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# Soil organic matter in podzol horizons of the Amazon region: Humification, recalcitrance, and dating

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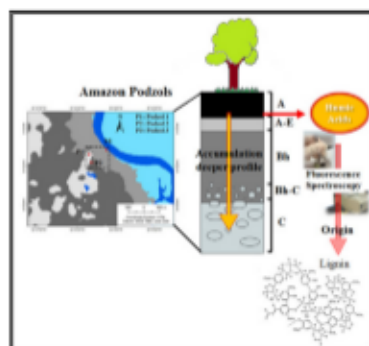
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## HIGHLIGHTS

- Structural variation in the humic acids for the depth profile.
- Humic acids originated from lignin-derived compounds.
- Lignin can accumulate in some Bh horizons.
- Amazonian podzols there are four kinds of the organic matter.
- Humification process has not direct association with the dating of organic matter.

## GRAPHICAL ABSTRACT



## ABSTRACT

Characteristics of soil organic matter (SOM) are important, especially in the Amazon region, which represents one of the world's most relevant carbon reservoirs. In this work, the concentrations of carbon and differences in its composition (humification indexes) were evaluated and compared for several horizons (0 to 390 cm) of three typical Amazonian podzol profiles. Fluorescence spectroscopy was used to investigate the humic acid (HA) fractions of SOM isolated from the different samples. Simple and labile carbon structures appeared to be accumulated in surface horizons, while more complex humified compounds were leached and accumulated in intermediate and deeper Bh horizons. The results suggested that the humic acids originated from lignin and its derivatives, and that lignin could accumulate in some Bh horizons. The HA present in deeper Bh horizons appeared to originate from different formation pathways, since these horizons showed different compositions. There were significant compositional changes of HA with depth, with four types of organic matter: recalcitrant, humified, and old dating; labile and young dating; humified and young dating; and little humified and old dating. Therefore, the humification process had no direct relation with the age of the organic matter in the Amazonian podzols.

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## 1. Introduction

The Amazon forest provides important services to humanity, such as high biodiversity, climate regulation, carbon sequestration, and the regulation of water and nutrient cycles (Coe et al., 2013). Climate changes can directly affect the physical integrity of soils in the Amazon region, causing important alterations in their water balance, evapotranspiration, and carbon transport (Coe et al., 2013; Neill et al., 2013). In particular, any modification of the soil composition and water cycle due to anthropogenic and/or natural sources is expected to affect the amount and chemical nature of the soil organic matter (SOM) present in these natural ecosystems and, consequently, influence the CO<sub>2</sub> concentration in the atmosphere.

Understanding the dynamics of SOM and its chemically and biologically active fractions, including humic acids (HA), is fundamental for evaluating its vulnerability to changes in physical and chemical properties when subjected to different weather and water conditions (Stevenson, 1994; Senesi and Loffredo, 1999). Lignin is recognised as one of the main precursors of humic substances, including HA (Burgues et al., 1963; Flaig, 1972; Hatcher et al., 1981; Hedges and Ertel, 1984; Senesi and Loffredo, 1999; Lehmann and Kleber, 2015). The transformation of lignin into HA can proceed by small modifications of the constituent biopolymers that increase their solubility, such as by the degradation of lignin into simple phenols, which can subsequently re-polymerize (Hedges and Ertel, 1984; Schmidt et al., 2011; Jex et al., 2014; Lehmann and Kleber, 2015). Modified lignin is often present as a major HA component, especially in the SOM of flooded areas, such as peats, sediments, and hydromorphic soils, rather than in well-drained soils (Hedges and Ertel, 1984). Lignin is much more resistant to microbial degradation, compared to other HA precursors such as polysaccharides, amino acids, and other plant constituents. However, several physical, chemical, and biological factors can contribute to lignin degradation during its percolation through the soil, and can modify its structure (Jex et al., 2014).

The organic carbon stored in the surface layers (0 to 1.0 m) of hydromorphic podzols in the upper Rio Negro basin amounts to  $87 \pm 7 \text{ kg m}^{-2}$ , which corresponds to a total of  $14 \pm 1 \text{ Pg}$  of carbon in these soils (Cerri et al., 2000; Montes et al., 2011). Hence, the effects of soil textural and mineralogical characteristics on SOM dynamics are especially important in this ecosystem, which is one of the most relevant global carbon reservoirs (Cerri et al., 2000).

Fluorescence spectroscopy is a technique widely used for differentiating and classifying SOM and HA according to their origin, genesis, and nature (Senesi et al., 1991). Emission and/or excitation fluorescence methods have been extensively applied to assess HA humification processes (Zsolnay et al., 1999; Kalbitz et al., 1999; Milori et al., 2002; Tadini et al., 2015). Among these, laser-induced fluorescence spectroscopy (LIFS) is a valuable tool for use in the structural and functional analysis of SOM. This technique is of interest due to its rapidity, simplicity, sensitivity, and performance under natural conditions (Milori et al., 2006, 2011; Martins et al., 2011; Tadini et al., 2015; Senesi et al., 2016). Milori et al. (2006) evaluated the humification index ( $H_{LIFS}$ ) of SOM in whole soils using LIFS with fluorescence excitation in the blue region. The presence and conservation of structural units such as conjugated aromatic rings and substituted quinones were suggested to be directly related to humification processes.

Few studies (Bravard and Righi, 1991a, 1991b; Santos et al., 2015a, 2015b) have investigated HA isolated from Amazon podzols. Therefore, the main aim of this work was to perform a comparative evaluation of the characteristics of SOM and its HA in the different horizons of three podzols from the Amazon region, using elemental analysis (CHN) and fluorescence spectroscopy.

## 2. Materials and methods

### 2.1. Study area

The study site was located in the north of Barcelos City, near the Demeni River (0° 15' 18"N; 62° 46' 36"W) in the middle watershed of

the Negro River region in the Amazon plains, Brazil (Fig. 1a). According to the Brazilian Institute of Geography and Statistics (IBGE, 2008), ferralsols, podzols, and gleysols are the most common soil types in this area. The climate is typically equatorial and is characterised by an average temperature of 25 °C and high rainfall (around 3000 mm) throughout the year, with no pronounced dry season.

### 2.2. Soil sampling and preparation for analysis

Soil samples were collected close to the Demeni River, from different horizons of three podzols developed on the Pleistocene sedimentary substrata of the Branco and Negro rivers (IBGE, 2008). The samples were from: (a) a permanently waterlogged podzol covered by herbaceous meadow of shrubs and grass, locally named "campina" (P1); (b) a seasonally waterlogged podzol covered by caatinga forest and rainforest (P2); and (c) a well-drained podzol under rainforest vegetation (P3) (Fig. 1b) (Pereira et al., 2015). Soil sample collection was carried out by hand auger drilling at different depths from 0 to about 390 cm, without replication. The vertical downward pedologic successions of layers sampled in the three podzol profiles were as follows:

- P1: A 0–15, A-E 15–30, Bh 40–50, Bh-C 240, Bh-C 260, and C 350;
- P2: A 0–15, Bh 165–175, Bh 180–183, Bh 210, Bh 280–310, and Bh 335–350;
- P3: A 0–20, A 20–30, A 30–40, Bh 110–120, Bh 170–180, Bh-C 270–280, Bh 370–380, and Bh 380–390.

In the above classification, P1, P2, and P3 represent the type of podzol; A, E, A-E, Bh, Bh-C, and C are the pedologic horizon types (Santos et al., 2015a, 2015b; Embrapa, 2006); and the numbers represent the upper and lower sampling depths (in cm).

The soil samples were oven-dried at 35 °C with air circulation and renovation, crushed, and sieved to <0.106 mm before analyses by laser-induced fluorescence spectroscopy (LIFS) and classical fluorescence spectroscopy.

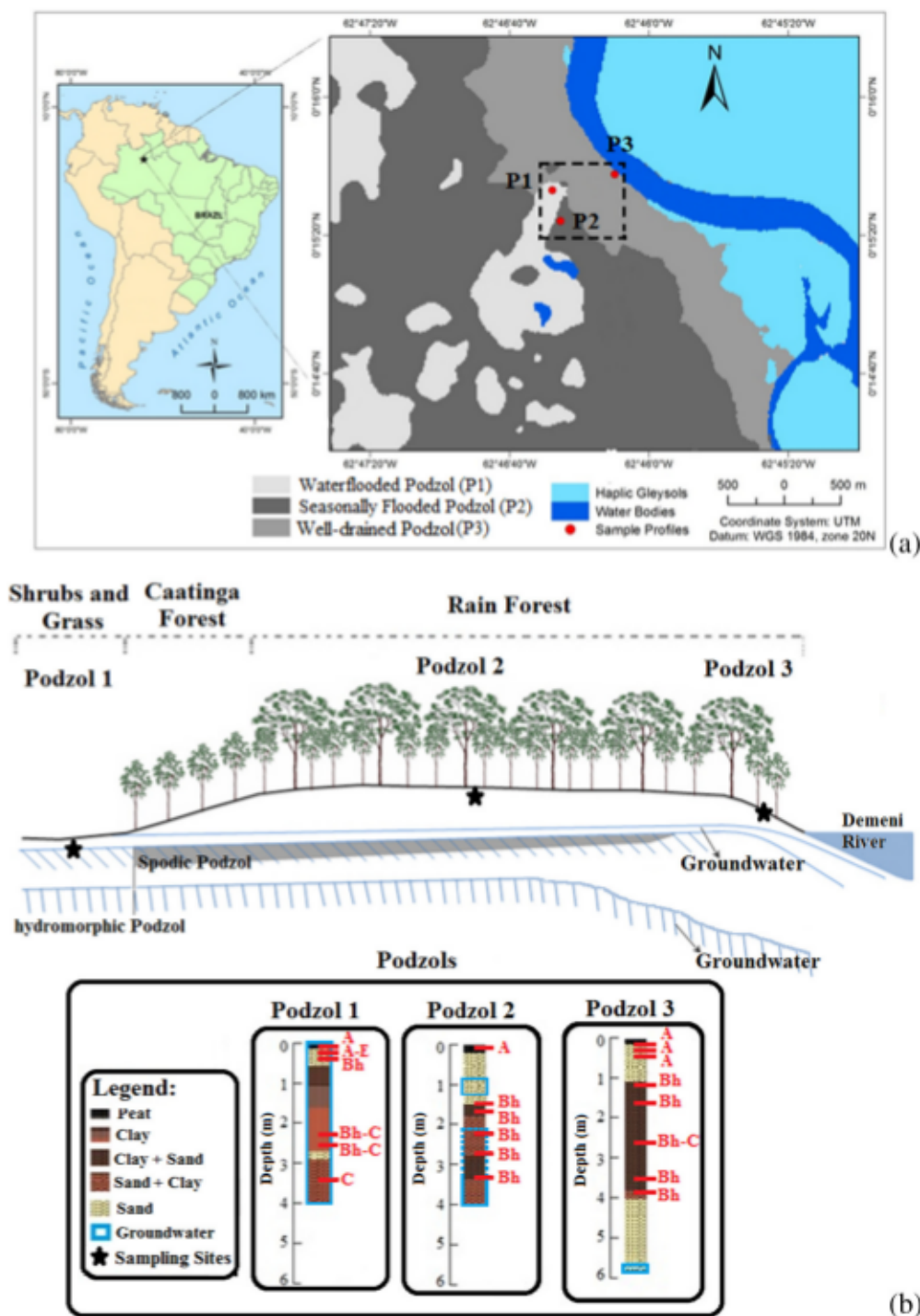
### 2.3. Isolation of humic acids

The HA fractions were isolated from the soil samples using the procedure recommended by the International Humic Substances Society (Swift, 1996). Briefly, the HAs were extracted with 0.1 M NaOH (Synth) at a soil:extractant ratio of 1:200 (volume in L:mass in g). After centrifugation, the HA fraction was separated from the supernatant by precipitation at about pH 2.0 with 6 M HCl. The HAs were then suspended in a solution of 0.1 M HCl/0.3 M HF to remove dissolved mineral impurities. After centrifugation, the HAs were dialysed at pH 8 (cellulose membrane dialysis tubing, 43 mm, cut-off of 14,000 Da, Sigma-Aldrich) and finally lyophilised.

### 2.4. Soil analysis

The textures of the soils were analysed in a previous work (Pereira et al., 2013), using the Robinson pipette method (Santos et al., 2005).

The LIFS measurements were performed using soil pellets. The soil samples were first dried at room temperature and passed through a 5 mm sieve, followed by preparation of mixtures containing 70% soil and 30% boric acid. Pellets about 1 cm in diameter and 2 mm thick were obtained by applying 10 tons of pressure for 2 min. The LIFS analysis was carried out in duplicate for each sample. An additional LIFS analysis was performed for sample P3 Bh-C 270–280, with addition of 1, 5, and 10% of lignin standard (Sigma-Aldrich). In this procedure, mixtures were prepared using 360 mg of soil and 40 mg of lignin (10%), 380 mg of soil and 20 mg of lignin (5%), and 396 mg of soil and 4 mg of lignin (1%).



**Fig. 1.** Soil sampling sites in the Amazon region, Brazil. (a) Map of the region (adapted from Pereira et al., 2013) and (b) topographic and vertical profiles of the soils studied.

The LIFS spectra were acquired with application of a continuous wave laser (20 mW power) at 405 nm, using homemade equipment (Santos et al., 2015a, 2015b). The laser beam was directed onto the pellet surface through six external optical fibres and the fluorescence emission resulting from the decay of excited species was conducted to the spectrometer through a central optical fibre inside the optical bundle. The emission wavelength used ranged from 420 nm to 800 nm, the maximum emission intensity was 1000 counts, and the selected integration time, averaging, and boxcar settings were 400 ms, 3, and 3, respectively, for all measurements. Four replicates were recorded for each sample and the final averaged spectrum was used to calculate the values of  $H_{LIFS}$  as the ratio between the area of the fluorescence

emission spectrum in the range 420–800 nm and the amount of carbon (%C) in the soil sample (Milori et al., 2006).

## 2.5. Soil organic matter and humic acid analyses

The carbon contents (%) were measured in duplicate using 10 mg of the whole soil and 3 mg of HA, after homogenisation and grinding to produce particles smaller than 0.106 mm, using a CHNS elemental analyser (Model 2400, PerkinElmer).

Radiocarbon dating of the soils was carried out at the Poznań Radiocarbon Laboratory, Poland, as described by Doupoux et al. (2017).

Fluorescence measurements were performed with a luminescence spectrometer (Model LS50B, PerkinElmer), using aliquots of 12.5 mg L<sup>-1</sup> (pH 8.0) solutions of HA dissolved in 0.05 mol L<sup>-1</sup> NaHCO<sub>3</sub>. An additional sample containing 3 mg L<sup>-1</sup> of lignin (Sigma-Aldrich) was also analysed (pH 8.0). The emission spectra were acquired at an excitation wavelength of 465 nm, in the scan range 480–700 nm, using an open filter, excitation and emission slit widths of 10 nm, and a speed of 500 nm min<sup>-1</sup>. The humification indexes ( $H_{465\text{ nm}}$ ) of HA and lignin were determined as the total area under the fluorescence emission spectrum recorded at the excitation wavelength of 465 nm (Milori et al., 2002) in 12.5 mg L<sup>-1</sup> HA solution. It was assumed that when SOM is transformed into HA, the quantity of conjugated aromatic and phenolic moieties increases, leading to a red-shift effect for both excitation and emission fluorescence peaks. Therefore,  $H_{\text{LIFS}}$  and  $H_{465\text{ nm}}$  should increase with increasing contents of these moieties considered as indicators of the progress of the humification process.

## 2.6. Colour corrections

Colour is widely used for characterization and classification of soil in the field. Generally, soil colour standardisation is achieved by means of the Munsell colour chart (Munsell, 2000). An empirical correction factor can be used to improve the  $H_{\text{LIFS}}$  determination. Here, the  $H_{\text{LIFS}}$  index was divided by the Munsell colour value of the bulk sample, with a darker sample resulting in a lower value. For example, for soils with the colour “dark brown”, the corresponding 10YR3/3 value was 3; for “brown and pale brown”, the corresponding 10YR5/3 and 10YR6/3 value was  $(5 + 6) / 2 = 5.5$ ; for “black and very dark brown”, the corresponding 10YR2/1 and 10YR2/2 value was  $(2 + 2) / 2 = 2$ .

## 3. Results and discussion

### 3.1. Characteristics of the soil organic matter

The textural characteristics of the soil samples are presented in Table 1. The three podzols studied showed a predominantly sandy texture, with different distributions of coarse and fine sand, silt, and clay. The profile of P1 lacked the development of a well-differentiated E horizon, probably due to the permanently water-logged conditions that prevailed in the open field area. The profile of P2 was that of a typical

Amazonian podzol with a permanent water table located on the top of the Bh horizon and another one at depth. The profile of P3 reflected a truncated podzol, where the E horizon and the upper part of the Bh horizon had been removed by erosion caused by the development of a neighbouring meander of the Demeni River.

The  $H_{\text{LIFS}}$  index values calculated from the LIFS spectra, as proposed by Milori et al. (2002) (Table 1), showed a low correlation ( $r = 0.67$ ) with the  $H_{465\text{ nm}}$  index values (Fig. 2a). It is possible that the soil samples could have exhibited fluorescence self-absorption, with a darker colour resulting in a lower  $H_{\text{LIFS}}$  index value. Therefore, a colour correction factor was applied to the LIFS fluorescence emission in order to take this into account. The corrected index, denoted  $H_{\text{LIFS}_M}$ , showed a satisfactory correlation ( $r = 0.85$ ) with the  $H_{465\text{ nm}}$  index (Fig. 2b).

Milori et al. (2002) showed previously that the 465 nm excitation resulted in data with high correlations with the aromaticity measured by NMR and the number of semiquinone free radicals evaluated by EPR. Hence, the good correlation obtained here between the  $H_{\text{LIFS}_M}$  and  $H_{465\text{ nm}}$  values confirmed that the most humified organic matter in these soils belonged to the HA fraction.

The reproducibility of the  $H_{\text{LIFS}_M}$  values was excellent, as shown in Fig. 3, which compares two sets of values obtained for samples from the same site, analysed in 2012 and 2014. This confirmed the reliability of the proposed correction factor and its applicability to soil samples with horizon colour variability.

The samples from Bh horizons below 240 cm (soil P1) and 210 cm (soil P3) showed higher humification index values, compared to the corresponding surface horizons and soil P2. This could be attributed to the accumulation of greater amounts of leached humified SOM in these horizons. For soil P3, the highest  $H_{\text{LIFS}_M}$  value was found for horizon Bh-C 270–280, while in the horizon below (Bh 370–380), carbon accumulation was observed, but the humification index value was lower, indicative of a smaller quantity of condensed aromatic carbon in this horizon.

For soils P1 and P3, variation of  $H_{\text{LIFS}_M}$  was observed with increasing depth, whereas this was not the case for soil P2, for which the  $H_{\text{LIFS}_M}$  values showed little variation ( $1.3\text{--}6 \times 10^3$ ). The former findings were in agreement with the results of González-Pérez et al. (2007), who showed that there was greater accumulation of conjugated aromatic and humified SOM in the deepest horizons of soils under no-tillage and native vegetation, with lower SOM humification in the surface

**Table 1**

Textural composition (Pereira et al., 2015), carbon dating, Munsell colour, carbon %, and  $H_{\text{LIFS}}$  and  $H_{\text{LIFS}_M}$  values of the whole soil in the different horizons; carbon % and  $H_{465}$  of HA in the different horizons.

Sample description	Whole soil						Humic acid					
	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Textural class	Carbon dating (years)	Munsell colour	Carbon (%)	$H_{\text{LIFS}}$ ( $\times 10^3$ )	$H_{\text{LIFS}_M}$ ( $\times 10^3$ )	Carbon (%)	$H_{465}$ ( $\times 10^3$ )
P1 A 0–15	a	a	a	a	a	a	10YR3/2	15.2 $\pm$ 0.1	0.5 $\pm$ 0.3	0.2 $\pm$ 0.1	45 $\pm$ 3	3.8
P1 A-E 15–30	13.24	45.13	40.63	1.00	Sandy loam	2645 $\pm$ 30	10YR4/3	1.00 $\pm$ 0.02	10 $\pm$ 6	2 $\pm$ 1	51 $\pm$ 1	7.8
P1 Bh 40–50	14.47	45.25	37.26	3.02	Sandy loam	3240 $\pm$ 35	10YR3/2	2.74 $\pm$ 0.02	7 $\pm$ 1	4 $\pm$ 1	56.2 $\pm$ 0.2	7.5
P1 Bh-C 240	a	a	a	a	a	a	7.5YR4/4	0.46 $\pm$ 0.01	89 $\pm$ 18	22 $\pm$ 5	46 $\pm$ 2	15.9
P1 Bh-C 260	40.92	48.96	5.26	4.86	Sandy	a	7.5YR4/4	0.45 $\pm$ 0.00	68 $\pm$ 7	17 $\pm$ 2	47 $\pm$ 1	12.0
P1 C 350	48.80	46.90	2.79	1.50	Sandy	a	2.5Y4/3	0.47 $\pm$ 0.00	58 $\pm$ 6	15 $\pm$ 2	51 $\pm$ 3	9.1
P2 A 0–15	12.91	55.56	30.53	1.00	Sandy loam	105.99 $\pm$ 0.34	7.5YR3/2	2.06 $\pm$ 0.03	14 $\pm$ 2	2.8 $\pm$ 0.4	53.9 $\pm$ 0.8	2.1
P2 Bh 165–175	37.72	41.07	15.66	5.55	Loamy sand	5060 $\pm$ 30	7.5YR2/1	2.17 $\pm$ 0.03	5 $\pm$ 1	3 $\pm$ 1	46.8 $\pm$ 0.1	3.2
P2 Bh 180–183	a	a	a	a	a	5860 $\pm$ 35	7.5YR3/3	2.36 $\pm$ 0.02	7 $\pm$ 1	1.3 $\pm$ 0.2	54 $\pm$ 6	2.9
P2 Bh 210	a	a	a	a	a	8480 $\pm$ 40	10YR5/4	0.88 $\pm$ 0.01	191 $\pm$ 18	6 $\pm$ 4	45 $\pm$ 1	4.8
P2 Bh 280–310	70.08	23.74	0.33	5.84	Sandy	6165 $\pm$ 35	5YR2.5/1	1.42 $\pm$ 0.01	11 $\pm$ 1	5 $\pm$ 1	53 $\pm$ 3	7.8
P2 Bh 335–350	9.65	81.49	4.31	4.54	Sandy	5590 $\pm$ 40	5YR2.5/1	1.59 $\pm$ 0.02	8 $\pm$ 1	4 $\pm$ 1	54.2 $\pm$ 0.8	5.4
P3 A 0–20	64.62	30.53	4.35	0.50	Sandy	107.23 $\pm$ 0.35	10YR4/2	1.45 $\pm$ 0.01	14 $\pm$ 1	2.7 $\pm$ 0.2	45.2 $\pm$ 0.3	2.7
P3 A 20–30	48.35	44.30	5.85	1.51	Sandy	105.21 $\pm$ 0.35	10YR2/2	1.04 $\pm$ 0.03	20 $\pm$ 3	10 $\pm$ 2	49.36 $\pm$ 0.01	6.4
P3 A 30–40	a	a	a	a	a	460 $\pm$ 30	10YR2/2	1.18 $\pm$ 0.03	22 $\pm$ 3	11 $\pm$ 2	50 $\pm$ 2	11.6
P3 Bh 110–120	a	a	a	a	a	2510 $\pm$ 30	10YR4/2	0.47 $\pm$ 0.01	97 $\pm$ 12	24 $\pm$ 3	50.0 $\pm$ 0.1	11.7
P3 Bh 170–180	a	a	a	a	a	7570 $\pm$ 50	10YR2/2	0.54 $\pm$ 0.01	39 $\pm$ 3	23 $\pm$ 2	52 $\pm$ 6	14.9
P3 Bh-C 270–280	a	a	a	a	a	a	2.5YR6/4	0.39 $\pm$ 0.01	661 $\pm$ 220	132 $\pm$ 44	51 $\pm$ 3	13.3
P3 Bh 370–380	30.31	59.52	7.16	3.02	Sandy	9980 $\pm$ 60	10YR2/2	0.71 $\pm$ 0.01	39 $\pm$ 2	20 $\pm$ 1	52.8 $\pm$ 0.3	24.8
P3 Bh 380–390	a	a	a	a	a	a	7.5YR3/3	0.58 $\pm$ 0.01	102 $\pm$ 10	37 $\pm$ 4	54.5 $\pm$ 0.5	21.8

<sup>a</sup> Not analysed.

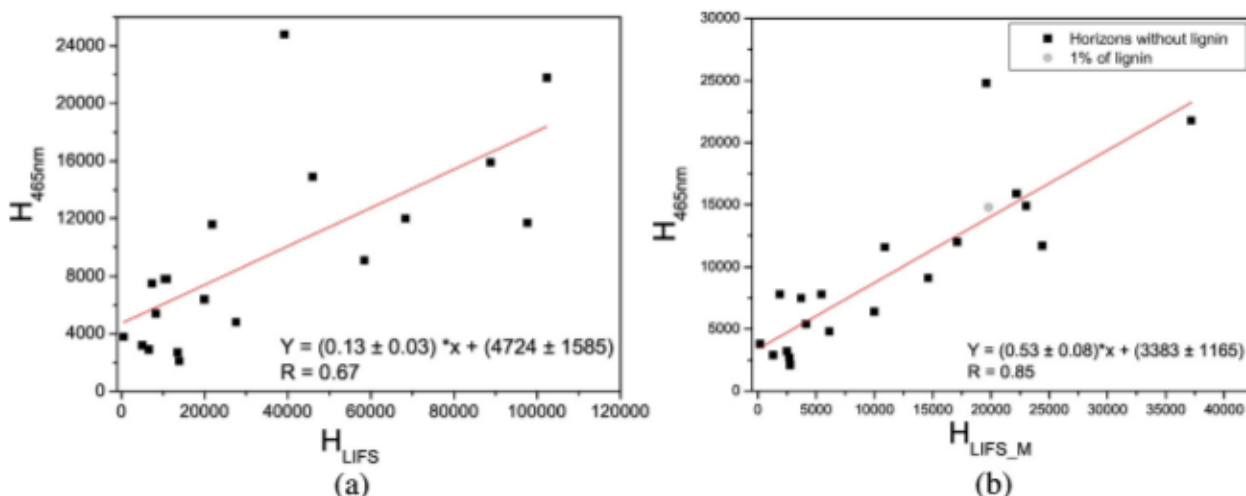


Fig. 2. Correlations for (a)  $H_{465\text{ nm}}$  vs  $H_{\text{LIFS}}$  and (b)  $H_{465\text{ nm}}$  vs  $H_{\text{LIFS}_M}$ .

horizons. According to Bardy et al. (2008), the aromatic character observed in Bh horizons of Amazonian podzols could be acquired during the humification and/or percolation processes of humic material. This material of low molecular size and high solubility could migrate and accumulate in macroporous horizons, suggesting that this process was likely to have occurred in the deeper Bh horizons of soils P1 and P3.

According to Senesi et al. (2016), the stabilisation of SOM due to humification processes occurs not only by the incorporation of recalcitrant SOM compounds such as lignin and phenolic and aliphatic components, but also smaller and more labile molecular fragments derived from soil microbial activity. Hence, the low humification index values obtained for the SOM in the surface horizons could be attributed to the presence of more labile organic matter (plant litter and/or non-humic compounds from microbial activity), while SOM decomposition increased with increasing soil depth. This increase could be explained by the movement of the most soluble molecules of smaller molecular size to deeper horizons, due to both physical (size) and chemical (mineral affinity) processes (Bayer et al., 2000; Senesi et al., 2016).

### 3.2. Characteristics of the HA fractions

Preliminary fluorescence experiments conducted to evaluate, minimize, and correct the possible internal filter effect (Luciani et al., 2009; Guo et al., 2015) showed that it was clearly evident at HA concentrations  $> 15\text{ mg L}^{-1}$  (Fig. 4). Consequently, an HA concentration of

$12.5\text{ mg L}^{-1}$  was selected for the fluorescence experiments described in this paper.

Table 1 shows the HA carbon content (C%) and humification index ( $H_{465}$ ) values used to evaluate the humification degree of the soil. The C% values for the HA fractions ranged from 45 to 56.2%, with the highest values obtained for the Bh horizons of the three soils: P1 Bh 40–50, P2 Bh 180–183, P3 Bh 170–180, P3 Bh-C 270–280, and P3 Bh 380–390. The HA isolated from the P1 soil horizons showed a gradual increase of C% from the surface horizon down to horizon P1 Bh 40–50, and a subsequent decrease in the deepest Bh horizons. The C% of HA from soils P2 and P3 showed a discontinuous pattern in the upper horizons and higher values in the deepest Bh horizons. In general, the  $H_{465}$  values for HA from the surface horizons were significantly lower than those for deeper horizons, and progressively increased with depth. These results suggested that the presence of conjugated aromatic compounds increased with the depth of the Bh horizons (Allard, 2006; Zhang et al., 2011).

Fig. 5 shows the  $H_{\text{LIFS}_M}$  values of the soils (Fig. 5a) and the  $H_{465}$  values of the HAs (Fig. 5b), as a function of the radiocarbon dating age of the SOM in the horizons, from which four soil groups can be discerned.

In Fig. 5a and b, the first group includes soils and HA from surface horizons with low values of  $H_{\text{LIFS}_M}$  and  $H_{465}$  and a more recent carbon age, which were therefore richer in labile compounds.

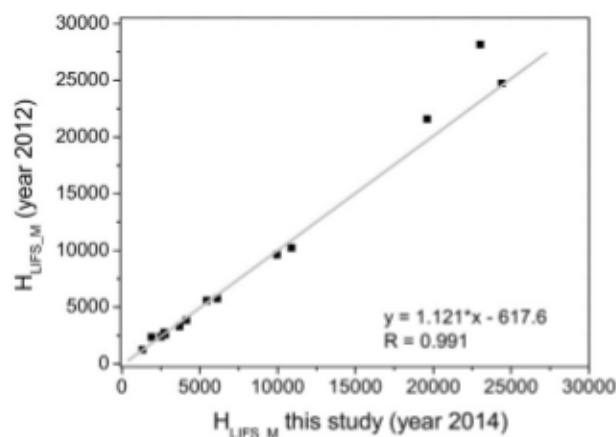


Fig. 3. Reproducibility of the correction factor proposed in this study.

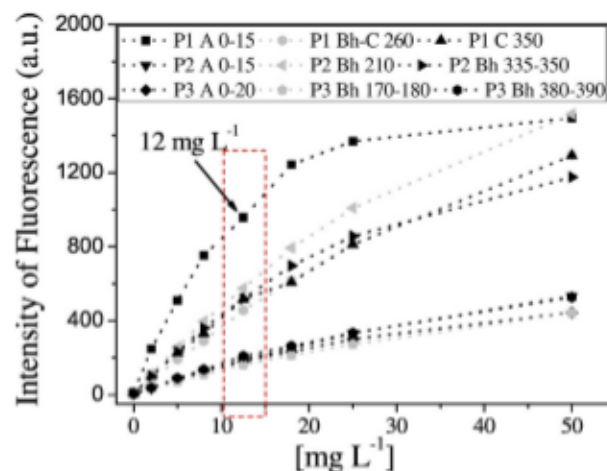


Fig. 4. Fluorescence with excitation at 465 nm, according to humic acid concentration.

The second group of soil samples consisted only of surface horizons with young organic matter, but intermediate humification ( $H_{LIFS\_M}$ ). This finding demonstrated the rapid contribution of carbon structures with condensed aromatic rings, possibly as a result of recent microbial activity leading to the condensation of aromatic substances in young organic matter. The same horizon grouping was observed for  $H_{465}$ , as shown in Fig. 5b. It can be hypothesized that this organic material originated from the formation of polyphenols (in condensation reactions) and their transformation into quinones, which can react with other compounds (Wu et al., 2017).

The third group was directly influenced by groundwater (Fig. 1b) and included the horizons P1 A-E 15–30, P1 Bh 40–50, and all the Bh horizons of P2. The  $H_{LIFS\_M}$  and  $H_{465}$  values reflected low humification (Fig. 5a and b). However, the carbon in this group was older than in the surface horizons, which could have been due to little structural modification. The possible presence of high contents of lignin derivatives was evaluated as described below (Section 3.3).

The fourth group included samples with the oldest carbon and the highest  $H_{LIFS\_M}$  and  $H_{465}$  values (Fig. 5).

These results suggested that the SOM in the surface horizons included simple and labile compounds and less humified HA, while more complex humified compounds were leached and were accumulated or retained in deeper horizons. Small and less condensed molecules were

transported to the river in floodwater (Lucas et al., 2012). It could be concluded that there were four types of organic matter: recalcitrant, humified, and old dating; labile and young dating; humified and young dating; and little humified and old dating. Therefore, the humification process did not have a direct relation with the dating age of the organic material.

### 3.3. Lignin experiment

The accumulation of material with a greater degree of humification in the third group of horizons was hypothesized to be associated with the presence of structures containing lignin and derivatives of lignin. Therefore, an exploratory experiment was performed by adding controlled amounts of lignin to the podzol sample that showed the highest humification index value (P3 Bh-C 270–280).  $H_{LIFS\_M}$  decreased with greater addition of lignin (Fig. 6a), with values of 19,798, 6279, and 2740 obtained for addition of 1, 5, and 10% of lignin to 400 mg of soil. Analysis of the  $H_{LIFS\_M}$  values for the soil with 1% added lignin and for other horizons without lignin (Fig. 2b) resulted in correct prediction of the corresponding  $H_{465}$  values (15,200 to 20,000) for the HA, using the relation  $H_{465} = f(H_{LIFS})$  (Table 1). Fig. 6b shows the fluorescence emission spectrum of a lignin solution, normalised to the quantity of material, with  $H_{465}$  of the same order of magnitude as obtained for HA in the Bh horizons from 240 to 350 cm (soil P1) and from 170 to 280 cm (soil P3) (Table 1). The signals for the humic acids present in

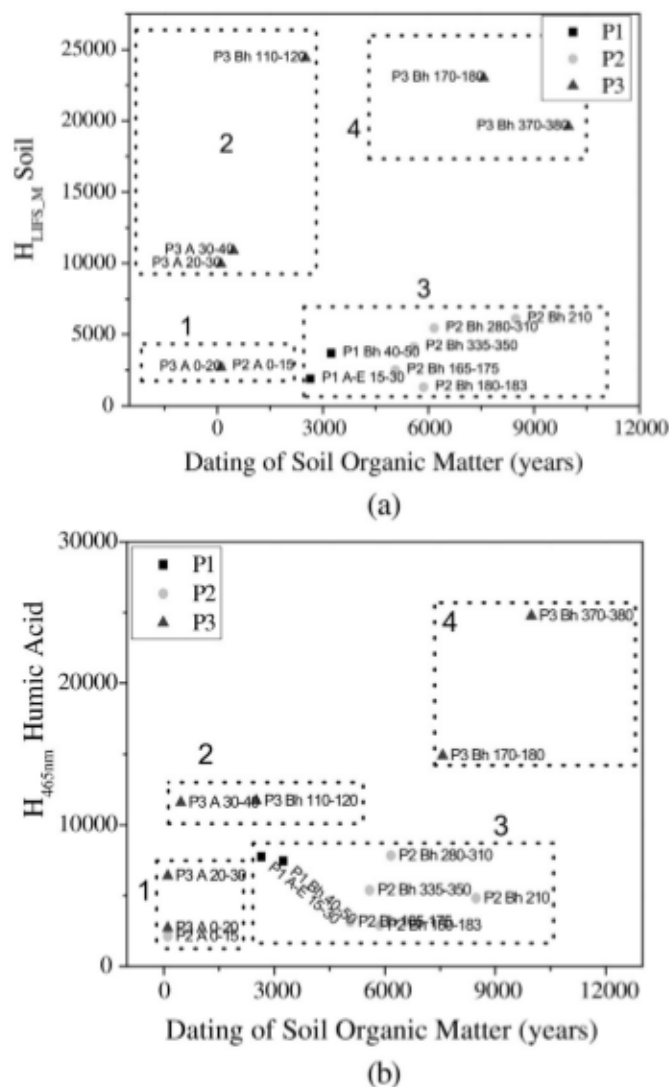


Fig. 5. (a)  $H_{LIFS\_M}$  values of the soils and (b)  $H_{465}$  values of the HA, as a function of the carbon dating age of the SOM in the horizons.

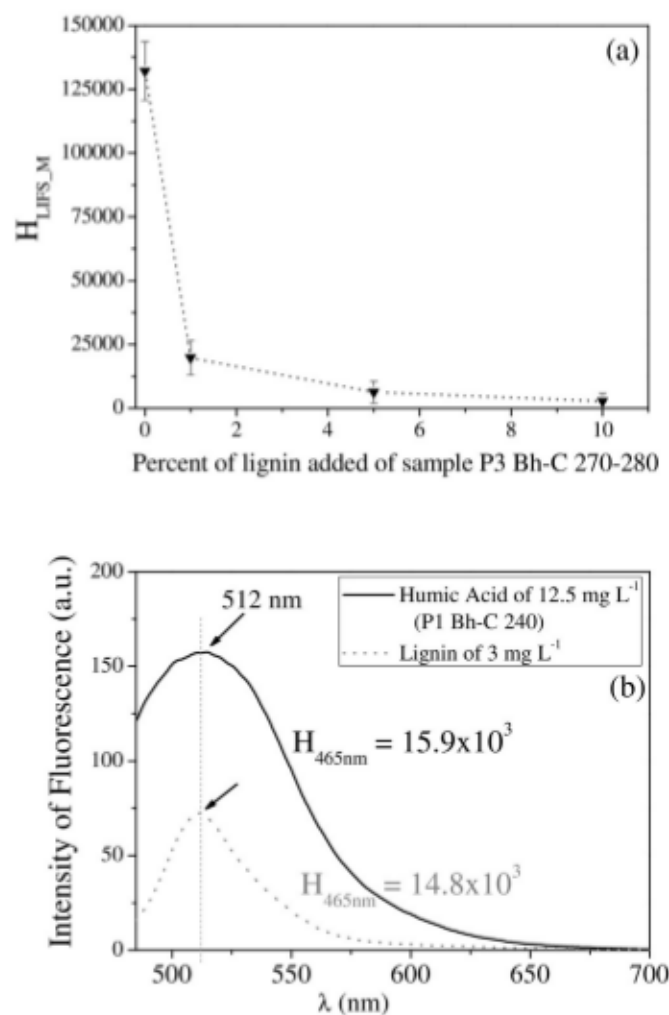


Fig. 6. (a)  $H_{LIFS}$  values as a function of the amount of lignin added to sample P3 Bh-C 270–280, and (b) fluorescence emission spectra of solutions of 3 mg L<sup>-1</sup> lignin and 12.5 mg L<sup>-1</sup> humic acid (sample P1 Bh-C 240).

the Bh horizons (P1 Bh-C 240, P1 Bh-C 260, P1 C 350, P3 Bh 170–180, and P3 Bh-C 270–280) were very similar to the lignin signal, indicating that the samples probably contained large amounts of lignin. The influence of lignin on the composition of the humic acids was indicated by the presence of peaks at the same fluorescence wavelength (512 nm) (Fig. 6b). The higher fluorescence intensity of humic acids, compared to lignin, can be explained by the presence of carboxylic, phenolic, and amine groups in the former.

It could be concluded from the fluorescence spectroscopy measurements, dating of the SOM, and elemental analyses that the structures of the humic acids were derived from lignin.

Overall, the results obtained using lignin in soil pellets and in solution suggested that the characteristics of lignin were very similar to those of the humic acids present at the intermediate state of humification (Fig. 2b, Table 1), which had intermediate humification index values (from 14,000 to 22,000) and intermediate ages. Another interesting feature was that the HA present in soil P2 had different characteristics and a low humification index value, suggesting that the HA was derived from other formation routes. This leads to the hypothesis that the humic acids present in the Amazonian podzols were formed in two routes, one (from modified lignin) that followed the classical theory, and another that followed the polyphenol pathway (condensation of polyphenols).

These findings enabled the proposal of a model for the three Amazonian podzol profiles. In the P1 profile, there was an accumulation of fresh organic material with a low degree of humification, which was displaced with depth according to physical and chemical processes. The groundwater present in the profile assisted in the translocation of older and more humified organic material to deeper horizons. Similar behaviour was observed for the P3 profile up to a depth of 280 cm (P3 Bh 270–280), while below this horizon there were only small amounts of humified organic material, which could be attributed to the presence of more recalcitrant compounds derived from lignin. The P2 profile showed some interesting features, with the carbon accumulated in horizon P2 Bh 180–183 being slightly humified and relatively old. This could be explained by the presence of an impermeable horizon above horizon P2 Bh 180–183, which consequently resulted in the organic material below this horizon deriving from older material with previous characteristics. This extremely old organic material with a low degree of humification was associated with the presence of lignin-like structures in this horizon. Furthermore, the groundwater level in this soil could have facilitated the contribution of microbiological activity (decomposition) and the displacement of molecules with greater solubility, smaller molecular size, and greater humification to deeper Bh horizons, as found for horizons P2 Bh 280–310 and P2 Bh 335–350.

These findings demonstrated that LIFS and classical fluorescence spectroscopy could be used as sensitive techniques to observe structural changes in the composition of organic matter and humic fractions extracted from Amazonian podzols. An additional consideration is that these techniques contribute to the use of green chemistry in environmental studies.

#### 4. Conclusions

Humic acids from Amazonian podzols showed structural characteristics that depended on the pedologic process, as observed using two humification indexes ( $H_{LIFS-M}$  and  $H_{465}$ ), dating of soil, and tests employing lignin. The SOM ages and humification index values indicated that the surface horizons contained simple and labile compounds, with less humified HA, while more complex humified compounds were leached and accumulated in deeper horizons. The characteristics of lignin were similar to those of the humic acids present in the intermediate state of humification (the third group identified in the SOM dating). The hypothesis proposed was that two processes contributed to the genesis of the humified SOM: one associated with the formation of

modified lignin derivatives, and the other involving the transformation (condensation) of polyphenols.

Four types of organic matter were present in these soils: recalcitrant, humified, and old dating; labile and young dating; humified and young dating; and little humified and old dating. Factors including soil texture, presence of water, microorganisms, and age influenced the processes of formation and humification of the organic material. In addition, lignin type organic compounds appeared to be preserved for many years in the deeper horizons of the soil. Therefore, there was no direct association between the humification process and the age of the organic matter in the Amazonian podzols.

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