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## ORGANIC MATTER IN AREAS UNDER SECONDARY FORESTS AND PASTURE

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**ABSTRACT:** The objective of this study was to evaluate the soil carbon stock (Stock C) and bulk density, the chemical and granulometric fractions of the organic matter and the isotopic signal of the soil  $\delta^{13}\text{C}$  in forest fragments and a mixed managed pasture (MMP). The study was carried out in the municipality of Pinheiral, State of Rio de Janeiro. The evaluated areas were: fragment of secondary forest in initial stage (SFIS) with 20 years of regeneration; fragment of secondary forest in intermediate stage (SFINS), with 25 years of regeneration; advanced secondary forest fragment (SFAS) with 60 years of regeneration and mixed pasture managed (MHP). The attributes related to soil carbon showed significant responses to the effects of land degradation / recovery processes, especially for the following indicators: total organic carbon, organic matter stock and particulate organic matter. No significant alterations for humic substances, and prevalence of the humin fraction was found. The most significant changes in  $\delta^{13}\text{C}$  values occurred up to the depth of 60 cm. In the grassland area, at 0-10 cm, 67% of the carbon stock comes from  $\text{C}_4$  plants, reducing in the subsequent layers. In SFINS and SFAS areas, at 0-10 cm, the contribution of  $\text{C}_3$  plants was significant, with minor changes in depth.

## MATÉRIA ORGÂNICA EM ÁREAS DE FLORESTAS SECUNDÁRIAS E PASTAGEM

**RESUMO:** O objetivo deste trabalho foi avaliar a densidade do solo e o estoque de carbono (EstC) do solo, as frações químicas e granulométricas da matéria orgânica e o sinal isotópico do  $\delta^{13}\text{C}$  do solo em fragmentos florestais e uma área de pastagem mista (MMP). O estudo foi realizado no município de Pinheiral, Estado do Rio de Janeiro. As áreas avaliadas foram: fragmento de floresta secundária em estágio inicial (FSEI) com 20 anos de regeneração; fragmento de floresta secundária em estágio intermediário (FSEI), com 25 anos de regeneração; fragmento de floresta secundária em estágio avançado (FSEA) com 60 anos de regeneração e uma pastagem mista manejada (PMM). Os atributos relacionados ao carbono do solo mostraram respostas significativas aos efeitos dos processos de degradação / recuperação da terra, especialmente para os seguintes indicadores: carbono orgânico total, matéria orgânica e matéria orgânica particulada. Não houve alterações significativas para as substâncias húmicas e foi encontrada a prevalência da fração húmica. As mudanças mais significativas nos valores de  $^{13}\text{C}$  ocorreram até a profundidade de 60 cm. Na área de pastagem, a 0-10 cm, 67% do estoque de carbono vem de plantas  $\text{C}_4$ , reduzindo nas camadas subsequentes. Nas áreas FSEI e FSEA, em 0-10 cm, a contribuição das plantas  $\text{C}_3$  foi significativa, com pequenas alterações na profundidade.

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## INTRODUCTION

Deforestation for agricultural crops, followed by pastures in bustling relief regions, that is, with deep and dissected narrow valleys, can give rise to a landscape with different degrees of degradation, and the main indicators of this process are the decreases in the capacity of soil water retention, low soil stability favoring an increase in the erosion process, and reductions in fertility levels and soil capacity to store carbon (MACHADO et al., 2010; MACEDO et al., 2008; GUARESCHI et al., 2014). However, Guareschi et al. (2014) has verified that the plant successions in the areas of forest under pasture promote the improvement of the physical and chemical attributes of the soil through the addition of organic matter.

According to Schedlbauer and Kavanagh (2008), reductions in soil carbon content from conversion of rainforest to pasture can often be restored through the development of secondary forests. However, Coutinho et al. (2010) when evaluating the impact of pasture substitution by eucalyptus plantation and secondary forest area, did not observe differences in soil C stocks in the forest and eucalyptus areas compared to a pasture area. Differences in the results found may be occurring, due to the time of installation of adopted management or forest recovery. Substantial losses of soil carbon resulting from the conversion of tropical forest into pasture can often be restored through the development of secondary forest (SCHEDLBAUER; KAVANAGH, 2008).

As for soil physical attributes in pasture areas and secondary forests in the Atlantic Forest, studies have shown higher values of bulk density (Bd) in pasture areas in relation to secondary forest (SANTOS et al., 2010; MELLONI et al., 2008). These results are justified by compaction of the soil by the trampling of the animals in the pasture area and by the greater and constant accumulation of vegetal residues on the soil surface (SVR) of the forest area in relation to pasture area. However, studies by Coutinho et al. (2010) and Oliveira et al. (2008) did not find a significant difference for Bd. This pattern may be due to the small recovery time of the secondary forest, that is, its regeneration has not yet been able to reduce the impacts on Bd caused by the previous PA (OLIVEIRA et al., 2008).

Another technique widely used in the detection of landscape changes is the isotopic analysis of soil C compartments. The organic matter decomposition process shows that there is practically no change in the  $\delta^{13}\text{C}$  signal between the source material and the soil organic matter (MOS). Thus, based on the analysis of variation in abundance of  $\delta^{13}\text{C}$ , it is possible to identify the origin of MOS. In this way, the isotopic determinations of the natural abundance of  $\delta^{13}\text{C}$  of the MOS are being

used as an indicator of the pre-existing vegetation type, and of the modifications that an area has undergone in the past (SALIMON et al., 2007; COUTINHO et al., 2010; GUARESCHI et al., 2012; LOSS et al., 2014).

Thus, the aim of this study was to characterize the organic matter and quantify its stocks in areas under secondary forests and grassland in hill environments in the municipality of Pinheiral (RJ).

## MATERIAL AND METHODS

The study was conducted at the municipality of Pinheiral, state of Rio de Janeiro, in the Middle Paraíba Fluminense region, sub-basin of the Cachimbal river, which comprises the basin of the Paraíba do Sul River, located at 22°29'03" and 22°35'27"S and 43°54'49"W and 44°04'05"W.

According to the Köppen climate classification, the climate was identified in two different ways in Cwa - temperate climate with dry winter and rainy summer, and Am - tropical rainy climate with dry winter. Data from the old weather station of Pirai, indicate that the region has an annual rainfall around 1300 mm and average annual temperature of 21°C.

The region is inserted into the ecological dominance of the Atlantic Forest, whose original vegetation is called Submontane Semideciduous Seasonal Forest, with characteristic of areas with altitudes between 300 and 800 meters. The current dominant vegetation in the region consists of grasslands, implemented and not managed spontaneous vegetation, which are at several degradation stages, level of use and / or abandonment, giving rise to other forms of vegetation in the area, such as abandoned pastures and brushwood at different succession stages. The predominant soils on the slopes are Typic Haplustults, Typic Haplustox and Typic Dystrocryepts.

To characterize the organic matter contents and carbon storage, areas with different succession stages were selected, which were inserted in an Atlantic Forest remnant with about 190 ha. For purposes of statistical comparison of the areas to be studied, they should be in the same slope position (upper third), located under the same soil and climatic conditions such as slope position (exposure face and slope) and soil class (Typic Dystrocryepts).

From this analysis, secondary forest areas were selected, which had their vegetal coverage classified into different succession stages of the Atlantic Forest, namely: secondary forest at initial stage (SFIS), (approximately 20 years of regeneration); forest at intermediate stage (SFINS) (approximately 25 years of regeneration) secondary forest at advanced stage (SFAS) (approximately 60 years

of regeneration) and a mixed managed pasture (MMP). A plot of a 20 x 20m was delimited in each area, opening five trenches up to one meter of depth, where soil samples were collected (with the aid of a kopeck ring) and deformed at regular intervals of 10 cm up to 100 cm deep. After collected, the samples were taken to the laboratory, being air dried and harrowed, resulting in the air-dried fine soil (ADFS), material on which the analyses were carried out.

Total organic carbon contents were determined by oxidizing organic matter with potassium dichromate in a sulfuric acid medium and titration with ammonium ferrous sulfate 0.1 mol·L<sup>-1</sup>, as recommended by (DONAGEMA et al., 2011).

The organic matter grain size fractionation was determined only in samples from the first three layers (0-10, 10-20 and 20-30 cm). The method described by (CAMBARDELLA; ELLIOTT, 1992) was adopted, where, as the first stage, approximately 20 g of soil and 70 ml of sodium hexametaphosphate (5 g·L<sup>-1</sup>) were homogenized for 15 hours in horizontal shaker. Then, the suspension was sieved through 53 mm sieve with the aid of water jet. The material retained in the sieve, which consists of particulate organic matter, was dried in an oven at 50°C, measuring the mass, and being ground in a porcelain mortar and analyzed in relation to the organic matter content. The carbon associated with minerals was calculated as the difference between total organic carbon and particulate organic carbon contents (> 53 mm).

The quantitative extraction and fractionation of humic substances was carried out according to differential solubility technique established by the International Humic Substances Society. The organic carbon contents of fulvic acids (C-FAF), humic acids (C-FAH) and humin fractions (HUM-C) were quantified. The C-FAH/C-FAF and C-AL/C-HUM ratios were calculated, as well as the percentage of each fraction in relation to the total organic carbon (TOC), as %FAF, %FAH, %HUM.

Samples were collected at regular intervals from 10 cm to 100 cm of depth and sent to the Stable Isotope Laboratory of the Center for Nuclear Energy in Agriculture (CENA / USP), where the natural abundance of <sup>13</sup>C was analyzed.

The results obtained with the average of three replicates were expressed by the relative unit “δ”, determined in relation to international standard PDB (limestone of Pee Dee formation) according to the following equation (1), where:  $R_{\text{sample}}$  is the <sup>13</sup>C \ <sup>12</sup>C isotope ratio and  $R_{\text{standard}}$  of the sample and <sup>13</sup>C \ <sup>12</sup>C is the isotope ratio of the standard.

$$\delta^{13}\text{C}\text{‰} = 10^3 \cdot (R_{\text{sample}} - R_{\text{standard}}) \cdot (R_{\text{standard}})^{-1} \quad (1)$$

The contribution of the remaining forest carbon ( $C_3$ ) and that introduced by grassland ( $C_4$ ) in each area was calculated using the following equations (2) and (3), where:  $C_4$  (%) is the carbon coming from grassland and  $C_3$  (%) is the carbon coming from the forest in the soil of the study unit to be assessed,  $\delta^{13}\text{C}$  represents the  $\delta^{13}\text{C}$  values of soil samples from the area to be assessed,  $\delta^{13}\text{C}_{\text{sfas}}$  represents the  $\delta^{13}\text{C}$  of the soil of the secondary forest at advanced stage (SFAS) and  $\delta^{13}\text{C}_{\text{grass}}$  represents the  $\delta^{13}\text{C}$  of grass plant material collected in the grassland area (10.16‰).

$$C_4(\%) = ((\delta^{13}\text{C}_{\text{area}} - \delta^{13}\text{C}_{\text{sfas}}) \cdot (\delta^{13}\text{C}_{\text{pasture}} - \delta^{13}\text{C}_{\text{sfas}})^{-1}) \cdot 100 \quad (2)$$

$$C_3(\%) = 100 - C_4(\%) \quad (3)$$

The carbon stock was evaluated at the different soil layers up to one meter deep. Soil organic carbon density data, established according to (DONAGEMA, 2011), were used in its determination.

The carbon stock (Cstock) was calculated from the following expression (4), where Cstock is the carbon stock (Mg·ha<sup>-1</sup>), C indicates the organic carbon content in the layer evaluated (g·kg<sup>-1</sup>), Bd is the bulk density (Mg·m<sup>-3</sup>) and E is the thickness (cm) of the layer under analysis.

$$\text{Cstock} = (C \cdot \text{Bd} \cdot E) \cdot 10^{-1} \quad (4)$$

The results were analyzed for normal distribution of errors (Lillifors test / SAEG 5.0) and homogeneity of variances (Cochran and Bartlett tests / SAEG 5.0), and whenever necessary, their logarithmic transformation was performed for further comparison of mean values using the Bonferroni T test, using the Sisvar 4.6 statistical program.

## RESULTS AND DISCUSSION

In general, the trend of lower bulk density values for all areas was observed at a depth of 0-10 cm, with an increase in the lower layers, but with no significant difference (ANOVA) in depth. The average soil density values ranged from 1.43 to 1.63 Mg·m<sup>-3</sup> in SFIS, 1.29 to 1.63 Mg·m<sup>-3</sup> in SFINS, 1.31 to 1.57 Mg·m<sup>-3</sup> in SFAS and 1.41 to 1.65 Mg·m<sup>-3</sup> in MMP; without, however, significant differences (ANOVA) for these attributes between areas in any of the depths studied. Similar results are presented in the studies of Coutinho et al. (2010) and Oliveira et al. (2008), which also did not find a significant difference for Bd. This pattern may be due to the small recovery time of the secondary forest, that is, its regeneration has not yet been able to reduce the impacts on the Bd caused by the pasture area that preceded it.

Although no significant differences (ANOVA) were observed, the lowest bulk density values were observed in SFAS and SFINS, contrasting with the lowest values found in SFIS and MMP. For units under forest

formations, variations in these attributes are associated with the greater amount of litter found in these areas.

The total organic carbon levels (TOC), particulate organic carbon (POC) and carbon associated with fine mineral particles (silt and clay) (CAM) are shown in Table 1. The percentage that these levels represent of the total soil organic carbon (TOC) (between 15 and 30% for POC and 70 to 85% for CAM) are similar to those found in literature (OKORE et al., 2007), in studies on soil organic fractions at different regions of subtropical and tropical climates.

Unlike TOC results, the POC values showed significant differences only in the surface layer (0-10 cm), and as the CAM values, they showed no difference in any of the layers studied. In the 0-10 cm layer, coinciding with the pattern of TOC values, the highest POC values were found in soil under secondary forest at intermediate stage (SFINS), intermediate in those under secondary forest at initial stages (SFIS) and mixed managed pasture (MMP) and lower under secondary forest at advanced stage (SFAS). Comparing the values between areas under forest cover at different succession stages, the lowest TOC and POC values were found in the surface layer (0-10 cm) under SFAS, which can be attributed to the carbon associated with the vegetal biomass and to the high decomposition rates of the litter-forming material.

The highest relative alterations (with SFAS as reference) of POC values in relation to TOC and CAM stood out (Table 2), confirming the findings of different authors (OKORE et al., 2007), who studied the soil organic matter dynamics in regions of tropical and subtropical climates and concluded that this fraction (POC), for being more labile, seems to be more sensitive to the management or vegetal coverage of soils than the other fractions.

The carbon content of soil organic matter fractions - carbon of fulvic acid (C-FAF), humic acid (C-FAH) and humin fractions (C-HUM), their percentages and humic

acid and fulvic acid fractions ratio (C-FAH/FAF) and carbon of the alkaline layer and humin (C-AL/C-HUM) are shown in Table 3.

There were no significant differences (ANOVA) of humic substances in the three soil layers between the study areas. However, alterations between fractions, especially at the surface layer (0-10 cm) were observed.

These results are similar to those found in areas with different soils under forest vegetation and grassland (GIÁCOMO et al., 2008; LOSS et al., 2010; ARAÚJO et al., 2011) showing the general dominance of the humin fraction over the others, followed by fulvic acid and humic acid fractions.

**TABLE 1** Total carbon (TOC) values<sup>(1)</sup>, carbon in particulate organic carbon fractions (POC) and organic matter associated with silt and clay (CAM) at the different study areas and depths.

Areas <sup>(2)</sup>	TOC	POC	CAM	$\frac{POC}{TOC} \cdot 100$	$\frac{CAM}{TOC} \cdot 100$
	g·kg <sup>-1</sup>	g·kg <sup>-1</sup>	g·kg <sup>-1</sup>	%	%
Depth (cm)					
0-10					
SFIS	18.03 ab	4.36 ab	13.67 a	24	76
SFINS	21.01 a	5.81 a	15.20 a	28	72
SFAS	16.45 b	3.73 b	12.72 a	23	77
MMP	17.52 ab	5.29 ab	12.23 a	30	70
10-20					
SFIS	14.11 a	2.83 a	11.28 a	20	80
SFINS	13.57 ab	3.44 a	10.13 a	25	75
SFAS	12.48 ab	1.86 a	10.62 a	15	85
MMP	10.49 b	2.38 a	8.11 a	23	77
20-30					
SFIS	10.40 a	2.84 a	7.56 a	27	73
SFINS	9.42 ab	2.86 a	6.56 a	30	70
SFAS	8.22 ab	1.41 a	6.81 a	17	83
MMP	6.50 b	1.33 a	5.17 a	21	79

<sup>1</sup> Average of five replicates. Values with same letter in column do not differ through the Bonferroni T test at 5%. <sup>2</sup> Legend: SFIS - secondary forest at initial stage; SFINS - secondary forest at intermediate stage; SFAS - secondary forest at advanced stage; MMP - mixed managed grassland.

**TABLE 2** Total carbon (TOC) values<sup>(1)</sup>, particulate organic carbon (POC) and carbon associated with silt and clay (CAM) at different areas<sup>(2)</sup> and depths, with relative alterations ( $\Delta$ )<sup>(3)</sup>.

Areas	TOC	POC	CAM	$\Delta$ TOC	$\Delta$ POC	$\Delta$ CAM
	Depth (cm)					
0-10						
SFAS	16.45 b	3.73 b	12.72 a	-	-	-
SFINS	21.01 a	5.81 a	15.20 a	4.56	2.08	2.48
SFIS	18.03 ab	4.36 ab	13.67 a	1.58	0.63	0.95
MMP	17.52 ab	5.29 ab	12.23 a	1.07	1.56	- 0.49
10-20						
SFAS	12.48 ab	1.86 a	10.62 a	-	-	-
SFINS	13.57 ab	3.44 a	10.13 a	1.09	1.58	- 0.49
SFIS	14.11 a	2.83 a	11.28 a	1.63	0.97	0.66
MMP	10.49 b	2.38 a	8.11 a	- 1.99	0.52	- 2.51
20-30						
SFAS	8.22 ab	1.41 a	6.81 a	-	-	-
SFINS	9.42 ab	2.86 a	6.56 a	1.2	1.45	- 0.25
SFIS	10.40 a	2.84 a	7.56 a	2.18	1.43	0.75
MMP	6.50 b	1.33 a	5.17 a	- 1.72	- 0.08	- 1.64

<sup>1</sup> Average of five replicates. Values with same letter in column do not differ through the Bonferroni T test at 5%. <sup>2</sup> Legend: SFIS - secondary forest at initial stage; SFINS - secondary forest at intermediate stage; SFAS - secondary forest at advanced stage; MMP - mixed managed pasture. <sup>3</sup> Different letters between EUs have SFAS vales as reference.

**TABLE 3** Parameters estimates of the omnidirectional and cross semivariograms Vol = volume, Exp = Exponential.

Areas	TOC	C-FAF	C-FAH	C-HUM	FAF	FAH	HUM	C-FAH/C-FAF	C-AL/C-HUM
	Depth (cm)								
0-10									
SFAS	18.1 (2.7)	3.7 (0.4)	2.0 (0.4)	7.9 (0.8)	21	11	44	0.5 (0.1)	0.7 (0.1)
SFINS	21.0 (3.2)	3.8 (0.7)	2.9 (0.5)	10.5 (2.4)	18	14	50	0.8 (0.1)	0.7 (0.1)
SFIS	16.5(5.0)	3.4 (0.4)	1.7 (0.6)	7.3 (0.6)	22	11	48	0.5 (0.2)	0.7 (0.1)
MMP	17.8 (5.1)	3.5 (0.5)	2.0 (0.5)	8.2 (0.7)	21	11	49	0.6 (0.2)	0.7 (0.1)
10-20									
SFAS	14.1 (3.2)	3.1 (0.7)	1.0 (0.3)	5.8 (0.5)	23	8	42	0.4 (0.1)	0.7 (0.2)
SFINS	13.6 (3.2)	2.8 (0.6)	1.5 (0.7)	5.0 (2.7)	21	11	37	0.5 (0.3)	1.1 (0.6)
SFIS	12.5(2.4)	2.6 (0.6)	0.7 (0.3)	5.3 (0.9)	22	6	45	0.3 (0.1)	0.6 (0.2)
MMP	10.5 (3.1)	2.6 (0.6)	0.7 (0.4)	5.2 (1.4)	26	6	52	0.2 (0.1)	0.6 (0.1)
20-30									
SFAS	10.4 (2.6)	2.3 (0.4)	0.4 (0.3)	3.9 (0.9)	23	4	38	0.2 (0.2)	0.7 (0.1)
SFINS	9.3 (2.8)	2.3 (0.5)	1.0 (0.9)	4.3 (1.9)	26	9	47	0.4 (0.4)	0.8 (0.3)
SFIS	8.2 (1.6)	2.7 (0.5)	0.3 (0.2)	3.4 (0.9)	33	3	43	0.1 (0.1)	0.9 (0.4)
MMP	6.5 (1.4)	2.3 (0.5)	0.5 (0.4)	3.6 (0.8)	37	7	55	0.2 (0.2)	0.8 (0.1)

<sup>1</sup> Average of five replicates. Values with same letter in column do not differ through the Bonferroni T test at 5%. <sup>2</sup> Legend: SFIS - secondary forest at initial stage; SFINS - secondary forest at intermediate stage; SFAS - secondary forest at advanced stage; MMP - mixed managed pasture.

The analysis of the carbon content of humic substances in the four areas and layers studied indicates predominance of the humin fraction (37-50%) on C-FAF (18 to 37%) and C-FAH (3 to 14%) fractions, with C-FAF presenting percentages greater than C-FAH. In Rhodic Hapludox under continuous *Brachiaria decumbes* pasture in the city of Maracajú - MS (FONTANA et al., 2006) found predominance of humin and higher C-FAH values compared to C-FAF.

Only the humin fraction showed higher and significant values in forest areas compared to grassland area in three layers evaluated and the humic acids fraction in the 10-20 cm layer.

The predominance of the humin fraction can be attributed to the low solubility and resistance to biological degradation due to the formation of metal complexes and to the constant input of organic material and less human interference (FONTANA et al., 2006; EBELING et al., 2011) characteristic of the systems studied.

The low and similar values of the C-AL/C-HUM ratio confirm the predominance of the humin fraction (more resistant) compared to fulvic and humic acid fractions in the three layers of the four areas. The low and similar values of the C-FAH/C-FAF ratio confirm the higher percentage of fulvic acids on humic acids, which may indicate that systems of the different areas are, similarly, favoring the degradation of more stable fractions or even impairing their formation (FONTANA et al., 2006).

The total carbon stock values in the different soil layers at different areas are shown in Table 4. No significant differences were observed in the carbon

stocks in the first two layers (0-10 and 10-20 cm) of the different areas. However, significant differences were found from the 20-30 cm layer, where the lowest values were observed in MMP when compared to those found in SFAS, SFINS and SFIS, as well as a tendency of higher values in SFIS and SFINS, especially at the 20-30 cm layer.

The total soil carbon stock values in the depth assessed (100 cm) showed an increasing trend from MMP area (100.53 Mg ha<sup>-1</sup>) to SFAS (111.07 Mg ha<sup>-1</sup>) SFINS (127.5 Mg ha<sup>-1</sup>) and SFIS areas (132.47 Mg ha<sup>-1</sup>).

The carbon stock results when correlated with total organic carbon (Table 1) allow inferring that the carbon contribution from the vegetation covers of the

**TABLE 4** Carbon stock (Mg·ha<sup>-1</sup>) values <sup>(1)</sup> in the different study areas and depths.

Depth (cm)	Areas <sup>(2)</sup>			
	SFIS	SFINS	SFAS	MMP
0-10	25.97 a	26.71 a	21.37 a	25.03 a
10-20	21.48 a	20.05 a	17.45 a	16.03 a
20-30	16.16 a	13.67 ab	12.40 b	10.38 c
30-40	12.69 a	12.74 a	10.12 ab	9.74 b
40-50	11.17 a	10.34 ab	10.09 ab	8.36 b
50-60	10.13 a	9.11 a	9.31 a	6.81 b
60-70	10.08 a	8.72 ab	8.93 ab	7.04 b
70-80	9.17 a	8.17 ab	8.28 ab	5.71 b
80-90	7.91 a	7.19 ab	6.50 ab	5.44 b
90-100	7.81 a	10.18 a	6.62 a	5.94 a
Total	132.47 a	127.50 a	111.07 ab	100.53 b

<sup>1</sup> Average of five replicates. Values with same letter in column do not differ through the Bonferroni T test at 5%. <sup>2</sup> Legend: SFIS - secondary forest at initial stage; SFINS - secondary forest at intermediate stage; SFAS - secondary forest at advanced stage; MMP - mixed managed pasture.

four areas do not differ in the first layers due to the important contribution of litter provided by the forest systems and to the abundant and dynamic root system of grasses in the grassland area. However, the comparison of the total carbon stock between the four areas has indicated the importance of the developing secondary forest formations (SFIS and SFINS) in stocking larger amounts of carbon in the soil.

In areas under secondary forest in advanced succession stage (SFAS) and intermediate stage (SFINS), the  $\delta^{13}\text{C}$  values increased from 0-10 to 10-20 cm layers (SFINS = -26.7 ‰ to -25.2 ‰ and SFAS = 27.2 ‰ to -26.5 ‰) leveling off in the other layers up to 100 cm. Dissimilar patterns are observed in area under secondary forest in initial stage (SFIS), where there is a decreased  $\delta^{13}\text{C}$  in depth with a tendency to level off from the 20 to 30 cm layer (from -21.8 ‰ at 0-10 cm to -23.5 ‰ in 20 to 30 cm) and under managed grassland (MMP), where the decrease is more significant and reaches greater depth (from -16.1 ‰ at 0-10, -18.6 ‰ at 10-20, -21.1 ‰ at 20-30 and -22.4 ‰ at 30-40 cm). This pattern in depth, as well as the  $\delta^{13}\text{C}$  values, similar to those found by other authors in Atlantic forest (TARRÉ et al., 2001) and Amazon areas (MELO, 2003; VIDOTTO et al., 2007) express the higher influence of  $\text{C}_3$  photosynthetic cycle plants in the contribution of organic matter in the soil surface layers in areas under intermediate and advanced stages of forest succession and of  $\text{C}_4$  cycle plants in areas under initial succession stage and grassland.

The delta  $^{13}\text{C}$  values between the different layers up to the depth of 60 cm are shown in Table 5. The results show in the first two layers (0-10 and 10-20 cm) significantly higher values in MMP, intermediate values in SFIS and lower values in SFINS and SFAS; in the two subsequent layers (20-30 and 30-40 cm), a similar behavior, but with greater tendency to equality between the values found in SFINS, SFAS and SFIS; and in the last two layers (40-50 and 50-60 cm), the results show a similarity of soil carbon isotope values between the four areas. These results confirm the assumption that at the extent that the secondary forest formations, which are encroaching on abandoned pasture areas, developed, there is a progressive decrease of  $\delta^{13}\text{C}$  values in the soil surface layers. This fact is due to the increased input of organic material from  $\text{C}_3$  plants (poorer in  $^{13}\text{C}$ ) that predominate in the floristic composition of these formations.

The pattern observed in depth is confirmed by results of other studies (TARRÉ et al., 2001; MELO, 2003), showing that the most significant alterations of  $\delta^{13}\text{C}$  values are observed up to the depth of 60 cm, with a tendency to stabilize in subsequent layers.

**TABLE 5** Natural abundance of  $^{13}\text{C}$  <sup>(1)</sup> and respective standard errors <sup>(2)</sup> up to the depth of 60 cm in the different areas <sup>(3)</sup>.

Depth (cm)	Areas <sup>(1)</sup>			
	SFIS	SFINS	SFAS	MMP
0-10	-21.8 (0.5) b	-26.7 (0.3) c	-27.2 (0.1) c	-16.1 (0.3) a
10-20	-22.1 (0.4) b	-25.2 (0.4) c	-26.5 (0.1) c	-18.6 (0.5) a
20-30	-23.5 (0.6) b	-25.0 (0.3) bc	-26.0 (0.1) c	-21.1 (0.2) a
30-40	-24.1 (0.2) b	-25.2 (0.1) bc	-26.0 (0.1) c	-22.4 (0.2) a
40-50	-24.6 (0.1) b	-25.4 (0.1) b	-25.9 (0.0) b	-23.3 (0.2) a
50-60	-24.9 (0.0) b	-25.5 (0.1) b	-25.8 (0.1) b	-23.8 (0.4) a

<sup>1</sup> Average of five replicates. Values with same letter in column do not differ through the Bonferroni T test at 5%. <sup>2</sup> Legend: SFIS - secondary forest at initial stage; SFINS - secondary forest at intermediate stage; SFAS - secondary forest at advanced stage; MMGP - mixed managed pasture. (*Brachiaria decumbens* and *Paspalum notatum*).

<sup>3</sup> Values between parenthesis show the mean standard error of three replicates.

Studying different succession stages of tropical forests in Costa Rica (SCHEDLBAUER; KAVANAGH, 2008) found results that confirm those observed in this study. By using isotope analysis, the authors showed that the increase in carbon stock from younger succession stages to more advanced ones was due to the increased contribution of organic matter by  $\text{C}_3$  photosynthetic cycle species, which typically dominate tropical forest formations. The same authors also emphasized that the onset of the secondary succession development on abandoned pasture areas leads to a rapid decrease in  $\delta^{13}\text{C}$  values, but the continuity of this development does not significantly change these values. This finding can be confirmed by the high evolution of  $\delta^{13}\text{C}$  data from soil under SFIS to SFINS and to the low trend of evolution from this to SFAS.

The source of carbon ( $\text{C}_3$  or  $\text{C}_4$  cycles) stored in the soil under the different areas is shown in Table 6. The study area mixed managed pasture (MMP) has in the surface layer (0-10 cm) 67% of the total soil carbon stock coming from  $\text{C}_4$  photosynthetic cycle plants (29.23  $\text{Mg}\cdot\text{ha}^{-1}$ ). In subsequent layers, there is a reduction of this contribution, with a consequent increase in the  $\text{C}_3$  contribution. The initial succession stage of the forest on the pre-existing grassland, SFIS, shows in the surface layer (0-10 cm) a decrease in the contribution of the  $\text{C}_3$  photosynthetic cycle plants in the amount of carbon in the soil, represented by 33% (15.07  $\text{Mg}\cdot\text{ha}^{-1}$ ), which gradually decreases in depth.

In areas under more mature succession stages (SFAS and SFINS), the increased contribution of  $\text{C}_3$  cycle plants is significant, represented by 97% in SFINS and 100% in SFAS (reference area), with minor changes in depth.

These results are similar to those found by Melo (2003) in area under 20 years of *Brachiaria Brizanta* cultivation on a deep Oxisol in the state of Acre, where the author found  $\text{C}_4$  contribution values of 80.03% in 0-5 cm, 63.1% in 5-10 cm, 42.3% in 10-20 and 29.09% in 30-

**TABLE 6** Origin of the carbon stored in soil <sup>(1)</sup> under the different areas <sup>(2)</sup> and depths.

Depth (cm)	Photosynthetic cycle			
	C <sub>3</sub>		C <sub>4</sub>	
	Areas			
	SFIS			
	%	Mg·ha <sup>-1</sup>	%	Mg·ha <sup>-1</sup>
0-10	67	30.56	33	15.07
10-20	72	20.32	28	7.73
20-30	84	16.63	16	3.27
30-40	88	14.99	12	2.03
40-50	91	14.43	09	1.44
50-60	93	13.42	07	0.94
	SFINS			
0-10	97	48.40	03	1.70
10-20	92	33.71	08	2.86
20-30	93	27.75	07	1.94
30-40	95	25.86	05	1.33
40-50	97	18.70	03	0.63
50-60	98	17.61	02	0.42
	SFAS			
0-10	100	39.72	00	0.00
10-20	100	25.25	00	0.00
20-30	100	21.26	00	0.00
30-40	100	15.42	00	0.00
40-50	100	15.68	00	0.00
50-60	100	10.38	00	0.00
	MMP			
0-10	33	14.46	67	29.23
10-20	50	14.62	50	14.62
20-30	68	14.25	32	6.75
30-40	77	14.19	23	4.28
40-50	83	13.61	17	2.87
50-60	86	12.66	14	1.98

<sup>1</sup> Average of five replicates. Values with same letter in column do not differ through the Bonferroni T test at 5%. <sup>2</sup> Legend: SFIS - secondary forest at initial stage; SFINS - secondary forest at intermediate stage; SFAS - secondary forest at advanced stage; MMP - mixed managed pasture. (*Brachiaria decumbens* and *Paspalum notatum*).

40 cm, with native forest being considered as reference area. This study did not consider the contribution of carbon originated from grassland.

Schedlbauer and Kavanagh (2008) reported that with increasing time of pasture abandonment and consequent growth of forest cover, a slow and gradual decrease of the contribution of soil carbon originated from C<sub>4</sub> cycle plants is expected to occur. However, this behavior was not observed by these authors in the study that assessed the origin of forest carbon formed under abandoned pasture in northeastern Costa Rica, when they found a rapid decrease in the contribution of C<sub>4</sub> cycle plants. On the other hand, when grassland encroach native forest, there is a progressive decrease of the forest carbon with the time of land use, since there is no longer input of organic matter from C<sub>3</sub> cycle plants, while carbon introduced by grassland increases. For these authors, comparisons between grassland areas indicate that those formed for longer times show higher proportion of carbon originating from C<sub>4</sub> plants.

## CONCLUSIONS

The attributes related to soil carbon showed significant responses to the effects of land degradation/

recovery processes, especially for the following indicators: total organic carbon, organic matter stock and particulate organic matter.

As for the chemical fractionation of soil organic matter, there were no significant alterations for humic substances, and prevalence of the humin fraction was found.

The most significant changes in  $\delta^{13}\text{C}$  values occurred up to the depth of 60 cm. In the grassland area, at 0-10 cm, 67% of the carbon stock comes from C<sub>4</sub> plants, reducing in the subsequent layers. In SFINS and SFAS areas, at 0-10 cm, the contribution of C<sub>3</sub> plants was significant, with minor changes in depth.

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