Vegetation influence on organic matter source of black soils from high altitude rocky complexes traced by $^{13}$C and $^{15}$N isotopic techniques

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ABSTRACT

The organic soil layer (0–35 cm) of seventeen sites under high altitude rocky complexes (HARC) was sampled in eastern Brazil to evaluate the relationship of vegetation type and carbon and nitrogen isotopic composition of bulk soil and humic acid (HA). The $\delta^{13}$C and $\delta^{15}$N values obtained for bulk soil and for HA (extracted with NaOH 0.1 mol L$^{-1}$) were not statistically different (p<0.05). Also, the high C to N ratio, and lower E/Eg and TGI corroborate with the hypothesis that bulk soil organic matter (SOM) and HA have similar characteristics and high recalcitrance. The majority of sites in the HARC presented a soil organic matter originated from C3-vegetation and demonstrated an intense N cycling based on the $\delta^{15}$N data. Even though charcoal could be suggested as a component of soil genesis for most of the HARC sites, the raised data was not consistent to consider fire a key process for soil organic matter existence in these areas.

1. Introduction

High altitude rocky complexes (HARC) are sheltering fragments of Atlantic Forest and then represent hotspots in terms of biodiversity (Martinelli, 1996; Myers et al., 2000). In addition, this marginal vegetation bordering the rainforest is believed to contribute for the function of the whole ecosystem (Scarano, 2002).

The geographical isolation leads HARC to a high endemism, with a flora well adapted to both nutritional and water limitations (Joly, 1970). Fire-tolerant plants are commonly found in these environments (Safford, 1999), suggesting a long-term evolution of vegetation under a natural fire pressure, or even by fire dependence (Rizzini, 1979). This sort of disturbance regime seems to be a key-control of soil organic matter (SOM) formation (Benites et al., 2005). According to Benites et al. (2005), the high aromatic and condensation degree of SOM from HARC may be associated to charcoal, which could figure as the main source of C for the humic substance genesis in these soils. Hence, the knowledge on how vegetation type and disturbance events can contribute to SOM formation would help in understanding how potential changes in disturbance regimes (anthropogenic or natural) would affect soil functions as the support of the environment resilience.

The natural abundance of $^{13}$C and $^{15}$N in the soil can be used to evaluate the origin of soil organic matter and the relative importance of N sources to plant nutrition (Balesdent et al., 1987; Martinelli et al., 1999; Reis et al., 2010; Robinson, 2001). The photosynthetic process in plants discriminates $^{13}$C isotope against the lighter isotope $^{12}$C at the carboxylation step (Farquhar et al., 1982; McCarrol and Loader, 2004). This discrimination is higher in C3 (Calvin cycle) than in C4 (Hatch–Slack cycle) plants, which brings about a $^{13}$C isotopic signature for each material that is relatively well preserved after decomposition in soil (Natelhoffer and Fry, 1988). Based on these differences, $^{13}$C natural abundance analysis of soil organic matter (SOM) can be associated or not to soil age (radiocarbon dating) to identify the chronology and variation in vegetation cover (Balesdent et al., 1988; Campos et al., 2010; Horak et al., 2011; Neill et al., 1997; Sisti et al., 2004). Furthermore, different sources of N accessed by plants or the relative contribution of different N cycle processes (loss or supply) could be identified by $^{15}$N isotopic analysis (Högberg, 1997; Peoples et al., 1989; Robinson, 2001).

Little is known about the biogeochemistry cycles in HARC environments, especially on how plants and the environment affect C stabilization in soil. As natural abundance of $^{13}$C of soils is mostly a consequence of C source, this isotopic technique can help in evaluating the...
hypothesis that open fields (as rock outcrops), dominated by grass and shrubs present soil organic matter strongly influenced by fire events (natural or anthropogenic) that are known to occur with a certain frequency.

The objective of this study was to evaluate the relationship of vegetation type and soil organic matter properties of seventeen Brazilian HARCs by the use of natural abundance of $^{13}$C and $^{15}$N of bulk soil and soil humic acids.

2. Material and methods

2.1. Site and soils characterization

The study was performed by sampling 17 upper horizons of soils from High Altitude Rocky Complexes (HARC) existing in four conservation units at the Espinhaço and Mantiqueira Ranges, which have, respectively, quartzite and igneous rocks (granites and gneisses) as the predominant lithology (Benites et al., 2001; Benites et al., 2005; Simas et al., 2005). Some other characteristics could be observed in Table 1.

Six composite soil samples were taken from the Mitra do Bispo (SV), nine from the National Park of Itatiaia (IT), one from the National Park of Chapada Diamantina (CD) and another one from the State Park of Diamantina (DI). Soils under SV and IT showed a slightly higher silt and clay content than soils developed in CD and DI. Both soils are characterized by the low levels of soil fertility, which is related to nutrient losses by leaching, especially in the case of quartzite areas (Benites et al., 2007).

2.2. Vegetation

Forest, rocky outcrop and field were the three different phytosociomorphological samples. The forest comprised a mosaic of shrubs, especially species of Asteraceae family such as Baccharis spp. and Vernonia spp., and several representatives of Eupatorieae and Melastomataceae tribes, such as Tibouchina and Leandra, as well as species of the Myrtaceae family and small trees, such as Escallonia (Convulvulaceae), Weinmannia (Cunoniaceae), Rapanea (Myrsinaceae), Symplocos (Styrelaceae), Maytenus (Celastraceae) and Roupala (Proteaceae) (Safford, 1999). Rocky outcrops were dominated by shrubs and subshrubs of Velloziaceae, Asteraceae and Melastomataceae families, and some species of Poaceae, with herbaceous vegetation of about 1 m height. For the field phytosociology a large numbers of individuals belonging to Poaceae, Cyperaceae and Eriocaulaceae, and some Xyridaceae and Velloziaceae were observed.

2.3. Humic acids extraction and purification

Humic acids (HA) extraction was described by Benites et al. (2005), and followed the International Humic Substances Society procedure (Swift, 1996). Briefly, a quantity of dried soil sieved to 2 mm were shacked for 24 h with 0.1 M NaOH under N$_2$ atmosphere to extract the HA. Then, the material was centrifuged at 10,000 g for 30 min, with the supernatant being separated and mixed with a needed volume of 6 M HCl to adjust the pH to 2.0. After 18 h, the excess of supernatant was discarded and the remaining material centrifuged at 5000 g for 10 min, and only reserving the precipitate. The precipitate was dissolved again in 200 mL of 0.1 M NaOH under N$_2$ atmosphere and centrifuged at 10,000 g for 30 min. The solution was collected and the pH was immediately adjusted to 2 with 6 M HCl. The acidified solution was centrifuged at 5000 g for 10 min. The precipitated HA was mixed with 0.5% HF + HCl solution, left to rest for 24 h and centrifuged at 5000 g, discarding the supernatant. This process was repeated twice. The purified samples were washed with 200 mL of 0.01 M HCl, followed by centrifugation at 5000 g and transferred for 100 mL cellophane bags, dialyzed and lyophilized.

2.4. Carbon and nitrogen isotopic analysis

Bulk soil and HA samples were oven dried at 65 °C, ground in ball mill, and analyzed for C and N contents and $^{13}$C and $^{15}$N natural abundances, using an elemental analyzer coupled to a mass spectrometer Finnigan Mat Model Delta-E. Results of natural abundance of $^{13}$C and $^{15}$N were expressed in delta units, calculated as $\delta^{13}C$ or $\delta^{15}N = [(R_{Sample}/R_{Standard}) - 1] \times 1000$ (‰), where R$_{Sample}$ and R$_{Standard}$ are the ratios of $^{13}C/^{12}C$ (or $^{15}N/^{14}N$) of the study samples and the reference standard, respectively.

2.5. Other attributes

Apart from the C, N and the respective stable isotopes, soil organic matter and HA were characterized by optical density analysis also known as E4/E6 ratio and by thermogravimetry index (TGI). Aliquots of 100 mg of bulk soil or HA were taken to prepare 1 L solution in 0.1 M NaHCO$_3$. Optical densities were recorded spectrophotometrically at 465 and 665 nm ($E_4$ and $E_6$, respectively) and the second derivative was obtained from the spectra, from which the $E_4/E_6$ ratio for each sample was calculated (Miralles et al., 2012). Low $E_4/E_6$ ratios are associated with the presence of high aromatic characteristics of organic compounds. The TGI was obtained for bulk soil and HA by thermo-decomposition curves performed in a thermo-gravimeter TGA-50 (Shimadzu), with the initial sample weight stabilized at 30 °C. A heating curve was obtained after incrementing the temperature at 5 °C min$^{-1}$ up to 105 °C, which was held for 10 min, and a further heating at 5 °C min$^{-1}$ up to 650 °C. The weight loss of samples relative to the two burning events is the TGI (Benites et al., 2005), which represents the resistance of the HA or soil organic matter to thermal degradation.

2.6. Statistical analysis

The obtained data were analyzed using descriptive statistics (mean and standard error) and Pearson's correlation. Comparisons between mean values of C, N, $\delta^{13}C$ and $\delta^{15}N$ of bulk soil and humic acid were performed by the Student t-test at 0.05 probability.

3. Results and discussion

The average soil C content in samples taken from the different sites was 173 g C kg$^{-1}$, with a high variation among the three phytosociomorphological samples (79 g C kg$^{-1}$ mean standard error), which was probably influenced by contrasting soil texture and fertility (Table 2) and also by climate constraints related to altitude (Table 1).
In the forest soils, soil C content was 148 ± 23 g C kg⁻¹ and in the altitudinal fields 77 ± 20 g C kg⁻¹, the latter significantly different from the former (p<0.05). Net primary production was not evaluated for the specific sites but it is certainly the reason for the differences in soil C stocks intra and inter vegetation physiognomies (McCulley et al., 2004).

Soil N contents for the altitudinal field, rocky outcrop and forest were 5.0 ± 1.2 g C kg⁻¹, 9.4 ± 1.3 g C kg⁻¹ and 8.0 ± 2.9 g C kg⁻¹, respectively. These figures tended to follow the differences observed for soil carbon. The C to N ratios of soil organic matter were about 15 for soil C stocks intra and inter vegetation physiognomies (McCulley et al., 2000; Bustamente et al., 2004; Natelhoffer and Fry, 1988), presented a δ¹³C signal for the bulk soil lower than −20.0‰ (Fig. 1) that can be grouped as sites under predominant influence of C₃ vegetation (Deines, 1980). The other sites were grouped in function of the less negative δ¹³C signals, which is explained by the existence of a mixture of species presenting C₄, C₃ and crassulacean acid metabolism (CAM) pathways, but with little C₃ influence (Benites et al., 2003; Conceição and Pirani, 2007). These differences in signs could be attributed to recent vegetation dynamics once some peat bog at the Mountain Range of Espinhaço Meridional have been presenting a vertical growth rate (Campos et al., 2010). In addition, soil samples were taken mainly from soil layers not deeper than 20 cm, where δ¹³C changes are likely to be more easily detected. Fire and soil transformations are normally responsible for slight differences in the δ¹³C signal in soils (Bird et al., 2000; Bustamente et al., 2004; Natelhoffer and Fry, 1988), but considering the composition of vegetation of the sampled areas and the wide range of δ¹³C signal among the sampled sites, it seems feasible to assume that the enrichment caused by both process are less important than the nature of plant residues being deposited onto the soils.

The values of δ¹³C obtained for the bulk soil and for HA were similar (p<0.05), corroborating the hypothesis that partially decomposed organic matter and humus of HARC have the same origin. Also, the results suggest the procedure used for HA extraction do not provoke a significant discrimination of ¹³C for the HARC soils. The sandy texture together with the relatively high C content of the studied soils is an important factor to consider the ¹³C fractionation was insignificant (Prentice and Webb, 2010).

The thermogravimetric index (TGI) and E⁰/Eₑ ratio that describe the resistance of organic compounds to thermal degradation and complexity of molecules that composites it, demonstrate greater variability among sites, and no pattern of vegetation and recalcitrant soil organic matter formation could be highlighted (Fig. 1). Fire frequency, position on the landscape and floristic composition are possible confounding factors and are interesting research lines to be explored. Nonetheless, the characteristics of high aromaticity with nucleus of high condensation degree revealed by the E⁰/Eₑ ratio (Table 2), especially for the soil formed on quartzite (Benites et al.,

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**Table 2**

Sites characteristics, soil and humic acids properties.

<table>
<thead>
<tr>
<th>Identification</th>
<th>Vegetation</th>
<th>Lithology</th>
<th>Soil¹</th>
<th>Depth</th>
<th>Bulk soil</th>
<th>Humic acids (HA)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>¹³C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td>g kg⁻¹</td>
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<td>0–18</td>
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<td>Histic epipedon</td>
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<td>5.9</td>
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<td>Umbric epipedon</td>
<td>0–25</td>
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<td>Histic epipedon</td>
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<td>−24.9</td>
<td>4.0</td>
</tr>
<tr>
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<td>Histic epipedon</td>
<td>0–20</td>
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<td>Umbric epipedon</td>
<td>0–35</td>
<td>−19.3</td>
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<td>Histic epipedon</td>
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</tr>
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<tr>
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<td>Histic epipedon</td>
<td>0–35</td>
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<td>5.0</td>
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<td>−24.7</td>
<td>1.6</td>
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<tr>
<td>CD7</td>
<td>Rocky outcrop</td>
<td>Quartzit</td>
<td>Umbric epipedon</td>
<td>0–15</td>
<td>−25.9</td>
<td>0.3</td>
</tr>
</tbody>
</table>

¹ Classification according to the soil taxonomy (Soil Survey Staff, 1999).
² C: carbon; N: nitrogen; E₀/Eₑ: visible ratio; and TGI: thermogravimetric index.

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**Fig. 1.** Dispersion of thermogravimetric index (TGI) and natural abundance of ¹³C (%). Squares indicate average values from two main pattern of vegetation (values below −20‰, predominance of plant with C₃ metabolism and above predominance of plants with C₄). Standard deviation for each variable are also indicated.
suggest charcoal could be a key-component in these soils. Other authors (Haumaier, 1995; Kumuda, 1983) also discussed that fragments form charcoal added to soil may be a source of carbon for humic substances genesis. The natural abundance of ¹⁵N in the bulk soil ranged between 0.31 and 6.74‰, with the predominance of values higher than 3.88‰ (15 of 17 samples). The two exceptions were samples from DI and CD, under rock outcrop fields and quartzite, which presented signals of 1.59 and 0.31‰, respectively. Several authors cited that a more opened nitrogen cycle generates more ¹⁵N enriched soils due to fractionation processes related to N losses (nitrification, ammonia volatilization, nitrate leaching and denitrification) (Martinelli et al., 1999; Penuelas et al., 1999). Therefore, most of the studied areas showed an intense N cycling. On the other hand, Martinelli et al. (especially in sandy soils) are normally depleted in ¹⁵N and this pattern could be associated to the presence of nitrogen fixing species. Salati et al. (1982) and Peoples et al. (1989) demonstrated previously that areas receiving significant contributions of N₂ fixing have a δ¹⁵N close to zero. It is possible that for both sites at DI and CD, where C₄ plants are the major vegetation component (−24.71 and −25.87‰ δ¹³C), the N₂ fixation had been influenced by oligothrophic conditions. It was not easily seen from the species composition at the moment of soil sampling, and studies associating spatial distribution of vegetation with photosynthetic pathways and soil characteristics should be encouraged to the better knowledge of the role of vegetation evolution and biogeochemical cycles.

A negative correlation found (−0.52, p = 0.026) between δ¹⁵N and soil C to N ratio indicates that the ¹⁵N enrichment of soil N can be related to the humification of soil organic matter. This trend was also confirmed with the E₄/E₆ ratio data that also correlated negatively with δ¹⁵N (−0.67, p = 0.002) as the increase in E₄/E₆ ratio is an indication of weakly-condensed molecules (Miralles et al., 2012) that could indicate low microbiologically processed soil organic matter.

4. Conclusion

The majority of sites in the HARC presented a soil organic matter originated from C₄-vegetation, as depicted from δ¹³C data. But there were some sites where the soil organic matter was formed also from species of C₃ and CAM metabolic pathways suggesting a simultaneous occurrence of such species or a recent change in composition due to natural or anthropogenic disturbance. Evidences of calcitrant organic matter came from the E₄/E₆ and TGI data suggesting high persistence in these soils. The C to N ratio was variable and the greater values seemed to be associated to the presence of low processed organic C more than to charring. Also, the majority of areas sample demonstrated an intense N cycling based on the δ¹⁵N data which suggests that soil C is a result of humification independent of charcoal formation.

Even though charcoal could be suggested as a component of soil genesis for most of the HARC sites, the raised data was not consistent to consider natural or anthropogenic fire as a key process for soil organic matter formation in these areas.

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References


References (Continued)

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