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# Carbon Stocks in Compartments of Soil Organic Matter 31 Years after Substitution of Native *Cerrado* Vegetation by Agroecosystems

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**ABSTRACT:** Changes in carbon stocks in different compartments of soil organic matter of a clayey *Latossolo Vermelho Distrófico* (Typic Haplustox), caused by the substitution of native savanna vegetation (*cerrado sensu stricto*) by agroecosystems, were assessed after 31 years of cultivation. Under native vegetation, a stock of 164.5 Mg ha<sup>-1</sup> C was estimated in the 0.00-1.00 m layer. After 31 years of cultivation, these changes in soil C stocks were detected to a depth of 0.60 m. In the case of substitution of *cerrado sensu stricto* by no-tillage soybean-corn rotation, a reduction of at least 11 % of the soil C pools was observed. However, the adoption of no-tillage as an alternative to tillage with a moldboard plow (conventional system) reduced CO<sub>2</sub> emissions by up to 12 %.

**Keywords:** conventional tillage, no-tillage, microbial biomass, particulate organic matter.

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## INTRODUCTION

Soil organic matter (OM) is all the organic material in the fraction  $<2$  mm, which contains 40 to 60 % C, and which, depending on composition and age, is often assumed to be, on average, 58 % (Nelson and Sommers, 1982). The role of soil OM in sustaining human societies includes the local (maintenance of fertility) and the global (mitigation of C emissions into the atmosphere) scale. In this context of global climate change, agroecosystems can play the role of CO<sub>2</sub> source or sink (Lal et al., 2010; Feller et al., 2012), since the rate of C storage in the soil can be expanded by the adoption of specific management practices (Smith et al., 2008; Lal et al., 2010). However, to suitably monitor these estimates in terms of magnitude, methodological standardization and periodic monitoring must be addressed in long-term studies.

Recent investigations showed that methodological differences are a source of variability in estimates of soil C storage (Batlle-Bayer et al., 2010), particularly with regard to conversion of land use, which always brings about changes in bulk density (Ellert et al., 2002; Don et al., 2011). In addition, C accumulation is usually measured in terms of total C stored in the soil; however, the “potential storage amount” and “potential storage time” depend on the turnover (residence time) of the C reservoirs.

Some conceptual models have been developed in an attempt to describe the formation and decomposition processes of OM. The Century Ecosystems model (Parton et al., 1987; Smith et al., 1997) assumes that OM carbon (TOC) is stored in pools or compartments designated as: the “active” compartment, with a rapid turnover [one to five years], usually represented by microbial biomass (Jenkinson and Powlson, 1976) and soluble organic C in the soil (Paul, 1984; Kaiser and Guggenberger, 2000). The “slow” or “intermediate” compartment, with a cycling time of 20 to 40 years. This C compartment has been represented by particulate OM, the organic matter fraction size  $>53$   $\mu$ m (Cambardella and Elliot, 1992). Finally, the “passive” compartment, with a cycling time of 200-1,500 years (Duxbury et al., 1989). Thus, long-term experiments are important instruments to consistently monitor changes in soil C pools due to changes in agricultural management.

In the *Cerrado* (the Brazilian savanna), a consistent set of soil and environmental data was compiled, collected over the period of 31 years in a long-term experiment of Embrapa Cerrados, Distrito Federal (Corazza et al., 1999; Neufeldt et al., 1999, 2002; Mendes et al., 2003; Oliveira et al., 2004; Ferreira et al., 2007; Jantalia et al., 2007; Resck et al., 2008; Figueiredo et al., 2007, 2010).

The hypothesis of this study is that the conversion from native *cerrado sensu stricto* vegetation to agroecosystems after 31 years of cultivation changes the C stocks in different compartments of soil organic matter. The purpose of this study was to evaluate the changes in the carbon stocks in different compartments of organic matter of a clayey *Latossolo Vermelho Distrófico* (Typic Haplustox) caused by the conversion from native *cerrado sensu stricto* vegetation to agroecosystems after 31 years of cultivation.

## MATERIALS AND METHODS

### Description of the study area

The study was carried out in experimental fields of Embrapa Cerrados in Planaltina, Distrito Federal, Brazil (15° 35' 30" S and 47° 42' 00" W, 1.014 m a.s.l.). According to the Köppen classification, the climate is Aw (tropical rainy), with dry winters and rainy summers and average annual rainfall of 1,400 mm from 1974 to 2003 (Malaquias et al., 2010). All treatments were carried out in a clayey *Latossolo Vermelho Distrófico* (Santos et al., 2013), a Typic Haplustox (Soil Survey Staff, 2014), with approximately 500 g kg<sup>-1</sup> clay.

The long-term experiment, set up in 1979, consisted of different management systems with a soybean-corn rotation. After removing native vegetation, the area was divided into two equal parts, and lime ( $7 \text{ Mg ha}^{-1}$  of dolomitic lime) and fertilizers ( $80 \text{ kg ha}^{-1} \text{ N}$ ,  $100 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$ ,  $100 \text{ kg ha}^{-1} \text{ K}_2\text{O}$ ) were incorporated into the soil in each part with a moldboard plough or a disk plow. In the second year, four treatments were established in areas of  $1,250 \text{ m}^2$  each: conventional tillage with a disk plow (CT-DP); no-tillage, after the first year of tillage with disk plow (NT1); conventional tillage with moldboard plow (CT-MP); and no-tillage, after the first year of tillage with moldboard plow (NT2). Native vegetation, *cerrado sensu stricto* (NV), was adjacent to the experimental field, and the NV treatment was included as a “management system” characterized by native vegetation protected from fire in the *Cerrado* region (a biome or morphoclimatic domain and therefore spelled with a capital letter). The sequence of crops in the entire experiment was identical and consisted of rice (*Oriza sativa*; 1979/1980, 1980/1981); soybean (*Glycine max*; 1981/1982); pigeon pea (*Cajanus cajan*; 1982/1983); fallow (1984-1986); soybean (1986/1987, 1987/1988); corn (*Zea mays*; 1988/1989); fallow (1989/1990); corn (1990/1991); soybean (1991/1992); corn (1992/1993); soybean (1993/1994); rice (1994/1995); soybean (1995/1996); corn (1996/1997); soybean (1997/1998, 1998/1999) and corn (1999/2000). After 2000, the soil was used for annual soybean-corn rotation (one crop by year) until sampling in November 2009. The soil properties and record of land use until 2002 were described in detail by Jantalia et al. (2007). In the 2008/2009 cropping season, soybean was planted in a  $0.45 \text{ m}$  row spacing with  $15 \text{ plants m}^{-1}$ . *Bradyrhizobium japonicum* was supplied as a seed coat inoculant and fertilizers were applied at rates of  $60 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$  and  $60 \text{ kg ha}^{-1} \text{ K}_2\text{O}$ . The soybean crop was harvested in April 2009 and the ground lay fallow until sampling time.

In each  $1,250 \text{ m}^2$  plot, three equidistant sampling points in a diagonal line were chosen, representing three pseudo-replicates from each treatment, according to Hurlbert (1984). Each composite sample consisted of five samples per replication and depth, collected from within a radius of  $1.5 \text{ m}$  around the central sampling point. Disturbed and undisturbed soil samples were collected after the first rains (October/November) when the soil was friable from layers down to a depth of  $1.00 \text{ m}$  ( $0.00\text{-}0.05$ ,  $0.05\text{-}0.10$ ,  $0.10\text{-}0.20$ ,  $0.20\text{-}0.30$ ,  $0.30\text{-}0.40$ ,  $0.40\text{-}0.60$ ,  $0.60\text{-}0.80$ , and  $0.80\text{-}1.00 \text{ m}$ ).

### Total carbon and particulate organic matter

All sampled soil was oven-dried at  $45\text{-}50 \text{ }^\circ\text{C}$  for  $24 \text{ h}$  and passed through a stainless steel sieve ( $2 \text{ mm}$ ). The organic matter (OM) was physically fractionated, as described by Cambardella and Elliott (1992), with adaptations of Bayer et al. (2004) and Figueiredo et al. (2010). This method consists of extracting particulate organic matter (POM) by sieving after shaking in hexametaphosphate solution. The material  $>53 \text{ }\mu\text{m}$  retained on the sieve, consisting of organic waste and the sand fraction, was defined as “total particulate matter”. Since the clay and silt fractions were removed by sieving, it was necessary to correct the values of particulate organic matter C (POM-C) by calculating the difference in relation to the total soil mass. The aliquots of total soil and POM were dried at approximately  $50\text{-}60 \text{ }^\circ\text{C}$ , weighed, ground in a mortar, and passed through a  $100$  mesh sieve ( $0.149 \text{ mm}$ ). The levels of soil C (TOC and POM-C) were determined by dry combustion with a CHN elemental analyzer in a closed combustion chamber at  $900 \text{ }^\circ\text{C}$  (model PE 2400 Series II CHNS/O, PerkinElmer, Norwalk, USA).

### Carbon in soil microbial biomass

After sampling, the soil sample was immediately stored in a refrigerator at approximately  $4 \text{ }^\circ\text{C}$  until analysis. To estimate the C in the soil microbial biomass (BIO-C), we used the fumigation-extraction method (Vance et al., 1987) by which microbial C is extracted after the death of microorganisms and rupture of the cell membrane by fumigation with  $\text{CHCl}_3$  and release of the cellular constituents, which were extracted at a soil:extractor ratio of  $1\text{:}2.5$  (De-Polli and Guerra, 2008).

The concentration of BIO-C was determined as described by Joergensen (1996). A 10 mL aliquot of the extract was filtered and assessed after combustion in a total organic C analyzer (Shimadzu TOC-V CSH/CSN). The amount of MIC-C was determined by applying the correction factor ( $K_{EC}$ ) 0.45, recommended by Joergensen (1996) for BIO-C recovered by combustion. The BIO-C contents were expressed based on the soil mass that was oven-dried at 50-60 °C for 48 h.

The residual C (residual C) was determined after subtraction of the fractions POM-C and BIO-C from TOC using the formula: residual-C ( $\text{mg kg}^{-1}$ ) = TOC - (POM-C + BIO-C).

### Soil texture and bulk density

The soil texture was analyzed by the pipette method (Day, 1965). The clay fraction includes particles  $<2 \mu\text{m}$  and silt from 2-50  $\mu\text{m}$ . The size of the sand fraction varied from 50 to 2,000  $\mu\text{m}$  (Christensen, 1992). Bulk density was determined by the volumetric ring method, with a mean volume of 313.9  $\text{cm}^3$  (Oliveira et al., 2004).

### Carbon stock

The total C stocks (TOC) were calculated for the 0.00-0.30, 0.30-0.60 and 0.60-1.00 m soil layers as the sum of the stocks of each sampled layer (0.00-0.05, 0.05-0.10, 0.10-0.20, 0.20-0.30, 0.30-0.40, 0.40-0.60, 0.60-0.80, and 0.80-1.00 m) by the following equation:

$$C (\text{Mg ha}^{-1}) = \sum (C \times \text{Bd} \times z)$$

where C is the C stock in the equivalent layer (*Layer*), C is the C concentration ( $\text{dag kg}^{-1}$ ), Bd is the bulk density ( $\text{Mg m}^{-3}$ ), and z is the thickness of the sampled layer (m).

To correct the C stock to the equivalent mass (*Mass*), we used the method proposed by Ellert and Bettany (1995) and described by Sisti et al. (2004). To calculate the stock, the mass equivalent to the native reference area (NV) in the corresponding layers (0.00-0.30 and 0.00-0.60 m, 0.00-1.00 m) was used as a reference. The same line of thought was used to calculate the POM-C and BIO-C stocks in the 0.00-0.30 m depth range.

Changes in C stocks between the current land use and the reference area with native *cerrado* vegetation (NV) were compared in pairs, and the differences between the two units were presented as: absolute difference (absolute  $\Delta C$ ), the difference between the reference area and the management system, expressed in  $\text{Mg ha}^{-1}$ , and the relative difference (relative  $\Delta C$ ), compared to the control, expressed in %.

Data were tested for homogeneity of variance (F test) and the assumption of normality (Shapiro-Wilk). The data are normally distributed below this critical level. For the comparison between the averages of two sample populations, the Student "t" test was used. All analyses were performed using SAS software, version 9.1.2.

## RESULTS

Under all agricultural experimental plots, soil bulk density (Bd) was significantly ( $p < 0.05$ ) higher than under native *cerrado* vegetation (VN) to a depth of 0.20 m, probably owing to agricultural machinery (plowing, seeding, and harvesting), (Table 1). The Bd under the experimental plots was an average of 8 % higher (mean of all depths and treatments) than under native vegetation.

The conversion of NV to agricultural systems induced reductions of 26, 20, and 15 % in the C content in the 0.00-0.30 and 0.00-0.60 m, 0.00-1.00 m layers, respectively (Table 2). In all treatments, the C contents were lower in the deeper layers, but the differences between the agricultural management systems decreased with depth. Whereas in the upper 0.30 m, reductions of 9 and 12 % were observed in the conventional systems (CT-DP and CT-MP), respectively, compared to the corresponding no-tillage treatments (NT1 and NT2), there was no significant difference between agricultural systems in the 0.30 to 1.00 m layer.

**Table 1.** Soil bulk density of a clayey *Latossolo Vermelho Distrófico* (Typic Haplustox) in the 0.00-1.00 m depth range under different management systems in the *Cerrado*

| Layer     | CT-DP <sup>(1)</sup> | NT1 <sup>(2)</sup> | CT-MP <sup>(3)</sup> | NT2 <sup>(4)</sup> | NV <sup>(5)</sup> |
|-----------|----------------------|--------------------|----------------------|--------------------|-------------------|
| m         | Mg m <sup>-3</sup>   |                    |                      |                    |                   |
| 0.00-0.05 | 1.02 a               | 1.06 a             | 1.05 a               | 1.06 a             | 0.92 b            |
| 0.05-0.10 | 1.17 a               | 1.08 a             | 1.23 a               | 1.17 a             | 0.95 b            |
| 0.10-0.20 | 1.04 a               | 1.06 a             | 1.06 a               | 1.09 a             | 0.92 b            |
| 0.20-0.30 | 1.05 (0.03)          | 1.11 (0.04)        | 1.10 (0.04)          | 1.06 (0.08)        | 1.11 (0.06)ns     |
| 0.30-0.40 | 1.09 (0.03)          | 1.09 (0.08)        | 1.18 (0.08)          | 1.03 (0.08)        | 1.12 (0.04)ns     |
| 0.40-0.60 | 1.15 (0.09)          | 1.04 (0.03)        | 1.14 (0.03)          | 1.10 (0.06)        | 1.09 (0.13)ns     |
| 0.60-0.80 | 1.14 (0.04)          | 1.12 (0.14)        | 1.12 (0.11)          | 1.17 (0.05)        | 1.11 (0.01)ns     |
| 0.80-1.00 | 1.14 (0.13)          | 1.12 (0.04)        | 1.09 (0.01)          | 1.17 (0.01)        | 1.04 (0.03)ns     |

<sup>(1)</sup> CT-DP: disk plow; <sup>(2)</sup> NT1: no-tillage, after the first year with disk plow; <sup>(3)</sup> CT-MP: moldboard plow; <sup>(4)</sup> NT2: no-tillage, after the first year with moldboard plow; <sup>(5)</sup> NV: *cerrado sensu stricto* protected from fire. Means followed by the same letter for the same depth interval are not significantly different at  $p < 0.05$  by the  $t$  test; ns: means for the same depth interval not significantly different at  $p < 0.05$  by the  $t$  test. Standard deviation in parentheses.

**Table 2.** Carbon content in a clayey *Latossolo Vermelho Distrófico* (Typic Haplustox) after 31 years under different tillage systems in the *Cerrado*

| Layer     | CT-DP <sup>(1)</sup> | NT1 <sup>(2)</sup> | CT-MP <sup>(3)</sup> | NT2 <sup>(4)</sup> | NV <sup>(5)</sup> |
|-----------|----------------------|--------------------|----------------------|--------------------|-------------------|
| M         | g kg <sup>-1</sup>   |                    |                      |                    |                   |
| 0.00-0.30 | 26.2 (1.3) Aa        | 19.6 (0.5) Ac      | 21.6 (1.1) Ab        | 17.2 (1.4) Ad      | 19.5 (0.5) Ac     |
| 0.30-0.60 | 16.1 (0.6) Ba        | 13.6 (1.0) Bb      | 13.7 (0.6) Bb        | 11.7 (0.6) Bc      | 12.3 (1.0) Bbc    |
| 0.60-1.00 | 11.5 (0.4) Ca        | 10.0 (0.1) Cb      | 10.2 (0.5) Cb        | 9.0 (0.8) Cb       | 9.7 (0.8) Cb      |

<sup>(1)</sup> CT-DP: disk plow; <sup>(2)</sup> NT1: no-tillage, after the first year with disk plow; <sup>(3)</sup> CT-MP: moldboard plow; <sup>(4)</sup> NT2: no-tillage, after the first year with moldboard plow; <sup>(5)</sup> NV: *cerrado sensu stricto* protected from fire. Means followed by the same uppercase letter in the column and by lowercase letters in the row indicate no significant difference from each other by the  $t$  test ( $p < 0.05$ ). Standard deviation in parentheses.

These results suggest that after 30 years, no agricultural management system was as efficient as the *cerrado* in terms of soil C concentration in the 0.00-1.00 m layer range. Among the management systems, the tillage effect was restricted to the 0.00-0.30 m layer.

The soil C stocks estimated by two methods: equivalent layer (*Layer*), in which C stocks are calculated based on the equivalent soil layer, considering a fixed layer for all systems and therefore the same soil volume; and equivalent weight (*Mass*) in which C stocks are corrected by the soil mass equivalent to the natural reference system [treatment NV], considering the change in Bd after soil cultivation (Table 3).

In the 0.00-0.30 m layer of NV, there was a stock of 70.5 Mg ha<sup>-1</sup> C. In the cropping systems, variations from 50.1 to 60.7 Mg ha<sup>-1</sup> C and 55.2 to 65.1 Mg ha<sup>-1</sup> C were found using the *Mass* and *Layer* method for this same layer, respectively (Table 3). In the 0.30-0.60 m layer, the C stock in NV was 46.4 Mg ha<sup>-1</sup> C, corresponding to an estimated accumulated stock of 116.9 Mg ha<sup>-1</sup> C in the 0.00-0.60 m layer. In the 0.00-0.60 m layer, the C stock in the cultivation systems ranged from 85.7 to 101.6 Mg ha<sup>-1</sup> C and from 94.7 to 110.5 Mg ha<sup>-1</sup> C using the *Mass* and *Layer* method, respectively.

In the 0.60 and 1.00 m layer, an accumulation of 47 Mg ha<sup>-1</sup> C was observed in NV, amounting to a total pool of 164.3 Mg ha<sup>-1</sup> C in the 0.00-1.00 m layer. In the cultivation systems, variations from 125.8 to 145.8 Mg ha<sup>-1</sup> C and 139.8 to 157.5 Mg ha<sup>-1</sup> C were found for *mass* and *layer*, respectively. Except for NT1, the C stocks in all management systems were significantly lower than in NV.



**Table 3.** Estimates of carbon pools by different methods of bulk density correction in a clayey *Latossolo Vermelho Distrófico* (Typic Haplustox) after 31 years of cultivation under different tillage systems in the *Cerrado*

| Method               | Layer     | CT-DP <sup>(1)</sup> | NT1 <sup>(2)</sup> | CT-MP <sup>(3)</sup> | NT2 <sup>(4)</sup> | NV <sup>(5)</sup> |
|----------------------|-----------|----------------------|--------------------|----------------------|--------------------|-------------------|
|                      | m         | Mg ha <sup>-1</sup>  |                    |                      |                    |                   |
| Layer <sup>(6)</sup> | 0.00-0.30 | 62.3 (0.7) bc        | 65.1(3.3) ab       | 55.2 (5.0) c         | 60.6 (2.7) bc      | 70.5 (3.5) a      |
|                      | 0.30-0.60 | 42.0 (2.6) bc        | 45.3 (2.0) ab      | 39.5 (3.0) c         | 41.6 (3.6) bc      | 46.5 (1.9) a      |
|                      | 0.60-1.00 | 46.1 (0.7) a         | 47.0 (0.5) a       | 45.1 (3.7) a         | 47.8 (3.5) a       | 47.4 (1.3) a      |
|                      | 0.00-1.00 | 150.5 (3.6) b        | 157.5 (2.7) ab     | 139.8 (11.6) b       | 150.0 (6.4) b      | 164.3 (6.5) a     |
| Mass <sup>(7)</sup>  | 0.00-0.30 | 56.7 (1.3) b         | 60.7 (3.1) b       | 50.1 (4.0) c         | 55.7 (1.6) b       | 70.5 (3.5) a      |
|                      | 0.30-0.60 | 40.5 (1.9) b         | 40.9 (1.0) b       | 35.6 (2.3) c         | 37.2 (3.0) bc      | 46.5 (1.9) a      |
|                      | 0.60-1.00 | 43.0 (0.6) a         | 44.2 (2.1) a       | 40.2 (4.1) a         | 42.9 (3.4) a       | 47.4 (1.3) a      |
|                      | 0.00-1.00 | 140.2 (3.1) b        | 145.8 (3.2) b      | 125.8 (9.7) c        | 135.8 (6.8) b      | 164.3(6.5) a      |

<sup>(1)</sup> CT-DP: disk plow; <sup>(2)</sup> NT1: no-tillage, after the first year with disk plow; <sup>(3)</sup> CT-MP: moldboard plow; <sup>(4)</sup> NT2: no-tillage, after the first year with moldboard plow; <sup>(5)</sup> NV: *cerrado sensu stricto* protected from fire. <sup>(6)</sup> Layer: C stocks calculated based on the equivalent layer, without correction by the soil mass equivalent to the natural system of reference [NV]; <sup>(7)</sup> Mass: C stocks corrected by the soil mass equivalent to the natural system of reference [NV]. Means followed by the same letter in the row indicate no significant differences by the t test ( $p < 0.05$ ). Standard deviations in parentheses.

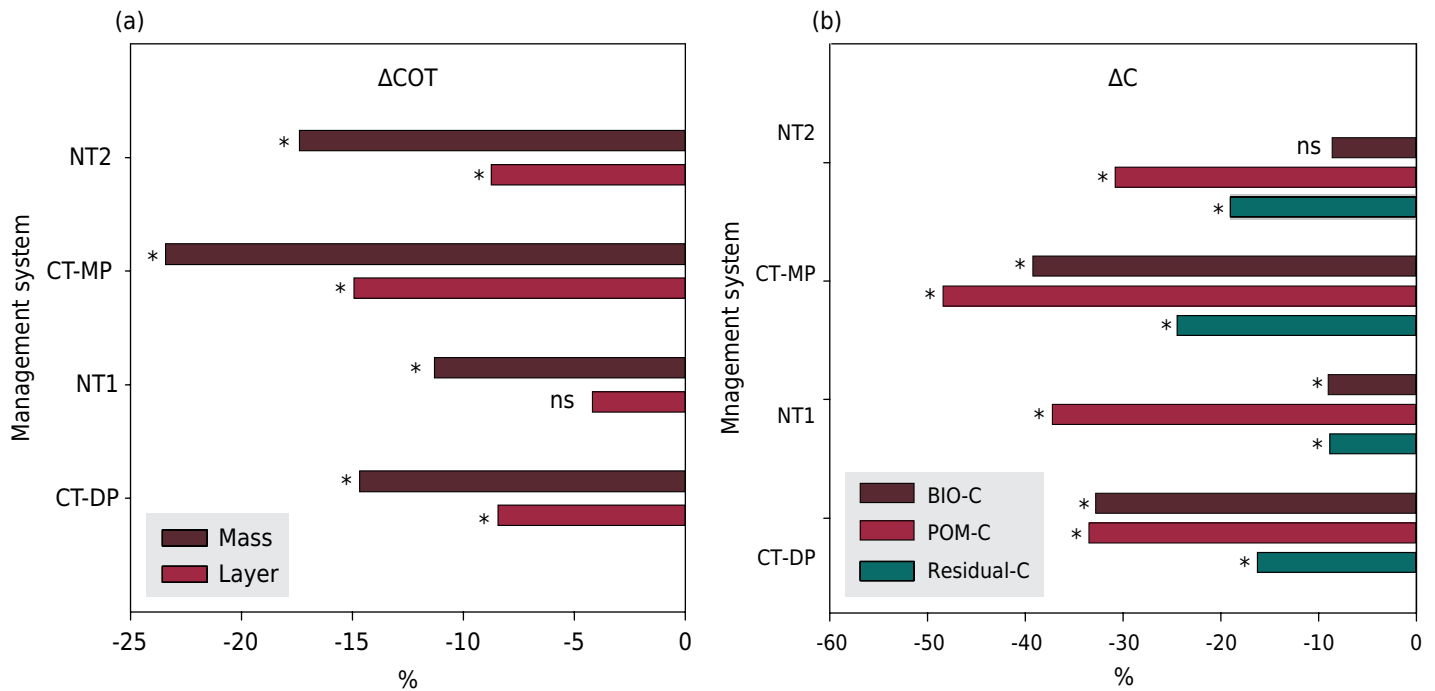
After 31 years of conversion of *cerrado*, no significant differences were observed between NT1 and NV by the *layer* method, and these results were consistent down to a depth of 1.00 m. However, due to correction by soil mass, there was a significant reduction (14, 12, and 7 %, for 0.00-0.30, 0.00-0.60, and 0.00-1.00 m layers, respectively) in the C stocks of NT1 (Table 3). Thus, the absence of a significant difference between NV and no-tillage soil C stocks could be related to the calculation method that disregarded the effects of increased soil density due to management.

After 31 years of cultivation, the reductions in soil C pools were greatest in the conventional system, where the soil is plowed annually with a moldboard plow (CT-MP). Regardless of the calculation method, the C stocks in CT-MP were always smaller than in NT1, both in the 0.00-0.30 and 0.00-1.00 m layers (Table 3). After adjusting for the equivalent soil mass, the C stocks in the 0.00-0.30 m layer were reduced as follows: MP-CT: -29 % (-20.4 Mg ha<sup>-1</sup> C); NT2: -21 % (-14.8 Mg ha<sup>-1</sup> C); CT-DP: -20 % (-13.8 Mg ha<sup>-1</sup> C); and NT1: -14 % (-9.8 Mg ha<sup>-1</sup> C). In the first meter of depth, the reductions in C stock in CT-MP were 38.5 Mg ha<sup>-1</sup> C, equivalent to reductions of 23 % from the original soil C stocks (Figure 1a).

In the 0.60-1.00 m layer, no effect of conversion of native vegetation to annual cropping was detected in the C stocks by either method. Therefore, it can be suggested that the impact of the conversion and of the agricultural management systems occur mostly down to a 0.60 m depth. The effect of conventional management (i.e., moldboard plow) on C stock reductions was invariably limited to a depth of 0.60 m and was more pronounced after correction by the *mass* method (Figure 1a).

With regard to the differences between the C pools of native vegetation and the cultivation systems, it was found that the reductions in soil C in CT-MP occurred at rates of 0.68, 1.04, and 1.28 Mg ha<sup>-1</sup> yr<sup>-1</sup> C in the .00-0.30, 0.00-0.60, and 0.00-1.00 m layers, respectively. The lowest reductions were found under NT1, at rates of 0.33, 0.51, and 0.62 Mg ha<sup>-1</sup> yr<sup>-1</sup> C in the same layers, respectively.

Based on these assumptions, the CT-MP system was adopted as a reference for calculation of reductions in CO<sub>2</sub>-C emission under NT1. Thus, after 31 years of cultivation in the 0.00-0.30 m layer, adjusting by the *mass* method, reductions in C emissions under NT1 corresponded to 10.6 Mg ha<sup>-1</sup> C at a linear rate of 0.35 Mg ha<sup>-1</sup> yr<sup>-1</sup> C. In the 0.00-1.00 m layer, this conservation management contributed to a reduction of 12 % of CO<sub>2</sub>-C emissions, or 20 Mg ha<sup>-1</sup> C, at a linear rate of 0.67 Mg ha<sup>-1</sup> yr<sup>-1</sup> C. In contrast, the replacement of native vegetation by NT1 led to a reduction in soil C stocks of 11 %, or 18.5 Mg ha<sup>-1</sup> C, at a linear rate of 0.6 Mg ha<sup>-1</sup> yr<sup>-1</sup> C (Table 2, Figure 1a).



**Figure 1.** Relative differences of C stock: (a) soil C stock in a clayey *Latossolo Vermelho Distrófico* (Typic Haplustox) in the 0.00-1.00 m depth range, based on the reference NV. \* and ns: significant and not significant, respectively, by the t test ( $p < 0.05$ ). NV: CT-DP, NT1, NT2, CT-MP. Losses are indicated by a minus sign (-). Layer: C stocks calculated by the equivalent layer, without correction for the soil mass equivalent to the natural system of reference [NV]; Mass: C stocks corrected by the mass of soil equivalent to the natural system of reference [NV]. (b) soil C compartments in the 0.00-0.30 m layer, based on the reference NV; BIO-C: microbial biomass; POM-C: particulate organic carbon; residual C = TOC-(POM-C + BIO-C). CT-DP: disk plow; NT1: no-tillage, after the first year with disk plow; CT-MP: moldboard plow; NT2: no-tillage, after the first year with moldboard plow; NV: *cerrado sensu stricto* protected from fire.

This shows the NT1 acting as a CO<sub>2</sub>-C source or sink, according to the calculation method and system considered as baseline. Both the magnitude and the rate of C reduction or accumulation were related to the respective sampling depth.

The C content of the >53 μm fraction of OM (POM-C) ranged from 7.36 to 2.21 mg kg<sup>-1</sup> (Table 4). The replacement of native vegetation by agricultural management systems caused average reductions of 5.9 mg kg<sup>-1</sup> in POM-C in the whole 0.00-0.30 m layer. Interestingly, the POM-C concentration decreased with increasing depth only in the treatments without tillage, including NV.

The levels of microbial biomass C (BIO-C) ranged from 557 to 203 mg kg<sup>-1</sup> C (Table 4). In the 0.00-0.05 m layer, *Cerrado* conversion resulted in a greater reduction in BIO-C in all cropping systems (47, 32, 54, and 27 % in CT-DP, NT1, CT-MP, and NT2, respectively). In the same layer, among the agricultural management systems, the levels of BIO-C were highest under no-tillage, decreasing in the following order: NT2 = NT1 > CT-MP > CT-DP (Table 4). In all management systems, the soil depth had a significant effect on BIO-C (Table 3).

The magnitude of the soil C stock per OM compartment (residual-C, POM-C, BIO-C) in the 0.00-0.30 m layer is at table 4. Under NV, approximately 1.1, 12.5 and 56.9 Mg ha<sup>-1</sup> or 1.4, 17.8 and 80.8 % was in the form of residual C, microbial biomass and POM-C, respectively.

Figure 1a shows the relative changes in the TOC stock (%) in the 0.00-1.00 m layer, with and without correction by soil mass (*mass* and *layer*, respectively). Figure 1b shows the relative changes in C stocks (%) in the OM compartments in the 0.00-0.30 m layer after correction for *mass*. In both cases, the reference system was NV.

In general, the replacement of native vegetation by crop systems caused reductions in the C content in all OM fractions, including TOC, after correction by the *mass* method (Figure 1b). The only exception was BIO-C, with non-significant reductions (8.6 %)



under NT2 in the 0.00-0.30 m layer (Figure 1b). In terms of BIO-C, the NV conversion to agroecosystems had a much greater impact on the treatments with soil disturbance (plowing). The plowing induced BIO-C reductions of approximately 33 and 39 % under CT-DP and CT-MP, respectively. Under NT1, the decrease in BIO-C was approximately 10 %.

In the 0.00-0.30 m layer, the replacement of NV by cultivation with moldboard plowing reduced the C pools in the residual form by about 24 % or 13 Mg ha<sup>-1</sup> (Table 2) and by 48 % or 6.1 Mg ha<sup>-1</sup> in the POM-C (Figure 1b). This pattern was confirmed in all other cultivation treatments; the C reductions in POM-C were an average of 2.5 times higher than in residual C.

The conversion of NV to conventional management systems led to reductions of 8 to 40 % in microbial C.

## DISCUSSION

After analysis of 31 years of management, in view of the evidence of reductions of 18 Mg ha<sup>-1</sup> C under NT1 in the 0.00-1.00 m layer, this study did not confirm the potential of no-tillage as a C sink as described by Corazza et al. (1999) at the same experimental site. Based on an area of native *cerrado* vegetation as reference, the authors evaluated the same tillage system (NT1) after 15 years of cultivation and found an accumulation of 21.4 Mg ha<sup>-1</sup> C at rates of 1.43 Mg ha<sup>-1</sup> yr<sup>-1</sup> C. However, our results are in agreement with estimates of Jantalia et al. (2007), who, at the same experimental site, observed a reduction of 26 Mg ha<sup>-1</sup> C in soil under NT1 after 20 years (Table 5).

The studies were consistent in terms of the changes in C stocks ( $\Delta C$ ) in the 20<sup>th</sup> year (Jantalia et al., 2007) and in the 31<sup>st</sup> year. Under NT1, these relative C losses after 20 and 31 years were estimated at 13 and 11 %, respectively. The same occurred in CT-DP after 20 and 31 years, with reductions of 13 and 14 %, respectively (Table 5). These values indicate that changes in C accumulation and reduction in these systems have been relatively stable in the last 11 years of management.

**Table 4.** Carbon compartments (particulate organic matter – POM-C, soil microbial biomass – BIO-C, and residual C) in a clayey *Latossolo Vermelho Distrófico* (Typic Haplustox) under different management systems in the *Cerrado*

| Fraction                  | Layer     | CT-DP <sup>(1)</sup> | NT1 <sup>(2)</sup> | CT-MP <sup>(3)</sup> | NT2 <sup>(4)</sup> | NV <sup>(5)</sup> |
|---------------------------|-----------|----------------------|--------------------|----------------------|--------------------|-------------------|
|                           | m         | g kg <sup>-1</sup>   |                    |                      |                    |                   |
| POM-C                     | 0.00-0.05 | 7.36 Aa              | 2.75 Ca            | 3.95 Ba              | 2.36 Ca            | 4.13 Ba           |
|                           | 0.05-0.10 | 4.97 Ab              | 3.22 BCa           | 2.75 Bb              | 2.21 Ca            | 3.29 BCab         |
|                           | 0.10-0.20 | 3.67 Ab              | 2.98 Ba            | 2.37 Bb              | 2.36 Ba            | 2.70 Bb           |
|                           | 0.20-0.30 | 3.23 Ab              | 2.58 Ba            | 2.23 BCb             | 1.94 Ca            | 2.41 BCb          |
|                           |           | mg kg <sup>-1</sup>  |                    |                      |                    |                   |
| BIO-C                     | 0.00-0.05 | 557 Aa               | 295 Ca             | 383 Ba               | 256 Da             | 408 Ba            |
|                           | 0.05-0.10 | 409 Ab               | 257 Cab            | 332 Bab              | 222 Cab            | 356 Aab           |
|                           | 0.10-0.20 | 348 Ab               | 223 Bb             | 315 Bab              | 206 Bab            | 318 Aab           |
|                           | 0.20-0.30 | 259 Ac               | 222 Abc            | 288 Ab               | 203 Ab             | 272 Abc           |
|                           |           | Mg ha <sup>-1</sup>  |                    |                      |                    |                   |
| Residual C <sup>(6)</sup> | 0.00-0.30 | 56.92                | 47.66              | 51.88                | 43.00              | 46.07             |
| POM-C                     | 0.00-0.30 | 12.53                | 8.34               | 7.87                 | 6.46               | 8.67              |
| BIO-C                     | 0.00-0.30 | 1.05                 | 0.70               | 0.95                 | 0.64               | 0.96              |

<sup>(1)</sup> CT-DP: disk plow; <sup>(2)</sup> NT1: no-tillage, after the first year with disk plow; <sup>(3)</sup> CT-MP: moldboard plow; <sup>(4)</sup> NT2: no-tillage, after the first year with moldboard plow; <sup>(5)</sup> NV: *cerrado sensu stricto* protected from fire. <sup>(6)</sup> Residual C = TOC – (POM-C + BIO-C). Means followed by the same uppercase letter in a row and lowercase letter in a column indicate no significant difference by the t test (p<0.05).

**Table 5.** Absolute and relative changes in C stocks in a clayey *Latossolo Vermelho Distrófico* (Typic Haplustox) in the 0.00-1.00 m depth range after 31 years of a long-term experiment in the *Cerrado*, according to different studies

| Management system    | Years <sup>(1)</sup> | Stock <sup>(5)</sup><br>Mg ha <sup>-1</sup> | ΔC                              |               | Rate   |               |
|----------------------|----------------------|---|---------------------------------|---------------|--|---------------|
|                      |                      |   | Absolute<br>Mg ha <sup>-1</sup> | Relative<br>% | Absolute<br>Mg ha <sup>-1</sup> yr <sup>-1</sup> | Relative<br>% |
| Conventional tillage | 15 <sup>(2)</sup>    | 128.8                                       | -4.90                           | -3.60         | -0.32  | -0.24         |
|                      | 20 <sup>(3)</sup>    | 176.4                                       | -26.7                           | -13.1         | -1.34  | -0.66         |
|                      | 31 <sup>(4)</sup>    | 140.2                                       | -24.1                           | -14.7         | -0.78  | -0.47         |
| No-tillage           | 15                   | 155.0                                       | 21.4                            | 16.0          | +1.43  | +1.07         |
|                      | 20                   | 177.1                                       | -26.0                           | -12.8         | -1.30  | -0.64         |
|                      | 31                   | 145.8                                       | -18.5                           | -11.3         | -0.60  | -0.36         |
| Native vegetation    | 15                   | 133.6                                       | -                               | -             | -  | -             |
|                      | 20                   | 203.1                                       | -                               | -             | -  | -             |
|                      | 31                   | 164.3                                       | -                               | -             | -  | -             |

<sup>(1)</sup> Experimental periods: 15, 20, and 31 years of cultivation; <sup>(2)</sup> Corazza et al. (1999) (geographic coordinates -15.605827, -47.707295); <sup>(3)</sup> Jantalia et al. (2007) (geographic coordinates -15.591228, -47.736006); <sup>(4)</sup> This study (geographic coordinates -15.593078, -47.737709) <sup>(5)</sup> Jantalia: *Cerrado*>DP=NT (t test p<0.05); Corazza: NT>*Cerrado*=DP (Tukey, p<0.05). Absolute ΔC: difference between the soil C stock of NV and of the cultivated systems; Relative ΔC: the same difference, expressed in percentage; Absolute rate: absolute annual linear rate of reduction (-) or increase (+) of C; Relative rate: the same rate expressed in percentage.

Divergent results in similar management systems, as observed between the 15<sup>th</sup> and 20<sup>th</sup> year in the C stock estimates under no-tillage management, may be associated with methodological differences, possibly related to analytical methods – wet oxidation with external heating used by Corazza et al. (1999) and dry combustion in an elemental analyzer used by Jantalia et al. (2007); and, or, differences in the arithmetic calculation of soil C stocks – equivalent layer used by Corazza et al. (1999) and correction by mass used by Jantalia et al. (2007).

In addition to the different methods, changes from 136 to 203 Mg ha<sup>-1</sup> in the soil C stocks of native vegetation between our study and that of Corazza et al. (1999) and of Jantalia et al. (2007) can be attributed to the heterogeneity of the *Cerrado* vegetation in the experimental area of Embrapa Cerrados at different geographic coordinates (Table 5: footnote). At the Embrapa Cerrados site, the physiognomies “Cerradão”, a forest vegetation where Corazza et al. (1999) collected soil samples, and “cerrado sensu stricto”, a typical savanna vegetation (consisting of trees, shrubs, and grass layers), can be found. In addition, 20 years earlier there were no protection programs against fire and accidental burning, which may have caused variations in the biomass and soil C stocks and interfered with the differences between the values of the first and last evaluations. In the case of different *Cerrado* physiognomies, the amount of C stored in soils under NV can vary from 87 to 210 Mg ha<sup>-1</sup> C (Bustamante et al., 2006).

In the absence of correction of the stocks by mass under NT1, we calculated 157 Mg ha<sup>-1</sup> C (ΔTOC = -4 %) as C stock in the first meter of depth (Figure 1). The stock reported for this same system, Corazza et al. (1999) estimated 155 Mg ha<sup>-1</sup> C in the 15<sup>th</sup> year (Table 5). Although these three studies used other analytical methods of C recovery, the data suggest that in the system under NT, the soil C pool may have reached the saturation point between the 15<sup>th</sup> and 20<sup>th</sup> year, and C stocks were stable until the 31<sup>st</sup> year of cultivation, as proposed by West and Post (2002) for tropical soils.

In this study, no-tillage (NT1) contributed to a reduction in CO<sub>2</sub> emissions of 20 Mg ha<sup>-1</sup> C, at a linear rate of 0.67 Mg ha<sup>-1</sup> yr<sup>-1</sup> C. This finding is consistent with the overall data of 67 long-term experiments reported by West and Post (2002), which, with regard to the conventional system, concluded that the no-tillage system increased C pools at rates of 0.57 Mg ha<sup>-1</sup> yr<sup>-1</sup>, re-establishing an equilibrium between the 15<sup>th</sup> and 20<sup>th</sup> year.

After 31 years of management, the maximum reduction in C stocks from the *cerrado* to conventional management systems was 23 %. This result was lower than the 40 % reduction from forests converted to agriculture, according to a publication on the effect of land use on global soil C pools (Guo and Gilford, 2002). These reductions in C stocks were also lower than the 60 % reported for soils of native tropical forests (Brown and Lugo, 1990) converted to cropland.

In the *Cerrado* in western Bahia, where rainfall is 15-20 % lower than in Planaltina, Distrito Federal, after five years of cultivation, 41 to 80 % of C was lost from soil gradients with clayey to sandy texture, respectively (Silva et al., 1994). These differences between the central *Cerrado* region (Planaltina, DF) and western Bahia can be explained by the results of a meta-analysis of C dynamics in tropical soils by Don et al. (2011). These authors found that, in tropical soils, tillage systems in interaction with climatic factors and clay mineralogy can explain up to 55 % of the variance in soil C stocks. On a nationwide level, 50 % of the variance can be explained by temperature and clay content (Assad et al., 2013).

These lower reductions in soil C stocks observed in the conventional systems of this study compared to the global indices were probably due to the effect of soil texture and controlled experimental conditions, resulting in optimal production conditions. The plowing and harrowing operations were carried out when the soil was friable, and liming and maintenance fertilization were applied according to the official recommendations for this soil type (Resck et al., 2000). In particular, the effect of the texture (~50 % clay) may have prevented the loss of organic C by the formation of stable complexes from humic substances and inorganic soil constituents (Oades, 1993; Stevenson, 1994; Zinn et al., 2005; Tristram and Six, 2007). In this context, it is worth remembering that the soil of our study is a clayey kaolinitic Oxisol (Typic Haplustox) [weathering index  $ki$  ( $1.7SiO_2/Al_2O_3$ ) = 1.3 and  $Fe_2O_3$  = 86 g kg<sup>-1</sup>], described by Oliveira et al. (2004). In such soils, the oxides, hydroxides, and oxyhydroxides of Al and Fe (FeOx and AlOx) contribute to stabilization of organic matter (Kaiser and Guggenberger, 2000; Bruun et al., 2010).

The results indicate OM protection in the organo-mineral complexes, resulting in greater degradation resistance of C in residual form. With regard to distribution of C in the SOM compartments, we observed that 13 to 18 % was stored in the sand fraction (>53 µm), and 1.2 to 1.7 % in the microbial biomass (Table 4). Therefore, more than 80 % of C of the OM is in the residual form, which can, in principle, be attributed to mineral-associated C. In general, the soluble C concentration was less than 0.09 and 1 % of the total C (Killham, 1994; Rosa et al., 2003).

Some authors have also shown differences in OM composition between the silt and clay fractions (<53 µm), and that part of this OM can be more readily available for decomposition (Baldock et al., 1992; Gregorich et al., 1995; Balesdent et al., 1998). The fractions <53 µm, however, are largely dominated by organo-minerals (Feller and Beare, 1997) and therefore more resistant to decomposition.

In a tropical climate, frequent soil tillage with a disk plow increased the relative share of mineral-associated C in clayey Oxisols with high levels of Fe oxides (Bayer et al., 2004; Figueiredo et al., 2010), which was attributed to the high adsorption energy of OM to mineral particles which not even the disk plow was able to break (González-Péres et al., 2004). There was also evidence that there is no change in the absolute amounts of stable microaggregates or in the C stored in that fraction when these soils were disturbed by plowing (Bayer, 1996; Resck et al., 2000, 2006; Mendes et al., 2003).

In the experimental area of this study, there are reports that in the *cerrado sensu stricto*, mineralizable C and activity of the enzyme β-glucosidase are higher (almost double) in macroaggregates than in microaggregates (Mendes et al., 2003). The authors attributed this difference to the presence of material rich in simple organic compounds that are

therefore easily mineralizable after the rupture of macroaggregates, when the soil is disturbed by plowing (Mendes et al., 2003).

The C stored in POM represented less than 18 % of TOC. Similar values and proportions were reported in tropical regions, where decomposition is accelerated by high temperatures and humidity. Generally, the coarser OM (>20 or 53  $\mu\text{m}$ ) represents 20 % of the total soil OM in such environments (Pillon et al., 2002; Salton, 2005). Similar observations were recorded in different soil types, in which the POM-C represented between 11 and 35 % of TOC (Bayer et al., 2004; Conceição et al., 2005; Gregorich et al., 2006; Sequeira et al., 2011).

After replacing the native *cerrado* vegetation by agroecosystems, the 30-50 % reductions of C in the particulate fraction can be attributed to the decrease in the contribution of plant residues (primary resources) in this compartment. The same line of thought can be applied to agroecosystems, where the POM-C levels in the upper soil layers of no-tillage are often higher than in the conventional systems, due to residue accumulation (Bayer et al., 2004; Figueiredo et al., 2010).

Both the absolute and relative portion of the microbial properties assessed in this study were consistent with the decrease of up to 64 % in BIO-C induced by conversion of *Cerrado* NV to annual cropping shown in a meta-analysis of soils in Brazil (Kaschuk et al., 2011). In the clayey Oxisol (Typic Haplustox) of our study, the reduction of over 30 % in microbial biomass in conventional tillage systems can be attributed to the lack of readily available substrate in the <53  $\mu\text{m}$  fractions, representing more than 85 % of OM. In this silt-clay fraction, the high energy adsorption to minerals in the soil matrix can promote long-term stabilization, leading to turnover rates of more than 200 years in microaggregates (Monreal et al., 1997).

However, Anderson and Domsch (1990) suggested that the decrease in the contribution of microbial C in conventional tillage systems can be attributed to a loss of the ability to sustain biological activity and probably biodiversity. Neufeldt et al. (2002) reported that, In the *Cerrado*, the origin of 85 % of the polysaccharides in the clay fraction is microbial and 75 % is extracellular. The authors completed that these polysaccharides are fundamental for the process of soil aggregation, which does not occur in the products of lignin oxidation. The glycoproteins derived from hyphae of arbuscular mycorrhiza (i.e., glomalin) and peptides adsorbed to the mineral oxides also contribute to the formation of aggregates relatively resistant to microbial degradation (Knicker, 2010).

Although the loss of microbial biomass is much lower than the magnitude of  $\text{CO}_2$  emissions, it is important to consider the multiple roles of microbial diversity in the functioning of ecosystems through their involvement in biogeochemical cycles and biotic interactions between plants and microorganisms. In addition, microorganisms are sources of polysaccharides, glycoproteins, and peptides, which, adsorbed to mineral oxides, contribute to the formation of aggregates that protect the OM from microbial decomposition. Ultimately, aggregate stability protects the soil-water-plant system against erosive processes.

## CONCLUSIONS

After 31 years of management, the changes in C storage in the soil due to the replacement of native *cerrado sensu stricto* vegetation by annual crops were restricted to the upper 0.60 m depth.

The substitution of *cerrado sensu stricto* by tillage in soybean-corn rotation caused a decrease of at least 11 % in soil C stocks. However, using no-tillage as an alternative to conventional tillage with a moldboard plow reduced  $\text{CO}_2$  emissions by up to 12 %.

After 31 years of management, we observed a relative increase in OM storage in the organo-mineral fraction, acceleration of mineralization of the OM fraction  $>53 \mu\text{m}$ , and a reduction of over 30 % in microbial biomass in conventional tillage systems.

The methods of determination and correction of C pools based on bulk density affect comparisons between different forms of management. Not considering these differences in the estimation of C emission or removal from the soil could lead to inaccurate results.

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