

Carbon stock of tree biomass, litter and soil in rubber based agroforestry systems

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ABSTRACT: Agroforestry systems (AFSs) that have rubber tree as the main tree component are important productive systems in the Brazilian Amazon. This work aimed to evaluate carbon stock (CS) of tree biomass, litter and soil in rubber based AFSs in South-Western Amazon. The analytical observational study was carried out in Rolim de Moura (RO) from June 2018 to March 2019 and it consisted of five areas: three AFSs, a forest fragment (FL), and a pasture (PA). CS of tree biomass was determined using allometric equations, while litter and soil carbon stocks were performed through material collection and chemical analysis. Total carbon stock of AFS 1, AFS 2, and AFS 3 was 181.36, 165.77, and 99.08 Mg ha⁻¹, and soil was the compartment that most contributed to the EC of these systems, with stocks of 108.06, 88.71, and 72.68 Mg ha⁻¹, respectively. Total stock of FL was 188.02 Mg ha⁻¹ and PA was 88.19 Mg ha⁻¹. The older AFSs, with greater floristic richness, greater basal area and with the presence of cupuassu tree as a secondary crop had total carbon stock similar to the forest area, evidencing the potential of these systems to sequester atmospheric CO₂.

Key words: climate changes; forestry systems; organic matter; South-Western Amazon

Estoque de carbono da biomassa arbórea, serapilheira e solo em sistemas agroflorestais com seringueira

RESUMO: Os sistemas agroflorestais (SAFs) que têm a seringueira como componente arbóreo principal são importantes modelos produtivos na Amazônia brasileira. O objetivo deste trabalho foi avaliar o estoque de carbono (EC) da biomassa arbórea, serapilheira e solo em SAFs com seringueira na Amazônia Sul-Ocidental. O estudo observacional analítico foi realizado no município de Rolim de Moura (RO) no período de junho de 2018 a março de 2019 e consistiu de cinco áreas: três SAFs, um fragmento florestal (FL) e uma pastagem (PA). O EC da biomassa arbórea foi determinado por meio de equações alométricas, enquanto da serapilheira e do solo foram realizados por meio de coleta de material e análises químicas. O estoque total de carbono do SAF 1, SAF 2 e SAF 3 foi de 181,36, 165,77 e 99,08 Mg ha⁻¹, sendo que o solo foi o compartimento que mais contribuiu para o EC nesses sistemas, com estoques de 108,06, 88,71 e 72,68 Mg ha⁻¹, respectivamente. O estoque total do FL foi 188,02 Mg ha⁻¹ e da PA foi 88,19 Mg ha⁻¹. Os SAFs mais velhos, com maior riqueza florística, maior área basal por hectare e com a presença do cupuaçuzeiro como cultura secundária tiverem EC semelhante ao fragmento florestal, evidenciando o potencial desses sistemas para o sequestro de CO₂ atmosférico.

Palavras-chave: mudanças climáticas; sistemas silviagrícolas; matéria orgânica; Amazônia Sul-Ocidental



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Introduction

The increasing concentration of greenhouse gases (GHGs) in the Earth atmosphere, such as carbon dioxide (CO_2) , methane (CH_4) , and nitrous oxide (N_2O) , raises global average temperatures and is the main cause of climate change evidenced in recent decades (<u>Abbas et al., 2017</u>). Among anthropogenic GHGs, CO_2 is the most significant, accounting for up to 80% of global warming (<u>IPCC, 2022</u>).

Agriculture is one of the anthropic activities that contribute the most to carbon emissions in the earth atmosphere, mainly due to deforestation and soil degradation. Studies have shown that the conversion of native forests into areas of agricultural crops significantly reduces the carbon stock of tropical ecosystems in the Amazon, releasing CO_2 into the atmosphere (Petter et al., 2017; Rittl et al., 2017).

Due to the climate change scenario and the negative impacts of agricultural activities, we seek to propose practices that can associate production with the conservation of natural resources, reducing GHGs emissions. An example of these practices are the agroforestry systems (AFSs), recognized by the United Nations (UN) and by the Kyoto Protocol as one of the strategies for mitigating environmental impacts.

Agroforestry systems comprise the integrated cultivation of trees, agricultural crops, and animals, following a certain spatial and temporal arrangement (Nair et al., 2021). They are related to several ecosystem services and, in particular, to atmospheric carbon sequestration and storage in different above- and below-ground compartments (Abbas et al., 2017; Couto et al., 2016). Above-ground carbon comprises all plant components, such as trunk, leaves, fruits, flowers, and the decaying material deposited on the surface (litter). Belowground carbon encompasses roots, soil microorganisms, and carbon present in the different soil horizons (Abbas et al., 2017).

In the Amazon region Arc of Deforestation, which is the region characterized by the recent advance of the agricultural frontier, agroforestry systems have been indicated as an alternative to reconcile agricultural production with the conservation of natural resources. <u>Villa et al. (2020)</u> point out that the implementation of AFSs is one of the main strategies to reduce deforestation in the Amazon, ensure sustainable food systems, and effect the United Nations Reducing Emissions from Deforestation and Forest Degradation (REDD+) policy.

The AFSs that have the rubber tree (*Hevea brasiliensis* Müll. Arg.) as the main tree component are common in the Brazilian Amazon and have significant potential for carbon sequestration. The rubber tree is a fast growing heliophytic species, reaching 20-30 m in height with a trunk diameter of 30-60 cm (Lorenzi, 1992). The root system is vigorous and well distributed, favoring the incorporation of organic carbon into the soil. The tree forms leafy canopy that deposits expressive amount of plant material on the soil surface (Carmona et al., 2018).

In most agroforestry systems with rubber trees, the only economic use of the species is the extraction of natural latex, destined mainly for the tire, shoe, glove, and condom industries. Therefore, since there is no logging of the species, the carbon stored in the AFSs is stored for a long period, constituting true carbon reservoirs.

Despite the potential of AFSs with rubber trees, studies evaluating carbon stocks in different compartments of the system are still incipient. In addition, wherever possible this potential should be assessed for specific environmental models and contexts. In this sense, the objective of this work was to evaluate the carbon stock of tree biomass, leaf litter and soil in agroforestry systems with rubber trees (*Hevea brasiliensis* Müll. Arg.) in southwestern Amazonia.

Materials and Methods

The study is defined as analytical observational and was conducted in the municipality of Rolim de Moura, located in the Rondonian Forest Zone, South-Western Amazon, in the period from June 2018 to March 2019. The region climate is classified as Am, according to the Köppen-Geiger climate classification, being characterized by an average annual temperature of 25 °C, altitude between 200 and 400 m, and total annual precipitation between 2,200 and 2,500 mm (Alvares et al., 2013). The areas are located in the Pimenta Bueno geological formation (SDpb) and the predominant soil class is Dystrophic Red-Yellow Argisol (PVAd) (Santos et al., 2011).

Three agroforestry systems were studied, named AFS 1, AFS 2, and AFS 3, which have the rubber tree as the main tree component (<u>Table 1</u>). For comparison purposes, a forest fragment (FL) and a pasture (PA) were also evaluated.

The soil chemical attributes of these areas are described in <u>Table 2</u>. The analyses were performed following methodology of <u>Teixeira et al. (2017)</u>.

The vegetation inventory was conducted in the three AFSs and the FL during the period from June to August 2018. For each study area, five 30.0×30.0 m plots were drawn by simple random sampling, totaling 4,500 m² inventoried per area. In each plot, taxonomic identification was performed and the diameter at 1.3 m (DBH) and total height of all individuals with DBH greater than or equal to 5 cm were measured. The sampling sufficiency of this inventory was verified using a species-area curve plot (Felfili et al., 2011).

To characterize the floristic composition of the AFSs and FL, the species richness (S), which indicates the quantity of species present in the area, the Shannon Diversity Index (H'), which measures the diversity of the plant community (Equation 1), and the Pielou Equability Index (J'), which indicates the uniformity of the distribution of species in an area, were calculated (Equation 2). Regarding phytosociology, the basal area per hectare (G) was calculated, which indicates the spatial occupation of the arboreal individuals

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Table 1.	General characteristics,	history and	management	of the agrofo	prestry system	areas (AFS	1, AFS 2,	and A	AFS 3),	forest
fragmen	t (FL), and pasture (PA).									

System	Area (ha)	Implementation year	Age (years)	History and management
AFS 1	1.4	1982	40	 1982: Suppression and burning of native vegetation. Application of agricultural corrective and NPK 4-14-8 fertilizer. Planting of rubber tree at 7.0 × 3.0 m spacing. 1982-1986: Rubber tree cultivated together with the agricultural crops rice (<i>Oryza</i> sp.), beans (<i>Phaseolus</i> sp.), and corn (<i>Zea</i> sp.). 1995: Planting of cupuassu tree (<i>Theobroma grandiflorum</i>) at 7.0 × 5.0 m spacing. Besides the rubber and cupuassu trees, there are other forest species in the area, such as <i>Cedrela odorata</i>, <i>Eugenia uniflora</i>, and <i>Stryphnodendron guianense</i>, which appeared due to natural regeneration and were maintained by the farmer. The only management of the AFS was annual weeding in the rainy season and the last fertilization occurred in mid-2013 with the application of NPK 4-14-8.
AFS 2	3.0	1979	43	1979: Suppression and burning of native vegetation. Rubber tree were planted at spacing of 6.0×3.0 m (555 ind ha ⁻¹) and coffee tree (<i>Coffea</i> sp.) in between the rows. 1994: Removal of the coffee bushes and planting of the cupuassu tree at $6.0 \times$ 7.0 m spacing. Besides the rubber and cupuassu trees, there are some other forest species in this AFS, such as <i>Jacaranda copaia</i> , <i>Bellucia grossularioides</i> , <i>Tabebuia</i> sp., and <i>Nectandra</i> sp., which appeared spontaneously and were maintained. In recent years, the only economic exploitation of the AFS has been the commercialization of the fruit of the cupuassu tree. In this area there has never been any correction of the soil acidity nor application of chemical fertilizers.
AFS 3	2.3	1997	25	 1997: Suppression and burning of native vegetation. Rubber tree planting at 9.0 × 4.0 m spacing (277 ind ha⁻¹) and coffee tree (<i>Coffea robusta</i> and <i>Coffea canephora</i>) at 3.0 × 2.0 m spacing. 1997-1999: Growing rice in between the rows of trees and bushes. At no time were there any practices such as soil preparation, acidity correction, and fertilization. Besides harvesting the coffee trees for commercialization, the only management applied in the area was the annual trimming during the rainy season.
PA	3.6	1990	32	 1990: Suppression and burning of native vegetation. Installation of the pasture with planting of <i>Urochloa brizantha</i> grass. There has never been any correction of soil acidity or application of chemical fertilizers. The stocking rate of the pasture is approximately 1 animal unit (AU) per hectare. In addition, the pasture showed constant forage formation and there was no evidence of erosive processes.
FL	4.0			The forest area comprises a nine-hectare fragment of secondary forest, classified by IBGE Technical Manual of Brazilian Vegetation as Alluvial Open Ombrophylous Forest (IBGE, 2012). The area had a history of logging, but since 1980 there has been no intervention in the area.

Table 2. Soil chemical attributes for the 0-0.20 m layer.

Aroo	pН	Са	Mg	К	SB	Al	Al + H	СТС	Р	V	m
Alea	(H ₂ O)				(cmol _c dm ⁻³	⁴)			(mg dm⁻³)	(%	%)
AFS 1	4.82	0.49	0.29	0.26	1.04	0.80	8.53	9.57	2.36	10.92	43.93
AFS 2	4.92	0.34	0.24	0.26	0.84	0.42	6.35	7.19	1.04	11.67	33.52
AFS 3	4.65	0.25	0.25	0.17	0.67	1.04	5.66	6.33	1.58	10.65	60.91
PA	5.66	1.24	0.90	0.54	2.68	0.00	6.02	8.70	0.96	30.66	0.00
FL	4.70	0.39	0.25	0.29	0.93	1.08	8.26	9.19	4.10	10.15	53.78

 $pH_{_{H20}} = pH \text{ in water; SB} = sum \text{ of bases } (Ca^{+2} + Mg^{+2} + K^{+}); CTC_{_{pH7,0}} = SB + H+AI; V\% = base \text{ saturation } [(SB/CTC) \times 100]; m\% = aluminum \text{ saturation } [(Al^{+3}/CTC) \times 100].$

number of individuals per hectare (Equation 4). All floristic described in Felfili et al. (2011).

(Equation 3), and the density of individuals (D), which is the and phytosociological analyses followed the methodology

- Shannon Diversity Index (H')

$$H' = -\sum_{i=1}^{S} \left[pi \ln \left(pi \right) \right]$$
(1)

- Pielou Equability Index (J')

$$J' = \frac{H'}{\ln(S)}$$
(2)

- Basal area per hectare (G)

$$G = \sum_{i=0}^{n} \frac{Asi}{A}$$
(3)

- Density of individuals (D)

$$D = \frac{N}{A}$$
(4)

where: S = total number of species; pi = relative abundance of each species; Asi = sectional area of the i-th tree sampled; A = total area in ha; N = number of individuals.

Carbon stock of the tree biomass

The tree biomass was determined by the indirect method, using allometric equations (<u>Table 3</u>). Species specific equations were used and developed for trees in agroforestry systems with environmental characteristics similar to the study region. However, for many species it was not possible to find specific equations, and in such cases the generic equation developed by <u>Chave et al. (2014)</u> for estimating tree biomass of tropical species was used. To obtain the carbon stock, the biomass values of the individual trees were multiplied by a factor of 0.5 (<u>IPCC, 2022</u>).

Carbon stock in the forest litter

The carbon stock of the litter was determined only for the AFSs and FL, since no litter was observed on the soil surface of the PA (probably due to the time of year and the intense grazing by the animals). Collections were conducted in the month of January 2019, the peak of the rainy season in the region. In each of the plots installed for the forest inventory, a square wooden template measuring 0.5×0.5 m was dropped twice, following the methodology described in <u>Arevalo et al. (2002)</u>. All plant material (such as leaves, branches, flowers, fruits, and seeds) found within the template was considered litter, and was carefully removed and placed in plastic bags.

Then, the material was separated into the following fractions: leaves (LE), branches (BR), reproductive material (RM), and amorphous material (AM). Organic debris in advanced stages of decomposition that was not identified for the other fractions was considered as AM. After sorting, drying was performed in a forced air circulation oven at 60° C until constant dry mass was reached and weighing of the material to determine the total biomass and litter fractions (Scoriza et al., 2012).

Subsequently, a subsample of the material was used to determine the total organic carbon content by oxidation method with potassium dichromate $(K_2Cr_2O_7)$ in strongly acidic medium, following the analytical march described in <u>Bezerra Neto & Barreto (2011)</u>. With the carbon contents and the values of the litter biomass, the carbon stocks of the litter (of the fractions and total) were calculated, according to <u>Equation 5</u>:

$$\mathrm{CS}(\mathrm{Mg ha}^{-1}) = \frac{\mathrm{BL} \times \mathrm{C}}{1,000}$$
(5)

where: BL = biomass of the litter (Mg ha⁻¹); C = carbon content (g kg⁻¹).

Soil carbon stock

Soil sampling was conducted in January and February 2019 and carbon stock was determined up to the 0.60 m layer for all areas studied (AFSs, FL, and PA). In each area five trenches measuring $0.4 \times 0.7 \times 0.6$ m were opened to collect undeformed soil samples with metal cylinders in the layers 0 to 0.10, 0.10 to 0.20, 0.20 to 0.40, and 0.40 to 0.60 m. These samples were used for soil density (SD) determination by the volumetric ring method (Teixeira et al., 2017).

To determine the soil organic carbon (SOC) content, deformed samples were collected, using a dutch type auger, in the layers from 0 to 0.10, 0.10 to 0.20, 0.20 to 0.40, and 0.40 to 0.60 m. Identical amounts of deformed samples from the 0 to 0.10 and 0.10 to 0.20 m layers were homogenized to form a sample corresponding to the 0 to 0.20 m layer, which was used for grain size determination by the pipette method (Teixeira et al., 2017).

Soil organic carbon was determined by the oxidation method with potassium dichromate $(K_2Cr_2O_7)$ in an acid medium and with an external heat source (<u>Yeomans & Bremner, 1988</u>). With the SD and SOC values, soil carbon stock

Table 3. Allometric equations used to estimate tree biomass.

Species	Equation	R ²	Source
Coffea sp.	Log ₁₀ (TB) = -0.779 + 2.338 × Log ₁₀ (th)	0.82	Segura et al. (2006)
Hevea brasiliensis	$TB = 0.0419 \times DBH^{2.316} \times th^{0.478}$	0.98	Yang et al. (2017)
Theobroma grandiflorum	TB = 4.1194 × DBH – 5.7818	0.92	Brancher (2010)
Theobroma cacao	TB = 3.3973 × DBH – 4.8961	0.92	Brancher (2010)
Other species	$TB = 0.0673 \times (d \times DBH^2 \times th)^{0.976}$	0.85	Chave et al. (2014)

Where: R² = coefficient of determination; TB = total biomass (kg); th = total height (m); DBH = diameter at 1.3 m from the ground (cm); d = wood density (g cm⁻³).

was calculated using the methodology of <u>Veldkamp (1994)</u> for each of the layers sampled (<u>Equation 6</u>) and for the entire profile (0 to 0.60 m) by summing the individual values.

SOC Stock =
$$\frac{(C \times SD \times t)}{10}$$
 (6)

where: SOC Stock = soil organic carbon stock (Mg ha⁻¹); C = organic carbon content (g kg⁻¹); SD = soil density (g cm⁻³); and, t = soil layer thickness (cm)

Total carbon stock

With the sum of the carbon stocks of the tree biomass, litter, and soil, the total carbon stock (TCS) was calculated for all the areas analyzed. In the case of the pasture where there was no determination of carbon stock of the tree biomass and the litter, the TCS corresponds to the soil carbon stock.

Statistical analysis

The study was analytical observational, in which the comparison areas were: the three agroforestry systems (AFS 1, AFS 2, and AFS 3), the forest fragment (FL), and the pasture (PA). Each sample plot allocated within these areas, used to

perform the forest inventory and soil and litter sampling, was considered as a repetition. In the case of PA, each trench was considered as a sample plot.

For tree and soil biomass carbon stocks, analysis of variance (ANOVA) was performed and Tukey test was applied at 5% probability. For the carbon stocks in the litter, Tukey test was used at 10% probability. For the soil carbon stock analysis, the layers were considered in the subdivided plot scheme. We checked the normality of the residuals with the Shapiro-Wilk test and the homogeneity of variances with the Bartlett test. When necessary, the transformation V(x) was applied. All statistical analyses were run in R Software and graphs were constructed in SigmaPlot 12.0.

Results and Discussion

Floristic and phytosociology composition

In total, 1,141 individuals were sampled in the agroforestry systems, distributed among 11 botanical families and 20 species. In the forest fragment, 370 individuals were sampled, distributed among 29 botanical families and 107 species. AFS 1, AFS 2, and AFS 3 were composed of 7, 13, and 4 species, respectively (Table 4).

Table 4. Floristic survey of agroforestry systems (AFS 1, AFS 2, and AFS 3) and forest (FL).

Area	Scientific name	Family	N
	Hevea brasiliensis (Willd. ex A. Juss.) Müll. Arg	Euphorbiaceae	458
	Theobroma grandiflorum (Willd. ex Spreng.) K. Schum.	Malvaceae	133
	Theobroma cacao L.	Malvaceae	8
AFS 1	Cedrela odorata L.	Meliaceae	6
	Eugenia uniflora L.	Myrtaceae	3
	Stryphnodendron guianense (Aubl.) Benth.	Fabaceae	3
	Not identified 1		3
	Hevea brasiliensis (Willd. ex A. Juss.) Müll. Arg	Euphorbiaceae	356
	Theobroma grandiflorum (Willd. ex Spreng.) K. Schum.	Malvaceae	236
	Jacaranda copaia (Aubl.) D. Don	Bignoniaceae	22
	Bellucia grossularioides (L.) Triana	Melastomataceae	13
	Tabebuia aurea (Silva Manso) Benth. & Hook.f. ex S.Moore	Bignoniaceae	16
	Tabebuia sp.	Bignoniaceae	16
AFS 2	Guarea guidonia (L.) Sleumer	Meliaceae	2
	Dipteryx oppositifolia (Aubl.) Willd.	Fabaceae	2
	Schefflera morototoni (Aubl.) Maguire, Steyerm. & Frodin	Araliaceae	4
	Nectandra sp.	Lauraceae	2
	<i>Mezilaurus itauba</i> (Meisn.) Taub. ex Mez	Lauraceae	2
	Not identified 1		2
	Not identified 2		2
	<i>Coffea</i> sp	Rubiaceae	1156
	Hevea brasiliensis (Willd. ex A. Juss.) Müll. Arg	Euphorbiaceae	207
AF3 5	Schefflera morototoni (Aubl.) Maguire, Steyerm. & Frodin	Araliaceae	4
	Not identified 1		2
	Ocotea puberula (Rich.) Nees	Lauraceae	20
	<i>Guatteria olivacea</i> R. e Fr.	Annonaceae	14
	Inga laurina (Sw.) Willd	Fabaceae	11
	Apeiba echinata Gaertn.	Malvaceae	11
E11	Pseudolmedia laevis (Ruiz & Pav.) J.F.Macbr.	Moraceae	10
	Pseudolmedia sp	Moraceae	10
	Vismia guianensis (Aubl.) Choisy	Hypericaceae	9
	Thyrsodium spruceanum Benth.	Anacardiaceae	8
	Stryphnodendron pulcherrimum (Willd.) Hochr.	Fabaceae	8
	Xvlopia emargingta Mart.	Annonaceae	7

Where: N = individual density (ind ha^{-1}); ¹ = Ten species with the highest N.

The phytosociological attributes varied considerably among the three agroforestry systems (<u>Table 5</u>). Regarding the density of individuals (D), in AFS 1 it was 614 ind ha⁻¹ and in AFS 3 it was 1,369 ind ha⁻¹, which correspond to the lowest and highest D, respectively. The highest density of individuals in AFS 3 was due to the coffee bushes (*Coffea* sp.), which are smaller plants and were planted at a high density (1,156 ind ha⁻¹).

Regarding G, AFS 1 and AFS 2 showed the highest values (27.17 and 29.02 m² ha⁻¹, respectively), demonstrating the high degree of occupation of space by arboreal individuals. These values were even higher than that of FL (18.34 m² ha⁻¹) due to the denser planting of trees in the agroforestry systems and to the past of logging in the forest area, which prioritized the suppression of large-diameter individuals.

Among all areas, AFS 3 had the lowest species richness (S). This is an area with homogeneous floristic composition, consisting basically of the species used in the implementation of the AFSs (rubber and coffee tree). In this case, the management practiced did not favor the preservation of native species that were in the area or that emerged through natural regeneration, which is why this system also showed the lowest diversity (H') and uniformity (J') indices.

Compared to AFS 3, AFS 1 and AFS 2 had higher values of H' and J', mainly due to the management applied to the areas, where native forest species, such as *Cedrela odorata*, *Jacaranda copaia*, *Bellucia grossularioides*, and *Tabebuia* sp., were preserved. Farmers have kept these species in agroforestry systems for reasons such as food production for wildlife, scenic beauty, and timber interest. The FL had higher values of S, H', and J', evidencing the high floristic diversity and heterogeneity typical of forest ecosystems.

 Table 5. Floristics and phytosociology of the agroforestry systems (AFS 1, AFS 2, and AFS 3), and forest fragment (FL).

Area	D (ind ha ⁻¹)	G (m² ha ⁻¹)	S	H'	J
AFS 1	614	27.17	7	0.72	0.37
AFS 2	676	29.02	13	1.21	0.47
AFS 3	1369	9.11*	4	0.46	0.33
FL	822	18.34	107	4.26	0.91

Where: D = individual density; G = basal area per hectare; S = species diversity; H' = Shannon diversity index; J' = Pielou equability index. * Basal area of the trees only, since the diameter of the coffee bushes was not measured.

Carbon stock of the tree biomass

For AFS 1, AFS 2, and FL areas, there was no statistically significant difference between tree biomass carbon stock means (Table 6). The agroforestry systems with the greatest number of species, the oldest, and with the greatest basal area per hectare (AFS 1 and AFS 2), showed a significant potential to accumulate carbon in the tree component, with values similar to native vegetation.

The lowest value found in AFS 3 (22.92 Mg ha⁻¹) is mainly due to the age of this system, 25 years, in relation to the others: 40 years (AFS 1) and 43 years (AFS 2). Because they are younger, the trees in this area have smaller diameters, smaller basal areas per hectare, and, consequently, smaller

Table 6. Carbon stock of tree biomass in agroforestry systems	
and in the forest.	

Área	Carbon stock of the tree biomass (Mg ha ⁻¹)
AFS 1	69.22 ± 10.25 a
AFS 2	74.46 ± 15.47 a
AFS 3	22.92 ± 5.92 b
FL	79.20 ± 26.41 a

Values are averages \pm standard deviation (n = 5). Averages followed by the same letter in the column do not differ by Tukey test at 5% probability.

carbon stocks in the tree biomass. <u>Orjuela-Chaves et al. (2014)</u> also reported this association between age and tree biomass carbon stock for AFSs in the northeastern region of Colombia.

Another factor that determined the lower carbon stock of the tree biomass in AFS 3 was the secondary crop coffee, which is a small/medium-sized shrub that does not store large amounts of carbon (Segura et al., 2006). Therefore, even though they have a high density of individuals (1,156 ind ha⁻¹), coffee bushes do not contribute as much to the carbon stock of the tree biomass. In AFS 1 and AFS 2 the secondary crop is the cupuassu tree, which is a medium/high-sized species with expressive carbon storage capacity in the tree biomass (Ramos et al., 2018).

The two AFSs with cupuassu trees had similar values, mainly due to the similarity in age, density of individuals per hectare, and spatial occupation of the area (<u>Table 5</u>). The carbon stock in these systems (AFS 1 and AFS 2) is close to that of the FL, showing the great potential of the AFSs with rubber and cupuassu trees in storing carbon in the tree biomass.

Both the rubber and the cupuassu tree have leafy canopies, with many branches and twigs, favoring the production of tree biomass. Even as the secondary species in the AFSs, the cupuassu tree is very well adapted to shaded environments and can reach up to 15 m in height and up to 35 cm in diameter (Lorenzi, 1992), and is therefore an important species for carbon stocks in agroforestry systems. In addition, the only economic use of the cupuassu tree is the extraction of fruits, ensuring that the tree remains in the system during its entire production cycle.

In addition to the two main species, these AFSs also featured other forest species that emerged with natural regeneration, were maintained by the farmers, and contributed to the carbon stock of the tree biomass. In AFS 1 there are a few individuals of *Cedrela odorata*, which is a large species that can reach 35 m in height and up to 150 cm DBH (Lorenzi, 1992). In AFS 2 there were many individuals of *Tabebuia* sp. and *Jacaranda copaia*, which are also large trees that store a lot of carbon in the tree biomass.

Carbon stock in the forest litter

The total carbon stock of the litter (sum of the fractions leaves, twigs, reproductive material and amorphous material) was higher in AFS 1 (4.10 Mg ha⁻¹) and AFS 3 (3.50 Mg ha⁻¹), with no statistical difference between these areas (Figure 1). The lowest stock was found in AFS 2 (2.60 Mg ha⁻¹), which did not differ statistically from AFS 3 and FL (2.80 Mg ha⁻¹).



Averages followed by the same letter do not differ by the Tukey test at 10% probability. Bar refers to the standard deviation of the average.

Figure 1. Carbon stock of the fractions leaves (LE), branches (BR), reproductive material (RM), amorphous material (AM), and total litter in the agroforestry systems (AFS 1, AFS 2, and AFS 3), and forest fragment (FL).

For the FO fraction, the highest value was in AFS 1 (1.90 Mg ha⁻¹), followed by AFS 2 (1.40 Mg ha⁻¹), while AFS 3 and FL had the lowest values (1.0 and 0.8 Mg ha⁻¹, respectively). The highest values in AFS 1 and AFS 2 probably occur due to the cupuassu tree depositing a significant volume of leaves. This species is suitable for agroforestry systems because of its adaptability to shaded environments and high biomass production. The leaves of the cupuassu tree are subcoriaceous and large, approximately 20-40 cm long by 6-12 cm wide (Lorenzi, 1992), contributing to a thick layer of litter on the soil surface.

For the GA and RM fractions there was no statistical difference between the areas. The carbon stock of reproductive material (fruits, flowers, and seeds) was low, especially in AFS 2 (0.30 Mg ha⁻¹) and FL (0.30 Mg ha⁻¹). It is believed that if the litter collection were done in the dry season (June to November in the study region), the amount of RM would be greater, due to the phenological patterns of many native forest species, which generally flower and fruit during the dry season.

For the AM fraction, the highest value was in AFS 3, possibly due to the large volume of coffee tree leaves that decompose rapidly and form organic detritus at advanced stages of decomposition (<u>Schmitt & Perfecto, 2021</u>). This rapid degradation of leaves is important for the formation

of organic matter and improvement of soil quality in this agroforestry system. Like the cupuassu tree, the coffee tree is also a species well suited as a secondary component in AFSs, due to its adaptation to shaded environments.

In general, the agroforestry systems studied showed a similar capacity for carbon stock in the leaf litter as FL. Or even higher, if you compare only AFS 1 and AFS 2, which had 46 and 25% more carbon in the litter than FL. For this reason the rubber tree is an appropriate tree for the AFSs in the region, since it is a semi-deciduous species that loses its leaves during a certain period of the year (in the Amazon biome it is usually from July to September) and contributes significantly to the formation of litter.

Although regular pruning is not performed in these systems, most species deposit a significant amount of plant material on the soil surface, mainly foliage, branches, fruits, and reproductive structures. This condition is very important for the sustainability of AFSs, because it contributes to the sequestration of atmospheric carbon, improvement of edaphic attributes, and nutrient cycling, among other environmental services.

Soil carbon stock

The soil organic carbon stock was the highest among the three compartments evaluated (biomass, litter, and soil). AFS 1 and FL had statistically the highest average SOC for the total profile from 0 to 0.60 m: 108.06 and 106.00 Mg ha⁻¹, respectively (<u>Table 7</u>). AFS 2 (88.71 Mg ha⁻¹) and PA (88.19 Mg ha⁻¹) had averages that did not differ statistically, while AFS 3 (72.68 Mg ha⁻¹) had the lowest average.

The higher SOC stocks in AFS 1 and FL, are related to the large amount of organic material observed in the topsoil layer. Even this AFSs also had the highest carbon stock in the litter (4.08 Mg ha⁻¹), which indicates the continuous contribution of SOC, since the litter is the main way of transferring carbon from the tree biomass to the soil.

AFS 1 is composed of rubber trees in association with cupuassu tree, besides other forest species in lower density such as *Theobroma cacao*, *Cedrela odorata*, and *Stryphnodendron guianense*. These species contribute to the deposition of plant remains, which decompose and favor the contribution of organic carbon to the soil.

The lower amount of SOC in AFS 3 (72.68 Mg ha⁻¹), being even lower than PA (88.19 Mg ha⁻¹), is mainly related to the sand content (Table 8). Sandy soils, such as in AFS 3, naturally

Table 7. Carbon stocks of soil layers and total in agroforestry systems (AFS 1, AFS 2, and AFS 3), forest fragment (FL), and pasture (PA).

			Carbon stock (Mg ha ⁻¹)		
Layer (m)	AFS 1	AFS 2	AFS 3	FL	PA
0-0.10	23.60 ± 2.93 CDab	18.81 ± 1.66 Cbc	15.32 ± 2.71 Cc	25.81 ± 3.09 BCa	18.06 ± 2.53 CDc
0.10-0.20	21.09 ± 2.12 Da	16.23 ± 2.20 Cb	16.15 ± 2.86 Cb	19.64 ± 1.66 Cab	17.23 ± 1.32 Dab
0.20-0.40	32.91 ± 2.32 Ba	27.27 ± 2.10 Babc	22.44 ± 2.91 Bc	30.16 ± 3.95 Bab	27.07 ± 4.09 Bbc
0.40-0.60	30.45 ± 1.74 BCa	26.40 ± 4.79 Ba	18.77 ± 2.34 BCb	30.38 ± 5.69 Ba	25.84 ± 4.49 BCab
Total	108.06 ± 6.95 Aa	88.71 ± 6.08 Ab	72.68 ± 4.03 Ac	106.00 ± 4.00 Aa	88.19 ± 7.55 Ab

Values are averages ± standard deviation (n = 5). Averages followed by the same capital letter in the column and lower case in the row do not differ by Tukey test at 5% probability.

Table 8. Particle size for the 0-0.20 m layer in the agroforestry systems (AFS 1, AFS 2, and AFS 3), pasture (PA), and forest fragment (FL).

Area	Clay	Silt	Sand
Area		(g kg ⁻¹)	
AFS 1	409.5 b	69.8	520.7 b
AFS 2	547.8 a	66.5	385.9 c
AFS 3	334.7 c	56.8	608.5 a
PA	591.5 a	58.5	350.0 cd
FL	581.7 a	87.9	330.4 d

Averages followed by the same letter in the column do not differ by Tukey test at 5% significance level. Averages that do not follow a letter do not show statistical difference by the Tukey test at 5% significance level.

have lower carbon storage capacity due to lower specific surface area and lower potential for organo-mineral complex formation (<u>Rasmussen et al., 2018</u>).

With the exception of AFS 3, the other AFSs under clayey soils showed a potential for SOC stock similar to the forest fragment, which indicates that agroforestry systems are able to store carbon in the soil in the same magnitude as natural ecosystems. <u>Couto et al. (2016)</u>, studying different land use systems in the RECA Project in Nova California, RO, Brazil, also obtained soil carbon stocks in AFSs with values close (in some cases even higher) to that of native forests.

The PA had SOC values close to those of AFS 2 in almost all soil layers, even though it is an area with less floristic diversity and no presence of arboreal components. According to <u>Segnini et al. (2019)</u>, properly managed pastures can show considerable values of SOC, especially for the more superficial layers, mainly due to the large volume of forage grass roots that continuously deposit organic residues in the soil profile.

Considering the soil profile (0-0.60 m), there is a natural tendency for a greater amount of carbon to occur in the upper soil layers, due to the constant deposition of organic material (leaves, branches, fruits, seeds, among other plant and animal residues). In all areas studied, the 0-0.20 m layer presented the highest values of SOC, corresponding, on average, to 41.50% of the total carbon in the analyzed profile.

Although the depth considered in the study was only up to 0.60 m, the potential of agroforestry systems with rubber trees to store carbon in the soil is much greater, given that the species root system is vigorous and well distributed, and can reach several meters deep (Yang et al., 2017). Besides the rubber tree, the cupuassu tree and the other forest species present in AFS also have pivotal roots and lateral roots that favor the continuous incorporation of SOC in deep soil layers.

As the economic exploitation of these AFSs is based exclusively on non-timber products (fruits, grains, and natural latex) and there is no thinning of trees, the permanent maintenance of the vegetation cover avoids erosive processes and favors the conservation of carbon stored in the soil. In pasture, as well as in other agricultural and forest monocultures, the eventual removal of the vegetation cover can cause soil degradation processes, releasing CO₂ into the atmosphere.

Total carbon stock

The total carbon stock ranged from 88.19 Mg ha⁻¹ in PA to 188.02 Mg ha⁻¹ in FL (Figure 2). The averages for AFS 1, AFS 2, and FL were the highest, in contrast to AFS 3 and PA that had the lowest stocks. In all areas the soil was the most expressive compartment, corresponding to 59.60% (AFS 1), 53.51% (AFS 2), 73.35% (AFS 3), 56.38% (FL), and 100% (PA) of the TCS.

It is noteworthy that the TCS of AFS 1 and AFS 2 did not differ statistically from FL. Thus, these agroforestry systems, which have a vegetation structure similar to natural ecosystems, have a carbon storage potential similar to that of native forests. In contrast, agricultural grazing has been shown to be a land use system with the least potential for atmospheric carbon sequestration.

One of the main characteristics of AFSs is the diversification of plant species, which results in multiple tree strata and intensifies the spatial use of the area (both vertically and horizontally). In the AFSs analyzed, the predominance of rubber trees and other forest species was observed in the upper stratum, the cupuassu and coffee trees in the intermediate stratum, and seedlings of natural regeneration in the lower stratum of the system. This plant diversification enhances the use of available natural resources (water, light, and nutrients) and increases energy flows and the total amount of biomass and carbon (<u>Nair et al., 2021</u>).

Another characteristic is that these agroforestry systems with rubber trees have progressive increases in carbon stocks in the different compartments, especially in the first decades after implementation, due to the continuous increase in diameter, volume, and biomass (<u>Abbas et al., 2017</u>). Therefore, in terms of carbon sequestration and fighting climate change, these AFSs can be considered efficient forms of land use.

This potential is even greater if the floristic composition of these AFSs is considered. The commercial species (rubber, cupuassu, and coffee trees) are long-cycle and the economic exploitation is based exclusively on non-timber products. Therefore, even if there are carbon outputs from the system, such as latex and fruit extraction, in the final balance the results are always favorable, since these systems favor



Averages followed by the same letter do not differ by Tukey test at 5% probability. **Figure 2.** Total carbon stocks, considering soil, litter and tree biomass compartments for the agroforestry systems (AFS 1, AFS 2, and AFS 3), forest fragment (FL), and pasture (PA).

contribution of organic material to the soil.

Conclusion

The carbon stock in agroforestry systems with rubber trees (Hevea brasiliensis Müll. Arg.) ranged from 99.08 to 181.36 Mg ha⁻¹, with the soil being the compartment that contributed most to the total carbon stock.

The older agroforestry systems, with greater floristic richness, greater basal area per hectare, and with the presence of the cupuassu tree (Theobroma grandiflorum) as a secondary crop have total carbon stock close to the forest fragment, evidencing the potential of these systems for CO₂ sequestration.

Compliance with Ethical Standards

Author contributions: Conceptualization: LHVL, AATF; Data curation: LHVL; Formal analysis: JB; Methodology: AATF; Investigation: LHVL; Supervision: AATF, OLSW; Writing - original draft: LHVL; Writing - review & editing: OLSW, EADH, SFC.

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