Soil aggregation and organic carbon fractions in the Río Grande de Arecibo watershed^{1,2}

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ABSTRACT

Cultivation affects soil organic matter loss through decreased soil structural stability. Sparse information is available for highly weathered soils. The objective of this study was to evaluate the effects of soil order (Ultisols and Inceptisols) and land use (agriculture and forest) on the formation of water stable aggregates, concentration of aggregate-associated C, and guality of C in aggregates from selected soils in a humid tropical watershed. Ultisols and soils under forest had increased soil C as a result of increased C concentrations in aggregates. Nearly 90% of the soil C was found in macroaggregates of soils under forest and in Ultisols. In forest and agriculture land use, soil silt+clay content was an important determinant for C storage in the bulk soil but not in aggregates. Cultivation reduced the percentage of soil mass in large macroaggregates (>2,000 µm) relative to that in forest soils, whereas Ultisols had greater soil mass percentage in large macroaggregates than Inceptisols. Overall, macroaggregates have higher labile and stable C than microaggregates. Ultisols had greater amounts of labile C but similar proportions of stable C. As has been found in other soils dominated by mixed-mineralogy and 1:1 clays and oxides, aggregate-associated C is not the sole determinant for the formation of macroaggregates.

Key words: Soil organic carbon, soil organic nitrogen, soil aggregates, watershed

RESUMEN

Agregación y reservas de carbono en suelos de la cuenca del Río Grande de Arecibo

La labranza del suelo afecta el contenido de materia orgánica a través de una reducción en la estabilidad estructural del suelo. El objetivo del estudio fue evaluar los efectos del orden de suelos (Ultisol e Inceptisol) y el uso de la tierra (agricultura y bosques) sobre la formación de agregados estables al agua, y en la concentración y calidad del carbono (C) en agregados en suelos de una cuenca hidrográfica tropical. Los Ultisoles y los suelos bajo bosque secundario han aumentado su C debido a un incremento del C en los

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agregados. El contenido de limo y arcilla fue un factor determinante para el almacenaje de C en suelos bajo bosque secundario y agricultura, pero no en los agregados. Cerca del 90% del C total del suelo estuvo asociado a los macroagregados bajo bosque y en los Ultisoles. Los suelos bajo uso agrícola tuvieron menor proporción de la masa de suelo en macroagregados grandes (>2,000 μ m) con relación a los suelos bajo bosque secundario, mientras que los Ultisoles tuvieron mayor masa de suelo en los macroagregados grandes. La concentración de C lábil y estable fue mayor en los macroagregados que en los microagregados. Los agregados de Ultisoles tuvieron mayor cantidad de C labil pero igual proporción de C estable que los del Inceptisol. El C asociado a los agregados en suelos dominados por mineralogía mixta, arcillas 1:1 y sesquióxidos.

Palabras clave: Carbono orgánico del suelo, agregados del suelo, fracciones de carbono en suelos, cuencas hidrográficas

INTRODUCTION

Soil organic matter (SOM) is an important stable carbon (C) reservoir, and its depletion reduces soil quality, increases dependence on inorganic fertilizer, and can impact water quality through increased erosion and nutrient losses (Gregorich et al., 1997; Magdoff, 1998; Sparling et al., 2006). Atmospheric C sequestration in soil occurs through the transfer of CO₀-C into terrestrial pools via primary production, subsequent decomposition of biomass and vegetative litterfall. and formation of SOM. Soil conditions that increase soil C residence time include lower soil temperature, high soil moisture, high base status, and increased silt plus clay content (Silver et al., 2000; Ingram and Fernandes, 2001). Increases in plant biomass C production and external C additions or decreased net C output, such as reduced oxidation and erosion, will lead to soil C accretion (Lugo and Brown, 1993; Silver et al., 2000). Specific management practices leading to increased soil C include the use of cover crops, application of organic residues, conservation tillage, adequate irrigation, and crop rotations (Halvorson et al., 1999; Silver et al., 2000; Carter, 2001; Sá et al., 2001).

Organo-mineral surface sorption, physical protection within stable macroaggregates by aggregation and biochemical recalcitrance, are mechanisms by which SOM can be protected from decomposition (Benny et al., 1989; Denef et al., 2004; Plante et al., 2006). Several studies have elucidated the relationship between aggregates and SOM (Tisdall and Oades, 1982; Beare et al., 1994b; Six et al., 1998). Tisdall and Oades (1982) formulated a hierarchical model for aggregate formation and organic C stabilization in soils dominated by 2:1 high activity clays in that silt+clay mineral particles are bound into microaggregates <50 µm which in turn are bound into macroaggregates >250 µm. Binding agents within microaggregates are considered more humified or re-

calcitrant than when outside the microaggregates. Microaggregates are bound together by roots, fungal hyphae, organic acids and polysaccharides (which are considered more labile or decomposable) to form macroaggregates. The SOM that binds microaggregates into macroaggregates (intraaggregate-C) is considered the main source of organic matter lost upon cultivation. Cultivation generally results in reduced proportion of soil mass as macroaggregates and reduced C concentration in macroaggregates. Hence, macroaggregates are less stable than microaggregates and thus susceptible to disruptive forces. An alternative conceptual model describes microaggregates and particulate organic matter as being in the center of macroaggregates. During decomposition of the particulate organic matter, the organic material becomes encrusted with microbial mucilage and clay particles, leaving the organic material inaccessible to microbial attack (Beare et al., 1994a: Six et al., 1999). The above mentioned models may not be as important in soils dominated by low activity clays (1:1 mineralogy and oxides), as aggregate formation is less dependent on the presence of SOM because of the electrostatic attraction of positive charges in oxides which attract the negative charges of clay minerals (Denef et al., 2004: Zotarelly et al., 2005).

Microbial communities and their associated activities are a key component in soil aggregate formation, whereas aggregate structure disruption by erosion, raindrop impact, or tillage can result in exposure of labile fractions of organic matter (particulate organic matter, enzymes and microbial biomass) to air and oxidation as CO_2 -C (Cambardella and Elliot, 1993, 1994). Reduction in the proportion of macro-aggregates and an increase in microaggregates (Chan, 1997; Six et al., 1998) can result in soil function alterations such as decreased water infiltration, increased runoff and erosion, and increased nutrient losses.

It is important to examine to what extent soil organic C levels can be restored in case of a shift in land use from cropland cultivation to forest and pasture land use in the tropics, and to examine how the organic C is distributed among the aggregate size fractions. There is limited information on the impact of land use practices and management on the formation and stability of organic matter of soils in the tropics, especially at the watershed scale. The objectives of this paper were to (i) determine the magnitude and distribution of C and nitrogen in aggregates (aggregate sizes >2,000; 250 to 2,000; 53 to 250; and 20 to 53 μ m); and to (ii) assess the magnitude of labile and stable C within macroand micro-aggregates as influenced by soil order (Inceptisols and Ultisols) and by land use (agriculture, forest, pasture) in a tropical watershed. This approach permits an assessment of the factors influencing formation, retention and stability of SOM.

MATERIALS AND METHODS

Soils from the Río Grande de Arecibo (RGA) watershed, located in north-central Puerto Rico, were sampled. Description of the area's land use, vegetation, location, and soils has been published elsewhere (Acosta-Martínez et al., 2007: Sotomayor-Ramírez et al., 2010). Briefly, the watershed has an area of 45.067 ha. 39.361 ha of which are classified as secondary forest, managed and unmanaged pasture, or agricultural land. After the 1950s most of the land area under cultivation was gradually abandoned with succession to pastures and secondary forests (Aide and Grau, 2004). At the time of sampling, the forest land consisted of secondary forests of between 30 to 50 years of age, and the agricultural land consisted primarily of coffee (Coffea spp.), plantains (Musa spp.), and citrus (Citrus spp.) (Sotomayor-Ramírez et al., 2010). Within the watershed, there are 35 soil series subdivided into 79 mapping units based on slope and level of erosion. Five soil series predominate (two of which are Ultisols and three are Inceptisols) (Acevedo, 1982: Beinroth et al., 2003). Soils from throughout the watershed were sampled as described by Sotomayor-Ramírez et al. (2010). Of the 104 sites sampled, 17 sites at two depths (i.e., 0- to 15- and 15- to 30-cm depth) were randomly selected (Table 1) from among the pool of the most representative soils and land uses.

Aggregate size separation was performed on the <4.75-mm soil fraction, by wet sieving air-dried soil (100 g soil) through a series of sieves (2,000; 250; and 53 µm) (Elliot, 1986; Cambardella and Elliot, 1994). Details of the procedure are given in Sotomayor-Ramírez et al. (2006). Four aggregate fractions were obtained: (i) >2,000 µm (large macroaggregates); (ii) 250 to 2,000 µm (small macroaggregates); (iii) 53 to 250 µm (microaggregates); and (iv) <53 µm (silt + clay). Floating plant residues and roots larger than 1 mm were removed from the soil subsamples by using forceps.

Aggregates were expressed on a sand-free basis because the concentrations of C and N can be influenced by the different proportions of sand in each size class using the equation:

Sand-free aggregate $_{\text{fraction}} =$ (aggregate + sand) $_{\text{fraction}} \times [1 - (\text{sand proportion})_{\text{fraction}}]$ [1]

The sand-free C concentrations within aggregates (g/kg sand-free aggregate) were calculated as (Six et al., 1998):

Sand-free C _{fraction} = C _{fraction} / $[1 - (sand proportion)_{fraction}]$ [2]

Total organic C and N content in ground soil and aggregate subsamples (<0.05 mm) were quantified by automated dry combustion using a

Order	Series	Land use ¹	Predominating mineralogy	Taxonomic classification ²
Inceptisol	Pellejas	(3) Forest / (3) Agric	Mixed	Fine-loamy over sandy or sandy-skeletal, mixed, subactive, isohyperther- mic Typic Dystrudepts
Ultisol	Humatas	(4) Forest / (2) Agric	Sesquic	Very-fine, parasesquic, isohyperthermic Typic Haplohumults
Inceptisol	Caguabo	(3) Forest	Mixed	Loamy, mixed, active, isohyperthermic, shallow Typic Eutrudepts
Ultisol	Consumo	(1) Agric / (1) Forest	Mixed	Fine, mixed, semi- active, isohyperthermic Typic Haplohumults

 TABLE 1. Description of soils selected for evaluation.

¹Number in parenthesis is the number of soil samples evaluated. ²Beinroth et al. (2003).

LECO C and N analyzer (Leco Corp., St. Joseph, MI)⁵ at the Soil, Plant and Water Laboratory of the College of Agricultural and Environmental Sciences, University of Georgia.

The large and small microaggregates within each of the micro- and macro-aggregates were pooled into macro- and micro-aggregates in order to further explore the magnitude of labile C, such as mineralizable C (C_o) and labile polysaccharides, and stable C pools. Mineralizable C, which is considered a major portion of labile C or active soil C, was quantified by measuring CO_2 -C evolved from aggregates re-wetted to approximately field capacity in a 24-h period and incubated in a closed vessel for 28 d at 25° C. The gas headspace was sampled for CO_2 -C at weekly intervals and quantified in a Series 6890 gas chromatograph (Agilent Technologies, Inc., Wilmington, DE), equipped with a Porapak Q column (250-µm mesh size) and a thermal conductivity detector (150° C). The two-pool model was used to estimate C_o using the relationship between C_{min} and time (t) as:

$$C_{\min} = C_0 * [1 - \exp(-k_1 * time - k_2 * (time^2)/2)]$$
 [3]

using PROC NLIN of SAS (SAS version 8.01, SAS Institute, Cary, NC).

⁵Company and trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

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Stable soil C was estimated as the difference between total organic C and labile C, as measured by mineralizable C. Total labile polysaccharides (P_L) were quantified in aggregates by using the colorimetric procedure of Lowe (1993), which involves the heating of the soil in a dilute acid solution followed by colorimetric quantification of sample hydrolysates at 490 nm in a spectrophotometer. This analysis recovers most polysaccharides other than cellulose, and includes those polymers most active in aggregate formation.

The data were arranged in a completely randomized design for statistical analysis. An analysis of variance was performed on measured and estimated parameters by using SAS to evaluate the effects of soil order, phase, land use and their interactions. Means separation was performed by using LSMeans with a significance level of P < 0.05.

RESULTS AND DISCUSSION

Soil TOC and TN: The TOC and TN concentrations in the bulk soil at 0- to 15- and 15- to 30-cm depths were not affected by the interaction of soil order (Ultisol vs. Inceptisol) and land use (forest versus agriculture). The same pattern was observed for TOC and TN contents at both depths. Soils under forest (0 to 15 cm) had higher TOC and TON contents than those under cultivation, with values of 5,332 and 2,073 g C/m² and 496.3 and 212.2 g N/m², respectively (Table 2). Ultisols (0 to 15 cm) had higher TOC and TN contents than Inceptisols with values of 5,065 and 2,340g C/m² and 475.0 and 233.6 g N/m², respectively. Land use did not affect TOC and TN concentrations and contents at 15- to 30-cm depth. The values and trends obtained were similar to those reported by Sotomayor-Ramírez (2010), suggesting that the samples selected were representative of the overall C and N status within the RGA watershed.

Soil TOC stocks and texture: Soils of the order Ultisol had greater silt+clay content than those grouped as Inceptisols with mean (standard deviation in parenthesis) values of 81.3 (±4.9) and 55.4% (±16.1), respectively. In general, the silt +clay content of the bulk soil (<4 mm fraction) was a better predictor (displaying higher correlation coefficients) for TOC and TN concentrations than the soil clay content, as previously reported in other studies (Feller et al., 2006). The Ultisols also had greater TOC and TN content, but the silt+clay content within Ultisols was not an important predictor of TOC and TN since correlations with silt+clay were not significant (P > 0.05). In contrast, TOC content to a depth of 0 to 15 cm was strongly dependent on the silt+clay content for Inceptisols and for forest and agriculture soils, where the following equations were obtained (P < 0.05):

	TOC	TON	TOC	TON			
Effect	g /	g/kg		g/m^2			
	(Order (0 to15 cm)					
Inceptisol	$12.02 \mathrm{b}$	$1.20 \mathrm{\ b}$	2,339.8 b	233.6 b			
Ultisol	29.46 a	2.75 a	5,065.2 a	475.0 a			
	Land-use (0 to 15 cm)						
Agriculture	$12.03 \mathrm{b}$	$1.22 \mathrm{\ b}$	2,072.8 b	212.2 b			
Forest	29.45 a	2.72 a	5,332.2 a	496.3 a			
		Order (15-30 cm)					
Inceptisol	3.91 a	0.52 a	795.0 b	104.2 b			
Ultisol	15.18 b	1.37 b	3,251.2 a	297.2 a			

TABLE 2. Soil organic C and N concentrations and contents in soils of the RGA watershed.

 $TOC_{Incentisel} = 121.3^{*}(silt+clay) - 4045; r^{2} = 0.70$

 $\text{TOC}_{\text{Forest}} = 127.4^{*}(\text{silt+clay}) - 3911; r^2 = 0.68$

 $TOC_{Agriculture} = 79.2^{*}(silt+clay) - 2371; r^2 = 0.74$

Similar trends were observed for soil TN content. The domain defined by the upper (forest vegetation) and lower (cultivation) limits represents the maximum potential C sequestration possible in this watershed for a given texture. Our data demonstrate that the potential maximum sequestration for a given land use and for the Inceptisol order is dependent on soil texture. Soils under agriculture with silt+clay contents in the range of 50 to 90% could store from 860 to 2,788 g C/m² with the proper organic matter input and management practices. These amounts account for up to an additional 58% storage capacity per area basis above present agricultural soil TOC levels.

Aggregation and SOC storage: For the 0- to 15-cm depth, Ultisols had a greater percentage of large macroaggregates than Inceptisols, but the opposite was observed for small macroaggregates; microaggregate size distribution percentages were similar in Inceptisols and Ultisols (Figure 1). Both Ultisols and Inceptisols had a greater percentage of soil mass in the macroaggregate size class than in the microaggregate size class. For the 15- to 30-cm depth, aggregate size percentages were not influenced by the main effects or interactions (P > 0.05).

Soils under forest had greater percentage of macroaggregates than microaggregates, but the magnitude of this trend was lower in soils under agriculture. Forest soils had a higher percentage of large macroaggregates than agriculture soils. Soils under cultivation have been found



FIGURE 1. Aggregate (sand-free) size distribution as influenced by (A) soil order, and (B) land use at 0 to15 cm in soils of the Río Grande de Arecibo watershed, Puerto Rico. An asterisk (*) within an aggregate size indicates statistical difference (P < 0.05) between soil order or land use. Different letters among aggregate size classes and within soil order or land use indicate significant difference (P < 0.05).

to have rates of macroaggregate formation similar to those of soils under native sod or no-tillage, but higher rates of macroaggregate turnover (Six et al., 1998). Cultivation apparently causes a reduction in the accumulation of stabilizing fine intraaggregate particulate organic matter affecting the stability of macroaggregates. Unprotected particulate organic matter is subjected to oxidation leading to reduced macroaggregate formation (Six et al., 1999). This mechanism may serve as a conceptual model to explain the increase in microaggregates at the expense of macroaggregates in our soils under cultivation. Soils under forest and agriculture had similar silt+clay content, and this parameter was not correlated to the presence of large macroaggregates in forest soils. Zotarelli et al. (2005) reported that conventional tillage of Oxisols reduced the proportion of the largest macroaggregate class (>2,000 μ m), a finding which coincides with our observations in soils under agricultural management.

Land use did not affect TOC and TN concentrations among aggregate size class (P = 0.1), although there was a strong tendency for aggregates in forest to be higher than in agriculture, with mean values of 35.45 and 23.66 g C/kg and 3.22 and 2.11 g N/kg, respectively. In both Inceptisols and Ultisols there was a positive, though non-significant, tendency for TOC and TN concentrations to increase with increasing macroaggregate size class at both 0- to 15- and 15- to 30-cm depths (Figure 2). The TOC concentrations were greater in Ultisols than in Inceptisols for the macroaggregates and large microaggregates at 0- to 15-cm depth and for the small macroaggregates at 15- to 30-cm depth. Ultisols had significantly greater TN concentrations than Inceptisols



FIGURE 2. Aggregate (sand-free) carbon concentrations at (A) 0 to 15 cm, and (B) 15 to 30 cm; and N concentrations at (C) 0 to 15 cm, and (D) 15 to 30 cm as influenced by soil order in soils of the Río Grande de Arecibo watershed, Puerto Rico. An asterisk (*) within an aggregate size indicates statistical difference (P < 0.05) between soil orders.

within each of the aggregate size classes at both 0- to 15- and 15- to 30- cm depths.

Concentrations of TOC and TN for each of the aggregate fractions were converted to mass per unit area after correcting for sand content and bulk density (Figure 3). Land use did not influence TOC and TN contents within aggregate classes (data not shown). Soil TOC and TN were higher in large macroaggregates of Ultisols than in those of Inceptisols. Therefore, the greater capacity to sequester C in Ultisols is due to its storage in large macroaggregates. The percentage of total aggregate C found in macroggregates was 83 and 90% for Inceptisols and Ultisols, respectively, and 78 and 91% in agricultural and forest soils, respectively. On average, macroaggregate (large and small) associated C was 47% less in the Inceptisols, and accounted for nearly 99% of the aggregate-associated C difference between Inceptisols and Ultisols. Agricultural soils had 65% less aggregate associated C than the forest soils; the difference was due to the C in the large macroaggregates. Six et al. (1998) also reported that C and N concentrations tended to increase



FIGURE 3. Aggregate (sand-free) carbon contents at (A) 0 to 15 cm, and (B) 15 to 30 cm; and N contents at (C) 0 to 15 cm, and (D) 15 to 30 cm as influenced by soil order in soils of the Río Grande de Arecibo watershed, Puerto Rico. An asterisk (*) within an aggregate size indicates statistical difference (P < 0.05) between soil orders. Aggregate size classes with different letters within soil order indicate significant difference (P < 0.05).

with aggregate size for no-tillage and conventional soils, except between the large and small macroaggregates. Six et al. (1998, 1999) reported that tillage reduced intraaggregate C in the large microaggregates, but not in the larger size aggregates. They suggest that the rates of aggregate formation are similar in both conventional and no-tillage with native vegetation, but that more C accumulates in macroaggregates because of slower macroaggregate turnover in the absence of soil disturbance from tillage.

The fact that Ultisols had greater soil C concentration than Inceptisols may be due to their having greater C concentration in all aggregate size classes (especially in macroaggregates) than Inceptisols. Yet the differences in C concentration in aggregates alone cannot account for the decreased aggregate percentages in the large macroaggregate size class (>2,000) of Inceptisols relative to those of Ultisols, because the relative differences in TOC concentration between Ultisols and Inceptisols were similar among all of the aggregate size classes. Further exploration of the data reveals that the silt+clay content in aggregate size classes was similar among them and among land uses and soil orders. Therefore, silt+clay differences within aggregates of the soils were not an important determinant for the storage of bulk soil C. The lack of differences in C and N concentrations observed in our soils may be due to their not expressing an aggregate hierarchy as occurs for 2:1 clay soils (Tisdall and Oades, 1982). Our findings suggest that the high non-crystalline inorganic forms of Fe and Al in combination with kaolinite are important binding agents, with organic carbon being a less important factor for microaggregate formation into macroaggregates (Denef et al., 2004). The reduced citrate-dithionate extractable Fe and Al in the Inceptisols relative to those of the Ultisols (Mount and Lynn, 2004) may account for the reduced macroaggregate formation of Inceptisols relative to that of Ultisols.

Fractions of soil C in aggregates: The amounts of labile C as measured by labile polysaccharides were significantly higher in Ultisols than in Inceptisols (Figure 4A) and tended to be higher for forest soils (data not shown). In turn, labile polysaccharides were greater in the macroaggregates than in the microaggregates for each soil order (Figure 4A). In a similar manner, there was a non-significant tendency for labile polysaccharides to be higher in macroaggregates of forest soils than in those of agriculture. Mineralizable C was similar among aggregate size class, order, and land use, although there was a tendency for greater mineralizable C in macroaggregates than in microaggregates (Figure 4B). There was much higher variability in the labile C



FIGURE 4. Labile polysaccharides and mineralizable C in macro- and micro-aggregates at 0 to 15 cm as influenced by soil order in soils of the Río Grande de Arecibo watershed, Puerto Rico. An asterisk (*) within an aggregate size indicates statistical difference (P < 0.05) between soil orders. Aggregate size classes with different letters within soil order indicates significant difference (P < 0.05).

estimate when using soil respiration than when using labile polysaccharides as an index. We propose that labile C contributes to the formation of macroaggregates in these soils.

As occurred for labile C, the amount of stable C was significantly higher in Ultisols than in Inceptisols in both macro-and micro-aggregates (Figure 5). We estimate that about 2% of the total C is mineralizable in a 28-d incubation, with the rest being of non-labile nature (stable). The percentage of stable C in the microaggregates ranged from 93 to 95% and was approximately 98% in macroaggregates of Inceptisols and Ultisols. Labile C fractions (consisting of simple sugars, organic acids, microbial biomass and metabolic compounds of plant residues) have been estimated to have laboratory mean residence time of approximately 25 days and to account for from 3 to 5% of the total C in temperate soils (Cochran et al., 2007), and 2 to 4% in tropical soils (Espinoza, 2004; Espinoza et al., 2007).



FIGURE 5. Concentrations and proportions of stable C in macro- and micro-aggregates as influenced by soil order in soils of the Río Grande de Arecibo watershed, Puerto Rico. An asterisk (*) within an aggregate size indicates statistical difference (P < 0.05) between soil orders.

CONCLUSIONS

Although aggregate silt+clay content did not predict soil TOC, the silt+clay content in bulk soil was a predictor of soil TOC in soils under forest and cultivation. The difference between the upper and lower C levels for a given texture represents an estimate for the maximum potential C sequestration possible of soils in this watershed. Soils under agriculture and those classified as Inceptisols had decreased soil C content probably as a result of the lower soil C concentration within the aggregates as compared to those under forest and classified as Ultisols. Inceptisols and Ultisols of the RGA watershed have similar C concentrations in the smaller aggregate size classes, except in the large macroaggregates. The decreased aggregation observed in the large macroaggregates of Inceptisols could not be explained by reduced C concentration, nor decreased silt+clay content in aggregates but rather because of presumably lower proportion of 1:1 clays and oxides which serve as binding agents for aggregate formation. Cultivated soils had a lower proportion of large macroaggregates relative to that of forest soils and had lower soil organic C content, probably as a result of increased macroaggregate turnover due to soil disturbance.

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