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Soil carbon stocks and origin under different cacao agroforestry systems in Southern Bahia, Brazil

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ABSTRACT

Cacao agroforestry systems (AFS) are characterized by accumulating large amounts of soil organic carbon (SOC). However, information on the influence of shade trees on SOC stock up to 100 cm depth is scarce in the literature. The objectives of this study were to quantify the SOC storage under different cacao AFS, natural forest and pasture to a depth of 100 cm; and to evaluate the origin of SOC using the ^{13}C isotopic ratio as an indicator of relative contribution of trees (C_3 plants) and grass (C_4 plants), after 4 years of pasture conversion into cacao and rubber AFS. SOC was determined by dry combustion in six layers (0–10, 10–20, 20–40, 40–60, 60–80 and 80–100 cm). The natural abundance of ^{13}C technique was used only on cacao and rubber lines in contrast to the SOC accumulated by pasture before conversion into AFS. The SOC stock in a depth between 0–100 cm was significantly high in the pasture and in both younger cacao and rubber AFS (4 years old). Systems that were more than 20 years old 'cabruca', cacao and *Erythrina* and cacao and rubber system (20 years) had lower SOC stocks and did not differ significantly from the natural forest. In the surface layers of soil there were significant differences in the SOC stock. However, below 20 cm all the systems were similar to each other. The SOC stock in the first 20 cm accounted between 31 and 44% of total SOC stored in the 100 cm in the seven systems, followed by 19–23% at a depth of 20–40 cm, 15–18% at a depth of 40–60 cm and 11–14% in the last two depths. After four years of establishment cacao and rubber AFS were the most efficient systems in the accumulation of SOC in the first 20 cm of soil and consequently up to 100 cm deep. Cacao was more efficient than rubber tree to accumulate C_3-derived C. While the rubber increased by almost 70% of C_3, cacao increased 131%. After the 40 cm of depth the SOC is still from the original natural forest.

1. Introduction

Cacao (*Theobroma cacao* L.) is a main agricultural commodity in many tropical countries (Hartemink, 2005). The main cacao-producing countries include Ivory Coast, Ghana, Indonesia, Nigeria, Cameroon and Brazil, which account for 82% of the world's cacao production (FAOSTAT, 2015). Since the 2010/2011 crop, Africa accounted approximately for 70% of the total produced in the world, Oceania around 13%, the American continent (South and Central America) ranges between 13 and 16% and Brazil contributes approximately 5% (CONAB, 2014; Midlej and Santos, 2012). However, in the late 80s and mid 90s there was a significant decrease in cacao production in Brazil, resulting from the witch's broom disease, caused by *Moniliophthora perniciosa*, and also by reduced commodity prices. As a result, there was the progressive impoverishment of the cacao region, where producers have abandoned the producing areas of cacao and converted them into pasture, and also by illegal exploration of shade trees with high value timber (Johns, 1999; Marques et al., 2012). However, in the last 20 years, the recovery of cacao production with plantation of resistant genetic material to witches' broom disease, combined with the rise of the commodity value are changing this negative picture, with an increase in production of almost 100% (CONAB, 2014).

In Brazil, the main cacao-producing areas are: south of Bahia, Amazon region, and coastal tableland of southern Bahia and northern of Espírito Santo. Specifically in southern Bahia, the largest planted area in Brazil, cacao crops are grown on about

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700,000 ha (Fontes et al., 2014) and are inserted in the central corridor of the Atlantic Forest, one of the main centers of endemism of this biome, which has gone through a continuous process of elimination and fragmentation (Lobão et al., 2012). Two typical cacao production systems are used both by smallholder (5–8 ha) and large farmers (approximately 300 ha) in this region: (1) traditional cultivation system, an area of approximately 399,000 ha, wherein the cacao plantations are implanted under natural forest; and herbaceous, shrub and individuals of the upper canopy are eliminated to provide increased light input, resulting in extensive agroforestry called 'cabrucas' with 600 cacao ha^{-1} (Fontes et al., 2014); (2) cacao plantations are established in areas where all native forest has been removed; cacao plants, in a density of 1100 cacao ha^{-1}, are shaded with banana and *Erythrina glauca* mainly because is a N_2 fixing tree legumes, rapid growth, proper height and little dense crown (Müller and Gama-Rodrigues, 2012; Lobão et al., 2012; Gama-Rodrigues et al., 2010). This plantation system grew out of the 1960s, when CEPLAC (Executive Committee of the Plan of Cacao Farming) initiated a broad program aimed a significant increase in cacao production at the expense of massive introduction of fertilizers, chemical control of insects and diseases and also reducing the cacao shading, eliminating 50–70% of the shade trees of the Atlantic Forest (Johns, 1999).

The cacao crop is now in an expansion and renovation phase, in which more productive and resistant (to diseases) varieties are used. Furthermore, in attempt to raise the cacao production, CEPLAC has recommended that *Erythrina* should be replaced by rubber trees, because this type of tree does not generate any additional revenue to cacao producers. The high economic value of rubber tree is due to latex and honey production and also by wood for multiple uses, which improves and diversifies farmer income sources. In addition, rubber tree shows rapid growth, little competition with other species and allows planting spacing with density that enables sustainable use of the two commodities (Marques et al., 2012).

The current scenario of southern Bahia cacao region is the following: cacao was renewed by grafting with clones tolerant to witches' broom in areas where *Erythrina* trees are being replaced by rubber trees, covering an area of approximately 50,000 ha, or, cacao being introduced in rubber tree plantations already established in the region, an area of around 30,000 ha. In addition, the possibility of expansion with the establishment of new cacao and rubber tree plantations (Marques et al., 2012). Despite all the initiative to change the cacao farming system in southern Bahia searching for higher yields, today there are yet a significant amount of 'cabrucas'. The historical fluctuation of the price of cacao on the world market made many farmers give up on the proposed changes, as they learned from previous generations experience, the risk of reduced shading in increasing susceptibility to drought and, or dependence on fertilizers and other agrochemicals (Johns, 1999). In this context, cacao cultivation in Bahia is a good example for agroforestry approach and can be a socio-economic-ecological component appropriate to reduce human pressure on the remaining of Atlantic Forest in southern Bahia (Lobão et al., 2012; Müller and Gama-Rodrigues, 2012).

Cacao agroforestry systems (AFS) with continuous and significant contribution of both above- and belowground biomass accumulation and subsequent turnover of leaf litter, roots, and woody material from the shade species and from cacao provide a continuous stream of organic inputs most of which, following decomposition, is stored into the soil and represent a substantial addition of soil C (Gama-Rodrigues et al., 2011). Besides, cacao AFS maintains many of others ecosystem functions includes control soil erosion because they protect the soil against raindrop impact and reduce runoff velocity and enhance soil fertility by an efficient nutrient cycling (Gama-Rodrigues et al., 2011; Tscharntke et al., 2011; Siebert, 2002) and also conserve the forest biodiversity (Schroth and Harvey, 2007; Gama-Rodrigues et al., 2010, 2011). All these possible environmental service can increase the income for the farmers of these crops, the majority of whom are smallholders, through the incentives from payment-for-ecosystem services and certification schemes.

Effects of land use changes are starting to be included in estimates of life-cycle greenhouse gas (GHG) emissions, so-called carbon footprints (CFs), from food production (van der Werf et al., 2009). In Brazil, for example, deforestation and forest burning account for about 75% of the greenhouse gases (GHG) emissions. The increase in atmospheric concentrations of CO_2 and other GHG is the major cause of global warming. Soil carbon (C) sequestration is a mechanism of reducing the CO_2 concentration in the atmosphere and depositing it in long term pools of C through afforestation, reforestation, and restoration of degraded lands (Nair et al., 2009a). In this scenario, agroforestry systems play an important role as a land-use system that allows for the mitigation of GHG emissions and helps reduce deforestation and restore degraded soils (Rita et al., 2011; Gama-Rodrigues et al., 2011, 2010). Several studies in the literature suggest agroforestry systems as a system with high potential to accumulate C both aboveground and belowground. For example, Verchot et al. (2007) predicted that the global carbon stock potential (C) of AFS would reach nearly 600 Mt C $year^{-1}$ by 2040 as there is a large area susceptible to land use changes. The carbon storage potential of cacao canopies in AFS range from 33 to 90 Mg C ha^{-1} (Somarriba et al., 2013; Gama-Rodrigues et al., 2011; Cotta et al., 2008). In addition, Barreto et al. (2011) found that the soil organic carbon (SOC) content in the top 50 cm of soil was nearly 100 Mg C ha^{-1} in a cacao + *Erythrina* AFS in southern Bahia. Furthermore, Gama-Rodrigues et al. (2010) observed soil SOC values of more than 300 Mg ha^{-1} in the 0–100 cm soil layer in a cacao AFS (*Erythrina* + cacao and 'cabruca').

An important aspect to be considered in the study of SOC stock which has received increasing interest from the scientific community is soil depth because the main source of C belowground comes from roots (Rasse et al., 2005). Particularly in AFS, the depth is very important, because the root system of shade trees can reach 2 m deep or more (Tscharntke et al., 2011; Lehmann, 2003). Cacao, for example, has a high concentration of roots in the first 60 cm, reaching 1–2 m deep (Silva Neto et al., 2001; Müller and Gama-Rodrigues, 2012). Additionally, according to Rumpel and Kögel-Knabner (2011), most subsoil horizons contribute to more than half of the total soil C stocks. This is because the input of organic material through these horizons is plant roots and root exudates, dissolved organic matter and bioturbation. Some authors even claim that the C subsoil may be more important in terms of source or sink for CO_2 than topsoil (Batjes, 1996; Paul et al., 1997; Lorenz and Lal, 2005). There is no consensus in the literature about the sources of C, processes involved in the stabilization and consequent stock of this C in depth, especially in cacao agroforestry systems in which these studies are scarce.

Many species that are commonly found in natural forests and the trees present in cacao AFS use the C_3 cycle as a mechanism for assimilating C. By contrast, the C_4 cycle is typical of grasses. Regarding the C assimilation pathway, the conversion pathways of natural forests into pastures and pastures into AFS differ, and stable C isotope studies may be useful for understanding the origin of soil organic matter. 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. 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. Several authors have used the natural ^{13}C abundance to determine the actual contributions of different species in SOC (Mendonça et al., 2010;

Takimoto et al., 2008; Zhang et al., 2007; Lemma et al., 2006). For example, Oelbermann et al. (2006) reported reduced $\delta^{13}C$ values in the 0–40-cm soil layer of 19- and 10-year-old alley cropping systems due to the incorporation of *Erythrina poeppigiana* (C_3 plant) residues from pruning. Takimoto et al. (2009) observed higher C_3-origin C contents at the soil surface and near live fence trees when studying C sources in soils in AFS, pastures and fallow fields. By contrast, the fodder area had less C_3-origin C, even in the presence of trees, and C_4-origin C prevailed in the fallow soil. Maggiotto et al. (2014) evaluated the effects of introducing rubber trees to pasture soils and observed that the rubber trees doubled the C_3-origin C levels to soil depths of 60 cm after 15 years according to the natural ^{13}C abundance method. The authors attributed the increase in the C stock to rubber tree plantation's root biomass and annual leaf deposition.

The objectives of this study are to (1) quantify the SOC stocks under different cacao agroforestry systems, natural forests and pastures to a depth of 100 cm and (2) evaluate the origins of the SOC by using the natural ^{13}C abundance 4 years after converting the pasture into a cacao + rubber AFS. Studying the soil C stock at depth and also the assessment of the contribution of cacao and rubber tree (replacing pasture) in the organic matter formation is an important step to advance in the understanding about the potential of tropical soils under cacao agroforestry systems as C reservoir.

2. Materials and methods

2.1. Study sites

Soil samples were collected at the Porto Seguro Farm in the municipality of Uruçuca (14°35′34″S 39°17′02″W) in the southern state of Bahia, Brazil. According to the Köppen classification system, the climate is classified as Af and is characterized by an annual precipitation of 1500 mm without a water deficit. The Porto Seguro Farm has an area of about 1100 ha, where around 300 ha there is cacao plantation and at 800 ha rubber tree, and primary and secondary forest. The farm soils were classified as a yellow Argisol (Ultisol) (Chepote et al., 2012).

Seven land use systems, each corresponding to an area of approximately 5 ha, were used in this study:

System 1: 30-year-old unfertilized pasture area.

System 2: secondary forest.

System 3: 35-year-old cacao agroforestry system in thinned forest, known as 'cabruca', cacao spaced 4 m × 2 m with a density of 1250 plants per hectare.

System 4: 35-year-old cacao and *Erythrina* (*Erythrina glauca*) agroforestry system with cacao spaced at 3 m × 3 m and *Erythrina* spaced at 25 m × 25 m in a quincunx planting pattern at densities of 1111 and 16 plants per hectare, respectively.

System 5: cacao and rubber agroforestry system with cacao (20 years) at 2.5 m × 2.5 m spacing and rubber (40 years) at 5 m × 5 m spacing.

System 6: cacao and rubber (4 years) agroforestry system replacing pasture and established in double rows of rubber (17 m × 3.0 m × 2.5 m) with 400 plants per hectare alternated with 5 rows of cacao (3 m × 3 m) at 833 plants per hectare and a row of gliricidia (*Gliricidia sepium)* in the cacao planting area.

System 7: cacao and rubber agroforestry system (4 years) replacing the natural forest and established in double rows of rubber (15 m × 3 m × 2.5 m) with 400 plants per hectare alternated with 4 cacao rows (4 rows of 3 m × 3 m) at 784 plants per hectare and a row of gliricidia (*Gliricidia sepium)* in the cacao planting area.

The systems were not fertilized from 1990 to 2002. All systems were fertilized every 3 years from 2003, with 18–18–18 and 16–24–16 (220–270 g plant $^{-1}$ year $^{-1}$). In 2011, fertilization was conducted after soil sampling. In the systems older than 25 years (3, 4 and 5) the plantation of banana was done in the gaps where there is no shade. In the other systems (6 and 7) banana and gliricidia were planted in the first three years, interspersed with cacao and rubber tree. Cacao, banana and gliricidia were pruned annually and the plant material was left on the ground in all systems. Rubber tree was pruned annually in the beginning of growth and after each two years.

2.2. Sample treatment and analyses

Four plots (30 × 30 m) were defined in the center of each land use system that were uniform in terms of soil homogeneity, slope, historical land use, density and tree age and were separated by at least 100 m. Trenches (1 × 1 × 1.5 m) were dug between the plant rows 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 multicountry study on soil C sequestration in AFS (Gama-Rodrigues et al., 2010; Saha et al., 2009; Haile et al., 2008; Takimoto et al., 2008). In System 6, soil samples were collected from four trenches in the cacao rows and four trenches in the rubber rows. These samples were collected to determine the isotope ratio of soil ^{13}C in the cacao and rubber rows relative to the SOC accumulated in the pasture before implementing the system.

Soil samples from each depth were air dried and passed through a 2-mm sieve. Soil particle size was determined using the pipette method (EMBRAPA, 1997) (Table 1). The volumetric ring method was used to determine soil bulk density (EMBRAPA, 1997) (Table 1). The SOC was determined by using dry combustion in an automated elemental analyzer (CHNS/O analyzer). 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 corrected by the clay content of the soil under the natural forest and used as a reference. SOC stock was corrected for clay content as suggested by Moraes et al. (1996) because the variations in the SOC levels were closely related to soil texture. Therefore, the SOC content was corrected for each depth by using the following equation:

$$C\,(\text{corrected}) = C\,(\text{measured g }100\,\text{g}^{-1}) \times \frac{\text{clay content (reference)}}{\text{clay content (treatment)}}$$

Since SOC levels of a given soil layer also depend on the compaction of the soil (Ellert and Bettany, 1995), we corrected the SOC levels for compaction, using natural forest soils as reference.

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

$$E_{\text{ad/sub}} = \frac{(M_{\text{ref.}} - M_{\text{treat.}})/D_s}{100}$$

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

2.3. Natural ^{13}C abundance in the soil

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 the rows of the cacao and rubber agroforestry system (4 years) (System 6) because this system was implemented in an area that was previously cultivated with pasture. The $^{13}C/^{12}C$ ratio ($\delta^{13}C$) was

Table 1
Particle size fractions and bulk density, up to 100 cm, in different cacao AFS, natural forest and grassland in the southern Bahia, Brazil.

Depth (cm)	Particle size fraction (%) and bulk density—BD ($g\,cm^{-3}$)	Systems						
		1	2	3	4	5	6	7
0–10	Sand	38	27	39	21	29	53	61
	Silt	17	13	8	13	9	11	8
	Clay	45	60	53	66	62	36	31
	BD	0.98	1.11	1.11	1.05	1.14	1.09	1.10
10–20	Sand	36	23	30	17	28	53	53
	Silt	19	12	10	12	16	12	9
	Clay	45	65	60	71	56	35	38
	BD	1.06	1.06	1.09	1.10	1.13	1.08	1.08
20–40	Sand	28	19	24	14	22	45	43
	Silt	17	13	9	11	15	13	10
	Clay	55	68	67	75	63	42	47
	BD	1.06	1.06	1.09	1.10	1.13	1.06	1.02
40–60	Sand	25	16	21	13	22	39	37
	Silt	15	10	8	9	14	12	11
	Clay	60	74	71	78	64	49	52
	BD	1.00	1.02	1.09	1.07	1.09	1.26	1.43
60–80	Sand	25	17	21	13	23	34	35
	Silt	14	10	9	13	13	9	13
	Clay	61	73	70	64	64	57	52
	BD	1.01	1.02	1.15	1.08	1.17	1.14	1.14
80–100	Sand	26	17	22	23	23	31	34
	Silt	13	9	11	12	12	9	9
	Clay	61	74	67	65	65	60	57
	BD	0.99	1.04	1.12	1.01	1.12	1.12	1.14

1—pasture (30 years old); 2—natural forest; 3—cacao ‘cabruca’ system; 4—cacao and *Erythrina* AFS (20 years old); 5—cacao and rubber AFS (20 years old); 6—cacao and rubber (4 years old) AFS replacing pasture; 7—cacao and rubber AFS (4 years old) replacing the natural forest.

determined by using a continuous-flow isotope ratio mass spectrometer (Finnigan Delta Plus) coupled with an automated C and N analyzer (Carlo Erba EA 1108) at the Embrapa/National Center for Research in Agrobiology (Centro Nacional de Pesquisa em Agrobiologia). The results were expressed in ‰ relative to the Pee Dee Belemnita (PDB) international standard and calculated by 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 following equation (Vitorello et al., 1989) 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):

$$\%C - C_4 = \frac{\delta - \delta_a}{\delta_p - \delta_a},$$

where δ = natural ^{13}C abundance in the samples; δ_a = natural ^{13}C abundance in the soil samples without C_4 plant residues (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 the C_3 carbon:

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

2.4. Statistical analyses

The data was analyzed by analysis of variance (ANOVA) as a completely randomized design with four replicates. Trenches opened in each plot were treated as pseudo-replication in each land use system. This assumption was previously described for cacao agroforestry and forest systems (Fontes et al., 2014; Rita et al., 2011; Dawoe et al., 2010; Gama-Rodrigues et al., 2010; Isaac et al., 2007; Lima et al., 2006; Dechert et al., 2005). As each plot was located at least 100 m from any other plot; we assumed that the plots were independent from each other, which validated our use of analysis of variance (ANOVA) (Lima et al., 2006). The data were analyzed using StatSoft Inc. (1974–2009) and STATISTICA 8.0 software, and the Scott–Knott test was used to compare the means at a 5% probability level. SIGMAPLOT 11.0 (Systat Software inc., 2010) was used to build the graphics.

3. Results

3.1. Soil organic carbon (SOC) content

The SOC content at a depth of 0–100 cm were significantly higher ($P = 0.05$) in the pasture and cacao and rubber systems that replaced pasture (System 6) and natural forest (System 7), respectively, than the other four land-use systems. The systems older than 20 years, including the ‘cabruca’ (System 3), cacao and *Erythrina* (System 4) and cacao and rubber system (System 5) had smaller C stocks and were not significantly different from the natural forest (System 2) (Fig. 1).

Significant differences in SOC contents were observed in the surface soil layers among the studied systems. However, all systems had similar SOC contents in the deeper soil layers (below 20 cm) (Fig. 2).

The SOC stock in System 7 at a depth of 0–10 cm was 57.10 Mg ha^{-1}, it means 27% of the SOC stored at 100 cm. At the same depth, the second largest SOC contents were observed to Systems 6 and 1, and were significantly different from those older systems, which exhibited lower values. Similar to what was observed in the SOC stock up to 100 cm: ‘Cabruca’ (System 3), 35-year-old cacao and

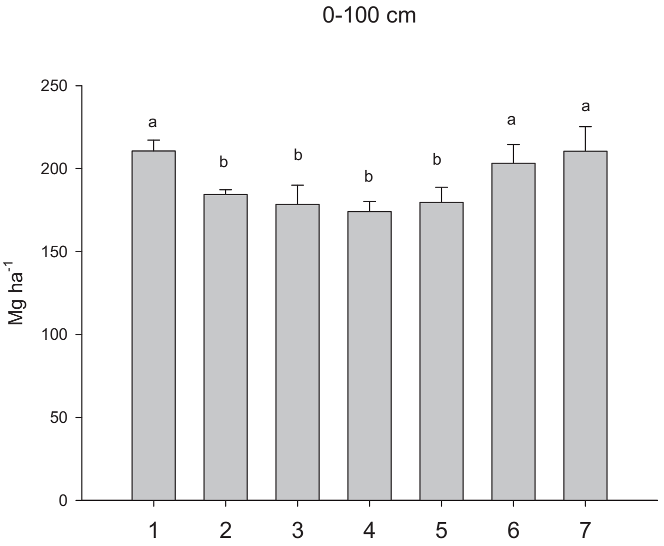

Fig. 1. SOC stocks in the 0–100-cm soil layer in different cacao AFS, natural forest and grassland in the southern Bahia, Brazil.
1—pasture (30 years old); 2—natural forest; 3—cacao 'cabruca' system; 4—cacao and *Erythrina* AFS (20 years old); 5—cacao and rubber AFS (20 years old); 6—cacao and rubber (4 years old) AFS replacing pasture; 7—cacao and rubber AFS (4 years old) replacing the natural forest. Values followed by the same letters are not statistically different according to the Scott–Knott test ($P = 0.05$); vertical bars represent ± standard error.

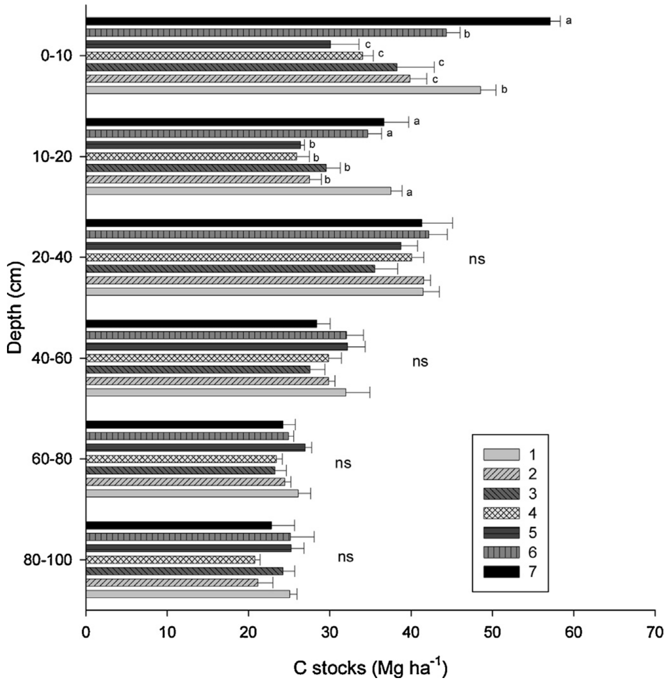

Fig. 2. SOC stocks at different depths of soils under different cacao AFS, natural forest and grassland in the southern Bahia, Brazil.
1—pasture (30 years old); 2—natural forest; 3—cacao 'cabruca' system; 4—cacao and *Erythrina* AFS (20 years old); 5—cacao and rubber AFS (20 years old); 6—cacao and rubber (4 years old) AFS replacing pasture; 7—cacao and rubber AFS (4 years old) replacing the natural forest. Values followed by the same letters are not statistically different according to the Scott–Knott test ($P = 0.05$); vertical bars represent ± standard error.

Erythrina (System 4) and cacao and rubber system (System 5) were not significantly different from the natural forest (System 2) (Fig. 2).

The results for a soil depth of 10–20 cm were similar to those observed at the greatest depths. Systems 1, 6 and 7 had the largest C contents and accounted for approximately 18% of the SOC in the soil profile. The older cacao agroforestry systems showed the smallest SOC stocks and did not differ from each other or the natural forest and the amount of C represented 15% of the SOC stock in the entire 0–100 cm soil layer (Fig. 2). The amount of C in the first 20 cm accounted for 31–44% of the total carbon stored in the top 100 cm of the soil in the seven systems, followed by 19–23% at a depth of 20–40 cm, 15–18% at a depth of 40–60 cm, and 11–14% at the deepest depths.

3.2. Variation of δ^{13}C with depth in the rubber and cacao systems replacing pasture

Different δ^{13}C values mainly occurred in the surface layers (0–20 cm) and decreased at a depth of 20 cm. This trend was similar to the trend observed for the SOC stock values (Figs. 2 and 3). In addition, this decrease was mainly observed below depths of 40 cm, where differences between the cacao and rubber rows were no longer observed. The differences in the δ^{13}C values between the cacao and rubber rows were 3.02‰ and −1.19‰ at depths of 0–10 cm and 10–20 cm, respectively (Fig. 3).

The δ^{13}C values of the pasture soils (System 1) were higher than those of the natural forest (System 2) at all depths. The δ^{13}C values of the pasture soils were −17.33‰ at a depth of 0–10 cm and −19.40‰ at a depth of 10–20 cm and continued to decrease with increasing depth, as observed in the rows in System 6. Furthermore, no additional differences in the δ^{13}C values were observed below a depth of 40 cm. By contrast, the natural forest (System 2) exhibited δ^{13}C values of −26.70‰ at a depth of 0–10 cm, −25.85‰ at a depth of 10–20 cm, and slightly greater values at deeper depths. The δ^{13}C values in the rubber row were higher than those in the surface layers of the cacao row. Similar to that observed in the pasture and cacao rows, the δ^{13}C values no longer differed at depths greater than 40 cm in the rubber row. In fact, the systems were indistinguishable at a depth of 80 cm (Fig. 3).

The amounts of C_3- and C_4-origin SOC are shown in Fig. 4 at a depth of 0–20 cm. Below 0–20 cm, the variations between the C_3 and C_4-origin SOC were less expressive in all systems, and C_3-origin SOC was prevalent (Fig. 3). The C_3-origin SOC decreased according to the following sequence: cacao row > natural forest > rubber row > pasture. In addition, the C_4-origin SOC decreased according to the following sequence: pasture > rubber row > cacao row. Overall, 81.7 Mg ha^{-1} of accumulated SOC in the cacao row and of 59.9 Mg ha^{-1} in the rubber row was derived from C_3 plant residues. At a depth of 0–20 cm, the C_3-origin SOC accumulated in the cacao row and surpassed the natural forest accumulation by 14.3 Mg ha^{-1}. By contrast, the C_4-origin SOC in the cacao row was 34.4 Mg ha^{-1} lower than the C_4-origin SOC in the pasture row and 20.9 Mg ha^{-1} lower than the C_4-origin SOC in the rubber row. Of the 86.0 Mg ha^{-1} of SOC in the pasture system, 50.7 Mg ha^{-1} of SOC originated from C_4 plant residues and 35 Mg ha^{-1} originated from C_3 plant residues (Fig. 4).

4. Discussion

4.1. Soil organic carbon (SOC) content

The SOC stock at a depth of up to 100 cm suggests that the cacao and rubber systems with 4 years of establishment (Systems 6 and 7) and the pasture system were more efficient for accumulating SOC in the soil than the natural forest and the older cacao agroforestry systems which were similar to each other. High SOC accumulation rates may occur during the initial period of AFS establishment and gradually become relatively stable (Isaac et al.,

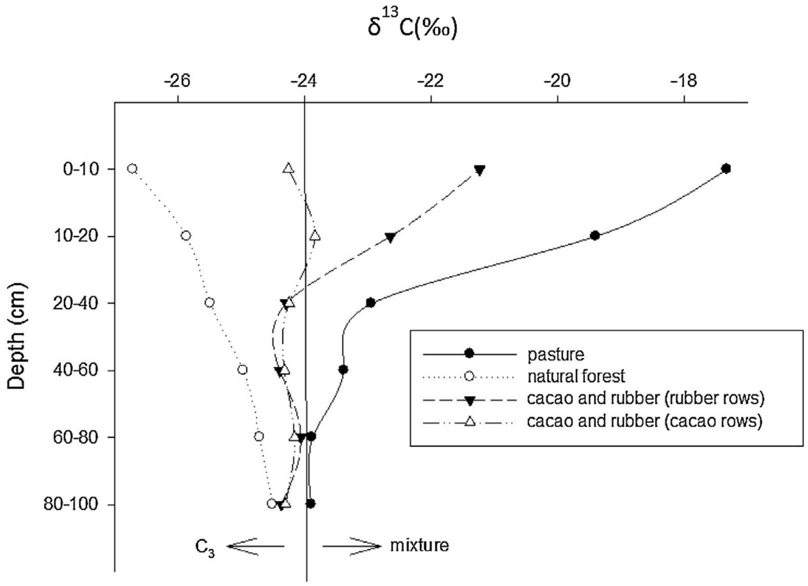

Fig. 3. δ^{13}C values at different depths of soils under pasture, natural forest and rubber and cacao AFS replacing pasture.

2005; Albrecht and Kandji, 2003). Mature forests results from slow growth, lower dry matter accumulation rates, larger amounts of decomposing residues, and well-balanced root systems (i.e., no fluctuations in the root distribution and turnover) (Montagnini and Nair, 2004).

Somarriba et al. (2013) also showed that SOC accumulation rates (shoots, leaf litter and soil) were higher in AFS that were less than 10 years old and that the values decreased until AFS that were 35 years old. The literature shows that reductions in soil SOC stock occur soon after the conversion of natural forests into simplified systems (Lemma et al., 2006; Rhoades et al., 2000; Fearnside and Barbosa, 1998). However, when AFS are established, a portion of the initial C content may be recovered quickly (Dawoe et al., 2014; Frazão et al., 2014; Nair et al., 2008; Mutuo et al., 2005).

The conversion of natural forests to 30-year-old cacao AFS did not reduce the accumulation of C above a depth of 100 cm. Gama-Rodrigues et al. (2010) studied two 30-year-old cacao AFS ('cabruca' and cacao + *Erythrina*) in southern Bahia and reported no differences in the SOC stocks of these systems and the natural forest until a depth of 100 cm. These results emphasize the soil as a significant reservoir of carbon and reinforce the importance of preserving forests and introducing trees as part of agricultural landscapes, both to increase productivity and obtain environmental services. According to Guo and Gifford (2002) a meta-analysis of five hundred and thirty-seven observations from 74 publications suggested that land use changes from native forest and pasture to crop reduced total C stocks 42% and 59%, respectively, whereas changes from native forest to pasture, crop to pasture, crop to

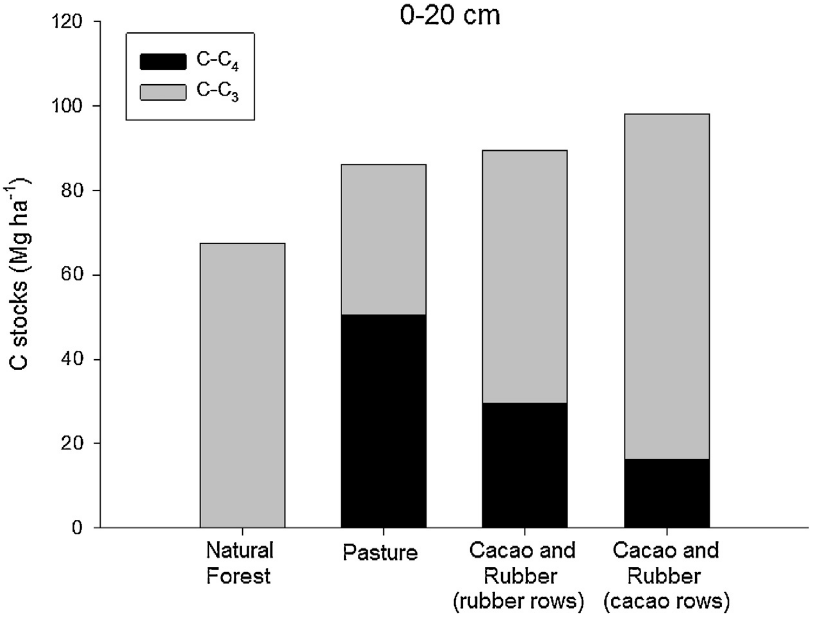

Fig. 4. Whole soil C, divided into C_3 plants (tree)-origin and C_4 plants (pasture)-origin, in depth 0–20 cm of soils of soils under pasture, natural forest and cacao and rubber AFS replacing pasture.

plantation and crop to secondary forest increased total C stocks 8%, 19%, 18% and 53%, respectively. Integration of trees, agricultural crops, and or animals into an agroforestry systems provide a number of ecosystem services (Jose, 2009; Haile et al., 2010; Takimoto et al., 2008; Tonucci et al., 2011). Both trees of native forests and in agroforestry systems would enhance soil C sequestration compared with treeless land-use systems based on the premise that the tree components can be significant sinks of atmospheric C due to their long-term storage of high amounts of C in biomass, especially in the deep root systems (Haile et al., 2010; Rasse et al., 2005). Furthermore, tree-based systems can enhance soil fertility by adding significant amounts of organic matter above and belowground and releasing and recycling nutrients; conserving soil biological diversity also plant species biodiversity and provide habitat for many species of animals and birds, as well (Schroth et al., 2011; Moço et al., 2009). Another important aspect of tree-based systems is the complementarity hypothesis, which states that a larger array of species in a system leads to a broader spectrum of resource utilization making the system more productive and implies that plant species in a mixed system use resources in a complementary way (Nair and Nair, 2014).

The accumulated C in the soil reflects a long-term balance between C absorption and release mechanisms. Plants synthetize CO_2 into biomass and portions of the plant biomass accumulate as organic soil carbon through decomposition. In addition, roots are an important part of the soil C balance for storing large amounts of C. More than one third of the C assimilated by plants during growth becomes soil C through root turnover, root exudates (organic substances), and the deposition of plant residues (Nair et al., 2009a; Montagnini and Nair, 2004). According to Tate et al. (1993), root turnover is the largest carbon source, and the decomposition of dead roots results in the highest input of organic matter into the soil. Hertel et al. (2009) concluded that fine roots were more important for C inputs into the soil in cacao agroforestry systems than in natural forests, especially in 10-year-old cacao agroforestry systems because of a particularly high fine root production in cacao and planted shade tree species.

Cacao, rubber and pasture areas have well-developed root systems. The biomass of fine cacao roots in the upper soil layers can reach up to 2.82 Mg C ha^{-1} (Fontes et al., 2014). These roots have a high turnover rate and are a significant source of soil organic matter (Müller and Gama-Rodrigues, 2012; Ogunkunle and Awotoye, 2011; Muñoz and Beer, 2001). In addition, rubber trees produce an average of 70 kg of root dry matter when the primary and lateral roots are both considered (Cotta et al., 2008). Pastures have greater fine root biomass, longer roots, and a greater root cycling rate than planted forests (i.e., 9.7 Mg dry matter ha^{-1} year^{-1}, with 2.7 Mg C in the top 15 cm of soil) (Guo et al., 2007). Solly et al. (2013) observed higher biomass and quick root turnover in pasture soils than forest soils at a depth of 0–10 cm.

In addition, practices that enable returning large amounts of plant residues to the soil could increase the soil C stock (Tscharntke et al., 2011; Gama-Rodrigues et al., 2010; Haile et al., 2008; Isaac et al., 2005; Montagnini and Nair, 2004). The cacao and rubber systems (4 years of establishment) both used gliricidia (*Gliricidia sepium*) and banana as a cover crop interspersed with cacao and rubber tree, which provides shading to the cacao plants during rubber tree growth (Alvim and Nair, 1986). During this period, banana and gliricidia were pruned, and the residues were left on the ground. Thus, the residues and roots of both plants served as sources of organic matter and resulted in SOC accumulation (Deheuvels et al., 2012; Haggar et al., 1993). The high rate of cacao plant material added to the soil, especially leaves, which may reach approximately 10 Mg ha^{-1} year^{-1} (Müller and Gama-Rodrigues, 2012), of which 2.7 Mg C ha^{-1} year^{-1} forms a thick layer of plant residue on the soil (Fontes et al., 2014) and the rubber tree that adds 2.5 Mg C ha^{-1} year^{-1} (Cotta et al., 2008).

When stratifying the C stock in the soil profile, we observed that the 4-year-old cacao and rubber AFS (Systems 6 and 7) and the pasture system (System 1) favored the accumulation of SOC at a depth of 0–20 cm depth. For example, System 7 resulted in the accumulation of 26.4 Mg C ha^{-1} more SOC than the natural forest (System 2) (i.e., an increase of nearly 40% and a mean accumulation rate of 6.6 Mg C ha^{-1} year^{-1}). The pasture (System 1) resulted in the accumulation of 27% more SOC and System 6 resulted in the accumulation of 20% more SOC than the natural forest. In addition, a mean accumulation rate of 2.9 Mg C ha^{-1} year^{-1} was observed for System 6. The other cacao AFS considered in this study (all over 20 years old) accumulated SOC concentrations that were equal to or less than those in the natural forest soils. These differences, which were observed in the top 20 cm of the soil, explained the highest SOC stock values that were recorded in Systems 1, 6 and 7 (to a depth of 100 cm) relative to the AFS with cacao that were more than 20 years old and the natural forest. Norgrove and Hauser (2013) observed SOC stock values in the soil profile of a 35-year-old AFS (cacao shaded with *Ricinodendron heudelottide*,*Ceiba pentandra*, and other plants) that were similar to the values observed in a secondary forest. In addition, these authors observed greater differences in the SOC stock in the AFS at a depth of 0–10 cm and smaller differences (1.1 Mg ha^{-1}) at a depth of 0–100 cm.

The density of fine roots is higher in surface soils and gradually decreases with soil depth under pasture or forest and agroforestry plantations (Guo et al., 2007). The annual contribution of rhizo deposition to SOC in forest soils at a depth of 0–15 cm was more than twice that at a 15–60 cm depth (Richter et al., 1999). According to Cadima and Alvim (1973) and Gama-Rodrigues and Cadima-Zevallos (1991), an average of 80% of cacao root systems are located at a depth of 0–30 cm, and the rootlets are concentrated at a depth of 0–5 cm (Kummerow et al., 1981). In addition, root longevity in the surface soil is smaller than in the deeper soil. Consequently, the turnover rate is higher in the surface soil layers (Schroth, 1999).

Thus, we suggest that the root systems of these newly established agroforestry systems and the plant residues produced from pruning the gliricidia and banana were significant sources of soil SOC. However, the root system characteristics and the contributions of gliricidia and banana residues were not considered regarding their roles as potential SOC sources in this study. Future research regarding root biomass production and root turnover, and litter deposition from cacao and shade trees as a substantial input to belowground C (including isotopic studies) will be useful to improve our understanding about the origin of the C accumulated in soils (Nair et al., 2009a; Rasse et al., 2005).

The accumulation and subsequent cycling of plant residues, fine roots and the woody materials of shade and cacao trees provide a continuous flow of organic material inputs and result in the return of SOC to the soil surface (Dawoe et al., 2014). Therefore, our results suggest that the natural forest and the original vegetation in these areas remain the source of SOC in the deeper soil layers. This finding could explain the absence of a difference between the cacao AFS, natural forest soils and pasture soils at depths of more than 20 cm.

4.2. Depth variation of $\delta^{13}C$ in the rubber cacao system

The natural forest and pasture had $\delta^{13}C$ values in the surface layers that were similar to the reference values (mean of −28‰ for C_3 and −12‰ for C_4) (Balesdent and Balabane, 1996; Vogel, 1993). The natural forest and pasture system and the cacao and rubber system implemented after pasture had different C assimilation pathways and usually have different $\delta^{13}C$ values. The degree of discrimination against ^{13}C isotopes is lower in C_4 plants than in C_3 plants because the phosphoenolpyruvate carboxylase of the C_4 pathway has a smaller isotope discrimination effect than rubisco in the C_3 pathway (Farquhar et al., 1989). Therefore, the higher $\delta^{13}C$ values found in the pasture soil indicate the accumulation of C–C_4 (Figs. 3 and 4).

The C–C_4 mainly influenced the pasture soils at depths of 0–40 cm, and C_3 plant residues were predominant at depths of more than 40 cm (Fig. 3). In the pasture environment at a depth of 0–20 cm, 59% of the SOC stock was derived from grasses, and 41% was derived from the natural forest that existed before the pasture was established. 30 years after pasture establishment the accumulation of C–C_4 derived from grasses was 50.70 Mg ha^{-1}. In southern Bahia, Tarré et al. (2001) observed that the C–C_4 was slightly influenced at depths of 40–100 cm when *Brachiaria* pastures were established after removing forest vegetation. The authors also observed that C–C_4 reached 44% of the SOC at a depth of 0–5 cm but did not exceed 7% below 40 cm. Most of the C–C_4 that was input by the pasture resulted from rapid cycling of the root system (Lima et al., 2006). In addition, the increase in the variability of soil moisture that resulted from altered inter-rainfall dry intervals increased the root/shoot ratio (Fay et al., 2003). The development of roots in deeper soil layers would be a disadvantage for grasses in regions where rainfall is more evenly distributed (i.e., without very long dry periods), such as in southern Bahia (annual precipitation of 1500 mm). Gama-Rodrigues and Cadima-Zevallos (1991) observed that the fasciculate root systems of grasses are concentrated in the upper 0–20 cm of yellow latosol soils ('tableland') in southern Bahia.

The cacao and rubber plants had different C_3-origin C accumulation abilities. The C_3-origin C in the rubber row increased by 59.9 Mg ha^{-1}, and the C_3-origin C in the cacao row increased by 81.7 Mg ha^{-1} at a depth of 0–20 cm after 4 years of establishment. The C_4–C_3 replacement rate in the rubber and cacao rows were 5.25 Mg ha^{-1} $year^{-1}$ and 8.60 Mg ha^{-1} $year^{-1}$, respectively. Deposition of cacao plant material occurred throughout the year and resulted in a thick layer of litter that provided a favorable environment for grass roots decomposition and leaf residues after conversion (Gama-Rodrigues et al., 2011). In addition, cacao produces high amounts of cellulose-rich and lignin-rich residues that regulate residue decomposition and act as substrates for the formation of organic matter in soils (Fontes et al., 2014). Thus, the organic matter in grasses is slowly compensated by the deposition of litter in the cacao rows, which results in the replacement of C–C_4 by C–C_3 in the surface soil horizons. Litter deposition and root cycling, which are typical in cacao and rubber plantations, are hypothesized to help replace soil organic matter. This hypothesis is confirmed by the C_4:C_3 ratios of the accumulated C in the cacao and rubber system (Fig. 4). Thus, the cacao rows had a lower C_4:C_3 ratio (0.20) than rubber row (0.49), which indicated that the C_3 residue from cacao was more efficient than the rubber residue at replacing C_4-origin C.

Rubber plant was effective in replacing C_4-origin C from pasture, however to a lesser amount than cacao. Studies have indicated that rubber trees produce approximately 5.6 Mg ha^{-1} of litterfall per year in tropical regions (Cheng et al., 2007; Schroth et al., 2002), which is nearly half the amount produced by cacao trees. In addition, the C stock in rubber tree can reach nearly 20 times the C stock in cacao (Cotta et al., 2008), which explains the reduced efficiency of rubber trees for replacing C_4 with C_3. The rubber and cacao AFS is favored by fewer weeds, lower fertilizer use, and other cacao management practices (Alvim and Nair, 1986). The rubber trees in AFS are planted at a low tree density of 400 trees ha^{-1} in double rows. This tree density is lower than that in rubber monoculture, which easily reaches 500 trees ha^{-1} (5 m × 4 m spacing). In rubber monoculture, the accumulation of organic matter is sufficient for providing a larger C_3-origin C stock.

Overall, AFS are known to effectively capture C in the trees (Mutuo et al., 2005). Little is known regarding C sequestration in deep soils, most likely due to the lack of standardization and protocol used to estimate C stocks (Gama-Rodrigues et al., 2011; Nair et al., 2009a,b). The results presented in this study showed few changes in SOC at depths greater than 40 cm in the soils under cacao AFS and also pasture. However, these systems directly affected the soil surface layers, and the influences of the deposited materials of the introduced crops were significant, as previously reported. Over relatively short periods (4 years), a 38% increase in SOC stock was observed in AFS relative to the natural forest. However, the results indicated that the accumulation of SOC with depth was still derived from the natural forest (i.e., from vegetation of natural forests).

5. Conclusions

The 4-year-old cacao and rubber AFS were the most efficient for accumulating SOC in the top 20 cm of the soil and the accumulation of SOC decreased from 20–100 cm. The cacao AFS that were older than 20 years were similar to the natural forest regarding the accumulation of SOC along the soil profile.

The cacao, rubber and pasture systems altered the origin of the accumulated SOC. After 30 years of establishment, 59% of the C_3 carbon from the natural forest was replaced with C_4 in the pasture system. The cacao system was more efficient than the rubber system for replacing the pasture C_4-origin C with C_3-origin C. The rubber tree resulted in a C_3-origin C increase of nearly 70% and the cacao resulted in a C_3 increase of 131%, 4 years after the systems were established. The accumulated SOC is still derived from the natural forest at a depth deeper than 40 cm.

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