



Semina: Ciências Agrárias

ISSN: 1676-546X

semina.agrarias@uel.br

Universidade Estadual de Londrina
Brasil

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Semina: Ciências Agrárias, vol. 37, núm. 2, marzo-abril, 2016, pp. 595-610
Universidade Estadual de Londrina
Londrina, Brasil

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Densimetric fractionation of organic matter in an agricultural chronosequence in no-till areas in the Cerrado region, Brazil

Fracionamento densimétrico da matéria orgânica em uma cronossequência de agricultura em áreas de plantio direto no Cerrado

Roni Fernandes Guareschi^{1*}; Marcos Gervasio Pereira²; Adriano Perin³

Abstract

Mineralization of organic residues deposited on the soil surface in a no-till system (NT) maintains a continuous flow of different forms of carbon (C), which might interfere with densimetric fractions of soil organic matter (SOM). Currently, there are few studies on variations in these fractions in NT areas with different deployment times in the Cerrado region. Thus, the objective of this study was to evaluate the total C, nitrogen (N), and ¹³C in the soil, as well as to quantify the mass and contents of C, N, and ¹³C in the light and heavy fractions of SOM extracted using different solutions (water, sodium iodide [NaI], and sodium polytungstate [SPT]) in areas of Cerrado, pastures, and NT with different deployment times with Distroferic Red Latosol soil. The study areas are located in Montividiu, Goiás (GO), Brazil: (1) pasture of *Brachiaria decumbens* (PA); (2) NT with three years of deployment with soybean in summer and fallow instead of a second crop (NT3); (3) NT with 15 years of deployment with soybean in the summer and maize/sorghum as the second crop (NT15); and (4) NT with 20 years of deployment with soybean in the summer and maize as the second crop (NT20). These areas were compared to an area of native Cerrado *sensu stricto* (CE). The NT according to the deployment time showed an increase in the contents of C (0-0.05 m) and N (0-0.2 m) in the soil. The origin of C in the soil of the NT areas is associated with C4 plants; however, in up to 0.2 m of the soil profile, the ¹³C contents reduced according to NT deployment years. Extraction of light-fraction organic matter (LFOM) with SPT better represents this SOM fraction quantitatively and qualitatively than extraction of LFOM with water and NaI. This pattern is evident because of a greater consistency in the mass, C, N, and ¹³C contents of the LFOM extracted with SPT among the evaluated areas and the lower C content in residual particulate organic matter among the extractants.

Key words: Water. Sodium iodide. Red Latosol. Light-fraction organic matter. Sodium polytungstate. Soil management systems.

Resumo

A mineralização dos resíduos orgânicos depositados na superfície do solo no sistema de plantio direto (SPD) mantém um fluxo contínuo de diferentes formas de carbono, podendo interferir nas frações densimétricas da matéria orgânica do solo (MOS). Atualmente, são escassos resultados de pesquisa que avaliem as variações destas frações em áreas de SPD com diferentes anos de implantação na região do cerrado. Dessa forma, o objetivo deste trabalho foi avaliar o C, N e ¹³C total do solo, bem como,

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quantificar a massa, C, N e ^{13}C das frações leves e pesadas da MOS extraídas com diferentes soluções (água, iodeto de sódio – NaI e politungstato de sódio – PTS) em áreas de Cerrado, pastagem e SPD com diferentes anos de implantação sob um Latossolo Vermelho Distroférrico. As áreas de estudo encontram-se localizadas em Montividiu (GO), e apresentam as seguintes características: 1) pastagem de *Brachiaria decumbens* (PA); 2) SPD com 3 anos de implantação com cultivo de soja no verão e pousio na safrinha (SPD3); 3) SPD com 15 anos de implantação com rotação soja no verão – milho/sorgo safrinha (SPD15) e 4) SPD com 20 anos de implantação com rotação soja no verão – milho safrinha (SPD20); sendo estas áreas comparadas a uma área de cerrado nativo stricto sensu (CE). O SPD em função do tempo de implantação aumentou os teores de C (0,0-0,05 m) e N (0,0-0,20 m) do solo. A origem da carbono do solo nas áreas de SPD é referente à plantas do ciclo fotossintético C4, no entanto, verifica-se que até os 0,20 m do perfil do solo, os resultados de ^{13}C estão reduzindo em função dos anos de adoção do SPD. A extração da matéria orgânica leve (MOL) com PTS melhor representa quantitativamente e qualitativamente essa fração da MOS, quando comparada à extraída com água e NaI. Esse padrão fica evidente, devido a maior coerência nos resultados de massa, C, N e ^{13}C da MOL extraída com PTS entre as áreas avaliadas e pelo seu menor conteúdo de carbono na matéria orgânica particulada residual (MOPres) entre os extratores.

Palavras-chave: Água. Iodeto de sódio. Latossolo Vermelho. Matéria orgânica leve. Politungstato de sódio. Sistemas de manejo do solo.

Introduction

Landscape of the state of Goiás, located in central-western Brazil, has undergone significant changes, mainly because of the replacement of the native vegetation by extensive agriculture and livestock. By 2010, it was estimated that only 24.6% of the original area occupied by the Cerrado biome in Goiás still existed, and the remaining area has a great potential to be deforested for new agricultural and livestock projects (FERREIRA et al., 2007; BROCHADO, 2014).

In addition to deforestation in Cerrado, another aggravating factor due to the deployment of agriculture and livestock in these areas is soil degradation because of the lack of appropriate management. In Goiás, areas with degraded pastures represent approximately 4.75 million hectares (SASSINE, 2009). Most of these pastures have the potential to be, or are being, converted into grain production areas. Therefore, in agriculture chronosequence studies performed in the state of Goiás, it is important to take into account the pasture areas in order to demonstrate the potential of other management systems, such as the no-till system (NT), to revert soil degradation in these areas. In addition, intensive use of soil under conventional tillage (CT) represents another very common means

of degradation in the state of Goiás (GUARESCHI, 2013; GUARESCHI et al., 2014).

Given this scenario, NT emerged as an alternative in Cerrado to minimize impacts on the soil (GUARESCHI et al., 2014). Previous studies have indicated that the adoption of NT in Cerrado promotes the accumulation of organic carbon (C) and nitrogen (N), triggering improvements in the physical and chemical properties of the soil (SIQUEIRA NETO et al., 2010; COSTA JUNIOR et al., 2011; LOSS, 2011; GUARESCHI et al., 2012, 2014). However, the benefits of NT are observed after a long period, for example, 10-15 years (CARVALHO et al., 2010). Thus, it is necessary to obtain more information about the pattern of C and N accumulation in areas of NT with different deployment times and soybean-maize succession (GUARESCHI et al., 2014).

Another important analysis used in NT chronosequences is variation in the natural abundance of ^{13}C , because it confirms the history of cultivation in the study areas, as well as shows how plant residues of the crops seeded in the areas are contributing to the formation of soil organic matter (SOM) in a particular environment (SIQUEIRA NETO et al., 2010; GUARESCHI et al., 2014).

In addition, analysis of densimetric fractions of SOM has been used to evaluate possible quantitative and qualitative short-term alterations in SOM due to changes in soil management (SOHI et al., 2001; DIEKOW et al., 2005; CONCEIÇÃO et al., 2007, 2008; LOSS, 2011). This analysis, which is based on the density difference between the free organic fractions and organomineral complexes, enables the extraction of SOM fractions with distinct stabilization mechanisms (ZECH et al., 1997). However, studies have shown that the fractionation efficiency is influenced by the density and composition of the solution (usually water, sodium iodide [NaI], and sodium polytungstate [SPT]), resulting in the separation of different fractions of SOM, both quantitatively and qualitatively (CONCEIÇÃO et al., 2007, 2008; DEMOLINARI et al., 2008; LOSS, 2011).

Since studies on the densimetric fractions of SOM in NT areas with different deployment times are scarce, there is a need for more studies in order to standardize a densimetric fractionation method that better represents such SOM fractions for comparing results obtained by different research groups (CONCEIÇÃO et al., 2007). Thus, the hypotheses of this study are as follows: (1) C, N, and ^{13}C contents and densimetric SOM fractions increase according to the NT deployment time and relative to the areas of pastures for signal grass, *Brachiaria decumbens* (Stapf) (PA), or that of the pastures for native Cerrado *sensu stricto* (CE) that preceded them; (2) composition and density of the extractant alter the quantity and quality of extracted densimetric fractions, modifying the results for the comparison between areas of NT chronosequences.

Thus, the objective of this study was to evaluate the total C, N and ^{13}C of the soil, as well as to measure the mass, C, N and ^{13}C contents of the light and heavy fractions of SOM extracted with different solutions (water, NaI, and SPT) in areas of Cerrado, pastures, and NT regions with different deployment times and Distroferic Red Latosol soil.

Material and Methods

For this study, samples of the soil surface (0-0.05, 0.05-0.1, and 0.1-0.2 m) were collected from the Montividiu Tiúba Farm, which is located in the city of Montividiu (GO) (17° 27' 52, 2" S; 51° 10' 33, 1" W; altitude, 890 m). The average annual pluviometric precipitation in the region is 1,740 mm, and the region has a warm tropical climate (Aw in Köppen's system) with well-defined rainy and dry seasons (GUARESCHI, 2013). The land is relatively flat, and the soils of the study areas were classified as Distroferic Red Latosol (EMBRAPA, 2006).

Five areas of 0.10 ha each were selected, with the following management systems: (1) CE, taken as the reference; (2) PA; (3) NT3 with three years of deployment (soybean, *Glycine max* L., in the summer and fallow instead of a second crop); (4) NT15 with 15 years of deployment (soybean in the summer and maize, *Zea mays* L., or sorghum, *Sorghum bicolor* L., as the second crop) and (5) NT20 with 20 years of deployment (soybean in the summer and maize as the second crop).

Because it is a system without anthropic action, the CE area was used as the reference. The analyzed CE is located within the preservation area of the Montividiu Tiúba Farm (S 17 27' 52, 2"; W 51 10' 33, 1"; altitude, 890 m). The PA area (17° 25' 58, 5" S; 51° 09' 39, 4" W; altitude, 804 m) has been cultivated with an approximate stocking rate of 1.5 animals per hectare. After the removal of CE vegetation with a bulldozer and steel chains, the NT3 area (17° 27' 20, 9" S; 51° 10' 16, 3" W; altitude, 858 m) was cultivated as a pasture for 20 years, followed by rice cultivation for one year; for three years, it has been cultivated under NT with soybean. The NT15 area (17° 28' 16, 8" S; 51° 11' 20, 4" W; altitude, 899 m) has a history of 27 years of cultivation, with the NT adopted in 1995 after 10 years of soybean in summer and maize as the second crop. Finally, NT20 (17° 28' 31, 7" S; 51° 10' 43, 6" W; altitude, 898 m) has the same history as NT15, differing by five years of CT and NT implantation in 1990.

Basic fertilization of the main crops for the agricultural systems was as follows: (1) NT3 and NT15, (a) soybean (summer) – 458 kg ha⁻¹ of the formulation 02-20-20 and (b) maize as the second crop – 312 kg ha⁻¹ of the formulation 12-15-15 and 120 kg ha⁻¹ of urea, in a coverage of 25 days after emergence (DAE); (2) SPD 20, (a) soybean (summer) – 200 kg ha⁻¹ of the formulation 02-20-20 + 60 kg ha⁻¹ of K₂O and P₂O₅ in coverage and (b) maize as the second crop – 30 kg ha⁻¹ of N + 60 kg ha⁻¹ of P₂O₅ + 70 kg ha⁻¹ of K₂O in the groove and 120 kg ha⁻¹ of urea, in a coverage of 25 DAE. NT3 was limed in 2006 and 2010; NT15, 2007; and NT20, 2008. Finally, the PA area was limed in 2009. Liming was performed by surface soil application in all areas.

A representative farmland of 2.25 ha (150 × 150 m) was outlined in each area, and five trenches of approximately 1 × 1 m and 0.20-m depth in random positions were dug. The design was entirely randomized, with five repetitions for each area. Subsequently, sampling of undisturbed samples was performed in each trench in different areas by using a volumetric ring (EMBRAPA, 1997) in the layers of 0-0.05, 0.05-0.1, and 0.1-0.2 m. In addition, disturbed samples were collected with a straight blade from these layers, and they were air-dried, ground, and sieved through a 2-mm mesh, resulting in dry-air thin soil (DATS) for which chemical characterization and granulometric analysis were performed (EMBRAPA, 1997) (Table 1).

Table 1. Chemical attributes and granulometric composition of the areas of native Cerrado *sensu stricto* (CE), pasture of *Brachiaria decumbens* (PA), and no-till system (NT) with three (NT3), 15 (NT15), and 20 (NT20) deployment years.

Area	pH H ₂ O	P mg kg ⁻¹	Ca	Mg	K	Al	H+Al	T	V	Clay	Silt	Sand
----- cmol _c kg ⁻¹ -----												
--%--												
----- g kg ⁻¹ -----												
0.0-0.05 m												
CE	4.4	9.5	0.8	1.9	0.2	0.5	13.3	16.2	17.8	410	170	420
PA	5.7	2.1	3.9	4.7	0.2	0.0	7.3	16.3	54.6	600	190	210
NT3	5.2	26.7	1.6	2.0	0.3	0.1	3.6	7.7	52.9	590	270	140
NT15	5.0	33.6	2.6	2.9	0.3	0.1	4.6	10.6	56.4	630	170	200
NT20	6.2	32.1	2.6	2.8	0.3	0.0	2.8	8.6	67.1	410	120	470
0.05-0.10 m												
CE	3.6	5.8	0.0	1.1	0.1	0.9	9.9	11.0	10.7	390	200	410
PA	5.6	1.5	3.0	3.0	0.1	0.0	5.0	11.2	54.8	570	240	190
NT3	5.1	20.9	0.9	1.7	0.3	0.1	3.8	6.6	42.0	610	270	120
NT15	4.9	29.1	1.9	1.9	0.3	0.2	4.0	8.2	52.6	670	160	170
NT20	5.3	29.2	1.8	1.8	0.3	0.1	3.9	7.9	50.3	460	110	430
0.10-0.20 m												
CE	3.8	4.8	0.0	0.9	0.1	0.8	10.1	11.1	9.1	380	170	450
PA	5.5	1.7	2.5	1.7	0.1	0.0	7.7	12.0	35.5	590	220	190
NT3	4.3	18.4	0.1	0.8	0.2	0.1	2.2	3.4	34.9	610	280	110
NT15	4.5	28.7	1.4	1.3	0.3	0.3	3.3	6.3	48.3	660	180	160
NT20	4.4	28.4	1.3	1.3	0.3	0.2	3.4	6.4	47.1	470	100	430

C and N contents in the soil were quantified using DATS by dry combustion with a CHNS analyzer (Elementar analysensysteme GmbH, Hanau, Germany). The natural abundance of ^{13}C ($\delta^{13}\text{C}\text{‰}$) in the soil was quantified with a mass spectrometer (Finnigan Delta Plus), in the Isotopic Ecology Laboratory of the University of São Paulo (Laboratório de Ecologia Isotópica – CENA-USP) in Piracicaba, São Paulo, Brazil. The results were expressed as delta ^{13}C (‰), according to the International Standard Pee Dee Belemnite.

Light-fraction organic matter (LFOM) in water was quantified in the 0-0.05-m and 0.05-0.1-m layers by using the method of flotation in water (ANDERSON; INGRAM, 1989). For this method, 50 g of DATS was weighed in a 250-mL beaker and 100 mL of 0.1 mol L⁻¹ NaOH was added. The suspension was left standing overnight and then stirred with a glass rod and sieved through a 0.25-mm mesh to eliminate the entire clay fraction.

Subsequently, the material retained on the sieve (LFOM and sand) was transferred again to the beaker, and the volume was maintained with deionized water. All the float materials were sieved using a 0.25-mm mesh, carefully separating LFOM from the sand. Water was added again to the beaker, and the remaining LFOM was manually agitated to resuspend it; the resulting solution was slowly poured in a 0.25-mm mesh sieve. This procedure was repeated until the entire float material was removed by stirring in water. The material retained on the sieve (LFOM) was transferred to aluminum cans (previously weighed), incubated in an oven at 65 °C until a constant weight was achieved (72 h), and then the entire set was weighed (LFOM + aluminum cans). C and N contents of this fraction were determined by dry combustion in a Shimadzu TOC V CSH equipment. Analysis of $^{13}\text{C}\text{‰}$ natural abundance of LFOM was also performed.

Sand and possible residues of particulate organic matter (POM) retained on the sieve were transferred to aluminum containers and dried in an oven at 60

°C; this residual fraction was named residual POM ($_{\text{res}}$ POM) (CONCEIÇÃO et al., 2008). Analysis of C and N contents (dry combustion) and natural abundance of ^{13}C was also performed with this fraction. The C content in the heavy fraction (HF) was calculated as the difference between total soil C and C of LFOM; therefore, the C of $_{\text{res}}$ POM fraction is considered as part of HF (CONCEIÇÃO et al., 2008). Thus, C of $_{\text{res}}$ POM, although quantified separately, was accounted for as belonging to HF (CONCEIÇÃO et al., 2007).

Light fractions of SOM were obtained using the procedure described by Sohi et al. (2001). The light fractions were extracted from the soil by using two different solutions: NaI and SPT, both with a density of 1.80 Mg m⁻³ (± 0.02). Five grams of DATS was weighed and added to 50-ml centrifuge tubes and 35 ml of NaI or SPT was added. The tubes were shaken by hand for 30 s so that the less dense organic fractions remained on the surface of the solution. Then, the samples were centrifuged at 18,000 rpm for 15 min at a temperature of 18° C, in order to promote the sedimentation of the mineral particles of the soil. The supernatant organic fraction present in the solution (free-light fraction [FLF]) was suctioned together with the NaI or SPT solution and immediately separated by vacuum filtration (Sterifil Aseptic System, 47 mm; Millipore) by using previously weighed fiberglass filters (47 mm diameter, 2 microns; Whatman type GF/A)).

The separate fractions were washed with distilled water to eliminate the excess NaI present in the fraction and filter. The organic fraction, together with the filter, was subsequently dried at 65 °C, weighed, and macerated using a mortar and pestle. FLF is composed primarily of microbial debris and plant residues, such as fungal hyphae; spores; and fragments of roots, seeds, and charcoal.

After the removal of FLF, the intra-aggregate light fraction (ILF) or light-occluded fraction (LOF) was extracted, the centrifuge tube was disposed in a container with ice and water, and the suspension

was dispersed using ultrasonic energy 1096 J mL⁻¹; (TOMAZI et al., 2011). After treatment with ultrasonic energy, the samples were centrifuged again at 18,000 rpm for 15 min, and the ILF was retained on filters (47 mm diameter, 2 microns; type Whatman type GF/A) that were processed as described for FLF. ILF is incorporated and physically stabilized between aggregates, and it is composed of plant residues, fecal pellets, pollen grains, root hairs, and fungal structures and with a reduced size and more advanced degree of decomposition when compared to FLF.

Three laboratory replicates were obtained for each fraction and combined into a single sample for determination of total C and N per dry via.

Analysis of total C and N contents in the light fractions was performed by dry combustion in a Shimadzu TOC V CSH by using approximately 40 mg of material previously macerated in a ball mill. Analysis of the natural abundance of ¹³C was also performed using these fractions.

After obtaining ILF, 0.5 g of sodium hexametaphosphate was added to the material remaining in the tube, which corresponds to HF. Subsequently, the volume of the suspension was increased to 40 ml with distilled water and homogenized for 6 h at 250 rpm. At the end of this stage, the suspension was transferred to a 0.53-mm mesh sieve and distilled water was squirted to separate the sand fraction. After washing, the remaining materials retained on the sieve (sand fraction + _{res}POM) were dried at 60 °C. Analysis of the C and N contents (dry combustion) and the natural abundance of ¹³C was performed using this fraction. C of HF was calculated by the difference between the total C of the soil and the C of FLF+LOF, therefore representing the C of _{res}POM fraction considered as belonging to HF.

For all data, the normality was calculated using Lilliefors' test, and the homogeneity of error variance was assessed using Cochran's and Bartlett's tests. Subsequently, the results were evaluated

using analysis of variance with the F test, and the average values were compared using the t test at 5% significance level with the software ASSISTAT (SILVA; AZEVEDO, 2002).

Results and Discussion

In the 0-0.05-m layer, C and N contents increased in proportion to the NT deployment time. In NT20, C and N values were higher than those of CE and PA (Table 2). In this layer, the C content of NT15 was similar to that of CE. Similar results were found by Matias et al. (2009), who showed that NT areas in a Latosol soil in Uruçuí – PI (Piauí, Brazil), exhibited an increase in C and N contents when compared to CE areas. Likewise, Corazza et al. (1999), who studied C in the soil of different management systems in relation to CE in a Dark Red Latosol soil in Planaltina – DF (Distrito Federal, Brazil), observed that the NT increased C storage in comparison with the CE area. According to these researchers, such an increase can be attributed to the higher rate of C addition to the soil due to the increased production of crop residues and roots in the topsoil and lower soil tillage.

The increase in the N content of the soil in the NT areas was also observed by Buso and Kliemann (2003), who evaluated the 0-0.12-m layer in a Dystrophic Red Latosol soil in Rio Verde – GO, and observed that the NT led to an increase, albeit slow, in the stock of total N in the soil.

In the other analyzed layers (0.05-0.1 m and 0.1-0.2 m), the CE area showed the highest C and N contents in comparison with those of other cropping systems. Finally, in the NT areas, the C contents of NT15 and NT20 did not differ and were higher than NT3, while the N content increased according to the NT deployment time. According to Siqueira Neto et al. (2009), the areas of “cerradão” have higher C and N contents in the soil because of the constant supply of plant residues and non-disturbance of the system. In comparison to the NT areas, there was an increase in the C and N contents according

to the deployment time, although it was less significant than that observed in the surface layer (0-0.05 m). According to Bayer and Mielniczuk (1999), accumulation of SOM in the NT occurs slowly; thus, it can be inferred from the results of

the present study that 20 years of NT deployment were still not sufficient for the accumulation of C and N in the subsurface layers, in comparison with CE vegetation.

Table 2. Total soil carbon (C), total soil nitrogen (N), and soil ^{13}C in the evaluated areas.

Depth (m)	Evaluated systems					
	CE	PA	NT3	NT15	NT20	CV(%)
C (g kg ⁻¹)						
0.0-0.05	30.8 b*	15.4 c	15.9 c	29.6 b	33.9 a	3.1
0.05-0.10	36.1 a	14.1 c	15.4 c	27.1 b	26.7 b	2.8
0.10-0.20	31.9 a	12.04 c	12.5 c	25.4 b	24.8 b	3.7
N (g kg ⁻¹)						
0.0-0.05	1.9 b	1.0 d	0.9 d	1.7 c	2.4 a	2.9
0.05-0.10	2.2 a	0.8 e	0.9 d	1.5 c	1.8 b	4.7
0.10-0.20	2.0 a	0.7 d	0.7 d	1.4 c	1.5 b	5.4
^{13}C (‰)						
0.0-0.05	-25.3 a	-14.4 d	-16.7 c	-16.6 c	-18.8 b	1.4
0.05-0.10	-25.4 a	-14.5 e	-16.0 d	-16.3 c	-18.6 b	0.9
0.10-0.20	-25.1 a	-14.6 d	-14.6 d	-15.7 c	-18.0 b	1.8

*Means followed by the same lowercase letter on the line do not differ significantly between the different land use systems by t test at 5% significance level.

In general and independent of the analyzed layer, the PA and NT3 areas had the lowest C and N values in comparison to the other areas. The results obtained in these areas (NT3 and PA) are attributable to the small NT deployment time and, in the PA area, to the fact that it is characterized by intensive grazing and lack of management (fertilization). Another factor is that there is a low supply of plant residues in the PA area (GUARESCHI, 2013), which contributes to the lowest C and N values.

The natural abundance of $\delta^{13}\text{C}$ (‰) in the soil profile showed that the CE area had a higher supply of plant residues from C3 plants in all examined layers (Table 2). This result is consistent, since the CE vegetation had always consisted mainly of C3 tree species and small bushes. During photosynthesis, C3 and C4 plants discriminate the ^{13}C isotope with different degrees of intensity. On the basis of the literature (ALVES et al., 2008), it is

known that C3 plants show $\delta^{13}\text{C}$ values between -33 and -22‰, whereas C4 plants show values between -16 and 9‰. The PA area showed $\delta^{13}\text{C}$ values near the normal discrimination range for C4 plants (Table 2), as it received a supply from plant residues originating from grasses.

Among the NT areas, the $\delta^{13}\text{C}$ (‰) signal originated from C4 plants up to a depth of 0.2 m. However, the $\delta^{13}\text{C}$ (‰) signal decreased according to the NT deployment time. In NT20, the $\delta^{13}\text{C}$ (‰) signal was closer to the average values established for C3 plants, probably because the organic matter in this area receives a large contribution of plant residues derived mainly from C3 plants (soybean) (GUARESCHI et al., 2012).

There was a statistical difference between systems with respect to the C and N mass and contents of LFOM extracted using water, NaI, and SPT in the soil layers of 0-0.05 and 0.05-0.1 m (Table

3). The results of LFOM extracted with water in the 0-0.05-m layer of the soil profile demonstrate that an increase in C and N accumulation and content of this fraction occurs according to the NT deployment time (from 3 to 20 years), whereas for the NT20 area, these contents did not differ in comparison to those of the CE area. This result shows the great importance of NT in this environment because the maintenance of LFOM is critical to the sustainability

of agricultural systems, since it represents, in short and medium terms, a high potential for nutrient cycling (COMPTON; BOONE, 2002). According to Loss et al. (2010), LFOM is affected by the management method and soil coverage in areas under NT, and because it is found in the straw on the soil surface, there is an increase in the light fraction of SOM.

Table 3. Quantity (mass), carbon content (C), nitrogen content (N), and natural abundance of ^{13}C in the free-light fraction (FLF), light-occluded fraction (LOF), and light-fraction organic matter (LFOM = FLF + LOF) of the evaluated areas extracted with different extractants.

Area	Fractions	Water				Sodium iodide				Sodium polytungstate			
		Mass	C	N	¹³ C	Mass	C	N	¹³ C	Mass	C	N	¹³ C
		g kg ⁻¹				g kg ⁻¹				g kg ⁻¹			
		0.0-0.05 m											
¹ CE	LFOM	4.21a*	1.41a	0.09a	-20.85	31.43a	1.57a	0.09a	-	34.43a	3.44a	0.13a	-
	FLF	-	-	-	-	22.07a	1.30a	0.07a	-27.02	22.08a	1.97a	0.12a	-25.92
	LOF	-	-	-	-	9.36a	0.27a	0.02a	-26.33	12.35a	1.47a	0.01a	-26.62
PA	LFOM	1.23c	0.38d	0.02d	-14.43	14.86c	0.30d	0.01c	-	16.04d	0.36d	0.02c	-
	FLF	-	-	-	-	10.41d	0.25d	0.01c	-15.54	10.60d	0.23d	0.02c	-15.91
	LOF	-	-	-	-	4.45c	0.05d	0.003c	-15.88	5.43c	0.13d	0.007c	-15.13
NT3	LFOM	1.80b	0.56c	0.03c	-21.10	14.91c	0.30d	0.01c	-	15.95d	0.36d	0.02c	-
	FLF	-	-	-	-	10.53d	0.25d	0.01c	-19.92	10.56d	0.23d	0.02c	-20.97
	LOF	-	-	-	-	4.37c	0.05d	0.003c	-19.29	5.39c	0.13d	0.007c	-19.62
NT15	LFOM	1.84b	0.70b	0.04b	-20.54	20.31b	0.73c	0.03b	-	22.93c	0.68c	0.04b	-
	FLF	-	-	-	-	14.96c	0.66c	0.03b	-19.76	14.79c	0.39c	0.03b	-21.73
	LOF	-	-	-	-	5.35b	0.07c	0.005b	-19.45	8.13b	0.29c	0.017b	-19.67
NT20	LFOM	4.04a	1.46a	0.09a	-20.63	21.76b	0.92b	0.06a	-	24.65b	1.86b	0.06a	-
	FLF	-	-	-	-	16.48b	0.82b	0.05a	-20.55	16.53b	1.47b	0.04b	-21.97
	LOF	-	-	-	-	5.28b	0.10b	0.01a	-19.69	8.12b	0.39b	0.02a	-21.07
0.05-0.10 m													
CE	LFOM	1.76a	0.68a	0.04a	-20.21	25.97a	0.98a	0.03a	-	26.98a	2.09a	0.07a	-
	FLF	-	-	-	-	19.71a	0.83a	0.03a	-25.83	19.59a	1.75a	0.054a	-26.12
	LOF	-	-	-	-	6.26a	0.15a	0.008a	-25.74	7.39b	0.34a	0.019a	-25.49
PA	LFOM	0.36c	0.11c	0.01c	-14.12	12.47d	0.12d	0.008c	-	12.65d	0.23d	0.02c	-
	FLF	-	-	-	-	9.01d	0.09d	0.005c	-15.96	9.07d	0.16d	0.020c	-15.18
	LOF	-	-	-	-	3.45c	0.03d	0.003c	-15.22	3.58c	0.07d	0.004b	-15.15
NT3	LFOM	0.31c	0.10c	0.01c	-20.79	12.50d	0.12d	0.008c	-	12.61d	0.23d	0.02c	-
	FLF	-	-	-	-	9.07d	0.09d	0.005c	-19.75	9.06d	0.16d	0.020c	-18.41
	LOF	-	-	-	-	3.43c	0.03d	0.003c	-20.34	3.55c	0.07d	0.004b	-20.00
NT15	LFOM	0.44c	0.14c	0.01c	-20.40	16.27c	0.20c	0.01b	-	18.40c	0.86c	0.03b	-
	FLF	-	-	-	-	11.03c	0.14c	0.01b	-20.11	11.04c	0.70c	0.025b	-19.11
	LOF	-	-	-	-	5.23b	0.06c	0.004b	-20.03	7.36b	0.16c	0.010a	-20.42
NT20	LFOM	0.90b	0.29b	0.02b	-20.19	22.31b	0.40b	0.04a	-	25.95b	1.38b	0.05a	-
	FLF	-	-	-	-	17.04b	0.31b	0.04a	-20.59	17.11b	1.09b	0.048a	-20.62
	LOF	-	-	-	-	5.27b	0.08b	0.004b	-21.83	8.84a	0.29b	0.017a	-20.43

¹ Areas of cerrado (CE), pasture (PA) and no-tillage system with 3 (NT), 15 (NT15) and 20 (NT20) years of implementation. * Means followed by the same lowercase letters column comparing the areas assessed in each extractor evaluated separately by t test at 5% significance level. – The methodology does not allow review.

The smaller values of C and N mass and contents of LFOM in the layers 0-0.05 and 0.05-0.1 m of the NT15, NT3, and PA areas than in those of the CE and NT20 areas may be due to the lower supply of plant residues deposited on their soil surface (GUARESCHI et al., 2012). In NT15 and NT3, the time of NT deployment may not have been sufficient for the modification of C and N mass and contents of LFOM, whereas in the PA area, the management adopted did not favor large additions of residues and consequent increases in LFOM contents. In an assessment of the effect of different soil management systems, Loss et al. (2010) observed higher contents of LFOM in areas with the highest deposition of residues.

It is also possible to infer that the highest C and N contents of LFOM extracted using water from NT20, amongst the NT areas, are associated with the quantity (GUARESCHI et al., 2012) and quality of the plant residues added to the soil. Plant residues with different C/N ratios increase the recalcitrance of LFOM, leading to C and N accumulation according to the NT system deployment time. On the other hand, the LFOM values extracted with water that were similar between CE and NT20 in the 0-0.05-m layer and higher in CE in comparison to the NT in the 0.05-0.1-m layer may also be attributed to the quality of the material of LFOM in the CE area. Usually, this type of vegetation presents a great diversity of plants that deposit soil organic residues of different sizes and with a high amount of lignin (CIANCIARUSO et al., 2006).

Regardless of the studied area, the LFOM mass extracted with NaI and SPT was on average 8.9 and 9.7 (0-0.05 m), and 30.3 and 32.4 (0.05-0.1 m), respectively, times greater than the LFOM extracted with water (Table 3). This result can be attributed to the different densities of the extractants (SOHI et al., 2001). Similar results were reported by Demolinari et al. (2008) and Loss (2011): by extracting only FLF with water and NaI, they found that the mass of the fraction separated with NaI was on average 4.86

and 4.63 times higher than that extracted with water.

The mass LFOM extracted with SPT was on average 9.15% (0-0.05 m) and 6.33% (0.05-0.1 m) greater than that extracted with NaI (Table 3). This pattern shows that, even using the same extraction density (1.8 Mg m^{-3}), the use of solutions with different compositions may result in the separation of a distinct LFOM, both quantitatively (SOHI et al., 2001) and/or qualitatively. The difference in extraction efficiency between NaI and PTS was higher for LOF (Table 3); the SPT solution extracted on average 26.07% (0-0.05 m) and 18.33% (0.05-0.1 m) more than the NaI solution. Probably, the lower recovery of the light fraction of SOM by NaI is due to the formation of complexes of the I^- ion with MOS, which increases the density of the light fraction and, consequently, decreases its recovery (CONCEIÇÃO et al., 2007).

The increased amount of LFOM extracted with NaI and SPT affects the results obtained for the evaluated areas, when comparing the results with each other and with the extraction of LFOM with water (Table 3). For example, if we observe the amount of LFOM extracted with NaI compared to the one extracted with water in the most superficial layer (0-0.05 m), it is possible to detect different results between the evaluated areas. In the extraction with NaI, CE presented the highest values of LFOM, followed by the oldest NT areas (15 and 20 years), which were similar and showed higher values in comparison to PA and NT3 (Table 3). In the 0.05-0.1-m layer, the LFOM extracted with NaI was shown to be a variable with a higher sensitivity in differentiating the NT areas when compared to LFOM extracted with water; thus, LFOM extracted with NaI distinguished such areas according to the NT deployment time (Table 3). The greatest amount of LFOM mass extracted with SPT provided more consistent results, since it allowed the identification of the CE area. Besides, the amount of LFOM mass extracted with SPT allowed us to distinguish the NT areas according to the deployment time in the 0-0.05-m and the 0.05-0.1-m layers (Table 3).

The low capacity of extraction of LFOM with water either underestimated or was not sensitive enough to differentiate the NT areas in a chronosequence, whereas extractions with NaI and SPT (1.8 Mg m^{-3}) best represented the theory of dynamics of this fraction. Loss (2011) evaluated the LFOM extracted with NaI (SOHI et al., 2001) and by flotation in water (ANDERSON; INGRAN, 1989) in Latosol soil in NT, NT with crop-livestock integration (CLI), and CE. In this study, the LFOM extracted with NaI was shown to be a variable with a higher sensitivity for the differentiation of the management systems than the LFOM extracted with water. In this study, extraction with NaI showed higher amounts of LFOM and differentiated areas ($\text{CE} > \text{CLI} > \text{NT}$), whereas in the extraction with water, the LFOM values were as follows: $\text{CE} > \text{CLI} = \text{NT}$.

The comparison between the extraction of LFOM with NaI and SPT, regarding the amount of extracted mass, indicated that the SPT showed better results among the areas and in both evaluated layers (Table 3). Conceição et al. (2007) also reported higher extraction of LFOM with SPT than with NaI, mainly of LOF.

The SPT solution was able to extract, in most areas and in both layers (0-0.05 and 0.05-0.1 m), the LFOM with the highest C concentration, whereas the extraction with water and NaI showed values very close to the C of LFOM (Table 3). This pattern, consistent with the pattern observed for the mass values, was due to higher C contents observed in comparison with those quantified with the extraction with water and was relative to the density difference between the solutions. Regarding NaI, the complexation of the I^- ion with SOM hinders extraction. Another factor that may help to explain the results is visual observation, during extraction of LFOM with SPT, of a greater amount of finely grounded charcoal than LFOM obtained with the remaining extractants, especially in the areas with the highest total C contents in the soil (CE and NT20). This may indicate that the SPT solution is

more effective in the recovery of this component of LFOM (charcoal); however, further studies must be conducted to validate this observation. The highest values of C in LFOM extracted with SPT did not alter the results among the areas (0-0.05 m and 0.05-0.1 m) when compared with NaI, i.e., the highest values of C in LFOM were observed in the CE area with both extractants, followed by an increase of C in LFOM according to the NT deployment time. However, when comparing the contents of C in the LFOM between areas after extraction with water or SPT, the SPT shows larger amounts of C extracted from areas where there is a greater supply of plant residues (CE and NT20).

The N content in LFOM was hardly affected by the extractants (Table 3). Regardless of the extractant, lower N content was observed in LFOM in the areas with the lowest supply of crop residues (PA and NT3) (GUARESCHI, 2013). There was an increase in the values of N in the LFOM according to the NT deployment time, with the oldest area (NT20) showing similar values to those of the CE in the 0-0.05-m (extracted with water, NaI, and SPT) and 0.05-0.1-m layers (extracted with NaI and SPT).

^{13}C analysis of LFOM extracted with water, NaI, and SPT (Table 3) showed that, in the areas under NT and CE, this fraction consists mainly of residues from C3 plants, whereas in PA areas, this fraction consists mainly of residues of C4 plants. These data are consistent with the type of vegetation observed in each area: in CE, the plant residues for LFOM are attributable to C3 tree species; likewise, soybean, which is a C3 plant, is grown for most of the year soybean crop in NT (TAIZ; ZEIGER, 2004). In the PA area whose exploitation is characterized by 20 years of grass culture, the ^{13}C contents in LFOM were elevated to a greater extent, since grasses are less able to discriminate ^{13}C than leguminous plants (ALVES et al., 2008).

The mass and contents of C in FLF in different areas and layers followed the same pattern of the total mass and contents of C in LFOM extracted

with the extractants, that is, highlighting the CE area with higher amounts of this fraction compared to other areas and still differentiating the NT areas according to their time of deployment (Table 3). The larger amounts of FLF in the CE and NT20 areas are associated with a higher deposition of plant residues on the soil surface and, still in relation with the NT20 area, because of the larger NT deployment time. Moreover, Conceição et al. (2008) reported that increases in C contents in FLF under NT probably arise from a decrease in soil temperature, which would reduce the microbial decomposition rate of this labile fraction of SOM. Similar to this study, other studies have shown that, in native areas, the high diversity of plants and the amount of burlap favor increases in the contents of C and/or N in FLF from SOM (SOUZA et al., 2006; RANGEL; SILVA, 2007; CARNEIRO et al., 2013).

The smaller values of mass, C, and N in FLF and LOF extracted with NaI and PTS in the PA and NT3 areas follow the same pattern observed for the values of LFOM extracted with water, that is, PA due to the lack of management and low addition of plant residues to the soil and NT3 by the short system deployment time and because it is characterized by 20 years of degraded PA. Similar results have been reported by Lima et al. (2008): when evaluating different management systems (degraded pasture, *Eucalyptus*, and native forest) in Latosol soil, they also found lower C contents in FLF of the degraded pasture area.

With respect to the contents of N in FLF extracted with NaI and SPT, the results were similar to those previously observed for the contents N in LFOM. However, LOF in the 0.05-0.1-m layer showed different results for the evaluated areas according to the extractant used (Table 3). This difference was most evident in the areas with the highest total C values (CE, NT15, and NT20). The mass of LOF extracted with SPT was higher than that extracted with NaI, and consequently showed higher N content. Therefore, the contents of N in the LFOM extracted with SPT in the NT areas (15 and 20 years)

were similar to those of the CE area, whereas these areas showed lower values after extraction with NaI. These results show the divergence between these extractants, mainly with respect to LOF.

In LOF, the SPT solution extracted on average 26.07% (0-0.05 m) and 18.33% (0.05-0.1 m) more mass than NaI solution, affecting the values of the areas evaluated for 0.05-0.1-m layer. In this layer (0.05-0.1 m), LOF extracted with NaI was characterized by higher amounts of mass in the CE, followed by the NT15 and NT20 areas, which were statistically similar to each other and superior to the PA and NT3 areas (Table 3). In the extraction with SPT, there was an increase of LOF according to the NT deployment time, wherein the oldest area (NT20) presented a higher amount of this fraction in the 0.05-0.1-m layer than in the CE area (Table 3). On the basis of the results of LOF extracted with NaI, the effect of soil aggregation can be masked depending on the NT chronosequence evaluated, because the extraction of this fraction with the solution was not sensitive enough to distinguish between these areas; however, the extraction of LOF with SPT can reveal the effect of NT in the aggregation and protection of this fraction of organic matter according to the time of deployment. Similar results were observed by Conceição et al. (2008), who observed differences in LOF values after extraction with NaI and SPT in Latosols and considered that SPT best highlights inter- and intra-aggregate physical protection in the stabilization of MO in Brazilian soils under NT systems.

By analyzing the averages of the evaluated areas, we observed that the contents of C in the FLF and LOF extracted with SPT were 14.78% and 273.75% (0-0.05 m), and 183.6% and 170.33% (0.05-0.1 m), respectively, higher than the values obtained after the extraction of these fractions with NaI. However, extraction with SPT did not change the results of the evaluated areas (Table 3).

According to Conceição et al. (2007), quantification of $_{res}^{POM}$ only serves to characterize

the solutions in terms of their efficiency in the separation of FLF from the organic matter (MO) of the soil and does not represent a suitable alternative to be used complementarily with densimetric

fractionation. Therefore, the results were compared only among the extractants, and the differences in this fraction ($_{res}POM$) from distinct areas were not compared (Table 4).

Table 4. Carbon content (C) and natural abundance of ^{13}C in residual particulate organic matter ($_{res}POM$) and the heavy fraction (HF) and percentage of C in $_{res}POM$ relative to the C of LFOM (POM/LFOM) in the evaluated areas extracted with different extractants.

Area	Fractions	Water			Sodium iodide			Sodium polytungstate		
		C	POM/LFOM	¹³ C	C	POM/LFOM	¹³ C	C	POM/LFOM	¹³ C
		g kg ⁻¹	%	‰ ₀	g kg ⁻¹	%	‰ ₀	g kg ⁻¹	%	‰ ₀
----- 0.0-0.05 m -----										
¹ CE	^{res} POM	0.40	28.37	-22.32	0.65	41.40	-22.43	0.03	0.87	-24.33
	HF	29.35 b	-	-	29.23b	-	-	27.32b	-	-
PA	^{res} POM	0.29	76.32	-15.20	0.25	83.33	-15.52	0.14	38.89	-15.70
	HF	15.06 c	-	-	15.10c	-	-	15.10c	-	-
NT3	^{res} POM	0.28	50.00	-15.21	0.13	43.33	-16.08	0.12	33.33	-16.03
	HF	14.41 c	-	-	15.60c	-	-	15.62c	-	-
NT15	^{res} POM	0.35	50.00	-16.49	0.02	2.74	-16.87	0.01	1.47	-17.51
	HF	28.95 b	-	-	28.87b	-	-	28.97b	-	-
NT20	^{res} POM	0.82	56.16	-16.59	0.65	70.65	-18.76	0.04	2.15	-17.89
	HF	32.48 a	-	-	32.98a	-	-	32.09a	-	-
----- 0.05-0.10 m -----										
CE	^{res} POM	0.28	41.18	-22.90	0.66	67.35	-22.86	0.02	0.02	-21.62
	HF	35.37 a	-	-	35.12 a	-	-	33.96a	-	-
PA	^{res} POM	0.26	236.36	-15.22	0.30	250.00	-16.62	0.17	73.91	-15.24
	HF	13.97 c	-	-	13.98 c	-	-	13.85c	-	-
NT3	^{res} POM	0.26	260.00	-15.43	0.24	200.00	-16.91	0.12	52.17	-17.24
	HF	15.33 c	-	-	15.28 c	-	-	15.20c	-	-
NT15	^{res} POM	0.28	200.00	-15.92	0.66	330.00	-17.81	0.02	0.02	-17.57
	HF	26.97 b	-	-	26.90 b	-	-	26.25b	-	-
NT20	^{res} POM	0.27	93.10	-16.28	0.03	0.03	-19.14	0.02	0.02	-18.52
	HF	26.42 b	-	-	26.30 b	-	-	25.35b	-	-

¹ Areas of cerrado (CE), pasture (PA) and no-tillage system with 3 (NT), 15 (NT15) and 20 (NT20) years of implementation. * Means followed by the same lowercase letters column comparing the areas assessed in each extractor evaluated separately by t test at 5% significance level. – The methodology does not allow review.

It is difficult to discuss the results obtained for $_{res}POM$ with respect to the extraction of LFOM performed with flotation in water, since there is a scarcity of studies that relate subsequent steps of this method in an attempt to determine the C contents in the sand fraction, that is, the reports finalize the LFOM extraction. According to Conceição et al. (2007), $_{res}POM$ should have been, to a great extent, separated by the solutions used for densimetric fractionation, and the decrease in the contents of C in this fraction indicates an increase in the efficiency

of the solution in separating the light fraction of SOM.

Regardless of the layer and area evaluated, the lowest contents of C in $_{res}POM$ were found after extraction with SPT, whereas the highest contents were observed after extraction with water and NaI (Table 4). This pattern is consistent with previously discussed results and indicates that the SPT solution was more efficient in separating LFOM from the soil. Similar results were observed by Conceição et

al. (2007), who detected a reduction in the contents of C in $_{res}POM$ after extraction with SPT. This situation is more evident when the POM/LFOM ratio between the extractants in the evaluated areas is compared, since extraction using SPT also resulted in lower values (0 in some areas, Table 4). Such results justify the higher mass and contents of C in LFOM extracted with SPT. According to Conceição et al. (2007), a greater efficiency in the extraction of LFOM with SPT can be achieved when a density of 2 kg L⁻¹ is used, further reducing the C concentration in $_{res}POM$ without significantly increasing the contamination of the light fraction by clay carbon.

On the basis of the POM/LFOM ratios, extraction of LFOM with water resulted in a low recovery efficiency of this fraction, since the $_{res}POM$ fraction represented on average approximately 52% of the C (0-0.05 m) and 166.13% of the C (0.05-0.1 m) of LFOM in the evaluated areas (Table 4). These results indicate that the difference in the mass and contents of C in LFOM extracted with NaI and SPT in comparison with the amounts extracted with water contributes to the $_{res}POM$ fraction, and that the contents of C and N in LFOM are underestimated when extracted with water. This same explanation is also plausible for the differences in the mass and contents of C and N between the LFOM extracted with NaI and SPT, i.e., the lower efficiency of the extraction of LFOM with NaI leads to higher C values in the $_{res}POM$ fraction. Similar results were reported by Roscoe et al. (2001), who found that the C recovered in the sand fraction with NaI (1.7 Mg m⁻³) was up to four times higher than that observed in the light fraction.

The values of $\delta^{13}C$ in $_{res}POM$ extracted with water, NaI, and SPT (Table 4) were close to those of $\delta^{13}C$ of the soil (Table 2) and somewhat distant from the LFOM values after separation with the extractants (Table 4). These results indicate that the CE area continued to show traces of C3 plants and the PA area, traces of C4 plants, and the NT areas showed a slight tendency to reduce the $\delta^{13}C$ signal

from NT3 to NT20 (Table 4). Regardless of the analyzed layer, these values were more positive for the NT areas, indicating a greater contribution of the C4 plants. The increase in the $\delta^{13}C$ signal relative to the LFOM extracted with water, provided by the LFOM fraction that was not extracted by this method, may be related to intra-aggregate LFOM residues, which preserved this fraction when the vegetation that occupied the area was of the C4 type and/or due to the contribution of residues of corn and possibly invasive offseason grasses.

The contents of C in HF after extraction of LFOM with water, NaI, and SPT showed the same pattern of total C in the soil (Table 4). Similar to Guareschi et al. (2013), our results suggest an increase of the more stable fractions of SOM according to the NT deployment time. These results also show that in the HF of the soil in the studied areas, C is prevalent, representing on average 96% and 99% of the C (extraction with water), 97% and 98% of the C (extraction with NaI), and 95% and 96% of the C (extraction with SPT) in the layers of 0-0.05 and 0.05-0.1 m, respectively. Similar results were reported by Conceição et al. (2007) and Demolinari et al. (2008) who observed that, after extraction of LFOM with SPT and/or NaI and water in Latosol regions, most of the SOM is associated with the mineral components of the soil, i.e., HF.

Differences were observed for the results between the fractions of LFOM (FLF and LOF) extracted with different solutions (NaI and PTS), both at the same and different densities (water); extraction with SPT was the most appropriate. Even representing 2-5% of SOM, we observed that LFOM is very sensitive to the way the soil is handled and used. Changes in the soil-plant system directly affects the quantity and quality of LFOM, and, therefore, also affects nutrient cycling and the population of soil organisms that use this fraction for their maintenance. This fact has also been reported by several studies, which mention that LFOM is an attribute that is very sensitive to variations in soil use and management (SOUZA et al., 2006; FARIA et al., 2008; CARNEIRO et al., 2013).

Conclusions

The NT according to the deployment time elevated the C (0-0.05 m) and N (0-0.2 m) contents of the soil. The origin of C in the soil of the NT areas is associated with C4 plants; however, at a depth of 0.2 m, the ^{13}C contents reduce according to the NT deployment time.

Extraction of LFOM with SPT better represents this SOM fraction quantitatively and qualitatively than extraction with water and NaI. This pattern is evident because of a greater consistency in the C, N, and ^{13}C contents and mass values of LFOM extracted with SPT among the evaluated areas and by the lower C content in $^{\text{res}}$ POM among the extractants.

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