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Characterization of Carbon and Nitrogen Distributed in Density Fractions and Humic Substances from Oxisols under Different Land Uses in the Atlantic Forest Biome in Southeast Brazil

Doctor Geraldo César Rocha

Professor do Programa de Pós Graduação em Geografia da Universidade Federal de Juiz de Fora. Campus Universitário, Bairro Martelos. Juiz de Fora, MG, Brazil. CEP 36036-330. **E.mail:** geraldo.rocha@ufjf.edu.br

Doctor Xiaozeng Han

Pesquisador do Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Haping Lu 138, Harbin 150081, China. **E.mail:** xzhan@mail.neigaeherb.ac.cn

Doctor Haibo Li

Pesquisador do Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Haping Lu 138, Harbin 150081, China. **E.mail:** harbourlee@126.com

ABSTRACT

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This work deals with the characterization of soil organic carbon and nitrogen of oxisols in southeast Brazil. It was studied three soil A horizons located in different spots with distinct land uses. The methodology was based on the different densities of organic soil carbon, using NaI solution (density 1.8 g.cm⁻³) for the separation of the free light fraction, occluded light fraction and heavy fraction. It was concluded that predominates the soil organic carbon in the heavy fraction and that cultivation diminished the carbon in all oxisols studied. The free light fraction carbon was major in the secondary forest, pointing out this fraction as an ecological indicator. The protected humin portion of the heavy fraction may be considered an important soil carbon pool.

PALAVRAS CHAVE:

Carbono orgânico do solo
Frações de densidade do carbono
Carbono em latossolos

RESUMO: CARACTERIZAÇÃO DO CARBONO E NITROGÊNIO EM FRAÇÕES DE DENSIDADE DISTRIBUÍDAS E SUBSTÂNCIAS HÚMICAS DE LATOSSOLOS SOB DIFERENTES USOS DA TERRA NO BIOMA MATA ATLÂNTICA NO SUDESTE DO BRASIL. Foram caracterizados carbono e nitrogênio orgânicos de três horizontes superficiais de latossolos sob diferentes usos no município de Juiz de Fora, Minas Gerais. A metodologia se baseou na extração do carbono usando uma solução de NaI com densidade de 1,8 g.cm⁻³ para separação das frações leve livre, leve oclusa e pesada. Concluiu-se que o carbono orgânico predomina na fração pesada e que o cultivo diminuiu o carbono orgânico em todos os latossolos estudados. A fração leve livre predominou na mata secundária, destacando essa fração como um indicador ecológico importante. A humina da fração pesada mostrou-se como uma efetiva seqüestradora do carbono orgânico.

MOTS-CLÉS:

Carbone organique du sol
Fraction de densité du carbone
Carbone en latossolos

Resumé – CARACTÉRISATION DE CARBONE ET AZOTE DE LA FRACTION DISTIBUE ET MASSE VOLUMIQUE DE SUBSTANCES OXISOLS HUMIQUES SOUS DIFFÉRENTES UTILISATIONS DES TERRES DANS LE BIOME DE LA FORÊT ATLANTIQUE DU SUD-EST DU BRÉSIL. On été caractériser carbone et nitrogène organiques de trois horizons superficiels de sol profond (latossolos) sur les différents usages dans la ville de Juiz de Fora, état de Minas Gerais. La méthodologie s'est basé dans l'extraction du carbone en utiisant une solution de NaI avec la densité de 1,8 g.cm⁻³ pour la séparation de fraction légère libre, légère ocluse et lourde. De cette manière s'est conclu que le carbone organique predomine dans la fraction lourde et que le cultive a diminué le carbone organique dans tous les latossolos étudiés. La fraction légère libre predomine dans le bois secondaire, en détachant cette fraction lourde comme une indicateur écologique importante. La humina de la fraction lourde a s'est montré comme une effective ravisseuse de carbone organique.

Introduction

It is a well known subject that the understanding of the dynamics of organic matter in the soil system is a key parameter to manage the soil carbon (John et al., 2005; Six et al., 2001; Kahle et al., 2003). This management is important due to the facts that, besides disturbing the earth's heat budget, emission of C as CO₂ from the soil results in diminution of soil organic carbon pool, soil fertility and productivity (Rastogi et al., 2002; Lal, 2005). So, it is environmentally interesting to enhance soil C sequestration, which could be achieved reducing C turnover and

increasing its residence time in soils, being the physicochemical protection one of the major mechanisms to maintain the soil C in place (Jastrow et al., 2007).

In this context, the soil aggregates (or its structure) have a special meaning, because they can protect different kinds, or pools, of organic matter. They can be subdivided into macroaggregates (>250 μm) and microaggregates (<250 μm). The actual accepted model that is applied in the analysis of soil density fractionation (Six et al., 2001; John et al., 2005; Paul et al., 2008) considers that the so called light free fraction of organic matter (LFF, with density <1.8 $\text{g}\cdot\text{cm}^{-3}$) is located between macroaggregates, and it is composed basically of materials chemically very close to litter, and has, in general, a very fast decomposition rate. The light occluded fraction (LOF, also with density <1.8 $\text{g}\cdot\text{cm}^{-3}$, but separated only after ultrasonic dispersion) is considered to occupy the space intra-macroaggregates, or the place between microaggregates, showing a more advanced degree of decomposition and a slower turnover. And finally, the so called heavy fraction of the organic matter (HF, density >1.8 $\text{g}\cdot\text{cm}^{-3}$) can, theoretically, be found binded to the clay minerals, and would be composed mainly of highly decomposed material and humus, with a very slow decomposition rate.

It is a matter of concern that the soil organic light fractions are more sensitive to detect changes in the carbon cycle after land use modifications (Freixo et al., 2002; Macedo et al., 2008; Jinbo et al., 2007; Post & Kwon, 2000; Paul et al., 2008; Rangel et al., 2007; Roscoe & Burman, 2003; Cookson et al., 2008) and in Brazil, there are some studies applying the density fractionation method for the separation of soil organic matter components, but focusing mainly in the light fractions to detect those changes.

These studies are concentrated in the Cerrado / Brazilian Savanna biome (Alcântara et al., 2004; Boeni, 2007; Roscoe et al., 2001; Roscoe & Burman, 2003; Zinn et al., 2007; Bayer et al., 2004), Atlantic Forest (Macedo et al., 2008; Freixo et al., 2002; Oliveira Júnior et al., 2008), Amazonia (Koutika et al., 1999; Piendo & Falcão, 2006) and the Pampa biome (Vieira et al., 2007; Dumig et al., 2008). Otherwise, in all of them, except for the work of Oliveira Júnior et al. (2008), the researches don't focus on the carbon content of the separated humic substances (present in the heavy fraction), still being this material the one that holds, for the majority of authors, the higher carbon content of the soil organic matter (Jinbo et al., 2007; Freixo et al., 2002; Cookson et al., 2005; John et al., 2005; Li et al., 2007; Rangel e Silva, 2007; Alcântara et al., 2004). This humic substances, represented by humic acids, fulvic acids and humin, as well as the rates between them, play a key role as parameters related to soil quality (Oliveira Júnior et al., 2008).

The objective of this work was to determine and discuss the carbon and nitrogen content of the density fractions and humic substances of A horizons of Brazilian oxisols under different land uses.

Material and Methods

Study area

The site of this study is located at the southeast region of Brazil, in the municipality of Juiz de Fora city, southeast of Minas Gerais State (figure 1). The climate is tropical of altitude, with fresh winters and mild summers (Cwa after Koppen classification). The topography consists basically of mountains, with flat valleys in "U", and the altitude varies from 900 meters (a.s.l.) until 650 m at the river level. The rocks are old metamorphic gneisses (2,5 b.y.b.p.), and the soils are predominantly red yellow oxisols. The mean yearly rainfall is 1500 mm, and the mean yearly temperature is about 19 °C; the mean in summer is 24 Celsius degrees, with a highest of 35 degrees, and the mean in winter is 12 degrees, with a lowest of 5 °C.

Soil sampling

Three samples of A horizons (0-20 cm) of Red Yellow Oxisols (Latossolos Vermelho Amarelos, Brazilian Soil Classification) were collected in three different locations and land uses at the southeast region of Brazil, Minas Gerais State, municipality of Juiz de Fora town, inside the Atlantic Forest Biome.

The first sample (Profile A) is from a soil located under grassland field, inside the *Campus* of the Federal University of Juiz de Fora. The second, profile B, was collected of a soil situated under secondary forest of a Municipal Ecological Park of Juiz de Fora town, and the third one, profile C, was sampled from a soil under Eucalyptus (*Eucalyptus globulus*) plantation, in the countryside.

Density fractionation

The density fractionation followed the method of Golchin et al. (1994), modified by Li et al. (2007). All the samples were air-dried and grounded in a mortar and sieved (<0,25 mm). In the first step, 10 gr of each one was put in a 100 ml centrifuge tube with 50 ml of a solution of NaI (1,8 g.cm⁻³). The tube was gently turned upside down by hand for five times and left standing at room temperature overnight. After centrifugation (centrifuge Jouan C3) for 15 min at 3500 r/min, the supernatant was filtered through a membrane filter (0,45 µm) into a milipore vacuum unit. The fraction recovered on the filter was washed with 50 ml of a solution of CaCl₂ 0,01 mol/L, and 100ml of distilled water; after that it was transferred to a pre-weighed beaker of 50 ml. The precipitate was resuspended in 40 ml of NaI solution, centrifuged, and filtered as described above. The obtained fraction was added to the previous one, left standing for 24 hrs, and dried in water-bath at 50 °C to constant weight; this was called the free light fraction (free- LF). In the second step, the precipitate was resuspended in 50 ml of NaI solution and shaken for 2 hrs (shaker SHZ 82 A China) at 175 r/min, instead of sonication (Oorts et al. 2005). After that, the tubes were left standing overnight. The centrifugation and filtration procedure (without shaken) was repeated several times as described above, and finally the fraction recovered from the supernatant was transferred to a pre-weighed beaker and water-bath dried at 50 °C to constant weight; this was called the occluded light fraction (occluded-LF). In the third and last step the final precipitate was resuspended in 50 ml of distilled water, shaken for 20 min and centrifuged for 20 min at 4000 r/min. The precipitate was washed with absolute ethanol for several times, until the

supernatant became clear and transparent; then it was transferred to a pre-weighted beaker and oven dried at 50 °C to constant weight. This was called the mineral-associated heavy fraction, H



Figure 1. Location of the studied area, situated southeast of Minas Gerais state.

Subsamples of the density-separated soil fractions, as well as samples of the whole soil (bulk soil) were grounded and passed through a 0,25 mm sieve for organic carbon and nitrogen analysis by dry combustion. It was used a Vario EL III CHN Elemental Analyser (Germany), and the total carbon was considered equivalent to total organic carbon because there is no carbonate present in the soil.

Extraction of humic substances

The chemical extraction of humic substances of the heavy fraction was done using the analytical procedures suggested by Lu (2000). To a 5g subsample of the heavy fraction was added 50 ml of 0.1 mol.L⁻¹ NaOH (pH12-13) solution, kept at a 30°C for 1 hr in an oven, and then centrifuged. The supernatant was transferred to a 250 ml PVC bottle, and this procedure was repeated for 4 times, being the latter extracted solution added to the former one. This obtained fraction was called the loosely combined humus, a mixture of humic acid (HA) and fluvic acid (FA). Secondly, a combined solution of 0.1 mol L⁻¹ NaOH and 0.1 mol L⁻¹ Na4P2O7 was added to the precipitate and kept at constant 30°C for 1 h, then centrifugated; this procedure was also repeated for 4 times and transferred to a 250ml PVC bottle as described above; the extracted fraction was called stably combined humus, composed of HA and FA. Finally, the precipitate, washing off the alkali solution, was called the tightly combined humus, also known as humin (Hm). The loosely and stably combined humus solution was acidified and precipitated by addition of 6 mol L⁻¹ of HCl solution (pH 1-1.5) to separate HA from FA. The extracted humus solution and HA precipitate were water bath dried at 60°C to constant weight. OC content in the fractions was determined by dry combustion as already explained. OC content of FA was determined by subtracting HAOC from the extracted humus fractions.

Results and Discussion

The studied oxisols support three different land uses in the region, i.e., grassland, forest and plantation, that are the main kinds of soil utilization in the area. Table 1 shows the main characteristics of the soil horizons studied.

Table 1. Properties of the A horizons (0-20 cm) of the tested soils.

Plot	BD [‡]	pH (H ₂ O)	SOC	TN	CS	NS
	g cm ⁻³		g kg ⁻¹		Mg hm ⁻²	
PA [†]	0.98	4.8	26.25	2.07	51.4	4.06
PB	0.97	4.7	25.21	1.72	48.9	3.34
PC	1.0	4.9	18.17	1.62	36.3	3.25

[†] PA, PB and PC represent soil profiles in, respectively, grassland, secondary forest and eucalyptus plantation. [‡]BD: bulk density; SOC: soil organic carbon; TN: total nitrogen; CS: carbon stock; NS: nitrogen stock.

It can be observed that the bulk densities are similar, and the soils are very acid in surface. Otherwise, the soil organic carbon denotes differences among the land uses, confirming the general assumption that cultivation reduces its content in oxisols (Freixo et al., 2002; Rangel & Silva, 2007; Roscoe & Buurman, 2003), as can be seen in the smallest value of carbon of plot PC (eucalyptus cultivation), as compared with the non cultivated horizons.

Table 2 shows the organic carbon and nitrogen contents among the different density fractions, as g.kg^{-1} of the bulk soil. It can be seen that the carbon, as well as the nitrogen, predominate in the heavy fraction. It is interesting to point out that the occluded light fraction exhibited the smallest content of organic carbon, in accordance with the results of Roscoe & Buurman (2003) for oxisols from Brazil. Those authors explain that the microstructures of oxisols, having a limited internal volume in relation to their surface, reduce its occluding capacity, a fact that seems to also happens in the soils studied in this work. And among the plots studied it can be seen that the area of plantation (PC) has the smallest values of organic carbon for all fractions, confirming the effect of cultivation on the soil organic carbon (Freixo et al., 2002; Rangel & Silva, 2007). Comparing the free light fraction carbon between the different plots studied, it can be noted that the highest value lies in the area of forest (PB), confirming the fact that this fraction is normally higher in regions with predominantly aboveground organic inputs (Oliveira Junior et al., 2008).

Table 2 . OC and N content in different density fractions (as g kg^{-1} of bulk soil)

Plots [†]	FrLF [‡]		OcLF		HF	
	OC	N	OC	N	OC	N
	g kg^{-1}					
PA	1.33	0.06	0.35	0.02	25.1	2.00
PB	1.96	0.12	0.34	0.02	22.9	1.61
PC	0.70	0.04	0.21	0.01	15.4	1.40

[†] PA, PB and PC represent soil profiles in, respectively, grassland, secondary forest and eucalyptus plantation.

[‡]FrLF, free light fraction; OcLF, occluded light fraction; HF, heavy fraction.

In table 3 it is shown the organic carbon, as g.kg^{-1} of the whole soil, found in the humic substances of the heavy fraction of soils. It is useful to observe that in the heavy fraction it is found the humic and fulvic acids, as well as the humin.

Table 3 . Variation of OC distributing in combined humus extracted from heavy fraction of Brazilian Oxisols (as g kg^{-1} of whole soil.)

Plots [†]	LCH [‡]			SCH ^{**}		TCH
	HA	FA	HA/FA	HA	FA	Hm
	g kg^{-1}					
PA	4.48	7.08	0.63	----	----	13.49
PB	7.00	4.88	1.43	----	----	11.01
PC	3.64	4.37	0.83	----	----	7.41

[†] PA, PB and PC represent A horizons (0-20 cm) of soil profiles developed, respectively, under grassland, secondary forest, and eucalyptus plantation.

[‡]LCH: loosely combined humus; SCH: stably combined humus; TCH: tightly combined humus; HA: humic acid; FA: fulvic acid; Hm: humin.

**The amount of HA and FA extracted by using NaOH together with Na₄P₂O₇ was too small to conduct the determination of C and N.

It can be observed the predominance of humin in all plots, a fraction that was separated as tightly combined humus. This expression means that this kind of carbon is tightly linked to the clay fraction of the soil, a fact extremely important to the carbon sequestration by the soil, as also noted Cabria et al. (2005) and Laird (2001). Looking the data of the so-called loose combined humus, which can be divided in humic and fulvic acids, it is possible to see that there are differences between the plots. So, in the PA and PC horizons, respectively under grassland and eucalyptus cultivation, the carbon of the fulvic acids predominate, while the contrary occurs in the PB plot (secondary forest), with predominance of humic acid. It is in accordance with other researchers, for example Oliveira Jr et al. (2008), who postulated that the highest values of carbon in the humic acids, compared to the carbon found in the fulvic acids indicate more preserved and well managed soils. The predominance of humic acids carbon in the secondary forest shows that in this environment would be a major ecological equilibrium, compared to the other areas, more managed by man. This fact is confirmed when we look the values of the relations between humic and fulvic acids, also displayed in the table 3; the highest relation is observed in the secondary forest. Oliveira Jr et al. (2008) justified that this highest value indicates a condensation more significant of the humic compounds, and consequently a major content of organic carbon inside the soil.

Conclusion

The study of the oxisols organic carbon under different kinds of use showed that the cultivation has diminished its content. Among the different density fractions, the carbon of the heavy fraction has predominated, followed by the free light fraction, and finally by the occluded light fraction. The free light fraction, considered as an indicator of the variations of the carbon due to cultivation, was major under secondary forest, confirming its value as an ecological indicator. At the humus, the humin fraction carbon has predominated, pointing to the importance of this fraction in the sequestration of carbon by the soil.

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