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C and N Content in Density Fractions of Whole Soil and Soil Size Fraction Under Cacao Agroforestry Systems and Natural Forest in Bahia, Brazil

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Abstract Agroforestry systems (AFSs) have an important role in capturing above and below ground soil carbon and play a dominant role in mitigation of atmospheric CO_2. Attempts has been made here to identify soil organic matter fractions in the cacao-AFSs that have different susceptibility to microbial decomposition and further represent the basis of understanding soil C dynamics. The objective of this study was to characterize the organic matter density fractions and soil size fractions in soils of two types of cacao agroforestry systems and to compare with an adjacent natural forest in Bahia, Brazil. The land-use systems studied were: (1) a 30-year-old stand of natural forest with cacao (cacao cabruca), (2) a 30-year-old stand of cacao with *Erythrina glauca* as shade trees (cacao + erythrina), and (3) an adjacent natural forest without cacao. Soil samples were collected from 0-10 cm depth layer in reddish-yellow Oxisols. Soil samples was separated by wet sieving into five fraction-size classes (>2000 μm, 1000–2000 μm, 250–1000 μm, 53–250 μm, and <53 μm). C and N accumulated in to the light (free- and intra-aggregate density fractions) and heavy fractions of whole soil and soil size fraction were determined. Soil size fraction obtained in cacao AFS soils consisted mainly (65 %) of mega-aggregates (>2000 μm) mixed with macroaggregates (32–34%), and microaggregates (1–1.3%). Soil organic carbon (SOC) and total N content increased with increasing soil size fraction in all land-use systems. Organic C-to-total N ratio was higher in the macroaggregate than in the microaggregate. In general, in natural forest and cacao cabruca the contribution of C and N in the light and heavy fractions was similar. However, in cacao + erythrina the heavy fraction was the most common and contributed 67% of C and 63% of N. Finding of this study shows that the majority of C and N in all three systems studied are found in macroaggregates, particularly in the 250–1000 μm size aggregate class. The heavy fraction was the most common organic matter fraction in these soils. Thus, in mature cacao AFS on highly weathered soils the main mechanisms of C stabilization could be the physical protection within macroaggregate structures thereby minimizing the impact of conversion of forest to cacao AFS.

Keywords Aggregate protection · Soil carbon stabilization · Soil fractionation · Free light fraction · Intra-aggregate light fraction · Heavy fraction

Introduction

Interest in studying organic C in soils has increased recently because of its well-known beneficial effect on dynamics of nutrient and soil structure as well as its potential role as a sink for carbon dioxide (CO_2) (IPCC 2000). The increase in atmospheric concentrations of CO_2,

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as well as other greenhouse gases (GHGs), is the major cause of global-climate change or global warming. 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. Carbon sequestration can be achieved through improved silvicultural techniques to increase growth rates and through implementation of agroforestry practices on agricultural lands (Nair and others 2009; Montagnini and Nair 2004). Agroforestry is of special importance as a carbon-sequestration strategy because of its applicability in agricultural lands as well as in afforestation and reforestation programs that have been approved as GHG-mitigation strategies under the Kyoto Protocol (Nair and others 2009).

In Brazil, deforestation and forest burning account for about 75% of the GHG emissions, and fossil fuel use accounts for the remaining 25% (Lal and others 2006). In this scenario, cacao agroforestry systems (AFSs) 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. Cacao plantations in southern Bahia, Brazil, are grown under two AFSs: the traditional cabruca in partially cleared forests and the more recent forest, in which the original forest trees are eliminated before planting and the cacao plants are generally shaded with introduced leguminous *Erythrina* trees (Müller and Gama-Rodrigues 2007). Therefore, in order to understand the C sequestration potential of cacao AFS, it is important to know the extent of soil C storage across different organic matter fractions.

Soil organic matter (SOM) is a heterogeneous mixture of organic compounds of plant, animal and microbial origin in various stages of decomposition (Stevenson 1994). Soil organic matter is a large and dynamic reservoir of C and is a major part of the global C cycle (Ussiri and Johnson 2007; Poirier and others 2005; Montagnini and Nair 2004). Restoring soil C is essential to enhancing soil quality, sustaining and improving food production, maintaining clean water, and reducing increases in atmospheric CO_2 (Lal and others 2004). The world soil C pool is estimated to be around 1300 to 1500 Gt C, which is about twice the pool in the terrestrial plant biomass and three times the atmospheric pool. Approximately 32% of these soil C stocks occur in tropical soils (Cadish and others 2006). The C pool represents a dynamic equilibrium of gains and losses, and it is known to be influenced by edaphic factors (soil texture, structure and biological diversity), rainfall, temperature, farming system, and soil management (Lal 2004). However, studies about C accumulation in soils under cacao AFSs are scarce in Brazil.

Aggregates are secondary particles formed through the combination of mineral particles with organic and inorganic substances (Bronick and Lal 2005) and represent a significant pool of soil C (Six and others (1998, 1999). This is because the inclusion of organic materials within soil aggregates reduces their decomposition rate (Oades 1984; Elliott and Coleman 1988), improves C sequestration, and reduces the rate of increase in CO_2 concentration in the atmosphere and associated global warming (Bronick and Lal 2005). Aggregates are often grouped by size: macroaggregates (>250 μm) and microaggregates (<250 μm). Different aggregate size groups differ in C lability levels: C associated to macroaggregates (>250 μm) is more labile and represents the light organic matter, while the C associated with microaggregates (<250 μm) is more recalcitrant and represents the stable fraction (Cadish and others 2006; Bronick and Lal 2005; Gupta and Germida 1988; Tisdall and Oades 1982).

Density fractionation divides soil into a small number of mutually exclusive soil organic matter fractions using density and ultrasonic dispersion. Key fractions are: (1) free light organic matter (free light fraction—FLF), which is isolated before the breakdown of stable aggregates, (2) intra-aggregate organic matter (intra-aggregate light fraction—ILF), which is isolated after ultrasonic dispersion to break up aggregates and (3) organomineral material, which is recovered as the residual material (heavy fraction—HF) (Poirier and others 2005; Roscoe and Buurman 2003; Sohi and others 2001). Density fractionation emphasizes the role of soil minerals in organic matter stabilization and turnover and is considered chemically less destructive than chemical fractionation procedures. The results are more related to the in situ structure and function of soil organic matter (John and others 2005; Christensen 1992).

The free light fraction (FLF) is a dynamic fraction in mineral soil composed partially of plant and animal residues, and the only protection mechanism of this fraction is material recalcitrance (Cambardella and Elliot 1994; Golchin and others 1997). This fraction decomposes easily and is linked to the organic residue supply in the system (Schwendenmann and Pendall 2006). The intra-aggregate light-fraction (ILF) is occluded in soil aggregate, but it is not strongly associated with soil particles (John and others 2005; Christensen 1992; Roscoe and Buurman 2003). This fraction is a diversified group of organic compounds, including plant residues with a reduced size, and it has a more advanced degree of decomposition in comparison with the free light fraction. This fraction has two protection mechanisms: material recalcitrance and occlusion inside aggregates (Christensen 2000). The heavy fraction consists of more processed material, has a slower turnover rate and is generally a more stable and high-density organomineral fraction with a higher degree of physical protection. It can be a major sink for C storage in soil because it has little mineralizable C (Christensen 1992, 2000; Golchin and others 1995; Hassink 1995). According to John and others (2005) and Roscoe and Buurman (2003), heavy fractions store 90% of total soil carbon.

This article is a complement to Gama-Rodrigues and others (2010), which concerns the same study areas as the present study and sought to characterize soil organic carbon storage in relation to soil fraction-size classes in cacao agroforestry systems. Soil samples were collected from four depth classes to 1 m depth (0–10, 10–30, 30–60 and 60–100 cm), 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 objective of this study was to characterize the organic matter density fractions in whole soil and soil size fraction (0 to 10 cm layer) in two types of cacao agroforestry systems compared to an adjacent natural forest in Bahia, Brazil. Our present study focuses exclusively on the 0 to 10 cm later to characterize the organic matter density fractions that differ in nature, turnover time and biological function and, in turn, act as a pool of C on the soil surface (John and others 2005). We selected a depth of 0 to 10 cm because the continual addition of plant residues and the presence of root hairs and fine roots to the soil surface contributes to the biological activity and the continuous transformation of organic matter, which can lead to different soil organic matter pools. This study could improve the understanding of soil organic matter dynamics in soils under cacao AFSs and act as a guide in the search for sustainable practices.

Study Area

This study was conducted on the Research Station of the MARS Center of Cocoa Science, Itajuípe, located in the southern region of Bahia, Brazil (14°0′ S and 39°2′ W). The research farm is situated in a humid tropical climate with a well-distributed annual mean rainfall of 1500 mm. The soils are highly weathered, reddish-yellow Ferralsols (USDA: Oxisols) with a predominance of low-activity clays, such as kaolinite and gibbsite, and iron oxi-hydroxides, and they contain no carbonates (Resende and others 1997). The three land-use systems were a 30-year-old stand of cacao cabruca (Cacao was planted in partially cleared forestland where some large trees were retained (20 different species of trees)), a 30-year-old stand of cacao + erythrina (*Erythrina glauca*) (Erythrina was planted in a 24 × 24-m space with one tree in the center of that space (quincunx planting system)), and an adjacent natural forest which had 33 different species of trees (Gama-Rodrigues and others 2010).

Methods

Soil Sampling

Soils from each land-use were sampled from a 0 to 10 cm soil depth. Before the samples were taken, the litter layer was removed. All of the samples were taken in a 1 m^2 pit dug to a depth of 0.4 m. To minimize compression and to obtain a representative sample for the aggregation state of the soil, samples were taken using a bricklayer's trowel inserted into the soil at the lower level of the sampling depth. Four composite samples were prepared in each land-use system by compositing equal amounts of soils from four pits for each replicate (16 pits in each land-use system). Each soil sample was passed through a 19 mm sieve at the site of sampling by gently breaking apart the soil. Clods and aggregates larger than 19 mm diameter were discarded (Madari and others 2005). The soil samples were air dried for 24 h in the shade (humidity 15 ± 2%). Very dry aggregates can lead to a falsely high resistance to breakdown and result in higher stability indices (Castro Filho and others 2002). Chemical and physical soil characterization are described in Table 1 (Gama-Rodrigues and others 2010).

Physical Fractionation

Fraction-size separation was performed according to the procedure of John and others (2005) and Six and others (1998). This procedure is briefly summarized here. Soil (100 g) was moistened using capillarity by placing the soil on filter paper at the top of the sieve. The water volume was then raised inside the water tank for 15 min to wet the filter paper and the soil. The filter paper was then removed, and the wet sieving process (2000 μm; 1000 μm; 250 μm; 53 μm) was carried out, which was moved up and down in

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

	Natural forest	Cacao cabruca	Cacao + erythrina
pH (H_2O)	4.17	4.81	4.27
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^{-1})	377.20	501.60	395.60
Silt (g kg^{-1})	23.60	26.30	34.00
Clay (g kg^{-1})	599.20	472.10	570.40

Source: Gama-Rodrigues and others (2010) water by about 3 cm with 50 repetitions. The aggregates >2000 µm were collected and the same sieving procedure was repeated for the <2000 µm classe with the 1000, 250 and 53 µm sieves. Then, the classes of 1000 to 2000 µm, 250 to 1000 µm, 53 to 250 µm and less than 53 µm were obtained by sieving. All soil size fractions were air dried at 25°C. The total carbon and nitrogen contents of the soil size fraction (except <53 µm) were determined by dry combustion in a Perkin Elmer CHNS/O Analyzer. The overall average recovery mass percentage of soil fractions after the wet sieving procedure was about 99 % of the initial soil mass.

Density Fractionation

Soil organic matter fractionation followed a protocol described by Sohi and others (2001) and Freixo and others (2002). Two aliquots (5 g each) of whole soil or soil size fraction (<250 µm classes were not used) were separately transferred to 50 ml centrifuge tubes and dispersed in 35 ml of NaI (SG = 1:80 ± 0:01). The tubes were gently swirled for 30 s and then centrifuged for 15 min at 18,000×*g*. The suspended material (free light fraction, FLF) was siphoned from the surface of the suspension and isolated on a single pre-weighed filter (1.6 mm retention, 47 mm diam., Whatman GF/A) using a Millipore vacuum filtration unit (Millipore, Bedford, MA). Once the FLF had been collected, the soil sample suspended in the NaI solution was gently returned to the centrifuge bottles and then dispersed ultrasonically with an energy input of 400 J ml^{-1}, for three minutes, using a Branson Sonifier 250 generator fitted with a probe. The centrifuge bottles were packed with crushed ice to minimize any temperature increase. Centrifugal density separation was repeated, and the intra-aggregate light fraction (ILF) was isolated in a similar method to the FLF. Both the FLF and ILF were dried at 60°C, weighed and finely ground for the determination of total C and N. Meanwhile, the heavy fraction remaining in the centrifuge tube was washed three times with 40 ml DI-water and centrifuged for 15 min at 18,000×*g*. Finally, the residue (or heavy fraction) was dried at 60°C overnight, weighed and ground for C and N determinations. The total carbon and nitrogen contents of FLF, ILF and HF of whole soil and the soil size fraction were determined by dry combustion in a Perkin Elmer CHNS/O Analyzer.

Statistical Analyses

The data were analyzed by analysis of variance (ANOVA) as a completely randomized design with four replicates. The four composite samples were treated as replicates with respect to the land-use systems, each of which was more than 1000 m^2 in area, using 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 C density fractions (free light fraction, intra-aggregate light fraction and heavy fraction) in whole soil and soil size fraction at three sites. Statistical analyses were performed with SAEG 9.1 (Funarbe 2007), and differences were considered significant at $P < 0.05$.

Results

Density Fractions of Whole Soil

The amount of the free light fraction (FLF) of soil was significantly ($P = 0.05$) higher in the natural forest, which did not differ from cacao + erythrina, and the lowest amount was found in the cacao cabruca. The highest SOC and total N of the FLF of soil were found in the cacao + erythrina system. However, the SOC, total N and the weight of the intra-aggregate light fraction (ILF) and heavy fraction (HF) of soil did not exhibit any significant difference between land-use systems (Table 2). The organic C-to-total N ratio of density fractions were not altered by the land-use systems. However, the organic C-to-total N ratio of the heavy fraction (average 5.9) of soil was consistently lower than that of the light density fraction (average 28.7) of soil (Table 2).

Density Fractions of Soil Size Fraction

On average, 99% of these soils consist of the macro-sized aggregate class of soils (>250–2000 µm). In natural forest, 45% was >2000 µm, 54% was 250–2000 µm and a small amount was microaggregates. The soil size fraction obtained in cacao AFS soils consisted mainly (65%) of mega-aggregates (>2000 µm) mixed with macroaggregates (32–34%) and microaggregates (1–1.3%) (Table 3). The highest SOC and total N content were found with increasing soil size fraction in all land-use systems. However, in each soil size fraction, SOC and total N content did not vary between land-use systems. The ratio of organic C-to-total N was higher in the macroaggregate than the microaggregate class (Table 3).

The organic C and total N content in the FLF, ILF, and HF did not vary between soil size fraction in the natural forest; the N content in FLF was an exception, which was significantly lower in the >2000 µm soil size fraction and did not differ from the 1000 to 2000 µm class (Table 4). However, in cacao AFS soils, the highest C and total N content in FLF, ILF and HF were found in the 250 to 1000 µm aggregate size classes. The C and N in the ILF

Table 2 Weight, C and N in density fractions separated from soils (0–10 cm) under three land-use systems in Bahia, Brazil

	W-FLF (g)	W-ILF[a] (g)	W-HF (g)	C-FLF (g kg^{-1}) soil	N-FLF (g kg^{-1}) soil	C-ILF (g kg^{-1}) soil	N-ILF (g kg^{-1}) soil	C-HF (g kg^{-1}) soil	N-HF (g kg^{-1}) soil	CN-FLF	CN-ILF	CN-HF
Natural Forest	0.18 (0.02) a	0.04 (0.00) a	4.26 (0.57) a	2.34 (0.18) b	0.09 (0.00) b	0.60 (0.07) a	0.01 (0.00) a	6.37 (0.69) a	0.90 (0.08) a	26‡	60	7
Cacao cabruca	0.10 (0.00) b	0.04 (0.01) a	5.72 (0.10) a	3.13 (0.31) b	0.13 (0.02) b	0.79 (0.04) a	0.02 (0.00) a	6.27 (0.90) a	1.20 (0.28) a	24	39	5
Cacao + erythrina	0.12 (0.01) ab	0.03 (0.00) a	5.94 (0.52) a	4.87 (0.56) a	0.21 (0.02) a	0.89 (0.10) a	0.03 (0.00) a	7.44 (1.81) a	1.35 (0.22) a	23	30	6

W-FLF weight of free light fraction, *W-ILF* weight of intra-aggregate fraction, *W-HF* weight of heavy fraction, *C-FLF* carbon of free light fraction

N-FLF nitrogen of free light fraction, *C-ILF* carbon of intra-aggregate light fraction, *N-ILF* nitrogen of intra-aggregate light fraction, *C-HF* carbon of heavy fraction

N-HF nitrogen of heavy fraction, *CN-FLF* C:N ratio of free light fraction, *CN-ILF* C:N ratio of intra-aggregate fraction, *CN-HF* C:N ratio of heavy fraction

[a] Values followed by the same letter(s) within column are not significantly different according to the Tukey test ($P = 0.05$)

‡ No statistical difference among land-use systems

Table 3 C and N content and organic C-to-total N ratio of soil size fraction and whole soil (0–10 cm) under three land-use systems in Bahia, Brazil

μm	DWSA (%)	SOC[a] (g kg^{-1})	Total N (g kg^{-1})	C:N ratio
Natural forest				
>2000	44.9 (5.60)	16.86 (2.84) a‡	1.55 (0.22) a	11‡
1000–2000	30.4 (2.69)	9.94 (0.92) ab	0.96 (0.07) ab	10
250–1000	23.3 (5.16)	7.65 (2.30) bc	0.80 (0.20) b	10
53–250	1.0 (0.25)	0.26 (0.07) c	0.03 (0.00) c	9
<53	0.4 (0.02)	–	–	–
Whole soil		39.90	2.90	9
Cacao cabruca				
>2000	67.0 (6.62)	21.02 (2.23) a	2.69 (0.39) a	8
1000–2000	19.7 (2.61)	5.95 (1.12) b	0.71 (0.11) b	8
250–1000	12.0 (3.27)	2.93 (0.88) bc	0.24 (0.06) b	12
53–250	1.0 (0.08)	0.20 (0.03) c	0.04 (0.01) b	5
<53	0.3 (0.03)	–	–	–
Whole soil		33.38	2.82	9
Cacao + erythrina				
>2000	64.8 (7.48)	27.34 (6.45) a	2.94 (0.43) a	9
1000–2000	25.2 (4.05)	7.10 (1.13) b	1.13 (0.25) b	6
250–1000	9.0 (1.77)	2.28 (0.48) b	0.43 (0.09) bc	5
53–250	0.6 (0.06)	0.14 (0.00) b	0.02 (0.00) c	7
<53	0.4 (0.02)	–	–	–
Whole soil		42.30	2.50	9

DWSA distribution of water stable aggregate size classes

[a] Values followed by the same letter(s) within column are not significantly different according to the Tukey test ($P = 0.05$)

‡ No statistical difference among land-use systems in each aggregate class

did not vary among the soil size fraction in cacao cabruca (Table 2). In general, the contribution of C and N in the light and heavy fraction were similar in the natural forest and cacao cabruca systems. However, in cacao + erythrina, the heavy fraction was the most common, contributing to 67% of C and 63% of N (Table 4).

Discussion

Density Fractions of Whole Soil

The FLF in the soil varied more than the ILF and HF (Table 2). These results suggest that the FLF was the most dynamic and most sensitive to changes in C and N content. Similar observations were reported irrespective soils and crops in systems such as leguminous tree forest in in southeast Brazil by Macedo and others (2008); soybean crop systems in northeast China by Zhang and others (2007) and by Freixo and others (2002) in crop rotation

Table 4 C and N content and organic C-to-total N ratio in density fractions of aggregate size classes of soils (0–10 cm) under cacao agroforestry systems and natural forest

μm	C-FLF[b]	C-ILF[a]	C-HF[a]	N-FLF[a]	N-ILF[a]	N-HF[a]	CN-FLF	CN-ILF	CN-HF
Natural forest									
>2000	0.50 (0.06) a	0.33 (0.04) a	2.26 (0.34) a	0.02 (0.00) b	0.01 (0.00) a	0.41 (0.03) a	25‡	33	6
1000–2000	0.82 (0.19) a	0.39 (0.05) a	3.21 (0.86) a	0.03 (0.00) ab	0.01 (0.00) a	0.45 (0.07) a	27	39	7
250–1000	1.63 (0.49) a	0.73 (0.22) a	4.42 (1.67) a	0.06 (0.01) a	0.02 (0.00) a	0.64 (0.25) a	27	37	7
Cacao cabruca									
>2000	0.31 (0.05) b	0.21 (0.02) a	1.16 (0.20) b	0.01 (0.00) b	0.01 (0.00) a	0.19 (0.01) b	31	21	6
1000–2000	1.38 (0.34) b	0.69 (0.19) a	2.72 (0.54) ab	0.06 (0.02) b	0.03 (0.00) a	0.71 (0.17) a	23	23	4
250–1000	3.81 (0.86) a	0.98 (0.28) a	3.79 (0.56) a	0.18 (0.04) a	0.04 (0.01) a	1.17 (0.11) a	31	25	3
Cacao + erythrina									
>2000	0.64 (0.10) b	0.22 (0.03) b	1.20 (0.25) b	0.03 b	0.01 b	0.20 (0.03) b	21	22	6
1000–2000	1.89 (0.58) b	0.82 (0.33) ab	4.20 (2.04) ab	0.07 b	0.03 ab	0.83 (0.28) ab	27	27	5
250–1000	9.14 (1.90) a	1.98 (0.43) a	11.03 (2.88) a	0.47 a	0.09 a	1.77 (0.37) a	19	22	6

[a] The units are in g kg^{-1} dry weight of aggregate

[b] Values followed by the same letter(s) within column are not significantly different according to the Tukey test (P = 0.05)

‡ No statistical difference among land-use systems in each aggregate class

system soils in southern Brazil. The HF was the most common density fraction in the soils of all land-use systems with the highest C and N concentration. In the continuous crop surface soil, C-HF was about 7.5 g kg^{-1} (Tan and others 2007). Jin and others (2008) and Yin and others (2005) reported that the HF was the most representative organic matter fraction in several agricultural soils from China. Overall, C-HF is a more stable pool of C, with turnover times ranging from decades to centuries (Yamashita and others 2006) and has a high amount of protected C in the soils. The lower organic C-to-total N ratio in the heavy fraction of whole soil and soil size fraction lends support to the idea that this fraction is composed of highly processed and persistent organic matter (Table 3 or 4). In contrast, the high organic C-to-total N ratio of the light density fraction indicates that the light fraction is composed of plant material in the early stages of decomposition (Liao and others 2006; Freixo and others 2002; Oades and others 1987). During the decomposition of organic matter, the consumption of C is greater than N; this difference causes the relative increase in N concentration, decreasing the organic C-to-total N (Oades 1988; Sollins and others 1996), which explains the highest and lowest organic C-to-total N ratio of LF and HF, respectively.

Density Fractions of Soil Size Fraction

Macroaggregates were the most abundant soil size fraction and were the major soil C and N pool in these soils (Table 3). The decreasing organic C-to-total N ratio (Table 4) with decreasing soil size fraction lends support to the idea that the macroaggregate size class is held together by labile binding agents. Some studies have shown high organic C-to-total N ratio in macroaggregates, suggesting that organic matter associated with this aggregate size class is less processed than that associated with microaggregates (Tan and others 2007; Liao and others 2006).

The amount of C in different soil aggregate classes varied: 49% of the C content and 46% of the total N content were in the >2000 μm aggregate size class in the natural forest. On average, 72% of the C and 69% of the total N were in the >2000 μm in cacao AFS soils. Less than 1% of the C was found in microaggregates (Table 3). These results do not agree with Zotarelli and others (2005, 2007), Six and others (2000) and Oades and Waters (1991), who reported no differences in C and N content across aggregate-size classes in crop and pasture systems on Oxisols. The constant replacement of organic materials in these land-use systems probably maintains the binding effect and increases the number of water stable macroaggregates and the protective effect of the fallen leaf litter against the beating action of raindrops (Gupta and others 2009). Furthermore, the higher root density of trees and the greater leaf biomass results in increased soil organic matter and hence a higher percentage of mega and macro-sized classes (Takimoto and others 2009; Chan and Heenan 1996); leguminous plant roots also increase aggregation (Haynes and Beare 1997). In addition, the lack of disturbance in the cacao AFS, which is a no-till system, the continuous input of organic material to the soil via litterfall, and the presence of leguminous plant roots (Haynes and Beare 1997) or sloughed-off roots (Kummerow and others 1982; Gama-Rodrigues and

Cadima-Zevallos 1991) help maintain the binding effect and increase the number of water-stable macroaggregates (Gama-Rodrigues and others 2010; Isaac and others 2005; Müller and Gama-Rodrigues 2007). Haile and others (2008) have observed high C content in the largest aggregate size classe, similar to data in this study, in silvopastoril systems on Spodosols and Ultisols from Florida. Gupta and others (2009) found that the >250 µm aggregate size classe dominated in poplar-based agroforestry system soils (Typical Ustifluvents) and accumulated high amounts of soil C when compared with the crop alone.

In our study, the soils are from stabilized land-use systems that are more than 30 years old (characterized by no soil disturbance and mature trees with a decreasing rate of growth—zero or close to it—but with continual additions of leaf and root litter to the soil). The Cacao cabruca system has high plant diversity, the cacao + erythrina system has high inputs of cacao leaf fall and nitrogen-rich litter from erythrina shade trees, and the natural forest has high plant diversity but no cacao leaf-fall input. In addition, all of these land-uses are no-tillage systems with accumulation and subsequent turnover of leaf litter, roots, and stump material that facilitates the maintenance of a high level of soil organic matter (SOM). Thus, there was a clear long-term effect of organic matter build up in the these soils that could explain the HF as a most representative density fraction and the low organic C-to-total N ratio in the whole soils and in HF from these soils (Tables 2 and 4).

Conclusions

This study indicated that the vast majority of the C and N content in the three land-use systems studied lies in macroaggregates, particularly in the 250 to 1000 µm aggregate size class. The heavy fraction was the most representative organic matter fraction in these soils. In mature cacao AFS in highly weathered soils, the main mechanisms of C stabilization could be physical protection within aggregate structures minimizing the impact of conversion of forest to cacao AFS. Thus, 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 soils in the humid tropics.

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