Resistance of Soil Organic Matter in “Humic A Horizons” of the Mountainous Region in the State of Rio De Janeiro

Ademir Fontana¹, Marcos Gervasio Pereira², Fabiano de Carvalho Balieiro¹, Cesar da Silva Chagas¹, Guilherme Kangussu Donagemma¹, Lucienne Silva de Oliveira³

¹Embrapa Solos – CNPS, Rio de Janeiro/RJ, Brasil
²Universidade Federal Rural do Rio de Janeiro – UFRRJ, Seropédica/RJ, Brasil
³Independent researcher, Mangaratiba/RJ, Brasil

ABSTRACT
Soils with high organic carbon content, such as those with “humic A horizon”, occur in different regions of Brazil. This study aimed to determine the quantitative and qualitative characteristics of organic matter regarding the humic A horizon under different land uses in Bom Jardim, in Rio de Janeiro State, Brazil. Samples from forest, pasture, Eucalyptus plantation, coffee cultivation, olericulture, and also passion fruit soils were sampled and analyzed. In October 2011, undisturbed samples from humic A horizons, were collected for soil density analysis. In addition, disturbed samples were collected for the determination of organic carbon (C_{org}) content, total carbon, humic substance fractions contents and natural abundance of {^{13}}Carbon (δ^{13}C). Low C_{org} contents were observed in areas under olericulture, pasture and passion fruit cultivation. The fulvic acid fraction was higher than other fractions, regardless of land use. The small variability in δ^{13}C indicated maintenance of original C_{org} and suggest high resistance of Soil Organic Matter in humic A horizons.

Keywords: organic carbon, humic substances, isotopic composition.
1. INTRODUCTION

Mountain farming systems are very common worldwide. Although generally small-scale and family-run systems ensure food security for thousands of people, as well as protect the environment, in other words, beautify rural landscapes, these systems also provide various ecosystem services (Kohler & Romeo, 2014). Mountain areas, which vary greatly in terms of relief patterns, soils, and cultures, are vulnerable to degradation. Some soil classes are more resilient to organic matter loss owing to agricultural interventions and low-temperature effects (Buol & Eswaran, 2000). Cultural practices, such as no-tillage farming, crop rotation and agroforestry, stabilize macroaggregates in the soil and reduce carbon loss.

In the state of Rio de Janeiro, Brazil, mountain farming is frequently carried out in soils with humus-rich topsoil, that is, in humic A horizons. According to the Brazilian Soil Classification System, the humic A horizon is a thick, dark-colored layer with high organic carbon content and low base saturation (Santos et al., 2018). These unique characteristics are a reflection of soil formation, landscape evolution and soil properties and processes, such as aeration, gas exchange, microbial activity and diversity, root growth, cation exchange capacity (CEC), and nutrient cycling (Doran & Parkin, 1994; Feller & Beare, 1997; Haynes, 2000; Dominati et al., 2010).

This study hypothesizes that the resilience of the humic A horizon is affected by agricultural practices, leading to modifications in the level and composition of SOM in areas subject to high-impact management practices. To test this hypothesis, the aim of this work was to evaluate the quantitative and qualitative characteristics of SOM in the humic A horizon under different uses in the state of Rio de Janeiro, Brazil.

2. MATERIAL AND METHODS

This study was carried out in Bom Jardim, located in the Pito Aceso microbasin (22°15’17” and 42°18’09”) in the mountainous region of the state of Rio de Janeiro, Brazil. The local relief is characterized by rugged topography including slopes of various altitudes and narrow valleys; the elevation varies between 640 and 1,270 m above sea level. More information on local relief, geology, climate, vegetation, and land uses (P05, Eucalyptus plantation; P09, olericulture; P12, pasture; P13, coffee cultivation; and P35, passion fruit cultivation) can be found in Chagas et al. (2012) and Fontana et al. (2017).

Six areas under different uses were selected: 1) eucalyptus (Eucalyptus sp.) - a 3-year-old plantation associated with leaf litter covering the soil surface; 2) olericulture - “cassava” (Manihot esculenta) cultivation was being managed using mechanical tillage and weed control practices were established; 3) pasture (Brachiaria decumbens) that was put out under moderate grazing; 4) coffee (Coffea arabica) planted in rows; 5) passion fruit (Passiflora edulis) planted in rows; and 6) a fragment of Atlantic forest at a medium stage of regeneration (Figure 1).

The soil sampling and the field evaluations were performed in October 2011. In each area, a trench was opened and the horizons were separated. Samples were collected and described morphologically according to Santos et al. (2013) and classified according to the Brazilian Soil Classification System (Santos et al., 2018). From each horizon, undisturbed samples were collected using a 100 cm$^3$ ring for the analysis of soil density, and disturbed samples were collected for the determination of organic carbon ($C_{org}$), content, total carbon (TC) content, humic substance fractions, and carbon-13 natural abundance ($\delta^{13}C$).
Undisturbed soil samples were oven dried at 105 °C for 24 h. Soil density was calculated by dividing soil dry weight by the ring volume. Disturbed samples were oven dried at 50 °C, de-clumped, and sieved through 2 mm mesh sieve. C$_{org}$ content was determined by wet oxidation with potassium dichromate ($K_2Cr_2O_7$) solution in acidic medium and titration of ferrous ammonium sulfate (Mohr's salt), both described in Teixeira et al. (2017). TC content was determined by the dry combustion method. Briefly, 0.5 g of the sample was combusted at 1200 °C under oxygen (O$_2$) atmosphere (99.97%). Carbon dioxide (CO$_2$) was quantified using a non-dispersive infrared detector (multi EA® 2000, Analytik Jena, Jena, Alemanha). Calcium carbonate (CaCO$_3$) with a carbon (C) content of 120 g kg$^{-1}$ was used as standard. The C stock was determined according to Ellert & Bettany (1995).

Chemical fractionation of soil organic matter was carried out in acidic and basic media, and C$_{org}$ content was determined in the Fulvic Acid (FAF), Humic Acid (HAF), and humin (HUMIN) fractions (Benites et al., 2017). Briefly, 1.0 g of soil sample was added to 20 mL of 0.1 mol L$^{-1}$ sodium hydroxide (NaOH) solution after 24 h, the alkaline extract was separated from the residue by centrifugation at 5,000 g for 30 min. Another extraction was performed, resulting in a final volume.
of alkaline extract of 40 mL. The pH of the alkaline extract was adjusted to 1.0 ± 0.1 using 20% of sulfuric acid (H₂SO₄) solution. The mixture rested for 18 h in a refrigerator, and the precipitate (HAF) was separated from the soluble fraction (FAF) by filtration. Samples were completed to 50 mL with distilled water.

The C₉org contents of FAF and HAF were determined by adding 1.0 mL of 0.042 mol L⁻¹ K₂Cr₂O₇ and 5.0 mL of concentrated sulfuric acid (H₂SO₄) to 5.0 mL of each extract, placing the solutions in a block digester at 150 °C for 30 min, and subsequently titrating with 0.0125 mol L⁻¹ ferrous sulfate ammonium. The insoluble fraction (HUMIN) was oven dried and added to 5.0 mL of 0.1667 mol L⁻¹ K₂Cr₂O₇ and 10.0 mL of concentrated sulfuric acid. Block digestion was carried out at 150 °C for 30 min, followed by titration with 0.25 mol L⁻¹ ferrous ammonium sulfate and ferroin indicator solution (Yeomans & Bremner, 1988). The C₉org contents of FAF, HAF, and HUMIN were determined, and the HAF/FAF ratio calculated (Benites et al., 2017). The percentage contribution of each fraction to TC content was also determined (%FAF, %HAF, and %HUMIN).

The natural abundance of ¹³C was determined using a mass spectrometer and the international standard Pee Dee Belemnite (PDB) was also used (Craig, 1957). Results are expressed as δ¹³C, according to the equation

\[ \delta^{13}C‰ = 10 \times \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \]

where \( R_{\text{sample}} \) is the ¹³C/¹²C ratio of the sample and \( R_{\text{standard}} \) is the ¹³C/¹²C ratio of the standard.

### 3. RESULTS AND DISCUSSION

#### 3.1. Variability in soil C₉org content and humic properties

C₉org contents in soil ranged from 10.5 to 30.1 g kg⁻¹ (Table 1). C₉org values were highest in shallow horizons, however, the C₉org values have been decreasing according to the soil depth (Table 1). It was found that the variation of the C₉org content is not consistent with land use or

| Uses                | Horizon | Depth cm | Corg g kg⁻¹ | FAF % | HAF % | HUMIN % | HAF/FAF |
|---------------------|---------|----------|-------------|-------|-------|---------|---------|
| Eucalyptus plantation | Ap      | 0-18     | 30.1        | 17    | 23    | 46      | 1.4     |
|                     | A1      | 18-45    | 22.8        | 12    | 26    | 45      | 2.1     |
|                     | A2      | 45-85    | 22.8        | 12    | 15    | 39      | 1.3     |
|                     | AB      | 85-100   | 14.8        | 11    | 16    | 39      | 1.4     |
| Olericulture         | Ap      | 0-40     | 15.6        | 22    | 20    | 29      | 0.9     |
|                     | A1      | 40-65    | 10.5        | 25    | 14    | 26      | 0.6     |
|                     | A2      | 65-80    | 11.1        | 11    | 16    | 66      | 1.5     |
|                     | AB      | 80-102   | 10.9        | 25    | 23    | 53      | 0.9     |
| Pasture              | Ap      | 0-23     | 15.9        | 15    | 18    | 26      | 1.2     |
|                     | A1      | 23-58    | 11.1        | 22    | 13    | 46      | 0.6     |
|                     | A2      | 58-95    | 11.0        | 15    | 18    | 25      | 1.2     |
| Coffee cultivation   | Ap      | 0-19     | 23.1        | 20    | 13    | 61      | 0.6     |
|                     | A1      | 19-32    | 20.5        | 18    | 13    | 43      | 0.7     |
|                     | A2      | 32-60    | 20.6        | 9     | 13    | 31      | 1.5     |
|                     | AB      | 60-79    | 12.9        | 26    | 20    | 25      | 0.8     |
| Passion fruit        | Ap      | 0-20     | 17.2        | 13    | 13    | 31      | 1.0     |
|                     | A1      | 20-38    | 15.8        | 15    | 15    | 29      | 1.0     |
|                     | A2      | 38-61    | 17.2        | 24    | 26    | 11      | 1.1     |
|                     | AB      | 61-85    | 10.9        | 15    | 23    | 43      | 1.6     |
| Forest               | A1      | 0-22     | 27.3        | 8     | 6     | 79      | 0.8     |
|                     | A2      | 22-40    | 27.1        | 8     | 6     | 76      | 0.7     |
|                     | A3      | 40-65    | 22.1        | 10    | 9     | 57      | 0.9     |
|                     | AB      | 65-75    | 12.8        | 13    | 15    | 49      | 1.1     |

Corg = organic carbon; FAF = fulvic acid fraction; HAF = humic acid fraction; HUMIN = humin fraction.
type of crop. For instance, $C_{org}$ values were in average 11%, 29%, 42% and 50% lower in soils under coffee cultivation, passion fruit, olericulture and pasture, respectively, than in soil under forest (Table 1 and Figure 2). In other hand, the humic A horizon from *Eucalyptus* plantation presented as an average of 3% more $C_{org}$ than soil under forest (Table 1).

These differences in $C_{org}$ content between soil layers can be attributed to variability in root contribution between areas (root/shoot ratio), such as, the effects of root exudates on soil microbial activity, and differences in water vapor permeability (Wang et al., 2018; Sokol & Bradford, 2019). It seems evident that a diverse forest ecosystem allows greater C stabilization at depth, and these data corroborated with the role of forests in C sequestration (Bonan, 2008). Nevertheless, $C_{org}$ contents in soils under pasture and crop correspond to SOM contents within the normal range for fertile soil (Freire et al., 2013), indicating good stability or resilience of $C_{org}$ in the sampled profiles.

In previous studies, Calegari (2008) and Chagas et al. (2012) observed in the same humic A horizon from the Southern, Southeastern and Northeastern regions similar variations and $C_{org}$ values between 5.9 and 68.9 kg g$^{-1}$ (Figure 2). In the Agreste region of Pernambuco in Brazil, Pessoa et al. (2012) also found variations in $C_{org}$ content among soils under different uses. In their study, $C_{org}$ values ranged from 15.0 to 43.7 g kg$^{-1}$ at the 10 cm depth. Native forest soils had the highest $C_{org}$ content, followed by soils under "capoeira" vegetation, 25- and 30-year old pastures and annual cropping systems.

Although the use of agriculture is admittedly deleterious to soil C stocks (Carvalho et al., 2010; Coutinho et al., 2014), in pastures, I could be found distinct results when compared to other authors who reviewed the world literature (Guo & Gifford, 2002), or even had been worked more locally (in the Atlantic Forest biome) (Tarré et al. 2001; Cerri et al., 2007), as they detected an increase in C levels and stocks after converting forest to pasture; on the other hand, this change has not occurred for soils with humic A horizon. The evidence of susceptibility to C-CO$_2$ loss from these soils seemed to be relevant for these soils, but more soils must be sampled and characterized.

The analysis of humic substance provides information on the degree of humification and C stability, complementing the results of SOM and $C_{org}$ analysis. There was wide variability in humic properties within and between soil fractions, regardless of land use or crop type. HUMIN varied from 11 to 79%, but most results were above 25%. HAF ranged from 6 to 26%, and FAF from 8 to 25% (Table 1). The HAF/FAF ratio is an indicator of the quality and origin of SOM. An HAF/FAF ratio greater than 1.0 for surface horizons indicates that soil use or changes in vegetation cover have been decreased the supply of fresh organic material into the soil. Surface horizons had lower HAF/FAF ratios than deeper horizons. Forest, olericulture, and coffee cultivation areas had topsoil HAF/FAF ratios below 1.0.

TC contents were used to estimate the C stock in humic A horizons (Table 2). The C stock varied greatly between sites: soil under passion fruit cultivation showed a C stock of 162.64 Mg ha$^{-1}$, whereas soil under *Eucalyptus* plantation had a C stock of 284.69 Mg ha$^{-1}$. These values are higher than those reported in previous studies for soils subjected to different uses in low-altitude regions.

![Figure 2. Depth distribution of the organic carbon contents in humic A horizons in "Pito Aceso" and in other municipalities of the state of Rio de Janeiro. (1) Present study; (2) Calegari (2008); (3) Chagas et al. (2012).](image-url)
The C stock changes in the humic A horizon can be a result of several factors, including inherent characteristics related to the formation of the soil, as well as past and present land use and management practices—from wood harvesting since colonization and coffee and cattle production days to modern times.

Calegari (2008) analyzed C contents in humic A horizon of other regions in the state of Rio de Janeiro and estimated higher TC stocks values than those found in this study: 368.84 Mg ha\(^{-1}\) for pasture and 613.82 Mg ha\(^{-1}\) for secondary forest. The author has also studied soil profiles submitted to different uses in other regions of the country, and this author could find TC stock values ranging from 251.12 to 500.82 Mg ha\(^{-1}\). This large variability in C stock shows that soils with humic A horizon differ greatly in C accumulation potential, in agreement with their differences in formation, vegetation cover, landscape evolution history, and water erosion susceptibility.

### 3.2. \(^{13}\)C natural abundance in Latossolos with humic A horizon

\(^{13}\)C values ranged from -25.73 to -21.33‰ (Figure 3). It was found that both past (deep horizons) and present vegetation (surface horizon) were formed predominantly, but not exclusively, by C\(_3\). A combination of C\(_4\) and C\(_3\) plants was detected in all soils. Calegari (2008) reported similar data in a study that evaluated the occurrence and paleoenvironmental significance of A humic horizon in Latossolos (Oxisols). That study, by evaluating the data of SOM isotope and phytolith assemblages indicated that this type of soil was formed under a less dense vegetation than the present one. Probably, owing to the mixture of C\(_3\) and C\(_4\) (\(-22\)%) plants and to the more contribution of C\(_3\) in the Southeast region. In addition, the author concluded that, from the late Holocene, it could be

Table 2. Total carbon and carbon stock from humic A horizons and total stock in each study area in the “Pito Aceso” microbasin, situated in Bom Jardim, in the state of Rio de Janeiro, Brazil.

| Uses         | Horizon | TC g kg\(^{-1}\) | BD Mg m\(^{-3}\) | Horizon Stock Mg ha\(^{-1}\) | Total Stock Mg ha\(^{-1}\) |
|--------------|---------|------------------|-----------------|-----------------------------|----------------------------|
| Eucalyptus   | Ap      | 45.0             | 0.79            | 63.99                       | 284.69                     |
|              | A1      | 31.4             | 0.82            | 69.52                       |                            |
|              | A2      | 30.3             | 0.92            | 111.50                      |                            |
|              | AB      | 22.8             | 1.16            | 39.67                       |                            |
| Olericulture | Ap      | 19.6             | 1.07            | 83.89                       | 201.47                     |
|              | A1      | 18.5             | 1.09            | 50.41                       |                            |
|              | A2      | 16.8             | 1.28            | 32.26                       |                            |
|              | AB      | 12.8             | 1.24            | 34.92                       |                            |
| Pasture      | Ap      | 19.2             | 1.25            | 55.20                       | 176.26                     |
|              | A1      | 13.3             | 1.15            | 53.53                       |                            |
|              | A2      | 16.9             | 1.08            | 67.53                       |                            |
| Coffee       | Ap      | 26.6             | 1.08            | 54.58                       | 208.10                     |
| cultivation  | A1      | 25.3             | 1.13            | 37.17                       |                            |
|              | A2      | 23.0             | 1.21            | 77.92                       |                            |
|              | AB      | 15.8             | 1.28            | 38.43                       |                            |
| Passion      | Ap      | 22.4             | 1.01            | 45.25                       | 162.64                     |
| fruit        | A1      | 20.6             | 1.00            | 37.08                       |                            |
|              | A2      | 22.7             | 0.91            | 47.51                       |                            |
|              | AB      | 13.4             | 1.02            | 32.80                       |                            |
| Forest       | A1      | 32.1             | 0.89            | 62.85                       | 185.51                     |
|              | A2      | 29.8             | 0.94            | 50.42                       |                            |
|              | A3      | 22.4             | 1.02            | 57.12                       |                            |
|              | AB      | 12.7             | 1.19            | 15.11                       |                            |

TC = total carbon; BD = bulk density.
found a more $^{13}$C depleted values ($\sim 25\%$), suggesting the expansion of the tropical and subtropical forests, probably associate to a humid and warm climate.

The small variability in $\delta^{13}$C indicated maintenance of original C, as even secondary forest areas converting into grass areas for 20 or 30 years had low C$_4$ contribution. Kuzyakov et al. (2000) described soil effects whereby changes in organic matter turnover that occurs due to soil interventions, such as mechanical disturbance or addition of fertilizers, crop residues, or organic compounds. According to an ecological perspective, negative priming effects are more important than positive one because they have an impact on original C conservation. Thus, it is important to consider the original C stocks equivalent to those found in forest fragments, a characteristic that can be observed is the resistance of these soils to C losses.

4. CONCLUSIONS

Soils under intensive agriculture, such as pasture and olericulture systems, had a greater C loss than soils under secondary forest cover or Eucalyptus plantation. There was wide variability in humic substance fractions between and within areas and horizons. Surface layers had higher FAF, regardless of land use or crop type. $\delta^{13}$C values showed little variability between soils under different uses, which indicates relative stability of the landscape and of the maintenance of soil C quality, with relative resistance of organic matter in the humic A horizon. Efforts in agriculture conservation practices should be encouraged to minimize C losses in these soils, especially regarding the perspective of global warming.

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CORRESPONDENCE TO

Ademir Fontana
Embrapa Solos – CNPS, Rua Jardim Botânico, CEP 22460-000, Rio de Janeiro, RJ, Brasil
e-mail: ademir.fontana@embrapa.br

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