

Soil organic matter of aggregates physicogenic and biogenic in areas under no-tillage system in the Cerrado, Brazil

Matéria orgânica do solo de agregados fisiogênicos e biogênicos em áreas sob sistema plantio direto no Cerrado, Brasil

Materia orgánica del suelo de agregados fisiogénicos y biogénicos en áreas de sistema de siembra directa en el Cerrado, Brasil

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Abstract

The aim of this study was to evaluate i) the different cover crops contribution used in no-tillage system (NT) to biogenic aggregation; and ii) the influence of aggregate formation pathways on the compartmentalization and the soil organic carbon origin. Two areas managed under NT with different implementation times (6 and 18 years, NT06 and NT18, respectively) and cover crops were evaluated, totaling six sampling areas: NT06, millet (NT06MD); NT06, brachiaria (NT06BR); NT06, sunn hemp (NT06SH); NT18, millet (NT18MI); NT18, brachiaria (NT18BR); NT18, and sunn hemp (NT18SH). In each sampling area, five pseudo-replicates were collected in the 0.00-0.05 and 0.05-0.10 m layers. The samples were air-dried and sieved using sieves with 9.7 and 8.0 mm mesh, and the aggregates retained within this interval were selected. The percentage of each type of aggregate (physicogenic and biogenic) was quantified. Total organic carbon (TOC) and the natural abundance of $\delta^{13}\text{C}$ (‰) were analyzed and the physical fractionations of SOM were performed: particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) and density fractionation (free light fraction carbon, FLFC). Physicogenic aggregates were quantified in greater proportion, except for the areas of NT06BR and NT18BR in the 0.00-0.05 m layer. The biogenic aggregates

showed the highest contents of TOC, POC, MAOC, FLFC and more negative values of $\delta^{13}\text{C}$. The use of grasses, especially *Brachiaria* spp., as cover plants in NT after 6 and 18 years of adoption favors the formation of aggregates through the biogenic pathway and they influence the compartmentalization and origin of stored organic carbon.

Keywords: Aggregate formation pathways; Cover plants; Adoption time.

Resumo

O objetivo do estudo foi avaliar i) a contribuição de diferentes plantas de coberturas utilizadas em sistema plantio direto (SPD) na agregação biogênica; e ii) a influência das vias de formação dos agregados na compartimentalização e origem do carbono orgânico do solo. Foram avaliadas duas áreas manejadas sob SPD com diferentes tempos de implantação (6 e 18 anos, SPD06 e SPD18, respectivamente) e coberturas vegetais, perfazendo um total de seis áreas amostrais: SPD06, milho (SPD06MI); SPD06, braquiária (SPD06BR); SPD06, crotalária (SPD06CR); SPD18, milho (SPD18MI); SPD18, braquiária (SPD18BR); SPD18, e crotalária (SPD18CR). Em cada área amostral foram coletadas cinco pseudorepetições (torrões) nas camadas de 0,00-0,05 e 0,05-0,10 m de profundidade. Após a coleta as amostras foram secas ao ar e posteriormente submetidas a peneiramento utilizando um conjunto de peneiras de 9,7 e 8,0 mm de malha, sendo selecionados somente os agregados retidos nesse intervalo. Nesses, quantificou-se o percentual de cada tipo de agregado (fisiogênicos e biogênicos). Foram realizadas as análises de carbono orgânico total (COT), abundância natural de $\delta^{13}\text{C}$ (‰) e os fracionamentos físico granulométrico (carbono orgânico particulado, COP; e carbono orgânico associado aos minerais, COAM) e densimétrico (carbono da fração leve-livre, CFLL). Os agregados fisiogênicos foram quantificados em maior proporção nas áreas de SPD06MI, SPD06CR, SPD18MI e SPD18CR nas duas camadas, sendo exceção as áreas de SPD06BR e SPD18BR na camada de 0,00-0,05 m. Nos agregados biogênicos foram verificados os maiores teores de COT, COP, COAM, CFLL e valores mais negativos de $\delta^{13}\text{C}$. O uso de gramíneas, em especial as *Brachiaria* spp, como plantas de cobertura em SPD após 6 e 18 anos de adoção favorece a formação de agregados da via biogênica e os mesmos influenciam na compartimentalização e origem do carbono orgânico armazenado.

Palavras-chave: Vias de formação dos agregados; Plantas de cobertura; Tempo de adoção.

Resumen

El objetivo del estudio fue evaluar i) la contribución de los diferentes cultivos de cobertura utilizados en el sistema siembra directa (SSD) en la agregación biogénica; y ii) la influencia de las vías de formación de los agregados en la compartimentación y origen del carbono orgánico del suelo. Se evaluaron dos áreas gestionadas bajo SSD con diferentes tiempos de implantación (6 y 18 años, SSD06 y SSD18, respectivamente) y coberturas de vegetación, haciendo un total de seis áreas de muestreo: SSD06, mijo (SSD06MI); SSD06, braquiaria (SSD06BR); SSD06, crotalaria (SSD06CR); SSD18, mijo (SSD18MI); SSD18, braquiaria (SSD18BR); SSD18 y crotalaria (SSD18CR). En cada área de muestreo, se recogieron cinco pseudo-repeticiones (terrones) en las capas de 0,00-0,05 y 0,05-0,10 m de profundidad. Después de la recogida, las muestras fueron secadas al aire y que fueron sometidos posteriormente a cribado mediante un juego de tamices de malla de 9,7 y 8,0 mm, solamente se seleccionan los agregados retenidos en este intervalo. En estos, se cuantificó el porcentaje de cada tipo de agregado (fisiogénico y biogénico). Se realizaron análisis de carbono orgánico total (COT), abundancia natural de $\delta^{13}\text{C}$ (‰) y fraccionamientos granulométrico y densimétrico de la materia orgánica del suelo (carbono orgánico particulado, COP, y carbono orgánico asociado con minerales, COAM; carbono de la fracción libre ligera, CFLL). Los agregados fisiogénicos se cuantificaron en mayor proporción en las áreas de SSD06MI, SSD06CR, SSD18MI y SSD18CR en las dos capas, con la excepción de las áreas de SSD06BR y SSD18BR en la capa de 0,00 a 0,05 m. En los agregados biogénicos se verificaron los niveles más altos de COT, COP, COAM, CFLL y valores más negativos de $\delta^{13}\text{C}$. El uso de gramíneas, especialmente *Brachiaria* spp, como plantas de cobertura en SSD después de 6 y 18 años de adopción favorece la formación de agregados de la vía biogénico y que influyen en la compartimentación y origen del carbono orgánico almacenado.

Palabras clave: Vías de formación de agregados; Plantas de Cobertura; Tiempo de adopción.

1. Introduction

Improvement in soil quality combined with decrease in production costs are characteristics associated with conservation agriculture. The use of conservation systems for soil management aims to increase the sustainability of agriculture in socioeconomic aspects, generate competitiveness for agribusiness, ensure food safety and quality, and preserve the edaphic environment. In Brazil, among these systems the most widely adopted is no-tillage (NT), mainly in areas of extensive agricultural production, such as the Cerrado.

The efficacy of NT is related, among other factors, to the quantity and quality of residues produced by cover plants and their persistence on the soil (Andrade et al., 2018). In the Cerrado, the rapid decomposition of plant residues compromises the maintenance of cover on the surface soil layer (Torres & Pereira, 2013). In this context, it has been sought to use cover

plants with high phytomass production and low decomposition rate to make up crop rotation or succession schemes, so that the mineralization of soil organic matter (SOM) and nutrient cycling is slower (Boer et al., 2008; Torres et al., 2008).

Another important factor regarding the analysis of NT, besides the use of different cover crops, is to evaluate the response over the time of implementation of the system. Studies have shown that NT can alter the edaphic attributes, with the succession of crops, due to the continuous supply of organic material on soil surface, derived from plant residues, the beneficial action of the root system of the introduced plants, and the protection of soil surface (Andrade et al., 2018). For this fact, it is interesting to highlight the importance of the time of adoption of NT for the occurrence of changes, such as the accumulation of SOM, improvement of aggregation, nutrient cycling and increased biological activity.

To evaluate these factors involved in the success of NT in Cerrado areas, some edaphic attributes have been used as indicators of soil quality, including the changes in SOM aggregation and dynamics. Aggregate formation mechanisms involve physical and chemical (physicogenic aggregates) and biological (biogenic aggregates) processes (Loss et al., 2014), and the differentiation between morphological types of aggregates is performed according to their genesis or formation pathways (Bullock et al., 1985; Pulleman et al., 2005; Batista et al., 2013).

By evaluating the SOM fractions, it is possible to verify how carbon contents are distributed in the different morphological types of aggregates. From the physical point of view, the SOM compartments that are evaluated are the granulometric fraction (particulate and mineral-associated organic carbon) (Cambardella & Elliott, 1992), and density fraction (light fraction organic carbon) (Sohi et al., 2001).

To complement the study of SOM fractions, the technique of natural abundance of $\delta^{13}\text{C}$ (‰) has been applied in aggregates. From this technique, it is possible to identify the origin of stored carbon that favored its formation, and these differences are due to the natural isotopic signature between plants of photosynthetic cycle C_3 (-24 to -34‰) and C_4 (-6 to -19‰) (Smith & Epstein, 1971).

From the above, and based on the hypothesis that different cover crops cultivated over time under conservation system of soil management can promote changes in the genesis of aggregates and in the organic matter associated with them, the aim of this study was to evaluate i) the contribution of different cover crops used in no-tillage system to biogenic aggregation; and ii) the influence of aggregate formation pathways on the compartmentalization and origin of soil organic carbon.

2. Methodology

The study was conducted in the city of Uberaba (19°39'10.17" S and 47°58'15.65" W), located in the state of Minas Gerais, Brazil. The sampled areas belong to the Federal Institute of the Triângulo Mineiro (IFTM) and are situated within the Cerrado biome, having as main characteristics: average altitude of 795 m; climate identified as hot tropical (Aw), according to Köppen's classification, with rainy season in summer and dry season in winter, and average annual precipitation of 1600 mm; flat to gently undulating relief; and soil classified as Latossolo Vermelho Distrófico (Oxisol), with medium texture (Santos et al., 2018).

For this study, two areas managed under no-tillage system (NT) with different implementation times were evaluated: NT area implemented 6 years ago (NT06), in transition phase; and NT area implemented 18 years ago (NT18), in the consolidation phase. In the areas under NT, three sub-areas with different cover plants were selected: pearl millet (*Pennisetum glaucum* L. cv. ADR500) (MI), brachiaria grass (*Urochloa brizantha* cv. Marandu) (BR) and sunn hemp (*Crotalaria spectabilis*) (SH), sampled in January 2019.

The management of cover plants is conducted in a similar way between the systems, which differ only for the time of implementation. In total, there were six sampling areas, described as: NT06, millet (NT06MI); NT06, brachiaria (NT06BR);

NT06, sunn hemp (NT06SH); NT18, millet (NT18MI); NT18, brachiaria (NT18BR); and NT18, sunn hemp (NT18SH). In all subareas, the same cover crops are used in the corn/soybean succession, cultivated on the residues of these crops in relation to the previous year. Soybean (*Glycine max* L.) was the annual crop that preceded the time of sample collection.

Fertilization of the annual crops is carried out according to the recommendations of Ribeiro et al. (1999), that is: for corn, 400 kg ha⁻¹ of 08-28-16 formulation at sowing (basal fertilization) with 140 kg ha⁻¹ of N and 80 kg ha⁻¹ of K as top-dressing, split and applied at 20 and 40 days after planting; and for soybean, 200 kg ha⁻¹ of 00-20-15 formulation + 2.5% of Zn + 2.5% of Mn at sowing (basal fertilization), corresponding respectively to 40 kg ha⁻¹ of P₂O₅, 60 kg ha⁻¹ of K₂O, 5 kg ha⁻¹ of Zn and 5 kg ha⁻¹ of Mn, with the inoculation of the seed.

For each sampling area, five pseudo-replicates (undisturbed samples) were collected in the 0.00-0.05 and 0.05-0.10 m layers, totaling 60 sampling units. Subsequently, the samples were air-dried and subjected to sifting with a set of sieves with 9.7 and 8.0 mm mesh, using only the aggregates retained within this interval.

These aggregates were taken to the laboratory, examined under binocular magnifying glass, manually separated and identified only two morphological types: biogenic aggregates - those in which it is possible to visualize rounded forms, with the intestinal tract of soil macrofauna individuals, mainly Oligochaeta (earthworms) or those in which it is possible to visualize the presence and activity of roots; and physicogenic aggregates - those that showed angular shapes resulting from the interaction between carbon, clay, cations and soil wetting and drying cycles (Bullock et al., 1985; Pulleman et al., 2005).

After identification, the percentage, relative contribution (mass), of each morphological type of aggregate was determined for each area. Subsequently, they were pounded to break up clods and passed through a 2.0 mm mesh sieve, to obtain the air-dried fine earth (fraction for SOM analysis). Total organic carbon (TOC) contents were quantified by wet oxidation of the organic material in the presence of potassium dichromate in acidic medium (Yeomans & Bremner, 1988).

Granulometric and density physical fractionations of SOM were performed using the methods proposed by Cambardella & Elliot (1992) and Sohi et al. (2001), respectively. Organic carbon of the particulate fraction (POC) and free light fraction (FLFC) of SOM were also determined according to Yeomans & Bremner (1988), whereas the organic carbon of the mineral-associated fraction (MAOC) of SOM was quantified by the difference between TOC and POC.

The natural abundance of $\delta^{13}\text{C}$ was evaluated with a Delta V Advantage mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Measurements of $\delta^{13}\text{C}$ and the determination of carbon percentage were performed in the Laboratory of Research in C and N Biotransformations (LABCEN), Santa Maria – RS, Brazil.

All data for each layer were evaluated for normality of residuals and homoscedasticity by the Shapiro-Wilk and Bartlett tests, respectively. When the assumptions of the tests mentioned above were not observed, the data were transformed by the Box Cox test. Subsequently, the results were analyzed as completely randomized design in a split-plot scheme, being subjected to analysis of variance with application of F test, and mean values were compared by Tukey test at 5% probability level using the R 3.3.1 program (R Development Core Team, 2015) and the package ExpDes.pt. (Ferreira et al., 2013).

3. Results

3.1 Relative contribution

Physicogenic aggregates were present in a greater proportion than biogenic ones in the areas of NT06MI, NT06SH, NT18MI and NT18SH in the two layers evaluated, except in NT06BR and NT18BR in the 0.00-0.05 m layer, where the opposite pattern was observed (Table 1). Between the areas of the NT06 and NT18 systems, it is possible to observe a reduction in the proportion of biogenic aggregates over the time of implementation in the two layers evaluated (Table 1).

Table 1. Distribution, in percentage, of physicogenic and biogenic aggregates in areas under no-tillage system (NT) with different times of implementation and cover crops at two depths in the Cerrado biome, Uberaba-MG, Brazil.

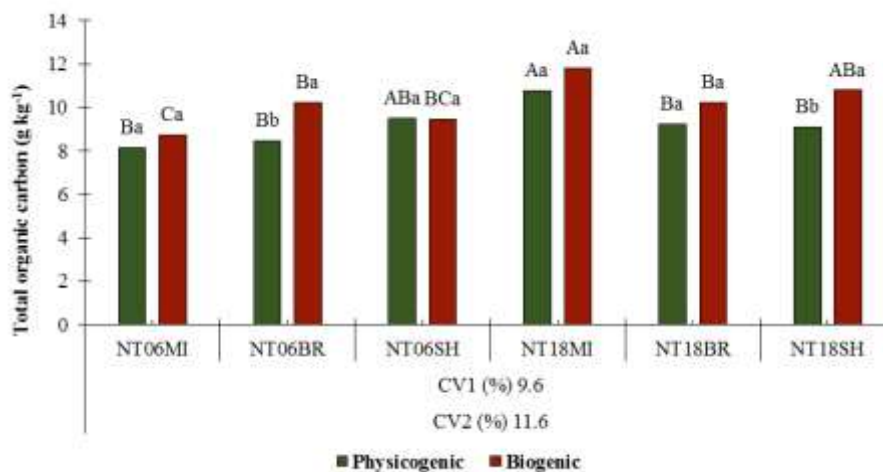
Areas	0.00-0.05 m		0.05-0.10 m	
	Physicogenic	Biogenic	Physicogenic	Biogenic
	%		%	
NT06MI	67	33	58	42
NT06BR	41	59	53	47
NT06SH	59	41	74	26
NT18MI	82	19	70	30
NT18BR	48	52	62	38
NT18SH	80	20	83	17

NT06MI: 6 years + millet; NT06BR: 6 years + brachiaria grass; NT06SH: 6 years + sunn hemp; NT18MI: 18 years + millet; NT18BR: 18 years + brachiaria grass; and NT18SH: 18 years + sunn hemp. Source: Authors.

3.2 Total organic carbon

Figures 1 and 2 show the results of total organic carbon (TOC). In the comparison between the areas, in the 0.00-0.05 m layer, the highest carbon contents were verified in the two types of aggregates of NT18MI (Figure 1). For the 0.05-0.10 m layer, considering both formation pathways, higher TOC contents were observed in the aggregates of NT06SH (Figure 2).

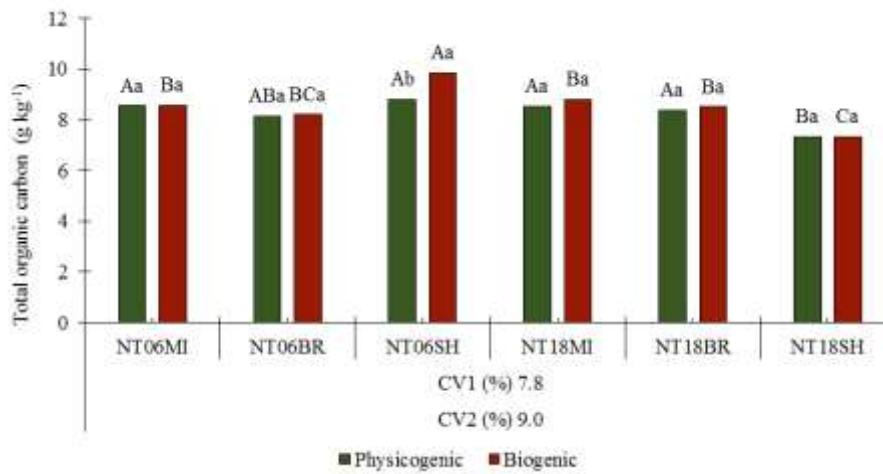
Figure 1. Total organic carbon (TOC) of physicogenic and biogenic aggregates in areas under no-tillage system (NT) with different times of implementation and cover crops in the 0.00-0.05 m layer in the Cerrado biome, Uberaba-MG, Brazil.



Same uppercase letter above the bar indicates no difference between the sampling areas for the same type of aggregate and same lowercase letter above the bar indicates no difference between the types of aggregates for the same sampling area (Tukey, at 5% probability level). NT06MI: 6 years + millet; NT06BR: 6 years + brachiaria grass; NT06SH: 6 years + sunn hemp; NT18MI: 18 years + millet; NT18BR: 18 years + brachiaria grass; NT18SH: 18 years + sunn hemp. CV1: Coefficient of variation for types of aggregates; and CV2: Coefficient of variation for sampling areas. Source: Authors.

For TOC, differences were observed between the formation pathways, and biogenic aggregates in the areas of NT06BR, NT18SH (0.00-0.05 m) and NT06SH (0.05-0.10 m) had the highest carbon contents (Figures 1 and 2, respectively).

Figure 2. Total organic carbon (TOC) of physicogenic and biogenic aggregates in areas under no-tillage system (NT) with different times of implementation and cover crops in the 0.05-0.10 m layer in the Cerrado biome, Uberaba-MG, Brazil.



Same uppercase letter above the bar indicates no difference between the sampling areas for the same type of aggregate and same lowercase letter above the bar indicates no difference between the types of aggregates for the same sampling area (Tukey, at 5% probability level). NT06MI: 6 years + millet; NT06BR: 6 years + brachiaria grass; NT06SH: 6 years + sunn hemp; NT18MI: 18 years + millet; NT18BR: 18 years + brachiaria grass; NT18SH: 18 years + sunn hemp. CV1: Coefficient of variation for types of aggregates; and CV2: Coefficient of variation for sampling areas. Source: Authors.

3.3 Organic carbon from particulate, mineral-associated and free light fractions of SOM

Table 2 shows the results for organic carbon of SOM physical fractions. Physicogenic aggregates of NT06SH and biogenic aggregates of NT18MI, in the 0.00-0.05 layer, showed higher POC contents in the comparison between the areas, but no differences were observed in the underlying layer. In the comparison between the types of aggregates, the highest POC contents were quantified in the biogenic aggregates of the areas of NT06BR and NT18MI (0.00-0.05 m), NT18MI and NT18BR (0.05-0.10 m).

Regarding the contents of mineral-associated organic carbon (MAOC), in the comparison between the areas, the physicogenic aggregates of NT18MI and biogenic of NT18MI and NT18SH had the highest contents of this fraction in the 0.00-0.05 m layer (Table 3). For the underlying layer, higher contents of MAOC were observed in the biogenic aggregates of NT06SH (Table 3). Among the morphological types of aggregates, differences were only observed in the first layer, in which the highest contents of MAOC were quantified in the biogenic aggregates of NT18SH (Table 3).

For the organic carbon of the free light fraction (FLFC), in the comparison between the areas, no differences were verified in the layer of 0.00-0.05 m (Table 3). In the 0.05-0.10 m layer, the highest contents of FLFC were found in the two types of aggregates of NT18MI (Table 3). Among the formation pathways, in almost all areas, except NT18SH, higher contents of FLFC were quantified in biogenic aggregates, for the two layers (Table 3).

3.4 Natural abundance of $\delta^{13}\text{C}$

Table 3 shows the values of $\delta^{13}\text{C}$ (‰) in the different types of aggregates, and differences were observed only in the 0.00-0.05 m layer. Among the areas, the least negative values of $\delta^{13}\text{C}$ were found in the physicogenic aggregates of NT06MI (-15.77‰) and NT06BR (-15.61‰) and in the biogenic aggregates of all areas, except NT18SH (-17.88‰). For the formation pathways, the physicogenic aggregates of the areas of NT06BR (-15.61‰) and NT18CR (-17.03‰) had less negative values compared to those of the biogenic aggregates (-16.43 and -17.88‰, respectively).

Table 2. Organic carbon from the physical fractions of physicogenic (Phy) and biogenic (Bio) aggregates in areas under no-tillage system (NT) with different times of implementation and cover crops in the Cerrado biome, Uberaba-MG, Brazil.

Areas	POC (g kg ⁻¹)		MAOC (g kg ⁻¹)		FLFC (g kg ⁻¹)	
	Phy	Bio	Phy	Bio	Phy	Bio
0.00-0.05 m						
NT06MI	1.58 ^{B ns}	2.19 ^{B ns}	6.58 ^{B ns}	6.56 ^{B ns}	0.56 ^{NS b}	0.80 ^{NS a}
NT06BR	1.90 ^{AB b}	2.73 ^{AB a}	6.56 ^{B ns}	7.51 ^{AB ns}	0.46 ^{NS b}	0.68 ^{NS a}
NT06SH	2.91 ^{A ns}	2.95 ^{AB ns}	6.59 ^{B ns}	7.25 ^{AB ns}	0.32 ^{NS b}	0.67 ^{NS a}
NT18MI	1.48 ^{B b}	3.60 ^{A a}	9.65 ^{A ns}	8.50 ^{A ns}	0.42 ^{NS b}	0.69 ^{NS a}
NT18BR	2.34 ^{AB ns}	3.00 ^{AB ns}	6.87 ^{B ns}	7.22 ^{AB ns}	0.44 ^{NS b}	0.88 ^{NS a}
NT18SH	1.95 ^{AB ns}	2.33 ^{B ns}	7.15 ^{B b}	8.85 ^{A a}	0.48 ^{NS b}	0.78 ^{NS a}
CV1%		25.0		12.0		19.6
CV2%		29.6		14.3		28.5
0.05-0.10 m						
NT06MI	1.44 ^{NS ns}	1.94 ^{NS ns}	6.75 ^{NS ns}	6.62 ^{AB ns}	0.46 ^{AB b}	0.75 ^{B a}
NT06BR	2.04 ^{NS ns}	2.35 ^{NS ns}	6.10 ^{NS ns}	5.85 ^{B ns}	0.46 ^{AB b}	0.65 ^{BC a}
NT06SH	2.05 ^{NS ns}	2.15 ^{NS ns}	7.06 ^{NS ns}	7.68 ^{A ns}	0.33 ^{B b}	0.57 ^{BC a}
NT18MI	1.54 ^{NS b}	2.52 ^{NS a}	6.74 ^{NS ns}	6.44 ^{AB ns}	0.58 ^{A b}	1.01 ^{A a}
NT18BR	1.79 ^{NS b}	2.95 ^{NS a}	6.60 ^{NS ns}	5.55 ^{B ns}	0.23 ^{C b}	0.44 ^{C a}
NT18SH	1.82 ^{NS ns}	2.26 ^{NS ns}	5.50 ^{NS ns}	5.07 ^{B ns}	0.51 ^{AB ns}	0.59 ^{BC ns}
CV1%		28.1		12.0		24.1
CV2%		25.8		15.1		23.1

Means followed by the same uppercase letter in the column indicate no difference between the sampling areas for the same type of aggregate, and means followed by the same lowercase letter in the row indicate no difference between the types of aggregates for the same sampling area (Tukey, at 5% probability level). NT06MI: 6 years + millet; NT06BR: 6 years + brachiaria grass; NT06SH: 6 years + sunn hemp; NT18MI: 18 years + millet; NT18BR: 18 years + brachiaria grass; NT18SH: 18 years + sunn hemp; POC: Particulate organic carbon; MAOC: Mineral-associated organic carbon; FLFC: Free light fraction organic carbon; CV1: Coefficient of variation for types of aggregates; and CV2: Coefficient of variation for sampling areas. Source: Authors.

Table 3. Natural abundance of $\delta^{13}C$ in physicogenic and biogenic aggregates in areas under no-tillage system (NT) with different times of implementation and cover crops in the Cerrado biome, Uberaba-MG, Brazil.

Areas	0.00-0.05 m		0.05-0.10 m	
	Physicogenic	Biogenic	Physicogenic	Biogenic
	‰		‰	
NT06MI	-15.77 ^{A ns}	-15.91 ^{A ns}	-15.55 ^{NS ns}	-15.93 ^{NS ns}
NT06BR	-15.61 ^{A a}	-16.43 ^{A b}	-15.68 ^{NS ns}	-15.60 ^{NS ns}
NT06SH	-16.57 ^{AB ns}	-16.72 ^{A ns}	-16.03 ^{NS ns}	-16.45 ^{NS ns}
NT18MI	-16.32 ^{AB ns}	-16.81 ^{A ns}	-15.99 ^{NS ns}	-16.18 ^{NS ns}
NT18BR	-16.82 ^{B ns}	-16.67 ^{A ns}	-15.87 ^{NS ns}	-16.10 ^{NS ns}
NT18SH	-17.03 ^{B a}	-17.88 ^{B b}	-15.97 ^{NS ns}	-16.22 ^{NS ns}
CV1%		2.4		1.7
CV2%		3.0		3.6

Means followed by the same uppercase letter in the column indicate no difference between the sampling areas for the same type of aggregate, and means followed by the same lowercase letter in the row indicate no difference between the types of aggregates for the same sampling area (Tukey, at 5% probability level). NT06MI: 6 years + millet; NT06BR: 6 years + brachiaria grass; NT06SH: 6 years + sunn hemp; NT18MI: 18 years + millet; NT18BR: 18 years + brachiaria grass; NT18SH: 18 years + sunn hemp; CV1: Coefficient of variation for types of aggregates; and CV2: Coefficient of variation for sampling areas. Source: Authors.

4. Discussion

4.1 Proportion of physicogenic and biogenic aggregates

In the relative proportion of the different pathways of aggregate formation (Table 1), other authors have found similar results for physicogenic aggregation (Loss et al., 2014; Silva Neto et al., 2016; Fernandes et al., 2017). When evaluating the proportion of aggregate formation by the different pathways in NT areas with different times of implementation and forest in two seasons in Guaíra-PR, Ferreira et al. (2020) observed that the proportion of physicogenic aggregates was higher than that of intermediate and biogenic aggregates after 23 years of NT in both seasons, with the opposite pattern for the forest area. The authors attributed the results to minimal soil mobilization and the cumulative effects of pressure of agricultural machines on soil surface.

The machines used in NT are heavier compared to those used in conventional tillage, which can cause changes in the edaphic environment, mainly in soil structure, favoring compaction in the surface layers, which was pointed out as one of the main negative effects observed in areas of NT (Andrade et al., 2018).

In the areas NT06BR and NT18BR in the 0.00-0.05 m layer, biogenic aggregates were found in a higher proportion than physicogenic ones (Table 1). These results corroborate those found by Loss et al. (2017), Mergen et al. (2019 a), Melo et al. (2019) and Schultz et al. (2019). Loss et al. (2014) observed higher amounts of biogenic aggregates in pasture area when compared to areas of NT and secondary forest, probably due to the root system of grasses, especially forage grasses.

The root system of the different cover crops directly influence the genesis of the different types of aggregates, being considered one of the main biological agents responsible for the formation of biogenic aggregates, as well as the edaphic macrofauna. Forage grasses, especially *Brachiaria* spp., promote a greater addition of carbon to the soil via root system renewal, providing better conditions for soil fauna and the formation of biogenic aggregates (Loss et al., 2014; Mergen Junior et al., 2019 a), hence justifying the results found in the study in the areas covered with brachiaria grass.

Regarding the decrease in the percentage of biogenic aggregates in the areas between the NT06 and NT18 systems (Table 1), Ferreira et al. (2020) point out that the reduction in the proportion of this class of aggregates as a function of the time of adoption of NT is an indication that some practices adopted in the system may be negatively affecting the structural quality of the soil or causing reduction in the activity of the edaphic macrofauna, by compaction, which is common in agricultural areas managed under NT.

4.2 TOC contents

The highest contents of TOC in the aggregates in the area of NT18MI in the 0.00-0.05 m layer (Figure 1) may be related to the higher phytomass production and low rate of decomposition of residues, verified for millet crop. In the municipality of Uberaba-MG, Torres et al. (2014) evaluated the phytomass production and decomposition of residues from different cover plants in NT. The authors observed that millet cultivars had the highest values of phytomass production and the lowest rates of residue decomposition. This is due to the adaptation of this crop to the climatic conditions of the study region associated with its C/N ratio, above 25:1, which promote a slower decomposition (Torres et al., 2005).

In a study evaluating TOC contents and stocks in the soil after different periods of cultivation under NT (6, 9 and 10 years), Rossetti & Centurion et al. (2015) verified an increase in carbon content in the soil surface layer as a function of the time of adoption. In the Cerrado of Goiás, Loss et al. (2011) quantified TOC contents in different classes of aggregate size in Oxisol (*Latossolo Vermelho*) in a Cerrado area and two areas of NT after 17 years of implementation, one with crop-livestock integration (CLI, NT-CLI) and the other without integration. The authors observed that, among the cultivated areas, the highest carbon contents in the aggregate classes were found in the NT-CLI. The results probably occurred due to the use of brachiaria,

which promotes the deposition of organic residues with higher C/N ratio, resulting in a slower decomposition and favoring the accumulation of TOC in the aggregates.

Evaluating TOC contents in management systems with different times of implementation in the Western region of Paraná, Rosset et al. (2014) observed a trend of increase in the contents as a function of NT implementation time, especially after 6 years (transition phase) and 22 years (maintenance phase) of adoption for the layers of 0.00-0.05 and 0.05-0.10 m, and reduction of the contents in subsurface. This reduction shows the contribution of carbon inputs in the most superficial layer compared to the deepest ones, a common fact in systems without intensive soil turning (Rosset et al., 2014). This pattern is similar to the results observed in the aggregates of the area under NT18MI (Figure 1).

In the Cerrado of Minas Gerais, Torres et al. (2019) evaluated SOM accumulation and TOC contents in areas of NT with 5 and 17 years (NT05 and NT17, respectively) and forest. The authors verified higher SOM accumulation in the area of NT17 (consolidation phase) compared to NT05 (transition phase) in the 0-0.40 m layer, and absence of significance in TOC contents between NT05 and NT17 for the other layers evaluated. This is due to the combined action between the addition and maintenance of crop residues that remain on the soil surface for a long period, with minimal disturbance of the environment and crop rotation, thus explaining the accumulation of SOM in NT17. In this study, the factors mentioned help justify the results of TOC in the aggregates of the area NT18MI in the 0.00-0.05 m layer (Figure 1).

In the 0.05-0.10 m layer, high carbon contents were also verified in the aggregates of NT06SH (Figure 2). Torres et al. (2014) found that brachiaria and sunn hemp showed similarity in the values of dry phytomass production (2.4 and 2.1 Mg ha⁻¹, respectively), and the highest decomposition rates were quantified in residues of sunn hemp compared to those of brachiaria (67.9 and 61.7%, respectively). In areas of NT under different cover crops, conventional tillage system (CTS) and fallow area in the Cerrado of Minas Gerais, Pereira et al. (2012) verified that in the 0.00-0.05 m layer, the highest TOC stocks were quantified in the areas of NT with brachiaria and fallow and NT with sunn hemp, respectively, whereas the lowest stocks were found in the CTS.

Quantifying the contents of TOC in different classes of aggregate sizes of a Oxisol (*Latosolo Vermelho*) in NT areas with different cover plants (corn + brachiaria and corn + sunn hemp) and Cerrado vegetation, Coutinho et al. (2010) verified that the area with brachiaria had the highest contents of TOC in the different classes of aggregates for the two layers evaluated. These results suggest that the root system of brachiaria is more efficient in increasing the carbon content of soil aggregates when compared to that of sunn hemp. These data are not in agreement with those found in the present study for the 0.05-0.10 m layer in the NT06SH area (Figure 2).

The results of TOC among the morphological types of aggregates (Figures 1 and 2) showed a pattern similar to those found by Loss et al. (2017), Ventura et al. (2018), Mergen Junior et al. (2019 b) and Schultz et al. (2019). Evaluating the effect of biogenic aggregation on chemical and structural dynamics in Oxisol (*Latosolo Vermelho*) in the city of Londrina-PR after nine continuous years of application of liquid pig manure or chicken manure, Melo et al. (2019) quantified higher contents of TOC in biogenic aggregates. For the authors, the higher the carbon input, the more intense the formation of biogenic aggregates.

In forest area, Ferreira et al. (2020) quantified higher TOC contents in biogenic aggregates compared to intermediate and physicogenic aggregates. In areas under native vegetation, due to the stability of this environment, a greater number and action of microorganisms, plants and edaphic animals responsible for biogenic aggregation is assumed. These results suggest that these aggregates, besides indicating greater biological activity, also favor carbon accumulation in the soil.

4.3 POC contents

In the Cerrado of Minas Gerais, Pereira et al. (2012) quantified higher stocks of POC in an area cultivated with corn +

sunn hemp and in the fallow area, and for the soybean crop with millet and brachiaria as cover crops, in the layer of 0.00-0.20 m. These results may have occurred due to the balance in the final C/N ratio of the mixture of the residues from the cover plants with those of the main crop, which caused the increase in POC stocks compared to areas cultivated only with grasses (millet or corn) and legumes (soybean or sunn hemp).

Increments in POC contents are generally verified in areas with legumes and grasses, since the biological nitrogen fixation (BNF) performed by legumes contributes to the growth and development of grasses, which in turn react with higher phytomass production and, consequently, greater amounts of dry matter are supplied to the soil (Pereira et al., 2012). Thus, part of this material, along the SOM decomposition process, will be transformed into POC. The results of POC in biogenic aggregates under NT18MI are in agreement with the TOC data (Figure 1), with a similar pattern between the variables in the 0.00-0.05 m layer, probably due to the characteristics inherent to the millet crop.

Evaluating seasonality in Guaíra-PR, Ferreira et al. (2020) found in the dry season higher contents of POC in physicogenic, intermediate and biogenic aggregates in NT after 23 years of implementation (maintenance phase) compared to other systems. According to the authors, these results were found because soils under NT remain with minimal disturbance and constant supply of organic residues, so aggregates are better preserved, enabling greater protection of carbon in this fraction of SOM.

Among the formation pathways, the results of POC are similar to those observed by Rossi et al. (2016) and Schultz et al. (2019). The higher contents of POC in biogenic aggregates (Table 3) indicate the predominance of material with greater lability compared to physicogenic aggregates (Loss et al., 2014), and the incorporation or maintenance of this material is favored in biogenic aggregates due to the agents responsible for its formation (soil fauna and plant roots) (Loss et al., 2014; Mergen Junior et al., 2019 a). These results indicate that biogenic aggregates can promote greater protection of this physical fraction of SOM.

4.4 MAOC contents

For MAOC, Ferreira et al. (2020) verified in the rainy season higher contents in the forest and NT areas after 23 years of adoption in the different types of aggregates. The favorable climatic conditions associated with the high availability of food sources found in systems with longer time of adoption, contribute for the edaphic environment to start housing more complex communities of soil fauna individuals, including decomposer organisms. These organisms convert POC into CO₂ and more stable forms of carbon in the soil.

Higher MAOC stocks were observed by Pereira et al. (2012) in areas of corn and soybean planted on millet and brachiaria residues, respectively, in the 0.10-0.20 m layer. According to the authors, these results demonstrate that the use of cover crops (grasses and legumes) before annual crops in the Cerrado under NT favors the increase of MAOC stocks.

Regarding the results of MAOC between the areas, the carbon contents quantified in the two types of aggregates in the areas of NT18MI in the layer of 0.00-0.05 m (Table 3) point to the presence of carbon in more recalcitrant fractions due to the longer time of adoption of NT, associated with a vegetation cover with lower rate of decomposition of its residues. For MAOC contents in biogenic aggregates in the NT06SH area, the data were similar to those of TOC in the 0.05-0.10 m layer (Figure 2).

As for the results of MAOC among the formation pathways (Table 3), these may be related to the capacity that biogenic aggregation has for protection and physical stabilization of the more labile fractions of SOM, such as POC. The highest contents of MAOC in biogenic aggregates indicates that this class is more favored in the formation of the most stable fractions of SOM.

Other authors have also found that higher contents of MAOC were quantified in biogenic aggregates, when compared

to physicogenic aggregates (Loss et al., 2014; Rossi et al., 2016; Schultz et al., 2019). Comparing the formation pathways, Ferreira et al. (2020) found differences in MAOC contents only in the rainy season, with higher values of this fraction in biogenic aggregates. However, MAOC does not always function as a good indicator in the measurement of soil quality, since changes in the contents of this SOM compartment require many years to be observed (Carmo et al., 2012), due to the high degree of stability of this physical fraction of SOM.

4.5 FLFC contents

The labile fractions of SOM are fundamental for cycling of carbon between compartments and of nutrients in a short period of time, in addition to their contribution to the formation and stabilization of soil aggregates (Santos et al., 2013). Evaluating the contents of light organic matter (LOM) in aggregates collected in areas of NT after 17 years of implementation, with and without crop-livestock integration (NT-CLI and NT without CLI, respectively) and Cerrado area, Loss et al. (2011) found MOL contents similar to those of the Cerrado area in the 0.05-0.10 m layer in the NT-CLI area. This demonstrates that the CLI contributed to the supply of root residues in subsurface and the renewal of the root system, so the supply was equivalent to that of litter produced in the Cerrado area and was more efficient than that of the area under NT without CLI in increasing the LOCM contents. These results are similar to those observed in this study for FLFC in subsurface (Table 3).

Among the morphological types of aggregates, the results of FLFC in biogenic aggregates corroborate the data of POC (Table 3), probably due to the greater lability in both fractions. The similarity of the organic structures present in the particulate organic matter and in the light fraction of SOM was confirmed by Conceição et al. (2007) through optical microscopy analysis. The authors demonstrated that both fractions are basically composed of root fragments and organisms of soil fauna, hyphae and decomposing plant residues. In this study, the results of FLFC and POC may be associated with the potential of the formation pathways for carbon sequestration, in which the type of carbon stored by biogenic aggregates is more soluble than the more recalcitrant carbon stored in physicogenic aggregates (Pinto et al., 2019).

4.6 Natural abundance of $\delta^{13}\text{C}$

In the aggregates of all areas, $\delta^{13}\text{C}$ contents ranged from -15.55 to -17.88‰, indicating predominance of carbon originated from plants with photosynthetic cycle C_4 (species that fix CO_2 through the PEPCase enzyme), with values between -6 and -19‰ (Table 3).

In areas of NT with and without crop-livestock integration (NT-CLI and NT without CLI, respectively), Loss et al. (2011) observed mean values of $\delta^{13}\text{C}$ among aggregate classes of -19.10‰ for NT-CLI, and -17.50‰ for NT without CLI. In Paraná, Brazil, Loss et al. (2014) verified $\delta^{13}\text{C}$ contents in pasture area ranging from -16.12‰ (0.00-0.05 m) to -17.89‰ (0.05-0.10 m) for biogenic aggregates and from -16.41‰ (0.00-0.05 m) to -18.12‰ (0.05-0.10 m) for physicogenic aggregates, corroborating the values observed in the present study (Table 3). The use of C_4 plants, such as corn, in rotation with soybean, and millet or brachiaria as cover crops, contributes to the formation and stabilization of aggregates, especially for areas with brachiaria (Loss et al., 2011).

In the comparison between the formation pathways, in the biogenic aggregates of the NT06BR and NT18SH areas, the most negative values of $\delta^{13}\text{C}$ were verified in the 0.00-0.05 m layer (Table 3). Loss et al. (2017) in Santa Catarina, Brazil, found no differences in $\delta^{13}\text{C}$ contents between biogenic and physicogenic aggregates. However, Loss et al. (2014) quantified more negative values in biogenic aggregates in areas of NT and secondary forest. The authors related these results to the high contents of TOC in these aggregates and to the isotopic signature of the predominant vegetation in these areas. The data of $\delta^{13}\text{C}$ in biogenic aggregates in this study are similar to those observed by Loss et al. (2014) and Jouquet et al. (2009).

5. Conclusion

The use of grasses, especially *Brachiaria* spp., as cover plants in no-tillage system after 6 and 18 years of adoption favors the formation of aggregates through the biogenic pathway. Both compartmentalization and origin of the stored organic carbon are influenced by the aggregate formation pathways.

Highest contents of TOC, POC, MAOC, FLFC and more negative values of $\delta^{13}\text{C}$ were found in biogenic aggregates. Mainly in areas covered with millet and sunn hemp under systems in consolidation and transition phases, respectively.

Biogenic aggregates were more influenced and benefited by the stabilization mechanisms of the different physical fractions of SOM.

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