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Carbon, nitrogen and the natural abundance of ^{13}C and ^{15}N in macro and microaggregates

Carbono, nitrógeno y la abundancia natural de ^{13}C y ^{15}N en macro y microagregados

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ABSTRACT

This study aimed to measure the concentrations of carbon (C), nitrogen (N) and the natural abundance of ^{13}C and ^{15}N in macro and microaggregates under systems of land use. We sampled the 0-5 and 5-10 cm layers in no-tillage system (NTS), conventional tillage system (CTS), secondary forest and pasture in southern Brazil. The largest variations of C and N concentrations were found for the 8-2 mm aggregate class, with the sequence pasture > forest = NTS > CTS, compared to the 0.25-0.105 mm class. The most negative $\delta^{13}C$ values were found in the microaggregates and the least negative were found in the macroaggregates. Plowing and harrowing in the CTS cause the fracturing of soil aggregates, accelerating the mineralization reactions of soil organic matter (SOM) and discouraging the occlusion of SOM in the aggregates, resulting in higher $\delta^{15}N$ values compared to the other systems evaluated. The larger variation in C and N content for the 8-2 mm class and smaller variation in the 0.25-0.150 mm class indicate that there is higher and lower sensitivity to the management system adopted in these areas and lesser and greater protection of the C and N by these aggregate classes, respectively.

Key words: aggregate classes, isotope composition, no-tillage system, conventional tillage system.

RESUMEN

Este estudio tuvo como objetivo medir las concentraciones de carbono (C), nitrógeno (N) y la abundancia natural de ^{13}C y ^{15}N en macro y microagregados en los sistemas de uso de la tierra. Tomamos muestras de las capas 0-5 y 5-10 cm en el sistema de siembra directa (NTS), sistema de labranza convencional (CTS), bosque secundario y pastizales en el Sur de Brasil. Se encontró más grandes variaciones en las concentraciones de C y N para la clase agregados 8-2 mm, con la secuencia de pastos > bosque = NTS > CTS, en comparación con la clase desde 0,25 hasta 0,105 mm. Además, se encontró que los valores de ^{13}C más negativos en los microagregados y el menos negativo se encontraron en los macroagregados. Arando y grada en el CTS causa la fractura de los agregados del suelo, lo que acelera las reacciones de mineralización de la materia orgánica del suelo (SOM) y desalentando la oclusión de SOM en los agregados, lo que resulta en valores ^{15}N altos en comparación con los demás sistemas evaluados. Las variaciones más grandes en C y N de contenido para la clase de 8-2 mm y las variaciones más pequeñas de la clase 0,25 hasta 0,150 mm indican que hay mayor y menor sensibilidad al sistema de gestión adoptado en estas áreas y la protección menor y mayor de la C y N por estas clases de agregados, respectivamente.

Palabras clave: *clases de agregados, la composición isotópica, sistema de labranza cero, sistema de labranza convencional.*

Introduction

Soil aggregation is mainly influenced by the following five factors: soil fauna, soil microorganisms, roots, inorganic agents and environmental variables. There is a multiplicity of interactions among these factors that ultimately lead to the formation and stabilization of aggregates (Bronick and Lal, 2005; Six *et al.*, 2004; Rillig and Mummey, 2006). In the factors mentioned above, the cultivation system (roots) used stands out, since it is directly related to the amount of carbon (C) and nitrogen (N) observed in the different soil aggregate classes (Salton *et al.*, 2008; Loss *et al.*,

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2009, 2011) and in the association with mycorrhizal fungi (Rillig and Mummey, 2006).

The impact of the soil management systems on the dynamics of soil organic matter (SOM), that is, on the C and N cycle in agroecosystems, deserves special attention. Management systems capable of maintaining and/or increasing the levels of C and N in the soil can contribute to the maintenance of productive capacity of soils and decrease CO_2 and NO_2 emissions into the atmosphere (Barreto *et al.*, 2009; Cerri *et al.*, 2010). Thus the availability of practical and efficient methods to assess the dynamics of these elements is necessary, especially in macro and microaggregates of soil (Cecillon *et al.*, 2010; Turbé *et al.*, 2010; Loss *et al.*, 2011; Costa Junior *et al.*, 2011a, b).

Reviewing the progress in our knowledge about soil aggregation made over the last 50 years reveals that there are still few studies that are quantitative and/or consider interactive effects among the five factors responsible for aggregation. The quantification of these interactions is clearly needed to improve the ability to predict changes in soil ecosystems due to agricultural management and climate change (Six *et al.*, 2004; Rillig and Mummey, 2006).

The technique of natural abundance of ^{13}C and ^{15}N in the soil aggregates is useful to examine more specifically the changes resulting from soil use systems, either regarding the change in vegetation coverage or between different crop systems such as NTS and CTS (Zotarelli *et al.*, 2007; Loss *et al.*, 2011, 2012; Costa Junior *et al.*, 2011a, b).

Considering the importance of maintaining C and N within the different soil aggregate classes and the changes promoted by the management systems used, this study aimed to measure the levels of C, N and the natural abundance of ^{13}C and ^{15}N of the soil aggregates in NTS and CTS areas and compare them with areas of forest and pasture in southern Brazil.

Material and Methods

Location, climate and soil in the study area

The study was performed in a rural property in Marmeleiro, southwest Paraná State, Brazil. Climate in the area is subtropical (Koppen Cfa climate), with well-defined seasons characterized by mild winters and hot summers. Rainfall is well distributed throughout the year. The soil is classified as an Alfisol (Soil Survey Staff, 2010) with clayey texture. According to the Sistema Brasileiro de Classificação do Solo (Embrapa, 2006), the soil is Nitossolo Vermelho.

The following areas under different soil management systems were evaluated: no-tillage system (NTS) for 15 years, conventional tillage system (CTS) for 56 years, secondary forest and pasture grass (*Axonopus compressus*) established for over 30 years. The original area was composed of Mixed Ombrophilous Forest. The NTS area (S 26° 14’ 43.3” and W 53° 10’ 20.18.4”, 753 m altitude) was cropped with successive soybean/ryegrass planting. The CTS area (S 26° 14’ 53,6’’ and W 53° 10’ 24.6”, 740 m altitude) had been managed with plowing and harrowing for 56 years and always planted with corn. In the last 14 years, the CTS area was planted with tobacco as main crop and corn as off-season crop, and after the corn harvest was sown with black oats as winter herbage for dairy cattle. The forest (S 26° 14’ 35.0” and W 53° 10’ 17.3”, 747 m altitude) and pasture (S 26° 14’ 59.1” and W 53° 10’ 30.3”, 713 m altitude) areas surrounding the croplands were considered as reference, representing the original soil features. In the pasture stocking density was approximately 1.4 animals ha^{-1}, with extensive grazing by dairy cattle. Each animal unit corresponds to 1 animal of 450 kg live weight.

The NTS area is fertilized with 290 kg ha^{-1} of formula 00:18:18 (N-P_2O_5-K_2O, respectively) at soybean sowing and limed every 5 to 6 years with 1,240 kg ha^{-1} of limestone. Ryegrass is sown in March and remains until October, when soybean is sown over ryegrass straw. The CTS area is fertilized with 850 kg ha^{-1} of formulated 10:18:20 (N-P_2O_5-K_2O, respectively) at tobacco planting and receives 400 kg ha^{-1} of urea as top dressing. When undisturbed soil samples were collected, the NTS area was covered by ryegrass while the CTS area had been plowed and harrowed 2 weeks before.

The areas under the different land use systems exhibited similar topography, soil conditions and climate, with only NTS and CTS differing in relation to the practices adopted (crop rotation and soil tillage). As such, a glebe or nearly 1.0 ha (100 m x 100 m) was established and undisturbed samples were collected in four trenches opened across the sowing lines. In the pasture area cattle grazing sites were avoided, and in the forest samples were collected in the central portion of the fragment, close to the other areas. The samples were collected among the trees, in places where there had been interference from the root system of trees. Soil samples were taken from the 0-5 cm and 5-10 cm layers, and a composite sample was formed with three undisturbed samples from each layer, with four replicates per area. The samples were identified, stored in plastic bags and taken to the laboratory, where they were air dried and sieved through 8.0 and 4.0 mm mesh to separate the aggregates used in the analysis.

To obtain water-stable aggregates, a method described in Embrapa (1997) was used. The levels of total C and N for the soil aggregates were determined by the method of dry combustion in a C and N autoanalyzer at 900 ºC (CHN-600 Carlo Erba EA-1110, Italy) from the Laboratory of Isotopic Ecology, CENA (Center for Nuclear Energy in Agriculture) in Piracicaba, Sao Paulo State, Brazil. Since no carbonate C is present in this Alfisol, the total C is referred as the organic C.

The ^{15}N and ^{13}C values were measured using a mass spectrometer (Finnigan Mat Delta Plus, Germany) from the Laboratory of Isotopic Ecology, CENA. We used aggregates of the following classes: 8.0 mm ≥ X > 2.0 mm, 2.0 mm ≥ X > 1.0 cm, 1.0 mm ≥ X > 0.5 cm, 0.5 mm ≥ X > 0.25 mm, and 0.25 mm ≥ X > 0.105 mm, in accordance with Embrapa (1997).

Homogeneity of soil characteristics such as source material, texture, and soil class were considered during the selection of the areas studied. Therefore we may assume that the environment or medium behaves uniformly in the experimental units, in such a way that the soil attributes are easily identified as homogeneous in order to receive the treatments. Consequently, the experimental design used can be considered a completely randomized design (CRD), composed of four areas (NTS, CTS, forest and pasture) or treatments, with four pseudo-repetitions. A CRD is used because it is considered the statistically simplest design. In this design, the experimental units are randomly distributed and the number of repetitions may be the same or different. To use a CRD it is only necessary that the environment or medium behave uniformly in all the experimental units and that these units can be easily identified in order to receive the treatment (Hurlbert, 1984; Costa Junior *et al.*, 2011a).

The results obtained were checked for normality by the Lilliefors test and for homoscedasticity by the Cochran & Bartlett test. After that, they were subjected to variance analysis with application of the F test and mean values were contrasted using the LSD-Student test at 0.05 significance level.

Results and Discussion

The highest concentrations of C and N were found in the pasture area for the 8-2 mm class. For other aggregate classes, the highest concentrations of C and N were found in the pasture, forest and NTS areas, the lowest values of these elements being observed in the CTS area for the two depths evaluated (Table 1).

The highest values of C and N in the pasture area are due to intense exploration of this area by the grass roots (missionary grass), which leads to higher C stocks, and also to higher values in the more labile portions of the C. According to Salton *et al.* (2008) and Denef and Six (2006), root growth increases microbial activity and the production of aggregation agents, in addition to mechanical forces that also stabilize the aggregates. As such, macroaggregates have been continuously forming with a consequent accumulation of C and N, as shown by the largest size class (8-2 mm, Table 1).

A contrary pattern was observed in the CTS, which had the lowest concentrations of C and N, showing that the management system used (plowing and harrowing) is unfavorable to maintaining the C levels in the soil aggregates, regardless of the class of aggregate assessed. Similar results were reported by Fabrizzi *et al.* (2009) who evaluated the stocks of C and N in aggregates of tropical (Brazil) and temperate (USA) soils. Compared to the CTS, the authors found higher C and N stocks in NTS areas with twenty years of adoption. This was due to the better maintenance of SOM in the NTS.

The mechanism that leads to the accumulation of SOM in an NTS is due to an optimum situation between the turnover of macroaggregates and mineralization of recent SOM occurring at average rates, which favors the occlusion of the SOM in this aggregate class with subsequent transfer and stabilization in the microaggregates. By contrast, higher turnover of macroaggregates (as in the CTS due to plowing and harrowing) does not allow the recent SOM to be occluded in macroaggregates (due to the breakdown), facilitating its microbial oxidation and consequently loss of SOM (Six *et al.*, 2004). This pattern can be seen in Table 1, where the CTS area showed the lowest concentrations of

Table 1. Total organic carbon and total nitrogen in the different soil use systems in Marmeleiro, Paraná, Brazil.

Management system	Aggregate classes (mm)					
	8-2	2-1	1-0.50	0.50-0.25	0.25-0.105	C.V.(%)
	Total organic carbon (g kg^{-1}) / 0-5 cm					
NTS	29.49 Bc	29.60 Ac	31.95 Abc	33.60 Ab	43.03 Aa	4.60
CTS	17.79 Db	20.17 Ba	20.48 Ca	20.52 Ca	21.15 Ba	3.18
Forest	26.84 Cb	28.99 Ab	32.84 Ab	34.48 Ab	46.53 Aa	16.24
Pasture	36.72 Aa	28.90 Ab	26.12 Bb	25.17 Bb	24.53 Bb	11.22
C.V.(%)	3.60	10.98	9.58	12.19	14.84	
	Total organic carbon (g kg^{-1}) / 5-10 cm					
NTS	22.90 Bc	23.59 ABc	23.00 ABc	27.52 Ab	30.04 Aa	8.55
CTS	16.99 Cb	19.32 Ca	19.68 Ca	20.04 Ca	21.67 Ba	10.51
Forest	24.06 Bb	20.58 BCc	21.74 BCbc	23.64 Bb	30.08 Aa	6.49
Pasture	40.81 Aa	25.84 Ab	26.87 Ab	26.72 Ab	31.58 Aab	17.40
C.V.(%)	9.05	10.96	15.46	13.34	10.67	
	Nitrogen (g kg^{-1}) / 0-5 cm					
NTS	2.77 Bc	3.02 ABbc	3.28 Abc	3.43 Ab	4.27 Aa	8.83
CTS	1.70 Cb	2.06 Ca	2.05 Ba	2.07 Ba	2.19 Ba	7.66
Forest	2.99 AB^{ns}	3.26 A^{ns}	3.77 A^{ns}	3.77 A^{ns}	4.69 A^{ns}	18.33
Pasture	3.12 Aa	2.53 BCb	2.26 Bb	2.21 Bb	2.18 Bb	12.14
C.V.(%)	5.15	11.71	13.54	16.94	16.30	
	Nitrogen (g kg^{-1}) / 5-10 cm					
NTS	2.33 Bb	2.39 BCb	2.43 ABb	2.79 Aab	3.18 Aa	10.22
CTS	1.77 Cb	2.07 Ca	2.12 Ba	2.13 Ba	2.23 Ba	6.91
Forest	2.66 Bb	2.52 Bb	2.65 Ab	2.88 Ab	3.57 Aa	12.66
Pasture	3.77 A^{ns}	2.87 A^{ns}	2.79 A^{ns}	2.81 A^{ns}	3.11 A^{ns}	14.85
C.V.(%)	11.02	7.36	14.34	15.51	11.65	

Means followed by the same upper case letter in one column do not differ between the systems of soil use for each class of aggregates and the same lower case letter in one row indicates no difference between classes of aggregates for each system evaluated (LSD-student test; $p < 0.05$). ns = not significant by F test ($p < 0.05$). CV = coefficient of variation.

C and N in all aggregate classes compared to the NTS area.

There were higher levels of C and N in aggregates of 8-2 mm and lower concentrations in the 0.25-0.105 mm class for the pasture area. For the other areas a pattern opposite to that of the pasture area was observed, with higher values of C and N in the smaller aggregate class (0.25-0.105 mm) and lower values in the largest size class (8-2 mm) (Table 1). Similar results to these were reported by Passos *et al.* (2007) who evaluated the C and N levels in macro and microaggregates of an Oxisol in the *cerrado*. The authors attributed this pattern to higher organic reserves present in the smaller aggregates, which are mainly associated with the clay fraction.

In the pasture area, the highest concentrations of C in the 8-2 mm aggregates, both between systems and between aggregate classes, may be caused by greater incorporation of fresh organic matter into the macroaggregates, especially in the 0-5 cm layer. In the pasture area, higher levels of light organic matter (LOM) arising mainly from the root system (visual observations in the field) were observed. In other words, there are more roots (missionary grass in the pasture) that promote aggregation and this larger quantity of roots is reflected in higher LOM values, because roots are recent organic matter in the soil. According to Denef *et al.* (2006), the entry of crop residues (LOM) stimulates recycling (maintenance) of aggregates > 0.25 mm, which are important for the stabilization of the soil organic matter over time.

In the CTS it was found that for the C and N levels there were differences only for the 8-2 mm class, where higher values were observed. For the other aggregate classes similar values were observed (Table 1). This pattern is a result of plowing and harrowing, which is done frequently in this area to prepare the soil. Through such operations, the breakdown of aggregates (mainly macroaggregates) occurs. And when the stability of water-stable aggregates is evaluated, most of the macroaggregates are unstable in water, which leaves the C and N exposed that had been protected inside the aggregates. Thus there are lower levels of C and N in the 8-2 mm class and homogeneous values in the other aggregate classes. To support this assertion, there is the homogenization of the ^{13}C and ^{15}N values in all aggregate classes at both depths evaluated; this pattern was not observed for the other areas (Table 2).

The ^{13}C(‰) values of the pasture area are an indication of the predominance of C_4 photosynthetic cycle plants, while the other areas show indication of C_3 photosynthetic cycle plants. For the CTS area, the influence of planting corn (C_4) can be seen, with values less negative than those for the NTS and especially the forest which best represents the isotopic signal of C_3 plants (Table 2).

Furthermore, in the CTS area the similar values of ^{13}C and ^{15}N in all aggregate classes and at the two depths evaluated are an indication of instability of the 8-2 mm aggregates in water, with posterior mixture of the C and N that were protected in the macroaggregates. Thus there is greater accessibility to these nutrients by microbial activity, with consequent increase in the emission of greenhouse gases into the atmosphere.

Table 2. Natural abundance of 13C and 15N in the different soil use systems in Marmeleiro, Paraná, Brazil.

Management system	Aggregate classes (mm)					
	8-2	2-1	1-0.50	0.50-0.25	0.25-0.105	C.V.(%)
	Natural abundance of ^{13}C(‰) / 0-5 cm					
NTS	–24.54 Ca	–25.08 Cab	–25.64 Cb	–25.55 Cb	–26.29 Cc	1.38
CTS	–23.48 B^{ns}	–23.49 B^{ns}	–23.14 B^{ns}	–23.03 B^{ns}	–23.11 B^{ns}	1.14
Forest	–26.12 D^{ns}	–26.17 D^{ns}	–26.37 D^{ns}	–26.26 D^{ns}	–26.99 C^{ns}	2.80
Pasture	–18.72 Aa	–19.01 Aa	–19.35 Ab	–19.78 Ac	–19.58 Abc	0.93
C.V.(%)	2.29	2.40	1.58	1.29	1.54	
	Natural abundance of ^{13}C(‰) / 5-10 cm					
NTS	–23.66 Ca	–24.01 Cab	–24.08 Bb	–24.36 Cb	–24.85 Cc	0.81
CTS	–22.65 B^{ns}	–22.84 B^{ns}	–22.86 C^{ns}	–22.78 B^{ns}	–22.86 B^{ns}	1.64
Forest	–25.24 D^{ns}	–24.88 C^{ns}	–24.99 C^{ns}	–25.38 C^{ns}	–25.51 C^{ns}	3.86
Pasture	–18.12 Aa	–19.32 Ab	–19.21 Ab	–19.40 Ab	–18.26 Aa	2.03
C.V.(%)	2.28	2.19	2.71	2.77	2.35	
	Natural abundance of ^{15}N(‰) / 0-5 cm					
NTS	7.07 Ba	6.12 Bbc	6.36 Bb	5.79 Bc	5.01 Cd	3.76
CTS	8.11 A^{ns}	8.26 A^{ns}	8.05 A^{ns}	8.04 A^{ns}	8.03 A^{ns}	2.36
Forest	5.51 Ca	4.72 Cabc	3.75 Cbc	4.76 Cab	3.71 Dc	12.52
Pasture	8.01 A^{ns}	7.82 A^{ns}	7.86 A^{ns}	7.82 A^{ns}	7.70 B^{ns}	4.55
C.V.(%)	3.56	5.53	8.83	5.22	1.83	
	Natural abundance of ^{15}N(‰) / 5-10 cm					
NTS	7.87 Aa	5.49 Bbc	6.22 Bb	5.04 Bc	4.92 Bc	6.86
CTS	7.82 A^{ns}	7.71 A^{ns}	7.73 A^{ns}	7.34 A^{ns}	7.38 A^{ns}	6.69
Forest	6.69 Aa	6.13 Bb	4.98 Cc	5.14 Bc	5.14 Bc	5.36
Pasture	7.08 A^{ns}	6.33 B^{ns}	6.04 B^{ns}	5.93 B^{ns}	5.68 B^{ns}	12.44
C.V.(%)	7.50	7.42	7.81	11.23	7.48	

Means followed by the same upper case letter in one column do not differ between the systems of soil use for each class of aggregates and the same lower case letter in one row indicates no difference between classes of aggregates for each system evaluated (LSD-student test; $p < 0.05$). ns = not significant by F test ($p < 0.05$). CV = coefficient of variation.

Among the aggregate classes, the most negative ^{13}C values were recorded in the microaggregates and the least negative were found in the macroaggregates (8-2 mm) (Table 2). This pattern was also reported by Costa Junior *et al.* (2011a) in a study on the origin of ^{13}C in macro, meso and microaggregates in *Cerrado* soil. These authors reported that the pattern observed suggests that the formation of these aggregates occurred in association with the newly incorporated C in the macroaggregates. According to Six *et al.* (2002) and De Galdo *et al.* (2003), the most negative ^{13}C results in the microaggregates and the least negative in the macroaggregates demonstrate that the C is more susceptible to oxidation in the macroaggregates (preferably), but more protected in the microaggregates. This pattern can be confirmed with the results in Table 1, where there were higher levels of C in the microaggregates compared to macroaggregates at the two depths studied, excluding the pasture area.

Looking at the C and N levels in the land use systems evaluated, one can see wider variations between the areas for the 8-2 mm macroaggregate class and smaller variations in the 0.25-0.105 mm microaggregate class (Table 1). These results suggest greater and lesser sensitivity, respectively, to the management adopted in these areas and lesser and greater protection of C by these aggregate classes.

The highest values of ^{15}N in the CTS area (Table 2) are indicative of the higher rate of decomposition and mineralization of the SOM, with a consequent decrease in the values of C and N (Table 1) compared to the other areas. At the 5-10 cm depth, except for the 8-2 mm class where there was no difference between the areas, it can be seen that the highest values of ^{15}N are in the CTS area (Table 2), with the lowest values of C and N (Table 1). These results are similar to those of Mendonça *et al.* (2010), who studied the identification of forest changes using isotopic techniques with ^{13}C and ^{15}N in soils of the Chapada do Araripe in Ceará, Brazil. They noted that the lowest values of ^{15}N were associated with greater amounts of SOM - this being found in the *Cerrado* soil. On the other hand, in the *Caatinga* area where the lowest C values were observed, the highest ^{15}N values were found, which is an indication of SOM decomposition.

According to Bustamante *et al.* (2004), as the reactions of mineralization, nitrification, denitrification and volatilization occur, the remaining SOM becomes enriched in ^{15}N atoms. In other words, the management adopted in the CTS area is accelerating these reactions, resulting in greater losses of C and N in the form of gases released into the atmosphere. This pattern is reflected in lower levels of these elements found in all aggregate classes assessed for the CTS (Table 1).

Among the aggregate classes, the NTS, forest and pasture areas showed higher ^{15}N values for the 8-2 mm class and a decrease in values in accordance with a reduction of the aggregate class for the two depths studied (Table 2). This pattern indicates that in the macroaggregates, particularly the 8-2 mm class, N is more susceptible to mineralization compared to the microaggregates (0.25-0.105 mm).

Conclusions

The CTS disfavors occlusion of SOM in the aggregates, as this had the lowest concentrations of C and N in all diameter classes compared to the NTS. The use of the practices of plowing and harrowing the soil of CTS cause the disruption of soil aggregates, accelerating the reactions of SOM mineralization, with higher ^{15}N values compared to other land use systems evaluated.

The more negative ^{13}C values in microaggregates (0.25-0.105 mm) indicate greater protection of C, while less negative values in macroaggregates (8-2 mm) indicate C recently incorporated.

Among the land use systems evaluated, the largest variations in the levels of C and N (pasture > forest = SPD > SPC) for the class of macroaggregates (8-2 mm) and minor variations in class microaggregates (0.25 - 0.150 mm) indicate that there is greater and lesser sensitivity to the management adopted in these areas and lesser and greater protection of C and N for these classes of aggregates, respectively.

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