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Effects of cover crops on the physical protection of organic matter and soil aggregation

Alberto do Nascimento Silva[1], Cícero Célio de Figueiredo[1*], Arminda Moreira de Carvalho[2], Daiane dos Santos Soares[1], Divina Cléia Resende dos Santos[1], Vivian Galdino da Silva[1]

[1]Faculty of Agronomy and Veterinary Medicine, University of Brasília (UnB), Brasília, Brazil
[2]Brazilian Agricultural Research Corporation (EMBRAPA), Embrapa Cerrados, Planaltina, DF, Brazil

***Corresponding author: cicerocf@unb.br**

Abstract

The objective of this study was to evaluate the effects of cover crops grown under no-tillage on the aggregation and physical protection of organic matter in soil macro and microaggregates. The experiment consisted of a randomized complete block design with three replications. The following cover crops were investigated in corn rotation systems: T1 = *Braquiária ruziziensis (Urochloa ruziziensis)*, T2 = *Canavalia brasiliensis* Mart. ex Benth., T3 = Pigeon pea 'BRS Mandarin' [*Cajanus cajan* (L.) Millsp.], T4 = millet 'BR05' [*Pennisetum glaucum* (L.) R.Br.], T5 = turnip-forage (*Raphanus sativus* L.), T6 = velvet bean (*Mucuna aterrima* Merr.) and T7 = native Cerrado vegetation as a reference environment. Soil was sampled at a depth of 0-10 cm in September 2015 for the determination of organic matter fractions in macro and microaggregates. There was a reduction in aggregate size and its stability when native Cerrado areas were converted into agricultural systems. Nevertheless, some cover crops such as velvet bean, millet and turnip-forage favored restructuring the soil, forming stable aggregates similar to the native Cerrado. Among the cover crops, millet was highlighted as presenting elevated capacity to accumulate labile organic carbon in macroaggregates (2.32 g C kg^{-1}) and microaggregates (2.34 g C kg^{-1}). These values are, on average, 60% higher than those presented by turnip-forage. In general, the conversion of land use under Cerrado vegetation to agroecosystems reduced the total organic carbon content, mainly due to macroaggregate breakup, resulting in a lower physical protection of soil organic matter.

Keywords: soil organic carbon; no-tillage; Cerrado; organic matter pools; conservation tillage.
Abbreviations: C_Carbon; OM_Organic Matter; SOM_Soil Organic Matter; NTS_No Tillage System; LC_labile carbon; MAC_Macroaggregates; MIC_Microaggregates; HS_Humic substances; FA_Fulvic Acid; HA_Humic Acid; HUM_Humin.

Introduction

Under the conditions of natural ecosystems, the formation of a well-defined soil structure is established over time due to chemical interactions and the role of biological agents. This good soil structure is dependent on and also protective of organic matter (OM), representing an efficient form of carbon (C) protection in the soil. Changes to natural environments caused by human activities impose drastic changes to the soil structure, reflecting the loss of OM and rupture of aggregates, especially macroaggregates (Wendling et al., 2011; Winck et al., 2014).

As an alternative to reduce the impacts on soil aggregation promoted by the incorporation of native areas into agricultural ecosystems, the use of cover crops in the no-tillage system (NTS) can reduce nutrient losses and increase the content of soil organic matter (SOM) (Crusciol et al., 2012; Nascente and Crusciol, 2012; Carvalho et al., 2012; Ensinas et al., 2016). The SOM directly influences the physical properties of the soil by increasing its aggregation and also promotes improvements in porosity, aeration, water infiltration and water retention in the soil (Garcia and Rosolem, 2010).

The use of cover crops increases plant residue inputs on the surface which increases protection of the soil, reducing erosion (Guedes Filho et al., 2013). In addition to increasing the SOM, plant residues on the soil surface serve as a substrate for microbial activity, mainly in the upper soil layers (Lima Filho et al., 2014; Ensinas et al., 2016), and consequently promotes increased stability of soil aggregates. Cover crops in the NTS improve soil aggregation over the cultivation years and the plant species interfere differently on aggregate stability (Loss et al., 2011; Loss et al., 2014).

For the formation and subsequent stability of the aggregates, there occurs a plurality of interactions between the following factors: macro and mesofauna, soil microorganisms, roots, inorganic agents, and environmental variables (Bronick and Lal, 2005; Rillig and Mummey, 2006). Among these factors, highlighted is the root system that is directly related to aggregation and accumulation of C and nitrogen (N) in different classes of soil aggregates (Kasper et al., 2009; Loss et al., 2015). Crops with aggressive root systems can minimize the negative effects of soil degradation through improvements to its structure. However, information on the action of different cover crop species on the aggregation and accumulation SOM are still incomplete (Lima et al., 2015).

To improve understanding of the relationship between OM and soil aggregation, fractioning of the SOM and analysis of the labile and recalcitrant fractions in different aggregates classes has been performed (Bimüller et al., 2016). Among the most recalcitrant fractions, humic substances are important in the process of formation and stability of aggregates due to its cementing action, which allows the formation of stable aggregates in water (Borges et al., 2015; Gazolla et al., 2015; Li et al., 2015). In the case of Latosols from the Brazilian Cerrado, the presence of microstructures may represent an important form of protection to the OM and they are generally neglected in evaluations of OM alterations promoted by soil management systems.

There are few studies that relate the use of different cover plant with the formation of aggregates and the protection of SOM. The hypothesis tested in this study was that different species of cover crops differently alter the stability of aggregates and the physical protection of SOM. In this context, the objective of this study was to evaluate the influence of different cover crops in a long term no-tillage system on the aggregation and protection of organic carbon in soil macro and microaggregates.

Results and Discussion

Effect of cover crops on aggregate stability

The effects of different cover crops on the formation and stability of aggregates, measured by the weighted average diameter (WAD) and the aggregate stability index in water are shown in Figures 1 and 2. The WAD ranged from 0.91 mm in *C. cajan* to 1.5 mm in the native Cerrado (Figure 1). Three distinct groups were formed with regards to capacity of the plants to form aggregates: the native cerrado vegetation presented the highest values, *M. aterrima*, *R. sativus*, *B. ruziziensis* and *P. glaucum* had intermediate capacity, and *C. cajan* and *C. brasiliensis* indicated the lowest capacity.

The largest WAD values in the area with native Cerrado are due to the higher quantity of plant material in that area and the total absence of soil disturbance. The differences among the groups with intermediate capacity (*M. aterrima*, *R. sativus*, *B. ruziziensis* and *P. glaucum*) and low capacity (*C. cajan*, *C. brasiliensis*) for aggregate formation are justified in function of the specific characteristics of each plant, such as aggressive root system and production of greater quantities of exudates, factors that promote soil aggregation. Grasses, for example, a group that includes *B. ruziziensis* and *P. glaucum*, are characterized by having a chemical aggregator effect due to higher root densities and better distribution of the root system in the soil which better distribute the root exudates and promote the union of small aggregates and formation of larger aggregates. However *R. sativus*, with its swiveling and aggressive root system, promotes a physical soil compaction effect as its root system develops, thus favoring the formation of larger and more stable aggregates (Guedes Filho et al., 2013). *M. aterrima*, because it is a legume and produces a large amount of dry matter, contributes to the incorporation of C and nutrients in the soil, increasing the growth and activity of microorganisms, which may influence the aggregate diameter and stability (Loss et al., 2015).

Studies on the effect of cover crops regarding aggregate formation and stability have shown that with the use of *B. ruziziensis*, *R. sativus*, *M. aterrima* and *P. glaucum* there occurred increases in the WAD and aggregate stability (Loss et al., 2011; Brandão and Silva 2012; Loss et al., 2015). Values of the aggregate stability index (ASI) were higher, ranging from 92% to 96% (Figure 2), indicating good soil structure, which confirms the positive influence of cover plants with regards to aggregate stability. The plants *R. sativus*, *M. aterrima* and *P. glaucum* presented a high capacity to form stable aggregates in water, similar to the Cerrado. *C. cajan* was the species that presented the lowest ASI. Similar results were found for the ASI by Torres et al. (2015), which justified the high ASI values as resulting from protection provided by the plant residues of different soil covers, which protect the soil against disaggregation caused by the impact of rain and sudden changes in humidity. In addition, the decomposition of cover plant residues enhances microbial activity, the accumulation of nutrients and organic matter on the soil surface layers, which favors greater stability of the aggregates (Lima Filho et al., 2014).

Effect of cover crops on the organic carbon fractions of the soil in macro and microaggregates

In general, the main form of labile carbon (LC) and total organic carbon (TOC) accumulation was observed in microaggregates (MIC) (Table 2). On average, the accumulation of LC and TOC in the macroaggregates (MAC) was 21% and 31% higher, respectively, than in the MIC. This increased C accumulation in the MAC may be due to use of the NTS, characterized by the absence of soil disturbance (Figueiredo et al., 2010). Similar results were obtained by Costa et al. (2012).

The sequence of LC accumulation in the MIC by the cover crops followed the following order: *P. glaucum* >native Cerrado= *C. cajan* = *M. aterrima* > *R. sativus* = *B. ruziziensis*. *C. brasiliensis* did not differ from *M. aterrima*, *C. cajan*, *B. ruziziensis* and *R. sativus*. Higher LC levels are usually observed in areas where there is greater MOS input from the crop residues (Leite et al., 2003). LC accumulation is also influenced by the chemical composition of the ground cover crops (Silva and Mendonça, 2007; Zhongkui et al., 2010). The addition of LC may be attributed to the production plant residues with lower C/N and lignin/N ratios, which more rapidly decompose and promote the increase of this fraction in soil aggregates. The high values observed for *P. glaucum* may be explained by its low C/N ratio (9.19) and low lignin/N ratio (1.95) when compared to the other species studied. Unlike *P. glaucum*, *R. sativus* due to its C/N (16.67) and lignin/N ratios (5.91), higher than the other species studied, showed lower LC values in macro and microaggregates.

The native Cerrado showed high LC content in MAC, not only significantly different from *P. glaucum*, but also superior to the other cover crops (Table 2). These results indicate that the larger aggregates are those most affected by the mechanical destruction that occurs when replacing the native Cerrado with agroecosystems (Figueiredo et al., 2010). Consequently, with the predominant rupture of macroaggregates, organic carbon that is physically protected within this class is exposed to the action of microorganisms, resulting in lower values in the cultivated areas (Loss et al., 2015). *P. glaucum* stood out as an LC accumulating species, both in MIC and in MAC, resulting from its chemical composition with low C:N ratio.

Analyzing the results of TOC in microaggregates, it was noticed that some plants (*M. aterrima*, *R. sativus*, *B. ruziziensis*, *P. glaucum*) were able to promote similar TOC levels to those found in the native Cerrado. These results demonstrated that the continuous supply of plant residues in different quantities and quality under the NTS makes TOC accumulation possible in soil microaggregates (Guareschi et al., 2012). Another explanation for the observed behavior refers to the ability of different species to influence the formation of microaggregates. According to the hierarchical clustering model, macroaggregates are formed by the union of microaggregates (Tisdall and Oades, 1982). Therefore, microaggregates are of fundamental importance in the physical protection of C and the soil structure.

Table 1. C:N ratio and concentration of hemicellulose, cellulose and lignin (g kg^{-1}) in the shoots of cover crops (average concentrations of flowering and maturation).

Cover crops	C:N	Hemicellulose		Cellulose		Lignin		Lignin:N	
C. cajan	12.50 (±2.2)	160.6 (±10.2)	bc	105.8 (±6.2)	d	59.5 (±4.2)	a	2.80 (±0.2)	a
C. brasiliensis	12.41 (±3.2)	196.9 (±13.0)	b	124.3 (±7.1)	cd	38.1 (±2.1)	bcd	1.89 (±0.3)	bc
M. aterrima	8.38 (±1.8)	137.6 (±8.2)	c	143.5 (±7.5)	bc	55.6 (±4.5)	ab	2.22 (±0.4)	ab
P. glaucum	9.19 (±1.2)	302.0 (±15.2)	a	178.2 (±9.2)	a	34.0 (±2.4)	cd	1.68 (±0.3)	c
R. sativus	16.67 (±3.1)	123.6 (±32.2)	c	164.6 (±8.2)	ab	42.0 (±4.2)	ab	3.33 (±0.6)	a
B. ruziziensis	10.23 (±2.1)	319.3 (±11.2)	a	105.7 (±6.3)	d	17.5 (±1.5)	d	0.85 (±0.1)	d

Source: Carvalho et al. (2012). The values are expressed as means ± standard deviation. Means followed by the same letter in the column do not differ by the LSD test ($P \leq 0.05$).

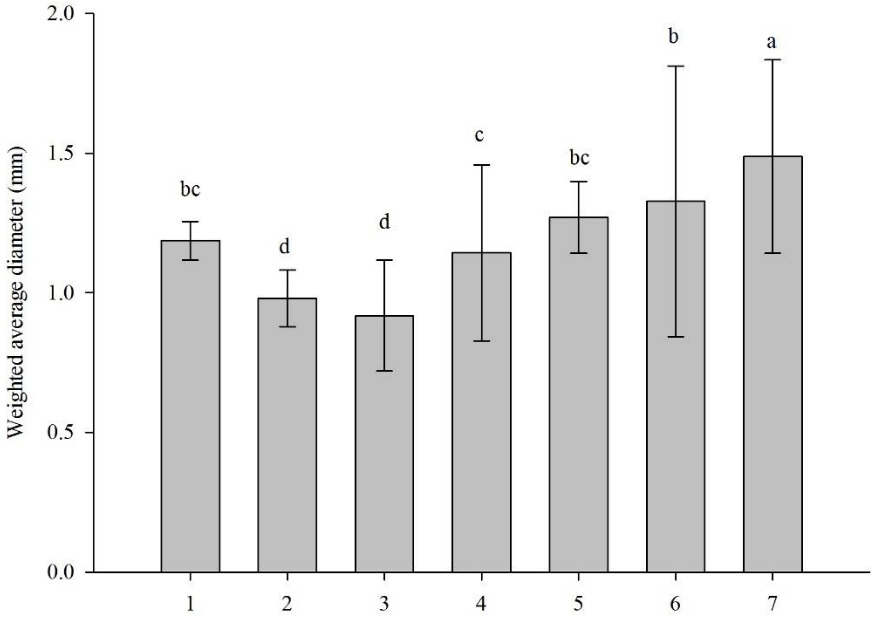

Fig 1. Weighted average diameter (WAD) of soil aggregates under cover crops and native Cerrado. Means followed by the same letters do not present statistical difference by the LSD test ($p \leq 0.05$). (1) *B. ruziziensis;* (2) *C. brasiliensis;* (3) *C. cajan;* (4) *P. glaucum;* (5) *R. sativus;* (6) *M. aterrima;* (7) native Cerrado.

Cover crops have differentiated capacity of soil aggregation through the promotion of greater exudation of polysaccharides and formation of humic substances, resulting from decomposition of its residues. Soil polysaccharides are mucilages resulting from the microbial metabolism and decomposition of roots, plant and animal residues and root exudation (Rangel et al., 2007). These substances act as transient bonding agents and are decomposed rapidly by microorganisms and associated predominantly with macroaggregates (> 250 um), while humic substances associated with iron, aluminum and aluminosilicates are persistent agents of microaggregates (<250 um) (Tisdall and Oades, 1982). Therefore, the species presenting TOC in MIC similar to the native Cerrado may cause an increase in humidification over time, which favors the formation of microaggregates.

The greatest impact of incorporating the Cerrado into agroecosystems was found in the loss of TOC in MAC. In this case, no cover crop was able to accumulate TOC in MAC at the same levels of native Cerrado. Furthermore, cover crops do not differ in their ability to accumulate in TOC in MAC. The elevated capacity of the Cerrado in accumulating carbon by means of the MAC was previously verified in other works (Figueiredo et al., 2010).

The native Cerrado showed the lowest levels of FA in microaggregates. Cover crops do not differ with regards to C in FA accumulation in microaggregates (Figure 3A). In microaggregates all species presented higher quantities than native Cerrado, which is attributed to the addition of plant residues with the highest proportion of labile constituents in relation to those recalcitrant. In macroaggregates, *C. cajan* and *B. ruziziensis* showed higher C levels in FA than in the native Cerrado, and there were no differences among the other plants. Regarding HA accumulation in microaggregates, *C. cajan* stood out by presenting levels higher than native Cerrado, *M. aterrima* and *C. brasiliensis*, and there was no difference between the other cover crops. In general, the C contents in FA and HA were lower in the microaggregates compared to macroaggregates, confirming the results found by Passos et al. (2007). These results demonstrate that both the chemical and physical protection have great importance in stabilizing these forms of C in the soil (Passos et al., 2007).

Different from the behavior observed for FA and HA, the native Cerrado showed higher HUM levels in MAC, greater than *M. aterrima*, *B. ruziziensis* and *C. cajan* (Figure 3A). In several studies of tropical soils the predominance of HUM was also observed in relation to other humic fractions (Conteh and Blair, 1998; Assis et al. 2006). A positive relationship between soil aggregation and humin content was observed both in macro and microaggregates. This relationship was also observed by Silva et al. (2014), where

Table 2. Labile carbon (LC) and total organic carbon (TOC) in microaggregates (MIC) and macroaggregates (MAC), in the 0-10 cm soil layer, for different land use systems.

Use system	LC				TOC			
	MIC		MAC		MIC		MAC	
	g kg^{-1}				g kg^{-1}			
1	1.23	d	1.90	b	15.43	ab	19.33	bc
2	1.31	cd	1.97	b	14.24	b	18.97	bc
3	1.64	bc	1.95	b	14.57	b	17.25	c
4	2.34	a	2.32	ab	16.43	ab	20.22	bc
5	0.96	d	0.92	c	15.30	ab	18.71	bc
6	1.62	bc	2.19	b	15.02	ab	19.19	bc
7	1.83	b	2.53	a	17.83	a	22.83	a
Mean	1.56		1.97		15.54		22.4	

(1) *B. ruziziensis;* (2) *C. brasiliensis;* (3) *C. cajan;* (4) *P. glaucum;* (5) *R. sativus;* (6) *M. aterrima;* (7) native Cerrado. Means followed by the same letter in the column do not differ by the LSD test (P≤0.05).

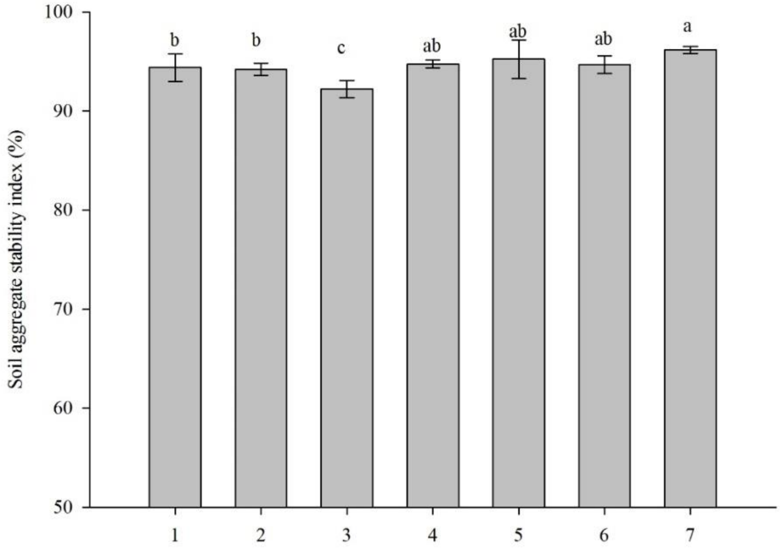

Fig 2. Soil aggregate stability index (ASI) under the influence of different cover crops, in the depth of 0-10 cm. Means followed by the same letters do not show statistical differences by the LSD test (p≤0.05). (1) *B. ruziziensis;* (2) *C. brasiliensis;* (3) *C. cajan;* (4) *P. glaucum;* (5) *R. sativus;* (6) *M. aterrima;* (7) native Cerrado.

the predominance of humin in the Cerrado is related to its insolubility and resistance to biodegradation, favored by the formation of stable complexes (Fontana et al., 2006). The relationship of macroaggregates and humic substances was also observed by Borges et al. (2015). According to these authors the high stability of humic substances is due to its complex chemical structure and its interactions with clay minerals and metal cations; the humin fraction is more stable when compared to fulvic and humic acids, which indicates greater recalcitrance and greater physical and chemical protection of carbon. This behavior demonstrates that the cover crops *C. cajan*, *P. glaucum* and *C. brasiliensis* favor the interaction between HUM and soil aggregation in these areas, presenting results similar to the natural conditions.

Materials and Methods

Experimental site and soil characteristics

The experiment was conducted at the experimental station of Embrapa Cerrados, in Planaltina, DF, Brazil (latitude 15° 35 '30 "S, longitude 47º 42' 30" W). The regional climate, according to the Köppen classification, is type Cwa (Alvares et al., 2013), with dry winters and wet summers. The average annual rainfall is 1400 mm and the average annual temperature is 21.3 °C. Soil of the experimental area is classified as dystrophic Red Latosol, with clayey texture.

The experiment was installed in an area with a history of corn cultivation in succession to cover crops in a NTS since 2005. At the time implementing the experiment, the soil of the 0-20 cm layer presented: pH (in water) = 6.0, OM = 21.7 g kg^{-1}, $P_{Mehlich1}$= 0.9 mg kg^{-1}, Al^{3+} = 0.1 $cmol_c$ kg^{-1}, Ca^{2+}+Mg^{2+} = 2.9 $cmol_c$ kg^{-1}, K^+ = 0.1 $cmol_c$ kg^{-1}. The mineralogical composition of the soil horizon diagnosis is as follows: kaolin (320 g kg^{-1}), gibbsite (496 g kg^{-1}), hematite (142 g kg^{-1}) and goethite (42 g kg^{-1}), as described by Reatto et al. (2009).

Experimental design and plant materials

The treatments consisted of the following cover crops: T1 = *Braquiária ruziziensis*, T2 = *Canavalia brasiliensis* Mart. ex Benth., T3 = Pigeon pea 'BRS Mandarin' [*Cajanus cajan* (L.) Millsp.], T4 = millet 'BR05' [*Pennisetum glaucum* (L.) R.Br.], T5 = turnip-forage (*Raphanus sativus* L.), T6 = velvet bean (*Mucuna aterrima* Merr.) and T7 = native Cerrado

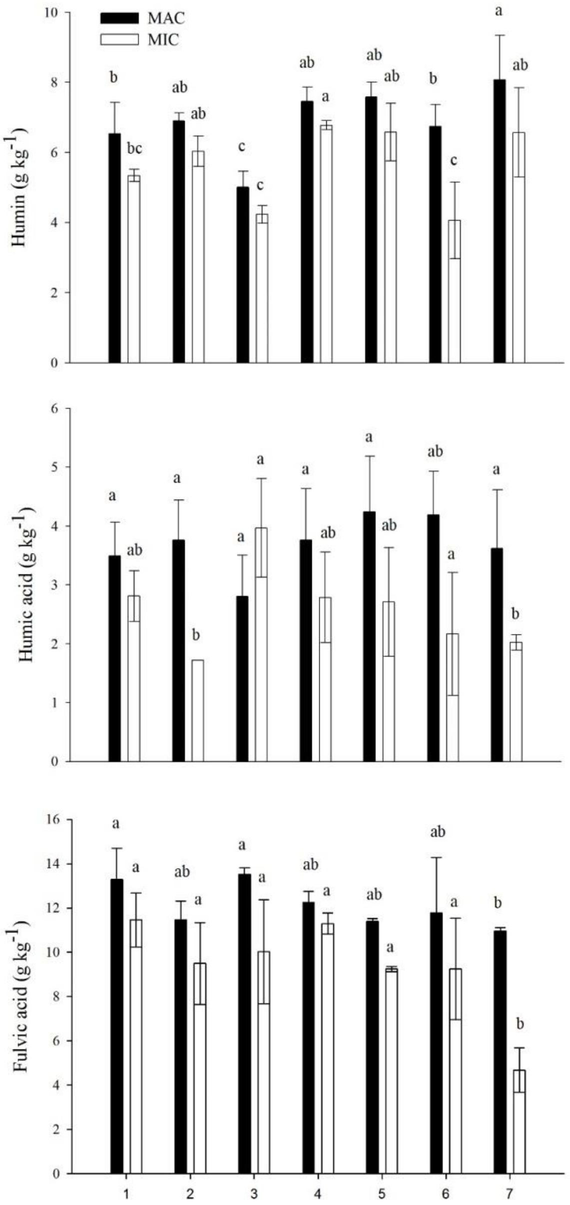

Fig 3. Carbon of the humin (HUM), humic acid (HA) and fulvic acid (FA) fractions in macroaggregates (MAC) and microaggregates (MIC) at the depth of 0-10 cm, under the effect of different cover crops. Means followed by the same letter in the same class of aggregates show no statistical difference by the LSD test ($p \leq 0.05$). (1) *B. ruziziensis;* (2) *C. brasiliensis;* (3) *C. cajan;* (4) *P. glaucum;* (5) *R. sativus;* (6) *M. aterrima;* (7) native Cerrado.

vegetation in an area adjacent to the experimental area. For *C. cajan* and *U. ruziziensis*, the sowing density was 20 plants linear m^{-1} and 10 plants linear m^{-1} for other species. A spacing of 0.5 m between the sowing rows was used for all species, as recommended by Carvalho and Amabile (2006). The treatments were arranged in a randomized block design with three replications. Each experimental plot, represented by different cover crops, comprised 48 m^2 (12m x 4 m). The chemical composition of the cover crops is presented in Table 1. The cover crops were sown in the first week of April in succession to the corn crop, which was sown in the second half of November. At corn planting, 20 kg ha^{-1} N, 150 kg ha^{-1} P_2O_5 and 80 kg ha^{-1} K_2O were applied to the planting furrow, and two applications of nitrogen were performed with urea (65 kg ha^{-1} N) when the plants emitted the fourth pair of leaves (V4) and the same dose of N when plants emitted the eighth pair of leaves (V6), totaling 150 kg ha^{-1} of N applied at planting and in coverage. The species used as cover crops were cut at the flowering stage.

Soil sampling and determination of aggregate size classes

In September 2015 the soil was sampled to determine aggregate stability, total organic carbon (TOC), labile carbon (LC), and carbon of the humic fractions (humic acid, fulvic acid and humin). Three sub-samples were collected per plot, in the 0-10 cm layer. The samples were appropriately packed in plastic bags and sent to the laboratory. Then, the samples were air dried, manually broken apart and passed through an 8 mm sieve for use in the separation of soil aggregate classes. From this 100 g samples were weighed, which were transferred to a 2 mm sieve, which comprised a set of sieves with decreasing mesh diameter as follows: 2.00, 1.00, 0.50, 0.25 and 0.106 mm, according to EMBRAPA (1997). The sample placed on the 2.00 mm sieve was moistened and subsequently the sieve assembly was subjected to wet vertical sieving for 30 minutes in a Yoder apparatus (Yoder, 1936). After this time, the material retained on each sieve was removed, placed in pre-weighed containers, identified and placed in an oven at 60 °C to obtain a constant dry weight.
From the aggregate mass the weighted average diameter (WAD) and the aggregate stability index (ASI) were calculated.

The weighted average diameter (WAD) was calculated by the formula:

$$WAD = \sum_{i=l}^{n} (xi.wi)$$

Where: wi = proportion of each class in relation to the total; xi = average diameter of the classes (mm);

The aggregate stability index in water was calculated by the expression:

$$\text{ASI} = \left(\frac{Weight\ of\ the\ dry\ sample - wp25 - sand}{Weight\ of\ the\ dry\ sample - sand}\right)$$

Where: wp25 = weight of the aggregates of the class < 0.25 mm.

After weighing and acquisition of the soil mass on each sieve, the aggregates were grouped in two classes (macro- and micro-aggregates). The macroaggregates (MAC) were considered those between 0.25 and 2.00 mm, obtained by combining the aggregate classes in this range. The microaggregates (MIC) were considered those between 0.25 and 0.106 mm.

Carbon analysis

In the two aggregate classes (MAC and MIC) the CL and C levels were determined in the humic substances (HS). Quantitative chemical fractionation of HS was performed according to Benites et al. (2003), using NaOH 1 mol L^{-1} as the extract. Obtained were the fractions of humic acid (HA), fulvic acids (FA) and humin (HUM), which was considered the insoluble residue in acid and base. Carbon determinations in extracts of the FA, HA and HUM fractions were performed by oxidation with potassium dichromate and titration of the excess with ammonium ferrous sulfate according to Yeomans and Bremner (1988).

The TOC was determined by oxidation with potassium dichromate and titration with ferrous ammonium sulfate, according to the method of Walkley and Black (1934). Another procedure based on the oxidation of C in the samples was performed according to Shang and Tiessen (1997), where the LC is regarded as oxidizable C by the solution of $KMnO_4$ 0.033 mol L^{-1}.

Statistical analysis

Data was submitted to analysis of variance and the averages were compared using LSD test at 5% significance ($p \leq 0.05$). The analyses were performed using the software XLSTAT 2013.

Conclusion

As established in the hypothesis, there was a reduction in aggregate size and its stability when native Cerrado areas were converted into agricultural systems. Nevertheless, the cover crops *M. aterrima*, *P. glaucum* and *R. sativus* favored restructuring of the soil, forming larger and stable aggregates, similar to the Cerrado. In general, the conversion of land use under Cerrado vegetation to agroecosystems reduced the total organic carbon content, mainly due to macroaggregate breakup, resulting in a lower physical protection of soil organic matter. *R. sativus* stood out as the cover crop with greatest capacity to accumulate labile organic carbon in microaggregates.

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