments with the NV at Passo Fundo, it was evident that mineral-associated C concentrations in each class of aggregates decreased in the order NV ≥ NT > CT (Table 2), suggesting that aggregate C behavior under NT was similar to that under NV. However, 14 years of NT compared to CT resulted in greater quantities of mineral-associated C in all classes of aggregates in the 0–5 cm depth layer (Table 2). The concentration of C was 40% greater in NT than CT. In contrast, no significant differences were observed for this parameter between tillage systems and NV and across aggregate size classes in the 5–20 cm depth interval. Furthermore, no effect of crop rotation on mineral-associated C was observed within tillage treatments and aggregate size classes. At Londrina, on the other hand, where the soil had been under NT for only 4 years, no significant differences were observed in the mineral-associated C between tillage treatments and aggregate size fractions and the C concentrations were very similar in both depth intervals studied. In contrast, mineral-associated C concentrations were much lower in both tillage treatments compared to NV (Table 2).

Considering the results obtained by Zotarelli et al. (2005) for Londrina and Passo Fundo, large amounts (79–90%) of the aggregate-associated C were found in the mineral fraction, but there were no differences in the proportion of C associated with minerals between NT and CT. Similar results were found by Six et al. (1999) and John et al. (2005) for temperate soils and Freixo et al. (2002) and Denef et al. (2004) for tropical soils.

In general, losses of mineral-associated C may be faster or slower depending on the soil management treatments and aggregate size classes. At Londrina, on the other hand, where the soil had been under NT for only 4 years, no significant differences were observed in the mineral-associated C between tillage treatments and aggregate size fractions and the C concentrations were very similar in both depth intervals studied. In contrast, mineral-associated C concentrations were much lower in both tillage treatments compared to NV (Table 2).

### Table 2

Mineral-associated carbon (g C kg\(^{-1}\)) from different depths of soil of different tillage systems and crop rotations at Passo Fundo and Londrina

<table>
<thead>
<tr>
<th>Site</th>
<th>Crop rotation</th>
<th>0–5 cm</th>
<th>5–20 cm</th>
<th>Site</th>
<th>Crop rotation</th>
<th>0–5 cm</th>
<th>5–20 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passo Fundo*</td>
<td></td>
<td></td>
<td></td>
<td>Londrina*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NV</td>
<td>–</td>
<td>36.7</td>
<td>28.1</td>
<td>25.9</td>
<td>20.7</td>
<td>23.7</td>
<td>20.8</td>
</tr>
<tr>
<td>NT</td>
<td>PF(_{R1})</td>
<td>29.0 a</td>
<td>29.7 a</td>
<td>31.3 a</td>
<td>21.3 a</td>
<td>20.1 a</td>
<td>21.0 a</td>
</tr>
<tr>
<td></td>
<td>PF(_{R2})</td>
<td>27.3 a</td>
<td>27.1 a</td>
<td>24.5 a</td>
<td>18.6 a</td>
<td>20.4 a</td>
<td>21.0 a</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>28.2 aA</td>
<td>28.4 aA</td>
<td>27.9 aA</td>
<td>19.9 aA</td>
<td>20.3 aA</td>
<td>21.0 aA</td>
</tr>
<tr>
<td>CT</td>
<td>PF(_{R1})</td>
<td>22.8 a</td>
<td>19.6 a</td>
<td>19.6 a</td>
<td>22.5 a</td>
<td>19.2 a</td>
<td>20.2 a</td>
</tr>
<tr>
<td></td>
<td>PF(_{R2})</td>
<td>17.4 a</td>
<td>20.3 a</td>
<td>20.9 a</td>
<td>15.1 a</td>
<td>23.6 a</td>
<td>22.1 a</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>20.1 aB</td>
<td>20.0 bA</td>
<td>20.2 bA</td>
<td>18.8 aA</td>
<td>21.4 aA</td>
<td>21.1 aA</td>
</tr>
<tr>
<td></td>
<td>C.V. (%)</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>11</td>
<td>29</td>
<td>31</td>
</tr>
</tbody>
</table>

|                      |               |         |         |                      |               |         |         |
| Passo Fundo*         |               |         |         | Londrina*            |               |         |         |
| NV                    | –             | 36.1   | 38.0    | 35.4       | 16.6         | 17.3    | 20.8    |
| NT                    | LA\(_{R1}\)  | 20.4 a  | 19.1 a  | 21.7 a     | 16.4 a       | 16.5 a  | 19.0 a  |
|                      | LA\(_{R2}\)  | 16.5 a  | 15.6 a  | 18.0 a     | 16.2 a       | 16.5 a  | 19.4 a  |
|                      | LA\(_{R3}\)  | 18.2 a  | 17.3 a  | 19.0 a     | 16.7 a       | 16.4 a  | 16.1 a  |
|                      | Mean         | 18.3 aA| 17.3 aA| 19.6 aA    | 16.5 aA      | 16.5 aA| 18.2 aA|
| CT                    | LA\(_{R1}\)  | 17.1 a  | 16.6 a  | 21.0 a     | 17.2 a       | 16.7 a  | 16.1 a  |
|                      | LA\(_{R2}\)  | 16.8 a  | 15.8 a  | 14.8 b     | 16.7 a       | 16.4 a  | 19.9 a  |
|                      | LA\(_{R3}\)  | 17.4 a  | 16.5 a  | 21.2 a     | 17.4 a       | 16.4 a  | 15.9 a  |
|                      | Mean         | 17.1 aA| 16.3 aA| 19.0 aA    | 17.1 aA      | 16.5 aA| 17.3 aB|
|                      | C.V. (%)     | 18      | 16      | 27         | 7            | 5       | 17      |

NV: native vegetation; NT: no-tillage; CT: conventional tillage; C.V.: coefficient of variation.\(^{a}\)Values followed by the same lowercase letter indicate that the means of C concentration are not significantly different (at \(P < 0.05\) according to the Student LSD test) between crop rotations within the same aggregate size fraction, tillage treatment, depth interval and agricultural site.\(^{b}\)Values followed by the same italic lowercase letter indicate that the means of C concentration are not significantly different (at \(P < 0.05\) according to the Student LSD test) between tillage treatments within the same aggregate size fraction, depth interval and agricultural site.\(^{c}\)Values followed by the same uppercase letter indicate that the means of C concentration are not significantly different (at \(P < 0.05\) according to the Student LSD test) between aggregate size classes within the same tillage treatments, depth interval and agricultural site.\(^{d}\)Londrina—(LA\(_{R1}\)): soybean/lupin–maize/oat; (LA\(_{R2}\)): soybean/wheat–soybean/lupin and (LA\(_{R3}\)): soybean/lupin–maize/wheat.
adopted. Conventional tillage increases aggregate disruption compared to NT, but this increase is mostly promoted in the surface layer and leads to increased decomposition of SOM due to the exposure of surface soil to erosion, faster oxidation of SOM, and increased macroaggregate turnover (Paustian et al., 1997; Six et al., 1998, 1999, 2000b). The history of management at both sites is also important in order to explain the observed differences in SOC losses. For all aggregate size classes, the magnitude of the differences of mineral-associated C concentration between NV and NT at Londrina were greater than those observed at Passo Fundo. At Londrina, the whole area was cultivated under CT for more than 20 years after the conversion from NV, which already depleted the SOC stocks present in the area. In contrast, at Passo Fundo, the natural ecosystem was directly converted to NT and CT tillage treatments, and in this case, the decline in the mineral-associated C concentration was more intense under CT than NT.

3.2. Free light fraction

In the 0–5 cm depth interval at Passo Fundo, the LF-C concentration under NV was greater than under the cropped plots for all aggregate size fractions (Table 3). Mean LF-C concentrations were significantly greater under NT than CT for 250–2000 μm and >2000 μm aggregate size classes, however, there were no differences between LF-C concentration in any aggregate size classes in the 5–20 cm depth interval and in the smallest aggregates size class (53–250 μm) of the 0–5 cm depth. Comparing the two crop rotations in the 0–5 cm depth interval at Passo Fundo, LF-C was lower under the soybean/wheat crop rotation (PF R1) in the 53–250 μm aggregate size class under both NT

| Table 3 | Light fraction carbon (g C m⁻²) from different depths of soil of different tillage systems and crop rotations at Passo Fundo and Londrina |
|-----------------|-------------------------------------------------|--|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | Site | Crop rotation | 0–5 cm | 5–20 cm | | | | | | |
| Passo Fundoa | NV | – | 560 | 1867 | 399 | 357 | 1324 | 333 |
| | NT | PF R1 | 124 b | 380 a | 36 a | 124 a | 483 a | 61 b |
| | | PF R2 | 302 a | 475 a | 68 a | 204 a | 513 a | 206 a |
| | | Mean | 213 aC | 427 aA | 52 aB | 164 aB | 498 aA | 134 aB |
| | CT | PF R1 | 91 b | 185 b | 23 a | 198 a | 650 a | 85 a |
| | | PF R2 | 308 a | 307 a | 33 a | 469 a | 605 a | 167 a |
| | | Mean | 199 aB | 246 bA | 28 bA | 333 aB | 628 aA | 126 aB |
| | C.V. (%) | 33 | 43 | 29 | 49 | 26 | 35 |
| Londrinae | NV | – | 187 | 1305 | 907 | 334 | 1434 | 384 |
| | NT | LA R1 | 272 a | 1292 a | 410 ab | 679 a | 1724 a | 402 a |
| | | LA R2 | 233 a | 1186 a | 301 b | 661 a | 1823 a | 387 a |
| | | LA R3 | 185 a | 711 a | 461 a | 509 a | 2006 a | 679 a |
| | | Mean | 230 aB | 1063 aA | 391 aB | 616 aB | 1851 aA | 489 AaB |
| | C.V. (%) | 27 | 32 | 29 | 19 | 23 | 33 |

NV: native vegetation; NT: no-tillage; CT: conventional tillage; C.V.: coefficient of variation.


b Values followed by the same lowercase letter indicate that the means of C concentration are not significantly different (at P < 0.05 according to the Student LSD test) between crop rotations within the same aggregate size fraction, tillage treatment, depth interval and agricultural site.

c Values followed by the same uppercase letter indicate that the means of C concentration are not significantly different (at P < 0.05 according to the Student LSD test) between aggregate size classes within the same tillage treatments, depth interval and agricultural site.

d Values followed by the same italic lowercase letter indicate that the means of C concentration are not significantly different (at P < 0.05 according to the Student LSD test) between tillage treatments within the same aggregate size fraction, depth interval and agricultural site.

and CT management, and in the 250–2000 μm size class under CT.

At Londrina, the LF-C concentration under NV was higher than under NT and CT in the largest aggregate size classes of the 0–5 cm depth interval. However, for the smallest aggregate size class (53–250 μm) in the 0–5 cm depth interval and all aggregate sizes in the 5–20 cm depth interval, the cultivated soils showed greater values of LF-C. The LF-C was similar between NT and CT across aggregate size classes except for the 250–2000 μm aggregate size class, for which the C concentration was significant higher under NT. No effect of crop rotation on LF-C concentrations was observed at Londrina. For both sites, the 250–2000 μm aggregate size class was significantly higher in LF-C concentration than other size classes except for the largest size fraction (>2000 μm) of the 0–5 cm depth interval. Furthermore, the LF-C associated with >2000 μm aggregate size class was greater in the LAR2 rotation versus LAR3 rotation under NT. This probably reflects the fact that the last two crops before sampling in the LAR2 were legumes (soybean and lupin) with residues that decompose rapidly, whereas in the LAR3 rotation, the last two crops were maize and wheat with residues that decompose slower.

As the LF is considered to be mostly influenced by C input levels (Six et al., 1999; Janzen et al., 1992), differences in LF-C were expected to be greater between NV and cultivated systems than between the two tillage treatments. This was illustrated by the drastic negative impacts of cultivation on LF-C concentrations in the 0–5 cm depth layer at Passo Fundo. In the 0–5 cm soil layer of the NV, approximately 16% of the stock of C was found in the LF, while for NT and CT the LF-C accounted for only 6 and 4% of the total C, respectively. Interestingly, at Londrina, the quantity of C found in the LF was around 10% and did not differ between NT, CT or NV. Nevertheless, in a similar Rhodic Ferralsol, Freixo et al. (2002) observed a decrease in whole soil LF-C of 40% for NT and 60% for CT compared to NV.

3.3. Intra-aggregate particulate organic matter

Six et al. (1998) suggested that macroaggregates (250–2000 μm) are formed around fresh residue, which

<table>
<thead>
<tr>
<th>Site</th>
<th>Crop rotation</th>
<th>53</th>
<th>250 f</th>
<th>250 c</th>
<th>2000 f</th>
<th>2000 c</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NV</td>
<td>–</td>
<td>8.29</td>
<td>1.08</td>
<td>0.34</td>
<td>1.51</td>
<td>0.91</td>
</tr>
<tr>
<td>NT</td>
<td>PF&lt;sub&gt;R1&lt;/sub&gt;</td>
<td>2.40 b</td>
<td>1.62 b</td>
<td>0.46 b</td>
<td>2.33 a</td>
<td>0.10 b</td>
</tr>
<tr>
<td></td>
<td>PF&lt;sub&gt;R2&lt;/sub&gt;</td>
<td>4.26 a</td>
<td>1.96 a</td>
<td>0.86 a</td>
<td>1.93 a</td>
<td>1.12 a</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>3.33 a</td>
<td>1.79 a</td>
<td>0.66 a</td>
<td>2.13 a</td>
<td>0.61 a</td>
</tr>
<tr>
<td>CT</td>
<td>PF&lt;sub&gt;R1&lt;/sub&gt;</td>
<td>0.83 a</td>
<td>0.44 a</td>
<td>0.27 a</td>
<td>0.44 a</td>
<td>0.19 b</td>
</tr>
<tr>
<td></td>
<td>PF&lt;sub&gt;R2&lt;/sub&gt;</td>
<td>1.03 a</td>
<td>0.54 a</td>
<td>0.13 a</td>
<td>0.64 a</td>
<td>0.37 a</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.93 b&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.49 b</td>
<td>0.20 b</td>
<td>0.54 b</td>
<td>0.28 b</td>
</tr>
<tr>
<td></td>
<td>C.V. (%)</td>
<td>13</td>
<td>23</td>
<td>15</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>5–20 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NV</td>
<td>–</td>
<td>4.10</td>
<td>0.57</td>
<td>0.18</td>
<td>0.54</td>
<td>0.31</td>
</tr>
<tr>
<td>NT</td>
<td>PF&lt;sub&gt;R1&lt;/sub&gt;</td>
<td>0.62 b</td>
<td>0.61 a</td>
<td>0.10 a</td>
<td>0.32 a</td>
<td>0.21 a</td>
</tr>
<tr>
<td></td>
<td>PF&lt;sub&gt;R2&lt;/sub&gt;</td>
<td>0.88 a</td>
<td>0.27 b</td>
<td>0.12 a</td>
<td>0.68 a</td>
<td>0.21 a</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.75 a</td>
<td>0.44 a</td>
<td>0.11 a</td>
<td>0.50 a</td>
<td>0.21 a</td>
</tr>
<tr>
<td>CT</td>
<td>PF&lt;sub&gt;R1&lt;/sub&gt;</td>
<td>0.70 b</td>
<td>0.64 a</td>
<td>0.15 a</td>
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<td>0.11 b</td>
</tr>
<tr>
<td></td>
<td>PF&lt;sub&gt;R2&lt;/sub&gt;</td>
<td>1.14 a</td>
<td>0.32 b</td>
<td>0.25 a</td>
<td>0.76 a</td>
<td>0.45 a</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.92 a</td>
<td>0.48 a</td>
<td>0.20 a</td>
<td>0.63 a</td>
<td>0.28 a</td>
</tr>
<tr>
<td></td>
<td>C.V. (%)</td>
<td>6</td>
<td>23</td>
<td>83</td>
<td>34</td>
<td>69</td>
</tr>
</tbody>
</table>

NV: native vegetation; NT: no-tillage; CT: conventional tillage; C.V.: coefficient of variation; 250 f and 250 c = fine (53–250 μm) and coarse (250–2000 μm) iPOM, respectively; 2000 f and 2000 c = iPOM in >2000 μm macroaggregates with a size 53–250 and >250 μm, respectively).

<sup>a</sup> Passo Fundo—(PF<sub>R1</sub>): soybean/wheat and (PF<sub>R2</sub>): soybean/hairy vetch–maize/oat–soybean/wheat.

<sup>b</sup> Values followed by the same lowercase letter indicate that the means of C concentration are not significantly different (at P < 0.05 according to the Student LSD test) between crop rotations within the same aggregate size fraction, tillage treatment, and depth interval.

<sup>c</sup> Values followed by the same italic lowercase letter indicate that the means of C concentration are not significantly different (at P < 0.05 according to the Student LSD test) between tillage treatments within the same aggregate size fraction, and depth interval.
becomes coarse iPOM. As the input of residues is practically the same for NT and CT (Paul et al., 1997; Zotarelli et al., 2005), the model of aggregate formation (Six et al., 1998) assumes that macroaggregate formation will be similar between NT and CT. Fine iPOM inside macroaggregates is derived from decomposition and fragmentation of coarse iPOM. Consequently, fine iPOM inside macroaggregates is older than coarse iPOM (Six et al., 2000b). In addition, Six et al. (2001) found that fine iPOM contained a greater proportion of functional C groups indicative of recalcitrant or microbial-C than coarse iPOM.

At Passo Fundo, the iPOM-C concentration for all aggregate size classes was strongly influenced by tillage treatments in the 0–5 cm depth interval. On average, iPOM-C concentration was approximately three times higher under NT than CT (Table 4). In the largest aggregate size class (>2000 μm) of NT, fine iPOM-C was 3.5 times greater than coarse iPOM-C, while under CT, fine iPOM-C was only two times greater than coarse iPOM-C. The highest concentration of iPOM-C was found in the 53–250 μm water stable aggregate size class (microaggregates). In the 5–20 cm depth interval at Passo Fundo, there were no significant differences in the iPOM-C concentration between NT and CT (Table 4). The concentration of iPOM was similar between agricultural systems and NV in the 5–20 cm depth interval, except for the 53–250 μm aggregate size class.

At Londrina, the order of total iPOM-C levels in the 0–5 cm depth interval was generally NV > NT > CT (Table 5), but the difference between NT and CT was much less than the difference observed in Passo Fundo. Nevertheless, in all aggregate size classes, iPOM-C was greater in NT than CT. At Londrina, the fine iPOM-C in > aggregates 2000 μm was greater under NT than under CT in the 0–5 cm depth layer. In the 5–20 cm depth layer, there were no differences in any of the iPOM-C concentrations between aggregates under either tillage system. Six et al. (1999) found similar results in temperate soils.

Table 5

<table>
<thead>
<tr>
<th>Site</th>
<th>Crop rotation</th>
<th>53</th>
<th>250 f</th>
<th>250 c</th>
<th>2000 f</th>
<th>2000 c</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5 cm</td>
<td>NV</td>
<td>–</td>
<td>3.54</td>
<td>0.71</td>
<td>1.07</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>LA R1</td>
<td>2.92 b</td>
<td>0.44 b</td>
<td>0.77 a</td>
<td>0.94 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LA R2</td>
<td>3.87 a</td>
<td>0.73 a</td>
<td>0.62 a</td>
<td>1.12 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LA R3</td>
<td>2.26 c</td>
<td>0.41 b</td>
<td>0.35 b</td>
<td>0.56 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>3.02 a</td>
<td>0.53 a</td>
<td>0.58 a</td>
<td>0.88 a</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>LA R1</td>
<td>2.32 a</td>
<td>0.59 a</td>
<td>0.36 a</td>
<td>0.60 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LA R2</td>
<td>2.24 a</td>
<td>0.57 a</td>
<td>0.34 a</td>
<td>0.44 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LA R3</td>
<td>2.16 a</td>
<td>0.40 b</td>
<td>0.47 a</td>
<td>0.57 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>2.24 b</td>
<td>0.51 a</td>
<td>0.40 b</td>
<td>0.53 b</td>
</tr>
<tr>
<td></td>
<td>C.V. (%)</td>
<td>15</td>
<td>17</td>
<td>25</td>
<td>30</td>
<td>43</td>
</tr>
<tr>
<td>5–20 cm</td>
<td>NV</td>
<td>–</td>
<td>0.85</td>
<td>0.13</td>
<td>0.18</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>LA R1</td>
<td>1.45 a</td>
<td>0.27 b</td>
<td>0.25 a</td>
<td>0.36 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LA R2</td>
<td>1.91 a</td>
<td>0.30 b</td>
<td>0.33 a</td>
<td>0.61 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LA R3</td>
<td>1.95 a</td>
<td>0.47 a</td>
<td>0.27 a</td>
<td>0.53 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>1.77 b</td>
<td>0.35 b</td>
<td>0.28 a</td>
<td>0.50 a</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>LA R1</td>
<td>2.11 a</td>
<td>0.44 a</td>
<td>0.27 b</td>
<td>0.52 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LA R2</td>
<td>2.33 a</td>
<td>0.50 a</td>
<td>0.32 ab</td>
<td>0.64 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LA R3</td>
<td>2.60 a</td>
<td>0.48 a</td>
<td>0.45 a</td>
<td>0.51 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>2.34 a</td>
<td>0.48 a</td>
<td>0.34 a</td>
<td>0.56 a</td>
</tr>
<tr>
<td></td>
<td>C.V. (%)</td>
<td>18</td>
<td>19</td>
<td>31</td>
<td>40</td>
<td>37</td>
</tr>
</tbody>
</table>

NV: native vegetation; NT: no-tillage; CT: conventional tillage; C.V.: coefficient of variation; 250 f and 250 c = fine (53–250 μm) and coarse (250–2000 μm) iPOM, respectively; 2000 f and 2000 c = iPOM in >2000 μm macroaggregates with a size 53–250 and >250 μm, respectively). 


b Values followed by the same lowercase letter indicate that the means of C concentration are not significantly different (at $P < 0.05$ according to the Student LSD test) between crop rotations within the same aggregate size fraction, tillage treatment, and depth interval.

c Values followed by the same italic lowercase letter indicate that the means of C concentration are not significantly different (at $P < 0.05$ according to the Student LSD test) between tillage treatments within the same aggregate size fraction, and depth interval.
The ratio of fine iPOM-C to coarse iPOM-C can be used as relative measurement of the turnover of aggregates, i.e. a higher ratio of fine iPOM to coarse iPOM indicates a slower breakdown or turnover of macroaggregates (Six et al., 2000b). At Passo Fundo, the ratio fine iPOM-C:coarse iPOM-C was lower in CT than NT (Fig. 1). This higher ratio was observed under NT compared to CT at Passo Fundo for both depth intervals, but it was only significantly different in the 0–5 cm depth layer. We conclude that for Passo Fundo, NT management generally reduced macroaggregate turnover compared to CT management. Furthermore, the concentration of fine iPOM-C was significantly higher (three times) under NT than CT in the 0–5 cm depth layer at Passo Fundo. Since microaggregate formation occurs inside of macroaggregates as fine iPOM is gradually encrusted with clay and microbial products (Six et al., 2000b), the higher concentration of fine iPOM-C under NT than CT is consistent with the concept of enhanced C stabilization in microaggregates due to a slower macroaggregate turnover in NT systems (Six et al., 1999).

At Londrina the ratio of fine iPOM-C: coarse iPOM-C (Fig. 1) was not significantly different between tillage treatments and was much lower than the ratio found for Passo Fundo. The difference in soil texture, amount of residue input (see Zotarelli et al., 2005), and time of adoption of NT were probably the major reasons for the difference in C distributions across fractions and overall soil C stock between Passo Fundo and Londrina.

Crop rotation and residue type influenced soil aggregation and iPOM-C accumulation. The size and distribution of the microbial community is related to the quantity and quality of residues, and the higher C:N ratio tends to favor the fungal community (Miller and Jastrow, 1990), which contribute to macroaggregate formation (Beare et al., 1993). At Passo Fundo, total iPOM-C recovery in PF_R1 was significantly lower than for the more diverse crop rotation (PF_R2) under NT. The same was observed in CT, but the difference was not significant. At Londrina, the LA_R3 rotation had the lowest total iPOM-C concentration. In the 0–5 cm depth interval of CT systems, the total iPOM-C concentration was not different between crop rotations. The iPOM-C concentration in the 0–5 cm depth layer of NT-LA_R2 was greater than in NT-LA_R3 for the 250f, 250c, 2000f and 2000c fractions. Across crop rotations under NT, the concentration of fine iPOM-C under LA_R2 (sampled after soybean/lupin) was greater than under LA_R1 and LA_R3 (sampled after cereals). These results suggest, first of all, that the deposition of fresh residues on the soil surface under NT favored aggregation and iPOM-C accumulation in surface layers, while, under CT, the incorporation of residues favored the iPOM-C accumulation in 5–20 cm depth layer (Table 4). Additionally, the adoption of NT integrated with the inclusion of a winter green-manure crop in the crop rotation increased soil C stock and iPOM-C concentration, confirming the synergism between NT and cover cropping for the enhancement of soil C stabilization (Bayer et al., 2000a,b; Sisti et al., 2004; Diekow et al., 2005). Furthermore, in the rotations where fixing vetch and lupin were planted at Passo Fundo and Londrina, respectively, both higher residue input and a positive N balance were observed (Sisti et al., 2004; Zotarelli, 2005).

4. Conclusions

Aggregate size fractionation showed that NT promotes conditions for aggregate formation, mainly in the 0–5 cm depth layer, and this effect was related to greater soil C accumulation under NT than CT. Especially the combination of NT and the inclusion of green manures promoted the stabilization of aggregate-associated C.

The concept of microaggregate formation from macroaggregates (Oades, 1984) explains the POM-C distribution in the aggregate size classes. Although, no aggregate hierarchy existed in these soils (Zotarelli et al., 2005), our results for Oxisols corroborate the conceptual model of macroaggregate turnover determining the stabilization of SOM as fine iPOM-C in microaggregates as proposed by Six et al. (1998, 1999). Consequently, we conclude this concept is not only...
valid for temperate soils dominated by 2:1 clay minerals, but also for tropical soils dominated by 1:1 clay minerals and oxides.

Acknowledgements

This work was supported by the National Science Foundation (DEB 0344971). We thank E. Torres (Embrapa Soja) and H.P. Santos (Embrapa Trigo) who maintained the long-term experiment at Londrina and Passo Fundo, respectively, used in this study. The contribution of D.A. Loni, E.G. Cardoso and M.S. Pires in soil sampling at Londrina is acknowledged.

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