

## Oxidizable organic carbon fractions in soils under leguminous nitrogen-fixing trees in a degraded pasture

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**ABSTRACT**: The chemical fractionation of C by an increasing oxidation gradient, has shown to be a fast and promising methodology to detect changes in C lability. The objectives of the present study presents were: to evaluate the level of lability of soil organic C after conversion of degraded pasture into leguminous trees; evaluate the influence of soil depth on the lability of soil organic C. The experimental area consisted of pure plantations of Acacia auriculiformis, Mimosa caesalpiniifolia and Inga sp., a pasture and a secondary forest. The oxidizable organic carbon was determined by wet oxidation and allowed separation of four fractions according to the lability level (labile, moderately labile, moderately recalcitrant, recalcitrant). Labile fraction was the predominant fraction in all vegetation covers and depths. The conversion of degraded pasture into forest legume plantations and the soil depth promoted changes in the chemical composition of C. The continuous deposition of vegetable residues 13 years of leguminous trees favored the distribution of labile and moderately labile fractions along the soil profile and the recalcitrant fraction in the topsoil. The reference covers contributed to the recalcitrant fraction in the soil below 20 cm depth. **Key words**: carbon, lability, organic matter, leguminous trees, forest soils.

# Frações oxidáveis do carbono em solos sob leguminosas florestais fixadoras de nitrogênio em pastagens degradadas

**RESUMO**: O fracionamento químico de carbono (C) por um gradiente crescente de oxidação, tem se mostrado uma metodologia rápida e promissora para detectar alterações na labilidade de C. Os objetivos do presente estudo foram: avaliar o nível de labilidade do C orgânico do solo após a conversão de pastagens degradadas em leguminosas; avaliar a influência da profundidade do solo na labilidade do C orgânico do solo. A área experimental consistiu em plantios puros de Acacia auriculiformis, Mimosa caesalpiniifolia e Inga sp., uma pastagem e uma mata secundária. O carbono orgânico oxidável foi determinado por oxidação úmida e permitiu a separação de quatro frações de acordo com o nível de labilidade (lábil, moderadamente lábil, moderadamente recalcitrante, recalcitrante). A fração lábil foi a fração predominante em todas as coberturas vegetais e profundidades. A conversão de pastagens degradadas em leguminosas florestais e a profundidade do solo promoveram mudanças na composição química do C. A deposição contínua de resíduos vegetais por 13 anos de leguminosas favoreceu a distribuição das frações lábil e moderadamente lábil ao longo do perfil do solo e da fração recalcitrante na camada superficial do solo. As coberturas de 20 cm de profundidade. **Palavras-chave**: carbono, labilidade, matéria orgânica, leguminosas florestais, solos florestais.

## **INTRODUCTION**

The Atlantic Forest is one of the most threatened biomes in the Brazil, covering an area of 100 to 120 million hectares, with only 12.5% of its original forests (http://www.inpe.br/). The situation is even more serious in the state of Rio de Janeiro, mainly due to the intensive cultivation of sugarcane and coffee. After the decline of these crops, the cultivated areas were replaced by pastures, which are currently the main rural activity in the region. The usage pressure is still high in other areas, leading to induced burning of regenerating vegetation or even forest in order to expand pasture areas (GAMA-RODRIGUES et al., 2008).

Tree planting is a viable alternative for both the recovery of degraded pastures and the composition of agroforestry systems in combination

Received 06.24.21 Approved 01.28.22 Returned by the author 04.01.22 CR-2021-0488.R1 Editors: Leandro Souza da Silva D Frederico Vieira with agricultural yield and pastures (NAIR et al., 2009). More promising results can be obtained with the use of leguminous trees, especially when inoculated with bacteria of the *Rhizobium* genus and/or with arbuscular mycorrhizal fungi, favoring the biological fixation of  $N_2$  and nutrient absorption, respectively (CHAER et al., 2011).

Forest systems are characterized as carbon (C) accumulators in the soil given the regular deposition of plant residues above and belowground. The roots are responsible for C accumulation both in relation to biomass accumulation, and in the turnover and release of exudates (RUMPEL & KOGEL-KNABNER, 2011). In this sense, studies on C dynamics in forest systems should consider the soil at depths aiming to know the contribution of the tree roots to the C stock in the deeper layers of the soil profile (VICENTE et al., 2016). Carbon stored in greater depths in different places of the world represents more than 50% of the total stored in the soil (SOUSSANA & LEMAIRE, 2014).

The chemical composition of the organic matter (OM) is also an important aspect in the research of organic soil C dynamics. This chemical composition can be influenced by the soil use, by the composition of the organic residues, and by the microbial products (HELFRICH et al., 2006). The chemical fractionation of C by an increasing oxidation gradient, has shown to be a fast and promising methodology to detect changes in the chemical composition of OM according to lability level of organic C. It has been used in several situations of soil use and management once that certain of these organic C fractions are more sensitive for detecting effects of land management practices (BLAIR et al., 1995; CHAN et al., 2001; BARRETO et al., 2014; BATISTA et al., 2018).

In this context, the present study presents the following hypotheses: (1) The leguminous nitrogenfixing trees as a result of N input produces a more labile soil organic C than the forest and pasture; (2) The topsoil present a more labile soil organic C than deep soils. Thus, the objectives were: to evaluate the level of lability of soil organic C (assessed in terms of degree of oxidizability) after conversion of degraded pasture into leguminous trees; evaluate the influence of soil depth on the lability of soil organic C.

## MATERIALS AND METHODS

## Description of the study area and sample collection

Samples were collected in an experimental area at Carrapeta farm, Conceição de Macabú, RJ, Brazil (21° 37' S and 42° 05' W). The climate of the region is Am (hot and humid) according to the Köppen classification, with an annual average temperature of 26 °C and an average annual rainfall of 1,400 mm. The relief is strong wavy, with a slope of around 0.35 m m<sup>-1</sup>. The soil is classified as a typical Dystrophic Red-Yellow Argisol according to the Brazilian Soil Classification System (SANTOS et al., 2018).

The study was carried out with an unfertilized pasture and revegetated with pure tree plantations of the leguminous trees Acacia auriculiformis A. Cunn. ex Benth., Mimosa caesalpiniifolia Benth. and Inga edulis Mart. plantations at 13 years of age. The leguminous trees (planted in December 1998) were inoculated with selected strains of atmospheric N<sub>2</sub> fixing bacteria and arbuscular mycorrhizal fungi (GAMA-RODRIGUES et al., 2008). The other two vegetation covers were a forest fragment of the Atlantic Forest in secondary ecological succession without presence of leguminous trees and a degraded pasture (dominance of Melinis minutiflora, Paspalum maritimum and Imperata brasiliensis). The forest fragment representing a condition of original vegetation and pasture a degraded area (burning cycles, without any type of management), both approximately 50 years old (GAMA-RODRIGUES et al., 2008).

In the middle of each experimental plot  $(1500 \text{ m}^2)$  three trenches (1 x 1 x 1.5 m) were dug between the plant rows (separated by at least 25 m). The soil samples were collected at six depths: 0-10; 10-20; 20-40; 40-60; 60-80 and 80-100 cm. The following determinations were executed: soil particle size; soil bulk density; pH; exchangeable Ca<sup>2+</sup>; Mg<sup>2+</sup>; Al<sup>3+</sup>; P; K; and total N using methods describe in EMBRAPA, (1997) (Tables 1 and 2).

#### Chemical carbon fractionation

The method used for C fractionation was adapted from CHAN et al., (2001). The soil samples were macerated ( < 0.5 mm). The soil subsamples with 10 ml of the 0.167 mol L<sup>-1</sup> K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution and increasing quantities of concentrated sulphuric acid (2.5, 5, 10 and 20 ml) were joined into digestion tubes, resulting in acidic aqueous concentrations of 0.25:1, 0.5:1, 1:1 and 2:1 (corresponding to 3, 6, 9 and 12 mol L<sup>-1</sup> respectively of H<sub>2</sub>SO<sub>4</sub>). The tubes were placed in the digester block at 135 °C for 30 minutes. The tubes were cooled to room temperature and then the volume was completed up to 70 ml with BaCl<sub>2</sub>. This mixture was remained overnight (approximately 12 hours). Afterwards, the supernatant was read in a spectrophotometer at 600 nm wavelength

Table 1 - Chemical and physical attributes of soil under different vegetation covers in Conceição de Macabú County, Rio de Janeiro State, Brazil.

Vegetation cover	pH	Р	Ν	Ca	Mg	K	Al	Sand	Silt	Clay
	$(H_2O)$	$(mg kg^{-1})$	$(g kg^{-1})$	(g kg <sup>-1</sup> )		l <sub>e</sub> kg <sup>-1</sup> )		(g kg <sup>-1</sup> )		
			0	-10 cm						
Secondary forest	4.0	1.8	1.3	0.3	0.4	0.1	1.8	606	62	332
Pasture	4.3	1.2	0.8	0.6	0.3	0.1	1.2	668	72	260
Acacia a.	4.6	4.8	1.4	1.2	0.6	0.1	0.3	742	56	202
Inga edulis	4.6	4.6	1.4	0.9	0.5	0.1	0.4	703	66	231
Mimosa c.	4.6	4.3	1.4	0.5	0.4	0.1	0.4	672	66	262
			1	0-20 cm-						
Secondary forest	4.2	1.8	0.0	0.11	0.40	0.1	2.2	546	71	383
Pasture	4.2	1.0	1.1	0.04	0.04	0.03	3.6	630	75	295
Acacia a.	4.2	1.6	1.2	0.02	0.32	0.1	2.4	658	68	274
Inga edulis	4.2	1.6	1.3	0.87	0.48	0.1	2.4	632	86	282
Mimosa c.	3.9	1.4	0.9	0.16	0.25	0.1	2.1	538	85	377
			2	0-40 cm-						
Secondary forest	4.1	0.6	0.9	0.02	0.2	0.03	2.1	434	81	485
Pasture	4.1	0.4	0.7	0.06	0.1	0.02	2.0	574	78	348
Acacia a.	4.1	0.6	0.8	0.5	0.6	0.06	1.9	507	83	410
Inga edulis	4.2	0.8	0.8	0.2	0.4	0.04	2.9	530	88	382
Mimosa c.	4.1	0.6	0.9	0.1	0.3	0.03	2.8	396	88	516
			4	0-60 cm-						
Secondary forest	4.2	0.4	0.6	0.1	0.2	0.01	0.01	404	78	518
Pasture	4.2	0.4	0.7	0.1	0.04	0.01	0.01	475	86	439
Acacia a.	4.3	0.4	1.1	0.5	0.5	0.02	0.02	405	63	532
Inga edulis	4.3	0.6	0.8	0.2	0.2	0.02	0.02	415	99	486
Mimosa c.	4.1	0.4	0.8	0.1	0.1	0.01	0.01	375	81	544
			6	0-80 cm-						
Secondary forest	4.0	0.4	0.5	0.1	0.1	0.01	0.01	371	80	549
Pasture	4.7	0.2	0.5	0.7	0.2	0.01	0.01	408	95	497
Acacia a.	4.1	0.6	0.6	0.3	0.3	0.01	0.01	387	67	546
Inga edulis	4.3	0.4	0.6	0.3	0.1	0.02	0.02	403	84	513
Mimosa c.	4.1	0.4	0.6	0.1	0.1	0.02	0.02	358	80	562
			8	0-100 cm						
Secondary forest	4.2	0.4	0.4	0.1	0.1	0.01	0.01	415	89	496
Pasture	4.9	0.2	0.5	0.7	0.2	0.01	0.01	402	103	495
Acacia a.	4.0	0.6	0.6	0.1	0.2	0.01	0.01	418	65	517
Inga edulis	4.3	0.4	0.6	0.1	0.1	0.02	0.02	407	85	507
Mimosa c.	4.2	0.4	0.6	0.1	0.03	0.01	0.01	338	72	590

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 Table 2 - Soil bulk density and oxidizable organic C fractions of soil under different vegetation covers in Conceição de Macabú County, Rio de Janeiro State, Brazil.

Vegetation cover	BD	F1	F2	F3	F4
	(Mg m <sup>-3</sup> )		(Mg ha)		
Secondary forest	1.17	0-10 cm 17.36 c	3.23 b	2.71 b	3.03 a
Pasture	1.17	30.05 b	5.34 ab	6.78 a	3.74 a
Acacia a.	1.28	40.13 a	6.45 a	6.27 a	4.06 a
Inga edulis	1.28	40.13 a 32.32 b	6.54 a	5.10 a	4.00 a 5.69 a
Mimosa c.	1.31	28.07 b	6.34 a	2.69 b	5.93 a
mimosu c.		10-20 cm		2.090	J.95 a
Secondary forest	1.14	13.03 c	3.84 bc	2.13 bc	3.06 ab
Pasture	1.53	27.28 ab	4.96 ab	6.12 a	3.15 ab
Acacia a.	1.56	31.29 a	4.90 ab	3.90 b	4.80 a
Inga edulis	1.30	22.93 b	5.03 a 5.12 ab	4.05 ab	4.80 a 1.55 b
Mimosa c.	1.31	22.93 b 15.78 c	3.00 c	4.03 ab	3.19 ab
mimosu c.		20-40 cm		1.57 C	5.19 au
Secondary forest	1.11	19.27 b	3.42 b	2.80 c	3.86 a
Pasture	1.11	19.27 b 35.88 a	5.33 b	2.80 c 8.15 a	4.12 a
Acacia a.	1.45	28.39 a	4.26 b	6.61 ab	4.12 a 0.93 b
Inga edulis	1.45	28.39 a 19.66 b	4.20 0 17.66 a	7.58 a	0.95 0 2.65 ab
Mimosa c.	1.36	20.24 b	4.79 b	3.71 bc	2.63 ab
mimosu c.		20.24 0		5.71 00	2.34 a0
Secondary forest	1.09	12.23 b	1.55 b	5.45 a	1.05 b
Pasture	1.26	12.25 o	3.91 a	4.56 ab	3.12 ab
Acacia a.	1.20	16.58 a	3.72 a	3.81 ab	1.54 b
Inga edulis	1.4	10.38 a 19.21 a	4.28 a	3.22 bc	3.61 a
Mimosa c.	1.23	12.67 b	4.60 a	1.77 c	1.55 b
<i></i>		60-80 cm	4.00 u	1.770	1.55 0
Secondary forest	1.11	8.99 b	1.15 c	4.57 a	0.87 c
Pasture	1.34	14.90 a	3.97 a	2.57 bc	2.79 a
Acacia	1.36	13.14 a	2.75 b	3.10 b	2.79 d
Inga edulis	1.29	14.09 a	4.08 a	2.48 bc	1.32 c
Mimosa c.	1.21	10.14 b	2.88 b	1.43 c	1.60 bc
	1.21	80-100 cm	2.000		
Secondary forest	1.11	8.39 a	1.93 b	1.38 a	4.17 a
Pasture	1.28	11.21 bc	3.43 a	1.48 a	2.46 b
Acacia	1.34	10.02 ab	2.39 ab	2.25 a	2.87 ab
Inga edulis	1.32	11.22 a	3.51 a	1.21 a	1.29 b
Mimosa c.	1.21	7.77 c	1.89 b	1.60 a	1.33 b

The values followed by the same letter are not subjected to differences between the vegetation covers by Tukey test, at 5% probability. BD = bulk density, F1 = labile fraction, F2 = moderately labile fraction, F3 = moderately recalcitrant fraction, F4 = recalcitrant fraction.

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(ANDERSON & INGRAM, 1996). Four oxidizable organic carbon (C) fractions were obtained:

LF - fraction constituted by the oxidizable organic C obtained from the solution of 3 mol  $L^{-1}$  H<sub>2</sub>SO<sub>4</sub>, and corresponds to the labile fraction;

MLF - fraction obtained by the difference between the oxidizable organic C extracted from the solutions of 6 mol  $L^{-1}$  and 3 mol  $L^{-1}$  H<sub>2</sub>SO<sub>4</sub>, corresponding to the moderately labile fraction;

MRF - fraction obtained by the difference between the oxidizable organic C extracted from the solutions of 9 mol L<sup>-1</sup> and 6 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>, which corresponds to the moderately recalcitrant fraction;

RF - fraction was obtained by the difference between the oxidizable organic C extracted from the solutions of 12 mol  $L^{-1}$  and 9 mol  $L^{-1}$  H<sub>2</sub>SO<sub>4</sub>, which corresponds to the recalcitrant fraction.

The oxidizable organic C fractions data were corrected by the clay content, using secondary forest as reference as suggested by MORAES et al., (1996) as the organic C levels variations were closely related to clay content. The following formula was used for the correction calculation for each depth:

organic C fractions (corrected) = organic C fraction (measured g 100g<sup>-1</sup>) x <u>clay content (reference)</u> clay content (treatment)

The oxidizable organic C fractions were also corrected by the levels of compaction. The following equation was used to calculate the thickness and compaction of the soil layer (ELLERT & BETTANY, 1995):  $E_{ad/sub} = (M_{ref.} - M_{treat.})/Ds/100$ 

where

 $E_{ad/sub}$  = depth to be added or subtracted in the stock of organic C fractions calculation (cm);

 $M_{ref.}$  = soil mass at the reference soil depth (Mg ha<sup>-1</sup>);  $M_{trat.}$  = soil mass at the assessed soil depth (Mg ha<sup>-1</sup>); and Ds = soil bulk density (g/cm<sup>3</sup>).

#### Statistical analyses

The data was subjected to the Lilliefors Kolmogorov-Smirnov and normality test and homoscedasticity and normal distribution residuals, which evaluates the normal distribution of analyzed variables. One-way analysis of variance (ANOVA) and the Tukey test was used to to evaluate the differences of oxidizable organic C fractions as a completely randomized design with three replicates. The data was analyzed using StatSoft inc. (1974-2009) and STATISTICA 8.0 software. Principal components analysis (PCA) was subsequently performed using the R<sup>®</sup>v.3.2.1 program (R Core Team 2015) and the Vegan package (OKSANEN et al. 2016). For this analysis, the oxidizable organic

C fractions of the different depths were grouped to obtain average values for two group of soil layers: 0-20 cm – average of oxidizable organic C fractions in the 0-10 cm and 10-20 cm layers; and 20-100 cm – average of oxidizable organic C fractions in the 20-40 cm, 40-60 cm, 60-80 cm and 80-100 cm layers. Thus, we obtained two sets of variables, one for the 0-20 cm layer (LF 0-20, MLF 0-20, MRF 0-20 and RF 0-20) and another for the 20-100 cm layer (LF 20-100, MLF 20-100, MRF 20-100 and RF 20-100). This separation had the objective to evaluate the dissimilarity between the vegetation covers considering the superficial soil layer and the deepest layer of the soil profile.

#### RESULTS

#### Oxidizable organic C fractions

LF fraction was the predominant fraction in all vegetation covers and depths. At depth of 0-10 cm, the soil under Acacia auriculiformis presented higher value than the other vegetation covers, while secondary forest had the lowest value. In the depth of 10-20 cm, Acacia auriculiformis and pasture showed the highest value and this last one did not differ from Inga edulis. Secondary forest and Mimosa caesalpiniifolia showed the lowest values. There was little variation in LF fractions between vegetation covers in the depth of 20-80 cm. However, below 80 cm the highest values of LF were observed in secondary forest, Inga edulis and Acacia auriculiformis. LF represented around 26 Mg ha<sup>-1</sup> of labile C stored up to 20 cm. From this depth we can see a reduction in the contribution of this fraction to the SOC formation (Table 2).

The MLF fraction presented smaller variation between the vegetation covers when compared to LF and without a defined differentiation pattern. MLF represented about 5 Mg ha<sup>-1</sup> of moderately labile C up to the first 20 cm and there was a slight increase up to 40 cm and a decrease below this depth in the contribution of this fraction in the SOC formation (Table 2).

MRF up to 60 cm depth represented around 4 to 6 Mg ha-1 and below this depth around 1.5 to 2.8 Mg ha-1 of moderately recalcitrant C. Up to the first 40 cm pasture, *Acacia auriculiformis* and *Inga edulis* generally presented higher values of MRF to the other vegetation covers. The secondary forest presented a higher value of this fraction between 40 and 80 cm, however there was no significant difference between the vegetation covers below 80 cm.

RF fraction did not differ between the vegetation covers in the first 10 cm, while there was slight variation in the other depths, but also with no defined pattern. A slight reduction in RF was observed only for secondary forest and pasture. This fraction represented around 4 Mg ha<sup>-1</sup> of recalcitrant C in the first 20 cm and decreased up to 100 cm depth (Table 2).

The averages of the oxidizable organic C fractions of the 0-20 cm and 20-100 cm layers were associated with the first two main components. Principal component 1 (PC1) explained 46.69 % of the variation between the vegetation covers and the principal component 2 (PC2) explained 32.85 %. The variables that contributed most to the formation of PC1 and therefore better explained the dissimilarity between the vegetation covers were: RF 0-20, LF 20-100, MRF 20-100, RF 20-100 and MRF 0-20. The most relevant variables in PC2 were the LF 0-20 and MLF 0-20. The MLF 20-100 variable was more associated to PC3, presenting a small contribution to explain the dissimilarity between the vegetation covers; and therefore was not considered in the present study (Table 3). The graphical dispersion of the vegetation covers and the set of variables given by the PCA suggested a dissimilarity between the leguminous trees and the reference covers. All leguminous trees were located to the left of the diagram, with the Acacia auriculiformis and Inga edulis in the upper quadrant, and the Mimosa caesalpiniifolia in the

lower quadrant. Secondary forest and pasture were located in the lower right quadrant (Figure 1).

#### DISCUSSION

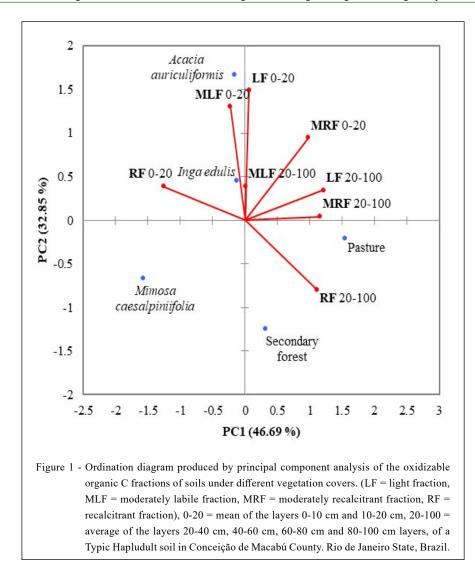
In all vegetation covers, the LF fraction predominated along the soil profile. These results suggested that the leaf litter, fine roots and deep roots (mass, turnover and exudates), and woody material during the decomposition process carried out by a complex interactions among soil biota, minerals and environment are metabolized in different molecules, in this case, predominantly labile form, along the soil profile. This recently formed soil organic matter may follow different pathways of mineralization and/or stabilization. Then, the degree and depth of incorporation of litter-root derived organic matter into the soil are a result of functional complexity derived from the interplay between spatial and temporal variation of molecular diversity and composition (LEHMANN et al., 2020).

Mainly the most labile fractions showed a decrease with depth increase. This tendency would be related to the spatially heterogenous distribution of fresh C and the soil microbial biomass along the soil profile and to differences in the C sources (more recalcitrant compounds from the roots), as well as the products resulting from the transformation of organic matter and the greater interaction with the mineral

Table 3 - Results of Principal Component Analysis (PCA) of the oxidizable organic C fractions of soils under different vegetation covers in Conceição de Macabú County, Rio de Janeiro State, Brazil.

Variables	Principal comp	Principal componentes				
vallables	1	2				
Factor loadings of major components						
F <sub>1</sub> 0-20	0.047	0.995				
F <sub>2</sub> 0-20	-0.171	0.872				
F <sub>3</sub> 0-20	0.735	0.634				
F <sub>4</sub> 0-20	<u>-0.942</u>	0.260				
F <sub>1</sub> 20-100	<u>0.910</u>	0.230				
F <sub>2</sub> 20-100	0.007	0.264				
F <sub>3</sub> 20-100	0.867	0.029				
F <sub>4</sub> 20-100	<u>0.836</u>	-0.534				
Variability	46.69	32.85				
% accumulated	46.69	79.54				

(F1 = labile fraction, F2 = moderately labile fraction, F3 = moderately recalcitrant fraction, F4 = recalcitrant fraction); 0-20 = mean of the layers 0-10 cm and 10-20 cm, 20-100 = average of the layers 20-40 cm, 40-60 cm, 60-80 cm and 80-100 cm layers.



fraction when compared to the topsoil (RUMPEL & KNABNER, 2011).

The dissimilarity of the leguminous trees in relation to the reference covers was verified through the PCA. The continuous deposition of vegetable residues 13 years after the conversion of degraded pasture into leguminous trees favored the formation of different proportions of C compounds into the soil via decomposition, and consequently an accumulation of soil organic C with different lability levels along the soil profile. COSTA et al., (2014), in the same vegetation covers of the present study, reported that Acacia, which presented higher levels of lignin, cellulose and polyphenols, accumulates more litter on the soil surface and Mimosa obtained a higher rate of decomposition and shorter nutrient residence time. Thus, providing greater protection of the soil by one species and greater availability of nutrients by another, respectively. According to LEHMANN & KLEBER, (2015) and LEHMANN et al., (2020), there is a *continuum* of many different organic compounds at various stages of decay at any time within a living soil. The decomposition pathways is a function of spatial arrangement of soil organic matter and environmental conditions once soil microrganisms change their activity in tandem with moisture and temperature fluctuations.

Additionally, the location of the secondary forest and pasture in the right quadrant of diagram was due to the relevance of the recalcitrant fraction (RF) in

deeper soils of these vegetation covers (Figure 1). Once again, COSTA et al., (2014) observed that secondary forest presented a lower coefficient of decomposition and higher levels of lignin, cellulose and polyphenols. The secondary forest presents high content of RF in the subsurface layer may be related to this vegetation cover being the oldest, which allowed the production of organic compounds with greater chemical stability. Another aspect that can corroborate this result is that a reduction in the density of fine roots and an increase in more suberized roots occur in the greater depths, and there is also greater contribution of C with microbial origin with the increase in depth, thereby increasing the recalcitrant level of C (OLIVEIRA et al., 2018). It is worth remembering that the vegetation cover before the pasture was this secondary forest. The  $\delta^{13}$ C values of the pasture soils below a depth of 40 cm (unpublished data) indicated that the accumulation of SOC with depth was still derived from the natural forest, which would explain the presence of a C recalcitrant in these deeper horizons.

These results reflect the relevance of searching for indicators which are sensitive and enable discriminating changes in soil organic matter levels after conversion of degraded pasture into leguminous trees. Attributes representing the labile compartment and fast cycling rate have been considered as more discriminating indicators of changes in soil organic matter. For these reasons, oxidizable organic C fractions which enable separating labile, moderately labile and recalcitrant reservoirs may be a more useful approach to characterize land-use changes in SOC.

## CONCLUSION

The integrated analysis of the oxidizable organic C fractions was adequate to assess the influence of different vegetation covers on the soil organic C lability.

The conversion of degraded pasture into forest legume plantations and the soil depth promoted changes in the chemical composition of C. The continuous deposition of vegetable residues 13 years of leguminous trees favored the distribution of labile and moderately labile fractions along the soil profile and the recalcitrant fraction in the topsoil. The reference covers contributed to the recalcitrant fraction in the soil below 20 cm depth.

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#### DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the research reported in this paper.

#### **AUTHORS' CONTRIBUTIONS**

The project to which this manuscript is inserted was conceived by Emanuela Forestieri da Gama-Rodrigues and Antonio Carlos da da Gama-Rodrigues. Lucas Luís Faustino performed the laboratory and statistical analyzes and prepared the draft of the manuscript. Emanuela Forestieri da Gama-Rodrigues supervised and coordinated the writing of the manuscript. Patrícia Anjos Bittencourt Barreto-Garcia collaborated with the writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

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