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Soil Organic Matter Responses to Anthropogenic Forest Disturbance and Land Use Change in the Eastern Brazilian Amazon

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Abstract: Anthropogenic forest disturbance and land use change (LUC) in the Amazon region is the main source of greenhouse gas emissions to the atmosphere in Brazil, due to the carbon (C) and nitrogen (N) emitted from vegetation clearance. Land use conversion associated with management practices plays a key role in the distribution and origin of C in different soil organic matter (SOM) fractions. Here, we show how changing land use systems have influenced soil C and N stocks, SOM physical fractions, and the origin of SOM in the Santarém region of the eastern Brazilian Amazon. Soil C and N stocks were calculated for the surface layer of 0–30 cm. Anthropogenic disturbances to the standing forest, such as selective logging and wildfires, led to significant declines in soil C and N stocks. However, in the long-term, the conversion of the Amazon forest to pasture did not have a noticeable effect on soil C and N stocks, presumably because of additional inputs from pasture grasses. However, the conversion to cropland did lead to reductions in soil C and N content. According to the physical fractionation of SOM, LUC altered SOM quality, but silt and clay remained the combined fraction that contributed the most to soil C storage. Our results emphasize the importance of implementing more sustainable forest management systems, whilst also calling further attention to the need for fire monitoring systems, helping to ensure the resilience of C and N stocks and sequestration in forest soils; thereby contributing towards urgently needed ongoing efforts to mitigate climate change.

Keywords: tropical forests; pasture; cropland; soil carbon; stable isotopes; SOM physical fractions

1. Introduction

Globally, soil organic matter (SOM) contains about 1550 Pg of C, which is three times more than that found in the atmosphere or terrestrial vegetation [1]. The current estimate of C stock in the world's forests is about 861 ± 66 Pg C, with 383 ± 30 Pg C (44%) in soil (to 1 m depth) and 471 ± 93 Pg C (55%) of which is stored in tropical forests [2]. Thus, soils in tropical forest regions form a vital component of the global C store, yet are increasingly threatened by land use change (LUC) and forest disturbance [2].

The role of forests as important stocks of soil carbon is of particular importance in the Brazilian Amazon, where LUC from tropical forest to agricultural land, continues to occur at a very high rate. The region of Santarém-Belterra in the Pará state, northern Brazil, has been the target for soybean expansion due to favorable topography and climate, and improvements of the port infrastructure and logistics for the transportation of grain to the river port of Santarém. The conversion of tropical forests is considered to be the main cause of CO_2 emissions to the atmosphere in Brazil. Approximately 17.4% of the global GHG emissions are associated with forestry activities, including logging, and 13.5% are related to agriculture. In Brazil, agriculture and land use changes are responsible for approximately 80% of national GHG emissions, and about 51% of Brazilian CO_2 emissions originate from the Amazon biome [3].

Soil organic matter plays a key role in shaping the physical structure of the soil, mainly through the formation of organo-mineral complexes that determine the arrangement and stability of soil aggregates. One of the most important characteristics of SOM is its cementing capacity [4,5]. Aggregates of organic matter can be found in different sizes and degrees of degradation in the soils, including the organic fraction (OF: 75–2000 µm), which is essentially comprised of plant residues (i.e., larger particles with lower degree of degradation); the mineral fraction (MF: 75–2000 µm), which is mainly formed of denser soil particles; and finally, the organo-mineral fractions (OMF: 53–75 µm), which can be split between soil micro-aggregates that act as a binding agent (called occluded fraction) or as a recalcitrant fraction, mainly linked to the clay fraction of soil [6–11].

Changes in land use and management practices can alter the SOM fraction in the soil [5,12,13]. When a forest is converted to pasture or cropland, the lighter fractions can decompose faster than the coarse inter-aggregate particulate organic matter—although all of the fractions derive from litter and plants, microbial alteration is more intensive in the enriched labile fraction [5]. Management practices adopted in croplands may significantly alter the particulate SOM fraction, and observed changes in this fraction can be used as an early indicator of levels of C sequestration in the soil. For example, small and more decomposed particles may indicate that the soil C is in a more recalcitrant stage [13]. As such, studies relating to LUC with SOM fractions can be extremely important tools for understanding the dynamics of SOM functioning, as a basis for more sustainable soil management practices [9,14–16]. Furthermore, measurements of natural stable isotopes (e.g., $\delta^{13}C$ and $\delta^{15}N$) also contribute to understanding how the ecosystems respond to environmental and anthropogenic changes [17]. Based on isotopic signals, it is possible to understand patterns of land use history, because depending on the type of plant material entering the soil, the SOM origin can be traced [18,19]. When the input of soil C is provided by C3 cycle plants, the $\delta^{13}C$ soil value remains at around −27‰ to −28‰, while the C introduced by C4 plants has a value of −12‰. Based on these values, it is possible to understand where the soil C originates from, and which kind of plants have contributed to the soil C stocks [18,20].

We addressed these issues by conducting a field study across a region of approximately one million hectares of mixed agricultural and forest land in the eastern Brazilian Amazon. We tested the hypothesis that forest disturbance and changes in land use can significantly change soil C and N stocks, resulting in a progressive decrease of forest-derived C in more intensively managed soils; especially in the areas where C4 cycle plants (i.e., grasses) were introduced. We addressed this objective by:

(i) assessing soil C and N stock responses to LUC in the Santarém-Belterra region; (ii) investigating the SOM origin and dynamic using $\delta^{13}C$ and $\delta^{15}N$ techniques and (iii) evaluating the LUC effects on SOM quality, by assessing physical fractionation.

2. Material and Methods

2.1. Study Area

The study was conducted in the eastern Amazon, close to the important BR-163 highway that connects Santarém (Pará state) and Cuiabá (Mato Grosso state) (Figure 1). In order to compare the effects of the different land use intensities on soil C and N stocks, we evaluated the main human-modified land uses that are characteristic of the eastern region of the Brazilian Amazon. Soils were sampled from seven different land uses, namely undisturbed forest (UF), logged forest (LF), burnt forest (BF), logged and burned forest (LBF), secondary forest (SF), pasture (PA), and cropland (CP). We classified areas of Primary forest (i.e., forest that has never been cleared) into Undisturbed, Logged, Burnt, or Logged and Burnt, based on evidence from either field observations (fire and logging scars) or the manual interpretation of satellite images, as described by [21,22].

Pasture areas are planted with introduced tropical grasses, especially *Brachiaria brizantha*, and are characterized by extensive cattle ranching, but in general, are poorly managed and demonstrate low levels of productivity. Croplands have been mainly cultivated with soybean and corn through annual mechanized agriculture. Anthropogenic modifications of the forest through time were measured using a time-series analyses for Landsat data, from 1990 to 2010 in the Santarém-Belterra region, while changes in pasture and cropland areas were obtained using a time series for MODIS data, from 2000 to 2010 [21].

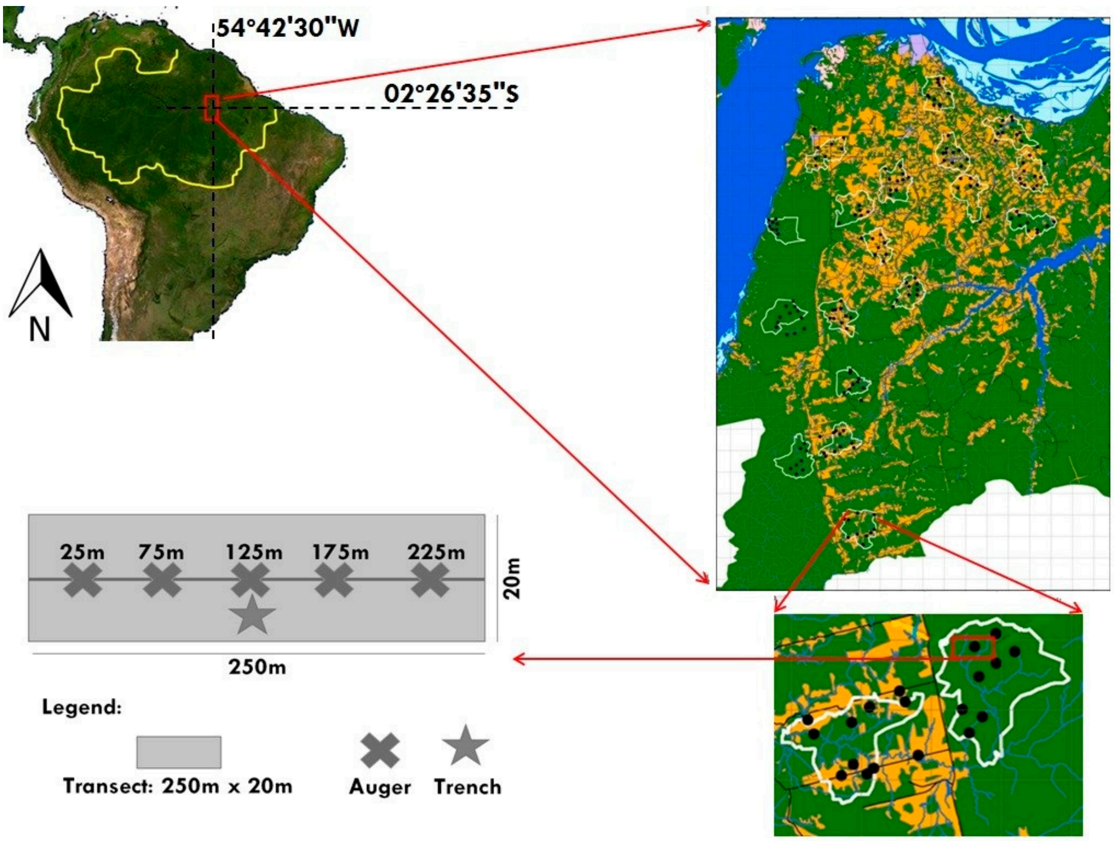

Figure 1. Geographic location of the study region in Santarém–Belterra, Pará state, eastern Brazilian Amazon, highlighting catchments, transects, and the soil sampling scheme used in this study.

2.2. Characterization of Study Catchments

The Santarem-Belterra region was divided into watersheds of 5000–6000 ha, which were delineated using a digital elevation model and SWAT (Soil and Water Assessment Tool) for ARCGIS 9.3 (ESRI, Redlands, CA, USA). Following this, 18 watersheds were selected to represent a gradient of deforestation, composed of areas ranging from c. 10% to 100% remaining forest cover. The final selection of 18 catchments was made to ensure the satisfactory representation of current land use practices, the spatial distribution of the rural population, and major soil types [21,22].

In each catchment, 250-m transects (between six and 15) were distributed across the landscape, based on a standard density of one transect per 400 m and which were in proportion to the percentage cover of forest and production areas (pastures and croplands). A minimum separation distance rule of 1500 m was employed, to minimize spatial dependence between points. In total, 173 transects were sampled, covering an area of 1 million hectares (Figure 1). In this region, Oxisols and Ultisols are the predominant soil types, accounting for 87.5% and 7.5%, of the landscapes sampled, respectively.

2.3. Soil Sampling

Five points were sampled within each transect, with a distance of 50 m between them (Figure 1). At each point, disturbed soil samples were collected at three depths: 0–10, 10–20, and 20–30 cm, providing a total of 2595 samples (i.e., 173 $\times$ 5 $\times$ 3) for C and N quantification. At the center of each transect, a 30-cm-depth trench was opened and undisturbed soil cores were collected using a volumetric ring (100 cm^{-3}), to determine the soil bulk density of each of the three evaluated depths, totaling 519 samples (i.e., 173 $\times$ 1 $\times$ 3).

Five transects were selected to perform physical fractionation of the SOM. These sites included the following land uses: (i) UF, considered as a reference SOM; (ii and iii) pastures with 20 years (PA 20) and 10 years (PA 10), cropped with tropical grasses, especially *Brachiaria brizantha*, and managed extensively with beef cattle ranching; (iv and v) and croplands with five years (CP 5) and one year of cultivation (CP 1), representing areas converted from pasture using intensive mechanization and currently being used for soybean and corn production. The choice of these land uses was made in order to assess the impacts of land use change on the SOM dynamic and functionality in the areas most affected by anthropic activities (PA and CP) in the Santarém region. The soil sampling was similar to that used for the quantification of C and N stocks. Thus, within each land use, five points spaced 50 m apart were sampled, to a depth of 10 cm.

2.4. Soil Analyses and Calculations

2.4.1. Soil Characterization

A soil chemical characterization was performed for each study site, through samples collected for the 0–10, 10–20, and 20–30 cm layers. The soil chemical attributes determined were: the pH of the water, available P, K^+, Ca^{2+}, Mg^{2+}, and Al^{3+}. In addition, we calculated the values of the effective and potential soil cation exchange capacity (CEC), base, and Al saturation percentage, for all soil samples (Tables S1 and S2). Soil particle-size analysis was performed for all samples, and the results are presented in Table S3.

2.4.2. Soil Bulk Density

The soil bulk density (BD, $Mg \cdot m^{-3}$) was determined by dividing the soil dry mass by the volume of the ring. The BD values presented in the Table 1 were used for calculating the C and N stocks.

Table 1. Soil bulk density across forest disturbance and land-use classes in the Santarém region, eastern Brazilian Amazon.

Land Use	Bulk Density (Mg·m^{-3})		
	0–10 cm	**10–20 cm**	**20–30 cm**
Undisturbed forest	0.89 ± 0.02 B c *	1.05 ± 0.02 A de	1.07 ± 0.02 A bc
Logged forest	0.86 ± 0.01 A c	1.02 ± 0.01 AB e	1.04 ± 0.01 B c
Burnt forest	1.02 ± 0.02 B b	1.16 ± 0.02 A ab	1.18 ± 0.02 A a
Logged + burnt forest	0.91 ± 0.01 C c	1.05 ± 0.01 B de	1.09 ± 0.01 A bc
Secondary forest	0.91 ± 0.01 B c	1.08 ± 0.01 A cd	1.10 ± 0.01 A bc
Pasture	1.11 ± 0.01 B a	1.17 ± 0.01 A a	1.18 ± 0.01 A a
Cropland	0.98 ± 0.01 B b	1.11 ± 0.02 A bc	1.12 ± 0.01 A b

* Means followed by the same capital letter (line—comparison among soil depth within same land use) and lowercase letter (column—comparison among land uses within same soil depth) do not differ among themselves according to Tukey's test ($p < 0.05$).

2.4.3. Contents of Soil C and N and Their Isotopes ($\delta^{13}C$ and $\delta^{15}N$)

Soil samples were further air-dried and sieved with a 2-mm mesh, to remove stones and root fragments. Sub-samples of 10 g were ground to a fine powder and sieved with 100 mesh (0.149 mm), prior to the total C and N determination by dry combustion in an elemental analyzer. The same sieved samples were used to establish the soil isotopic ratio of $^{13}C/^{12}C$ and $^{15}N/^{14}N$, which were determined by the release of gases (CO_2 or NxOy) from combustion at 550 °C in a Carbo Erba EA-110 elemental analyzer. Gases generated from this combustion were separated through gas chromatography and carried through continuous flux to the Finnigan Delta Plus mass spectrometer. The $^{13}C/^{12}C$ ($\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$) ratios of each sample are expressed in delta (δ) unit per million (‰), in relation to the international standard Vienna Pee Dee Belemnita (PDB), according to [18] (Equations (1)–(3)).

Soil isotopic ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$ are as follows:

$$\delta^{13}C = \left(\frac{\text{R sample} - \text{R standard}}{\text{R standard}}\right) \times 1000 \quad (1)$$

$$\delta(‰)^{13}C = \left[\frac{(^{13}C\ /^{12}C)\ \text{sample} - (^{13}C\ /^{12}C)\ \text{standard}}{(^{13}C\ /^{12}C)\ \text{standard}}\right] \times 1000 \quad (2)$$

$$\delta(‰)^{15}N = \left[\frac{(^{15}N\ /^{14}N)\ \text{sample} - (^{15}N\ /^{14}N)\ \text{standard}}{(^{15}N\ /^{14}N)\ \text{standard}}\right] \times 1000 \quad (3)$$

where R sample = ratio of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ of the sample; R standard = ratio of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ of the standard (PDB).

2.4.4. Calculation of C and N Stocks

For each soil layer, C and N stocks were calculated through the Equation (4):

$$\text{C or N stock} = \text{C or N} \times \text{LT} \times \text{BD} \quad (4)$$

where C or N stock is in Mg·ha^{-1}; C or N is the element content in %; LT is the soil layer thickness in cm; and BD is the bulk density in Mg·m^{-3}.

Samples were collected in the field from fixed layers and the stock calculations were adjusted in order to compare the equivalent mass of soil between the different land uses, according to the methodology described in [23].

2.4.5. Physical Fractionation of SOM

The SOM physical fractionation was performed using the particle size method described by [6]. Briefly, this method consists of the separation of soil after dispersion through a sieve with a mesh of 0.053 µm. In the first step of the method, 80 mL of distilled water was added to a 20 g sample of soil, and this solution was dispersed using ultrasound equipment (Sonics Vibracell) working at 70% power (500 W), providing approximately 13 J of energy to samples for 15 min. Samples were passed through a 75-µm mesh sieve for the separation of organic (OF) and mineral fractions (MF) of sizes between 2000–75 µm, before both fractions (OF and MF) were separated by flotation . The fraction with a size between 75 µm and 53 µm is called the organo-mineral fraction (OMF). Finally, the fraction that is not retained in the 53 µm sieve is called the fraction of silt and clay size (clay + silt). All samples were dried at 60 °C until they reached a constant mass.

2.4.6. Proportion of C Introduced by Pastures (C4) and the Remaining C Forest (C3)

Based on the results of $\delta^{13}C$, it was possible to determine the origin of C by the percentage of C derived from forest (C3—photosynthetic cycle plants) and the percentage introduced by pasture (C4—photosynthetic cycle plants) in each of the fractions. To accomplish this, we used two equations ((5) and (6)), proposed by [14]:

$$Cdp = \left(\frac{\delta^{13}C_P - \delta^{13}C_{UF}}{\delta^{13}C_{PA} - \delta^{13}C_{UF}}\right) \times 100 \quad (5)$$

where Cdp is the percentage of carbon derived from the pasture; $\delta^{13}C_P$ is the $\delta^{13}C$ value for grasses, obtained in the literature. In this case, we used a value of −14.3‰, as proposed by Moraes et al. (1996); $\delta^{13}C_{UF}$ is the $\delta^{13}C$ value of undisturbed forest area found in this study; and $\delta^{13}C_{PA}$ is the $\delta^{13}C$ value of the pasture areas found in this study.

Posteriorly, the proportion of remaining forest C (C3) was estimated using Equation (6):

$$Crf = 100 - Cdp \quad (6)$$

where Crf is the remaining carbon forest in percent and Cdp is the percentage of carbon derived from the pasture.

2.5. Statistical Analyses

An analysis of variance (ANOVA) was performed to test the effects of LUC on soil C and N stocks. If the ANOVA results were significant ($p < 0.05$), the mean values were compared using a Tukey's test ($p < 0.05$). The same statistical procedure was used to analyze the distribution of soil $\delta^{13}C$ and $\delta^{15}N$ within the different soil layers (0–10, 10–20, and 20–30 cm). Finally, the results from SOM physical fractionation were subjected to an analysis of variance using a Kruskal-Wallis' test, and the pairwise comparison was performed by a Bonferroni's (Dunn) test (α = 5%). All statistical analyses were carried out using the Statistical Analysis System—SAS v.9.3 (SAS Inc., Cary, NC, USA).

3. Results

3.1. Soil C and N Stocks

Undisturbed primary forests (UF) presented the highest stocks of C (56.2 ± 1.70 Mg·ha^{-1}) and N (4.61 ± 0.14 Mg·ha^{-1}) (Figure 2). Statistically similar C and N stocks to those found in UF were observed in the soils under logged forest (LF), and logged + burned forest (LBF). In contrast, burnt forest (BF) had the lowest soil C (39.73 ± 2.33 Mg·ha^{-1}) and N stocks (3.01 ± 0.20 Mg·ha^{-1}). Secondary forest (SF) showed higher soil C and N stocks compared to BF, and statistically similar stocks to UF, LF, and LBF.

The conversion of primary Amazon forest to pasture land (PA) did not affect soil C and N stocks. On the other hand, the conversion to cropland induced significant soil C and N stock losses, compared to UF. Soil C and N stocks for the 0–30 cm layer under pasture averaged 52.68 ± 1.06 Mg·ha^{-1} and 4.26 ± 0.08 Mg·ha^{-1}, respectively, whilst under cropland, C and N stocks averaged 46.21 ± 1.37 Mg·ha^{-1} and 3.81 ± 0.10 Mg·ha^{-1}, respectively.

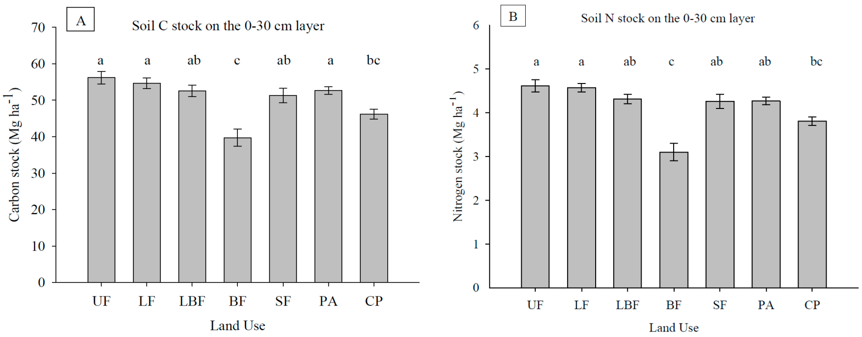

Figure 2. Soil C stock (**A**) and total N stock (**B**) (Mg·ha^{-1}) for the 0–30 cm layer under a sequence of land use and management change (UF: Undisturbed Forest; LF: Logged Forest; LBF: Logged and Burnt Forest; BF: Burnt Forest; SF: Secondary Forest; PA: Pasture and CP: Cropland) in the Santarém region, eastern Brazilian Amazon.

3.2. *Soil δ^{13}C e δ^{15}N*

The lowest δ^{13}C values were observed in the soils under forests, regardless of the degree of disturbance of these forests (i.e., UF, LG, LBF, BF, and SF) (Figure 3A). There was a slight increase in the δ^{13}C values of deeper forest soils. For example, under LF soils, the values ranged from −28.14‰ ± 0.08‰ in the 0–10 cm layer, to −27.26‰ ± 0.08‰ in the 20–30 cm layer. In contrast, PA soils predominantly planted with tropical grasses (C4) presented the highest δ^{13}C values (−24.37‰ ± 0.08‰), distinct from those found in forest and CP soils. In PA soils, we could more clearly observe a decrease in δ^{13}C values between top (0–10 cm) and deeper soil layers (10–20 and 20–30 cm).

A gradual decrease in the δ^{15}N signatures was observed among land use systems (Figure 3B). The greatest δ^{15}N value was 10.79‰ ± 0.12‰ in the UF, and the lowest was observed in SF, being equal to 9.53‰ ± 0.13‰ (Figure 3B), followed by PA and CP soils. In all land use systems, the δ^{15}N signatures showed a pronounced increase in ^{15}N enrichment with increasing soil depth. The δ^{15}N changes among land use systems were less significant in the deeper layers, with the greatest values in UF and LF soils (Figure 3B).

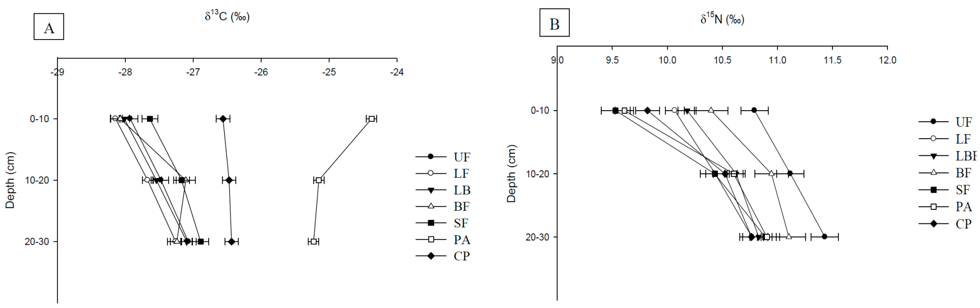

Figure 3. Soil δ^{13}C (‰) (**A**) and δ^{15}N (‰) (**B**) distribution in the three depths (0–10; 10–20, and 20–30 cm) under a sequence of land use and management change (UF: Undisturbed Forest; LF: Logged Forest; LBF: Logged and Burnt Forest; BF: Burnt Forest; SF: Secondary Forest; PA: Pasture and CP: Cropland) in the Santarém region, eastern Brazilian Amazon.

3.3. SOM Physical Fractionation

3.3.1. Soil Mass Proportion in Each Fraction

For almost all of the sampled sites, the fraction which presented the largest proportion of mass was the mineral fraction (MF 75–2000 μm), ranging from between 540 to 917 g·kg^{-1} soil at the sites with 20- and 10-year-old pasture, respectively. The only exception was for the 5-year-old cropland site (CP5), where the largest proportion of mass was observed in the silt + clay fraction (<53 μm) and the largest content of clay was observed in the same place. Organic and organo-mineral fractions (OF and OMF) did not differ from each other and they contributed the same magnitude in every sampled area, with approximately 3.0 to 7.0 g·kg^{-1} soil (Figure 4).

3.3.2. C Stock in Each SOM Fraction

The great majority of SOC was found within the silt + clay SOM fraction at the 0–10 cm layer, regardless of land use (Table 2 and Figure 4). In general, the conversion from UF to PA and CP, led to an increasing trend in C stocks within the silt + clay fraction. The highest C stock in that fraction was found under CP5 (16.8 ± 1.5 Mg·ha^{-1}), which only differed statistically from PA10 (8.1 ± 1.2 Mg·ha^{-1}). No significant differences were found between C stocks within other SOM fractions (OF, OMF, and MF).

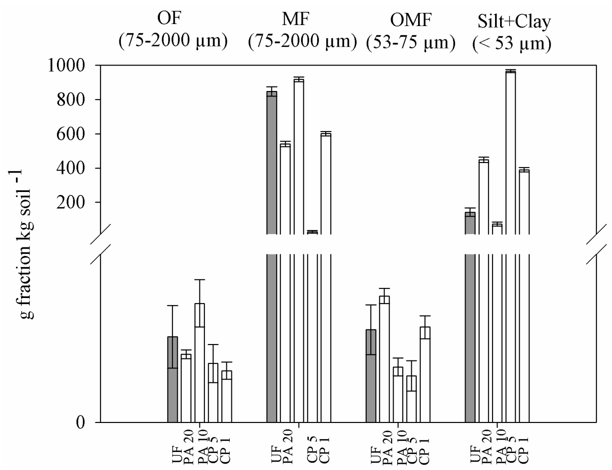

Figure 4. Mass proportion (g fraction kg·soil^{-1}) within each SOM physical fraction (0–10 cm depth) under a sequence of land use change (UF: Undisturbed Forest; PA 20: Pasture 20 years old; PA 10: Pasture 10 years old; CP 5: Cropland five years old; CP 1: Cropland one-year-old) in the Santarém region, eastern Brazilian Amazon. OF = Organic fraction; MF = Mineral fraction; OMF = Organo-mineral fraction.

3.3.3. δ^{13}C Values and C Derived from Forest and Pasture

Overall, undisturbed forest soils had the lowest δ^{13}C value, while PA20 had the highest value for the 0–10 cm layer (Table 2). A clear ^{13}C enrichment in SOM was observed according to the aging of pasture areas. Cropland soils (CP5 and CP1) presented δ^{13}C values closer to those found in UF soils. It is worth highlighting that CP5 presented numerically higher δ^{13}C values than CP1 sites, in all SOM fractions. The organic fraction (OF 75–2000 μm) presented the largest range of values, varying between −29.2‰ ± 0.40‰ in UF to −18.20‰ ± 0.60‰ in PA20, indicating that the presence of a C4 plant during at least the past 20 years had increased the δ^{13}C value of the OF fraction (Table 2). In addition, significant increases were observed even in the silt + clay fraction (−21.4‰ ± 0.5‰) in PA20, which is associated with more primitive and recalcitrant C fractions in the soil.

Based on the δ^{13}C signature, we observed that after 20 years of conversion to pasture (PA20), there is still C originating from the remaining forest vegetation, but there is also a large part of the C that was introduced from C4 plants, especially for the OM fractions (i.e., OF and OMF), where about 30% of the total carbon coming from the original forest vegetation (Table 3). In the mineral SOM fractions, MF and silt + clay, the C3-derived C still accounted for 68% and 49% of the total soil C, respectively. In general, under other land uses (i.e., PA10, CP5, and CP1) the great majority of C (73% to 97%) in the SOM fraction derives from C3 plants, indicating low inputs of C4-C in those soils. Relative proportions of C-C3 and C-C4 did not statistically differ among PA10, CP5, and CP1 land uses.

Table 2. Soil organic C stocks and $\delta^{13}C$ within each SOM physical fraction (0–10 cm depth) under a sequence of land use change (UF: Undisturbed Forest; PA 20: Pasture 20 years old; PA 10: Pasture 10 years old; CP 5: Cropland five years old; CP 1: Cropland one-year-old) in the Santarém region, eastern Brazilian Amazon.

Land Use	**OF**	**MF**	**OMF**	**Silt + Clay**
	75–2000 μm	**75–2000 μm**	**53–75 μm**	**<53 μm**
		SOC (Mg·ha^{-1})		
UF	1.2 ± 0.5 a B *	0.5 ± 0.2 a B	0.1 ± 0.04 b B	10.0 ± 1.9 ab A
PA20	0.9 ± 0.1 a B	0.1 ± 0.1 a B	0.2 ± 0.02 ab B	15.6 ± 1.2 a A
PA10	2.6 ± 0.9 a B	0.4 ± 0.3 a B	0.3 ± 0.10 ab B	8.1 ± 1.2 b A
CP5	1.2 ± 0.4 a B	0.3 ± 0.1 a B	0.5 ± 0.20 a B	16.8 ± 1.5 a A
CP1	0.6 ± 0.2 a B	0.1 ± 0.1 a B	0.1 ± 0.04 ab B	12.2 ± 2.3 ab A
		$\delta^{13}C$ (‰)		
UF	−29.2 ± 0.4 c A	−28.0 ± 0.9 b A	−28.9 ± 0.5 c A	−28.8 ± 0.1 c A
PA20	−18.2 ± 0.6 a A	−23.6 ± 0.3 a B	−18.7 ± 0.6 a A	−21.4 ± 0.5 a B
PA10	−25.3 ± 0.8 b A	−26.5 ± 0.2 b A	−25.1 ± 0.7 b A	−25.6 ± 0.4 b A
CP5	−28.5 ± 0.1 bc B	−26.9 ± 0.3 b A	−28.5 ± 0.2 c B	−27.4 ± 0.3 bc AB
CP1	−26.2 ± 0.6 bc B	−25.9 ± 0.2 ab A	−26.3 ± 0.8 bc B	−26.2 ± 0.6 b AB

* Means followed by the same capital letter (line—comparison among fractions within same land use) and lowercase letter (column—comparison among land uses within same fraction) do not differ among themselves according to Bonferroni's test ($p < 0.05$). $n = 5$. OF = Organic fraction; MF = Mineral fraction; OMF = Organo-mineral fraction.

Table 3. Relative proportion of carbon derived from C-C3 and C-C4 photosynthetic cycle plants in each soil organic matter fraction (i.e., organic (OF), mineral (MF), organo-mineral (OMF) and silt + clay fractions) due to land use changes (undisturbed forest (UF), pasture 20 (PA20) and 10 (PA10) years old and cropland 5 (CP5) and 1 (CP1) years old) in Santarém-Belterra region, eastern Brazilian Amazon.

Land Use	**OF**	**MF**	**OMF**	**Silt + Clay**
	75–2000 μm	**75–2000 μm**	**53–75 μm**	**<53μm**
		% C-C4		
UF	-	-	-	-
PA20	74.0 ± 4.2 a A *	31.8 ± 1.9 a C	70.0 ± 4.5 a A	51.0 ± 3.1 a B
PA10	26.4 ± 5.5 b A	11.2 ± 1.5 b A	26.0 ± 4.6 b A	22.6 ± 2.8 b A
CP5	5.0 ± 1.0 b A	8.0 ± 2.3 b A	2.7 ± 1.2 c A	9.3 ± 1.8 b A
CP1	20.0 ± 4.4 b A	15.0 ± 1.0 b A	17.8 ± 5.9 bc A	18.5 ± 4.3 b A
		% C-C3		
UF	-	-	-	-
PA20	26.0 ± 4.2 b C	68.2 ± 1.9 b A	30.0 ± 4.5 c C	49.0 ± 3.1 b B
PA10	73.6 ± 5.6 a A	88.8 ± 1.5 a A	74.0 ± 4.6 b A	77.4 ± 2.8 a A
CP5	95.0 ± 1 a A	92.0 ± 2.6 a A	97.3 ± 1.2 a A	90.7 ± 1.8 a A
CP1	80.0 ± 4.4 a A	85.0 ± 1.0 a A	82.3 ± 5.9 ab A	81.5 ± 4.3 a A

* Means followed by the same capital letter (line—comparison among fractions within same land use) and lowercase letter (column—comparison among land uses within same fraction) do not differ among themselves according to Bonferroni's test ($p < 0.05$). $n = 5$.

4. Discussion

4.1. Land Use and Management Changes vs. Soil C and N Stocks

Our results demonstrate that forest disturbance (especially from fire) and land use change in the Eastern Amazon have negatively affected soil C and N stocks. A combination of fire and logging can severely alter the forest structure and drastically change the above- and belowground C and N stocks [22,24,25]. During the burning of a forest, a large amount of C is transferred to the atmosphere (e.g., CO_2 and CO). Recently, controlled experiments of fire in the Amazon forest have shown that about 60 $Mg \cdot ha^{-1}$ of soil C is lost during a single burning event [26].

Vegetation clearance also interrupts the C and N inputs in the soil, resulting in an imbalance between the inputs and outputs of C and N, and releasing these elements to atmosphere as GHG emissions. Furthermore, uncovered soil increases the exposure of SOM to more intensive climatic factors (temperature and precipitation) that accelerate the rate of decomposition of SOM. Consequently, the levels of soil C and N decrease [15,27–29].

Secondary forests can play an important role in regional carbon balance [30–32], assimilating CO_2 through increased photosynthesis following the conversion of the original forest [33], and after 20 years, the aboveground biomass can recover an average of 122 megagrams per hectare ($Mg \cdot ha^{-1}$), corresponding to a net carbon uptake of 3.05 $Mg \cdot C \cdot ha^{-1} \cdot yr^{-1}$; eleven times the uptake rate of old-growth forests [34]. We show how this rapid regrowth of vegetation influences the soil (Figure 2), as SF sites presented soil C and N stocks which were statistically similar to undisturbed forest.

In addition to forest disturbance, the conversion of forests to agriculture is the major environmental threat facing the eastern Brazilian Amazon. We hypothesized that converting Amazon forest to either pasture or cropland would promote significant soil C and N losses, since those agricultural land uses result in intensive soil disturbance during the conversion process and subsequent management. However, our hypothesis was only partially accepted, since the conversion of forest to pasture did not result in any significant changes in soil C and N stocks, supporting the results of previous research [24]. In a recent meta-analysis, Fujisaki et al. [24] showed that the conversion of Amazon forest to pasture (mean age of 17.6 years) may promote slight increases in SOC stocks (6.8 $\pm$ 3.1 $Mg \cdot ha^{-1}$) in the top layers of the soil (0–20/30 cm) [24]. Moreover, the conversion from forest to pasture increased C stocks within deeper soil layers (0–100 cm) in the Brazilian Amazon region near the BR163 road, in the Mato Grosso state [25]. A regional survey of pastures that included other Brazilian biomes, such as Cerrado, Atlantic Forest, and Pampas, [35] found that the absolute change in the SOC stocks during the conversion of native vegetation to pastures, indicated an average gain of C of 6.7 $Mg \cdot ha^{-1}$ compared to native vegetation, or relative gains of 15%. However, it is worth mentioning that those authors also reported losses of SOC following the conversion to pasture in 17 paired sites, highlighting the uncertainties (e.g., soil type and management) associated with soil sample data.

One of the reasons why soil C stocks did not change in pastures is due to the introduction of perennial grasses, which are able to accumulate and redistribute C in subsurface soil - well-managed pastures, with a high biomass input and lack of soil disturbance, are able to sequester large amounts of C [36]. The Brazilian Amazon region comprises about 13 Mha of degraded pastures. Cerri et al. [27] estimated that, if these areas were restored under good management practices, they could potentially accumulate C at a rate of 0.27 $Mg \cdot C \cdot ha^{-1} \cdot year^{-1}$ in the 0–30 cm layer. Some studies reported that a new equilibrium in soil C stocks and potential C sequestration in pasture areas can only be reached after several years (probably more than 10 years) of improved management [37,38]. In the Santarém region, the average age of pastures is around 10 years (young pasture), and the high soil C and N stocks found in those areas illustrate the great potential of pastures for sequestering C in the soil; this could be further increased by adopting agricultural practice guidelines such as the integrated crop-livestock system (ICL) [39]. Despite this, converting primary Amazon forest to pasture precipitates a drastic loss of both aboveground C and biodiversity, both of which affect the conservation and delivery of several ecosystem services [40–42].

Croplands differed significantly from UF, with a loss of approximately 10 Mg·C·ha^{-1} following conversion (Figure 2), indicating that when the conversion to an annual agriculture occurs, there is a decline in soil C and N stocks, and consequently, an increase in the CO_2 and N_2O emissions from the soil. A meta-analysis showed that conversions from Amazon forest to croplands (mean cropland age of 8.7 years) decreases SOC stocks (-8.5 ± 2.9 Mg·ha^{-1}) [24]. In contrast to the results obtained in this study, Neto et al. [43] found no significant difference in the soil C stocks between cropland and native vegetation in the Cerrado region of Brazil.

On the other hand, after thirty years of the conversion from native vegetation to pasture, the original SOM from native vegetation decreased significantly and only a small quantity of new organic matter was introduced from tropical grasses into the soil, to offset the losses, reflected in a net C emission of 0.4 Mg·ha^{-1}·yr^{-1} [44].

Considering the results obtained from the isotopic signals in our study, it was possible to separate the studied land uses into three distinct situations. The first one is formed by one group of all the forests classes (UF, LF, BF, LBF, and SF), because they have similar values of δ^{13}C at all depths. The second situation is illustrated by the CP, with an intermediate stage of dilution, between forests and PA, with CP areas cultivated with a soybean and maize rotation – resulting in an expected isotopic signal between the values of C3 and C4 plants. Finally, the land use PA is only composed of plants with a C4 cycle and thus, has the higher values of δ^{13}C.

Pasture areas were also compared with forest areas by Bernoux et al. [20] in the Paragominas region, Para State of Brazil, and they found values similar to those found in this study. They observed a δ^{13}C in forests equal to -27.7‰ at 0–10 cm depth, and equal to -26.4‰ in the 20–30 cm layer. For PA, the values observed at 0–10 cm depth were -25.8 ‰, -23.9‰, and -22.4‰ in pastures with 4, 10, and 15 years, respectively. Thus, the higher values of δ^{13}C found in these land uses can be associated with the dynamic vegetation changes that are typical for our study region, and the eastern Amazon in general. Tarre et al. [45] studied the variation of δ^{13}C in a pasture of *Brachiaria* (C4 plants), established in an area previously occupied by forest (C3 cycle), and they observed that SOM was enriched by carbon from PA (-12‰) for a long time.

The δ^{13}C values obtained from 16 pasture chronosequences in the Amazon region indicated that the forest-derived SOC can vary among sites, while pasture-derived SOC varies less and was characterized by a dynamic accumulation plateau of 20 Mg SOC ha^{-1} after 20 years [24].

The δ^{15}N signatures showed a pronounced overall increase in ^{15}N enrichment with increasing soil depth in all land uses and field sites investigated (Figure 3). Increases in SOM ^{15}N enrichment have been described as a result of the progress in the mineralization, nitrification, denitrification, and volatilization processes [46,47], and are typically accompanied by reductions in SOM levels, indicating organic matter decomposition [44].

According to Zeller et al. [48] there is a high variability for both the liberation and incorporation of soil N between the different types of forest, which is strongly associated with the soil type and amount of organic matter in the soil. However, in the case of areas under cropland, δ^{15}N is enriched with fertilizers, such as ammonium sulfate. Using techniques that employ ion exchange resins, it is possible to obtain nitrogenous substances with a proportion of δ^{15}N greater than that found in nature [49].

According to Alves et al. [50], most of the δ^{15}N variation in Amazon forests is attributable to site-specific conditions, strongly influenced by extractable soil phosphorus and dry-season precipitation, suggesting a restricted availability of nitrogen in both young and old soils, and/or at low precipitation levels. The authors concluded that plant δ^{15}N levels indicate that low levels of nitrogen availability are only likely to influence Amazon forest function with immature or old weathered soils and/or where dry-season precipitation is low. In the case of our study, the ^{15}N signal decreased from native vegetation to secondary forest, suggesting that SFs accumulate more recalcitrant SOM.

4.2. Land Use Changes vs. SOM Quality

Initially, SOM physical fractions are highly influenced by the type of plant that is the origin of the organic fraction and controls whether the SOM has a low or a fast rate of decomposition, as well as how rich the SOM is in C and N. Depending on the type of material that provides the original SOM, this will increase C and N contents in a short-time period and will characterize the signature of δ^{13}C [6,9,51]. This was observed in our results in the organic fraction (OF 75–2000 µm), where there are still fresh materials deposited by the current vegetation on the soil surface. According to the δ^{13}C values, the highest δ^{13}C observed in pasture with 20 years indicates that time is also an important factor in determining the SOM origin and dynamics, and that OF is the fraction that is closest to the original C4 and C3 values. Here, we show that OF in PA 20 and PA 10 presented the highest δ^{13}C values, while the UF presented the lowest ones (Table 2).

The type of vegetation also influences the proportion of C3-C% remaining in the soil and it is clear that the more time a C4 plant occupies the land, the less C3-C% contributes to the SOM origins and composition; as we can see in all fractions under PA 20 site (Table 2). Pasturelands provide a good opportunity to view these differences, because they are always seeded with C4 plants, the grasses. On the other hand, croplands in the Santarém region are characterized by an annual agriculture which receives a system that rotates crops with soybean, corn, and rice being the main crops. Thus, the isotopic dilution under these land uses (CP1 and CP5) is still not well defined and the SOM under this land uses presents a high contribution of C3 plants to the SOM.

Another important result that was observed in this study is that SOM physical fractions are potentially influenced by soil texture [6]. The highest values of mass (g fraction kg^{-1} soil) were found under the silt + clay (<53 µm) fraction, where its associated with very clayey soils (Figure 4). As a consequence, the highest C stocks were also observed on the silt+clay fraction (Table 2). This is considered an important fraction as it retains a more recalcitrant C [9,52–54].

The organo-mineral fraction (FOM 53–75 µm) was present in a greater proportion under PA 20, while the lowest fraction was observed under CP 5. This fraction is the one that is bound between soil aggregates and functions, as a cementing agent keeping the soil structure stable and strong [54–56]. This was expected since pastures are considered as good keepers of soil aggregates, because this system does not require soil tillage and plowing. On the other hand most cropland systems use intensive methods of soil preparation, which break down soil aggregates and expose the soil C presented on the FOM fraction [52].

5. Conclusions

Anthropogenic disturbances in the Amazon forest, mainly through burning, promote significant declines in soil C and N stocks in shallow (0–30 cm) soils. The conversion of Amazon forest to pasture did not affect soil C and N stocks, probably because tropical grasses have a strong capacity to add C (C4-derived C) into the soil via aboveground biomass and vigorous root systems, gradually replacing native C (forest-derived C) and compensating for its loss. By contrast, the conversion from forest to cropland resulted in significant depletions of soil C and N, and consequently C and N emissions to the atmosphere. Land use change also induced alterations in SOM quality. Long-term conversion from Amazon forest to pasture (i.e., at least 20 years) had a greater effect on organic fractions of SOM, through the introduction of more recalcitrant C to the soil. Nevertheless, soil C storage is primarily controlled by a fine mineral fraction (i.e., silt + clay) content in the soil, which is relatively insensitive to land use and management practice changes.

The adoption of more sustainable conservation agricultural practices is needed for the Amazon region. In some situations, land use changes, and the associated impact on the soil condition, may decrease the capacity of the forest to provide multiple ecosystem services at both local scales (e.g., food source and habit for endemic soil organisms), and global scales (e.g., C sequestration and its impacts on global climate changes). Finally, our results provide support to ensure the implementation of appropriate forest management systems, whilst also calling further attention to the need for a fire monitoring system, helping to ensure the resilience of C and N stocks and sequestration in forest soils, thereby contributing towards urgently needed ongoing efforts to mitigate climate change.

Supplementary Materials: The following are available online at www.mdpi.com/2071-1050/9/3/379/s1, Table S1: Mean soil macronutrient contents for the primary land uses studied in Santarém-Belterra region, eastern Brazilian Amazon, Table S2: Mean soil acidity attribute values and effective and potential cation exchange capacity (CECpH7 and CECeffective) values for the primary land uses studied in Santarém-Belterra region, eastern Brazilian Amazon, Table S3: Mean soil clay, silt and sand contents for the primary land uses studied in Santarém-Belterra region, eastern Brazilian Amazon.

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