Research Article

Stable carbon in soils under rubber tree (*Hevea brasiliensis*) agroforestry systems in the south of Bahia, Brazil

Gerbeli Mattos Salgado[1] · **Emanuela Forestieri Gama-Rodrigues**[2] · **Laís Carvalho Vicente**[2] ·
Antonio Carlos Gama-Rodrigues[2] · **Seldon Aleixo**[2] · **José Raimundo Bonadie Marques**[3]

Abstract

The early natural forest conversion to pasture, rubber tree plantations or agroforestry systems (AFSs) will not negatively affect soil organic carbon (SOC) accumulation due to the lack of soil tillage and the continuous contribution of vegetal residues to the soil, over the years, which would favor C occlusion in soil aggregates. The objectives of this study were: to evaluate the potential of AFSs with rubber trees to accumulate SOC up to 100 cm; to evaluate the $\delta^{13}C$ variations over the soil profile after forest to pasture and rubber tree plantation (RTP) conversion; and to verify the C physical protection in aggregates as a mechanism of C stability in these soils. SOC was quantified up to 100 cm in whole soil and in three fraction-size classes (macroaggregates, microaggregates and silt + clay class). The occluded C was quantified in macro- and microaggregates by using an ultrasonic method. The relative contributions of C_3 and C_4 plants-derived C were quantified. The RTP system presented the highest SOC values, up to 100 cm depth, contributing with 20 Mg C ha^{-1} from belowground input, during 35-year old of system implantation. However, about 90% of SOC was not physically protected in the soil aggregates. The forest to rubber + cacao agroforestry system (AFS) conversion reduced SOC stock up to 100 cm approximately 72 Mg ha^{-1}, and the reduction in SOC stock after forest to rubber tree + açaí AFS was around 34 Mg ha^{-1}. Rubber tree + cacao and rubber tree + açaí AFSs had significant contributions of occluded C in soil aggregates.

Keywords Soil aggregates · $^{13}C/^{12}C$ · Cacao, açaí · Atlantic forest · Physical protection

1 Introduction

Agroforestry systems (AFSs) are sustainable land uses that combine, simultaneously or sequentially, agricultural crop production with tree or fruit tree plantations and/or animals using the same land unit and applying techniques which are compatible with the cultural practices of the local population [33]. AFSs are recognized as soil exploration models that are more ecologically similar to natural forests and function as environmental service providers, such as high potential for sequester soil organic carbon

(SOC) [32, 34]. The estimate of AFSs potential to sequester carbon (C) in a global scale is 1.1–2.2 Pg of C over 50 years, above and belowground [11]. The C credit sale due to the rubber tree C sequestration, in pure plantations and also in agroforestry systems, can be a source of income for the smallholder farmers and encourage the expansion of forest and agroforestry production in Brazil.

A rubber tree (*Hevea brasiliensis* Muell. Arg), which is one of the major tree crops of economic interest in tropical regions of the world [21], is an example of a tree species that has been used in AFSs with diverse crops, such as

açaí (*Euterpe oleracea*), cacao (*Theobroma cacao*), acerola (*Malpighia glabra*), guaraná (*Paullinia cupana* Kunth), red pepper (*Piper nigrum*), urucum (*Bixa orellana*), maize (*Zea mays*), bean (Phaseolus vulgaris), among others [41]. In Brazil, there are around 170 thousand ha of planted area with rubber trees, and this is expanding for several reasons: (1) Latex and wood are being produced for multiple purposes; (2) rubber tree is considered a viable production alternative to aid in the reduction in socioeconomic and environmental problems; (3) planting rubber trees supplies work for a large labor force; (4) rubber tree is highly adapted to stabilize and recover degraded areas; and (5) rubber tree contributes to climatic effects mitigation due to C sequestration, both in plant biomass and in soil, with an economic useful life of 20–40 years and the extension of this productive period being mainly determined by the intensity of latex extraction [27]. In this sense, rubber tree AFS can be considered an intelligent agricultural system for the climate (climate-smart agriculture).

There are three relevant aspects to be considered in SOC stock studies. The first is the soil's deepest horizons, which are responsible for more than half of the accumulated C in the profile. This C is derived from roots and remains stable in the soil due to its more lignified constitution, which makes this material decompose more slowly [19, 39]. The second aspect is the origin of C in soils where there were forest to pasture or pasture to forest plantations or agroforestry conversions. The ^{13}C isotopic tracking technique behaves as a digital identification of the C source being deposited in the soil. When replacing a secondary forest (C_3) with a $C–C_4$ source crop or pasture, it is possible to analyze how much the new source of C is contributing to the current stock in the soil [2, 4]. Plants with a C_3 cycle, a characteristic of forests, have a δ^{13}C value between -35 and $-20‰$, with an average value close to $-27‰$. Plants with a C_4 cycle, a characteristic of grasses and some agricultural systems, have a δ^{13}C value between -18 and $-8‰$, with an average value close to $-13‰$. These values are much more negative than those found in the CO_2 in the air, around $-7.4‰$, and are directly connected with their C source [4, 15]. Finally, C stabilization within the aggregates is an important mechanism to reduce the bioavailability and accessibility of organic matter for soil microorganisms and enhance the potential of C sequestration in soils [42]. The hypothesis of the present study was that in heavily weathered soils, early natural forest conversion to pasture or rubber tree plantations or rubber tree AFSs (rubber tree + cacao or rubber tree + açaí) will not negatively affect SOC accumulation due to the lack of soil tillage and the continuous contribution of vegetal residues to the soil over the years which would favor C occlusion in soil aggregates. Thus, the objectives were: (1) to evaluate the potential of rubber tree AFSs and pure RTP

to accumulate SOC up to 100 cm in comparison with an adjacent secondary forest and pasture; (2) to evaluate the variation in δ^{13}C along the soil profile after the conversion of forest to pasture, as well as in RTP with grass predominance in their understory composition; and (3) to verify the physical protection of C in the aggregates as a mechanism of C stability in these soils.

2 Materials and methods

2.1 Study sites

This study was performed in the areas of the Experimental Station Djalma Bahia (EDJAB) in the municipality of Una (15° 17′ 20.44″ S, 39° 3′ 39.44″ W) in the south region of Bahia, Brazil, in a flat land with 63 m of altitude. According to the Köppen classification system, the climate is classified as Af and is characterized by an annual precipitation of 2000 mm and an average annual temperature of 26° C (Climatological Station of CEPLAC, unpublished data). The soils were classified as Dystrophic Grayish Argisols (Ultisols) [8]. The previous vegetation was a secondary forest, which was felled for agroforestry systems, rubber tree plantations and pasture implantations, and the area of each land use was 1 ha. These AFSs and the rubber tree plantation, composed of different ages, were chosen because they were the most homogeneous and well-developed plantations:

(1) 35-year-old rubber tree (*Hevea brasiliensis*) plantation (RTP): spacing was 7 m × 3 m (476 plants ha^{-1}), with grasses (*Paspalum Notatum Fluegge* and *Cynodon dactylon*) as soil cover. This system has been without inorganic fertilization since 1982.

(2) Secondary forest—SF, classified as Dense Ombrophylle Lowland Forest, whose structural and physiognomic characteristics are similar to the Amazon Rain Forest. Approximately 30% of the trees with approximately 30 cm of diameter had been removed. Currently, these forest fragments are maintained preserved.

(3) 8-year-old unfertilized pasture (PAS) composed of *Brachiaria decumbens.* Before the pasture was installed, the area had remained fallow for approximately 30 years. We chose this pasture, even though it was much younger than the other land-use systems, because it was the only one available at the experimental station.

(4) 30-year-old rubber tree (*Hevea brasiliensis*) and açaí palm (*Euterpe oleracea* Mart) AFS − RT + A: rubber tree and açaí spacing was 7 m × 3 m (476 plants ha^{-1}) in a quincunx planting pattern. Açaí was planted to

produce palm hearts. Açaí fertilization was applied to the crown of each tree 67, 13 and 67 kg/ha of urea, potassium chloride and triple superphosphate, respectively. This system has been without inorganic fertilization since 1987 [1].

(5)   35-year-old rubber tree (*Hevea brasiliensis*) and cacao (*Theobroma cacao*) AFS – RT + C: cacao spacing was in double rows of 2 × 2 m, lines L1 and L2 were planted at a distance of 2.5 m from the hevea hedgerows and rubber tree spacing was 7 × 3 m. Cacao fertilization consisted of 1107, 778 and 932 kg/ha of limestone; 222, 821, 292 and 525 kg/ha of N:P$_2$O$_5$:K$_2$O as 11:30:17; 244 kg/ha of N:P$_2$O$_5$:K$_2$O as 13:35:10 and ammonium sulfate and micronutrients (181 and 56 kg/ha, respectively). This system has been without inorganic fertilization since 1987 [1].

Rubber tree fertilization in all land uses consisted of 22, 115, 78 and 100 kg/ha of triple superphosphate; 54, 19 and 38 kg/ha of potassium chloride; 91 and 70 kg/ha of urea; and 97 kg/ha of ammonium sulfate [1].

2.2   Soil sampling and analysis

Four fixed plots (30 × 30 m) were defined in the center of each land-use system (i.e., four pseudoreplicates), within the same original area with the same previous land-use history and also the same topographic position, separated by at least 100 m. Trenches (1 × 1 × 1.5 m) were dug randomly between the plant rows using a zigzag pattern in each plot. The soil was sampled at six depths (0–10, 10–20, 20–40, 40–60, 60–80 and 80–100 cm). These depth classes were chosen in accordance with the protocol used for a multi-country study on soil C sequestration in AFSs [30, 46]. Soil samples from each depth were air-dried and passed through a 2-mm sieve. Soil particle size was determined using the pipette method, and the volumetric ring method was used to determine soil bulk density (Embrapa [14]) (Table 1). The SOC and total N were determined by dry combustion in an automated elemental analyzer (CHNS/O analyzer). The following measurements were performed using the methods described in Embrapa [14]: pH was measured with a glass electrode in 1:2.5 water stirred suspension. Exchangeable Ca^{2+}, Mg^{2+} and Al^{3+} were extracted with 1 mol L^{-1} KCl. P and K were extracted with Mehlich-1. Ca^{2+}, Mg^{2+} and Al^{3+} in extract were determined by atomic absorption spectrophotometry. K concentrations were determined by flame emission spectrophotometry. P was determined by colorimetry with ascorbic acid by spectrophotometry (Table 2).

The soil bulk density at each depth was used to calculate the total amount of SOC stored to a depth of 1 m and was expressed in Mg ha^{-1}. The SOC stock was later

Table 1 Particle size fractions (sand. silt and clay) and bulk density at different depths in rubber tree agroforestry systems, secondary forest and pasture in Bahia, Brazil

	Land-use systems				
	SF	PAS	RT	RT + A	RT + C
pH (H$_2$O)	4.52	4.60	3.50	3.70	3.70
P (mg kg^{-1})	0.80	1.60	11.20	4.40	5.80
K (mg kg^{-1})	33.00	40.00	32.00	26.00	29.00
Ca (cmolc dm^{-3})	0.16	1.15	0.42	0.14	0.50
Mg (cmolc dm^{-3})	0.48	0.53	0.22	0.14	0.62
Al (cmolc dm^{-3})	1.40	0.50	2.20	1.20	1.80
H + Al (cmolc dm^{-3})	11.20	9.90	14.90	9.70	10.90
SB (cmolc dm^{-3})	1.42	2.30	1.72	0.95	1.69

BD bulk density, *SF* secondary forest, *PAS* pasture, *RT* rubber tree plantation, *RT + A* rubber tree and açai agroforestry system, *RT + C* rubber tree and cacao agroforestry system

corrected by the clay content of the soil under the natural forest and used as a reference, as suggested by Moraes et al. [31], as the SOC level variations were closely related to soil texture. Therefore, this correction is to remove the effect of clay content and to show the possible variations in soil C stock resulting from the differences between land-use systems.

The SOC content was corrected for each depth using the following equation:

$$C_{(corrected)} = C_{(measured\ g\ 100\ g^{-1})} * \frac{clay\ content_{(reference)}}{clay\ content_{(treatment)}}$$

Since SOC levels of a given soil layer also depend on the soil compaction, we corrected the SOC levels for compaction using natural forest soils as Ref. [13].

The following equation was used to calculate the thickness and the soil layer compaction that should be considered:

$$E_{ad/sub} = \frac{\left[\frac{(M_{reference} - M_{treatmeant})}{Ds}\right]}{100}$$

where $E_{ad/sub}$ = depth to be added or subtracted in the stock calculation (cm); M_{ref} = soil mass at the reference soil depth (Mg ha^{-1}); M_{trat} = soil mass at the assessed soil depth (Mg ha^{-1}); and Ds = soil bulk density (g/cm^3).

Natural ^{13}C abundance was determined to obtain the C isotope ratio (^{13}C/^{12}C) and distinguish between residues from C$_3$ (e.g., forestry and agroforestry systems, indicating the presence of trees) and C$_4$ plants (in this study a pasture with a predominance of *Brachiaria*). Thus, the natural ^{13}C abundance method was only used in secondary forest (C$_3$ plants), pasture (C$_4$ plants) and RTP (C$_3$ plants). Pasture

Table 2 Chemical attributes (0–10 cm) of soils under rubber tree agroforestry systems, secondary forest and pasture in Bahia, Brazil

Depth (cm)	Particle size fraction (%) and bulk density (g cm⁻³)	SF	PAS	RT	RT + A	RT + C
0–10	Sand	45.69	53.70	42.41	43.87	35.87
	Silt	14.20	3.66	9.21	7.17	6.82
	Clay	40.11	42.64	48.38	48.95	57.30
	BD	1.03	1.54	1.22	1.15	1.16
10–20	Sand	42.83	52.67	37.89	42.31	30.95
	Silt	19.94	9.10	7.25	7.40	7.56
	Clay	42.23	38.23	54.87	50.29	61.50
	BD	1.20	1.43	1.34	1.28	1.20
20–40	Sand	38.52	46.90	31.91	36.87	26.76
	Silt	7.97	7.38	7.18	6.88	6.53
	Clay	53.50	45.73	60.92	56.25	66.72
	BD	1.34	1.50	1.38	1.36	1.26
40–60	Sand	32.60	43.13	27.57	32.89	23.95
	Silt	5.98	5.03	3.89	7.84	5.06
	Clay	61.42	51.84	68.54	59.27	70.99
	BD	1.36	1.46	1.36	1.34	1.26
60–80	Sand	35.04	38.79	25.93	29.35	21.80
	Silt	1.05	5.04	8.85	4.71	6.81
	Clay	61.42	56.16	65.22	65.94	71.38
	BD	1.22	1.38	1.28	1.30	1.15
80–100	Sand	31.60	40.89	25.63	29.35	20.59
	Silt	2.17	5.35	5.11	4.71	3.76
	Clay	66.23	53.76	69.26	70.25	75.64
	BD	1.24	1.37	1.28	1.32	1.18

SF secondary forest, *PAS* pasture, *RT* rubber tree plantation, *RT + A* rubber tree and açai agroforestry system, *RT + C* rubber tree and cacao agroforestry system

system was implemented in an area that was previously cultivated with C_3 vegetation (secondary forest), and RTP had in its understory a grass predominance. The $^{13}C/^{12}C$ ratio ($\delta^{13}C$) was determined using a continuous-flow isotope ratio mass spectrometer (Finnigan Delta Plus) coupled with an automated C and N analyzer (Carlo Erba EA 1108) at USP/Nuclear Energy Center of Agriculture (Centro de Energia Nuclear na Agricultura). The results were expressed in ‰ relative to the Pee Dee Belemnite (PDB) international standard and calculated using the following equation: $\delta^{13}C = \frac{R_{sample} - R_{reference}}{R_{reference}}$ where R sample = $^{13}C/^{12}C$ ratio of the sample and R reference = $^{13}C/^{12}C$ ratio of the sample.

The next equation [47] was used to determine the proportion of C_4 plant-derived (pasture) C at a depth of 0–20 cm (where differences in the $\delta^{13}C$ were found):

$$\%C - C_4 = \frac{\delta - \delta_o}{\delta_p - \delta_o} * 100$$

where δ = natural ^{13}C abundance in the samples; δ_0 = natural ^{13}C abundance in the soil samples without C_4 plant

residues (a natural forest was used as a reference); and δ_p = natural ^{13}C abundance of the pasture plant material (−12.65‰).

The following equation was used to obtain the % of C_3 carbon:

$$\% C - C_3 = 100 - \% C - C_4$$

2.3 Physical fractionation

Aggregate fractionation was carried out according to the procedure of Elliot [12], adapted as described by Gama-Rodrigues et al. [17]. First, soil samples obtained from the field were air-dried and passed through a 2-mm sieve. The procedure consisted of submerging 100 g of the soil sample (< 2000 µm) in a 500-mL beaker of deionized water for about 5 min before placing it on top of a 250-µm sieve. The sieving was done manually, moving the sieve ~3 cm up and down during a period of 2 min. The samples were physically fractionated by wet-sieving through a series of two sieve sizes (250 and 53 µm), and three aggregate size fractions were obtained: macroaggregate (2000–250 µm), microaggregate (250–53 µm) and silt-and-clay size fraction

(< 53 µm). All fraction classes were air-dried at 60 °C, and the percent weight of each fraction was calculated.

2.4 Sonication

The purpose of sonification technique was to promote the complete aggregates disruption. The sonication procedure consisted of weighing 5 g of macroaggregate or 3 g of microaggregate in a 500-mL beaker to which 150 ml of deionizer water was added. The suspension was sonicated at 600 J ml^{-1} (chosen after a prior test) by using an amplitude of 40% and in 20-minute combinations with a Sonic Dismembrator. The pulse method (60 s on and 30 s off) and ice were used to keep the temperature below 35 °C to avoid cavitation reduction [38, 40]. The depth immersion of the sonificator probe was kept constant at 10 mm. After sonication, the suspension was passed through the same-sized sieve used to obtain the size fraction (e.g., 250-µm sieve for the macroaggregate and 53-µm sieve for the microaggregate). The fraction that remained on the sieve after sonication was not analyzed (fraction composed mainly of sand), and the fraction that passed through the sieve was termed aggregate-organic-matter fraction (AOM$_f$).

The occluded C (OC) in the AOMf was determined by dry combustion using an automated Perkin-Elmer Series II 2400 CHNS/O analyzer.

The OC stock in macro- and microaggregates stock (Mg ha^{-1}) was calculated as follows:

Step 1: C content in AOMf:
C AOMf = (C * W_{AOMf} * WF)/(100 * iWf)
where C = C concentration measured in AOMf (g 100 g^{-1}), W_{AOMf} = weight of AOMf (g), WF = weight of the size fraction, obtained in physical fractionation (g), iWf = initial weight of size fraction (5 g for macro- and 3 g for microaggregate)
Step 2: OC stock (Mg ha^{-1})
OC stock = (C content in AOMf * WF* BD* SDI) * Ci
where C content in AOMf is obtained in step 1, WF = weight of the size fraction, obtained in physical fractionation (g), BD = soil bulk density in kg dm^{-3}, SDI = soil depth interval (cm), Ci = 0,01 (convert unit to Mg ha^{-1})

3 Statistical analysis

Each site was considered a fixed-effect treatment due to the multiple sources of variation, such as land-use systems (agroforestry systems, pasture and rubber tree plantation), different management practices and the ages of the plantations [25]. This assumption was previously explained for cacao agroforestry and forest systems [10, 16, 30, 37, 46]. To evaluate the normal distribution of the variables analyzed, data were tested for normality by Kolmogorov–Smirnov and Lilliefors, and homoscedasticity and normal distribution residues. The data were transformed to √X. In sequence, the data were analyzed by analysis of variance (ANOVA) as a completely randomized design with four replicates. As each plot was located at least 100 m from each other, we assumed that they were independent from each one, which validated our use of analysis of variance (ANOVA) [24]. The data were analyzed using Assistat software (exception δ^{13}C). SigmaPlot 11.0 (Systat Software inc., [43] was used to build the graphics.

4 Results

4.1 Soil fertility level and soil organic carbon (SOC) stocks

All of the sites studied exhibited clayey to very clayey soils, high acidity, very low extractable P concentrations, low-to-medium base sum and Al concentration and high potential acidity (Tables 1, 2). At 100 cm depth, the RTP stored around 9% more SOC and AFSs between 21 and 44% less than the secondary forest (Fig. 1). In general, SOC stock varied among systems at all depths; however, while AFSs contributed similarly along the soil profile, the pasture accumulated 48% and 28% of the total SOC stock, respectively, in the first 20 cm and below 40 cm. On the
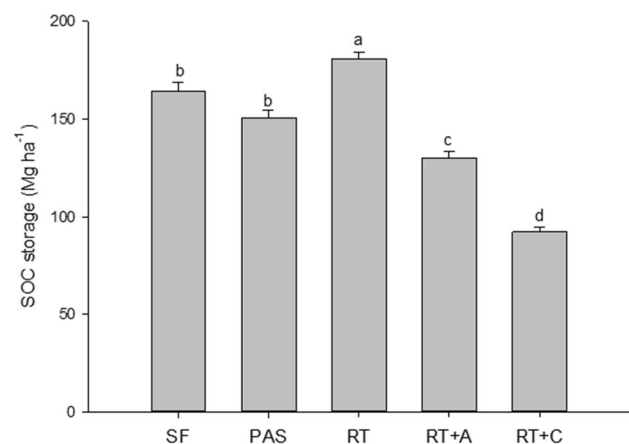

Fig. 1 Soil organic carbon (SOC; Mg ha^{-1}) stocks, upper to 1 m, in soils under rubber tree agroforestry systems, secondary forest and pasture in Bahia, Brazil. SF: secondary forest; PAS: pasture; RT: rubber tree plantation; RT+A: rubber tree and açai agroforestry system; RT+C: rubber tree and cacao agroforestry system. Values followed by the same letter(s) within land-use systems are not significantly different according to Scott–Knott test (*P=0.05*); vertical bars represent ± standard error

other hand, the rubber tree plantation in the first 20 cm contributed 29% to the SOC stock. Below 40 cm depth, the contribution was approximately 47% of the total SOC up to 100 cm (Table 3).

5 Variation in $\delta^{13}C$ with depth in secondary forest, pasture and rubber tree plantation

The highest $\delta^{13}C$ value variation occurred in the first 10 cm, where the secondary forest presented the most negative values, ranging around -28‰, followed by

Table 3 Occluded C in macroaggregates (OC_{Ma}) and in microaggregate (OC_{Mi}) at different depths in rubber tree agroforestry systems, secondary forest and pasture in Bahia, Brazil

Land-use systems	Depth (cm)	OC_{Ma} (Mg ha^{-1})	OC_{Mi}
SF	0–10	7.68[a]	0.93[b]
PAS		10.15[a]	1.44[b]
RT		5.23[b]	2.50[a]
RT+A		9.42[a]	2.47[a]
RT+C		8.41[a]	1.45[b]
SF	10–20	5.60[a]	2.43[a]
PAS		5.35[a]	1.27[b]
RT		3.57[a]	2.44[a]
RT+A		3.71[a]	2.88[a]
RT+C		5.15[a]	1.55[b]
SF	20–40	6.33[a]	1.77[b]
PAS		3.11[b]	3.72[a]
RT		5.52[a]	4.37[a]
RT+A		7.32[a]	3.52[a]
RT+C		6.69[a]	4.78[a]
SF	40–60	4.70[a]	3.23[a]
PAS		1.85[b]	3.85[a]
RT		4.00[a]	4.53[a]
RT+A		4.93[a]	4.30[a]
RT+C		4.39[a]	4.26[a]
SF	60–80	1.93[a]	11.99[a]
PAS		1.09[a]	12.80[a]
RT		1.88[a]	3.12[b]
RT+A		3.48[a]	3.83[b]
RT+C		2.61[a]	3.83[b]
SF	80–100	1.40[b]	9.56[a]
PAS		0.98[b]	5.87[b]
RT		2.53[a]	3.71[b]
RT+A		3.27[a]	3.45[b]
RT+C		2.84[a]	3.90[b]

SF secondary forest, *PAS* pasture, *RT* rubber tree plantation, *RT+A* rubber tree and açai agroforestry system, *RT+C* rubber tree and cacao agroforestry system

Values followed by the same letter in each depth (column) are not different according to Scott–Knott test (*P*=0.05)

rubber tree and pasture, with values close to -25 and -23‰, respectively. The secondary forest tended to present more positive results with depth increase. However, the RTP and pasture presented slightly more negative values and approached the forest values starting at 40 cm depth (Fig. 2). Below 40 cm, there was almost no change in $\delta^{13}C$ values between systems; while the RTP up to 1 m depth has values closer to C_3, the pasture and secondary forest have values closer to the C_3 and C_4 mix (Fig. 2). The accumulated C–C_3 at 0–10 cm depth was 37.95 Mg ha^{-1} in the secondary forest, 23.90 Mg ha^{-1} in the pasture and 21.85 Mg ha^{-1} in the rubber tree plantation. The accumulated C–C_3 from rubber tree was 162 Mg ha^{-1} up to 100 cm, with very little contribution of C–C_4, 3.4 Mg ha^{-1}. Overall, the C_4-origin C in the pasture was 23.40 Mg ha^{-1} and 127.36 Mg ha^{-1} was C_3-origin C along the soil profile (Fig. 3).

5.1 Aggregate classes and occluded C stock distribution at different depths

In all land-use systems, there was macroaggregate predominance up to 40 cm depth. Below this depth, there were a decrease in macroaggregates and an increase in the amount of microaggregates and in the silt + clay fraction. On average, in the first 40 cm, macroaggregates, microaggregates and silt + clay fraction represented 26, 19 and 5% of the soil mass, respectively, while below this depth the aggregate classes accounted for 19, 23 and 8%, respectively (Table 4). In general, along the 100 cm depth, there were variations among the aggregate classes without a defined trend between systems and/or depth (Table 4).

The average amount of OC_{Ma} in different land-use systems at 100 cm depth ranged from 22.50 to 32.15 Mg ha^{-1}, and around 20 Mg ha^{-1} was found up to 40 cm depth.
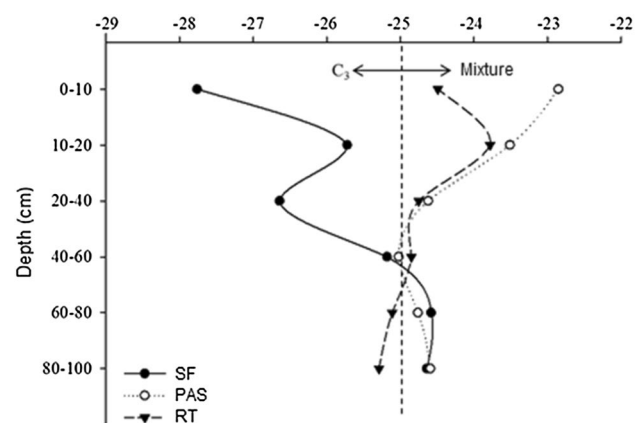

Fig. 2 $\delta^{13}C$ values at different depths of soils under rubber tree agroforestry systems, secondary forest and pasture in Bahia, Brazil. SF: secondary forest; PAS: pasture; RT: rubber tree plantation
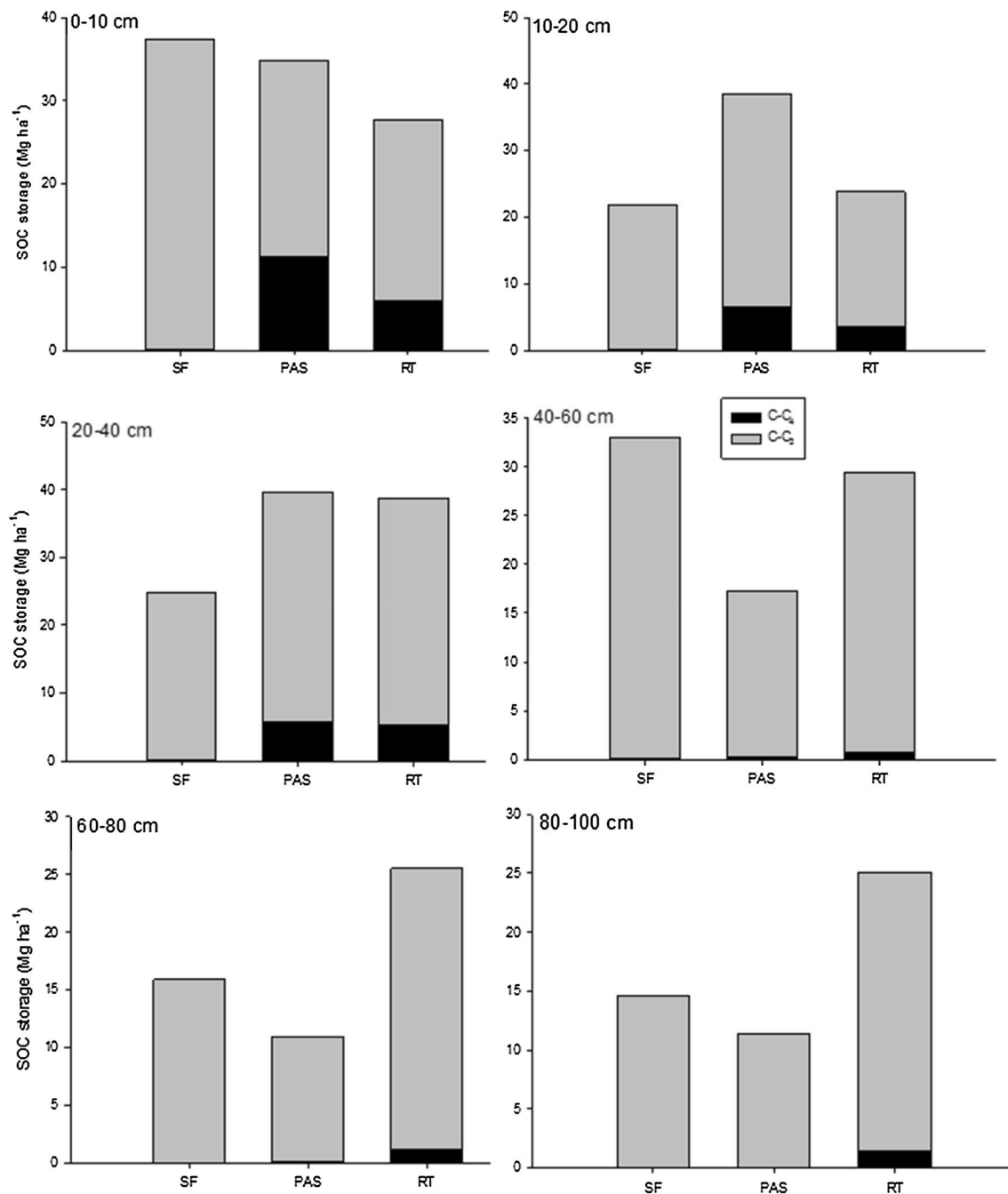
Fig. 3 SOC from C$_3$ and C$_4$ plants (trees and pasture, respectively) at different depths in rubber tree agroforestry systems, secondary forest and pasture in Bahia. Brazil. SF: secondary forest; PAS: pasture; RT: rubber tree plantation

Microaggregates, on the other hand, showed higher values from this depth, ranging from 12 to 25 Mg ha^{-1} (Table 3). In the first 40 cm, the amount of OC$_{Mi}$ ranged from 5.13 to 9.31 Mg ha^{-1}. Both occluded C in macroaggregates (OC$_{Ma}$) and in microaggregates (OC$_{Mi}$) showed differences between land-use systems (Table 3).

The secondary forest was the system that accumulated the most occluded C (OC$_{Ma}$ + OC$_{Mi}$) up to 1 m

(58 Mg ha^{-1}), representing 35% of SOC stock (Fig. 4). The rubber tree + açaí AFS and pasture accumulated around 53 Mg ha^{-1} and 52 Mg ha^{-1}, representing approximately 41% and 35% of the SOC stock, respectively. The rubber tree + cacao AFS presented 50 Mg ha^{-1} of occluded C, which represented 54% of SOC stock. The RTP showed the lowest value of occluded C, around 43 Mg ha^{-1}, representing only 24% of the SOC stock (Fig. 4).

Table 4 Depthwise distribution of different soil size fractions in rubber tree agroforestry systems, secondary forest and pasture in Bahia, Brazil

Depth (cm)	Particle size fraction (µm)	SF	PAS	RT	RT+A	RT+C
			Mass of soil	Fractions (%)*		
0–10	> 250	60.00[ns]	57.35	50.72	56.59	56.26
	> 53	28.75[ns]	32.27	36.80	32.83	30.52
	< 53	5.49[b]	7.31[b]	10.61[a]	8.71[a]	10.32[a]
10–20	> 250	51.22[ns]	51.97	47.04	50.09	50.48
	> 53	34.73[ns]	37.39	38.54	38.51	35.53
	< 53	8.79[ns]	7.89	11.72	10.98	11.41
20–40	> 250	51.61[a]	40.16[b]	43.31[b]	50.28[a]	43.66[b]
	> 53	33.08[ns]	45.02	39.94	36.72	39.66
	< 53	10.39[ns]	12.16	14.31	11.09	14.08
40–60	> 250	35.73[ns]	33.58	38.28	46.64	40.21
	> 53	45.62[a]	49.76[a]	41.76[b]	38.44[b]	41.53[b]
	< 53	12.63	13.91	17.21	12.85	15.90
60–80	> 250	32.59b	31.75[b]	32.68[b]	41.83[a]	35.17[b]
	> 53	47.09[a]	50.00[a]	44.85[b]	41.88[b]	43.42[b]
	< 53	15.00[ns]	15.70	19.19	14.31	17.49
80–100	> 250	30.77[ns]	32.44	34.01	38.91	36.06
	> 53	47.49[a]	48.56[a]	44.34[b]	42.53[b]	42.34[b]
	< 53	14.75[b]	16.37[b]	17.98[a]	15.66[b]	18.94[a]

SF secondary forest, *PAS* pasture, *RT* rubber tree plantation, *RT+A* rubber tree and açai agroforestry system, *RT+C* rubber tree and cacao agroforestry system

*Values followed by the same letter within size fraction and each depth are not different according to Scott–Knott test (*P = 0.05*)
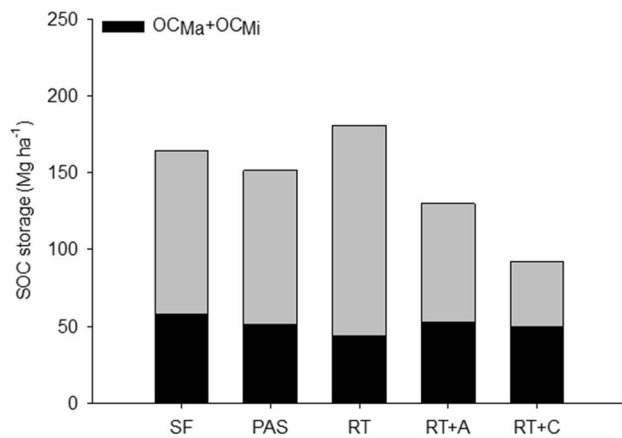

Fig. 4 Contribution of occluded C ($OC_{Ma}+OC_{Mi}$) in soil organic carbon (SOC), up to 1 m. SF: secondary forest; PAS: pasture; RT: rubber tree plantation; RT+A: rubber tree and açai agroforestry system; RT+C: rubber tree and cacao agroforestry system. There were no statistical differences of occluded C ($OC_{Ma}+OC_{Mi}$) among the land-use systems

6 Discussion

6.1 Soil organic carbon (SOC) stock

The forest to rubber + cacao AFS conversion reduced SOC stock up to 100 cm approximately 72 Mg ha^{-1}, and the reduction in SOC stock after forest to rubber tree + açaí AFS was around 34 Mg ha^{-1} (Fig. 1). This low SOC stock in AFSs was not expected, as these polyculture systems are able to accumulate similar or even higher values of SOC when compared to native forest systems or planted forests [45], even in soils with low fertility levels. The SOC stocks found in the present study were lower, mainly in agroforestry systems, than those shown by Monroe et al. [30] and Gama-Rodrigues et al. [17]. These authors found SOC stock up to 100 cm depth in the older agroforestry systems, similar to that of the secondary forest, and attributed this result as a consequence of a large litter deposition that occurs in these planted systems. Consequently, the following question came up: What factors could explain the reduced SOC stock found here, compared to other areas of AFSs in southern Bahia, Brazil? The C stock in soil is influenced by a number of factors, including plant residues deposition (leaves, branches and barks) that returns to the soil; root systems, soil fertility levels (which influence biomass production aboveground and belowground);

soil texture; decomposition rate (which is dependent on organic matter quality and on the soil's physical–chemical environment); and soil microbial activity. These factors interrelate, which makes it difficult to establish which one is the most preponderant. In the present study, soils in all systems presented high acidity and low fertility (Table 2) due to interruption of fertilization, which may have led to a reduction in the production of vegetal biomass and, therefore, a low return of vegetal residues to the soil. In addition, this soil chemical status may have negatively affected the activity and microbial diversity and, consequently, the organic matter decomposition, resulting in a reduction in the rates of nutrient and C transformation, leading to SOC stock reduction [22, 29].

The rubber tree plantation, on the other hand, was the most efficient system, storing approximately 17 Mg ha^{-1} more SOC than the secondary forest up to 100 cm. Of the total stored in the rubber tree plantation, the greater part (around 70%) was below 20 cm depth. The other systems, on the other hand, accumulated 70% of the SOC in the first 40 cm (Fig. 1 and Table 3). The total SOC stock in the RTP represented a C accumulation rate around 0.4 Mg C ha^{-1} year^{-1}, which is a less significant value, if compared, for example, with the rate of 1.1 Mg C ha^{-1} year^{-1} in soils up to 60 cm in depth under rubber tree plantations between 4 and 15 years of age in Brazil [27] and, in China, 0.72 Mg C ha^{-1} year^{-1} in soils up to 100 cm under rubber tree plantations with ages varying from 3 to 38 years old [49]. However, our result about the C accumulation rate found in the RTP was similar to that obtained by Wauters et al. [48]. They found a C accumulation rate in the order of 0.23 to 0.49 Mg C ha^{-1} year^{-1} in rubber tree roots aged 2 to 26 years. A rubber tree can reach 46.4 Mg ha^{-1} of root biomass, and its roots account for 15–22% of C in its biomass [9] because this root system is deep, well developed and can reach 4 m depth [27, 50]. Roots have a fundamental role in C sequestration in depth because root cycling and decomposition of dead roots represent a high input of organic matter in soil, and also because of roots' high soil stabilization potential [36, 39]. Vicente et al. [46] also found larger SOC stock in Ultisol under RTP (around 220 Mg ha^{-1}). These authors showed that compared to the secondary forest, the RTP increased around 46 Mg C ha^{-1} up to 1 m. The rubber plantation of the present study stored a significant amount of SOC. However, in the first 20 cm, we recorded a decrease of 14 Mg ha^{-1} and 21 Mg ha^{-1} in relation to the secondary forest and pasture, respectively (Table 3), probably due to rapid decomposition and low residence time of the rubber tree litter, which reduces substrate production for organic matter formation in soil. Despite C losses that can occur in the conversion of secondary forest to pasture [18], in the present study the SOC stock in the pasture was similar to that in the secondary forest, suggesting that it was able to

partially replace the C lost in surface in this 8-year period. We can attribute these results to a pasture's capacity to develop extensive root systems with high biomass production, as well as to maintain an active rhizosphere due to death and decomposition of root systems which creates a favorable biological condition for C storage [35].

7 Variation in $\delta^{13}C$ with depth in secondary forest, pasture and rubber tree plantation

The principle of the natural ^{13}C abundance method is based on the degree of discrimination between the two stable isotopes of carbon, ^{13}C and ^{12}C. The $^{13}C/^{12}C$ soil ratio (expressed in $\delta^{13}C$) varies based on the C assimilation pathway of the species that prevails in the system. From the total SOC accumulated up to 100 cm depth from the pasture, 85% was C_3-origin C probably derived from natural forest that existed before the pasture was established (Fig. 3), as well as from 30 years of grassland vegetation (i.e., *Brachiaria decumbens*) during fallow. In the RTP, there was also a predominance of C_3-origin C but we cannot affirm that this is only from rubber trees. The species that use the C_3 pathway discriminate against ^{13}C uptake more than the species that use the C_4 pathway. Therefore, the amount of $\delta^{13}C$ is lower (mean values of −28‰ for C_3 and −12‰ for C_4) when C_3 plants prevail. Thus, the natural ^{13}C abundance method can be used to determine the relative contributions of C_3 and C_4 plants-derived C in the total SOC and to estimate the turnover of SOM when the original photosynthetic pathway of organic inputs is changed, independent of the plant species involved in each of these groups (C_3 or C_4) ([3, 6, 5, 23, 28, 30, 46]; Paul 2016).

So, the C–C_3 could be both from prior (a secondary forest) and new vegetation. It is important to emphasize that, below 20 cm depth, the RTP started to accumulate more C than the secondary forest, with a C_3-origin C predominance, 119.57 Mg ha^{-1} against 99.51 Mg ha^{-1} from the secondary forest (Fig. 3). Thus, we can suggest that 20 Mg ha^{-1} of C represented rubber tree roots' contribution in C input to soil during the 35 years of this system's implantation and can explain partially the difference between the secondary forest in SOC stock up to 100 cm. A rubber tree has a well-developed root system with a high turnover rate, which is a significant source of soil organic matter [26]. The C–C_4 contribution in the rubber tree plantation, as a result of grasses in their understory, was relevant up to the first 40 cm depth and represented approximately 9% of SOC stocked up to 100 cm depth, and in pasture, this contribution was around 15%. Below this depth, the influence of C_4 was very low, not exceeding 2% of SOC in these two systems (Fig. 3). We can highlight that a period of 8 years after forest into pasture conversion allowed the

incorporation of 24 Mg ha^{-1} of C_4-derived C from grasses up to 100 cm depth which suggests a high carbon turnover time in this land-use system.

7.1 Aggregate classes' distribution and occluded C stock at different depths

Since the soils used in this study stored SOC in the range of 92–181 Mg C ha^{-1} to 100 cm depth, it is relevant to know how much of this accumulated SOC is actually physically protected and can be considered stabilized C in these soils. The macroaggregates predominance and larger OC_{Ma} stock in the first 40 cm depth (Tables 3, 4) suggest that macroaggregation was the main C stabilization mechanism up to this depth. Macroaggregation restriction to surface soil is the result of greater availability of fresh plant residues deposited in the soil by these systems. The surface residue induces the formation of macroaggregates because it is a C source for microbial activity and the production of microbial-derived binding agents [42]. Gama-Rodrigues et al. [17], on the other hand, found macroaggregation predominance and higher occluded C stock in this fraction up to 100 cm depth. The authors attributed these results to the constant addition of organic matter via litterfall and tree roots of both cacao and shade trees. Vicente et al. [46] also observed a macroaggregate predominance and significant occluded C stock in this large fraction (around 50 Mg ha^{-1}), which represented almost 30% of SOC storage up to 1 m depth. According to Tisdall and Oades [44], the deposited amount of organic matter, more than its quality, is important in soil aggregates formation.

The microaggregates, on the other hand, were predominant and had a higher occluded C stock below 40 cm (Tables 3, 4), probably due to a rise in clay content in deeper horizons, since clay is an important binding agent for soil microaggregates stabilization [20]. In addition, persistent agents involved in microaggregates formation are C from microbial origin [44], i.e., resulting from the continuous microbial cycling of labile material in deep soils [39]. In this case, below 40 cm depth microaggregates represent stable C and therefore are protected from decomposition in these soils.

Rubber tree + cacao AFS was the system that most contributed with occluded C in aggregates (almost 60%) in relation to the SOC stored up to 100 cm depth. Next were rubber tree + açaí AFS, pasture, secondary forest and finally the RTP that contributed with 24% of occluded C in soil aggregates (Fig. 4). The soils under rubber tree + cacao and rubber tree + açaí AFSs were the ones that presented high clay content along soil profile. Clay fraction, iron and aluminum oxides are fundamental for organomineral complex formation (clay-polyvalent metal–organic matter)

that form soil aggregates and, consequently, promote physical protection of SOC [44]. Additionally, the occlusion of C in soil aggregates could be attributed to the continuous deposition of plant residues, the presence of decomposing roots, root exudates (which act as a binding agent), root length and also the presence of fungal hyphae [44]. A rubber tree, as previously mentioned, produces litter with a high decomposition rate and reduced turnover time, which does not contribute to secondary compounds' formation in the soil aggregation process [7].

Then, the results of the present study suggest that the RTP favors more labile C production. On the other hand, during the transformation of organic matter in the rubber tree + cacao and rubber tree + açaí agroforestry systems, there is a preservation of the most recalcitrant compounds of C once cacao and açaí produce high amounts of cellulose-rich and lignin-rich residues [30] that regulate residue decomposition rates and act as substrates for the formation and stabilization of C within the aggregates.

8 Conclusion

The RTP system presented the highest SOC values, up to 100 cm depth, contributing with 20 Mg C ha^{-1} from belowground input, during 35-year old of system implantation. However, about 90% of SOC was not physically protected in the soil aggregates. The rubber tree + cacao and rubber tree + açaí AFSs stored the smallest amount of SOC with a significant contribution of stable occluded C in soil aggregates. Macroaggregation and microaggregates were the main mechanisms of C physical protection in the first 40 cm and below this depth, respectively. These results indicate that AFSs are more efficient in producing stable C in soil which is protected from decomposition, minimizing CO_2 emissions into the atmosphere. Thus, the present study supports the role of AFSs as environmental service providers that could supply an additional income through the C credit sale, which creates a favorable outlook for the small farmers and, therefore, could encourage the agroforestry expansion in Brazil.

Compliance with ethical standards

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