

Humic substances and oxidable fractions of organic matter in different environments in Amazônia Maranhense

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Abstract

The realization of soil management, without considering the limitations and potential of space, has contributed to the degradation of these environments, in different degrees, depending on the impact promoted on the soil attributes. This work investigated the levels of carbon (C), chemical, and oxidizable fractions of soil organic matter, in pasture areas with different recovery times, compared to a secondary forest. We evaluated the following forms of management: Secondary Forest (SF), Perennial pasture (PP), Perennial pasture recovered five years ago by the corn consortium + *Brachiaria brizantha* cv. Marandu (P5) and Perennial pasture recovered eight years ago by intercropping corn + *Brachiaria brizantha* cv. Marandu (P8). We determined the C contents of the oxidizable fractions (F1, F2, F3, and F4) and the contents and stocks of humic substances of organic matter in the layers of 0.00-0.10, 0.10-0.20, 0.20-0.30, and 0.30-0.40 m. We observed the highest levels of C in humic substances in areas SF, P8, and P5. Regarding the oxidizable fractions, we found in the P8 area the highest levels of C within the F1 and F3 fractions. Through the multivariate analysis employed, it was possible to evidence the following increasing order P8 > SF > P5 > PP of the impact on soil quality. The recovery of the pasture area, eight years ago, provided improvements regarding the accumulation of carbon in the soil in the oxidizable fractions and humic substances.

Introduction

The Amazon biome is the largest biodiversity region on the planet, covering 6.74 million km² and extending across nine countries: Brazil, Bolivia, Colombia, Ecuador, Guyana, French Guiana, Peru, Suriname, and Venezuela (Benchimol 2011). In this biome, there are several ecosystems that involve the largest tropical forest in the world, the Amazon Forest, and in the Maranhão State it originally comprised 81,208.40 km², however, due to deforestation, 25 to 24% of this total is found (Celentano et al. 2017, Silva Júnior et al. 2020, INPE, 2020).

The deforestation of the forest in the Maranhão State is due to the expansion of beef cattle that developed rapidly from the mid-1960s (Mesquita 2007, Silva et al. 2019). The conversion of the forest for exploitation of cattle raising has caused problems regarding soil degradation due to the lack of proper management and the successive use of fire, in order to control the natural regeneration, renewal, or recovery of the pasture and addition of nutrients to the soil, from burned plant material (Dias-Filho 2014, Reis et al. 2018).

The intensification of land use and management carried out in a way that does not consider the suitability of the space has contributed to the intensification of the degradation process in many areas, due to the non-maintenance of sustainability. The variation in the accumulation of soil organic matter (SOM), as well as the forms in which the organic matter is found in the soil, can work as an indicator of quality, due to the sensitivity of the SOM content to changes in an area subjected to a certain management system (Rosset et al. 2016, Moraes Rego et al. 2020, Piano et al. 2020).

SOM plays a fundamental role in the maintenance of soil quality, directly and indirectly influencing several attributes. In soils formed in tropical environments, it has greater importance due to its role in edaphic attributes, so the reduction of the SOM content also indicates the chemical, physical and biological degradation of the soil (Rosset et al. 2016). Most studies on SOM emphasize the influence of different management practices on carbon (C) levels and stocks in the soil, and the most specific way of evaluating such changes is related to a greater understanding of the stability of C in the soil. (Machado et al. 2019, Morais et al. 2021).

The evaluation of the dynamics and stability of SOM can be done through fractionation techniques, including chemical (Swift 1996) and oxidizable (Chan et al. 2001), which are important and sensitive tools capable of detecting changes associated with different forms of use and/or management of the soil and plant species used (Piano et al. 2020).

Through chemical fractionation, humic substances are obtained (fulvic acids - FA, humic acids - HA and humin - HUM) that constitute a compartment of the SOM with greater reactivity, presenting 70 to 90% of the soil C content. Furthermore, these substances participate in most edaphic chemical reactions, strongly related to soil fertility (Rosa et al. 2017; Mendonça and Matos 2017). As for the oxidizable fractionation, four fractions (F1 to F4) with decreasing degrees of oxidation are obtained. The first two are related to the availability of nutrients and the last are related to compounds with greater chemical and molecular stability. Consequently, such measurements result in information regarding the lability and resistance of C to degradation (Guareschi et al. 2013, Rosset et al. 2016).

Understanding the changes in C in the different fractions of the SOM makes it possible to measure the degree of soil quality, helping to detect possible impacts caused by a given management system. Given the above, this work starts from the hypothesis that pasture recovery modifies the humic substances and oxidizable fractions of SOM. Therefore, this work aimed to evaluate the C contents of the chemical and oxidizable soil fractions of pastures with different recovery years and compare them to the secondary forest.

Material And Methods

Study Location

The study was carried out at the Technological Reference Unit (TRU) of Embrapa Cocais and at the State University of Maranhão (UEMA), in the municipality of Pindaré-Mirim/MA. According to the Köppen classification, the climate of the region is classified as Aw, with a dry winter, with an average annual temperature of 26 to 27 °C and average annual precipitation between 1900 and 2100 mm (Araújo 2013, Alvares et al. 2014).

The soil of the TRU was classified as Plinthosols in the FAO classification system (IUSS Working Group WRB 2015), and Plintossolo Argilúvico according to the Brazilian Soil Classification System (Santos et al. 2018a), of medium texture (Table 1), originating from sediments of the Itapecuru formation and

composed of fine sandstones (Araújo 2013). The relief varies from smooth wavy to wavy. It is covered by Ombrophilous Forest associated with secondary forest vegetation, with a predominance of babassu palm (*Attalea speciosa* Mart.) (Mata dos Cocais), dominant in the Mid-North region of the state of Maranhão (Araújo 2013).

Table 1
Chemical and granulometric characterization of the soil in the different areas and layers of Pindaré-Mirim region, Maranhão, Brazil

Areas	Layer (m)	pH	OM	P	K	Ca	Mg	Al	H + Al
		CaCl ²	g kg ⁻³	mg dm ⁻³	—————cmolc dm ⁻³ —————				
SF	0.0-0.2	4.49	15.72	1.78	0.15	1.61	1.73	0.26	2.67
	0.2-0.4	4.18	7.59	1.74	0.14	0.97	2.21	0.91	3.65
PP	0.0-0.2	4.58	10.14	1.56	0.28	2.36	2.28	0.16	2.51
	0.2-0.4	4.25	6.85	1.37	0.29	2.42	3.45	1.37	4.97
P5	0.0-0.2	4.33	12.43	1.36	0.14	0.58	1.24	0.28	2.84
	0.2-0.4	4.15	5.04	0.92	0.10	0.38	1.33	0.62	3.09
P8	0.0-0.2	4.56	12.43	1.10	0.20	1.67	3.16	0.23	3.60
	0.2-0.4	4.38	6.05	0.95	0.23	1.25	4.22	1.04	5.96
Areas	Layer (m)	Sand	Silt		Clay				
		—————g kg ⁻¹ —————							
SF	0.0-0.2	746.7	135.3		118.1				
	0.2-0.4	707.2	124.3		168.6				
PP	0.0-0.2	579.3	343.0		77.8				
	0.2-0.4	616.1	260.4		123.7				
P5	0.0-0.2	745.5	103.5		150.5				
	0.2-0.4	704.5	169.5		125.5				
P8	0.0-0.2	741.9	120.8		137.3				
	0.2-0.4	723.1	97.3		179.7				

Legend: SF: Secondary Forest, PP: Perennial pasture, P5: Perennial pasture recovered five years ago and P8: Perennial pasture recovered eight years ago. pH: hydrogenonic potential, MO: Organic matter, P: Phosphorus, K: Potassium, Ca: Calcium, Mg: Magnesium, Al: Aluminium, H + Al: hydrogen and aluminium. P and K – Mehlich-1 Extractor; Al, Ca, and Mg – KCl extractor 1 mol L⁻¹; H + Al - pH SMP (7.5).

Evaluated systems and usage history

For the study, the following areas were selected for evaluation: Secondary Forest (SF), Perennial pasture (PP), Perennial pasture recovered five years ago by the corn consortium + *Brachiaria brizantha* cv. Marandu (P5) and Perennial pasture recovered eight years ago by intercropping corn + *Brachiaria brizantha* cv. Marandu (P8). The history and characteristics of the managements used are described by Rego et al. (2021).

Collection of samples and evaluations performed

In each land use system, five trenches with dimensions of 1 x 1 x 1 m were opened, randomly arranged. Therefore, we collected the samples in the summer period, when the highest rainfall occurs, contributing to the soil water content being close to field capacity. On opposite walls of the trench, we collected the undisturbed samples using a stainless-steel Uhland collector equipped with volumetric rings and with a known volume of 100 cm³, which comprised the layers of 0.0–0.10; 0.10–0.20; 0.20–0.30; 0.30–0.40 m and was used to determine soil density (SD) (Teixeira et al. 2017).

For the fractionation of the SOM, deformed samples were collected at three equidistant points, at ten metres each, in relation to each wall of the trench, with the aid of a Dutch auger. We collected a total of twelve simple samples to compose a representative composite sample in the same previously mentioned layers. The deformed samples were air-dried, crushed, and passed through a 2 mm mesh sieve, through which we obtained air dried soil (ADS).

At ADS, we performed the determination of total organic carbon (TOC) by the wet oxidation method, using 0.167 mol L⁻¹ potassium dichromate solution and concentrated sulfuric acid, with heating in a digester block, as described by Mendonça and Matos (2017) adapted from Yeomans and Bremner (1988).

Humic substances (HS) were obtained by extraction and separation through the differential solubility of organic matter in a basic or acidic medium (fulvic and humic acids) and the residue (humin), performed by the method proposed by the International Humic Substances Society (IHSS), described by Swift (1996) with adaptations by Mendonça and Matos (2017). We then separated the fractions into fulvic acid (FA), humic acid (HA) and humin (HUM). After this step, for the different fractions, we determined the carbon contents, according to Mendonça and Matos (2017). Subsequently, we calculated the alkaline extract (AE) (AE = HA + FA) and the HA/FA and AE/HUM ratios to verify the SOM humification processes, in which the first ratio indicates carbon mobility in the soil and the degree of humification. In the second relation, we verified the possible illuviation of organic matter in the soil (Benites et al. 2003).

The stocks of humic substances were calculated using the following equation: $Est = (C \times SD \times e)/10$, where Est represents the carbon stock in each layer expressed in Mg ha⁻¹; C represents the carbon content in the layer (g kg⁻¹); SD represents soil density (Mg m⁻³); and e represents the thickness of the soil layer (cm).

To adequately compare the stocks between the different managements, it would be necessary to make a comparison between equal masses of soil, adjusting the values of the layers used in the calculations. However, as no differences were found in the SD (Table 2), the stocks were calculated without using the correction for the equivalent mass. To verify trends in the accumulation or loss of C in the humic fractions in the 0-0.40 m section, the variation of the C stock (CS) of each fraction was calculated, compared to the secondary forest (reference) (ΔCS , $Mg\ ha^{-1}\ cm^{-1}$).

Table 2
Soil density ($Mg\ m^{-3}$) of an Argiluvic Plintisol under different areas in the Amazon of Maranhão

Areas	Layers (m)			
	0.00-0.10	0.10-0.20	0.20-0.30	0.30-0.40
SF	$1.33 \pm 0.06ns$	$1.41 \pm 0.03ns$	$1.43 \pm 0.02ns$	$1.48 \pm 0.01ns$
PP	1.42 ± 0.03	1.43 ± 0.02	1.43 ± 0.04	1.45 ± 0.03
P5	1.35 ± 0.03	1.48 ± 0.02	1.43 ± 0.01	1.43 ± 0.01
P8	1.37 ± 0.03	1.43 ± 0.02	1.43 ± 0.02	1.44 ± 0.03

Note. SF: Secondary Forest, PP: Perennial pasture, P5: Perennial pasture recovered five years ago and P8: Perennial pasture recovered eight years ago. $\pm$: Standard error, ns: not significant by the t test ($p < 0.05$).

The oxidizable fractions SOM were obtained using different degrees of oxidation through increasing concentrations of sulfuric acid (3, 6, and $9\ mol\ L^{-1}$), in which four fractions with decreasing degrees of lability F1, F2, F3, and F4 were determined. We obtained the values from the difference between the concentrations and from the TOC itself, as described by Chan et al. (2001) and adapted by Mendonça and Matos (2017). Due to the lability characteristics of the F1 fraction, the fraction is considered as labile carbon (LC), and the non-labile carbon (NLC) we obtained by difference: $NLC = TOC - LC$ (Rosset et al. 2016). Subsequently, to obtain indices that demonstrate the dynamics between the fractions obtained, we calculated the following relationships: F1/F4 and LC/TOC.

Statistical analysis

The resulting data were submitted to the Shapiro-Wilk and Bartlett normality and homogeneity tests, respectively. When these assumptions were met, the means were compared by the t-test at 5% probability. As a complementary analysis, the multivariate analysis of principal components was used, based on the Euclidean distance. Statistical analyses were performed using the R 4.0 software (R Core Team 2021).

Results

Differences were found between areas for TOC and C contents in soil humic substances (Table 3). For the areas of SF and P8, the highest levels of TOC were found in the surface layer (0.00-0.10 m). Higher values

in depth were verified in the areas P8, 0.10–0.20, and 0.30–0.40 m, and in the PP area in the depths of 0.10–0.20 to 0.30–0.40 m.

Table 3
Total organic carbon content and humic fractions of soil organic matter in the different managements

Areas	TOC	FA	HA	HUM	AE	HA/FA	AE/HUM
g kg⁻¹							
0.00-0.10 m							
SF	9.65 a	1.04 a	1.21 a	7.32 b	2.25 a	1.15 b	0.31 a
PP	7.27 b	0.52 c	0.66 b	5.22 c	1.18 d	1.28 b	0.23 b
P5	7.66 b	0.55 c	0.98 b	5.37 c	1.53 c	1.88 a	0.29 a
P8	9.86 a	0.76 b	1.08 a	9.13 a	1.84 b	1.42 ab	0.20 b
0.10–0.20 m							
SF	4.31 c	0.44 b	0.36 b	4.02 b	0.79 b	0.81 ab	0.21 ab
PP	5.38 a	0.35 b	0.26 b	4.24 b	0.61 b	0.76 b	0.14 c
P5	4.68 b	0.52 b	0.54 a	4.06 b	1.06 a	1.13 a	0.26 a
P8	5.63 a	0.72 a	0.35 b	5.24 a	1.07 a	0.51 b	0.20 b
0.20–0.30 m							
SF	3.75 c	0.43 b	0.26 a	3.74 a	0.69 b	0.61 b	0.19 b
PP	4.84 a	0.26 d	0.18 b	3.85 a	0.43 d	0.67 ab	0.11 c
P5	4.05 c	0.34 c	0.27 a	3.87 a	0.61 c	0.81 a	0.16 b
P8	4.49 b	0.71 a	0.26 a	3.50 a	0.97 a	0.37 c	0.28 a
0.30–0.40 m							
SF	3.79 b	0.34 b	0.17 a	3.47 a	0.52 b	0.51 b	0.15 c
PP	5.15 a	0.26 c	0.17 a	3.07 ab	0.43 c	0.67 a	0.14 c
P5	4.06 b	0.34 b	0.17 a	3.24 c	0.51 b	0.51 b	0.19 b
P8	4.81 a	0.94 a	0.17 a	3.15 b	1.11 a	0.19 d	0.36 a

Note. SF: Secondary Forest, PP: Perennial pasture, P5: Perennial pasture recovered five years ago, P8: Perennial pasture recovered eight years ago, TOC: Total organic carbon, AF: fulvic acid, AH: humic acid, HUM: humin, EA: alkaline extract. Means followed by the same letter in the column, for each layer, do not differ from each other by the t-test ($p < 0.05$).

Regardless of the area, among the HS, the predominance of the HUM fraction was observed in all the layers, ranging from 71.68 to 89.8% of the total C (Table 3). In the surface layer (0.00-0.10) the greater participation of HA fractions stands out, ranging from 9.84 to 12.61% of the total C, when compared to the FA fraction, 6.92 to 10.86%. For the other layers, an inverse pattern is observed, with the participation of 6.06 and 22.07% for the FA and 3.99 to 10.55% for the HA of the total C.

The study also found that in the 0.00-0.10 m layer in the SF area there was greater participation of fractions FA and HA, followed by areas P8 and P5. For HUM, the highest levels were found in P8, in the first two layers, followed by the SF area, in the last two layers (Table 3). For the FA, the highest values were found in the P8 area, in which we found average values between 39.36, 39.44 and 63.41% in the layers from 0.10–0.20 to 0.30–0.40 m, respectively, higher than those quantified in the SF area. The highest levels of HA were observed in areas P5, layers of 0.10–0.20 and 0.20–0.30 m, SF and P8, both in layers 0.10–0.20 and 0.10–0.20 m, with no differences in the last layer between the areas.

We observed the highest values for AE in the SF areas, layer 0.00-0.10 m, P5, layer 0.10–0.20 m, and P8, layers 0.10–0.20 and 0.30–0.40 m (Table 3). In the relationship between the HA/FA, we verified the highest values along the layers in the P5 area. For the AE/HUM ratio, the highest values were found in the SF area, layer 0.00-0.10 m, P5, layers 0.00-0.10 m and 0.10–0.20 m, and P8, layer 0.20 – 0.30 and 0.30–0.40 m.

When verifying the variation of the C stock of humic substances in depth (0.00-0.40 m) we found positive values in the P8 area for the $\Delta\text{CS-FA}$ (0.29) and for the $\Delta\text{CS-HUM}$ (0.82). Thus, we highlight an increase in the variation of the C stock of 199.86 and 187.76%, respectively, of these fractions when compared to the PP area. We also observed a negative variation in the C stock of humic substances at depth (0.00-0.40 m), for $\Delta\text{CS-HA}$, in all pasture areas. We highlight that in the P5 area (-0.01) we found the best results (Fig. 1).

The C contents of the oxidizable fractions and their respective relationships showed differences between the areas in the studied layers (Table 4). In general, and regardless of area and layer, this study verified the predominance of C in the most recalcitrant fractions (F3 and F4), ranging from 46.22 to 68.11% of the total, in relation to the most labile (F1 and F2), which varied between 31.89 and 53.78%. This pattern was confirmed by the high levels of NLC and low values of the F1/F4 and LC/TOC ratios.

Table 4
Carbon contents of oxidizable fractions, non-labile carbon (NLC), labile carbon/TOC ratio (LC/TOC), and between oxidizable fractions (F1/F4) in different areas in the Amazon of Maranhão

Areas	F1	F2	F3	F4	F1/F4	NLC	LC/TOC
	g kg ⁻¹					g kg ⁻¹	%
0.00-0.10 m							
SF	3.24b	0.76a	1.35b	4.30a	0.76b	6.41a	33.61b
PP	2.01d	0.31b	1.16ab	3.79a	0.54c	5.26b	27.71d
P5	2.31c	0.75a	0.94c	3.66a	0.63bc	5.35b	30.18c
P8	3.63a	0.59a	1.78a	3.86a	0.96a	6.23a	36.89a
0.10–0.20 m							
SF	1.27 b	0.76 a	0.97 a	1.31 c	0.99 b	3.04 b	29.55 bc
PP	1.41 b	0.47 a	0.51 b	3.00 a	0.48 c	3.97 a	26.16 c
P5	1.97 a	0.55 a	0.63 b	1.53 c	1.33 a	2.71 b	42.18 a
P8	2.01 a	0.51 a	0.57 b	2.54 b	0.80 b	3.62 a	35.80 b
0.20–0.30 m							
SF	1.02 c	0.67 ab	0.56 b	1.50 b	0.70 b	2.72 b	27.37 c
PP	1.38 b	0.81 a	0.71 ab	1.94 a	0.76 b	3.46 a	28.61 bc
P5	1.40 b	0.62 ab	0.48 b	1.55 ab	0.91 b	2.65 b	34.73 ab
P8	1.82 a	0.36 b	0.94 a	1.38 b	1.36 a	2.68 b	40.46 a
0.30–0.40 m							
SF	1.00 c	0.53 ab	0.42 bc	1.75 b	0.73 b	2.79 b	27.13 b
PP	1.43 b	0.31 b	0.30 c	3.10 a	0.46 b	3.71 b	27,88 b
P5	1.25 bc	0.58 ab	0.68 ab	1.55 b	0.85 ab	2.81 b	30.82 ab
P8	1.80 a	0.64 a	0.93 a	1.58 b	1.18 a	3.02 a	37.55 a

Note. SF: Secondary Forest, PP: Perennial pasture, P5: Perennial pasture recovered five years ago and P8: Perennial pasture recovered eight years ago. Means followed by the same letter in the column, for each layer, do not differ from each other by the t-test ($p < 0.05$).

Our research also verified increases from 12.22 to 79.41% of C contents of the F1 fraction in the P8 area, in relation to the forest area for all analyzed layers. This pattern was also verified by the study for the F3

fraction, even though it did not differ from the P5 and PP areas in some layers (Table 4). For the F2 fraction, we observed in the SF and P5 areas, both in the layer 0.00-0.10 m, P8, layers 0.00-0.10 and 0.30–0.40 m, and PP, layer 0.20–0.30 m, the highest C contents, with no differences between the areas in the 0.10–0.20 m layer. In fraction F4, the tests performed here did not detect differences between the areas in the 0.00-0.10 m layer. In the other layers in the PP area, we observed the highest levels of C, not differing from P5 in the layer of 0.20–0.30 m.

For the F1/F4 and LC/TOC ratio, we verified in areas P8, layers 0.00-0.10, 0.20–0.30 and 0.30–0.40 m, and P5, layer 0.10–0.20 m, the highest values when compared to the others. The mean values ranged from 78.81 to 176.96% and 33.14 to 61.24%, respectively, being higher than those quantified in the PP area (Table 4). This study found that the highest values of NLC were observed in the areas of SF, layer 0.00-0.10 m, P8, layers 0.00-0.10, 0.10–0.20 m, and 0.30–0.40 m, and PP, layers 0.10–0.20 to 0.20–0.30 m.

Through the analysis of principal components (PC) we found that the first two components (PC1 and PC2) explained more than 90% of the total variation of the data, with variations of 52.3 and 42.7% being observed for PC1 and PC2, respectively (Fig. 2 and Table 5). We verified that the variables used presented high contributions to the principal components 1 and 2, thus reducing the number of axes necessary to explain the variation of the data. We also observed the highest correlations (> 0.70) for the attributes TOC, F1, F3, FA, and HUM with PC1 and F2, F4, NLC, and HA with PC2 (Fig. 2 and Table 5).

Table 5

Weight coefficients (eigenvectors), eigenvalues, variance explained, correlation of variables by each principal component (PC1 and PC2) of the different soil organic matter fractions under different areas in the Amazônia Maranhense

Variance component	Principal component	
	1	2
Eigenvalues	4.706	3.841
Proportion (%)	52.285	42.682
Cumulative proportion (%)	52.285	94,966
Variables		
TOC	0,718	0,695
F1	0,931	0,120
F2	0,005	-0,924
F3	0,990	0,103
F4	-0,379	0,919
CNL	0,048	0,931
FA	0,996	-0,059
HA	0,488	-0,871
HUM	0,984	0,080

The areas are separated into different quadrants. By the distribution of areas, it is possible to evidence the following increasing order $P8 > SF > P5 > PP$ of the impact on soil quality (Fig. 2). When analyzing the components, we observed that PC1 is associated with areas P8 and SF in a positive way and with areas PP and P5, in a negative way. For PC2, we found positive correlations with areas PP and P8 and negative correlations for areas P5 and SF. In general, for the areas close to the vectors, the greatest contributions of these. This pattern corroborates the results observed for the individual analyses. We verified for the SF area the highest values of humic acids (HA) and of the moderately labile fraction (F2). In the PP area, we observed the highest recalcitrant fractions (F4). In area P5, we highlight the highest values for the moderately labile fraction (F2). As for the P8 area, the highest correlations were found for the attributes TOC, oxidizable fractions of greater lability, moderately recalcitrant (F1, F3), and humic substances (FA and HUM).

Discussion

The similarity of TOC contents between the P8 and SF areas in the surface layers and the predominance in depth for the PP and P8 areas may be associated with the slow deposition of carbon in the edaphic environment of secondary forests associated with water stress (Elias et al. 2020). Secondary forests are vulnerable to water stress, with carbon balance and lower growth rates in drier periods (Elias et al. 2020), which are common in the State of Maranhão, which has periods of low rainfall (Araújo 2013). Bastos et al. (2021) did not find differences between TOC contents in a Plintisol, in the State of Rondônia, when compared to pasture and forest areas. This pattern was also observed by Reis et al. (2018) and Santos et al. (2018b) for Plintisol in the State of Maranhão.

Pastures cultivated in the Amazon biome can deposit an average of 18.90 Mg ha^{-1} of dry biomass on the soil surface (Sanquetta et al. 2020), meaning an input of more than 7 Mg ha^{-1} of C on the soil surface (MCTI, 2014; Sanquetta et al. 2020). At depth, tropical grasses can produce about 10 Mg ha^{-1} of dry root biomass, of which about 21 and 12% of root and shoot biomass, respectively, are converted into TOC (Bayer et al., 2011; Oliveira et al., 2017).

Sarto et al. (2020) observed that after 8 years of pasture establishment, C levels like those observed in forest areas were found in the surface layer of the soil. This pattern can be attributed to several factors such as higher residual deposition of the aerial and root parts of plants, residues from animals that collaborate to increase biological activity, C/Nitrogen ratio of the material that tends to be higher for grasses, among other factors. In-depth, the best results in the P8 and PP areas can be attributed to the capacity of *Brachiaria brizantha* cv. Marandu forms a voluminous, fast-growing, and well-developed root system. Due to the constant renewals provided by grazing, dead roots are decomposed by soil microorganisms, releasing nutrients, and promoting the increase of organic compounds (Salton & Tomazi 2014, Oliveira et al. 2018).

The pasture recovery eight years ago contributed in a beneficial way to the increase of the humin fraction when compared to the PP area. This pattern becomes clear through the analysis of $\Delta\text{CS-HUM}$ and the analysis of principal components (Figs. 1 and 2). This pattern may have been provided, possibly, by the humin inherited from the forest, which was preserved from the action of biological activity by the initial development of the pasture, which tends to provide a greater amount of biomass that is easier for microorganisms to access (Ebeling et al. 2013, Salton and Tomazi 2014). The highest levels of C in the HUM fraction, in all areas and layers, confirmed by the low values of the EA/HUM ratio, are common in regions with tropical climates. In these, the dominance of the most stable and insoluble fraction is provided by both the intense mineralization of the residues and the edaphic restrictions on biological activity, which reduces the humification process, indicative of the strong stability of the SOM. (Cunha et al. 2005, Ebeling et al., 2011, Rosa et al. 2017).

There is a predominance of the humin fraction in all areas and layers, which may be associated with its recalcitrance, low solubility, and the association with iron oxides (Li et al. 2015), the latter very present in Plintosols (Santos et al. 2018a). Other factors that may help to understand the predominance of the humin fraction would be the essentially aliphatic chemical nature of the molecule, derived from

macromolecules highly resistant to biological degradation and coming from plants and/or microorganisms (Valladares et al. 2011). Also, due to the high molecular weight and a greater degree of stability of the humin fraction, when compared to the other fractions, there is a greater protection of the fixed C. This fraction is considered the most significant as a reserve of organic carbon in the soil (Ebeling et al. 2013).

Regarding the alkaline-soluble fractions (FA and HA) that presented the best results in the recovered pasture areas (5 and 8 years) when compared to perennial pasture, this fact may have been caused by the lower deposition of residues in the PP. This is possibly caused by the decrease in crop productivity (Pereira et al., 2020) after 20 years of implantation of perennial pasture, associated with the conditions of initial soil preparation and lack of proper management of the same. The recovery of pasture areas (5 and 8 years) provided an increase in the HA fraction levels, and although these were negative for $\Delta\text{CS-HA}$, they were better when compared to the PP area (Fig. 1). The greatest contribution to the PC2 axis of the principal component analysis is also highlighted (Fig. 2), which suggests greater stabilization of organic matter, with greater carbon content in the humified form (Ebeling et al. 2013).

The high C contents of the FA fraction in the P8 area, as well as the positive values in the $\Delta\text{CS-FA}$ (Fig. 2), can be attributed to the greater deposition of residues by the aerial and root biomass, in addition to animal residues, which contribute to the accumulation of material for decomposition and formation of this fraction. In-depth, the results can be attributed to the greater mobility and less condensation of the FA, compared to the HA, allowing them to be present in greater proportions in the more subsurface layers due to greater FA mobility. (Cunha et al. 2005, Valadares et al. 2011, Ebeling et al. 2013, Rosa et al. 2017). In relation to the predominance of the FA fraction compared to the HA, mainly in-depth, this pattern was observed in all areas, being confirmed by the low values of the HA/FA ratio. This fact indicates a limited evolution of organic matter due to edaphic reasons, probably because of the wetting and drying cycles present in the soil and, or, movement of the water table, as well as recent inputs of organic matter (Benites et al. 2013, Rosa et al. 2017).

Araújo et al. (2011), evaluating the impact of converting the Amazon Forest into a pasture, in the State of Acre/Brazil, found a behaviour like that observed in this study for humic substances. The authors observed higher levels of TOC in pasture areas with a predominance of the humin fraction over the others and greater formation of FA when compared to HA at depth.

Through the analysis of the fractionation of oxidizable organic carbon, the Predominance of the most recalcitrant forms of C (F3 and F4) and NLC is verified, a pattern that corroborates the predominance of the humin fraction observed. In addition to the predominance of low values of the F1/F4 and LC/TOC ratios, these indicate that even the low levels of C found in the soil are largely associated with compounds with greater stability and greater interaction with the mineral fraction of the soil. This hampers microbial attack, contributing to a higher molecular weight and permanence time of these products in soils (Rosset et al. 2016, Oliveira et al. 2018, Xu et al. 2021).

Pasture recovery eight years ago provided increases in C levels found in labile (F1), moderately recalcitrant (F3) and non-labile (NCL) fractions when compared to perennial pasture area. This was due to the greater initial contributions and greater vigour of the plants, which reflects in greater contributions of biomass on the surface by the shoot and into the soil by the root system (Pereira et al. 2020, Xu et al. 2021). In addition, this increase reflects on the recovery capacity of soil C contents, and in some cases, the quantified values were higher than those found in the Forest area. The increase in the accumulation of C in the P8 area for the fractions F1, F3 and NCL, as well as for the F1/F4 ratio, showed that pasture recovery contributes to an increase in the amount of carbon stored in the soil, reducing its emission to the atmosphere. The results observed for the labile fractions, provided by the recovery of pasture areas, can demonstrate the benefits of the changes promoted to the soil. The attributes used, due to greater sensitivity, were able to indicate the changes resulting from the forms of management used. Similar results were observed in other studies that found changes in soil attributes caused by a change in management (Luo et al. 2021, Xu et al. 2020), as well as for moderately recalcitrant and non-labile fractions, demonstrating how these contribute to the stabilization of soil organic carbon (Song et al. 2021, Xu et al. 2021).

Xu et al. (2020) also found increases in C levels in the labile fraction (F1) of the soil in relation to the benefits generated on soil structure and nutrient content. Additionally, an increase in the C reservoir, biological activity, and other attributes was observed. It is verified that the chemical transformations to which the SOM is submitted, associated with the physical protection of C, provide increases in the accumulation of this in the recalcitrant fractions, providing greater stability of C, which contributes to the increase of soil quality (Song et al., 2021).

Conclusions

The pasture recovery process eight years ago promoted improvements in the different labile (F1 and FA) and recalcitrant (F3 and HUM) fractions of soil organic matter, presenting itself as an alternative for soil conservation management and beneficial for the Amazon region in Maranhão.

By the analysis of principal components, the studied areas were classified in the following decreasing order of quality: perennial pasture recovered eight years ago, secondary forest, perennial pasture recovered five years ago, and perennial pasture, in the function of the positive impacts on the different fractions of the organic matter of the ground.

Declarations

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Conflicts of interest

The authors declare no conflicts of interest.

Financial interests

The authors declare they have no financial interests.

Non-financial interests: none

Consent to publish

The authors consent to the case manuscript being submitted to, and published in the journal.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions Statement

Conceptualization: Carlos Augusto Rocha de Moraes Rego and Paulo Sérgio Rabello de Oliveira; Project Administration: Carlos Augusto Rocha de Moraes Rego and Paulo Sérgio Rabello de Oliveira; Supervision: Carlos Augusto Rocha de Moraes Rego and Paulo Sérgio Rabello de Oliveira; Data Curation: Carlos Augusto Rocha de Moraes Rego, Luciano Cavalcante Muniz, Eloisa Mattei, and Bruna Penha Costa; Formal Analysis: Carlos Augusto Rocha de Moraes Rego, Paulo Sérgio Rabello de Oliveira, Jean Sérgio Rosset, and Marcos Gervasio Pereira; Methodology: Carlos Augusto Rocha de Moraes Rego, Jean Sérgio Rosset, And Marcos Gervasio Pereira; Validation: Carlos Augusto Rocha de Moraes Rego, Paulo Sérgio Rabello de Oliveira, Jean Sérgio Rosset, and Marcos Gervasio Pereira; Writing – Original Draft: Carlos Augusto Rocha de Moraes Rego; Writing – Review & Editing: Carlos Augusto Rocha de Moraes Rego, Paulo Sérgio Rabello de Oliveira, Luciano Cavalcante Muniz, Eloisa Mattei, Bruna Penha Costa, Jean Sérgio Rosset, and Marcos Gervasio Pereira

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Figures

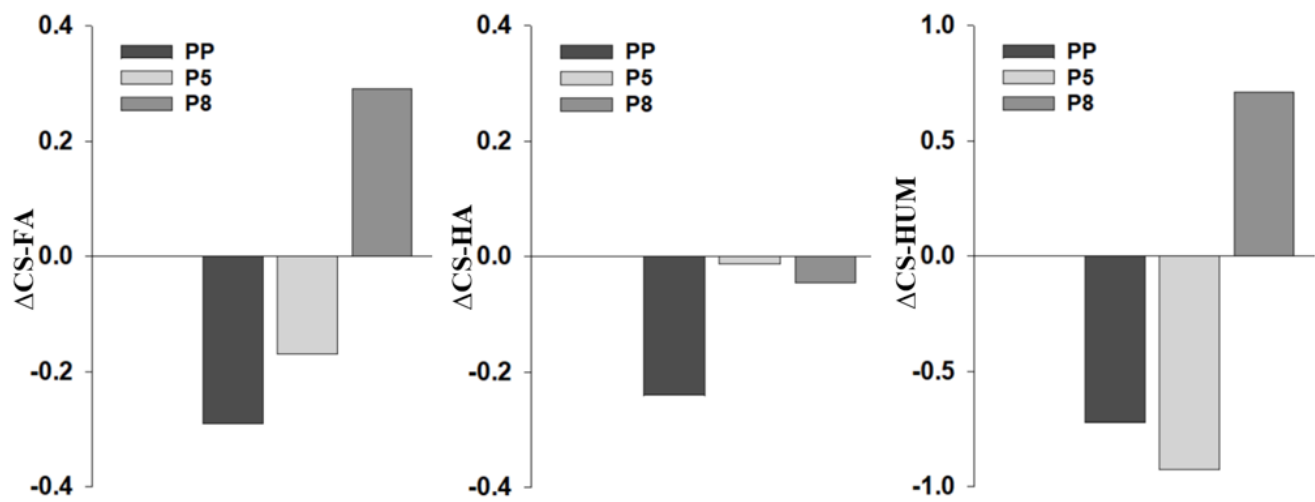


Figure 1

Variation in carbon stock (ΔCS) of the humified fractions of soil organic matter in different areas in the Amazon of Maranhão, 0.00-0.40 m section. FS: Secondary Forest, PP: Perennial pasture, P5: Perennial pasture recovered five years ago and P8: Perennial pasture recovered eight years ago

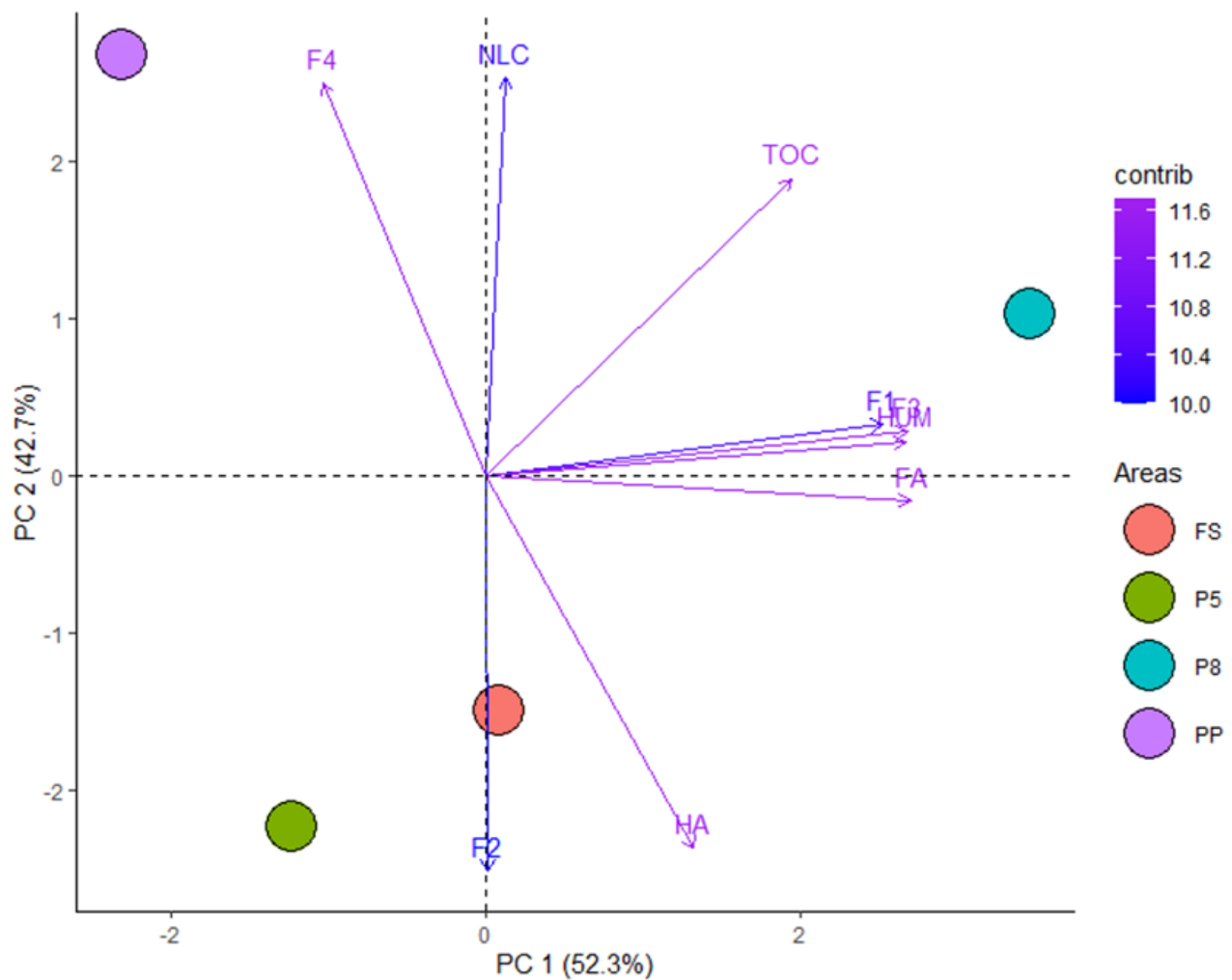


Figure 2

Analysis of principal components in different areas in the Amazon of Maranhão, 0.00-0.40 m section. SF: Secondary Forest, PP: Perennial pasture, P5: Perennial pasture recovered five years ago, P8: Perennial pasture recovered eight years ago.