

## Fluoridic Acid in the Infrared Spectroscopy Analysis of Chemical Composition of Organic Matter

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### Abstract

Fourier transform infrared spectroscopy (FTIR) assists in investigating functional groups of soil organic matter (SOM). However, the use of this tool is impaired given the low organic carbon levels and high content of oxides in tropical soils, resulting in low quality spectra and in turn requiring the use of hydrofluoric acid (HF). The objective of this study was to verify the efficiency of using HF in removing the mineral fraction and to increase the C concentration to enable visualizing the bands related to SOM in infrared spectra in soil samples under forest system. The HF treatment was efficient in removing mineral components and proportionally increasing C. The FTIR with HF enabled identifying differences between coarse and fine fractions. The spectra of the HF samples showed that the coarse fractions presented bands related to aromatic material and the fine fractions presented more labile components, with the absence of more recalcitrant components.

**Keywords:** FTIR, carbon stock, tropical soil, soil aggregates.

Forest systems play an important role in soil C accumulation through the continuous deposition of plant residues. The stabilization of this C occurs through interaction with mineral particles (organomineral complex) and physical protection in the aggregates (Vicente et al., 2019). Physical fractionation allows separation into different organic matter fractions, which can function as a C source or sink, depending on its accessibility by microbiota and the physical location (Six and Paustian, 2014). FTIR spectroscopy enables identifying the chemical composition and reactivity of C in these fractions. However, this technique is impaired by the low C levels in tropical soils. Thus, the removal of mineral components is essential for a proportional increase in the C concentration (Boeni et al. 2014).

The objective of this study was to verify the efficiency of HF in removing the mineral fraction, increasing the concentration of C and N and visualizing the bands related to SOM in the FTIR spectra of different soil samples (fine earth fraction (FEF, 2,00 mm); macroaggregates; microaggregates; silt+clay fraction; particulate and occluded fraction of macroaggregates

and microaggregates) under forest in the municipality of Uruauca, Southern Bahia, Brazil, at a depth of 0-10 cm.

Four fixed plots of 900 m<sup>2</sup> were delimited for soil sampling; the plots were separated by at least 100 m. Each composed soil sample was formed of 15 single soil samples taken at 0–10 cm depth. The soils were classified as Latossolo Amarelo according to the Brazilian System of Soil Classification (Embrapa, 2013). To obtain the fine earth fraction (FEF) the soil samples were air-dried and passed through a 2 mm sieve. Physical fractionation in aggregates classes was carried out following the method of Elliot (1986) and adapted by Vicente et al. (2019). 100 g of soil sample was sunk into 500 ml of deionized water for 5 minutes and fractionated by wet-sieving (two sieve sizes: 250 μm and 53 μm) and three aggregates classes were obtained: 2000-250 μm, 250-53 μm and <53 μm. The particulate and occluded organic matter fractions within macro and microaggregates were obtained by the sonication method (Sarkhot et al., 2007). The fractions were submitted to treatment with hydrofluoric acid (HF) 10% with 2 hours of

agitation on horizontal shaker (Tecnal TE-240), followed by 10 minutes of centrifugation (3000 rpm). In “Falcon” tubes, 10 g FEF, macro and microaggregates with 30 ml of HF, 8 times with HF and 3 times with water (Dick et al., 2008); 1 g of the silt+clay fraction with 40 ml of HF, 4 times with HF and 3 times with water; 0.5 g for particulate fraction and 0.3 g for occluded fraction, both with 20 ml of HF, 3 times with HF and 3 times with water (Boeni et al., 2014). The following determinations were carried out (before and after HF): Total Fe; R index  $((C/N)/(C/NHF))$ . The C (CE) and N (NE) enrichment and the C (CR) and N (NR) recoveries were calculated. Recoveries after HF were calculated using the initial mass and recovered mass (MR) values of the samples (Dick et al., 2008). C and N contents were determined by dry combustion using an automated analyzer. Fourier Transform Infrared Spectroscopy analyzes were conducted by Diffuse Reflectance using Shimadzu spectrometer (DRS-8000A). The fractions was mixed and ground with potassium bromide (KBr), after being dried in an oven at 60 °C, in the proportion 1 sample:100 KBr. The reading was performed with a diffuse reflectance accessory, using 40 scans and a resolution of 4 cm<sup>-1</sup>, in the spectral range of 4,000 to 400 cm<sup>-1</sup>. Spectra and band intensities data were obtained by the Shimadzu IR Solution 1.6 program (Gonçalves et al, 2003; Dick et al., 2008).

$M_R$  values were higher in particulate fractions and lower in silt+clay fraction. The lower  $M_R$  values in silt+clay fraction are the result of the higher clay contents, resulting from the dissolution of the mineral fraction after HF (Table 1). The analysis of functional groups by DRIFT is hampered by the low levels of carbon and high concentrations of Fe oxides, as in tropical soils, and the presence of silicates (Gonçalves et al., 2003). For this reason, it is important to remove these mineral and increase the carbon concentration to obtain adequate spectra. The higher  $C_E$  in the soil fractions (silt+clay,

particulate and occluded fractions) compared to FEF is due to the soil fractionation which makes the mineral matrix more accessible to HF, increasing specific surface and the C protection (Jindaluang et al., 2003). The increase in C and N in the samples is also associated with the removal of Fe ( $Fe_R$ ), one of the the clay fraction components. The samples presented R index that suggested negligible losses with HF (Table 1). According to Schmidt et al. (1997),  $R = 1.0 (\pm 0.2)$ , there were no preferential losses of C and N.

The bands in samples without HF referring to SOM were not able to stand out from those of minerals: 3,694, 3,622 and 3,173 cm<sup>-1</sup> (OH stretch in the Al–OH group of silicates) 1,097, 1,036 and 912 cm<sup>-1</sup> (stretch of the Si–O in kaolinite and quartz) (Dick et al., 2008). The treatment efficiency with HF in the samples was evidenced by the absence of bands related to minerals and the presence of those related to SOM by the decrease of Fe and increase of  $C_R$ . However, some bands related to the mineral fraction continued to be present, even after treatment: 3,300 cm<sup>-1</sup> (angular deformation H–O–H in mineral fractions); 1,994, 1,870 and 1,794 cm<sup>-1</sup> (Si–O stretch, quartz) and 799 and 694 cm<sup>-1</sup> (clay and quartz minerals) (Haberhauer and Gerzabek, 1999). The presence of these bands, especially quartz, is due to their high resistance to dissolution with HF (Gonçalves et al., 2003; Dalmolin et al., 2006). Not to mention the influence of the mineralogical composition of this soil. According to Silva (2008) these soils showed kaolinite as the main phyllosilicate, and goethite as the most representative oxide. Djomgoue and Njopwouo (2013) revealed that pure kaolinite presents four well resolved (-OH) bands in IR spectrum: the stretching vibrations of surface hydroxyl groups (3,652; 3,671, and 3,694 cm<sup>-1</sup>) and the vibrations of inner hydroxyl groups (3,620 cm<sup>-1</sup>). Furthermore, bands due to  $\nu(AlFeOH)$  at 865-875 cm<sup>-1</sup> and stretching at 3,607 cm<sup>-1</sup> are typical of Fe bearing kaolinites.

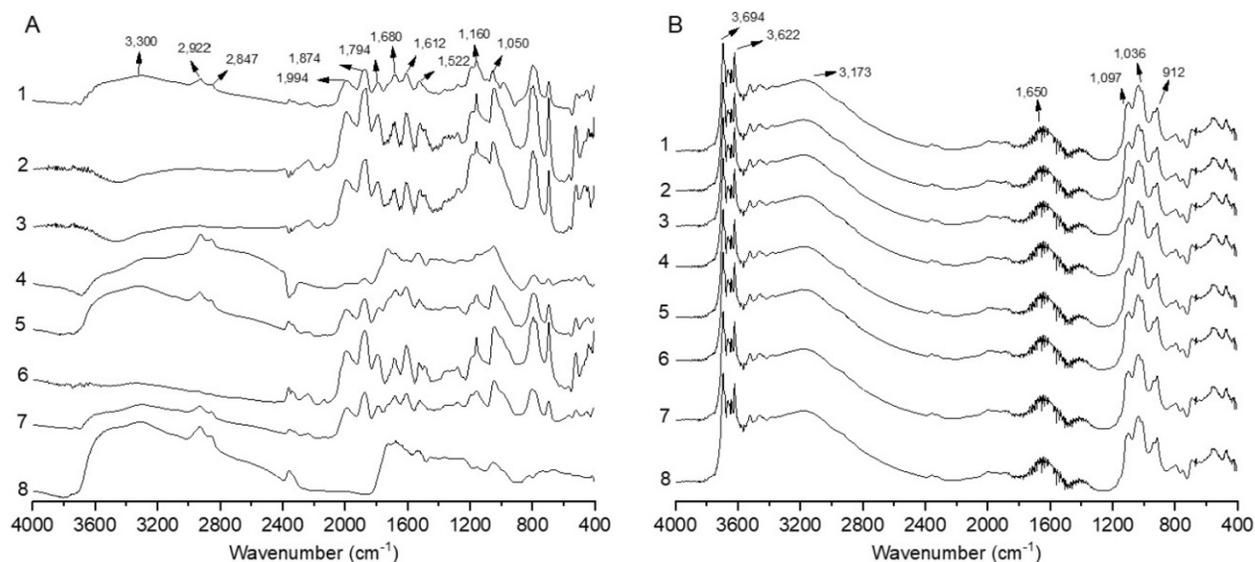
**Table 1.** Means of recovered mass (%Mr), carbon enrichment ( $C_E$ ) and nitrogen ( $N_E$ ), recovered carbon and nitrogen (% $C_R$  e % $N_R$ ), Iron removal (% $Fe_R$ ) and R index in soil samples (0 - 10 cm) under forest, in southern Bahia.

Soil Samples	Mr (%)	$C_E$	$N_E$	Cr (%)	Nr (%)	$Fe_R$ (%)	R
Fine earth fraction	28.3±5.8	1.5±0.1	1.9±0.5	41.7±10.3	56.0±21.5	89.9±5.6	1.0±0.1
Macroaggregates	32.1±7.8	1.4±0.1	1.2±0.1	45.4±12.3	37.5±9.7	92.0±8.0	0.8±0.04
Microaggregates	32.6±5.8	1.3±0.05	9.4±3.6	41.1±7.6	272.6±87.4	88.4±5.4	1.2±0.1
Silt+Clay fraction	9.8±2.4	6.0±1.6	5.0±1.4	58.4±17.8	45.1±11.3	96.1±2.1	1.0±0.1
Particulate fraction macroaggregates	66.7±9.5	4.6±2.3	3.9±2.1	326.8±195.1	279.2±176.1	81.2±10.7	0.9±0.1
Particulate fraction microaggregates	68.3±4.8	4.6±0.8	3.6±0.7	316.3±68.1	247.2±48.0	91.6±6.2	0.9±0.1
Occluded fractions macroaggregates	35.4±15.8	3.0±1.4	2.8±1.3	93.4±59.4	82.6± 42.6	83.0±16.7	1.0±0.1
Occluded fractions microaggregates	21.6±14.5	6.6±0.8	6.0±0.9	143.5±98.5	131.6±89.1	96.0±2.9	0.9±0.1

Bands at 1,050, associated to the presence of cellulose (Calderón et al. 2011) and 1,160  $\text{cm}^{-1}$  which represents aliphatic compounds linked to hydroxyl groups (C-OH) or can be assigned to C-O vibration of polysaccharides or other groups such as alcohols and esters (Janik et al. 2007), were observed in all samples. The 1,720, 1,680, 1,612 and 1,522  $\text{cm}^{-1}$  bands represent aromatics (the first two refer to the C=O of esters or carboxylic acids, while 1,612 and 1,522  $\text{cm}^{-1}$  refer to C=C in more condensed structures) (Calderón et al., 2011), and 1,250  $\text{cm}^{-1}$  band (stretch in the C-O bond) indicating the presence of carboxylic acids and phenols functional groups (Bornemann et al., 2010) were absent in the occluded fractions of macro and microaggregates and silt+clay fraction. However, were observed mainly in FEF, macro and microaggregates and particulate fraction samples (Figure 1). These coarser fractions present less protection of C to the action of the microbiota due to the lower complexation of organic matter to the mineral fraction (Lehmann and Kleber, 2015). Thus, labile forms of C were the first used by microorganisms, which led to the proportional enrichment of aromatic C from lignin (Calderón et al., 2011). The silt+clay and occluded (fine fractions) fractions showed a presence of aliphatic C (bands around 2,920 and 2,850  $\text{cm}^{-1}$ ) (Figure 1) which is associated

with the finer fraction (Pisani et al., 2014). In these fine fractions, even the most labile organic matter is protected from the action of microorganisms by the complexation with soil mineral fraction (Six et al., 2014).

HF was efficient in the removal of less resistant mineral components, with expressive removal of Fe and a proportional increase of C and N. HF was also efficient for observing bands related to the organic components present in the studied samples. The FTIR with HF enabled identifying differences between coarse and fine fractions of organic matter under forest soils. There was a proportional increase in aromatic content during decomposition in the coarse fractions free from chemical or physical protection, as evidenced by bands referring to aromatic C and more condensed structures with the presence of double bonds in the molecules. On the other hand, the fine fractions (occluded fractions of macroaggregates and microaggregates and silt+clay) had more labile components, evidenced by the presence of bands referring to aliphatic structures of C and N suggesting less degree of transformation into the protected organic matter in soil aggregates. The use of physical fractionation and the chemical characterization of C by FTIR provided relevant information about the stabilization process of organic matter under forest soils.



**Figure 1.** Spectra of Forest samples before HF treatment (A) and after HF treatment (B). (1) FEF fraction; (2) Macroaggregates; (3) Microaggregates; (4) Silt + Clay; (5) Particulate fraction of macroaggregates; (6) Particulate fraction of microaggregates; (7) Occluded fraction of macroaggregates; (8) Occluded fraction of microaggregates.

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