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# Protection of soil organic C and N in temperate and tropical soils: effect of native and agroecosystems

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**Abstract** Soil carbon sequestration is a viable short-term option to mitigate increased atmospheric $CO_2$. In agriculture, strategies to increase the soil carbon (C) sink include no-tillage, cover crops, and improved crop rotation. The objective of this study was to determine the influence of tillage systems on SOC and total N, soil aggregation and aggregate associated C and N in three soil types: Oxisol (Brazil), Vertisol (Argentina), and Mollisol (USA). Long-term tillage experiments included tilled (T) and no-till (NT) systems. A native grassland was included for comparison in each site. Soil samples were taken at 0–5, 0–15, and 15–30 cm depths. Water-stable aggregates (WSA) were separated using a wet-sieving method. Total C and total N were determined by dry combustion. A shift from native grassland to an agroecosystem decreased microbial biomass, but this decrease was less pronounced under NT. Cultivation reduced the mass of macroaggregates and the concentration associated C and N; however among agroecosystems, NT, regardless soil type, tended to be more similar to the native grassland sites. Agroecosystems reduced TOC and total N stocks, regardless of soil type, compared to the native grassland. This effect followed: Mollisol > Oxisol > Vertisol, and was more pronounced at the 0–5 cm soil depth than at deeper depths. This loss of C and N was associated with the decrease in the mass of macroaggregates and lower C and N concentrations of the aggregates. Macroaggregation was related to TOC and microbial biomass in the Mollisol, suggesting that the biological process of aggregate formation is the principal mechanism of C protection in these soils. The relationship between TOC and large macroaggregates showed lower values for the Oxisol and Vertisol, indicating that in these soils TOC has a complementary role in macroaggregation.

**Keywords** Aggregates · Tillage · Native grassland · No-tillage · Mollisol · Vertisol · Oxisol

**Abbreviations**

| | |
|---|---|
| C | Carbon |
| NT | No-tillage |
| T | Tillage |

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TOC Total organic carbon
Total N Total nitrogen

## Introduction

Soil C sequestration is a viable short-term option to mitigate increased atmospheric $CO_2$ (Post et al. 2004; Caldeira et al. 2004; Smith et al. 2008; IPCC 2007). In agriculture, strategies to increase soil C involve increasing C inputs or decreasing loses. Increasing plant C inputs include cover crops, and improved crop rotations; decreasing loses include reducing tillage intensity with no-tillage providing the lowest soil disturbance (Paustian et al. 2000; West and Post 2002; Lal 2004; Post et al. 2004; Smith et al. 2008).

Soil tillage decreases soil organic carbon (SOC) and promotes a reduction in soil aggregation (Tisdall and Oades 1980). Tillage results in direct and indirect effects on SOC dynamics. Indirect effects occur through changes in soil environment (temperature, moisture, and oxygen) thus affecting microbial activity and ecology; and direct effects through the disruption of soil structure (Balesdent et al. 2000). The immediate effect of tillage is the physical disruption of aggregates, incorporation of residues into the soil, and increase soil temperature and oxygen which lead to greater accessibility of the C for microbial decomposition. The second effect of tillage is the bare soil surface which permits more frequent wet-dry and freeze-thaw cycles (Beare et al. 1994; Paustian et al. 1997) thus making the aggregates more susceptible to disruption forces. Therefore, these changes in soil environment induced by tillage promote soil organic matter (SOM) decomposition (Cambardella and Elliott 1993), and affect the microbial community composition (Beare et al. 1993; Frey et al. 1999; Watson and Rice 2004).

Carbon stabilization in temperate and tropical soils is mediated by soil biota, soil structure and their interactions, and also by agricultural management (Six et al. 2002). Some of the factors that greatly differ among tropical and temperate soils are climate, parent material, and vegetation. Oxisols generally have low activity clays (1:1 clays) which are characterized by low specific surface area and cation exchange capacity (CEC). In addition, the tropical climate is generally characterized by high temperatures and high precipitation relative to the temperate climate. As a result of the interaction of soil and climate conditions, microbial activity is high through the year in Oxisols due to higher temperature and precipitation, consequently contribute to a lower capacity to stabilized C (Six et al. 2002, 2004). Conversely, Mollisols and Vertisols have predominance of high activity clays (2:1 clays), with high specific surface area and CEC and relatively lower temperature and precipitation. These conditions allow Mollisols and Vertisols a greater capacity to stabilized C (Six et al. 2002).

Soil organic matter and biological processes play a primary role in the aggregation of temperate soils dominated by 2:1 clay mineralogy. In this case, aggregation has been reported as the primary mechanism for C protection in temperate soils (Six et al. 2004). However, in weathered tropical soils, soil organic matter and biological processes may play a secondary role in the binding of aggregates (Six et al. 1999; Denef et al. 2002; Denef and Six 2005). The primary factor for aggregation in these soils is mineral–mineral bonding due to electrostatic interactions between oxides and 1:1 clay minerals, although SOM may still promote C stabilization.

Management practices can alter the composition and function of microbial communities thus affecting soil C dynamics. Bacteria and fungi play key role in organic matter decomposition (Six et al. 2006). The proportion of microbial biomass composed of fungi can increase with less soil disturbance such as NT (Beare 1997; Frey et al. 1999; Watson and Rice 2004). The degree of disturbance, pH, soil moisture and residue placement are factors controlling the proportion of bacterial and fungal biomass in agroecosystems (Six et al. 2006; White and Rice 2009).

Conservation tillage is a strategy to reduce soil erosion and C loss, improve soil structure, and maintain sustainable agriculture. Conservation tillage had been gaining importance, in different parts of the world specifically in North and South America. The United States, Brazil and Argentina have the greatest adoption of no-tillage systems in the world, with a total area estimated close to 70 millions of ha (Derpsch and Benites 2004). Soil management influences the dynamics of C in soils affecting the quantity and quality of SOM, soil aggregation, and microbial populations to different degrees according to soil type and climate. Thus, the objective of this study was to determine the influence of long-term conservation agriculture and native grassland protection of soil C in three representative soil types (Oxisol-Brazil, Vertisol-Argentina, and Mollisol-USA).

## Materials and methods

### Site description

This research was conducted at three sites with differences in soil and climatic conditions in South and North America. The main characteristics of the experimental sites are summarized in Table 1.

The Oxisol was located at the Center of Experimentation and Research FUNDACEP in Cruz Alta (RS), Brazil (28°36′ S, 53°40′ W). The experiment was initiated in 1985 on a clay Rhodic Hapludox with a predominance of 1:1 kaolinite and iron and aluminum oxides, and referred in the text as Oxisol. The average annual precipitation was 1,727 mm without a dry season and an annual mean temperature of 19.2°C. The climate was classified as cfa subtropical following Koppen classification. The crop rotation was: black oat (*Avena strigosa* Schreber); soybean (*Glycine max* (L.) Merr.); black oat + common vetch (*Vicia sativa* (L.) *Walp*.); maize (*Zea mays* L.); *radish oil* (*Raphanus sativus* L.); wheat (*Triticum* aestivum L.); soybean, under tilled (T) and no-till (NT) systems. Tillage consisted of disk plow followed by tandem disk. No-tillage consisted of planting directly into the crop residue, with minimum soil disturbance. The plots were amended with lime and fertilized with N, P, and K following soil analysis. The plots size are 13.3 m × 30 m. Details for the experiment were reported by Campos et al. (1995) and Campos (2006). The experimental design was a randomized block with three replications. A nearby native grassland site was included as a comparison, which represented the natural vegetation of the area with predominant species such as bluestem (*Andropogon lateralis*), bahiagrass (*Paspalum notatum*), asthmaweed (*Conyza bonariensis*), eryngo (*Eryngium horridum*), zarzabacoa comun (*Desmodium incanum*), flatsedge (*Cyperus* sp.) and crabgrass (*Digitaria* sp.). The native site was sampled with two replications.

The Vertisol was located at the Experimental Station INTA Paraná, Entre Ríos, Argentina (31°50′07″ S, 60°32′19″ W). The averaged annual precipitation was 995 mm and an annual mean temperature of 18.5°C. The soil was classified as an argic chromic Peludert (very fine, montmorillonitic slighly acaline, thermic Peludert) and referred in the text as Vertisol. The crop rotation, initiated in 1997, was wheat/soybean-maize, with two tillage systems: tilled (T) and no-till (NT). The plots size are 7 m × 40 m. Tillage at this site was less intensive which consisted of two or three operations with disk or chisel plow. No-tillage consisted of planting directly into the crop residue with minimum soil disturbance. A nearby native grassland site was

**Table 1** Soil characteristics at 0–5 cm of the three experimental sites evaluated from Brazil, Argentina, and Kansas (USA)

| Site | Soil type | pH | Bray-*P* (mg $kg^{-1}$) | $Ca^{2+}$ (mg $kg^{-1}$) | $K^+$ (mg $kg^{-1}$) | $Mg^{2+}$ (mg $kg^{-1}$) | $Na^+$ (mg $kg^{-1}$) | CEC (cmol $_{(+)}$ $kg^{-1}$) | Sand (%) | Silt (%) | Clay (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazil | Oxisol | | | | | | | | | | |
| | Tilled | 5.1 | 18.4 | 681 | 146 | 189 | 3.2 | 16.2 | 25 | 23 | 52 |
| | No-till | 5.1 | 26.8 | 1004 | 261 | 261 | 4.3 | 17.1 | 25 | 24 | 51 |
| | Native grassland | 5.3 | 3.7 | 826 | 185 | 336 | 4.8 | 20.3 | 28 | 28 | 44 |
| Argentina | Vertisol | | | | | | | | | | |
| | Tilled | 7.5 | 29.3 | 7011 | 804 | 374 | 24.3 | 41.2 | 8 | 49 | 44 |
| | No-till | 7.4 | 35.8 | 6290 | 994 | 389 | 11.6 | 43.3 | 8 | 49 | 43 |
| | Native grassland | 6.3 | 36.4 | 4340 | 527 | 544 | 49.7 | 39.2 | 6 | 52 | 41 |
| Kansas | Mollisol | | | | | | | | | | |
| | Tilled | 6.2 | 54.9 | 2260 | 371 | 297 | 14.5 | 17.1 | 10 | 70 | 20 |
| | No-till | 5.8 | 55.0 | 2137 | 318 | 265 | 10.2 | 18.4 | 12 | 68 | 20 |
| | Native grassland | 5.7 | 65.0 | 2472 | 659 | 412 | 19.6 | 24.7 | 9 | 59 | 32 |

included as a comparison. The native vegetation was a savanna with xenomorphic species such as mesquite (*Prosopis* sp.) and acacia (*Acacia* sp.) and grasses such as brome (*Bromus* sp.), bristlegrass (*Setaria* sp.), and needlegrass (*Stipa* sp.). The tillage treatments were arranged in a randomized complete block design with four replications. For the native grassland site two replications were taken at 0–5 cm, and only one replication at 0–15 and 15–30 cm.

The Mollisol was located on the North Agronomy Farm located at Kansas State University, Manhattan, Kansas, USA (Riley County; 39°13′12″ N, 96°36′0″ W). The soil was a moderated well-drained Kennebec silt loam (fine-silty, mixed, superactive mesic Cumulic Hapludoll) and referred in the text as Mollisol. The 30-year average annual precipitation was 800 mm, which was mainly concentrated in the spring-summer period, with an annual mean temperature of 11.4°C. The experiment, initiated in 1990, was a split-plot with four replications, with tillage as the main plot and nitrogen as the sub-plot. The plots size was 7.5 m × 6 m. For this study, samples were taken only in the tilled (T) and no-till (NT) plots receiving 168 kg N $ha^{-1}$ as ammonium nitrate. Tillage consisted of fall chisel plow and spring offset disk. No tillage consisted of planting directly into the crop residue. Similar to the other experiments, a native grassland site was included for comparison. The native vegetation was a tallgrass prairie dominated by warm-season grasses: big bluestem (*Andropogon* gerardii Vit.), indiangrass (*Sorgastrum nutans* (L.), and switchgrass (*Panicum virgatum* Michx.). For the native grassland two replications were taken at 0–5 cm, 0–15, and 15–30 cm.

## Soil sampling

Soil samples were taken from each plot at 0–5, 0–15, and 15–30 cm depth using a soil probe (2 cm diameter) or shovel. Samples were collected in the center of the plots. About 3–5 subsamples were taken for one composite sample from each plot. Samples were collected August 2005 for the Oxisol, November 2005 for Mollisol, and March 2006 for the Vertisol. Soil samples were passed through 8-mm sieve, roots removed, and stored at 4°C until analyzed.

Bulk density at each experimental site was determined using a soil-probe. Two intact soil samples were taken at 0–5, 5–15, and 15–30 cm and dried at 105°C and weighed. The volume of the soil was determined by precisely measuring the diameter of the core and the depth increment.

## Aggregate-size distribution

Water-stable aggregates (WSA) were separated using a wet-sieve method described by Yoder (1936) with modifications by Mikha and Rice (2004). Soil was air-dried and 50 g placed on the top of the sieve of each nest. To slake the air-dried soil, 1 L of water was rapidly added until soil was covered with water. Soils were submerged in water for 10 min following by 10 min of wet sieving. Four aggregate size classes were collected from each treatment >2,000, 250–2,000, 53–250, and 53–20 μm diameter. Water stable aggregates were dried and a subsample was used to determine sand content of each fraction (Mikha and Rice 2004). Sand-free WSA was measured using a subsample of intact aggregates (2–5 g) and combined with fivefold volume (10–25 ml) of 5 g $l^{-1}$ sodium hexametaphosphate, left overnight and shaken on an orbital shaker at 350 RPM for 4 h. The dispersed organic matter and sand was collected on a 53 μm mesh sieve, washed with deionized water, and dried at 105°C for 24 h, and the aggregate weights were recorded for estimating the sand-free correction.

Large macroaggregates were defined as >2,000 μm, small macroaggregates 250–2,000 μm, microaggregates 250–53 μm, and silt by 20–53 μm size fraction (Denef and Six 2005). The size fraction <20 μm was discarded but represented <10% depending on the soil type. The total recovery was 93–96% in Mollisols and 97–99% in Oxisols. The Vertisols had lower recovery of 88–95% which can be attributed to the presence of calcium carbonates in the soil. In each aggregate fraction, roots and plant material were removed. Free POM associated with the aggregate size fraction was not separated and would be included in the aggregate associated C or N fraction.

## Total C and N

Soil aggregate fractions samples were air-dried and ground to a fine powder using a mortar and pestle. Total C (TOC) and total N contents were determined by dry combustion using a C/N Elemental Analyzer (Flash EA 1112 Series ThermoFinnigan Italia S.p.A., MI, Italy). For the Vertisols, soil samples were treated with HCl 0.5 M to eliminate the presence of calcium carbonates.

Carbon and N concentrations in each aggregate-size fraction were adjusted for sand content.

### Total microbial biomass

Phospholipids (PLFA) analyses were determined following a modification of the Blight and Dyer (1959) method (White and Ringelberg 1998). Lipids were extracted with a single phase chloroform:methanol:phosphate buffer solution (Blight and Dyer 1959) for 2 h from 5 g of freeze-dried soil. Total lipid extracts were separate into neutral lipids, polar lipids and glycolipids using preconditioned silica gel disposable extraction columns (J. T. Baker, Phillipsburg, NJ, USA). Neutral and polar lipids were subject to alkaline methanolysis to cleave the fatty acids from the glycerol molecule and replace it with methyl groups, creating fatty acid methyl esters. Samples were analyzed by gas chromatography (HP 6890, Agilent Incorporated, Palo Alto, CA, USA). A 25-m Ultra-2 (J & W Scientific, Agilent Technologies, Palo Alto, CA, USA) column was used and he was the carrier gas at 1 ml $min^{-1}$. The initial temperature was 80°C for 1 min followed by an increase of 20°C $min^{-1}$ until 155°C, and a second increase at 5°C $min^{-1}$ until 27°C. Peaks were identify using retention times of fatty acid standards and by comparing spectra from a library (Wiley 138 K mass spectral database). Samples peak were quantified based on comparison of the abundance with an internal standard nonadecanoic acid methyl ester (19:0) in terms of nmol $g^{-1}$ dry soil or mol %. Fatty acids were grouped into gram positive bacteria (i15:0, a15:0, 10Me16:0, i17:0, and a17:0), gram negative bacteria (18:1$\omega$7c and cyclic 19:0), actinomycetes (10Me18:0 and 10Me17:0), and fungi (18:2$\omega$6,9c and 18:1$\omega$9c) (McKinley et al. 2005).

Total microbial biomass estimate by PLFA was determined as a sum of all the bacteria, actinomycetes and fungi indicators identified.

### Statistical analysis

Analysis of variance was performed using SAS PROC MIXED and PROC GLIMMIX (SAS Institute 2002) to assess differences in total C and N in soil, mass of aggregate fraction, C and N concentration in each aggregate fraction, and microbial biomass between treatments for each soil type. Because the native site treatment was not included in the experimental design of the agricultural experiments two different analyses were performed: (1) comparison between tillage treatments, NT vs T at each site; and (2) comparison between tillage treatments and the native grassland treatment at each site. Analysis was performed by soil depth.

Statistical comparison between the native grassland and tillage treatments for the Vertisol were only performed at 0–5 cm, because there was only one repetition for the native grassland at 0–15 and 15–30 cm depth.

Results were considered statistically significant at $P < 0.05$ unless noted otherwise. Means were compared using differences in LS means.

A correlation analysis was performed using SAS PROC CORR (SAS Institute 2002) to determine the correlations between the amount of aggregates and total C, microbial biomass and total C, and the amount of macroaggregates and microbial biomass.

## Results

### Total C and N

For all three soils, TOC was significantly greater in NT than the corresponding tilled system at 0–5 cm (Table 2). The greatest difference in TOC was in the Oxisol, where NT had 54% greater TOC than the tilled soil. Total organic C also was significantly greater in NT than T for 0–15 cm in the Oxisol; with a 14% increase compared to the tilled system. There were no differences between tillage systems for TOC at 15–30 cm, regardless of soil type (Table 2).

The native grasslands for the Oxisol and Mollisol had greater TOC stocks at 0–5 cm than the corresponding agroecosystems (Table 2). The decline of TOC as a result of agriculture relative to the native vegetation was 55% in the Mollisol and only 46% in the Oxisol. At 0–15 and 15–30 cm, the native grassland also had significantly more TOC than both agroecosystems in the Mollisol and Oxisol (Table 2). At 0–15 cm the decline in TOC in the tilled treatment relative to the native vegetation was 43% for the Mollisol and 29% for the Oxisol.

**Table 2** Total organic carbon (C) and total nitrogen (Total N) for cropped (tilled (*T*), no-till (NT)) and native grassland in an Oxisol, Vertisol, and Mollisol

| | Total organic C (Mg C ha$^{-1}$) | | | Total N (Mg N ha$^{-1}$) | | |
|---|---|---|---|---|---|---|
| | 0–5 (cm) | 0–15 (cm) | 15–30 (cm) | 0–5 (cm) | 0–15 (cm) | 15–30 (cm) |
| Oxisols | | | | | | |
| Tilled | 11.3 Cb | 37.6 Cb | 32.6 Ba | 1.15 Aa | 4.52 Ba | 2.94 Aa |
| No-till | 17.4 Ba | 42.9 Ba | 31.9 Ba | 1.75 Aa | 3.36 Bb | 3.16 Aa |
| Native Grassland | 21.1 A | 52.8 A | 39.2 A | 1.76 A | 5.67 A | 3.19 A |
| *P* values[a] | 0.0048 | 0.0043 | 0.5625 | 0.1100 | 0.0106 | 0.7941 |
| *P* values[b] | 0.0013 | 0.0004 | 0.0151 | 0.1675 | 0.0008 | 0.9296 |
| Vertisols | | | | | | |
| Tilled | 12.2 Ab | 31.7 Aa | 22.1 Aa | 0.85 Ab | 2.25 Aa | 1.62 Aa |
| No-till | 15.2 Aa | 30.9 Aa | 22.3 Aa | 1.09 Aa | 2.10 Aa | 1.63 Aa |
| Native Grassland | 14.2 A | 28.3 | 19.8 | 0.98 A | 1.96 | 1.28 |
| *P* values[a] | 0.0108 | 0.7107 | 0.8999 | 0.0070 | 0.4575 | 0.8834 |
| *P* values[b] | 0.1704 | 0.7531 | 0.7515 | 0.1448 | 0.5318 | 0.1946 |
| Mollisols | | | | | | |
| Tilled | 11.4 Cb | 33.4 Ba | 27.6 Ba | 1.03 Cb | 2.80 Ba | 2.16 Ba |
| No-till | 15.0 Ba | 35.7 Ba | 25.2 Ba | 1.44 Ba | 3.01 Ba | 1.99 Ba |
| Native Grassland | 25.2 A | 58.3 A | 41.4 A | 2.20 A | 5.16 A | 3.62 A |
| *P* values[a] | 0.0230 | 0.2131 | 0.1824 | 0.0059 | 0.2242 | 0.2756 |
| *P* values[b] | 0.0010 | 0.0006 | 0.0010 | 0.0003 | 0.0008 | 0.0013 |

[a] Comparisons between tilled and no-till systems. Lowercase letters indicate differences between tillages

[b] Comparisons between tilled and no-till systems with native grassland. Uppercase letter indicates differences among tillage and native grassland treatments

For the Mollisol and Vertisol, total N was significantly greater in NT than T for 0–5 cm (Table 2). There were no significant differences in total N between tillage systems at 0–15 and 15–30 cm, except in the Oxisol where T had greater total N than NT at 0–15 cm.

Total N in the agroecosystems declined in the Mollisol at 0–5 cm relative to the native grassland. The decline in total N was similar to the decline in C with 53% for the Mollisol (Table 2). At 0–15 cm, the decline in total N stocks was 20 and 45% for the Oxisol and Mollisol, respectively. The native grassland had greater total N stocks than the agroecosystems at all depths in the Mollisol.

## Aggregate distribution

Soil aggregation, as represented by the distribution of small and large aggregates, was greater for the Oxisol than the other two soils (Fig. 1). For the Oxisol at 0–5 cm, sand-free WSA was significantly affected by the interaction of tillage and aggregate size (Fig. 1; Table 3). No-till had significantly greater amounts of large macroaggregates than the tilled treatment; this came at the expense of the smaller aggregates. No-till resulted in similar amounts of large macroaggregates as the native grassland. The native grassland had significantly lower amounts of small macroaggregates and microaggregates than the tilled treatment in agreement with the greater proportion of macroaggregates (Fig. 1; Table 4). The differences in aggregates between tilled and no-till systems were observed at all depths, although they were not significant at 0–15 and 15–30 cm (Fig. 1; Table 3) indicating the response was confined to the shallow depth of the NT soil. The native grassland had greater amounts of large macroaggregates (> 2,000 μm) than the agroecosystems except for 0–5 cm where NT had significantly greater amounts of large macroaggregates than the native grassland. Consequently, the native grassland had significantly lower amounts of small macroaggregates and microaggregates at 0–15 cm (Fig. 1).

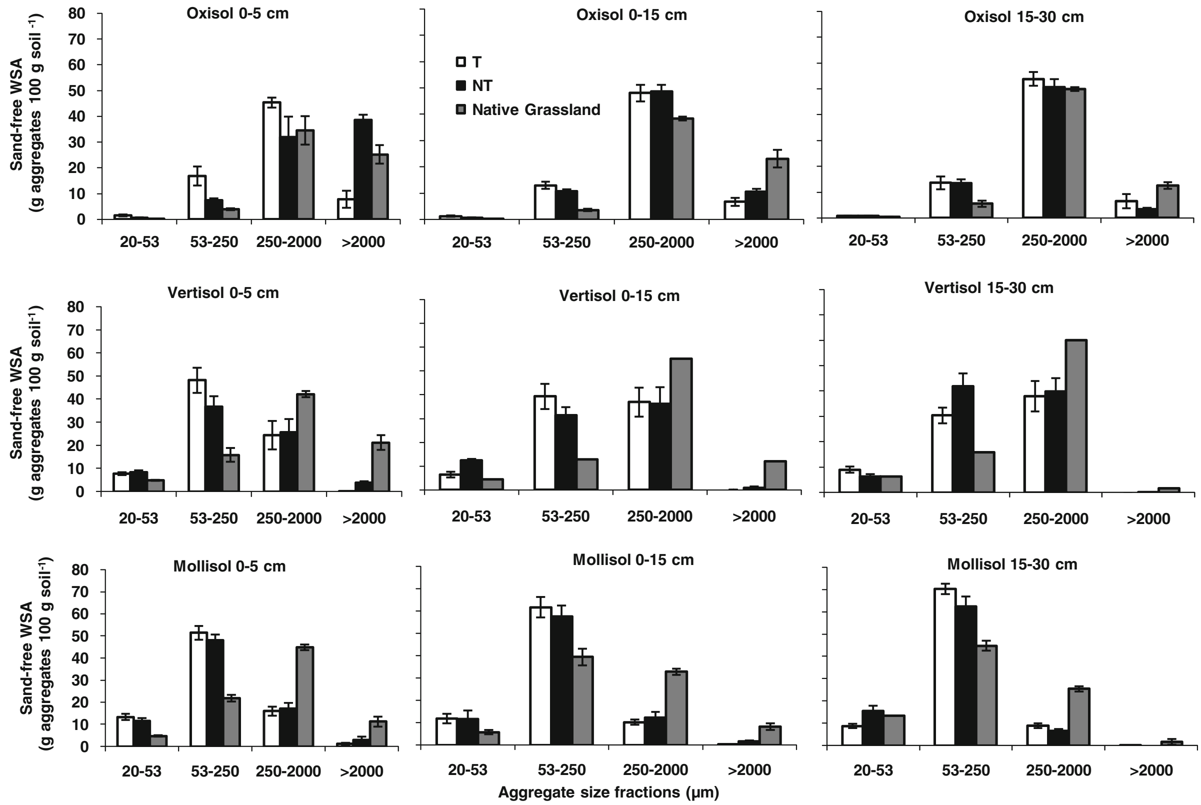



**Fig. 1** Distribution of sand-free water stable aggregates (WSA) under tilled (*T*), no-till (*NT*) and native grassland at 0–5, 0–15, and 15–30 cm for the Oxisol, Vertisol, and Mollisol sites: Error bars represent the standard errors of the means

For the Vertisol there were generally no differences in aggregate distribution ($P > 0.05$) between tillage systems at all depths (Fig. 1; Table 3). At 0–5 cm, native grassland had greater amount of large and small macroaggregates than tilled and no-till systems (Table 4; Fig. 1). At 0–15 and 15–30 cm, there were no statistical comparisons with native grassland due to lack of adequate replicates for this treatment (Table 4).

For the Mollisol, no significant differences in aggregates were detected between tillage systems at any depths (Fig. 1; Table 3). Nevertheless, the native grassland had significantly greater amounts of macroaggregates and lower amounts of microaggregates than the agroecosystems (Fig. 1; Table 4).

Summarizing, the effect of tillage on aggregate distribution was influenced by soil texture and mineralogy. For the Mollisol the most marked effect was the decrease of large macroaggregates and increase in microaggregates in all depths investigated when management changed from grassland to an annual agricultural system. For the Vertisol this effect was evident at 0–5 cm. For these soils, the greatest proportion of aggregates was in the microaggregates and small macroaggregates. In the Oxisol, for 0–15 and 15–30 cm there was a decrease in the large macroaggregates, and an increase in the small macroaggregates and microaggregates with the greatest proportion of aggregates in the small macroaggregates (250–2,000 μm). The Oxisol NT had the greatest proportion of large macroaggregates at 0–5 cm.

## Concentrations of C and N in aggregate fractions

For the Oxisol at 0–5 cm, a non significant tillage x aggregate size was detected for C concentration at 0–5, 0–15 and 15–30 cm (Fig. 2; Table 3). At 0–15 and 15–30 cm, no significant differences were observed between tillage systems for C concentrations at any size (Fig. 2). At 0–5 cm, N concentration was significantly greater under NT than T in all aggregate size fractions (Fig. 3; Table 3). At 0–15 and 15–30 cm,

**Table 3** Statistical analysis for mass of aggregates, total organic C and total N at the different depths. Comparison between tilled and no-till systems

| | *P* values | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mass of aggregates | | | Total organic C | | | Total N | | |
| | 0–5 (cm) | 0–15 (cm) | 15–30 (cm) | 0–5 (cm) | 0–15 (cm) | 15–30 (cm) | 0–5 (cm) | 0–15 (cm) | 15–30 (cm) |
| Oxisols | | | | | | | | | |
| Tillage (T) | 0.9331 | 0.7373 | 0.3145 | 0.0004 | 0.2718 | 0.2630 | 0.0001 | 0.0241 | 0.2196 |
| Size (S) | 0.0001 | 0.0001 | 0.0001 | 0.0213 | 0.0012 | 0.0045 | 0.1450 | 0.0014 | 0.0243 |
| T × S | 0.0001 | 0.3981 | 0.8121 | 0.9309 | 0.8666 | 0.3189 | 0.8398 | 0.7226 | 0.4441 |
| Vertisols | | | | | | | | | |
| Tillage (T) | 0.2265 | 0.5404 | 0.4249 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| Size (S) | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| T × S | 0.0129 | 0.2991 | 0.2168 | 0.0001 | 0.0001 | 0.0006 | 0.0001 | 0.0001 | 0.0007 |
| Mollisols | | | | | | | | | |
| Tillage (T) | 0.6991 | 0.9156 | 0.6751 | 0.2783 | 0.8166 | 0.0204 | 0.0333 | 0.5930 | 0.0204 |
| Size (S) | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| T × S | 0.5974 | 0.7701 | 0.0001 | 0.2134 | 0.9627 | 0.0178 | 0.2431 | 0.8231 | 0.0178 |

**Table 4** Statistical analysis for mass of aggregates, total organic C and total N at the different depths. Comparison between tilled, no-till systems and native grassland

| | *P* values | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mass of aggregates | | | Total organic C | | | Total N | | |
| | 0–5 (cm) | 0–15 (cm) | 15–30 (cm) | 0–5 (cm) | 0–15 (cm) | 15–30 (cm) | 0–5 (cm) | 0–15 (cm) | 15–30 (cm) |
| Oxisols | | | | | | | | | |
| Treatment (T) | 0.0162 | 0.6649 | 0.2300 | 0.0001 | 0.0001 | 0.0003 | 0.0002 | 0.0001 | 0.0001 |
| Size (S) | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0003 | 0.0005 | 0.0001 | 0.0010 |
| T × S | 0.0002 | 0.0001 | 0.0561 | 0.0001 | 0.0501 | 0.0004 | 0.0006 | 0.0604 | 0.0008 |
| Vertisols | | | | | | | | | |
| Treatment (T) | 0.0023 | – | – | 0.0001 | – | – | 0.0001 | – | – |
| Size (S) | 0.0001 | – | – | 0.0001 | – | – | 0.0001 | – | – |
| T × S | 0.0001 | – | – | 0.0001 | – | – | 0.0001 | – | – |
| Mollisols | | | | | | | | | |
| Treatment (T) | 0.2931 | 0.9612 | 0.6751 | 0.0005 | 0.0001 | 0.0001 | 0.0018 | 0.0001 | 0.0001 |
| Size (S) | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| T × S | 0.0001 | 0.0006 | 0.0001 | 0.0446 | 0.0811 | 0.0014 | 0.0476 | 0.0430 | 0.0067 |

in general no differences between tillage were observed (Fig. 3; Table 3).

C concentrations of the aggregates from the native grassland were greater than in NT in the microaggregate fraction but similar to NT in the macroaggregate fraction (Fig. 2; Table 4). Carbon concentration of the aggregates from the native grassland was significantly greater than T for all aggregate sizes, indicating that tillage accelerated the loss of C in all aggregates while NT preserved aggregate-associated C (Fig. 2). At 0–15 cm, native grassland had greater C concentration in all aggregate fractions >53 μm (Fig. 2). At 15–30 cm, the native grassland had significant greater C concentration only in the microaggregate fraction (Fig. 2).

For the Vertisol at 0–5 cm, the C and N concentration was significantly greater ($P < 0.05$) under NT

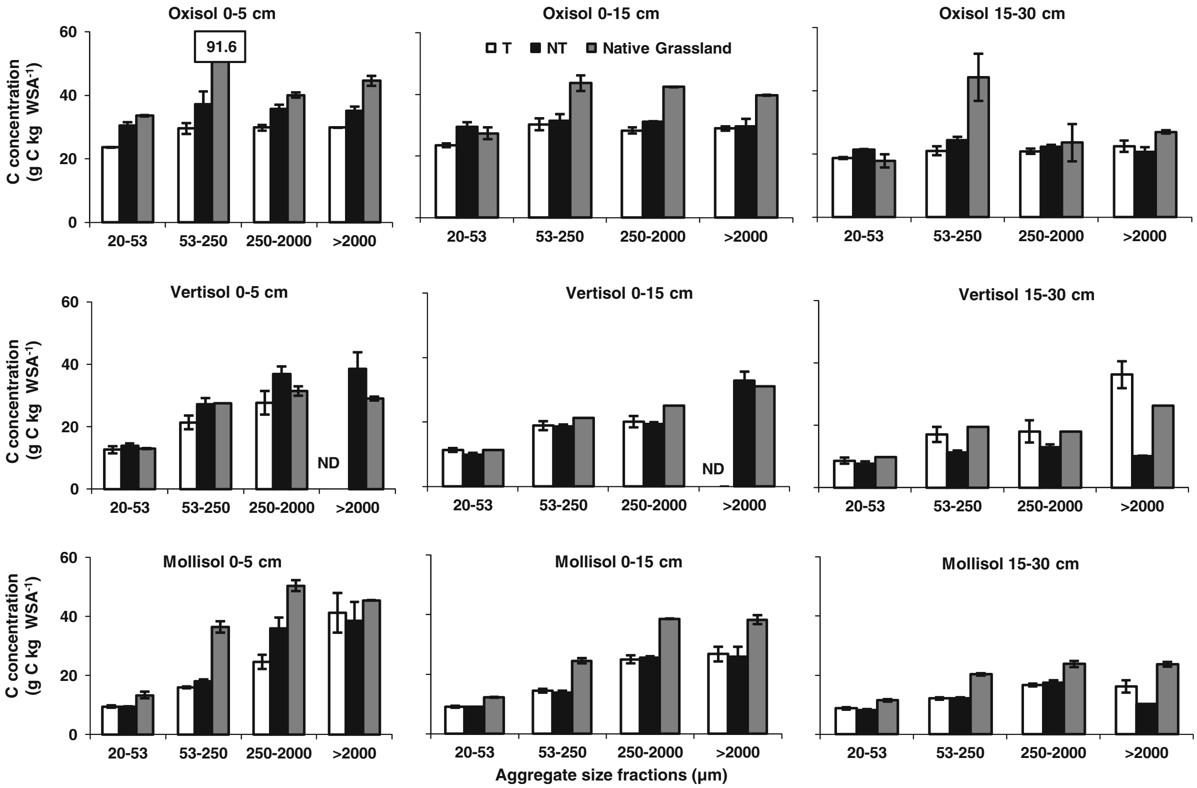



**Fig. 2** Total C in the sand-free water stable aggregates (WSA) under tilled (*T*), no-till (*NT*) and native grassland at 0–5, 0–15, and 15–30 cm for the Oxisol, Vertisol, and Mollisol: Error bars represent the standard errors of the means

than T in the macroaggregate fraction, with no differences in the microaggregate fraction (Figs. 2, 3). At 0–15 cm, NT had significantly greater C and N concentration in large macroaggregates than T (Figs. 2, 3; Table 3). Conversely, T at 15–30 cm had significantly greater ($P < 0.05$) C and N concentration than NT (Figs. 2, 3; Table 3). Carbon and N concentration in the native grassland was significantly lower than both tillage systems in the large macroaggregates (Figs. 2, 3; Table 4). At 0–15 and 15–30 cm, there were no statistical differences (Table 4).

The C and N concentration in the Mollisol were significantly greater under NT than T in the small macroaggregates at 0–5 cm, with no differences between agroecosystems in the microaggregates (Figs. 2, 3; Table 3). At 0–15 cm no differences were observed in C and N concentration between T and NT (Figs. 2, 3; Table 3). At 15–30 cm, T had greater C and N concentrations in the large macroaggregates than NT, but no differences were observed in the other size classes (Figs. 2, 3; Table 3).

In the Mollisol, the native grassland had greater C and N than the agroecosystems in the small macroaggregate and microaggregate fraction at 0–5 and 0–15 cm (Figs. 2, 3; Table 4). At 15–30 cm, C and N concentrations in the native grassland site were greater than agroecosystems for all aggregate size classes except in the 20–53 µm fraction (Figs. 2, 3; Table 4).

## Microbial biomass

Microbial biomass estimated by total PLFA was significantly greater ($P < 0.10$) under NT than T (Fig. 4) in the Oxisol and Mollisol. Microbial biomass was 60 and 44% greater in the no-till than the tilled soil, for the Oxisol and for the Mollisol, respectively.

When the native grassland was compared with the agroecosystems, tillage significantly reduced microbial biomass across all soil types. For the Oxisol, there were no significant differences ($P < 0.05$) with

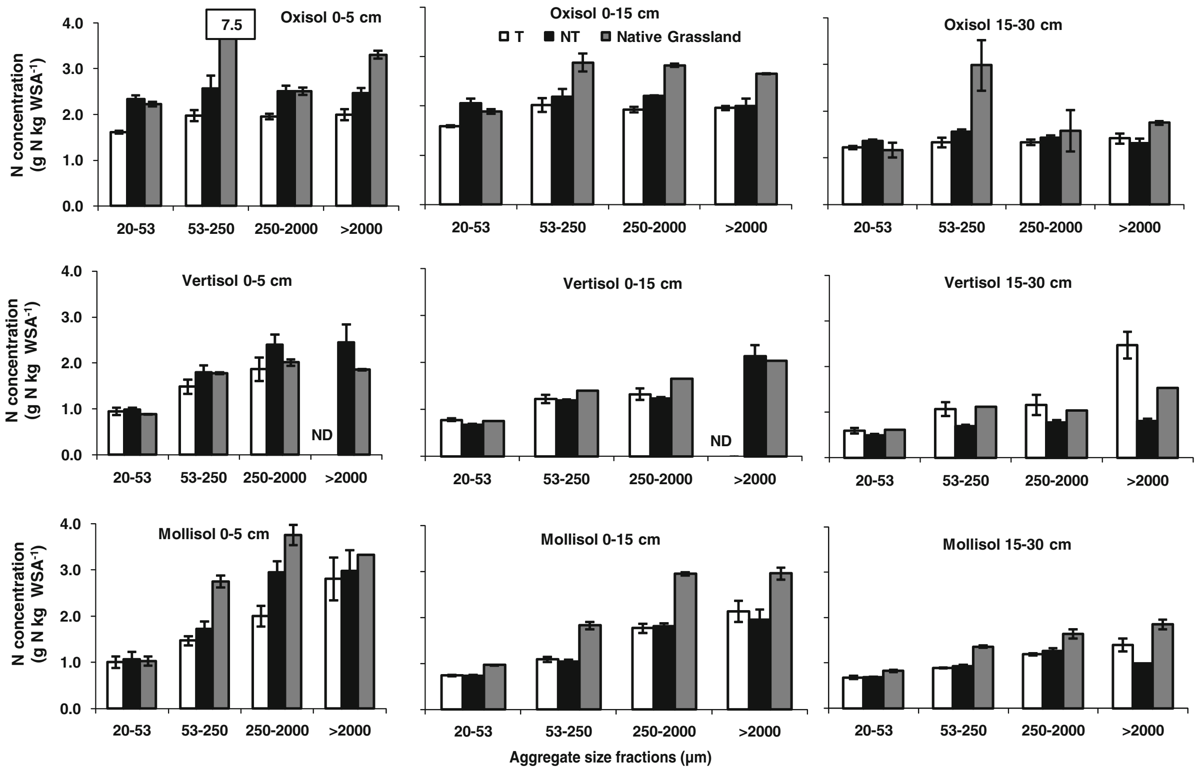



**Fig. 3** Total N in the sand-free water stable aggregates (WSA) tilled (*T*), no-till (*NT*) and native grassland at 0–5, 0–15, and 15–30 cm for the Oxisol, Vertisol, and Mollisol: Error bars represent the standard errors of the means

no-till (Fig. 4). For the Mollisol, when the native grassland site was compared with the agroecosystems, the native grassland had significant greater microbial biomass ($P < 0.05$) than both no-till and tilled systems (Fig. 4).

## Relationships among total C, soil aggregation and microbial biomass

For the Mollisol, both TOC and microbial biomass were strongly correlated with the mass of macroaggregates (Figs. 5, 6). In the Oxisol, there were weak relationships between TOC and microbial biomass with macroaggregates (Figs. 5, 6). However, there was a strong negative relationship between TOC and microaggregates for both the Mollisol and Oxisol (Fig. 5). For all the three soils investigated there were relationships between TOC and large macroaggregates. The order of these relationships was: Mollisol > Oxisol > Vertisol.

There was a significant correlation of TOC and microbial biomass in the Mollisol and Oxisol and no correlation in the Vertisol (Fig. 6a). In the Mollisol, for each g kg$^{-1}$ increase in C there was an increase of 1.6 nmol of microbial biomass while in the Oxisol the increase was 3.6 nmol (Fig. 6).

## Discussion

Despite the differences in soil and climate among sites, the NT had a higher concentration and total mass of C and N in the surface 5 cm relative to the tilled system (Table 2). Similar results have been reported in the literature (Deen and Kataki 2003; Fabrizzi et al. 2003; Mikha and Rice 2004; McVay et al. 2006; Wright and Hons 2004, 2005a, b; Amado et al. 2006). In the Oxisol, NT also had greater concentration and total mass of C stocks to a depth of 15 cm. Positive gains with NT management have been reported to a depth of 30 cm (Cambardella and Elliott 1994; Six et al. 1999; Fabrizzi et al. 2003); however, other studies have report no increase in TOC under NT systems (Angers et al. 1997;

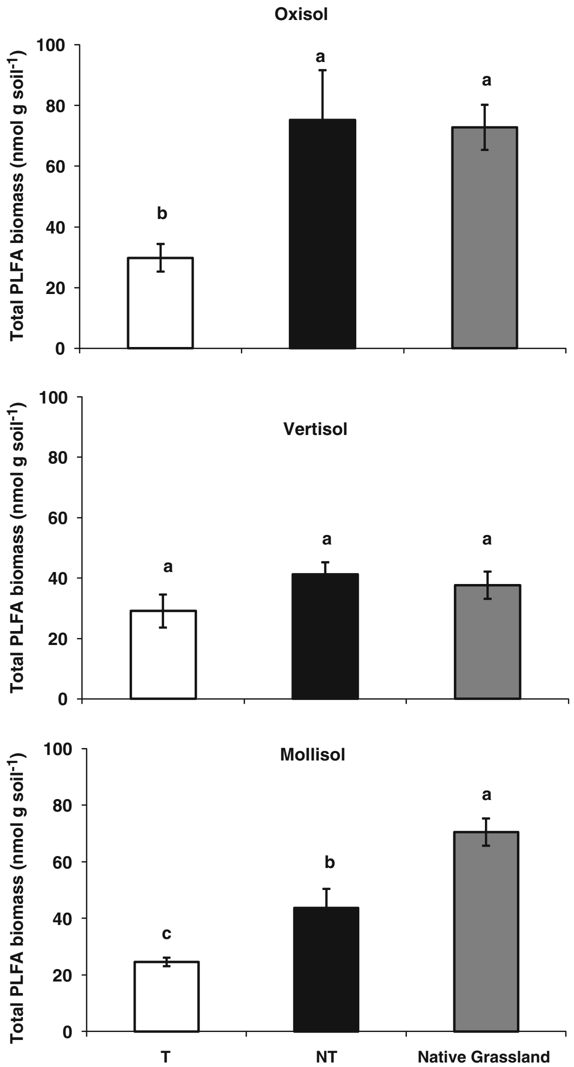



**Fig. 4** Microbial biomass estimated through the PLFA technique under tilled (*T*), no-till (*NT*) and native grassland at 0–5 cm, for the Oxisol, Vertisol and Mollisol: Error bars represent the standard errors of the means

Franzluebbers et al. 1999; Needelman et al. 1999; Puget and Lal 2005; Sainju et al. 2006). The higher concentration of C and N in NT suggest that the organic matter is protected to a greater extent than in the tilled environment. This may be due to the greater mass of aggregates thus protecting the organic matter associated with that size fraction.

The Vertisol had similar C and total N stocks as the native grassland, which may be a function of prior history. The tilled system in the Vertisol was less intensive than the tillage at the other sites. The lack of differences in the Vertisol may be due to the self-mixing of the shrink–swell clays that minimize stratification that would develop under NT facilitating the downward movement of C. Leinweber et al. (1999) and Schulten and Leinweber (2000) mentioned that the swell and shrink dynamics of Vertisols lead to pedoturbation and mixing of mineral-associated organic matter within the soil profile. This notion is supported by the fact that aggregate distribution is not affect by mechanical disturbance. Organic matter sorption on clay surfaces provided by the high CEC and clay content of the Vertisol could be an alternative explanation but the concentration was different in the surface. Further research is needed to separate the role of clay type on sorption and aggregation in these soils.

Several authors have reported an increase in the proportion of macroaggregates under NT systems (Beare et al. 1994; Mikha and Rice 2004; Wright and Hons 2005a). The mechanical disruption of aggregates in the tilled systems decreases the aggregate size distribution. Differences in soil aggregation induced by tillage systems in our study were more pronounced in the Oxisol at 0–5 cm. The greater quantity of macroaggregates in the Oxisol may be associated with soil mineralogy and texture. The Oxisol also had the most intensive cropping system (eight crops in 3 years) which may have accentuated differences in the balance between C inputs and C losses. In the Vertisol and Mollisol, the amount of large macroaggregate under T was low or even undetectable which may be a function of the frequency wetting and drying cycles and the lack of stability under the tilled environment while in the Oxisol the aggregates appeared to be more resilient to disruption even with mechanical disturbance. The greater response of the Oxisol to changes in management could be related to the direct and indirect impact of tillage and soils dominated by 1:1 clay and lower CEC that would promote faster turnover and less stabilized C (Six et al. 2004). Tillage also alters microbial communities, with a proportional reduction in fungal biomass that contribute to macroaggregate formation (Six et al. 1998).

The lack of change in C and N content following the increase in aggregate size classes for the Oxisol in 0–5 cm depth indicates that in this soil the macroaggregates do not follow the aggregate hierarchy (Tisdall and Oades 1982; Zoratelli et al. 2005). This behavior was different than the other soils and deserves future investigation. Organo-metal binding

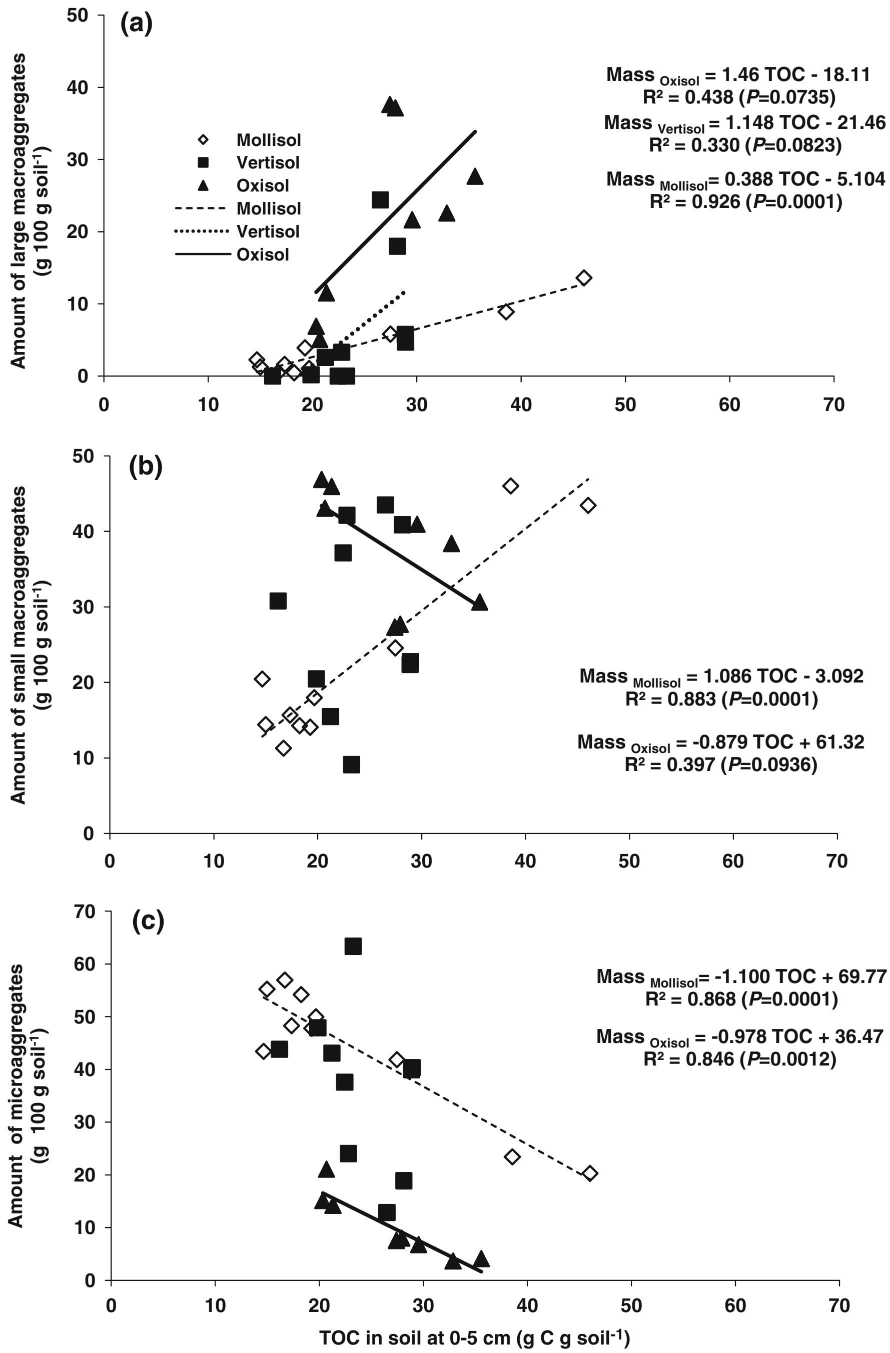
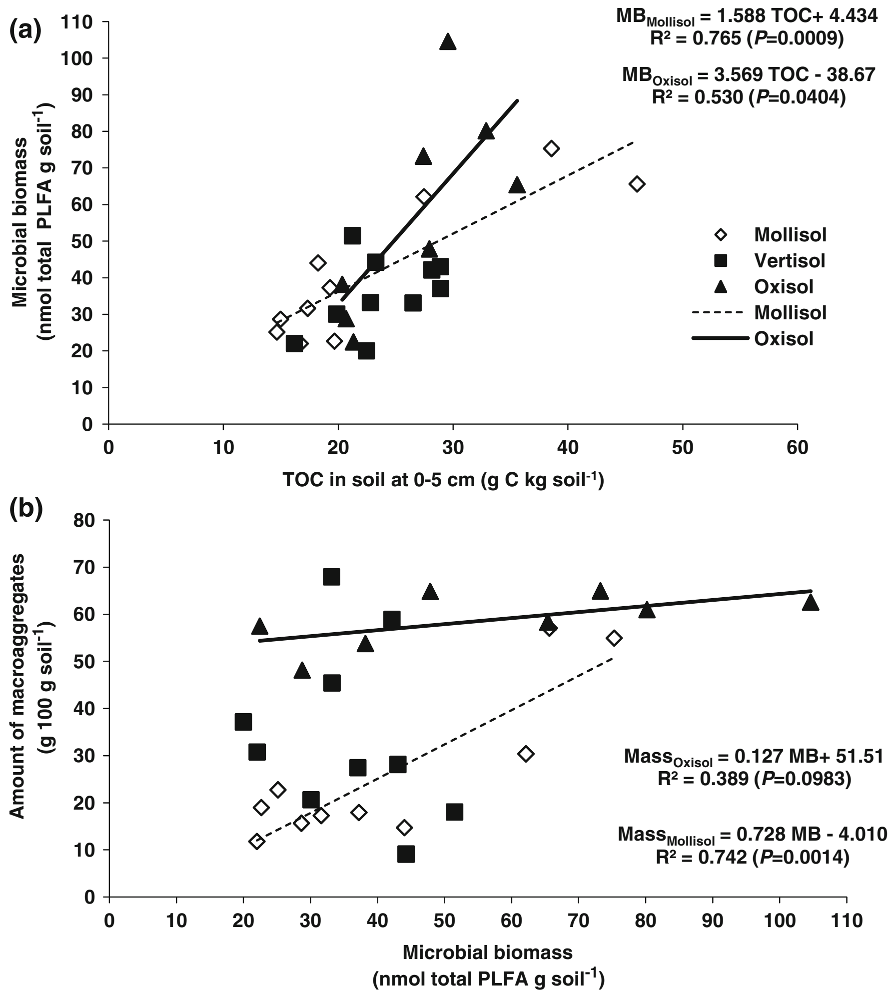



**Fig. 5** Relationship between total C (TOC) in soil at 0–5 cm and the amount of large macroaggregates (>2,000 µm) (**a**), small macroaggregates (2,000–250 µm) (**b**), and microaggregates (250–53 µm) (**c**) for the Oxisol, Vertisol, and Mollisol

from Al and Fe oxides are considered principal agents of aggregation in Oxisols; while SOM and microbial biomass play a secondary role as binding agents (Six et al. 2000a, 2000b, 2002; Denef et al. 2002; Zoratelli et al. 2005). Conversely, the Mollisol and Vertisol had increased C and N concentration with increasing aggregate size fraction in 0–15 cm depth supporting the aggregate hierarchy (Six et al. 2000b).

Microbial biomass was greater under NT than T in the Oxisol and Mollisol, but was similar among treatments in the Vertisol (Fig. 4). The magnitude of the difference between no-till and tilled may be

Fig. 6 Relationship between microbial biomass estimated through the PLFA technique and total organic C (TOC) in soil at 0–5 cm (**a**) and the amount of macroaggregates (>2,000 µm and 2,000–250 µm) and microbial biomass (**b**) for the Oxisol, Vertisol, and Mollisol




a function of the time of adoption of the systems, Oxisol (22 years), Mollisol (17 years) and Vertisol (10 years). The lack of differences in the Vertisol also could be related to the lower intensity of tillage disturbance. In the Oxisol and Mollisol, no-tillage systems maintained similar amounts of microbial biomass as the native grassland. This suggest that the active fraction of the organic matter responds more quickly to the reduction in tillage intensity that the more recalcitrant fractions of organic matter.

The greater slope of the linear relationship between TOC and microbial biomass suggest that a greater proportion of the total organic C in the Oxisol is available for supporting microbial populations than the Mollisol. In addition, TOC is correlated to macroaggregate mass.

While TOC is important in aggregate distribution in both the Oxisol and Mollisol the differences in the relationship with macroaggregates suggest that the binding mechanisms are different (Fig. 5). The greater amount of large macroaggregates in the Oxisol than in the Mollisol may be due to the formation of bridges between primary and secondary particles through the formation of a coat of oxides on the surface (Muggler et al. 1999). The binding of oxides to minerals reduce the CEC of kaolinite and increase positive charge, promoting aggregation through electrostatic binding (Dixon 1989). In this case the biological agents play a secondary role in the process. In the Mollisol there was a strong correlation between TOC or microbial biomass and the amount of macroaggregates suggesting a greater role of biological agents on aggregate formation. The slope was ~7 times higher in the Mollisol than in Oxisol. However, in the Vertisol there was not relationship.

The results from soils of contrasting mineralogy suggest that chemical binding could produce greater amounts of stable macroaggregates than binding by organic materials. More research is needed to elucidate the mechanisms and sensitivity of binding to environmental changes. The Mollisol experienced a greater loss of C as a result of tillage relative to the Oxisol. Thus any change in microbial activity or composition may have a greater impact on Mollisols. Furthermore, Oxisols may be less vulnerable to change.

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