

Carbon Storage in Soil Size Fractions Under Two Cacao Agroforestry Systems in Bahia, Brazil

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Abstract Shaded perennial agroforestry systems contain relatively high quantities of soil carbon (C) resulting from continuous deposition of plant residues; however, the extent to which the C is sequestered in soil will depend on the extent of physical protection of soil organic C (SOC). The main objective of this study was to characterize SOC storage in relation to soil fraction-size classes in cacao (*Theobroma cacao* L.) agroforestry systems (AFSs). Two shaded cacao systems and an adjacent natural forest in reddish-yellow Oxisols in Bahia, Brazil were selected. Soil samples were collected from four depth classes to 1 m depth and separated by wet-sieving into three fraction-size classes (>250 μm , 250–53 μm , and <53 μm)—corresponding to macroaggregate, microaggregate, and silt-and-clay size fractions—and analyzed for C content. The total SOC stock did not vary among systems (mean: 302 Mg/ha). On average, 72% of SOC was in macroaggregate-size, 20% in

microaggregate-size, and 8% in silt-and-clay size fractions in soil. Sonication of aggregates showed that occlusion of C in soil aggregates could be a major mechanism of C protection in these soils. Considering the low level of soil disturbances in cacao AFSs, the C contained in the macroaggregate fraction might become stabilized in the soil. The study shows the role of cacao AFSs in mitigating greenhouse gas (GHG) emission through accumulation and retention of high amounts of organic C in the soils and suggests the potential benefit of this environmental service to the nearly 6 million cacao farmers worldwide.

Keywords Cacao cabruca · Carbon sequestration · Ecosystem services · *Erythrina* spp. · Natural forest · Soil aggregates · Soil fractionation

Introduction

Agroforestry, the purposeful growing of trees and crops in interacting combinations on the same unit of land (Nair and others 2008), is recognized as an afforestation/reforestation activity for greenhouse gas (GHG) mitigation under the Kyoto Protocol (Nair and others 2009). Several authors have suggested that, compared with pastures and sole crops, agroforestry systems (AFSs) have higher potential to sequester carbon (C) in soils because of the increased rates of organic matter addition and retention (Kirby and Potvin 2007; Roshetko and others 2002; Sanchez 2000; Sharrow and Ismail 2004). Among the various AFSs, the shaded perennial systems [such as the cultivation of cacao (*Theobroma cacao* L.) and other shade-tolerant perennial species under or among tall-growing shade plants] are reported to have a high potential for sequestering C in soil (Nair and others 2009). This is based on the notion that the

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accumulation and subsequent turnover of leaf litter, roots, and woody material from the shade species as well as cacao provide a continuous stream of addition of organic materials to soil (Montagnini and Nair 2004; Muñoz and Beer 2001; Oelbermann and Voroney 2007). In Brazil, the shaded cacao system extends over more than 0.7 million ha. In the traditional cacao AFSs called “cabruca” of southern Bahia, Brazil, cacao is planted under a thinned natural forest and is shaded by forest trees; their vegetation structure and stratification are similar to those of natural forests. During the past 50 years, a new cacao system has been developed in which the original forest trees are completely eliminated before planting cacao; but the cacao plants are shaded with introduced shade trees, the leguminous *Erythrina* spp. trees being the most common. In this “new” system, the cacao stand density is about two times that of cacao cabruca systems (Müller and Gama-Rodrigues 2007).

Soil organic carbon (SOC) stocks under cacao AFSs are generally high (>150 Mg/ha) in most cacao-growing regions of the world (Fassbender and others 1991; Oelbermann and others 2006; Smiley and Kroschel 2008). This is because of the continuous deposition of plant litter to the soil (on average, 10 Mg/ha/year; Müller and Gama-Rodrigues 2007) and relatively little removal of C from the system via harvested products. Out of the C that is added to the soil, the amount that is sequestered depends on a variety of physical, chemical, and microbial processes (Sollins and others 1996). Depending on environmental conditions and land use, soils might act as a source or sink for C. In order to develop management strategies to increase C sequestration of soils, it is important to understand the mechanisms that control the stabilization and release of C (Marschner and others 2008). Three major factors of soil organic matter (SOM) stabilization have been proposed, but the relative contribution of each to C protection in soils is not well understood (Six and others 2002; Sollins and others 1996). Physical protection of SOM through occlusion within aggregates or small pores and chemical protection through interaction with mineral surfaces or with other organic molecules are considered as important mechanisms to reduce the bioavailability and accessibility of organic matter for soil microorganisms and soil enzymes. Another mechanism of SOC stabilization is the selective preservation of certain recalcitrant organic compounds due to their molecular-level characteristics such as elemental composition and molecular conformation that restricts their decomposition. Some reports indicate, however, that SOM recalcitrance due to composition is relatively less important compared to protection offered physically or through the formation of organo-mineral complexes (Marschner and others 2008; Mikutta and others 2006).

Soil aggregates and size fractions are known to have an important effect on the retention of C in soil (Six and others

2004). Aggregates are secondary particles formed through the combination of mineral particles with organic and inorganic substances (Bronick and Lal 2005). They range in size from microns to millimeters and are often classified according to their ability to resist slaking in water. Depending on their size, they are classified into macroaggregates (2000–250 μm) and microaggregates (250–53 μm). Aggregates physically protect SOM by (1) forming a physical barrier among microorganisms, microbial enzymes, and their substrates, (2) controlling food web interactions, and (3) influencing microbial turnover (Six and others 2000). It has been established that the inclusion of organic materials within soil aggregates reduces their decomposition rate (Elliott and Coleman 1988; Oades 1984). Increases in aggregation concomitant with increases in organic C have been observed in no-till systems (Paus-tian and others 2000; Six and others 2000). Tisdall and Oades (1982) found greater concentrations of organic C in macroaggregates than in microaggregates and suggested that the presence of decomposing roots and hyphae within macroaggregates not only increased C concentrations but also contributed to their stabilization. However, this might not be true for Oxisols, in which oxides are the main binding agents, rather than SOM (Oades and Waters 1991; Zotarelli and others 2005, 2007). Elliot (1986) fractionated SOM into decomposable and recalcitrant fractions on the basis of its location within aggregates of different sizes. Different procedures are available to divide SOM into several fractions based on the degree of physical protection and occlusion within aggregates (Cambardella and Elliot 1994; Golchin and others 1994; Sollins and others 1996; Swanston and others 2002).

In order to understand the C sequestration potential of cacao AFSs, it is important to know the extent of soil C storage in different soil aggregate classes at different soil depths. Furthermore, the deep-rooted nature of cacao and shade trees makes it imperative to extend the study below the surface soil to which most conventional soil studies are targeted. The objective of the research reported here was to determine the extent of soil C storage in different soil aggregate-size fractions and within larger fractions (2000–53 μm) in different soil depths up to 1 m in two types of cacao agroforestry systems in comparison with an adjacent natural forest in Bahia, Brazil.

Study Area

The study was conducted on the Research Station of MARS Center of Cocoa Science, Itajuípe, located in the southern region of Bahia, Brazil. The research farm is located at 14°0' S and 39°2' W, 101 m a.s.l. (above sea level), in a humid tropical climate with a well-distributed

Table 1 Chemical and physical properties at 0–10-cm depth of soils under three land-use systems in Bahia, Brazil

	Natural forest	Cacao cabruca	Cacao + erythrina
pH (H ₂ O)	4.17	4.81	4.27
Total N (g/kg)	2.90	2.82	2.50
P (mg/dm ³)	4.83	5.23	6.63
K (cmol _c /dm ³)	0.16	0.10	0.14
Ca (cmol _c /dm ³)	0.72	2.50	1.47
Mg (cmol _c /dm ³)	0.58	1.37	0.93
Al (cmol _c /dm ³)	1.55	0.38	0.83
Sand (g/kg)	377.20	501.60	395.60
Silt (g/kg)	23.60	26.30	34.00
Clay (g/kg)	599.20	472.10	570.40

mean annual rainfall of 1500 mm and mean annual temperature of 26°C (mean annual range: 13–32°C). The soils are highly weathered reddish-yellow Ferralsols (USDA: Oxisols) with predominance of low-activity clays, such as kaolinite and gibbsite, and iron oxi-hydroxides; they contain no carbonates (Resende and others 1997). The soil characteristics are described in Table 1.

Three land-use systems were used in the study:

1. A 30-year-old stand of a cacao cabruca system: Cacao was planted in partially cleared forestland where some large trees were retained (20 different species of trees) at an average stand density of 70 trees/ha as shade trees for cacao; average number of cacao plants = 550 ha⁻¹.
2. A 30-year-old stand of cacao + erythrina (*Erythrina glauca* Lour.): Erythrina was planted in a 24 × 24-m space with one tree in the center of that space (quincunx planting system) such that the erythrina stand density was 30 plants/ha and cacao was planted in a 3 × 3-m space (1111 plants/ha).
3. An adjacent natural forest with 33 different species of trees.

These land-use systems were maintained in a “natural state” without any special management practices such as fertilizer application or pruning of shade trees.

Methods

Sampling and Physical Fractionation of Soils

Soil pits were dug to a 2-m depth at six randomly chosen sites of ~4 m² area each in each land-use system. Soil samples were collected from each pit from four depth classes (0–10, 10–30, 30–60, and 60–100 cm). These depth classes were chosen in accordance with the protocol used

for a multicountry study at the University of Florida (UF) on soil C sequestration in AFSs (Haile and others 2008; Saha and others 2009; Takimoto and others 2008). A bricklayer’s trowel was inserted into the soil at the lower level of each sampling depth in order to minimize compression and to obtain a sample representing the current state of the soil. Three composite samples were prepared for each depth class of each land-use system by compositing equal amounts of soils from two pits for each replicate (in a total of six pits in each land-use system). Soil samples were air-dried for 24 h in shade and then passed through a 2-mm sieve according to the research procedures used for Brazilian Ferralsols (Madari and others 2005; Sá and others 2001).

Fraction-size separation was done according to the procedure of Elliot (1986) adopted in the earlier-mentioned UF studies. The procedure consisted of submerging 100 g of the composite soil sample 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 50 times 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) to obtain three aggregate size classes: macro (2000–250 μm), micro (250–53 μm), and silt + clay-sized fraction (<53 μm) resulting in 108 (4 depths, 3 land-use systems, 3 size fractions, and 3 replications) fractionated samples. The fraction remaining on the top of the 250-μm sieve was collected in a hard plastic pan. Water plus soil <250 μm were poured through the 53-μm sieve and the same sieving procedure was repeated. All size fraction classes were air-dried at 65°C and the percent weight of each fraction was calculated. The overall average recovery mass percentage of soil fractions after the wet-sieving procedure was about 98% of the initial soil mass. The mean weight diameter (MWD) was determined according to the procedure recommended by Embrapa (1997): $MWD = \sum(\text{weight \% sample on sieve} \times \text{mean intersieve size}/100)$, where the upper and lower limits were 2000 and 0 μm, respectively.

Sonication

The purpose of sonication was to quantify the amount of carbon occluded within aggregates in soils under cacao AFSs. The procedure consisted of disrupting the aggregates completely and separating the organic matter inside the aggregates (released after aggregate disruption) into two fractions (2000–250 and 250–53 μm) in a water–soil system; the organic matter remaining on the sieve (nonoccluded particulate organic matter) after sonication was not analyzed. Five grams of the larger size fraction (2000–250 μm) or 3 g of the smaller fraction (250–53 μm) of soil

were weighed into a 250-mL beaker, to which 100 mL of distilled water was added. The suspension was sonicated at 454 J/mL (Freixo and others 2002a; Macedo and others 2008) by using an amplitude of 65% and 15-min combinations with a Sonic Dismembrator (model 500; Fisher Scientific, Hampton, New Hampshire). The temperature was kept below 35°C during sonication to avoid reduction in cavitation (Roscoe and others 2000). The depth of immersion of the sonicator probe was kept constant at 10 mm. The suspension was then passed through the same-sized sieve used to obtain the size fractions (e.g., 250- μm sieve for the 250–2000- μm fraction and 53- μm sieve for the 250–53- μm fraction). The SOM passing through the sieve was termed aggregate organic matter released after aggregate disruption (Sarkhot and others 2007).

Chemical and Physical Analyses

Soil samples from 0 to 10 cm depth passed through a 2 mm sieve were analyzed for pH in water, available P and K^+ (Mehlich-1), Ca^{2+} , Mg^{2+} and Al^{3+} (1 mol L^{-1} KCl), total N (kjeldahl) and granulometry by pipette method (Embrapa 1997). Soil bulk density for each depth interval was measured by the volumetric ring method (Embrapa 1997). For calculating the dry bulk density, the initial weight of soil core from each layer was measured in the laboratory after collection, and soil moisture was determined by oven-drying a 30-g subsample at 105°C for 48 h. Rock volume was negligible; therefore, no adjustment was made for that. The bulk density of each soil depth interval was used to calculate the amount of SOC stored to 1 m depth and expressed as Mg ha^{-1} (assuming 1 m thickness).

For chemical analysis, whole and oven-dried fractionated soils were ground to fine powder using a QM-3A High Speed Vibration Ball Mill (MTI Corp., Richmond, California) for 10 min. Total SOC in whole and fractionated soil samples as well as inside the aggregate were determined by dry combustion using an automated Leco CHNS/O analyzer at the University of Florida. The C storage in fraction-size classes and also inside the aggregates were calculated by multiplying the C concentration (g/kg soil in aggregate size, and those obtained from the aggregates after sonication) with a bulk density of depth interval (kg/m^3) and the percent weight of the fraction.

Statistical Analyses

The data were analyzed by analysis of variance (ANOVA) as a completely randomized design with three replicates. The three composite samples were treated as replicates considering the land-use systems, each of which was more than 1000 m^2 in area, as fixed-effect treatments according to the procedure of Lugo and others (1990). Tukey's

Studentized range test was used to compare the mean differences between land-use systems on SOC in whole soil, macro-sized, micro-sized, and silt-and-clay-sized fractions at three sites. Statistical analyses were performed with SAS (SAS Institute, 2004), separately for all depth classes, and differences were considered significant at $P < 0.05$.

Results

Soil organic carbon stock of cacao AFSs was not statistically different from that of a natural forest in the entire 0–100-cm soil layer (Fig. 1) and the mean SOC stock in these soils was 302 Mg/ha . Cacao cabruca had significantly higher SOC stock than the other two land-use systems in the 0–30-cm soil layer. In the 30–60-cm soil layer, the cacao + erythrina system had ~30% higher SOC than the

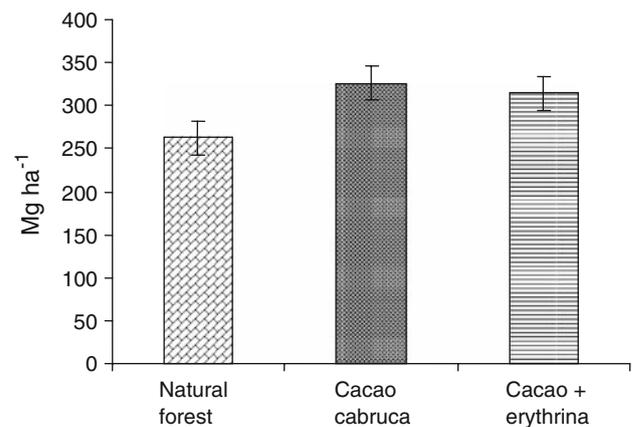


Fig. 1 SOC storage in the 0–100-cm soil layer in forest, cacao cabruca, and cacao + erythrina land-use systems in Bahia, Brazil. There were no statistical differences among the land-use systems

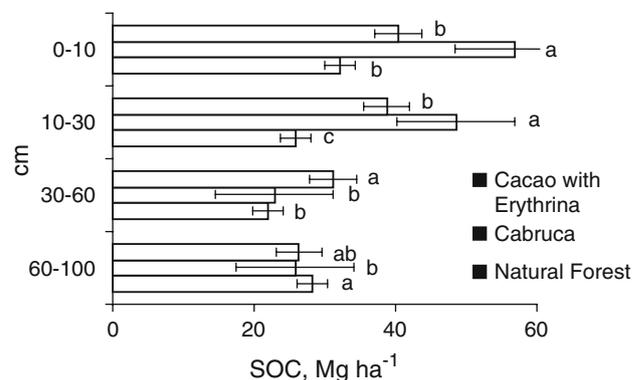


Fig. 2 SOC storage at different depths in three land-use systems in Bahia, Brazil. Values followed by the same letter(s) within each depth are not significantly different according to the Tukey test ($P = 0.05$)

Table 2 Depthwise distribution of different soil size fractions and their SOC content under three land-use systems in Bahia, Brazil

Depth (cm) and Bulk density (g cm ³)	Size fraction (μm)	Mass of soil fractions (%) ^a			SOC (Mg/ha to 1 m depth)		
		Natural forest	Cacao cabruca	Cacao + erythrina	Natural forest	Cacao cabruca	Cacao + erythrina
0–10	2000–250	76	77	80	29.5 b‡	54.5 a	33.7 b
	250–53	16	15	13	6.0 a	9.5 a	5.5 a
	<53	3.6	5.4	4.4	1.6 b	4.4 a	2.2 ab
Bulk density		0.684	1.198	0.746			
10–30	2000–250	74	72	72	23.0 b	43.0 a	36.6 a
	250–53	17	20	18	5.5 c	11.5 a	9.1 b
	<53	6.1	6.6	6.0	2.1 b	4.4 a	3.3 ab
Bulk density		0.690	1.343	1.130			
30–60	2000–250	70	68	70	21.0 b	21.0 b	2.82 a
	250–53	21	22	21	6.6 a	6.9 a	8.5 a
	<53	6.7	7.9	7.1	2.1 a	2.6 a	3.0 a
Bulk density		0.730	0.767	0.992			
60–100	2000–250	70	69	72	28.3 a	24.1 b	25.3 ab
	250–53	21	22	20	8.6 a	7.9 a	7.2 a
	<53	7.2	7.7	7.3	3.0 a	2.8 a	2.7 a
Bulk density		1.015	0.914	0.906			

Note: Values followed by the same letter(s) within size fraction and within each depth are not significantly different according to the Tukey test ($P = 0.05$)

^a No statistical difference among land-use systems within each size fraction and depth

cacao cabruca and natural forest systems. The natural forest had significantly higher C stock than cacao cabruca only in the 60–100-cm soil layer (Fig. 2).

The distribution of fraction-size classes did not vary between depths or land-use systems and the MWDs for the three land-use systems were 847 ± 36 μm. On average, in these soils, 73% were macroaggregate-size (2000–250 μm), 18% were microaggregate-size (250–53 μm), and 7% were silt-and-clay-size fractions (<53 μm). The cacao cabruca system had significantly higher C storage (Mg/ha) in the largest size fraction at the 0–10-cm depth compared to other two land-use systems; at 10–30-cm depths, both cacao AFSs (cacao cabruca and cacao + erythrina) had higher C storage in macro-size fraction than under natural forest (Table 2). In the 30–60-cm depths, the cacao + erythrina system had significantly more SOC storage than cacao cabruca and natural forest. At a soil depth below 60 cm, the three systems were not different in terms of SOC in the macro-size fraction (Table 2). The C accumulated in the micro-size fraction did not show any differences among the land-use systems at 0–10 cm and below 30 cm. In the 10–30-cm layer, the SOC accumulated was in the order cacao cabruca > cacao + erythrina > natural forest (Table 2). The C storage in the silt-and-clay-size fraction above 30 cm was almost 50% greater under the two cacao AFSs than under natural forest; below 30 cm

depth, however, the SOC values did not show any significant difference among land-use systems (Table 2).

The C content in soil aggregates of macro-size fraction (2000–250 μm) declined with increase in soil depth in all land-use systems. The mean values were 30.9, 22.2, and 18.7 g/kg at 0–10, 10–30, and 30–100 cm, respectively. For the micro-size (250–53 μm) fraction, the C content in soil aggregates did not show any differences among soil depths and the mean value across the three land-use systems was 2.91 g/kg (Table 3). The larger aggregate size accumulated a greater amount (g/kg) of fine aggregate occluded C than the smaller (micro-size) fraction. Overall, in the 0–100-cm layer, under natural forest and cacao + erythrina, the macro- and micro-size fractions accumulated 70% and 37% of total C in the aggregates, respectively; under the cabruca system, the larger fraction accounted for 66% and the smaller fraction accounted for 32% of fine aggregate occluded C (Table 3).

Discussion

The high amount of soil C stock in cacao AFSs (in excess of 300 Mg/ha) is likely a consequence of the deposition of high amounts of plant litter. The amount of such deposits will vary according to local climatic and management

Table 3 SOC content in soil size-fractions and aggregates at different depths under three land-use systems in Bahia, Brazil

Sites	Depth (cm)	Size fraction (μm)	C content in soil size fraction (g/kg soil)	C content in soil aggregates		
				(g/kg soil) ^a	% ^b	
Natural forest	0–0	2000–250	43.10	30.22	70	
		250–53	8.80	3.16	36	
	10–30	2000–250	33.42	24.37	73	
		250–53	7.96	2.79	35	
	30–60	2000–250	28.47	18.96	67	
		250–53	8.99	3.47	39	
	60–100	2000–250	27.87	18.55	67	
		250–53	8.48	3.05	36	
	Cacao cabruca	0–10	2000–250	45.54	29.60	65
			250–53	7.88	2.62	33
10–30		2000–250	31.94	20.30	64	
		250–53	8.55	2.90	34	
30–60		2000–250	27.24	17.98	66	
		250–53	8.97	2.83	32	
60–100		2000–250	26.42	17.82	67	
		250–53	8.57	2.40	28	
Cacao + erythrina		0–10	2000–250	45.29	33.00	73
			250–53	7.32	2.64	36
	10–30	2000–250	32.26	22.05	68	
		250–53	8.11	3.17	39	
	30–60	2000–250	28.46	19.55	69	
		250–53	8.55	3.22	38	
	60–100	2000–250	27.90	19.00	68	
		250–53	7.86	2.70	34	

^a No statistical difference among land-use systems at any depth

^b Mean of fine C occluded within aggregates for each size fraction

conditions; but 10 Mg/ha/year of plant litter, mainly cacao leaf fall, seems to be an average figure (Müller and Gama-Rodrigues 2007). In addition, up to 90% of root hair (Kummerow and others 1981) and 60% of fine root (Kummerow and others 1982; Muñoz and Beer 2001) are found in the top 15 cm of soil, and up to 80% of lateral roots (>5–10 mm) occur in the 0–60-cm depth (Gama-Rodrigues and Cadima-Zevallos 1991). With an average yield of 780 kg/ha/year cacao beans (Müller and Gama-Rodrigues 2007), the removal of C from the system via harvested product is comparatively low. Compared with clean-cultivated agricultural systems, other avenues of C losses such as via soil erosion are also comparatively low in cacao AFSs because of the soil cover offered by the presence of thick layers of dead and decaying leaf litter throughout the year. The SOC stock (0–100-cm soil depth) in cacao + gliricidia [*Gliricidia sepium* (Jacq.) Walp.] AFS in Indonesia amounted to 155 Mg C/ha (Smiley and Kroschel 2008) and SOC stock (0–45-cm depth) in cacao + erythrina [*Erythrina poeppigiana* (Walp.) Cook] in Costa Rica was 240 Mg/ha (Fassbender and others 1991). Under comparable conditions, the SOC storage in other land-use systems are lower than in cacao AFSs: A

Brazilian multistrata AFS had 113 Mg C/ha in the 0–200-cm soil depth (Schroth and others 2002), whereas the tropical forest systems of Bahia had 88–116 Mg C/ha in the 0–50-cm soil layer (Gama-Rodrigues and others 2008).

Overall, the distribution of C in the different soil layers, especially below 30 cm, was uniform in all three land-use systems (in the 0–30-cm soil layer, the cacao cabruca system had higher amounts of C than the other two systems). It needs to be noted, however, that the bulk density (BD) of surface soils (up to 30 cm depth) was higher in both cacao systems compared to the forest soils, whereas at the lower (60–100 cm) depths, the situation was reversed, with the forest soil having more BD than the cacao soils (Table 2). We did not investigate the reasons for the BD differences with depth. Considering these variations in BD values, the C data are presented in grams per kilogram of soil (Table 3); the data are also reported in megagrams per hectare for comparison with other reported data in the literature. The relatively minor differences among the systems in the amounts of C stored in different soil layers (Figs. 1 and 2) possibly reflect the differences in plant litter quality (Nair and others 1999). We did not study litter quality in these systems. It is well known, however, that the

rate of litter decomposition and therefore the amount of SOC content are dependent on plant litter quality, represented mainly by the content of lignin and polyphenols, and the nature and amount of root exudates (Palm and others 2004; Sá and Lal 2009).

The distribution of size fraction classes did not vary among soil depth classes and land-use systems. Macro-size fraction was the most abundant fraction, with a high amount of C in these soils in the 0–100-cm layer, followed by micro-size fraction and silt-and-clay-size fraction (Table 2). High C content in the largest aggregate class in this study were not in accordance with previous reports such as Zotarelli and others (2005, 2007) and Oades and Waters (1991), who found no differences in C and N content across aggregate-size fractions in Oxisols under crop and pasture systems. Probably, the constant addition of organic materials via litter fall and the presence of leguminous plant roots (Haynes and Beare 1997), coupled with the absence of tillage and use of machinery (no-till) in cacao AFSs, help maintain the binding effect and increase the number of water-stable macroaggregates. The fractionation of slaking-resistant aggregates of different sizes by wet-sieving and subsequent use of ultrasonic energy and the aggregate disruption allowed us to determine the amount of fine aggregate occluded C. Carbon associated with macroaggregates constituted the major share of C in this study. Overall, about 70% of SOC in these land-use systems were located inside the macroaggregates at all soil depths (Table 3). The formation and stabilization of macroaggregates play an important role in the protection and subsequent accumulation of SOC (Liao and others 2006). In land-use systems with no tillage, a slow macroaggregate turnover allows time for the formation of fine occluded particulate organic matter that gradually becomes encrusted with clay particles and microbial products to form microaggregates (containing young crop-derived C) within macroaggregates (Six and others 1998, 1999, 2000).

The occluded C that is physically protected within soil aggregates represents a relatively more stable pool of C, but not strongly associated to soil particles (Christensen, 1992; John and others 2005). Freixo and others (2002b) observed that occluded C ranged from 0.2 to 1.1 g/kg soil under forest and from 0.1 to 0.2 g/kg soil under no tillage and conventional tillage crop rotations, including wheat (*Triticum* sp.), soybean (*Glycine max* L.), hairy vetch (*Vicia sativa* L.), and maize (*Zea mays* L.) in a Brazilian Rhodic Ferrasol (Oxisol). In a forested Podsol (USDA: Spodosol) in northern Florida (USA). Sarkhot and others (2007) found, using a sonication method, that, on average, 45% of the total SOC was contained in macroaggregates and microaggregates. Although the methods used in those studies (density fractionation or sonication) were different from those of our study, the results show that the

proportion of soil C occluded in aggregates in the systems we studied was considerably higher than that of other systems reported in the literature. The constant replacement of the organic materials, as in these land-use systems, maintains the binding effect and increases the number of water-stable macroaggregates (John and others 2005). Furthermore, previous studies have reported that the high concentration of fine roots at the surface soil to 15 cm depth (Muñoz and Beer 2001) and lignified coarse roots at the subsurface soils to 100 cm (Gama-Rodrigues and Cadima-Zevallos 1991) contribute substantially to below-ground C stocks in cacao AFS. The presence of leguminous plant roots also promotes soil aggregation (Haynes and Beare 1997). Additionally, the cacao AFS is a no-till system that facilitates the maintenance of a high SOM level and macroaggregate formation due the continuous input of organic materials to the soil via litterfall (Isaac and others 2005; Müller and Gama-Rodrigues 2007) or sloughed-off roots (Gama-Rodrigues and Cadima-Zevallos 1991; Kummerow and others 1982). The location of organic matter within the aggregate is a key factor for the stabilization and storage of SOM (Six and others 2004). The protective effect of clay on SOM involves the interaction of SOM with the surface of the clay particles (cation bridges, hydrogen bonds, electrostatic and van der Waals interactions) and the occlusion of organic material in the matrix of soil aggregates (Hassink and Whitmore 1997). The silt-and-clay-protected C pool depends on the silt and clay proportions in soils; the 1:1 clay-mineral-dominated soils, such as Oxisols, have a low level of silt-and-clay-protected C pool (Six and others 2002). Therefore, clay content alone is not necessarily an appropriate measure for the protection of C in these soils. Oxisols do not show the same aggregate hierarchy as other soils. Oades and Waters (1991) reported that “aggregate hierarchy occurs in Alfisols and Mollisols because organic materials are the dominant stabilizing agents in larger aggregates but in the Oxisol oxides are dominant stabilizing agents and prevent the expression of aggregate hierarchy caused by organic materials.” Results from our study suggest that occlusion of C in the soil aggregates can be a major mechanism of C protection in these soils. A strong correlation between organic C and aggregate size in our study suggests that very high levels of organic matter could lead to a change in the dominant binding agents of these aggregates from oxides to organic molecules in these soils.

As suggested by Bronick and Lal (2005), the development of SOM conservation practices in agricultural systems such as no tillage, incorporation of trees, and continuous input of organic matter are important to improve soil aggregation and, consequently, the accumulation of soil C in highly weathered soil in the humid tropics. Barring minor differences among the three systems in

terms of the relative amounts of SOC stored in different soil layers, all three systems stored substantial amounts of SOC in the soil up to 1 m depth (Figs. 1 and 2). Although this is to be expected in undisturbed natural forest systems, the fact that such high amounts (in excess of 300 Mg/ha) of soil C stored in the two managed agroecosystems (cacao systems) is quite important in the context of mitigation of atmospheric CO₂.

In Brazil, deforestation and forest burning account for about 75% of the greenhouse gas (GHG) emission and fossil fuel use accounts for the remaining (Lal and others 2006). In that scenario, the cacao AFS plays an important role as a land-use system that allows mitigation of GHG emission and helps reduce deforestation and restore degraded soils. Considering that nearly 85% of world production of cacao is from small-holder agroforestry systems (Shapiro and Rosenquist 2004), trading of soil C could be of considerable economic advantage to these farmers. Furthermore, traditional cacao AFSs with diverse and structurally complex shade canopies are among the agricultural land uses that are most likely to conserve a significant portion of the original forest biodiversity (Schroth and Harvey 2007).

Conclusions

This study indicates that cacao AFSs are comparable to natural forests in accumulating high amounts of SOC. Seventy percent of C in the cacao AFS was fine C occluded within aggregates, a physically protected form of C. The vast majority of SOC was present in macroaggregate fractions throughout the soil layers up to 1 m depth. Although SOC inside the macroaggregates is more subjected to disturbance than that in microaggregates and silt-and-clay fractions, the extent of such disturbances is low in cacao systems; therefore, C contained in this fraction can be expected to become more stabilized in the system over time. Thus, the cacao AFSs seem to play an important role in environmental protection by mitigating GHG emission through the storage of high amounts of well-protected organic carbon in the soils. Trading of soil carbon could offer an economic incentive to the small-holder cacao farmers.

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References

- Bronick CJ, Lal R (2005) Soil structure and management: a review. *Geoderma* 124:3–22
- Cambardella CA, Elliot ET (1994) Carbon and nitrogen dynamics of soil organic matter fractions from cultivated grassland soils. *Soil Science Society of America Journal* 58:123–130
- Christensen BT (1992) Physical fractionation of soil and organic matter in primary particle size and density separates. *Advances in Soil Sciences* 20:1–90
- Elliot ET (1986) Aggregate structure and carbon, nitrogen and phosphorus in native and cultivated soils. *Soil Science Society of America Journal* 50:627–633
- Elliott ET, Coleman DC (1988) Let the soil work for us. *Ecological Bulletins* 39:23–32
- Embrapa. Serviço Nacional de Levantamento e Conservação de Solos (1997) Manual de métodos de análise de solos, 2nd ed. rev. atual. SNLCS, Rio de Janeiro, 412 pp
- Fassbender HW, Beer J, Heuvelod J, Imbach A, Enriquez G, Bonnemann A (1991) Ten year balances of organic matter and nutrients in agroforestry systems at CATIE, Costa Rica. *Forest Ecology Management* 4:173–183
- Freixo AA, Machado PLOA, Guimarães CM, Silva CA, Fadigas FS (2002a) Estoques de carbono e nitrogênio e distribuição de frações orgânicas de latossolo do cerrado sob diferentes sistemas de cultivo. *Revista brasileira de ciência do solo* 26:425–464
- Freixo AA, Machado PLOA, Santos HP, Silva CA, Fadigas FS (2002b) Soil organic carbon and fractions of a Rhodic Ferralsol under the influence of tillage and crop rotation systems in southern Brazil. *Soil Tillage Research* 64:221–230
- Gama-Rodrigues AC, Cadima-Zevallos A (1991) Efectos de fertilización sobre sistema radicular de cacao en suelos de “tabuleiros” del sur de Bahia, Brasil. *Turrialba* 41:135–141
- Gama-Rodrigues AC, Gama-Rodrigues EF, Barros NF (2008) Balanço de carbono e nutrientes em plantio puro e misto de espécies florestais nativas no sudeste da Bahia. *Revista brasileira de ciência do solo* 32:1165–1179
- Golchin A, Oades JM, Skjemstad JO, Clarke P (1994) Soil structure and carbon cycling. *Australian Journal of Soil Research* 32:1043–1068
- Haile SG, Nair PKR, Nair VD (2008) Carbon storage of different soil-size fractions in Florida silvopastoral systems. *Journal of Environmental Quality* 37:1789–1797
- Hassink J, Whitmore AP (1997) A model of the physical protection of organic matter in soils. *Soil Science Society of America Journal* 61:131–139
- Haynes RJ, Beare MH (1997) Influence of six crop species on aggregate stability and some labile organic matter fractions. *Soil Biology & Biochemistry* 29:1647–1653
- Isaac ME, Gordon AM, Thevathasan N, Oppong SK, Quashie-Sam J (2005) Temporal changes in soil carbon and nitrogen in west African multistrata agroforestry systems: a chronosequence of pools and fluxes. *Agroforestry Systems* 65:23–31
- John B, Yamashita T, Ludwig B, Flessa H (2005) Storage of organic carbon in aggregate and density fractions of silty soils in different types of land use. *Geoderma* 128:63–79
- Kirby KR, Potvin C (2007) Variation in carbon storage among tree species: implications for the management of a small-scale carbon sink project. *Forest Ecology Management* 246:208–221

- Kummerow J, Kummerow M, Alvim PT (1981) Root biomass in mature cacao (*Theobroma cacao* L.) plantation. *Revista Theobroma* 11:77–85
- Kummerow J, Kummerow M, Silva WS (1982) Fine-root growth dynamics in cacao (*Theobroma cacao*). *Plant Soil* 65:193–201
- Lal R, Cerri CC, Bernoux M, Etchevers J, Cerri E (eds) (2006) Carbon sequestration in soils of Latin America. Food Products Press, New York
- Liao JD, Boutton TW, Jastrow JD (2006) Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland. *Soil Biology & Biochemistry* 38:3184–3196
- Lugo AE, Cuevas E, Sanchez MJ (1990) Nutrients and mass in litter and soil of ten tropical tree plantations. *Plant Soil* 125:263–280
- Macedo MO, Resende AS, Garcia PC, Boddey RM, Jantalia CP, Urquiaga S, Campello EFC, Franco AA (2008) Changes in soil C and N stocks and nutrient dynamics 13 years after recovery of degraded land using leguminous nitrogen-fixing trees. *Forest Ecology Management* 255:1516–1524
- Madari B, Machado PLOA, Torres E, Andrade AG, Valencia LIO (2005) The effect of no-tillage and crop rotations on soil aggregation and organic carbon accumulation in a Rhodic Ferrasol from Southern Brazil. *Soil Tillage Research* 80:185–200
- Marschner B, Brodowski S, Dreves A, Gleixner G, Gude A, Grootes PM, Hamer U, Heim A, Jandl G, Ji R, Kaiser K, Kalbitz K, Kramer C, Leinweber P, Rethemeyer J, Schäffer A, Schmidt MWI, Schwark L, Wiesenberger GLB (2008) How relevant is recalcitrance for the stabilization of organic matter in soils? *Journal of Plant Nutrition and Soil Science* 171:91–110
- Mikutta R, Kleber M, Torn MS, Jahn R (2006) Stabilization of soil organic matter: association with minerals or chemical recalcitrance? *Biogeochemistry* 77:25–56
- Montagnini F, Nair PKR (2004) Carbon sequestration: an under-exploited environmental benefit of agroforestry systems. *Agroforestry Systems* 61:281–295
- Müller MW, Gama-Rodrigues AC (2007) Sistemas Agroflorestais com cacauero. In: Valle RR (ed) *Ciência, tecnologia e manejo do cacauero*. CEPLAC, Ilhéus, BA, Brazil, pp 246–271
- Muñoz F, Beer J (2001) Fine root dynamics of shaded cacao plantations in Costa Rica. *Agroforestry Systems* 51:119–130
- Nair PKR, Buresh RJ, Mugendi DN, Latt CR (1999) Nutrient cycling in tropical agroforestry systems: myths and science. In: Buck LE, Lassoie JP, Fernandes ECM (eds) *Agroforestry in sustainable agricultural systems*. CRC Press, Boca Raton, FL, pp 1–31
- Nair PKR, Gordon AM, Mosquera-Losada M-R (2008) Agroforestry. In: Jorgensen SE, Fath BD (eds) *Encyclopedia of ecology*, vol 1. Elsevier, Oxford, pp 101–110
- Nair PKR, Kumar BM, Nair VD (2009) Agroforestry as a strategy for carbon sequestration. *Journal of Plant Nutrition and Soil Science* 172:10–23
- Oades JM (1984) Soil organic matter and structural stability: mechanisms and implications for management. *Plant Soil* 76:319–337
- Oades JM, Waters AG (1991) Aggregate hierarchy in soils. *Australian Journal of Soil Research* 29:815–828
- Oelbermann M, Voroney RP (2007) Carbon and nitrogen in a temperate agroforestry system: using stable isotopes as a tool to understand soil dynamics. *Ecology and Engineering* 29:342–349
- Oelbermann M, Voroney RP, Naresh V, Thevathasan NV, Gordon AM, Kass DCL, Schlönvoigt AM (2006) Carbon dynamics and residue stabilization in a Costa Rican and southern Canadian alley cropping system. *Agroforestry Systems* 68:27–36
- Palm CA, Tomich T, Van Noordwijk M, Vosti S, Gockowski J, Alegre J, Verchot L (2004) Mitigating GHG emissions in the humid tropics: case studies from the Alternatives to Slash-and-Burn Program (ASB). *Environment, Development and Sustainability* 6:145–162
- Paustian K, Six J, Elliott ET, Hunt HW (2000) Management options for reducing CO₂ emissions from agricultural soils. *Biogeochemistry* 48:147–163
- Resende M, Curi N, Rezende SB, Correa GF (1997) *Pedologia: base para a descrição de ambientes*, 2nd ed. NEPUT, Viçosa, 367 pp
- Roscoe R, Buurman P, Velthorst EJ (2000) Disruption of soil aggregates by varied amounts of ultrasonic energy in fractionation of organic matter of a clay Latosol: carbon, nitrogen and δ^{13} distribution in particle-size fractions. *European Journal of Soil Science* 51:445–454
- Roshetko M, Delaney M, Hairiah K, Purnomosidhi P (2002) Carbon stocks in Indonesian homegarden systems: can smallholder systems be targeted for increased carbon storage? *American Journal of Alternative Agriculture* 17:125–137
- Sá JCM, Lal R (2009) Stratification ratio of soil organic matter pools as an indicator of carbon sequestration in a tillage chronosequence in a Brazilian Oxisol. *Soil Tillage Research* 103:46–56
- Sá JCM, Cerri CC, Dick WA, Lal R, Vesnke-Filho SP, Piccolo MC, Feigl BE (2001) Organic matter dynamics and carbon sequestration rates for a tillage chronosequence in a Brazilian Oxisol. *Soil Science Society of America Journal* 65:1486–1499
- Saha S, Nair PKR, Nair VD, Kumar BM (2009) Soil carbon stock in relation to plant diversity of homegardens in Kerala, India. *Agroforestry Systems* 76:53–65
- Sanchez PA (2000) Linking climate change research with food security and poverty reduction in the tropics. *Agriculture, Ecosystems & Environment* 82:371–383
- Sarkhot D, Comerford NB, Jokela EJ, Reeves III JB, Harris WG (2007) Aggregation and aggregates carbon in a forested southeastern Coastal Plain Spodosol. *Soil Science Society of America Journal* 71:1779–1787
- SAS Institute (2004) SAS user's guide: Statistics SAS/C OnlineDoc, Release 7.50. SAS Institute, Cary, NC
- Schroth G, Harvey CA (2007) Biodiversity conservation in cocoa production landscapes: an overview. *Biodiversity Conservation* 16:2237–2244
- Schroth G, D'Angelo SA, Teixeira WG, Haag D, Lieberei R (2002) Conversion of secondary forest into agroforestry and monoculture plantations in Amazonia: consequences for biomass, litter and soil carbon stocks after 7 years. *Forest Ecology Management* 163:131–150
- Shapiro H-Y, Rosenquist EM (2004) Public/private partnerships in agroforestry: the example of working together to improve cocoa sustainability. *Agroforestry Systems* 61:453–462
- Sharrow SH, Ismail S (2004) Carbon and nitrogen storage in agroforests, tree plantations, and pastures in western Oregon, USA. *Agroforestry Systems* 60:123–130
- Six J, Elliot ET, Paustian K, Doran JW (1998) Aggregation and soil organic matter accumulation in cultivated and native grassland soils. *Soil Science Society of America Journal* 63:1350–1358
- Six J, Elliot ET, Paustian K (1999) Aggregate and soil organic matter dynamics under conventional and no tillage systems. *Soil Science Society of America Journal* 63:1350–1358
- Six J, Elliot ET, Paustian K (2000) Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biology & Biochemistry* 32:2099–2103
- Six J, Conant RT, Paul EA, Paustian K (2002) Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. *Plant Soil* 241:155–176
- Six J, Bossuyt H, Degryze S, Denef K (2004) A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil Tillage Research* 79:7–31

- Smiley GL, Kroschel J (2008) Temporal change in carbon stocks of cocoa–gliricidia agroforests in Central Sulawesi, Indonesia. *Agroforestry Systems* 73:219–231
- Sollins P, Homann P, Caldwell BA (1996) Stabilization and destabilization of soil organic matter: mechanisms and controls. *Geoderma* 74:65–105
- Swanston CW, Caldwell BA, Homann PS, Ganio L, Sollins P (2002) Carbon dynamics during a long-term incubation of separate and recombined density fractions from seven forest soils. *Soil Biology & Biochemistry* 34:1121–1130
- Takimoto A, Nair PKR, Nair VD (2008) Carbon stock and sequestration potential of traditional and improved agroforestry systems in the West African Sahel. *Agriculture, Ecosystems & Environment* 125:159–166
- Tisdall JM, Oades JM (1982) Organic matter and water-stable aggregates in soils. *Journal of Soil Science* 33:141–163
- Zotarelli L, Alves BJR, Urquiaga S, Torres E, Santos HP, Paustian K, Boddey RM, Six J (2005) Impact of tillage and crop rotation on aggregate-associated carbon in two Oxisols. *Soil Tillage Research* 95:196–206
- Zotarelli L, Alves BJR, Urquiaga S, Boddey RM, Six J (2007) Impact of tillage and crop rotation on light fraction and intra-aggregate soil organic matter in two Oxisols. *Soil Science Society of America Journal* 69:482–491