Microaggregate-associated carbon as a diagnostic fraction for management-induced changes in soil organic carbon in two Oxisols

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Received 27 April 2006; received in revised form 5 December 2006; accepted 11 December 2006
Available online 22 January 2007

Abstract

Carbon stabilization by macroaggregate-occluded microaggregates (Mm) has been proposed as a principal mechanism for long-term soil organic carbon (SOC) sequestration in temperate alternative agricultural and (af)forested systems. The aim of this study was to evaluate the importance of the Mm fraction for long-term C stabilization in Oxisols and to validate its diagnostic properties for total SOC changes upon changes in land use. Soil samples were taken from the 0–5 and 5–20 cm soil layers of native forest vegetation (NV), conventional tillage (CT) and no-tillage (NT) systems at an experimental site near Passo Fundo and one near Londrina in Southern Brazil. After aggregate-size separations by wet-sieving, macroaggregate-occluded water-stable microaggregates (53–250 μm) (Mm) were isolated from large (>2000 μm) and small (<250 μm) macroaggregates. Particulate organic matter located inside the Mm (intra-Mm-POM) and the mineral fraction (<53 μm) associated with the Mm (mineral-Mm) were separated from the POM fraction located outside the Mm (inter-Mm-POM) by density flotation followed by mechanical dispersion. Sand-free Mm-C concentrations on a macroaggregate basis were generally greater under NV and NT compared to CT in the 0–5 cm depth at both sites. Our findings support the importance of Mm (especially the mineral-Mm fraction) as long-term C-stabilization sites in highly weathered tropical soils under sustainable agricultural and natural systems. At both sites, significant differences in total SOC stocks (g C m⁻²) among different land use systems were always accompanied by parallel Mm-C stock differences. Though total SOC did not differ among land use systems in the 0–20 cm depth at both sites, Mm-C stocks were greater under NT compared to the CT treatment in the 0–20 cm depth at the Londrina site. We concluded that in these highly weathered tropical soils the Mm-C fraction is a more responsive fraction to management changes than total SOC and represents a diagnostic fraction for present as well as potential total SOC changes upon land-use change.

Keywords: Microaggregates; C sequestration; No-tillage; Forest; Oxisols

1. Introduction

Soil organic matter (SOM) occupies a central role in agroecosystem function and its preservation is, therefore, crucial for agricultural sustainability. Furthermore, SOM plays a key role in the terrestrial C budget by acting as a sink for atmospheric CO₂ when appropriate management practices are used (Paustian et al., 1998; West and Marland, 2002). Conservation tillage, in particular no-tillage (NT), is considered as an agricultural practice to sequester C and to improve the sustainability of temperate (Kern and Johnson, 1993; Lal et al., 1997) as well as tropical (Sa et al., 2001; Bayer et al., 2006) agroecosystems. In temperate soils, increased soil organic carbon (SOC) levels with NT compared to conventional tillage (CT) management have often been observed concomitant with improved soil aggregation (e.g., Beare et al., 1994; Six et al., 1999; Jiao et al., 2006). Zotarelli et al. (2005) reported similar increases in soil aggregation upon

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conversion of CT to NT in two highly weathered Oxisols as generally observed in temperate soils. Yet, a lack of aggregate hierarchy (sensu Tisdall and Oades, 1982) suggested that the tight feedback between improved aggregation and SOM accumulation, found in temperate soils, would not exist in these tropical soils, where oxides are the dominant stabilizing agents and prevent the expression of aggregate hierarchy caused by organic materials (Oades and Waters, 1991).

According to Six et al. (2000), increases in total SOC under NT relative to CT management are attributed not only to a greater amount of C-rich macroaggregates (>250 µm), but also to a reduced rate of macroaggregate turnover under NT. The slower turnover of macroaggregates was suggested to enhance the formation of highly stable microaggregates (within macroaggregates) in which C is stabilized and sequestered in the long term. A previous study with three NT and CT experiments in widely varying soil types (Mollisol, Alfisol, Oxisol) and environments (i.e. Nebraska, Kentucky, Brazil) supported this theory by finding that over 90% of the difference in total SOC between NT and CT could be accounted for by the macroaggregate-associated C isolated from stable macroaggregates (Denef et al., 2004). These findings suggested that the macroaggregate-occluded microaggregate-associated C fraction (Mm-C) may be a highly accurate and general diagnostic fraction for changes in total SOC in response to changes in tillage and potentially other management practices (Six et al., 2000; Denef et al., 2004). The diagnostic potential of this Mm-C fraction for management-induced changes in total SOC has only been evaluated in a few selected soil types and management practices. Further examination of the C stabilization capacity of this Mm fraction across a wide range of soils with different responses to different management practices can help further elucidate mechanisms of SOM stabilization and turnover, and can validate Mm-C as a measurable and functionally-meaningful fraction that could be incorporated into SOM models (Elliott et al., 1996; Smith et al., 2002).

Therefore, the objective of this study was to examine the potential of the Mm-C fraction to serve as a diagnostic fraction for management-induced C changes in two highly weathered Oxisols showing different responses in total SOC to long-term cultivation of previously forested ecosystems and conversion to NT management. Our overall hypothesis was that management-induced changes in total SOC would be predominantly reflected by parallel changes in the Mm-C fraction.

2. Materials and methods

2.1. Site description

Soils were collected in July 2001 from two different long-term agricultural field experiments in Southern Brazil. One experiment was located near Passo Fundo (State of Rio Grande do Sul, Brazil; 28°15’S; 52°24’W; 687 m altitude) with mean annual temperature (MAT) of 17.5 °C and mean annual precipitation (MAP) of 1787 mm. The other experiment was located near Londrina (State of Paraná, Brazil; 23°23’S; 51°11’W; 566 m altitude) with MAT of 20.6 °C and MAP of 1622 mm. Both sites were characterized by highly weathered soil, classified as a very fine, kaolinitic, isothermic, Typic Haplorthox in the USDA classification, as a Rhodic Ferralsol in the FAO classification, and as a Latossolo Vermelho distroférrico in the Brazilian soil classification (Embrapa—Empresa Brasileira De Pesquisa Agropecuária, 1999). At both sites, the soils were of clayey texture with 24% sand, 13% silt and 63% clay at Passo Fundo; and 20% sand, 8% silt and 72% clay at Londrina. Soil clay mineralogy was dominated by kaolinite at both sites.

The experiment at Passo Fundo was established at the Experimental Station of the Embrapa Wheat Research Centre in Passo Fundo. The experiment was installed in 1986 in an area of native vegetation (NV) (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 thereafter. Two tillage treatments were installed: NT and CT. The sampled plots at Passo Fundo consisted of a 3-year crop rotation with two summer cash crops: soybean (Glycine max L.) and maize (Zea mays L.) which were planted in alternated years; and two winter cash crops, oat (Avena sativa) and wheat (Triticum aestivum L.) also planted in alternated years. Hairy vetch (Vicia villosa L.) was included as green manure, which was mowed during the flowering stage, and the residues were maintained on the soil surface. The crop sequence soybean/hairy vetch–maize/oat–soybean/wheat was repeated every 3 years.

The experiment at Londrina was established at the Experimental Station of the Embrapa Soybean Research Centre in Londrina. The area was originally forested (tropical semi-deciduous rainforest) but has been under conventional agriculture since the 1980s with a soybean/wheat rotation. 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. Between 1997 and 2001, the crop sequence of the sampled plots was maize/oat–soybean/wheat–soybean/lupin–maize/oat. The same management used for hairy vetch in Passo Fundo was adopted for lupin in Londrina.

At Passo Fundo, the CT and NT plots were arranged in a randomized split plot design with 3 replications; tillage treatments were in the main plots and crop rotations in the sub-plots of 4 × 10 m. At Londrina, experimental plots of 6 × 12 m were arranged in a 2 × 3 factorial design (2 tillage treatments × 3 rotations) with 4 replications. At Passo Fundo, the NV site (approximately 5 ha) was 1800 m away from the experimental plots. At Londrina, the NV
site (approximately 7 ha) was 200 m away from the experimental plots. In the NT treatments, both summer and winter crops were direct drilled. CT consisted of disc plowing (20 cm) followed by light disk harrowing before each of the cropping seasons in a year.

Further details on site and soil characteristics can be found in Sisti et al. (2004) for Passo Fundo, and Zotarelli et al. (2005) for Passo Fundo and Londrina.

2.2. Soil sampling

At both sites, soil compaction was severe when core sampling was attempted. Therefore, two soil samples were taken per plot, each from the side of one trench of approximately 50 cm × 50 cm square, from which a 10 cm by 10 cm monolith was taken at two depths (0–5 and 5–20 cm). The litter layer (where present) was removed by 10 cm monolith was taken at two depths (0–5 and 5–20 cm). The litter layer (where present) was removed before sampling. For sampling of NV, 3 plots were selected in the middle of the NV area at both sites, with a similar distance (~20 m) from each other as the distance between the replicate plots within each tillage treatment in the experimental areas.

Once in the laboratory, the field moist soil samples were passed through an 8 mm sieve by gently breaking apart the soil. The sieved soil samples were composited per plot and per depth, then air-dried, and stored at room temperature.

2.3. Microaggregate and associated particulate organic matter isolations

Microaggregates were isolated from macroaggregates, which were obtained through a standard wet-sieving procedure (adapted from Elliott (1986)). In brief, wet-sieving was conducted with 100-g subsamples of the 8-mm sieved air-dried soil samples to obtain four water-stable aggregate size fractions: (1) > 2000 µm (large macroaggregates); (2) 250–2000 µm (small macroaggregates); (3) 53–250 µm (free microaggregates); and (4) < 53 µm (silt-sized microaggregates plus silt and clay particles).

Microaggregates protected within large and small macroaggregates were mechanically isolated according to Six et al. (2000) and Denef et al. (2004). Briefly, macroaggregate subsamples of approximately 8 g were submerged in deionized water for 4 h, then transferred on top of a 250 µm mesh screen inside a cylinder and reciprocally shaken (120 rev min⁻¹) in water with 50 glass beads (diam. 4 mm) until the complete disruption of all macroaggregates. From the < 250 µm sized fraction, which was continuously flushed onto a 53 µm sieve, water-stable microaggregates were obtained by regular wet-sieving. The particulate organic matter (POM) and sand that was retained on the 250 µm mesh screen was transferred onto a 2 mm and 250 µm sized sieve when large macroaggregates were analyzed, and over a 250 µm sieve when small macroaggregates were analyzed. The proportion of micro-aggregates found within macroaggregates (Mm) was corrected for aggregate-sized sand:

\[
\text{Proportion Mm (％)} = \frac{\text{microaggregate weight} - \text{weight of 53 to 250} \mu \text{m sand}}{\text{macroaggregate weight} - \text{weight of macroaggregate-sized sand}} \times 100
\]

Since no > 2 mm-sized sand was found in any large macroaggregate size fraction, no macroaggregate-sized sand corrections were needed for this fraction. For the CT soils of Londrina, microaggregate isolations were only done on small macroaggregates from both 0–5 to 5–20 cm depths because the large macroaggregate proportion in both soil layers of the CT treatment was negligible (< 10%) and insufficient for analysis.

The fine POM (53–250 µm) fraction that was not occluded in the Mm (inter-Mm-POM) was isolated by density flotation in 1.85 g cm⁻³ sodium polytungstate. This was followed by a mechanical dispersion of the heavy fraction with glass beads to isolate the fine POM occluded inside the Mm (intra-Mm-POM) (Six et al., 2000).

2.4. Carbon analyses

Total C analyses were done on: (1) total soil, (2) the total macroaggregate fraction isolated from large and small macroaggregates, and (3) the inter- and intra-Mm-POM fractions. Total C analyses were done on a CHN analyzer (model LECO CHN-1000, Leco Corp., St. Joseph, MI), except for the C concentration of the C contents of the total microaggregate fraction isolated from the macroaggregates, because of the smaller size of these fractions.

In order to determine the concentration of C (g kg⁻¹ macroaggregates) associated with the microaggregates themselves (Mm-C), inter-Mm-POM-C was subtracted from the C content of the total microaggregate fraction isolated from the macroaggregates:

\[
\text{Mm-C} = \text{total microaggregate fraction C} - \text{inter-Mm-POM-C.}
\]

This Mm-C is a combination of intra-Mm-POM-C as well as C associated with the mineral fraction of the micro-aggregates (mineral-Mm-C) (g kg⁻¹ macroaggregates). The latter can be calculated by difference:

\[
\text{Mineral-Mm-C} = \text{Mm-C} - \text{intra-Mm-POM-C.}
\]

When evaluating microaggregate-associated C concentrations expressed on a macroaggregate basis, we corrected for the total sand content of the macroaggregates, because of slight textural differences between the soils and the fact that there is little or no binding of organic matter with sand particles (Elliott et al., 1991). Sand-free C concentrations (g kg⁻¹ sand-free macroaggregates) were
calculated as follows:

\[
\text{Sand-free } C_{\text{fraction}} = \frac{C_{\text{fraction}}}{[1 - (\text{sand proportion})_{\text{fraction}]}}.
\]  

(4)

where the sand proportion was calculated from the weight of the coarse sand retained on the 250 µm mesh in the microaggregate isolator combined with the weight of the fine (53–250 µm) sand obtained during the intra-Mm-POM isolation.

The diagnostic properties of the Mm-C fraction for total SOC differences between NV, NT and CT management were evaluated by calculating how much of the difference in the total SOC content (g C m\(^{-2}\)) was explained by the difference in Mm-C among treatments, using the same calculations as described by Denef et al. (2004). For calculations of total C content, expressed as g C m\(^{-2}\), bulk density values were used, which were measured and reported by Zotarelli et al. (2005).

### 2.5. Statistical analyses

All data for Mm-C concentrations, Mm proportions and C stocks were analyzed by one-way ANOVA at \(P < 0.05\) with management treatment as fixed effect, followed by Tukey’s test at \(P < 0.05\) using the software package S-Plus 7.0 for Windows. Where the criteria for ANOVA were not met due to heteroscedasticity (unequal variances), a power transformation was performed: \(y = y^p\), with \(p = 1 - a\), and \(a = \) slope of the linear regression of log (standard deviation) with log (mean). Management treatment had 3 replications at Passo Fundo (\(n = 3\)) and 4 replications at Londrina (\(n = 4\)). For the NV plots, 3 replicate soil samples (\(n = 3\) at both sites) were taken out of one native area per site (i.e. pseudo-replication), but from selected plots with similar distances between each other as between the experimental tillage replicate plots.

### 3. Results

#### 3.1. Macroaggregate-occluded microaggregates and associated carbon

At Passo Fundo, sand-free C concentrations of the Mm (on a macroaggregate basis, i.e. g Mm-C kg\(^{-1}\) sand-free macroaggregates) decreased in the order: NV > NT > CT in the 0–5 cm and NV > NT > CT in the 5–20 cm soil depths (Table 1). At Londrina, Mm-C concentrations showed the same trends in the 0–5 cm layer (NV > NT > CT), while in the 5–20 cm layer, Mm-C only differed between NT and NV (NT > NV) (Table 1). In both soils, depths and all three treatments, more than 90% of Mm-C was associated with the mineral-Mm fraction. The C concentration of this mineral-Mm fraction showed similar differences among the different treatments as the Mm-C. Intra-Mm-POM-C concentrations were also different among treatments, but did not follow a distinct trend among the different treatments, except in the 0–5 cm layer, where the intra-Mm-POM-C concentration was greater under NT compared to CT in both soils.

The proportion of macroaggregate soil mass found in the Mm fraction significantly differed between NV and CT at both sites in the 0–5 cm layer, but opposite trends were observed for large and small macroaggregates. Large macroaggregates had generally greater Mm proportions under NV than CT while small macroaggregate Mm proportions were larger under CT (Table 2). At both sites, Mm proportions were nearly equal under NT vs. CT in both large and small macroaggregates.

#### 3.2. Total soil and microaggregate-associated carbon stocks

At Passo Fundo, total SOC stocks (expressed in g C m\(^{-2}\)) were significantly greater under NV and NT compared to...
CT in the 0–5 cm layer (Table 3). At Londrina, SOC levels were significantly reduced under NT and CT relative to NV in the 0–5 cm layer, but no significant differences in total SOC stocks were found between NT and CT. In both soils, total SOC stocks did not differ among treatments in the entire 0–20 cm layer.

Total Mm-C and mineral-Mm-C stocks followed the same trends as total SOC stocks across treatments in the 0–5 cm at both soils, and in the 0–20 cm at Passo Fundo (Table 3). In the 0–20 cm depth at Londrina however, total Mm-C and mineral-Mm-C stocks were, in contrast to the total SOC stocks, significantly greater under NT than CT.

In the 0–5 cm, intra-Mm-POM-C stocks were also significantly greater under NT than CT in both soils. Greater POM-C stocks were also found under NV compared to both NT and CT, but mainly in the inter-Mm-POM-C. This inter-Mm-POM-C contributed more to the NV total SOC stocks than the intra-Mm-POM-C (Table 3).

### 4. Discussion

#### 4.1. Macroaggregate-occluded microaggregates as carbon stabilization sites

Recent studies have revealed that the Mm fraction can serve as an ideal indicator for C sequestration in sustainable agroecosystems, in particular in NT systems (Denef et al., 2004) and alternative cropping systems (e.g., low-input and organic farming systems) (Kong et al., 2005). Similarly, enhanced C sequestration through C stabilization within Mm has been confirmed in afforested...
(Six et al., 2002; Del Galdo et al., 2003) and forested soils (Six et al., 2002) compared to agricultural soils. For the two Oxisols of the present study, cultivation of previously forested sites resulted in a considerable decrease in Mm-C concentration relative to the NV treatment in the surface soil (0–5 cm), whereas NT greatly increased Mm-C concentration compared to CT. These findings validate for these two Oxisols the importance of Mm, in particular its mineral fraction (mineral-Mm), as long-term C-stabilization sites in improved agricultural or natural systems.

In contrast to the earlier findings and the conceptual model by Six et al. (2000), the difference in Mm-C in the soils of this study and others (Six et al., 2002; Del Galdo et al., 2003; Denef et al., 2004) was not always accompanied by a parallel difference in the proportion of water-stable Mm. In a previous study (Denef et al., 2004), the difference in amount of Mm between NT and CT soils decreased with increasing degree of weathering as follows: Mollisol > Alfisol > Oxisol, suggesting a decoupling between SOM and aggregate formation in more weathered kaolinitic and oxide-rich soils. Del Galdo et al. (2003) attributed similar observations for an afforested vs. agricultural site comparison in Italy to the increased C inputs in the forest soil and possibly the shift in quality of the woodland litter with respect to the maize residue of the agricultural sites. Therefore, we conclude that the original conceptual idea of a tight relationship between amount of Mm and Mm-C stabilization as influenced by management (Six et al., 2000) is not applicable for all soil types and/or management systems. Enhanced C stabilization within the Mm fraction under NV and NT compared to CT was therefore related to the dynamic ‘behavior’ rather than the ‘amount’ of the microaggregates (and the macroaggregates that protect them). In other words, similar to the conceptualized importance of both amount and turnover of macroaggregates for C sequestration upon reduced physical disturbance (Six et al., 1999), we postulate that differences in Mm-C concentration among management systems can be linked to differences in amount and stability as well as turnover of Mm. More explicitly, our hypothesis is that in natural or improved agricultural systems Mm has a slower turnover due to the protective environment within the macroaggregates. This slower turnover of the Mm allows greater protection of intra-Mm-POM and greater stabilization of mineral-bound C decomposition products in the Mm resulting in increased Mm stability.

4.2. Contribution of the microaggregate-C fraction to management-induced changes in total soil organic carbon stocks

At Passo Fundo, forest sites were cultivated under NT and CT management approximately 15 year prior to soil sampling. Where NT induced a significant increase in total SOC content (g C m$^{-2}$) relative to CT (i.e., in 0–5 cm), this increase was reflected in a parallel and predominant increase in Mm-C content, and mostly in the mineral-Mm-C. This supports the importance of the Mm (and in particular the mineral-Mm fraction) as C protection and stabilization sites under less soil disturbing agricultural practices such as NT.

For the Londrina soil, where no changes in total SOC content upon conversion from CT to NT were found, Mm-C and mineral-Mm-C stocks significantly increased from CT to NT in the 0–20 cm depth. These significant differences in both (mineral)-Mm-C contents as well as earlier reported (mineral)-Mm-C concentrations between NT and CT, regardless of concomitant total SOC differences, provide evidence that C sequestration under NT is preferentially initiated inside Mm, especially the mineral fraction inside the Mm.

The accumulation of SOC under NT is known to be a slow and gradual process. Results from Franzluebbers and Arshad (1996) have indicated that there may be little to no response in total SOC in the first 2–5 year after a change in management practice (Lal et al., 1998; West and Post, 2002). The absence of a difference in total SOC content between NT and CT at Londrina could thus be due to the young age of the experiment at this site. The NT vs. CT experiment was only established 4 year prior to sampling and after approximately 14 year of CT followed by 3 year of NT. During this period, also no differences in C input occurred between NT and CT (Zotarelli et al., 2005). Soil organic C data from a 2003 sampling event at Londrina (Zotarelli et al., unpublished data) show a 16% (0–5 cm) and 3% (0–20 cm) SOC increase under NT and a 14% (0–5 cm) and 7% (0–20 cm) SOC decrease under CT relative to the SOC stocks in 2001. This resulted in significant differences in total SOC stocks between NT and CT in 2003. This means that the observed 2001 differences in Mm-C stocks between NT and CT form an early indication for the potential of NT to sequester C through preferential long-term stabilization of C in the Mm fraction.

The potential of the Mm-C fraction to serve as a diagnostic fraction for management-induced SOC changes has so far only been demonstrated by Denef et al. (2004) for NT and by Kong et al. (2005) for alternative cropping systems. Furthermore, all soils used in those two studies were temperate soils, except for one. For the highly weathered soil from Passo Fundo, where a significant SOC difference between NT and CT was found in the 0–5 cm layer, Mm-C differences between NT and CT accounted for 60% of the total SOC difference (cf. Denef et al., 2004). For a forested compared to agricultural site in Ontario however, much lower contributions of Mm-C to the overall difference in SOC have been reported (Six et al., 2002). In that particular study, the contribution of total (free and macroaggregate-ocluded) microaggregate-C was greater than that of the Mm-C, but still only accounted for approximately 20% of the total difference in SOC. Those results suggest that other fractions besides the microaggregates contribute to total SOC stocks in forested systems. In our study, the contribution of Mm-C to any significant
total SOC change upon cultivation of native forested (NV) sites was 45% (NV to CT) at Passo Fundo and 44% (NV to NT) and 53% (NV to CT) at Londrina, all in the 0–5 cm depth. Yet, a small but noticeable part of the decrease in total SOC stocks upon cultivation of NV was also accompanied by a decrease in the POM fraction that was not occluded within the Mm (i.e. inter-Mm-POM) and this was the case for both soils. Inter-Mm-POM differences were also found between NT and CT, but were much smaller and generally not statistically significant compared to the inter-Mm-POM differences between NV and NT or CT. According to Six et al. (1999), free POM (i.e. not protected by aggregates) is mostly affected by input rates whereas aggregate-associated C fractions are rather affected by disturbance. CT and NT systems are characterized by similar C inputs and litter quality but drastically differ in disturbance regime, which has been linked to different macroaggregate turnover rates and different Mm-C stabilization between CT and NT (Six et al., 2000). Conversion from forest to cultivated systems on the other hand results not only in a greater physical disturbance of the soil but also in changes in the amount of residue- and root-C input as well as litter quality. Based on our findings and those of others, we postulate that the Mm fraction plays an important role in the long-term stabilization of SOC, but other fractions and perhaps the composition of the Mm-C also may help build-up the overall SOC stock in forested soils. In forested soils, other SOM pools (such as free POM) can contribute to the total increase in SOC stock due to the higher input rates, different litter quality and different plant growth cycles in addition to lower disturbance regime in forested compared to cultivated systems. In contrast, a greater similarity between total SOC sequestration and Mm-C stabilization is observed upon conversion of cultivated systems to systems where only the disturbance regime is altered, such as with the conversion from CT to reduced tillage systems.

5. Conclusions

The results of this study validate the importance of macroaggregate-occluded microaggregates (in particular the mineral-Mm fraction) as long-term C-stabilization sites in forested and NT systems in Oxisols. Moreover, it is concluded that this Mm fraction can serve as an early indicator for potential total SOC sequestration with conversion from CT to NT management. Finally, the results of this study also suggest that C sequestration due to preferential Mm-C stabilization in natural and improved agricultural systems is not directly related to a greater amount of Mm but rather caused by a greater stability and slower turnover of the Mm.

Acknowledgments

Thanks to Koen Blauwet, Dan Reuss, Blythe Ponce and Rosaline Danzman for laboratory assistance. We thank E. Torres (Embrapa Soja) and H.P. Santos (Embrapa Trigo) who maintained the long-term experiments at Londrina and Passo Fundo, respectively. This research was supported by a grant from the National Science Foundation. Karolien Denef acknowledges a grant from the Research Foundation–Flanders (Belgium) (FWO).

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