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STRUCTURAL CHARACTERISTICS OF SOIL HUMIC ACIDS IN COFFEE-CULTIVATION SYSTEMS: ^{13}C NMR AND ANALYTICAL PYROLYSIS

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Abstract – Structural characterization of soil organic matter is still poorly understood in Brazilian agroecosystems. We aimed to identify the qualitative changes of soil humic acids as influenced by agroforestry- and full sun-coffee systems in the Atlantic Coastal Rainforest domain. The study was carried out in the Zona da Mata located in the state of Minas Gerais, Brazil. The specific studied sites were: agroforestry-coffee (AGF), Full sun coffee (FSC) and Native forest (NF) which was selected and used to determine the native or steady state condition of soil. Soil humic acids were extracted by using basic-acid medium, purified, and freeze-dried to a solid state to perform ^{13}C NMR and analytical pyrolysis analyses. ^{13}C NMR assignment of soil HAs from FSC system suggests an intensification of the process of humification of HAs structure. The higher aromaticity index of HAs from FSC compared to the AGF system confirmed this result. The relative abundance in each specific pyrolitic group was similar among the different land use, with exception to the higher amounts of lignin-like and lipids compounds in AGF compared to FSC system, and the higher abundance of N-compounds in FSC (37,0%) than in AGF (21,5%) system. Combining ^{13}C NMR with Pyrolysis is an interesting tool to the study of structural features of HAs. Despite of the discrepancies, both techniques indicated that studied AGF systems favor the genesis of soil HAs with lower degree of humification when compared to FSC system, as a result of the influence of organic residues (fresh and old) from trees.

Keywords: agroforestry; carbon; spectroscopy; Zona da Mata.

INTRODUCTION

Agroforestry systems (AGF) have been shown to enhance soil organic matter (SOM) levels when compared to monoculture agricultural systems (Mendonça et al., 2001), and are considered an interesting option of land use to cope with soil degradation in the Zona da Mata (Souza et al., 2010). Nevertheless, information on the chemical and structural characterization of SOM in areas under agroforestry remains scarce.

There has been a great deal of interest in the utilization of more specific and accurate techniques for characterizing the chemical nature of SOM. Studies have shown that combining the techniques 13C nuclear magnetic resonance with cross-polarization magic angle spinning (^{13}C CP-MAS/NMR) and analytical pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) is a valuable tool for structural studies on humic substances (Zhang et al., 1999; Buurman et al., 2009).

The constant input and quality of organic residues derived from tree-component in AGFs, in contrast to monoculture systems (full sun coffee), is expected to have an important effect on the humification processes and consequently on the chemical and structural features of soil humic substances. Our objective was to identify the qualitative changes of soil humic acids as influenced by management (agroforestry coffee versus full sun coffee) in areas under coffee production in the Atlantic Coastal Rainforest domain in the state of Minas Gerais, Brazil.

MATERIALS AND METHODS

General description of studied sites

The study was carried out in the Zona da Mata (about 36,000 km^2), one of most important domain of the Atlantic Coastal Rainforest Biome, located in the state of Minas Gerais, Brazil. The region is characterized by a tropical highland climate (average temperature and precipitation of 18°C and 1500 mm, respectively, with 2 to 4 months of dry period), and altitudes ranging from 200 to 1800 m. The parental rock is gneiss and the dominant soil types are deep and well drained Oxisols.

The specific studied sites were: (a) Agroforestry-coffee (AGF): an area of approximately 3,150 m^2 with 600 coffee-trees (*Coffea arabica* L.) spaced in 3.5 m × 1.5 m. Native tree species were intercropped with coffee. The agroforestry had 15 years old and the main representative tree species was *Luehea grandiflora* Mart. (common name: Açoita-cavalo); (b) Full sun coffee (FSC): The area had approximately 2,000 m^2 with 1,000 coffee trees spaced in 2 m × 1 m. There were no trees intercropped with coffee. Since these areas are considered as smallholding production, intensive tillage or high fertilizers inputs were

not adopted; (c) <u>Native forest</u> (NF): area under native forest was selected and used to determine the native or steady state condition of soil.

13C NMR and Py-GC/MS analyses

Humic acids (HAs) from 0-5 and 5-10 cm soil depths were extracted and separated according to the International Humic Substances Society methodology (Swift, 1996). The precipitated HAs were purified by successive washes of a mixed-solution of 10% HF + 0.1M HCl. After acid treatment, the samples were dialyzed by using cellulose membranes (Visking dialysis tubing 36/32) until free of chlorine and then freeze-dried.

The solid-state ^{13}C NMR spectra of freeze-dried HAs were obtained using a Varian Unity 300 spectrometer operating at a ^{13}C resonance frequency of 75.42 MHz and a ^{1}H frequency of 300 MHz. The experiments were performed using cross-polarization with magic angle spinning (CP-MAS) technique applied with a spectral width of 50 kHz with 90º pulse of 6.7 ms, contact time of 1.5 ms, spinning speed of 4 kHz, a pulse delay of 4 s and acquisition time of 35 ms. For all samples it was performed 20,000 scans.

Pyrolysis (Py) was performed using a double-shot pyrolyzer (Frontier Laboratories, model Y2020iD) attached to a GC/MS system. The thermal desorption was carried out by increasing the initial temperature of 100ºC at a rate of 20ºC min^{-1} to a final temperature of 500ºC held for 1 min. The GC/MS system was composed by an Agilent 6890 gas chromatograph equipped with a fused silica capillary column DB17 J&W Scientific (30 m × 0.25 mm × 0.25µm film thickness), oven temperature was held at 50 ºC for 1 min and then increased up to 100 ºC at 30 ºC min^{-1}, from 100 to 300ºC at 10ºC min^{-1} and isothermal at 300ºC for 10 min using a heating rate of 20ºC min^{-1} in the scan modus. Injector and transfer line temperatures were 250 and 280ºC, respectively. The carrier gas used was helium with a controlled flow of 1 mL min^{-1}. The detector consisted of an Agilent 5973 mass selective detector (ion source temperature of 230ºC, mass range 40–800, and 0.7 scan s^{-1}) and mass spectra were acquired with a 70 eV ionizing energy. The pyrolytic fragments were identified after examining stored (NIST and Wiley libraries) and published data.

RESULTS AND DISCUSSION

Figure 1 shows the ^{13}C CP-MAS/NMR spectra of soil HAs extracted from different cultivated and native forest sites. ^{13}C assignment was dominated by the *O*-alkyl C resonance (45–92 ppm) followed by a resonance line assigned to alkyl C (0–45 ppm) (Table 1). Soil HAs extracted from the FSC system showed a decrease in the intensity of the peak centered at 56 ppm (methoxyl C), followed by a slight increase of the peak at 130 ppm in the aromatic C (92–150 ppm) resonance, suggesting that FSC management seems to favor the intensification of the process of humification of HAs structure. The higher aromaticity index of HAs from FSC compared to the AGF system (Figure 2) indicates

a higher degree of humification of the organic structure, confirming our previous results.

Figure 3 shows the Py-GC/MS Total Ion Chromatograms of soil HAs. More than 90 pyrolytic compounds were identified by Py-GC/MS (total ions abundance not shown). Table 2 shows the general abundance of products belonging to specific chemical groups.
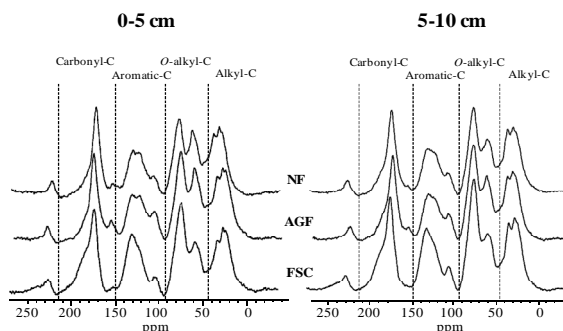
Figure 1: ^{13}C CP-MAS/NMR spectra of soil humic acids extracted from native forest (NF), agroforestry-coffee (AGF) and full sun-coffee (FSC) at 0-5 and 5-10 cm depths. Dotted vertical bars indicate integrated regions of the spectra and their respective group.

The highest abundances (% of total) were registered by non-specific aromatics and N compounds groups, followed by the lowest abundances of lignins, polysaccharides and lipid compounds (Table 2). The higher abundance of aromatics and N-compounds over the abundance of polysaccharides and/or lipids suggests that, under tropical environment, SOM structure is strongly influenced by the high intensity of oxidation processes. In general, the relative abundance in each specific group was similar among the different land use, with exception to the higher amounts of lignin-like and lipids compounds in AGF compared to FSC system, and the higher abundance of N-compounds in FSC (37,0%) than in AGF (21,5%) system. These results indicate that AGF system influenced the genesis of HAs by favoring a less condensed structure when compared to FSC system. The higher abundance of lignins compounds in the structure is an indicative of recent inputs of plants residues, probably as a result of the influence of organic material derived from trees. The higher abundance of lipids in AGF system might be related to as well the higher contribution of microorganisms (e.g. lipids derived from microbial cells) as from plants waxes (Bull et al., 2000).

There were some slight discrepancies in the relative amounts of various chemical groups as determined by ^{13}C NMR and analytical pyrolysis. Py-GC/MS products showed lower amounts of aliphatic compounds than NMR spectroscopy, which is in agreement with Buurman et al. (2009). These authors stated that a numerical coincidence of these two kinds of data should not be expected basically due to three main reasons: (i) Py-GC/MS is not an ideal method for distinction of highly polar compounds such as fatty acids; (ii) each pyrolytic fragment may contain C atoms that belong to different functional groups as identified by NMR; and (iii) NMR quantification is based on C environments, whereas each pyrolysis product may

have different C environments. Moreover, the inability of ^{13}C NMR to detect carbons far removed from protons, can results in an overestimate of aliphatic carbons (Poirier et al., 2000).

CONCLUSIONS

1. The combined use of ^{13}C CPMAS/NMR with Py-GC/MS was valuable to the study of structural features of HAs. Pyrolysis provides more detailed information about the molecular structure of SOM.

2. Despite of the discrepancies, both techniques indicated that AGF systems favored the genesis of soil HAs with lower degree of humification when compared to FSC system, probably due to the influence of organic residues (fresh and old) derived from trees.

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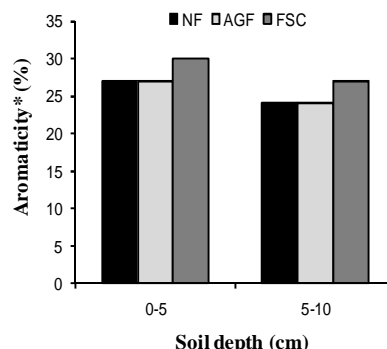
Figure 2. Aromaticity index of soil humic acids extracted from native forest (NF), agroforestry-coffee (AGF), and full sun-coffee (FSC) systems. *Aromaticity = [aromatic C (92-150 ppm)]/[Alkyl C + O-alkyl C (0-92 ppm) + aromatic C] x 100.
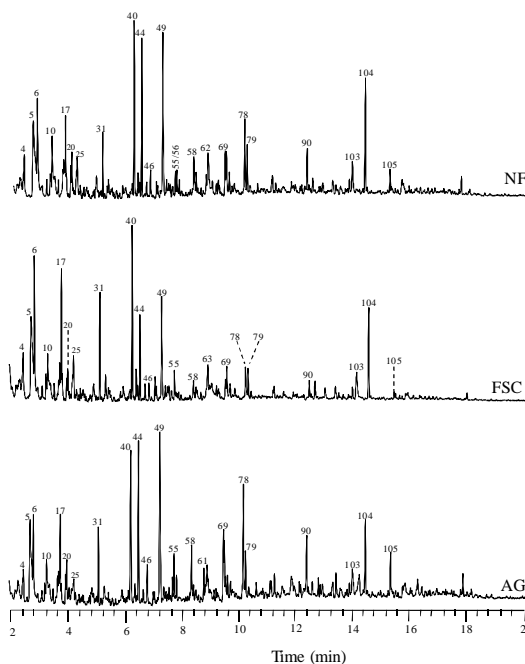
Figure 3. Py-GC/MS chromatograms of soil humic acids extracted from native forest (NF), agroforestry-coffee (AGF), and full sun-coffee (FSC) systems at 0-5 cm depth.

Table 1. Distribution of ^{13}C in soil humic acids from areas under native forest (NF), agroforestry system coffee (AGF) and full-sun coffee (FSC) systems as determined by ^{13}C CP-MAS/NMR

Systems	Alkyl C 0-45 ppm	*O*-alkyl C 45-92 ppm	Aromatic C 92-150 ppm	Carbonyl C 150-215 ppm
--- % [a] ---				
0-5 cm depth				
NF	26.4	30.9	21.3	21.4
AGF	26.7	31.3	21.8	20.3
FSC	23.6	29.8	22.5	24.1
5-10 cm depth				
NF	27.6	33.1	19.3	20.0
AGF	25.2	35.4	19.2	20.2
FSC	23.4	33.9	21.2	21.5

[a] values are expressed as percentage of the whole spectrum area.

Table 2. General abundance (% of Sum) of compounds belonging to specific chemical groups of soil humic acids extracted from native forest (NF), agroforestry system coffee (AGF) and full-sun coffee (FSC) systems at 0-5 cm depth as assessed by Py-GC/MS

Source	Systems		
	NF	AGF	FSC
--- % -----------------------------			
Polysaccharides	9.3	11.5	16.0
Non-specific aromatics	31.0	27.5	30.4
Lignins	27.2	35.6	15.4
N-compounds	31.2	21.5	37.0
Lipids	1.3	3.9	1.2
Total	100.0	100.0	100.0