



Article Carbon and Nitrogen Stocks and Soil Organic Matter Persistence under Native Vegetation along a Topographic and Vegetation Gradient in the Central Amazon Region

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Abstract: The Amazon Forest has a soil organic carbon stock (SOCS) potential of 126 to 141 Tg year⁻¹ and it depends on soil organic matter (SOM) accumulation factors and stabilization mechanisms. This study aimed to evaluate SOCS, soil nitrogen stocks (SNS), SOM fractions under the Amazon Forest along a topographic and vegetation gradient (Terra Firme, River Plain, and Terraces), and to evaluate the main mechanisms responsible for SOM stabilization. The study was developed using 35 study points (35 profiles) in Coari County, Amazon State, Brazil. In each profile, soil samples were collected from soil horizon for soil analysis. Of the 35 soil profiles, 10 were selected to evaluate the contribution of free light fractions (FLF) and intra-aggregate light fractions (ILF), C and N contents, and SOCS and SNS up to 1 m soil depth. SOCS and SNS are influenced by topographic and vegetation gradient, being statistically equal in the Terra Firme and River Plains areas (median of 92.5 and 92.2 Mg C ha⁻¹, respectively), but Terraces presented a greater median (157.9 Mg C ha⁻¹). There are relationships between SOCS and SNS and C, N, Al, clay content, t value, FLF, and ILF. SOCS, SNS, and SOM stabilization in Amazon soils are influenced by soil properties and landscape position. SOCS in the Terrace is mainly in FLF form. If vegetation cover loss continues, an amount of up to 98.05 Mg C ha⁻¹ of FLF can be lost, causing soil degradation and global warming.

Keywords: free light fractions; intra-aggregate light fractions; physical fractionation of SOM; soil carbon stabilization

1. Introduction

Throughout the 28th Conference of Parties (COP28), held in Dubai in 2023, national governments and organizations announced commitments to the need for sustainable development and payment mechanisms to reduce deforestation; an example is the Amazon Fund. At COP28, the Brazilian president said that Brazil can preserve its forests and increase production. It is essential to prevent the Amazon Forest from reaching a tipping point from which it will start emitting more carbon than it retains.

The Amazon Forest vegetation has a huge impact on carbon sequestration. The vegetation compartment is subjected to human interferences, such as forest fires and deforestation, which are closely associated to the advancement of animal husbandry and illegal mining.



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). According to the National Institute for Space Research (INPE) [1], the deforestation rate in the nine states of the Brazilian Legal Amazon was estimated at approximately 9.8 km² (data from August 2018 to July 2019). An increase in deforestation of \pm 34% was recorded in 2020 [1]. This means not only loss of biodiversity, but also the increase of emissions of soil C stored in an equilibrium environment as the Amazon Forest stores an amount of carbon equivalent to 15–20 years of global CO₂ emissions (150–200 Pg C) [2]. However, from January to August 2023, deforestation in the Amazon Forest declined by 48.9%, compared to the same period of the previous year, according to INPE [3]. Therefore, Brazil avoided the emissions of 200 million tons of carbon dioxide to the atmosphere. This win was strongly celebrated during the COP28, as all countries agreed that the Amazon cannot exceed the 'point of no return'. This means if deforestation exceeds 25%, the forest will enter a savannization process.

The role of soil in the biogeochemical carbon cycle is undeniable. According to the Intergovernmental Panel on Climate Change (IPCC), soil stores approximately four times more C than plant biomass and three times more than the atmosphere. An inadequate soil management does not only contribute to intensified greenhouse effects but also creates problems related to soil security [4]. Losses of soil organic matter (SOM), considered as a soil security indicator, result in the degradation of the physical and chemical quality of the soil, as well as soil biodiversity [5]. Organic material in the soil is easily decomposed when non-conservationist management practices are carried out [6]. According to [7], the Brazilian forests contribute greatly in mitigating the greenhouse effect, not only in the sequestration of C, but also in the maintenance of current C stocks. That is, avoiding the emission of CO_2 through accelerated degradation or burning organic material.

Understanding the main mechanisms and factors driving soil C persistence in soils is as important as its stocks. It is important to clarify in which compartment and forms (active or stable) soil C are in soils to indicate soil capability and management to maintain soil security. Soils under forests are reference areas for elucidating soil C accumulation and persistence in soils [8]. The persistence of SOM should not be restricted to total organic carbon. SOM has two main compartments, including a living component (composed mainly of microfauna, soil microorganisms, and plant roots), and a dead component [9]. This dead component can be further subdivided into the free light fraction (FLF), or macroorganic matter, intra-aggregate light fraction (ILF), and the heavy fraction [10,11]. The FLF is the most sensitive to changes caused by human interference, since it is composed of plant residues and other labile components that are quickly depleted after removing the vegetation and the top layers [4], while the ILF and the heavy fraction are protected by occlusion in soil aggregates and soil chemical interactions, respectively [12,13].

Regarding the control of SOM persistence in soils, it is also known that the five soil formation factors are involved [14]. Climate, vegetation, and parent material are known to be the main actors in SOM storage, but their interactions are still largely unknown [15,16]. The landscape of Amazonia is cut by a sequence of valleys and slopes, separated by plateau areas [17]. Soils on these topographic features have different physical and chemical properties, being sandier in valley bottoms and having a high clay content on plateaus [18–20]. Vegetation also changes across topographic gradients, and these differences are linked to differences in soil organic carbon (SOC) as well as SOC fractions [21].

While climate and soil geochemistry can be considered overarching drivers of SOM persistence because they largely control the two mechanisms responsible for long-term soil C storage (i.e., microbial physiological limitation and microbial access constraint, respectively), vegetation cover represents a significant driver of short-term SOM dynamics, controlling microbial communities and their functionality [21]. Further, the interplay of vegetation and associated microbial communities controls SOM persistence by affecting the distribution between active and stable SOM compartments [22].

Studies have shown that soil C persistence in soils is governed not only by the soil forming processes but also by the three carbon soil stabilization mechanisms: (i) recalcitrance; (ii) physical protection of SOM inside soil aggregates; and (iii) soil chemical protection [23]. Recently, [9] stated that SOM persistence will also depend on microbial activity inhibition, the degree of its limitation and carbon use efficiency, and microbial access constraints, primarily due to association to minerals and occlusions in fine aggregates.

In the Urucu River Basin, Central Amazon, SOC stock is higher in soils developed in relief forms exhibiting well-drained soils, which are covered by Upland Dense Tropical Rainforest compared to the high waterlogging, which are covered by Flooded Lowland Open Tropical Rainforest [24]. According to the authors, relief and vegetation are the main factors that can influence the variability of soil classes, as well as their chemical, physical, and biological attributes. Our hypothesis is that SOM labile fractions will also change along topographic and vegetation gradients, and the first input of SOM will contribute to SOCS, SNS, and C persistence in natural soils.

The present study aimed to evaluate soil organic carbon stocks (SOC) and soil nitrogen stocks (SNS), as well as SOM labile fractions (FLF and ILF) inputs under the Amazon Rainforest along a topographic and vegetation gradient. In addition, the study evaluated the main soil properties responsible for stabilizing SOC in soils. This knowledge is important to elucidate the main mechanism of soil C persistence in soils, its storage, and how much of the soil labile fraction can be lost if vegetation cover loss continues. The key question is: how can we manage soils for SOM restoration?

2. Materials and Methods

2.1. Study Area

The study site is in the central region of the Amazon State, in the Urucu River Basin, in the municipality of Coari, Brazil (Figure 1). The region belongs to the Phanerozoic Solimões sedimentary basin, which consists of Tertiary–Quaternary sediments. The climate is classified as Af (Koppen classification), characterized by the average temperature of the coldest month above 18 °C and a uniform distribution of rainfall throughout the year, with rainfall averages above 60 mm monthly. The Amazon flora is the result of climate changes that occurred in the Pleistocene, which allowed the entry of different biotypes [25]. Previous studies conducted by [24] presented a model of the relationship between soil, relief, and vegetation. According to the authors, the main vegetation types along the study site are Upland Dense Tropical Rainforest, Flooded Lowland Open Tropical Rainforest and Upland Open Tropical Rainforest [15]. The Upland Dense Tropical Rainforest is commonly found in areas with relief forms called "terra firme" (=solid earth), which are upland areas on flat-topped terrain located in river interfluves (Figure 2A). The soils in these relief forms are well drained, with no water deficits for plants, and have higher clay contents. The Upland Open Tropical Rainforests (Figure 2B) are commonly found in imperfectly drained flatlands ("Terracces"). The Flooded Lowland Open Tropical Rainforests are observed in relief forms called "floodplains", which are the river floodplains (Figure 2C).

Upland Dense Tropical Rainforest (Figure 2A) is the main type of vegetation in the study area [25]. Visually, the landscape is quite uniform but presents a large botanical variation of species. The presence of exposed roots is observed due to the presence of aluminum in the soil, which prevents the roots from deepening. The penetration of sunlight is prevented by tree leaves interfering with the development of smaller plants. Plant roots have a symbiotic association with some types of fungi. These fungi decompose organic matter deposited in the soil to absorb nutrients before they are leached, acting as primary, secondary, and tertiary decomposers. In relation to the Upland Open Tropical Forest (Figure 2B), it has an average plant biomass. Their size is much less significant than Upland Dense Tropical Rainforests and they differ considerably botanically. It covers poorly drained soils ("Terraces") since its species are more adapted to restricted soil aeration conditions, mainly in subsurface horizons [26]. The third type of vegetation (Figure 2C) also occurs in areas with poorly drained soils, called "River plains". In this environment, the vegetation type is Flooded Lowland Open Tropical Rainforest. In this vegetation type, tabular roots are common, as are pneumatophores roots that allow oxygen absorption. In



areas covered by this vegetation, it is common to observe a high density of species, such as palm trees.

Figure 1. Location of the study site. DEM: Digital Elevation Model.





Figure 2. Typical vegetation of the study area characterized as Upland Dense Tropical Rainforests (A), Upland Open Tropical Rainforests (B), and Flooded Lowland Open Tropical Rainforest (C) in Urucu, Central Amazon.

2.2. Soil Survey and Field Procedures

The soil survey was carried out from September to November 2018, during which trenches were opened for soil description. In each soil profile, a general and morphological description was conducted, including the separation of horizon/layers according to [27], where soil depth, color, texture, structure, consistency, and transition were evaluated. For

each horizon/layer, disturbed and undisturbed soil samples were collected for chemical and physical characterization, as well as soil bulk density (BD), respectively. The soils were classified according to the Brazilian Soil Classification System up to the fourth categorical level [28]. The number of profiles and the frequency of soil classes observed in the study area are presented in Table 1. In total, 35 soil profiles were classified, with emphasis on the Argissolos (Ultisoils/Acrisoils) class, which presented a contribution of 60.0% (21 profiles) of all profiles. Cambissolos (Inceptisoils/Cambisols) represent 14.3% (5 profiles), while Gleissolos (Entisols/Gleysols) and Espodossolos (Sposols/Podzols) account for 11.4% (4 profiles). One soil profile was classified as a Planossolos (Albaqualfs/Planosols) class, representing only 2.9% of all soil profiles.

Table 1. Number of soil profiles (*n*) and the frequency (%) of soil orders observed in the Urucu region, Central Amazonia.

Soil Classes (SiBCS—2018) [28]	Soil Classes (Soil Taxonomy, 2014) [29]	Soil Classes (WRB, 2022) [30]	n	Frequency (%)
Argissolos	Ultisols	Acrisols	21	60.0
Cambissolos	Inceptisols	Cambisols	5	14.3
Gleissolos	Entisols	Gleysols	4	11.4
Espodossolos	Spodosols	Podzols	4	11.4
Planossolo	Albaqualfs	Planosols	1	2.9
Total			35	100

SiBCS—Brazilian system of soil classification. WRB—world reference base for soil resources.

2.3. Soil Profile Selection to Evaluate Soil C Persistence

To evaluate the relationship between formation factors (relief and vegetation) and mechanisms for stabilizing C in the soil, ten representative profiles of the region were selected and organized according to three environmental classes (EC) (Table 2). The EC presented in this study followed the same criteria proposed by [24], which called the same environmental classes, as the soil–relief–vegetation model (SRV). For this study, three ECs are presented as follows: EC 1—Terra firme (=solid earth): refers to soils located in relief called and under Upland Dense Tropical Rainforests coverage, well-drained regions and relief varying from flat to strongly wavy; EC 2—River Plains: refers to soils located in lowland areas, close to watercourses with drainage ranging from imperfect to poorly drained and, under Flooded Lowland Open Tropical Rainforest coverage; EC 3—Terraces: soils located in regions with a wide top and flat elevation with drainage ranging from imperfect to poorly drained, under Upland Open Tropical Rainforests coverage. All 35 soil profiles were organized according to environmental classes, not just the 10 in Table 2. The images of these vegetation types are presented in Figure 3.

Table 2. The ten (10) selected profiles according to environmental classes (EC).

EC	Ν	Soil Classes (SiBCS) *	Code **	Relief	Vegetation Type
	01	ARGISSOLO VERMELHO-AMARELO Distrófico típico	PVdtip1	Terra Firme	Upland Dense Tropical Rainforests
EC1	02	ARGISSOLO VERMELHO-AMARELO Alumínico típico	PVAatip1	Terra Firme	Upland Dense Tropical Rainforests
	03	ARGISSOLO VERMELHO-AMARELO Alumínico plintossolico	PVAaplin	Terra Firme	Upland Dense Tropical Rainforests
EC2	04	ESPODOSSOLO FERRI-HUMILUVICO Órtico arênico	ESKare	River plains	Flooded Lowland Open Tropical Rainforest
EC2	05	ESPODOSSOLOS FERRI-HUMILUVICO Órtico durico	ESKodur	River plains	Flooded Lowland Open Tropical Rainforest

EC	Ν	Soil Classes (SiBCS) *	Code **	Relief	Vegetation Type	
	06	ARGISSOLO ACINZENTADO Distrófico abruptico	PACdab	Terraces	Upland Open Tropical Rainforests	
	07	GLEISSOLO HÁPLICO Tb Distrófico típico	GXbdtip2	Terraces	Upland Open Tropical Rainforestst	
EC3	EC3 08	ESPODOSSOLO FERRI-HUMILUVICO Órtico típico	ESKotip	Terraces	Upland Open Tropical Rainforestst	
09 10	09	GLEISSOLO MELÂNICO Tb Alumínico organossólico	GMbaorg	Terraces	Upland Open Tropical Rainforests	
	10	PLANOSSOLO HÁPLICO Distrófico gleisólico endoalumínico	SXdglei	Terraces	Upland Open Tropical Rainforests	

N—number of the soil profiles; * SiBCS—Brazilian system of soil classification. The nomenclature presented is maintained in the Portuguese language (SiBCS), once, at this level of classification (subgroups), there is not a direct correspondence of these soils classes with respective names using the soil taxonomy and the WRB system. ** Code—soil classes abbreviation according to SiBCS.



Figure 3. The schematic visualizations of environmental classes (EC) and the distribution of the 10 soil profiles.

2.4. Soil Analyses

In the laboratory, the soil analyses (chemical and physical) followed the procedures described in [31]. The depth collected in each soil profile and the numbers of soil horizons varied between soil classes. Disturbed soil samples from each soil horizon/layer were air-dried and subsequently screened with the aid of a 2.0 mm diameter sieve to obtain the air-dried fine earth fraction. From these samples, granulometric analysis was carried out using pipette method (total contents of sand, silt and clay). Particle density (PD) was determined using the volumetric flask method and using ethyl alcohol to expel air. The undisturbed samples to determine soil bulk density (BD) were collected using Kopecky rings, with dimensions of 5 cm in height and 2.5 cm in radius, totaling a total volume of 98.13 cm⁻³. BD was calculated using the Equation (1), as follows:

$$BD = Ms/Tv$$
(1)

The variables of the equation are as follows: BD—soil bulk density, Ms—oven-dried soil mass (105–110 °C, 48 h),

Table 2. Cont.

Tv—total soil sample volume.

In each horizon/layer, the pH, contents of Ca^{2+} , Mg^{2+} , Al^{3+} , K^+ , Na^+ , P, and potential acidity (H⁺ + Al⁺³) were determined. Base saturation (V%), sum of bases (SB), cation exchange capacity (CEC) and exchangeable aluminum saturation (m%) were also determined. The organic C content of the soil was determined using the Walkley and Black method [32]. The analysis of the total N content of the soil was carried out using the method proposed by [33], in which nitrogen compounds (proteins, amines) are converted into ammonia. So, a chemical and physical analysis was performed for all soil horizons.

Soil carbon and nitrogen stocks were quantified according to [34], and the data were calculated in the first 100 cm of depth (Equations (2) and (3), respectively).

$$SOCS = (C \times BD \times T) \times 10$$
 (2)

$$SNS = (N \times BD \times T) \times 10 \tag{3}$$

The variables of the equation are as follows:

SOCS—soil organic carbon stocks (Mg ha^{-1}),

SNS—soil nitrogen stocks (Mg ha^{-1}),

C—organic carbon content (Kg Mg^{-1}),

N—nitrogen content (Kg Mg $^{-1}$),

BD—soil bulk density (Mg m^{-3}),

T—thickness of the soil layer (m).

The light fractions of SOM were extracted from each soil horizon, namely: free light fraction (FLF) and intra-aggregate light fraction (ILF). Extractions were carried out in all soil horizons of the ten selected soil profiles (Section 2.3). A modified procedure based on the densimetric fractionation, proposed by [35], was used. FLF and ILF were extracted from the soil sample weighing 5 g, using 35 mL sodium iodide (NaI) solution (density of $1.80 \pm 0.02 \text{ g cm}^{-3}$) in a centrifuge tube. A Hielscher Ultrasound (Hieslscher, Teltow, Germany, model UP400S) was used to disperse mineral particles and obtain FLI. For this, energy of 600 J mL⁻¹ was applied. Fractions were separated by centrifugation at 3000 rpm for 15 min.

2.5. Statistical Analysis

The data were evaluated using multivariate analysis, applying principal component and hierarchical cluster techniques. Initially, the 35 profiles were evaluated for C and N stocks, and later correlated to physical and chemical attributes (clay, silt, total sand, soil and particle density, assortative complex values, exchangeable aluminum, hydrogen, total organic carbon, and total nitrogen). Thereafter, the ten selected profiles were evaluated in relation to the contents of light SOM fractions, total organic carbon and total nitrogen using the "AQP" package (algorithm for quantitative pedology) and its "SCP" (soil profile collection) function to generate graphical sketches of the profiles, based on their horizon limits. In addition, multivariate analyzes of these ten profiles were generated, associating the light fractions of SOM with the contents and stocks of C and N and physical attributes (clay, silt, total sand, and soil density) through the Factominer and Factoextra packages in R 3.6 software (p < 0.05).

3. Results

3.1. Soil Organic Carbon and Nitrogen Stocks in Soils

Considering all 35 soil profiles, SOCS and SNS values ranged from 27.4 to 230.3 Mg C ha⁻¹ and between 8.5 and 42.0 Mg N ha⁻¹, respectively (Figure 4A,B). It was observed differences in SOCS and SNS average when considering landscape position (Terra Firme, Terraces, and River Plains) and type of vegetation. The greatest amplitude of SOCS variation was observed in the Terraces (EC3), with minimum and maximum values of 27.4 and 230.3 Mg C ha⁻¹, respectively. The lowest value of SNS (Figure 4B) was found in the River

Plains (EC2) area (8.5 Mg N ha⁻¹). A box plot analysis of SOCS and SNS values, in each environmental class (EC1-Terra Firme = solid earth, EC-2 River Plains, and EC-3 Terraces) is presented in Figure 4C. SOCS were statistically equal in EC1 and EC2 (median of 92.5 and 92.2 Mg C ha⁻¹, respectively), but the EC3 area presented median statistically greater (157.9 Mg C ha⁻¹, Figure 4C). SNS followed the same pattern as SOCS, with similar median values for EC1 and EC2. SNS data variation was greater in EC3, with a median value higher than 30 Mg N ha⁻¹. SOCS and SNS also varied between soil classes; however, there is no pattern of SOCS and SNS in relation to soil classes. On the other hand, the highest SOCS values for the soil profile 9 (230.3 Mg C ha⁻¹) and soil profile 8 (204.7 Mg C ha⁻¹) soils stand out (Figure 4A).



Figure 4. The SOCS (**A**) and SNS (**B**) values (Mg ha⁻¹) of the 35 soil profiles and the average values for each environmental class (**C**).

The principal component analysis (PCA) of soil variables in the 35 soil profiles is presented in Figure 5. The axis Dim 1 and Dim 2, together, explained 53.8% of the variance, in which the first component (Dim 1 axis) explained 38.1% of the total variation, while the second component (Dim 2 axis) accounted for 15.7% (Figure 5). The variables which most contributed to the formation of the axes were effective exchange capacity (t), exchangeable aluminum (Al), hydrogen (H), base saturation (V value), sum of bases (S value), soil bulk density (Bd), and sand (sand) and clay content (clay). SOC and SNS are highly correlated with soil C, N, and exchangeable Al contents, t value and clay contents and are inversely correlated with sand content, particle density (PD), Bd, exchangeable hydrogen (H), and V and S values (Figure 5).



Figure 5. Principal component analysis (Dim1 and Dim2) of soil variables in 35 soil profiles. S, sum of bases; V, base saturation; H, H⁺; Sand, total sand; Pd, particle density; Bd, bulk density; Silt, total silt; C, total organic carbon; N, total nitrogen; Clay, total clay; SOCS, soil organic carbon stock; SNS, soil nitrogen stock; t, effective cation exchange capacity; AL, Al³⁺.

3.2. Light Fractions of SOM in the Selected Soil Profiles

The FLF and ILF (g kg⁻¹) inputs in the selected soil profiles (Table 2) are shown in Figure 6. In general, the contribution of FLF and ILF in all soil classes were higher than that observed for other soils in the Brazilian biomes [36,37]. In all soil classes, the amount of FLF and ILF was higher in the surface layer than in the subsurface horizons, except for the spodosols. As it was expected, the spodosols (soil profiles 4, 5, and 8) showed a slight increase in the input of FLF but a strong contribution of ILF at depth, in their spodic B horizons (Figure 6).



EC2 - River Plains



Figure 6. FLF and ILF inputs $(g kg^{-1})$ of the ten selected soil profiles.

FLF and ILF input were not homogenous along the topographic and vegetation gradient with higher values at EC3 followed by EC1 and then by EC2. It is important to highlight that at EC3, under poor drainage conditions, the amounts of FLF in the first

horizons were 842.4, 351.4, 91.2, 44.2, and 9.7 g kg⁻¹ in the (soil profiles 9, 10, 6, 7, and 8, respectively) (Table 2). ILF inputs were 15.3, 21.0, 3.2, 2.9, and 3.5 g kg⁻¹, respectively. FLF and ILF values are higher compared to other soils even those evaluated in Amazon soils [36,37].

Total organic C and N content in the selected soil profiles was also influenced by the topographic gradient (Figure 7). Total C content has the same behavior as FLF, being higher at EC3 and lower at EC2. Soil profile 9, situated in the EC3, presented the highest total soil C and N content compared to other evaluated soil class. The greater inputs of FLF and ILF were also observed in these same soil profile, resulting in greater total organic C and N contents and also the highest SOCS (230.3 Mg ha⁻¹), as shown in Table 3. Moreover, a straight relationship was also observed between total organic C and N contents with exchangeable Al and the t value (Figure 5).

N *	SOCS	SNS
	EC1	
01	149.2	12.8
02	66.2	28.6
03	113.5	36.8
	EC2	
04	110.1	14.4
05	57.3	8.5
	EC3	
06	181.2	34.2
07	27.4	21.1
08	204.7	22.0
09	230.3	39.7
10	163.0	42.0

Table 3. SOCS and SNS of the ten selected soil profiles (Mg ha⁻¹, 100 cm soil depth).

* Number of the ten soil profiles.

As settled in the literature, soil organic C and N contents decreased in soil depth, except for spodosols, with increases being observed in the spodic B horizons. Soil profile 8 showed high ILF and organic C contribution in the spodic horizons resulting the second highest SOCS (204.7 Mg ha⁻¹) at EC3. These results are supported by principal component analysis for the selected soil profiles (Figure 8). It evaluated the relationship between soil attributes (clay, sand, silt, BD, SOCS, SNS, total carbon (C) and nitrogen (N) content, FLF, and ILF) and soil classes. The two main components (Dim1 and Dim2) explain 71.8% of the variance. The first component (Dim1) explains 50% of the total variance, while the second component (dim2) explains 21.8% of the total variance. Soil profile 9 is in the same quadrant as clay, total C content, ILF and SOCS (Figure 8). Soil profile 8 has a stronger relationship with sand content. The high sand content (horizon E1 and E2) plays an important role in FLF, ILF, and total C content eluviation to the spodic horizon. Soil profile 8, however the highest SOCS and SNS (Table 3), is in the same quadrant as soil profile 8, however the highest C and N contents, FLF, and ILF are in the superficial depths. This similarity between soil profiles 8 and 6 is also presented in the dendrogram (Figure 9).



EC1 - Terra Firme









Figure 7. Distribution of carbon and nitrogen content (g kg^{-1}) of the ten selected soil profiles.



Figure 8. Principal component analysis for the ten selected soil profiles. Sand, total sand; BD, bulk density; Silt, total silt; N, total nitrogen; C, total organic carbon; Clay, total clay; SNS, soil nitrogen stock; SOCS, soil organic carbon stock; FLF, free light fraction; ILF, intra-aggregate light fraction.



Figure 9. Cluster analysis (cluster plot and dendrogram) of the ten selected soil profiles.

The similarity presented in the dendrogram (Figure 9) increases as the Y axis goes down. In other words, a height of ten implies that the soils are 100% dissimilar, while at

a height of two, the soils are 20% dissimilar (80% similar in Euclidean distance terms—X axis). Right from the start (at height 10), it is noted that the soil profile 9 stands out from all the others. In fact, it is a very peculiar soil in its high content of organic matter and light fractions in the first 50 cm of depth, coinciding with what is shown in Figures 6 and 7. As highlighted before, this soil profile presents a large amount of FLF and ILF in its surface horizons. It is located on a *plateau*, with moderate drainage, under the influence of Upland Open Tropical Rainforest that consists mainly of palm trees (EC3). According to [14], this vegetation has organic material with high lignin and polyphenol contents, resulting in high particulate organic carbon (POC). POC consists basically of FLF, ILF, and organic material associated with soil sand fractions. It is the main soil organic material contributing to soil C and N contents and stocks.

At a height close to six (60% dissimilarity), soils with different ECs are grouped together. For example, a group on the right is formed composed of the following soils profiles: 1, 2, 3, 6, and 8. The first three soils (1, 2, and 3) occur in the EC1 environment, while the remaining two (6 and 8) are in the EC3 environment.

Still at that same height (close to six), there is another group that incorporates soils from the environment EC3 (7 and 10) and EC2 (4 and 5).

Finally, below a height of four (more than 60% similarity), practically all grouped soils are those that belong to the same environmental class (EC1, EC2, and EC3). The only exception is the soil profile 1 (belonging to the EC1) and which continues to be grouped with soils from the EC3.

The cluster data demonstrate that there is a relative pattern of similarity between the soils in relation to the EC1, EC2, and EC3 environments. Environments with more sand are related to spodosols (soil profiles 4, 5, and 8—Figure 8). Analyzing Figure 9, it is noted that the soil profiles 8 and 9 occur in EC2, while the soil profile 8 is also found in EC3. The River Plains environment (EC2) occurs in relatively lower regions of the landscape and is influenced by rivers, with a notable presence of sandy sediment deposits, especially in rivers of greater hydrological order. In the EC3 environment, soil profile 8 is also found in regions closer to watercourses, but with lower hydrological orders.

4. Discussion

Soil Organic Carbon and Nitrogen Stocks

The present study aimed to evaluate SOCS and SONS, as well as SOC fractions (FLF and ILF), input in the Amazon Rainforest, along a topographic and vegetation gradient. In the Urucu River Basin, Central Amazon, [24] observed higher SOCS in soils developed in relief forms exhibiting well-drained soils, which are covered by Upland Dense Tropical Rainforest compared to the high waterlogging which are covered by Flooded Low land Open Tropical Rainforest. According to the authors, relief and vegetation are the main factors that can influence the variability of soil classes, as well as their chemical, physical, and biological attributes [24]. In this study, our hypothesis is that SOM fractions will also change along topographic and vegetation gradients and that the first input of the labile SOM will contribute to SOCS and SNS and C stabilization in natural soils.

Considering all 35 soil profiles studied, SOCS and SNS values changed in topographic gradient and, consequently, in soils class and vegetation types (Figure 4). However, the highest SOCS and SNS were observed in the Terraces area under Upland Open Tropical Rainforest. Our results demonstrate that when expanding the study area, especially in a complex territory such as the Amazon, some soil patterns are found that could not be detected in the previous work of [24]. In fact, this is due to the highest SOCS values of the studied soil profiles 9 and 8. In areas covered by this vegetation it is common to observe a high density of species, such as palm trees. This type of vegetation contributes to the recalcitrance of SOM, which is considered one mechanism for SOC persistence. This recalcitrance may be attributed to the high lignin and polyphenol contents that prevent soil organic material mineralization. As a result, these recalcitrance materials can be accumulated in soils as FLF and ILF. And this answers our hypothesis: SOM fraction

inputs are either heterogeneous along the topographic and vegetation gradient, presenting higher inputs at Terraces, followed by Terra Firme, and then by River Plain area. The Terraces area presents the largest amount of both fractions (FLF and ILF) in its surface horizons, higher soil organic C and N content, and higher SOC and SNS compared to other landscape positions.

A straight relation was also observed between total organic C and N contents with exchangeable Al and value t (Figure 5). Due to mineralogy, Amazonian soils contain high amounts of aluminum, which can contribute to C stabilization in soils by linking the mineral and organic fractions through cationic bridges. Soil nutrient availability can also affect SOM levels, i.e., SOCS relies upon the availability of stabilizing elements like N, P, and S, which are known to be essential components of the humus [38]. Ref. [39] concluded that the C:N:OP:S ratios were reasonably constant for humus, and these were observed across a wide range of global soils.

Cluster and PCA analyses grouped the soil profile 9 into one single group, differentiating it from other soil classes. Soil profile 9 is in the same quadrant as clay, ILF, total C content, and SOCS (Figure 8). It means that these soil attributes, and also aluminum, are contributing to the highest SOCS (230.3 Mg ha⁻¹) in poor drainage Terrace areas. The formation of organic horizons is probably not only a result of the recalcitrance of SOM but also comes from its physical protection inside soil aggregates (ILF) and the chemical bond between soil C and Al; it is also due to the continuous deposition of organic materials that are not removed due to surface runoff. Additionally, soil profile 9 has dense subsurface layers that significantly reduce water infiltration, resulting in a flooded horizon most of the time that prevents SOM mineralization. Due to the flooding conditions, the soil is colonized by microorganisms that are adapted to the hydromorphism and acidic conditions [38]. Soils that are more likely to support the regeneration of SOM include fine-textured soils, soils with higher reactive mineral/metal concentrations, areas with low disturbance, or even cold or waterlogged soils [9].

Soil profile 8, under moderate drainage, showed a high FLF and ILF contribution. An important contribution of ILF in the spodic horizons results in higher total soil organic C in this same horizon and the second highest SOCS (204.7 Mg ha⁻¹). The physical protection of the labile fraction (ILF) into soil aggregates is probably one important mechanism for soil C stabilization. ACP analysis also showed a strong relationship between the soil profile 8 and sand content. The sand export horizon (E1, E2) plays an important role in ILF and C content eluviation to the spodic horizon, resulting in another important mechanism, called chemical protection, for soil C persistence [9].

The permanence of C in soils has already been reported by several authors and most of them are considered to be related to the silt and clay fraction content in soils [38,40–45]. The presence of minerals with greater amounts of charges on their surfaces favors less soluble organic materials leaching and consequently greater soil C accumulation. Thus, clay content has a direct correlation with soil C and N contents, and their respective stocks [11,13,23,34]. However, according to [46], SOM protection is associated not only with clay content but also with the type of clay and the Al content present in the soil. The present study corroborates this study [46], as it is show in the ACP analysis exchangeable Al content and clay content are directly correlated to SOCS and SNS. This cation is commonly quantified in Amazonian soils in significant quantities, as it originates from the weathering of some minerals that have this element in their chemical and structural composition (Ex. gibbsite—Al(OH)₃). High aluminum content and low pH values tend to reduce C degradation in soils [47]. The explanation is the chemical protection mechanism of SOM through a cationic bridge linkage, where exchangeable aluminum binds organic and mineral particles, promoting the stabilization of C in soils. In addition, a low pH value is intrinsically related to a low metabolic activity of soil microorganisms, reducing SOC decomposition [9]. Even with a high correlation with C and N stocks, clay contents are not as significant as sand and silt fractions. Evaluating the quantification of particle size fractions in the study site, it is

possible to perceive a significant contribution from the fine sand and silt fractions (sand and silt contents exceeding 700 g kg⁻¹ in some soil horizons). The sand fraction does not present electrical charges on its surface, nor does it have a high specific surface area, resulting in low SOM chemical protection. This inverse correlation is demonstrated in the ACP analysis (Figure 5). Due to natural conditions, these soils have probably reached equilibrium. No direct correlation was observed between the C and N contents, and their respective stocks, with the physical attribute Bd, according to the ACP (Figure 5). Bd is affected by soil texture and the amount of SOM in soils. The more clayey the soil and the more organic material present, the lower the Bd values [45]. This phenomenon can be explained by a greater deposition of organic material on the soil surface, favoring higher levels of C and N. Therefore, C and N contents and their stocks showed opposite behavior with the Bd vector. The same is true for the Pd attribute, i.e., higher soil C and N contents contribute to lower Pd values.

The average values of SOCS and SNS of the 35 soil classes were higher than those verified by [48] in different biomes and soils in Brazil. In this study, for the Amazon biome, SOCS values ranged from 28.0 (Planososols), 42.1 (Ultisols); 52.2 (Cambisols), 55.6 (Gleisols) to 104.7 Mg C ha⁻¹ (Spodosols). This difference may be related to the fact that SOCS quantified in the soils of the Amazon Forest not only included classes under native vegetation, but also anthropized soils, which, when inadequately managed, can be unfavorable for soil C storage. The study of [49] evaluated the SOCS (100 cm soil depth) in three areas of the Amazon State, Brazil (anthropized, transition between anthropized and non-anthropic and non-anthropized areas). The SOCS was 232.0 Mg C ha⁻¹ for anthropized soils, while in the transition and non-anthropized areas, the values of SOCS were 159.0 and 128.0 Mg ha⁻¹, respectively. It is noted that the average SOCS in non-anthropized areas are close to the average value found in the present study (106.9 Mg C ha⁻¹). The author also found higher values of SOCS in the Anthropogenic dark earths (Terra Preta do Índio) than those in adjacent soils. The author justified these results due to the chemical recalcitrance of C in the form of pyrogenic C, which is found abundantly in these soils.

In areas of primary forest, without anthropogenic disturbances, in the Cueiras Biological Reserve (Central Amazon), [50] quantified SOCS values of 136.5 Mg C ha⁻¹ in plateau soils; 116.0 Mg C ha⁻¹ on slope; and 241.0 Mg C ha⁻¹ of C in lowland (valley) soils.

The average value of SNS verified in the soil classes of this study was 26.9 Mg N ha⁻¹. The research carried out by these authors aimed to present revised estimates of soil C and N contents and stocks in terrestrial ecosystems in the Amazon region. These authors [50] quantified average values of 31.3 Mg N ha⁻¹ for soils with a finer texture and 4.6 Mg ha⁻¹ in soils with a coarser texture. The average SOCS value verified in the study by [50] was 98.0 Mg C ha⁻¹, which is closer to the results observed in this study: 106.9 Mg C ha⁻¹ (Figure 4).

There were different contributions of FLF and ILF in different landscape positions. The total FLF contents at EC1 were lower than those found in the soils at EC3. The total inputs of FLF in soils of the EC2 were the lowest compared to other topographic gradients (Figure 6). This can be explained by the fact that organic matter, after deposition in soil surface, is removed by water reducing its stocks. Furthermore, the organic materials cycles occur more significantly in soils with good drainage, favored by the aerobic decomposition of SOM.

Again, clay fractions play an important role in stabilizing not only soil C and N contents but also the active SOM fractions. This strong relationship between light fractions and C and N contents and their stocks answers the hypothesis of the influence of FLF and ILF inputs on C and N persistence in soils (Figures 6 and 7). FLF, through organic material recalcitrance, remains longer in moderately/poorly drained areas, in the same way as ILF. The greater correlation between FLF and N stock can be explained by the fact that N comes first from litter deposition. Litter interaction with the mineral fraction and its transformation through fragmentation by edaphic macrofauna can result in FLF. This process released N and, consequently, an increase in soil N levels and its stocks. SOM

storage can be highly impacted by the removal of litter inputs [9]. The ILF eigenvector is closer to the eigenvector that represents C contents. Studies show that soil aggregates contain labile C that is physically protected against soil microorganisms [51]. Differences in composition and stability of C in intra-aggregated fractions are believed to be the results of recalcitrance and of soil-aggregate protection mechanisms [52–54].

FLF contents in EC1 soils were lower than those in EC3. In well-drained EC1 soils, part of the SOM can be removed by surface runoff reducing its stocks. This could become even more intense due to the high precipitation in the Amazon. Fragmentation by soil fauna and translocation of litter residues through water infiltration primarily form light fractions (FLF, ILF). However, the direct association of soluble and low-molecular-weight organic compounds or exudate compounds primarily form the stable compartment of SOM in well-drained soils [9]. Studies in contrasting ecosystems confirm that low molecular weight C inputs, as dissolved organic matter (DOM), are efficient precursors of SOM [9,55,56]. In particular, DOM derived from above ground litter residues is expected to contribute to the formation of stable SOM in the topsoil, while DOM derived from exudates and root litter decomposition would contribute to SOM formation at depths [57]. As soils located at EC1 have a more developed physical structure, the persistence of the light fraction is favored within the aggregates or directly linked to the mineral fraction. According to [55,56], the physical protection offered to SOM by occlusion may be as strong as that offered by organic–mineral chemical binding.

Moreover, soil profile 3, located at EC1, is in the same quadrant as silt, N content, SNS, and FLF (Figure 8). The low molecular N organic compounds, released from FLF by microbe-mediation decomposition or direct association with the mineral fraction, can contribute to SNS and, consequently, SOCS in well-drained soils. It is worth highlighting that the chemical bond between organic and inorganic fractions, mediated by exchangeable aluminum, is an important mechanism for soil C stabilization at EC1. However, this organic–mineral association is limited to the few clay charges, being lower in tropical soils, that can be saturated over time, causing SOC and SNS to come into balance.

The same pattern occurs for soil profile 1, also located in EC1, as it is homogeneous to soil profile 3, by cluster analysis (Figure 9). According to [9], SOM formed by this pathway tends to last longer in soil, has a higher density (when including the minerals it is associated with), contains less chemically complex compounds on average, and has a lower carbon-to-nitrogen (C:N) ratio.

Light fraction stocks (SFLF—stocks of free light fraction and SILF—stocks of intraaggregate light fraction) were calculated for the two soil orders that presented the highest and lowest SOM light fraction inputs (Table 4). Stocks were calculated considering the dry mass of soil (Mg ha⁻¹) and the inputs of FLF and ILF (g kg⁻¹) in the first 30 cm of depth, which is the layer most susceptible to the loss of organic materials. In the H1 horizon (soil profile 9), 84% of the total soil mass is SFLF, while only 0.97% of the A1 horizon (soil profile 8) refers to SFLF. It means that if a land use change occurs in the Amazon Forest, soils with an organic horizon, like the soil profile 9, are four times more susceptible to losing soil organic material when compared to soils with a mineral horizon like soil profile 8.

To quantify how much C can be lost, the C stocks of those fractions (SCFLF—soil carbon stock of free light fraction and SCILF—soil carbon stock of intra-aggregate light fraction) were calculated. After a vast literature review researching the proportion of C in the light fraction to the total soil carbon (%CFLF/CT), it was observed that the SOM light fraction has a huge variability (5–74%) in terms of C content. In tropical soils, it ranged from 5 to 57% and in temperate soils, it ranged from 5 to 74%. These values are very close to those observed by [58], who observed that the %CFLF/CT in the light fraction ranges from 8 to 74%. The chemical composition of FLF can be variable. Since it is a pool of organic matter largely in transition between fresh residues and humified, stable organic matter [59], its chemical composition can be somewhat variable. Carbon contents in the ILF to total soil carbon (%CILF/CT) are scarcer, but Refs. [60,61] observed values in the order

of 2.4 and 4.0% in subtropical and tropical soils, respectively. An average value of 31% was considered to estimate SCFLF, and 4% for SCILF.

Table 4. Stocks of the amount of light fractions (SFLFl and SILF), carbon stock in the light fractions (SCFLF and SCILF), and proportion of the light fractions to the total soil mass (SFLF/MS and SILF/MS) of two soil profiles selected.

Horizon	Depth	DS	FLF	ILF	VT	Ms	SFLF	SILF	SFLF/MS	SILF/MS	SCFLF	SCILF
	cm	Mg m ⁻³	${\rm g}{\rm kg}^{-1}$	g kg ⁻¹	m ³	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	%	%	Mg C ha ⁻¹	Mg C ha ⁻¹
Soil Profile 8												
A1	14	1.32	9.71	3.50	0.14	1848	17.94	6.47	0.97	0.35	5.56	0.26
AE	17	1.50	9.64	2.60	0.17	2550	24.58	6.63	0.96	0.26	7.62	0.27
Total	31	-	19.35	6.1	0.31	4398	43.92	13.10	-	-	13.18	0.53
Soil Profile 9												
H1	10	0.09	842.40	15.34	0.10	90	75.80	1.38	84	1.5	23.49	0.06
H2	14	0.16	787.51	11.89	0.14	224	176.4	2.6	79	1.1	54.68	0.10
H3	6	0.88	116.66	33.72	0.06	528	61.6	17.8	12	3.3	19.01	0.71
Total	30	-	1746.57	60.95	0.30	842	313.8	21.78	-	-	97.18	0.87

SFLF—stocks of free light fraction; SILF—stocks of intra-aggregate light fraction; SFLF/MS—ratio of the free light fraction to soil mass; SILF/MS—ratio of the intra-aggregate light fraction to soil mass; SCFLF—soil carbon stock in free light fraction; and SCILF—soil carbon stock in intra-aggregate light fraction.

Approximately 97.18 and 13.18 Mg C ha⁻¹ in SCFLF and 0.87 and 0.53 Mg C ha⁻¹ in SCILF were observed for soil profiles 9 and 8, respectively (Table 4). According to [62], some soils have lost as much as 20 to 80 tons Mg C ha⁻¹, mostly emitted into the atmosphere. Soil profile 9 has the potential to emit 7 times more CFLF and 1.6 times more CILF than soil profile 8, in the first 30 cm of depth. The highest C content susceptible to loss is in the FLF, as it has only one protection mechanism for C persistence in soils, which is recalcitrance. If vegetation cover loss continues, an amount of 98.05 Mg C ha⁻¹ can be lost from organic horizons to the atmosphere, decreasing soil security and contributing to global warming.

5. Conclusions

Relief, a modifying factor, plays an important role in stabilizing C and N in the natural soil of Urucu, Central Amazon. It acts directly by affecting soil drainage, and consequently, the input of labile (FLF and ILF) SOM, one of the first C sources in soils. Furthermore, relief provides diverse conditions for the establishment of different vegetation that provide different quantities and qualities of organic material to the edaphic system. As a result, FLF and ILF inputs, totally dependent on the vegetation factor, are not homogeneous along the topographic gradient, affecting not only soil C and N contents but also its stocks. There were observed differences in SOCS and SNS when considering landscape position, with the differences being statistically equal in EC1 and EC2, but with a statistically greater EC3 median.

SOCS and SNS also varied between soil classes; however, there is no pattern between them and the soil classes. However, there are relationships between SOCS, SNS, and soil attributes: C, N, and exchangeable Al contents, t value, clay content, FLF, and ILF. SOCS and SNS are inversely correlated with sand content, PD, Bd, H, V, and S values.

FLF contents in EC1 soils are lower than in EC3 soils. In EC1, SOM is removed by water or fragmented and decomposed by soil organisms, reducing its stocks. On the other hand, soils from EC3 provide organic material with high lignin and polyphenol contents that are difficult to decompose, resulting in higher inputs of FLF, ILF, and, consequently, higher SOCS and SNS. The recalcitrance of the organic material is preserved due to the lack of soil drainage. Considering landscape position, EC3 and EC1 are contributing to SOCS and C stabilization, but in different ways. EC3 soils have higher values of SOCS, but a considerable amount of it is in FLF and ILF compartments and is concentrated in the

surface horizon, which is the layer more susceptible to decomposition. If vegetation cover loss continues, an amount of $98.05 \text{ Mg C} \text{ ha}^{-1}$ can be lost from the soil to the atmosphere, decreasing soil security and contributing to global warming.

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