Soil fertility, humic fractions and natural abundance of ^{13}C and ^{15}N in soil under different land use in Paraná State, Southern Brazil

Fertilidad del suelo, fracciones húmicas y abundancia natural de ^{13}C y ^{15}N en el suelo bajo diferentes usos del suelo en el estado de Paraná, Sur de Brasil

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ABSTRACT

Humic fractions of soil organic matter (SOM) and measurements of ^{13}C and ^{15}N isotope can be used to highlight differences between management systems with different intensities of land use. This study characterized soil fertility, quantified carbon levels in the humic fractions and evaluated the natural abundance of ^{13}C and ^{15}N in systems cultivated under no-tillage system (NTS) and conventional tillage system (CTS) or used with secondary forest or perennial pasture in Marmeleiro, Parana State, Southern Brazil. NTS was more efficient than the conventional tillage system (CTS) in increasing pH (0.0-0.10 m layer), Ca (0.0-0.05 m layer), P (except 0.05-0.10 m layer) and N (0.0-0.10 m) levels, total organic carbon (TOC) stocks (0.0-0.20 and 0.0-0.40 m layers); carbon of the humin fraction (C-HUM) in 0.0-0.40 m; the fulvic acid fraction (C-FAF) and humic acid (C-HAF) in 0.0-0.05 m. The use of grasses, in NTS and pasture, increased TOC stocks compared to the other soil use or management systems evaluated in the 0.0-0.40 m layer. In the topsoil layer, the anthropogenic influence of plowing and harrowing in CTS promoted greater loss of carbon in C-HUM, C-FAF and C-HAF than NTS, forest and pasture. In CTS, growing corn for 42 years after the removal of forest cover did not alter the ^{13}C at 0.0-0.40 m. In pasture, the absence of legumes, constant deposition of cattle manure and a more stable organic matter favored high ^{15}N levels (except at 0.0-0.05 m in CTS). The decrease in ^{15}N values from the 0.0-0.10 to 0.10-0.20 m layer in CTS indicates that soil turnover (by plowing and harrowing) has the potential to disturb the depth-related variation in soil ^{15}N, accelerating decomposition and compromising N transformations. Among the variables analyzed, the determination of carbon in humic fractions and ^{15}N values were efficient in identifying soil changes produced by land use or management systems.

  Key word: humic substances, carbon stocks, isotopic composition, no-tillage, conventional tillage.

RESUMEN

El uso de las fracciones húmicas de la materia orgánica del suelo (MOS) y las mediciones isotópicas de ^{13}C y ^{15}N se puede utilizar para resaltar las diferencias entre los sistemas de gestión con diferentes intensidades de uso de la tierra. El estudio caracteriza la fertilidad del suelo, cuantifica los niveles de carbono en las fracciones húmicas y evaluó la abundancia natural de ^{13}C y ^{15}N en el sistema de siembra directa (NTS), sistema de labranza convencional (CTS), bosque secundario y pastizales en el Sur de Brasil. NTS es más eficaz en el aumento de pH (0,0 a 0,10 m), niveles de Ca (0,0 a 0,05 m), P (excepto 0,05-0,10 m) y N (0,0 a 0,10 m); los estoques de carbono orgánico total (TOC) (0,0-0,20 y 0,0 a 0,40 m), y los niveles de carbono de la fracción humina (C-HUM) en 0,0-0,40 m; carbono de la fracción de ácido fúlvico (C-FAF) y ácidos húmicos (C-HAF) en 0,0-0,05 m, que el sistema de labranza convencional (CTS). El uso de hierbas, en NTS y pastizales, aumenta las reservas de TOC en comparación con los otros sistemas de uso y manejo de suelos evaluados en 0,0-0,40 m. En la capa superior del suelo la influencia antropogénica de arar y desgarradora, en CTS promove una mayor pérdida de carbono en C-HUM, C-FAF y C-HAF en comparación con NTS, bosque y pastos. En CTS, el cultivo de maíz durante 42 años después de la eliminación de la cubierta forestal no alteró el ^{13}C en 0,0-0,40 m. En pastos, la ausencia de legumbres, la deposición constante de estiércol de ganado y una materia orgánica más estable favorece a los niveles altos ^{15}N (excepto en 0,0-0,05 m en CTS). La disminución de los valores ^{15}N en 0,0-0,10 a 0,10-0,20 m en CTS indica que la rotación del suelo (por el arado y desgarradora) tiene el potencial para perturbar la variación relacionada con la profundidad en el suelo ^{15}N, acelerando su descomposición y comprometiendo las transformaciones de N. Entre las variables analizadas, la determinación del carbono en fracciones húmicos y los valores de ^{15}N son eficientes en la identificación de cambios en el suelo producidos por el uso del suelo o de los sistemas de gestión.

  Palabras clave: *sustancias húmicas, reservas de carbono, composición isotópica, siembra directa, labranza convencional.*

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Introduction

The conversion of natural systems such as forests to an agricultural system modifies soil fertility and the soil organic matter (SOM) fraction (Kremen and Miles, 2012). Spaccini *et al*. (2006) reported a progressive decrease in humic fraction concentrations in soils that were converted from forest to arable farming. This decrease is attributed to microbial oxidation of the organic materials previously protected in the soil aggregates which are destroyed by cultivation.

However, the adoption of management systems using crop rotation and minimum soil disturbance can increase the levels of soil fertility and SOM fractions. The use of no-tillage systems (NTS) for cropping has increased in Brazil compared to conventional tillage systems (CTS), mainly because NTS are more sustainable in terms of soil conservation and nutrient cycling (Loss *et al*., 2013).

Determination of total organic carbon (TOC) in soil is usually used to assess changes produced by land use, but is unsuitable for detecting changes in soil quality depending on the time of adoption of the system of land and soil management use (Loss *et al*., 2009, 2010; Benites *et al*., 2010). To improve the sensitivity of methods that differentiate soil from different management systems, Loss *et al*. (2010) suggested that in addition to TOC, SOM fractions should be evaluated. Fractioning techniques are used to to this; chemical fractioning is used to separate and quantify carbon in the humic fractions of fulvic acid (C-FAF), humic acid (C-HAF) and humin (C-HUM) (Stevenson, 1994; Loss *et al*., 2010).

Benites *et al*. (2010) reported that the soil humic fractions reflect soil changes produced by anthropogenic activity and are more stable in response to short-term spatial and temporal variations compared to biological and biochemical variables usually evaluated. Thus the characterization of these fractions may serve as a reliable indicator of changes in soil quality.

In a study of an Oxisol (*Latossolo Vermelho-Amarelo in Brazilian classification*), Benites *et al.* (2010) determined carbon content of humic fractions in samples of surface horizon (0-15 cm) and applied discriminant analysis to differentiate soil in the Atlantic Forest and pasture from cropped soil (banana, coffee and annual crops). These authors concluded that TOC levels alone are not sensitive to identify the differences detected by fractioning SOM into C-FAF, C-HAF and C-HUM compounds.

In a study on humic fraction carbon quantification of soil cultivated with crop rotation in an agroecosystem in Seropédica, Brazil, Loss *et al*. (2010) found that fertility parameters (S value = Ca+Mg+K+Na and T value = S + H+Al) were correlated with C-FAF, C-HAF and C-HUM in areas under NTS, but not in areas under CTS. These results indicate that CTS does not favor the production and/or maintenance of carbon in the humic fractions of soil, whereas NTS promote soil fertility by enhancing the carbon content of humic substances.

Methods that evaluate stable SOM isotopes such as natural abundance of ^{13}C and ^{15}N have contributed to study the carbon and nitrogen cycles. They are important for investigating soil changes in areas where vegetation has changed, e.g. forest areas, where C_3 plants have been replaced by C_4 plants such as tropical grasses (Jantalia *et al*., 2007; Loss *et al*., 2012). These methods are also used to evaluate differences between agricultural practices such as NTS and CTS (Costa Junior *et al*., 2011).

The ^{15}N stable isotope is used to evaluate specific N transformation in soil, thereby estimating transformation rates and providing information on the system (Szpak, 2014). Mendonça *et al*. (2010) identified forest changes by applying isotope techniques (^{13}C and ^{15}N) in soil from *Chapada do Araripe* in Ceara state, Northwest Brazil. The lowest ^{15}N levels and highest SOM contents were found in the *Cerradão* (forest), whereas in the *Caatinga* (semi-arid area), which exhibited the lowest organic C levels, ^{15}N was high, indicating greater organic matter decomposition in the *Caatinga* compared to the *Cerradão* area.

Since modifications in areas with different vegetation composition such as the *Cerradão* and *Caatinga* can be assessed by isotope techniques, this procedure can also be used to evaluate soil changes produced by different land use or management systems. To that end, in addition to natural ^{13}C and ^{15}N abundance, chemical SOM fractioning with fertilization characterization can also be analyzed. Therefore, the present study aimed to characterize soil fertility, quantify carbon levels in the humic fractions of SOM, and evaluate the natural abundance of ^{13}C and ^{15}N in systems cultivated under NTS and CTS or used with secondary forest or perennial pasture in Marmeleiro, Paraná State, Southern Brazil.

Material and Methods

The study was conducted on a farm in Marmeleiro, southwest Paraná State, Southern Brazil. The climate in the area is subtropical (Cfa according to the Köppen classification), with well-defined seasons, mild winters and hot summers, and rainfall well distributed throughout the year. The soil is classified as Alfisol (*Soil Taxonomy according to Americam classification*) with clayey texture (*Nitossolo Vermelho according to the Brazilian classification*).

The study was performed in four systems characterized by soil land use or soil management: with crops under NTS for 15 years, crops under CTS for 56 years, perennial summer pasture (*Axonopus compressus*) for livestock husbandry and secondary forest (the last two areas formed over a 30-year period). The area of all systems was originally covered with Mixed Ombrophilous Forest. Only one area was sampled for each system. The area under NTS (S 26° 14' 43.3" and W 53° 10' 20.4", 753 m) was planted with successive soybean/ryegrass crops, and the area under CTS (S 26° 14' 53.6"and W 53° 10' 24.6", 740 m) was managed with plowing and harrowing and was planted with corn. In the previous 14 years, the area under CTS had been planted with tobacco (main crop) and off season corn crop; after the corn harvest, the field was sown with oats for dairy cattle grazing (1.4 animals ha^{-1}) in the winter and beginning of spring. Secondary forest (S 26° 14' 35" and W 53° 10' 17.3", 747 m) and perennial summer pasture areas (S 26° 14' 59.1" and W 53° 10' 30.3", 713 m) adjacent to the cropped areas were used as reference of original soil conditions. In the pasture, stocking density was approximately 1.4 animals ha^{-1}; there is extensive grazing with dairy cattle.

At sampling time, the area under NTS was covered with ryegrass and the CTS area had just undergone plowing and harrowing. The area under NTS received lime application (1,240 kg ha^{-1} of dolomitic limestone) every 5-6 years, and for soybean cropping it received 290 kg ha^{-1} of 00-18-18 (N-P_2O_5-K_2O) fertilizer before sowing. Ryegrass was sown in March and grew until October, when soybean was sown (using NTS) over dried ryegrass straw. The area under CTS received lime application (1,000 kg ha^{-1} of dolomitic limestone) every 3-4 years, and 850 kg ha^{-1} of 10-18-20 fertilizer for tobacco planting, with 400 kg ha^{-1} urea (45% N) as top dressing.

The cropped areas studied were under the same climatic and topographical conditions and differed only in terms of soil use/management. In July, 2008 we opened four trenches in all areas. Across the planting rows of 600 m[2]-plots in the areas under NTS and CTS to collect soil samples. In the pasture area cattle grazing sites were avoided, and in the forest we sampled soil from the central area of the fragment. Soil samples were collected at depths of 0.0-0.05, 0.05-0.10, 0.10-0.20 and 0.20-0.40 m, in triplicate for each layer, producing a composite sample with four pseudo-replications per system. To determine the natural balance of ^{15}N and ^{13}C in soil samples we collected three simple samples, forming a composite sample with four replications at depths of 0.0-0.10, 0.10-0.20, 0.20-0.30 and 0.30-0.40 m. Samples were identified, stored in plastic bags and taken to the laboratory. They were then air-dried and sieved in 2 mm open mesh to obtain air-dried fine soil (ADFS).

ADFS was used to characterize soil fertility (pH in water-saturated soil paste, exchangeable Ca^{2+}, Mg^{2+}, Al^{3+} and K^+ and extractable P) according to routine methods employed by Embrapa (1997). Total N was measured as described by Tedesco et al. (1995). Carbon content, determined according to Yeomans and Bremner (1988), was also used to calculate TOC stock using the equivalent soil mass method (Sisti *et al.*, 2004).

Humic fractions were separated into fulvic acid (FAF), humic acid (FAH) and humin (HUM) using the differential solubility technique established by the International Society of Humic Substances and according to adaptations suggested by Benites *et al*. (2003). To that end, 1.0 g ADFS was immersed in 20 mL NaOH (0.1 mol L^{-1}) for 24 h. The separation between the alkaline extract (AE = FAF + HAF) and the residue (HUM) was carried out by centrifugation at 5000 g for 30 min, after which the tubes were washed with the NaOH solution to a final volume of 40 mL. The residue was removed from the centrifuge tubes, stored in a Petri dish and dried completely at 65 °C. The pH value of AE was adjusted to 1.0 (±0.1) with 20% H_2SO_4 and decanted for 18 h in a refrigerator. The precipitate (HAF) was separated into soluble fraction (FAF) by filtration, and both HAF and FAF were topped up to 50 mL with distilled water.

Quantification of organic carbon in FAF (C-FAF) and HAF (C-HAF) was performed using 5 mL aliquots of the extract mixed with 1 mL

potassium dichromate (0.042 mol L^{-1}) and 5 mL concentrated H_2SO_4 in a digestion block at 150 °C (30 min) and titrated with ammonium iron sulfate (0.0125 mol L^{-1}). C-HUM was determined in oven-dried residue adding 5 mL potassium dichromate (0.1667 mol L^{-1}) and 10 mL concentrated H_2SO_4 in a digestion block at 150 °C (30 min), and titrated with ammonium iron sulfate at 0.25 mol L^{-1} using ferroin as indicator (Yeomans and Bremner, 1988). Measurements of $\delta^{15}N$ and $\delta^{13}C$ were evaluated using a mass spectrometer (Finnigan Mat Delta Plus, Germany) at the Laboratory of Isotope Ecology of CENA (Center of Nuclear Energy in Agriculture) – Piracicaba, São Paulo State.

The homogeneity of soil characteristics was verified before selecting the areas studied to ensure their similarity in terms of source material, soil texture and class. Considering that the areas were under similar environmental conditions, the experimental units were identified as homogeneous to receive the treatments. Therefore, the study adopted a completely randomized design (CRD) comprising four areas (NTS, CTS, Forest and Pasture) (treatments) with four pseudo-repetitions. This CRD is applicable because it is considered to be the simplest statistical design, thereby assuming a random distribution of experimental units, with equal or unequal repetitions per treatment. The use of CRD requires uniform action of environmental over experimental units, in addition to easy identification of these units and the respective treatments (Costa Junior *et al.*, 2011).

The results obtained were checked for normality by the Lilliefors test and for homoscedasticity by the Cochran and Bartlett test. They were then subjected to analysis of variance and the F test, and mean values were contrasted using the LSD-student test at 0.05 error probability.

Results and Discussion

Evaluation of soil fertility

The pasture exhibited the lowest pH in all soil layers evaluated (Table 1) probably because of the

Table 1. Characterization of soil fertility under different land use or management systems in Marmeleiro, Paraná State, Southern Brazil.

Land use/ management system	0.0-0.05 m						
	pH	Ca	Mg	Al	K	P	N
	---------	cmol$_c$ kg^{-1}			mg kg^{-1}		g kg^{-1}
NTS	6.55 A	9.81 B	5.07 B	0.00 B	214.53 C	13.89 A	3.42 B
CTS	5.67 C	6.58 C	6.03 A	0.00 B	221.52 C	8.29 B	2.04 C
Forest	6.34 B	14.83 A	4.84 B	0.00 B	253.55 B	9.10 B	3.13 B
Pasture	5.24 D	6.45 C	3.33 C	0.10 A	456.31 A	13.63 A	4.06 A
C.V. (%)	2.88	5.38	10.65	2.25	7.59	8.34	7.21
	0.05-0.10 m						
NTS	6.31 A	7.42 B	4.11 B	0.00 B	140.42 B	3.93 B	2.29 A
CTS	6.14 B	7.38 B	6.13 A	0.00 B	159.06 B	6.47 A	2.02 C
Forest	6.10 B	14.26 A	1.52 C	0.00 B	128.73 C	3.15 C	2.23 A
Pasture	4.81 C	1.63 C	1.64 C	0.86 A	374.44 A	4.27 B	1.95 B
C.V. (%)	2.98	10.35	21.30	2.02	11.30	12.41	5.12
	0.10-0.20 m						
NTS	5.98 A	5,75 C	3,36 B	0,00 B	156,01 A	5,07 A	1,63 B
CTS	6.15 A	6,48 B	4,48 A	0,00 B	109,12 C	3,43 B	1,82 A
Forest	5.66 B	8,83 A	3,32 B	0,00 B	54,62 D	3,33 B	1,45 C
Pasture	4.88 C	1,07 D	1,09 C	2,25 A	140,42 B	3,05 B	1,86 A
C.V. (%)	2.16	8,86	13,30	3,01	4,87	6,42	6,40
	0.20-0.40 m						
NTS	5.51 A	4.16 B	3.05 B	0.26 C	46.89 B	2.72 A	1.43 A
CTS	5.55 A	6.50 A	4.98 A	0.10 D	53.61 B	1.77 B	1.54 A
Forest	5.29 B	6.59 A	2.00 C	0.37 B	35.10 C	2.82 A	1.09 C
Pasture	4.83 C	1.04 C	0.64 D	2.36 A	89.71 A	2.64 A	1.27 B
C.V. (%)	2.72	6.03	17.31	7.91	8.48	5.40	6.93

Means followed by the same letter in a column within each layer do not differ among areas studied (LSD-student test, P<0.05). CV: coefficient of variation

high Al levels (except at 0.0-0.05 m). NTS, CTS and forest areas showed pH values over 5.6 up to a depth of 0.0-0.20 m, indicating the absence of Al and high Ca and Mg levels. The NTS had higher pH values compared to that under CTS up to the 0.0-0.10 m layer (Table 1). These results stem from the soil management used. In the NTS nutrients and lime were applied in the soil surface, creating greater concentration gradients to the lower layers in comparison to the CTS, where the liming and nutrients were incorporated into the 0.0-0.40 m layer (Almeida *et al*., 2005).

The highest levels of Ca were found in the forest (except at 0.20-0.40 m), the lowest in the pasture (except at 0.0-0.05 m). In the topsoil layer, Ca levels were higher in the NTS than in the CTS. In 0.10-0.40 m, Ca levels were higher in the CTS than in the NTS. The lowest Mg levels were found in all layers of the pasture (except at 0.05-0.10 m) and the highest in the CTS (Table 1). The high Ca levels, mainly observed in the forest, reflect the high natural fertility of this Alfisol, since it is derived from basaltic rock decomposition. It may contain interstratified kaolinite-smectite, thereby explaining the high cation exchange capacity (CEC) of these soils (Teske *et al*., 2013). According to Teske *et al*. (2013), the occurrence of these interestratified minerals in southern Brazil could account for the high CEC detected in a number of basalt-derived soils lacking smectite.

The lowest Ca levels in areas where NTS, CTS and pasture replaced forest can result from extraction of this Ca by soybean/ryegrass crops in NTS; tobacco, corn and oats in CTS and forage grass in pasture. The highest Mg values found in CTS are due to lime application (dolomitic limestone) every 3-4 years and the incorporation of this limestone in the topsoil (0.0-0.40 m layer). Soil fertility in Rio de Janeiro state, Brazil, is considered high according to the study of Freire et al. (2013), who obtained Ca + Mg above 6.10 cmolc dm^{-3} using Embrapa's (1997) method. Therefore, the areas evaluated in the present study, especially the forest, showed high Ca + Mg levels (Table 1). In pasture, the lower Ca + Mg values derive from the removal of forage. Pasture had been grazed daily by cattle and required water and nutrients such as Ca and Mg to resprout and grow.

The highest K levels were found in NTS (0.10-0.20 m layer) and pasture areas (0.0-0.05, 0.05-0.10 and 0.20-0.40 m layers), and the lowest in forest (except at 0.0-0.05 m). P levels were also higher in NTS and pasture areas than in forest (0.0-0.05 and 0.05-0.10 m layers). The highest levels of available P and exchangeable K were found in NTS and pasture areas, probably because of the high ability of grasses to absorb and accumulate P and K, thereby cycling these nutrients. This efficient nutrient uptake is likely related to straw deposition combined with the large, deep root system of grasses (Crusciol and Borghi, 2007), with emphasis on the maintenance of the straw in the NTS and root system renovation of grasses in pasture.

High K levels were found up to a depth of 0.20 m, irrespective of the system evaluated (Table 1), which is probably caused by the gradual release of small amounts of K in the form of mica and feldspar. Melo *et al*. (2004) studied K sources in basalt-derived *Latossolo Vermelho* (Oxisol) and *Nitossolo Vermelho* (Alfisol) in the state of Rio Grande do Sul and found that, although these soils are highly weathered and exhibit predominance of kaolinite in the clay fraction, they have small amounts of primary minerals such as micas and feldspars as well as hydroxy-Al interlayers in smectites, which are potential K sources. Comparing soil types, these authors found more evidence of K-containing minerals in *Nitossolo Vermelho* (Alfisol), and consequent higher potential for K release in this soil type than in *Latossolo Vermelho* (Oxisol).

K levels in the 0.0-0.20 m and 0.20-0.40 m layers were high in all systems (Table 1), probably because of the gradual nutrient release during crop residue decomposition and the use of chemical fertilizer in the NTS and CTS, and animal waste in the pasture. NTS had higher K levels than CTS (at 0.10-0.20 m) and P (except at 0.05-0.10 m). These results indicate that soybean/ryegrass crops with NTS is more efficient in increasing K and P levels than CTS with soil plowing and harrowing.

N content was higher in pasture in the 0.0-0.05 and 0.10-0.20 m layers, and in the NTS and CTS in the 0.20-0.40 m layer. NTS and forest had the highest N levels at 0.05-0.10 m. The forest had the lowest N levels at 0.10-0.20 and 0.20-0.40 m layers, and N levels in the CTS were lower in the topsoil layers (Table 1). The lower N values in CTS (0.0-0.10 m layer) compared to other systems are due to the incorporation of plant residues and practices of plowing and harrowing, accelerating the decomposition and mineralization of SOM as well as decreasing their physical protection.

In other systems, the constant contribution and accumulation of plant residues near the soil surface reduces erosion processes and favors the physical protection of organic matter in organo-mineral complexes, resulting in increased N levels in soil (Loss *et al*., 2009).

Nitrogen levels reflect the dynamics of TOC stocks (Table 2), especially in the NTS, where deeper layers exhibited higher N levels compared to forest and pasture systems. This result demonstrates that legume and grass cropping using NTS increases N levels in the deeper layers.

Total Organic Carbon (TOC) stocks

The highest TOC stocks were found in the NTS (0.0-0.05 m layer) and the lowest in layers of 0.0 to 0.10 m in the CTS. At 0.05-0.10, 0.10-0.20 and 0.0-0.40 m depth, the highest TOC stocks were observed in pasture followed by NTS (Table 2). The high TOC stocks in the top layer of the NTS area derives from ryegrass crop residues and absence of soil disturbance. In pasture, the grass (*Axonopus compressus*) cover explains the high TOC levels, given that it produces a large amount of plant matter with high C/N ratio, and the high rate of root renovation in the perennial grass. This is confirmed with the higher TOC content in deeper layers of this land use. The highest TOC stocks in NTS and pasture corroborate results obtained by Sisti *et al*. (2004), Jantalia *et al*. (2007) and Boddey *et al*. (2010).

In the CTS, the lower TOC stocks in the 0.0-0.10 m layer confirm that practices which result in high soil disturbance such as plowing and harrowing cause negative effects such as decreasing TOC stocks. Similar results were found by Rosa *et al*. (2011) in a study on TOC stocks in clayey soils in Southern Brazil managed with NTS and CTS. These authors found that NTS preserves TOC levels, whereas CTS promotes TOC loss in topsoil (0.0-0.05 m layer). In the 0.20-0.40 m layer, TOC stocks in CTS were as high as in NTS, probably because conventional management practices include soil turnover, which incorporates plant residues deposited on the soil surface into deeper layers, corroborating results obtained by Boddey *et al*. (2010) in soils managed with NTS and CTS.

The decrease in TOC stocks from the 0.05 m layer in forest is due to fact that the lesser influence of plant residues arising from the litter because of disposal of this waste is increased in the topsoil due to the continuous supply of leaves and branches. These results are corroborated by several authors (Loss *et al*., 2012; Teske *et al*., 2013) evaluating the TOC stocks in forest areas. These authors found a decrease of TOC stocks in forest areas compared to the subsurface soil layer with topsoil layer.

The lower TOC stocks in the topsoil layer (0.0-0.10 m) in the CTS than in NTS, pasture and forest systems indicate that the CTS does not promote C stocking over time, proving completely inefficient compared to the other systems studied. These results are confirmed by Loss *et al*. (2014), who evaluated the aggregation index, light organic matter (LOM) levels and mineralizable carbon in the same areas and depths as this study. These authors found the lowest mean geometric diameter and mean weight of the aggregates as well as lower LOM levels and mineralizable carbon in the CTS.

Chemical SOM fractioning

The highest C-HUM levels were found in pasture and forest (0.0-0.05 m layer) and pasture (0.05-0.40 m layers). The lowest C-HUM levels

Table 2. Total Organic Carbon (TOC) stock in an Alfisol under different land use or management systems in Marmeleiro, Paraná State, Southern Brazil.

Depth (m)	Land use/management system			
	NTS	Forest	Pasture	CTS
	Total Organic Carbon Stock (Mg ha^{-1})			
0.0-0.05	18.98 A	16.63 B	17.11 B	11.34 C
0.05-0.10	16.66 B	13.74 C	18.56 A	11.80 D
0.10-0.20	22.69 B	19.06 C	26.68 A	20.77 C
0.20-0.40	35.79 B	30.12 C	48.73 A	36.19 B
0.0-0.40	94.12 B	79.55 C	111.08 A	80.10 C

Means followed by the same letter in a row do not differ among areas studied (LSD-student test, $P<0.05$).

were found in CTS in the 0.0-0.10 m layer. Among the cultivated systems, NTS showed higher C-HUM levels than CTS at 0.0-0.20 m (Table 3).

Pasture also exhibited the highest C-FAF levels, except in the 0.20-0.40 m layer, where there was no significant difference between the areas. The lowest C-FAF content in topsoil was found in the CTS. The profile of C-HAF distribution was similar to that of C-HUM and C-FAF, with the highest levels detected in the pasture area (except the 0.20-0.40 m layer, where pasture did not differ from NTS and CTS) and the lowest in the CTS (at 0.0-0.05 m layer) and forest in 0.10-0.40 m layer (Table 3).

The lowest C-HAF levels in forest (0.10-0.40 m layers) are due to the smaller influence of the plant material (litter) in the subsurface soil associated with lower microbial activity. These results are corroborated by Loss *et al*. (2013), who evaluated C-HAF levels in cultivated areas (NTS and Integrated Crop-Livestock-CLI) and native forest (*Cerradão*). These authors found higher C-HAF values at 0.0-0.10

m and smallest values at 0.10-0.40 m layers in *Cerradão* area compared to NTS and CLI. They concluded that in deeper layers systems without human interference there is a minor contribution of vegetable intake and hence lower microbial activity and less formation of humic acids.

In pasture, the highest values of carbon from humic substances as well as the TOC stocks are associated with the use of the grasses. The permanent roots in the soil contributed to raise the levels of TOC stocks and carbon in the humic substances. According to Llreme *et al*. (2013), it is believed that the root system of pasture grasses explores a larger volume of soil in relation to the annual crops, which remain in the soil for shorter periods (lower cycle). For Roscoe *et al*. (2006), pastures can accumulate TOC that exceed the levels of the soil under native vegetation.

C content in the humic substances from pasture was generally higher, probably as a result of high TOC stocks. However, in the 0.0-0.05 m layer, the

Table 3. Humic SOM fractions in an Alfisol under different land use or management systems in Marmeleiro, Paraná State, Southern Brazil.

Land use/ management system	Humic fraction carbon (g kg^{-1})		
	C-HUM	C-FAF	C-HAF
	0.0-0.05 m		
NTS	23.06 B	4.19 B	3.83 B
CTS	12.00 C	2.23 C	2.90 C
Forest	25.06 A	4.40 B	3.57 B
Pasture	26.00 A	5.94 A	6.17 A
C.V.(%)	9.69	9.53	9.89
	0.05-0.10 m		
NTS	14.81 B	3.25 B	2.84 B
CTS	12.68 C	3.44 B	2.61 B
Forest	15.57 B	3.58 B	2.42 B
Pasture	19.41 A	5.03 A	4.45 A
C.V.(%)	11.35	19.12	15.16
	0.10-0.20 m		
NTS	11.87 B	3.37 B	2.95 B
CTS	9.92 C	3.35 B	2.74 B
Forest	10.90 BC	3.57 B	1.66 C
Pasture	14.74 A	4.50 A	3.65 A
C.V.(%)	9.20	12.16	14.59
	0.20-0.40 m		
NTS	8.98 B	3.73 ns	2.36 A
CTS	9.13 B	2.99	2.27 A
Forest	9.31 B	2.81	0.76 B
Pasture	10.72 A	3.47	2.33 A
C.V.(%)	9.16	16.16	22.41

Means followed by the same letter in a column within each layer do not differ among areas studied (LSD-student test, P<0.05). ns: not significant by the F test. C-HUM: carbon in the humin fraction; C-FAF: carbon in the fulvic acid fraction; C-HAF: carbon in the humic acid fraction. CV: coefficient of variation.

NTS exhibited the highest TOC stocks (Table 2), but C-HUM in this area was low and only higher than in CTS (Table 3). The humification rate was therefore lower in the CTS (72 %) than in pasture (97 %) and forest (87 %). In addition, human activity in the NTS (sowing, cropping and harvesting practices) and deployment time (15 years in NTS and 56 years in CTS) probably delays humification, although these interventions are minimum compared to those in the CTS. This explains why part of the TOC stock did not undergo humification. In areas protected from human interference, plant residue deposition and consequent cycling promoted humification. By the polymerization process linked by the soil microorganisms, lignin and phenol compounds are converted to the main components of humus (Stevenson, 1994). An opposite pattern was observed in the CTS, where the anthropogenic influence of plowing and harrowing hindered the maintenance of C-HUM, C-FAF and C-HAF, especially in topsoil. These results corroborate studies by Loss *et al*. (2010) and Llerme *et al*. (2013) on crop rotation under NTS and CTS.

C levels in C-FAF and C-HAF in the topsoil layer were similar between forest and NTS; CTS showed the lowest levels of C-FAF and C-HAF. This result indicates higher plant straw deposition (possibly associated with the ryegrass root system) in NTS and litter in forest, which increases C levels in labile soil fractions and produces humic and fulvic acids. These results corroborate studies by Loss *et al*. (2013) and Llerme *et al*. (2013).

For CTS, soil managed with plowing and harrowing compromises the maintenance of the SOM humic fractions, highlighting the C-HUM up to a depth of 0.20 m, C-FAF and C-HAF at 0.0-0.05 m layer compared to the other management systems (Table 3). In CTS, the soil disturbance increased the aeration in the soil environment and then accelerated organic matter oxidation. Furthermore, there is disruption of the soil aggregate with consequent exposure of SOM-decomposer microorganisms, thus promoting greater loss of carbon in humic substances. Similar results were obtained by Llerme *et al*. (2013) who studied the influence of soil use on carbon humic substances (C-HUM, C-FAF and C-HFA) of an Oxisol in pasture with *Brachiaria brizantha* for 35 years; grain production (soybean and maize) under CTS for 35 years and NTS (one with maize/maize succession, the other with soybean/maize succession, both for 20 years). The authors

observed that the CTS resulted in increased reduction of carbon humic substances in the 0.0-0.10 m layer compared to the other management systems.

Natural abundance of ^{13}C and ^{15}N in soil

Forest, NTS and CTS systems exhibited lower ^{13}C values at the deepest layers, ranging from -24.19 to -22.08 ‰ in the forest, -22.89 to -20.57 ‰ in the NTS and -22.89 to -22.84 ‰ in the CTS (Figure 1). These results indicate predominance of C_3 plants, especially in the forest, which showed $\delta^{13}C$ values typical of soil from other Brazilian forests. Seed-bearing plants that use RuBisCO (via C_3) for CO_2 fixation exhibit isotopic (^{13}C) composition ranging from -24 to -34 ‰, whereas plants using PEPcase (via C_4) range from -6 to -19 ‰ (Smith and Epstein, 1971).

NTS and CTS areas had ^{13}C values closer to those of forest, but not pasture areas. This is expected because, although NTS and CTS systems contained C_3 plants, they were covered with forest (C_3 plants) before being used for agriculture. After the removal of forest cover, NTS was implanted ones C_3 cycle crops (soybean and ryegrass). Thus, there was no significant change in the ^{13}C values up to 0.0-0.40 m layers. The intermediate values observed between forest and NTS in 0.0-0.40 m layer were probably determined by the isotopic signal of C_4 plants growing spontaneously. In CTS, after the removal of forest cover, C_4 plats (corn) were grown for 42 years, and over the past 14 years, the C_3 plants tobacco and oats. The ^{13}C values in CTS at 0.0-0.40 m indicated that the corn did not alter the ^{13}C signal in relation to forest, with C_3 plants.

Similar results were found by Jantalia *et al*. (2007), who studied the influence of different land use and soil management systems involving grazing and grain production for 12 years on Oxisol (also a *Latossolo Vermelho*) in Planaltina, Brazil. The authors reported that the profile of ^{13}C in depth was similar between cropped areas and cerrado, suggesting that the soil profiles displayed ^{13}C composition similar to that observed prior to the experiment.

In pasture, differing from the areas with NTS and CTS, the removal of forest cover to plant grasses (*Axonopus compressus*) for 30 years changed the isotopic signal of C_3 to C_4 plants in the 0.0-0.40 m layer. These results corroborate studies by Martinelle *et al*. (2009) and Costa Junior *et al*. (2011), where the authors found that removal of forest vegetation
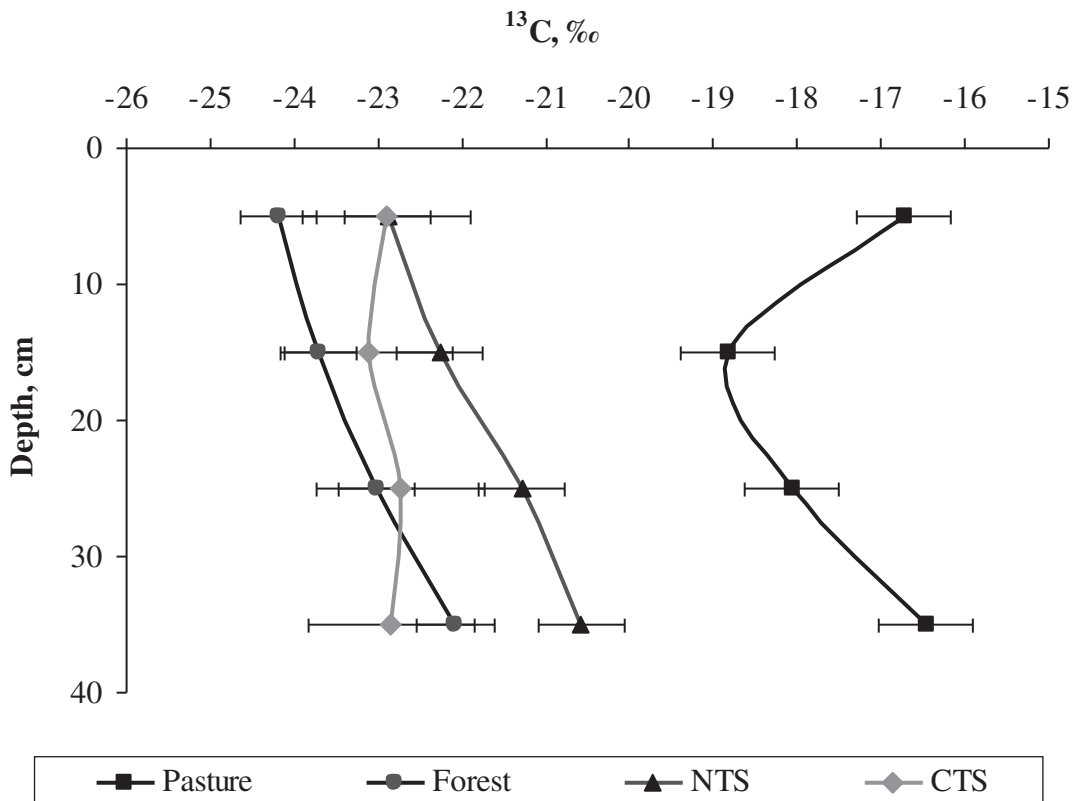
Figure 1. Measurement of ^{13}C isotope composition in an Alfisol under different land use or management systems in Marmeleiro, Paraná State, Southern Brazil (Error bars represent standard error; n=4).

for the establishment of pastures significantly altered the isotopic signal in the soil for C_4 plants.

Isotopic enrichment with ^{15}N increased down the soil profile, mainly in the pasture but also in NTS and forest areas. An opposite pattern was exhibited by the CTS, where ^{15}N decreased from the 0.0-0.10 to 0.10-0.20 m layer, increasing in deeper layers to values higher than those of the topsoil layer. ^{15}N values ranged from 8.29 to 9.81% in the pasture, from 7.58 to 9.40% in the NTS, from 7.20 to 9.58% in the forest and from 8.24 to 8.99% in the CTS (Figure 2).

The isotopic enrichment in ^{15}N in depth (Figure 2) and the reduction of nitrogen concentration with soil depth (Table 2) is a classic pattern of occurrence in soils (Nadelhoffer and Fry, 1994). These authors reported that this pattern is commonly attributed to the preference for ^{14}N over ^{15}N during nitrification and denitrification processes, resulting in lower ^{15}N abundance in the products of these

reactions, and consequently enrichment in abundance of derived waste.

According to Szpak (2014), ^{15}N increases with the age of SOM and the extent of decomposition. Nadelhoffer and Fry (1994), in studies with ^{15}N abundance as an indicator of SOM transformations in different land use or management systems, observed that as organic matter undergoes mineralization, the ammonium that is produced is relatively depleted in ^{15}N and the residual organic matter becomes increasingly ^{15}N-enriched. Organic matter decomposes, reduces in size and travels down the soil profile, creating the characteristic increase in ^{15}N with depth. These results corroborate studies by Martinelli et al. (2009).

The highest ^{15}N values were found in the pasture (0.0-0.40 m layer) and CTS (only 0.0-0.05 m layer). In the pasture, the absence of legumes and the constant deposition of cattle manure likely increased soil ^{15}N. Manures are typically more enriched in
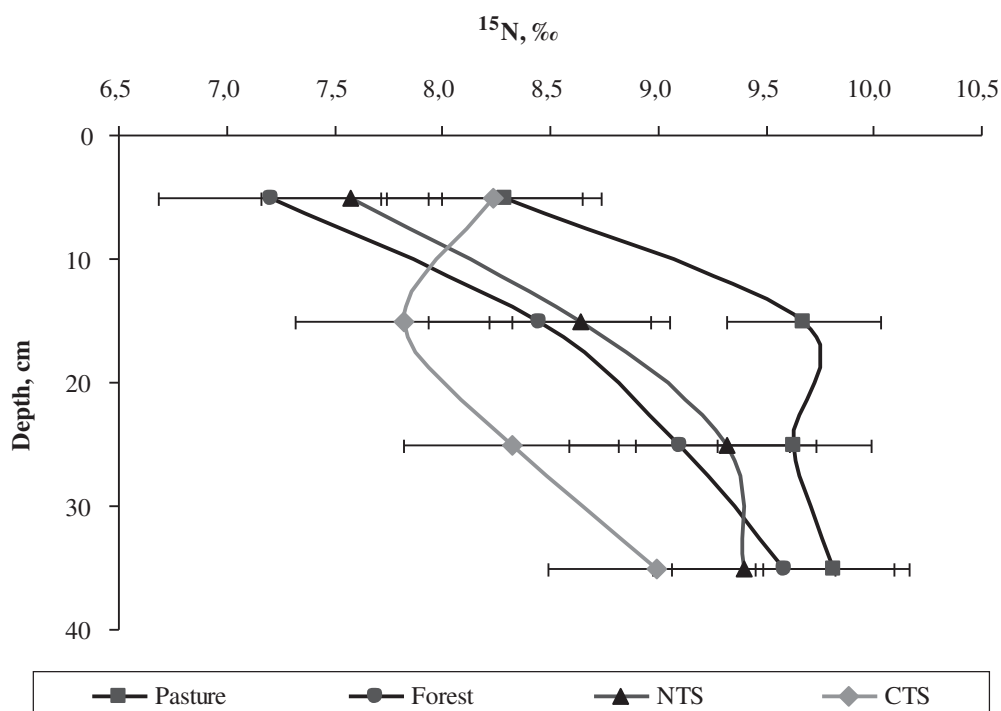
Figure 2. Variation in ^{15}N levels in an Alfisol under different land use or management systems in Marmeleiro, Paraná State, Southern Brazil. (Error bars represent standard error; n=4).

^{15}N than soil, and the deposition of this material increases soil ^{15}N (Szpak, 2014).

The higher ^{15}N levels in pasture can also be attributed to the high chemical stability of its SOM. The most humified SOM fractions usually show higher ^{15}N levels than poorly decomposed forms (Nadelhoffer and Fry, 1988). Thus the pasture area showed higher C-HUM content and humification rate (Table 3), which is the most recalcitrant SOM fraction. C-HUM is associated with the clay fraction of soil (Stevenson, 1994); in uncultivated soils the fine clay contains residual nitrogen derived from microbial biomass turnover and higher content of humified compounds that are enriched in ^{15}N (Zspak, 2014).

In CTS, the values were equal to those found in pasture and were greater than in NTS and forest (0.0-0.05 m), which can be attributed to the absence of legumes. According Trivelin (2002) soils with continuous cultivation of nitrogen fixing legumes (e.g. soybean in the NTS and tree legumes in forest) likely exhibit lower ^{15}N levels than those cropped with non-fixing cultures (e.g., the CTS area, without legumes). The decrease in ^{15}N values from the

0.0-0.10 to 0.10-0.20 m layer in CTS indicates that soil turnover (by plowing and harrowing) moves plant residues from the topsoil (0.0-0.10 m) to deeper layers (0.10-0.20 m), accelerating their decomposition and compromising N transformations.

According to Szpak (2014), the redistribution of N throughout the soil profile associated with tillage has the potential to disturb the depth-related variation in soil ^{15}N. The SOM is redistributed through the soil profile during tilling, consequently compromising N transformations.

Conclusions

The no-tillage system (NTS) is more efficient in increasing pH (0.0-0.10 m), Ca (0.0-0.05 m), P (except 0.05-0.10 m) and N (0.0-0.10 m) levels, TOC stocks (0.0-0,20 and 0.0-0.40 m layers) and humic fraction carbon content (C-HUM, in 0.0-0.40 m; C-FAF and C-HAF, in 0.0-0.05 m) than the conventional tillage system (CTS).

The use of grasses (ryegrass in NTS and *Axonopus compressus* as forage grass in the perennial pasture) increases TOC stocks compared to the

other soil use or management systems evaluated in the 0.0-0.40 m layer.

In the topsoil layer, the anthropogenic influence of plowing and harrowing in CTS promoted greater loss of carbon in humic substances (C-HUM, C-FAF and C-HAF) than NTS, forest and pasture.

In CTS, growing corn for 42 years after the removal of forest cover did not alter the isotopic signal (^{13}C) at 0.0-0.40 m.

In pasture, the absence of legumes, constant deposition of cattle manure and more stable organic matter (with a higher degree of humification) favors high ^{15}N levels (except at 0.0-0.05 m in CTS). The decrease in ^{15}N values from the 0.0-0.10 to 0.10-0.20 m layer in CTS indicates that soil turnover (by plowing and harrowing) has the potential to disturb the depth-related variation in soil ^{15}N, accelerating decomposition and compromising N transformations.

Among the variables analyzed, the determination of the carbon in humic fractions and ^{15}N values were efficient in identifying soil changes produced by land use or management systems.

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