



# Effects on the composition and structural properties of the humified organic matter of soil in sugarcane strawburning: A chronosequence study in the Brazilian Cerrado of Goiás State



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## ARTICLE INFO

### Article history:

Received 6 March 2015

Received in revised form 3 September 2015

Accepted 19 September 2015

Available online xxx

### Keywords:

Humic acids

Soil burning

Soil organic matter

Spectroscopic characterization

## ABSTRACT

The heating of soil organic matter after burning events can significantly change the composition and structure of humified organic matter. The impact of burning on the amount and quality of SOM depends on fire intensity, recurrence, the type of vegetation, soil texture and the local climate. The present study identified the composition and structural properties of humic acids (HA) extracted from a sandy loam textured *Latosolo Vermelho* (Oxisol) managed with the pre-harvest burning of sugarcane straw for chronosequences of 1, 5, 10 and 20 years in Rio Verde, Goiás (GO). Soil samples were collected at depths of 0–0.05, 0.05–0.10, 0.10–0.20 and 0.20–0.30 m. To obtain a reference of natural conditions, soil was also sampled in adjacent areas covered with native vegetation and Pasture grass. The HA samples from soil under different burning conditions and areas (Cerrado and Pasture grass) were analyzed using chemical-spectroscopic characterization and the chemiometric technique. The greatest modifications in HA composition after burning occurred in the most superficial soil layers. At a depth of 0.0–0.05 m, the main changes observed were a decrease in mean C and N levels and enrichment in O, irrespective of the length of time that burning practices had been adopted. Additionally, in the most superficial layer, the ultraviolet-visible spectroscopy assay showed that burning enhanced the aromatic properties ( $<E_4/E_6$ ) of HA, and FTIR spectroscopy coupled with PCA confirmed that the top soil layer shows higher variations in the functional groups as a function of burning. The PCA analysis of <sup>13</sup>C NMR spectra also showed that stronger aromatic properties were found in the most superficial layer, and the PCA of FTIR spectra reinforced the result by grouping HAs in these layers and thereby indicated that the most intense changes occurred in the 0.0–0.05 m layer. The most intense disturbances produced by sugarcane burning on the composition and structure of humified organic matter occurred in the most superficial layer. The combined use of the methods adopted was efficient and, along with the chemiometric techniques, consisted of useful tools that can support the development of procedures to study phenomena such as burning.

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## 1. Introduction

The final products of biomass combustion are water, CO<sub>2</sub> and minerals contained in the ash. Total biomass oxidation depends on a number of conditions to ensure adequate oxygen supply during combustion. This is not observed in nature, where incomplete combustion yields a number of compounds, such as carbon monoxide, methane and a wide variety of pyrolysis products,

including hydrocarbons and organic matter fractions. In addition to producing a substantial amount of different organic compounds, natural fire modifies other compounds through heating (González-Pérez et al., 2004).

The impact of fire on the amount and quality of soil organic matter (SOM) depends mainly on its intensity and recurrence, vegetation type, soil texture and local climate (González-Pérez et al., 2004; Knicker, 2007; Satín et al., 2008; Vergnoux et al., 2011; Granged et al., 2011). In general, SOM heating can considerably increase the aromaticity of the remaining organic matter to the detriment of carboxyl and aliphatic groups (Almendros et al., 1992). The structures formed are derived from carbon hydrates, lipids, alkylated macromolecules and peptides. As these structures are more recalcitrant, they are not readily humified and can be extracted as the humic fraction (Vergnoux et al., 2011).

Dry soil is an efficient insulator, and the effects of fire are usually restricted to the upper centimeters of the soil (Debano et al., 1998), which receives only a small fraction of the heat produced. The temperatures attained in the different soil layers, the heat exposure time and the soil stability determine the nature and intensity of the changes in the chemical and physical attributes of the soil.

In the short term, moderate fire becomes a mineralization agent because the ashes produced contain high levels of P, K and Ca, which increases the nutrient availability for plant growth, especially in layers up to 0.05 m (Coutinho, 1990; Kauffman et al., 1994; Rheinheimer et al., 2003; González-Pérez et al., 2004). In addition, vegetation fire can affect SOM stocks and quality (Roscoe et al., 2000; Knicker et al., 2005).

Pre-harvest sugarcane straw burning can compromise the maintenance of SOM levels by reducing the incorporation of crude organic matter and promoting mineralization of existing organic matter. Moreover, given that soil remains bare for a long period of time, erosion is accelerated and physicochemical attributes are affected (Molina, 1995; Mendoza, 1996).

The effect of burning suppression and maintenance of sugarcane straw on soil increases SOM levels as reported by Blair (2000) and Noble et al. (2003) in Australia, Dominy et al. (2002) in South Africa and Villegas et al. (1998) in Cuba. Studies conducted in Brazil found similar results in very clayey and medium-textured soil (Orlando Filho et al., 1998) but not in very sandy soil (Ball-Coelho et al., 1993).

The chemical and spectroscopic techniques applied to soil organic matter, particularly humic substances, permit acquisition of important information on the structural characteristics and properties of these compounds in addition to monitoring these features throughout events and phenomena of soil (Cunha, 2005). Recent studies on soil organic carbon are based on the spectrophotometric analysis of soil humic substances, particularly humic acid, which allows the most detailed investigations because it is easier to extract, exhibits a close relationship with soil identity and especially because of its capacity to express soil transformations including changes caused by degrading management practices (Fontana et al., 2006).

The present study aimed to identify the composition and chemical properties of humified SOM through spectrophotometry and chemometric determination of humic acids extracted from *Latossolo Vermelho* (Oxisol) and under a sugarcane crop chronosequence, a Pasture area and a natural Cerrado in Goiás.

## 2. Material and methods

### 2.1. Study area description

The study was performed using *Latossolo Vermelho* (Oxisol) (Embrapa, 2006) with a sandy loam texture in Rio Verde, Goiás

state, and managed with the pre-harvest burning of sugarcane straw for chronosequences of 1, 5, 10 and 20 years (areas 1Y, 5Y, 10Y and 20Y, respectively). The soil samples were collected at depths of 0–0.05, 0.05–0.10, 0.10–0.20 and 0.20–0.30 m. The adjacent areas, which were covered by native Cerrado vegetation and Pasture grass, were also sampled to serve as a reference of the natural soil conditions. The history of soil use in the last 40 years is described in Fig. 1.

Areas cropped with sugarcane received 500 kg ha<sup>-1</sup> of 5–30–20 NPK at the initiation of planting and 500 kg ha<sup>-1</sup> of 18–00–27 NPK at ratoon. Area 10Y was first renewed in 2010, and area 20Y was renewed on February 2, 2000 and after the 2010 crop season. In the 2009/2010 season, the sugarcane yield was 113 Mg ha<sup>-1</sup> in Y1, 111 in 5Y, 85 in Y10 and 96 in Y20. Area Y20 received annual inputs of 60 m<sup>3</sup> ha<sup>-1</sup> of vinasse (N=0.03, P=29, K=225, Ca=70, Mg=87, in mg L<sup>-1</sup>; pH (H<sub>2</sub>O)=3.5).

The humic acids (HA) were isolated and purified as recommended by the International Humic Substance Society- IHSS (Swift, 1996). The soil samples were pretreated with 0.1 mol L<sup>-1</sup> HCl and extracted in 0.1 mol L<sup>-1</sup> NaOH at 1:10 (v/v) soil:solvent ratio. The humic acids were precipitated using decreasing pH (1.0–1.5) with 6 mol L<sup>-1</sup> HCl. This procedure was performed twice. The humic acids were then treated with 0.1 mol L<sup>-1</sup> HCl and 0.3 mol L<sup>-1</sup> HF and then dialyzed with deionized water until a negative test against AgNO<sub>3</sub> was obtained, followed by freeze-drying. The total organic carbon (TOC) was determined in samples with 200–400 g of total carbon and using a Carlo Erba automatic C–N analyzer (EA 1108, Milan, Italy) coupled to a Delta Plus continuous-flow isotope ratio-mass spectrometer (Finnigan Mat, Bremen, Germany).

### 2.2. Elemental composition of humic acids

Determination of C, H, N and S in the humic acid samples was conducted using a Flash EA1112 Elemental Analyzer (ThermoFinnigan) at the University of Coruña. The samples were weighed in 9 × 5 mm tin capsules using an MX5 Microbalance (Mettler Toledo, 1 µg resolution) and analyzed by spontaneous combustion in a quartz tube containing Cu/WO<sub>3</sub> and maintained at 1020 °C. Sulfanilamide and BBot were used as standards for C, H, N and S quantification. The C, H and N levels (in percentage) were corrected to a dry ash-free base using data from thermogravimetric analysis and according to the equation: %corrected = %original × 100 / (100 – %humidity – %ash). The oxygen level was obtained by subtracting the corrected level of the other elements: %O = (100 – %C – %H – %N). The atomic ratios were calculated using the formulas: H/C = (%H/1)/(%C/12); O/C = (%O/16)/(%C/12); O/H = (%O/16)/(%H/1) and C/N = (%C/12)/(%N/14).

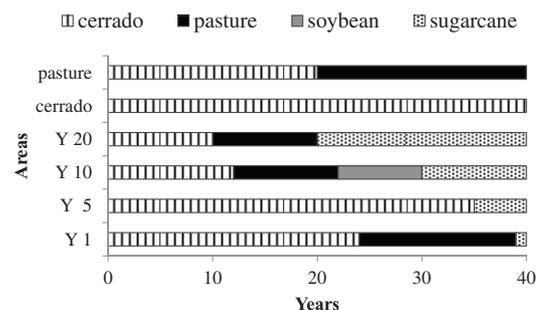


Fig. 1. Land use history in the study area. Areas cropped with sugarcane were managed with pre-harvesting burning for 1 (Y1), 5 (Y5), 10 (Y10) and 20 (Y20) years.

### 2.3. Ultraviolet–vis spectroscopy (UV–vis) and $E_4/E_6$ ratio

The UV–vis spectra were obtained in a Zuzi 4418 spectrophotometer at the University of Coruña (UDC). The samples were examined in the UV region (200–380 nm) and visible light (380–600 nm). The spectra were recorded at 25 °C with an optical constant of 1 cm and in a solution of 2 mg HA in 10 mL of 0.05 mol L<sup>-1</sup> NaHCO<sub>3</sub>. The  $E_4/E_6$  ratio (ratio of optical density at 465 nm and 665 nm) was also calculated.

### 2.4. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra between 400 and 4000 cm<sup>-1</sup> (4 cm<sup>-1</sup> resolution) were obtained in a Bruker vector 22 spectrophotometer at the *Servizo de apoio á investigación* (SAI) of the University of Coruña. The Mid-IR transmittance was obtained from samples embedded in KBr pellets. In this regard, 2 mg of freeze-dried HA was mixed with 200 mg of KBr and ground in an agate mortar and pressed in 13 mm pellet molds under an applied load of 10 Mg and using a hydraulic press.

### 2.5. Carbon-13 nuclear magnetic resonance (<sup>13</sup>C NMR)

A Bruker AV500 spectrometer equipped with a dual cryoprobe for <sup>1</sup>H and <sup>13</sup>C was used to perform the <sup>13</sup>C NMR spectroscopy at the

*Servizo de apoio á investigación* (SAI) at the University of Coruña. The humic acids were solubilized into an alkaline solution (0.1 mol L<sup>-1</sup> NaOH prepared with D<sub>2</sub>O). A power gated decoupling pulse sequence with a 45 degree pulse for <sup>13</sup>C was used. The acquisition time was 1.09 s in conjunction with 1 s of relaxation delay and producing 20,000–40,000 scans per sample (12–24 h).

For each treatment, the relative intensity of each band and the proportion of aliphaticity and aromaticity (in percent) were calculated based on the respective spectral range and according to Stevenson (1994):

$$\text{Aliphaticity (\%)} = \frac{\text{area of the aliphatic C signals (0 – 100)}}{\text{area of the C signals}} \times 100\%$$

$$\text{Aromaticity (\%)} = \frac{\text{area of the aromatic C signals (110 – 160 ppm)}}{\text{area of the C signals (0 – 160 ppm)}} \times 100\%$$

### 2.6. Statistical analysis of spectral data from elemental composition, FTIR and <sup>13</sup>C NMR

Samples of TOC and the elemental composition were confirmed by the Lilliefors test and homocedasticity by the Cochran &

**Table 1**  
Total organic carbon (TOC), Elemental composition of humic acids extracted from soil in areas managed with sugarcane chronosequences in a Cerrado area in Goiás.

System	Soil %TOC	Humic acids							
		% C	% H	% N	% O	H/C	O/C	O/H	C/N
0.0–0.05 m									
Y1	1.72 <sup>b</sup>	49.01 <sup>b</sup>	5.26 <sup>a</sup>	3.49 <sup>a</sup>	42.24 <sup>d</sup>	1.30 <sup>a</sup>	0.60 <sup>ns</sup>	0.50 <sup>b</sup>	16.40 <sup>c</sup>
Y5	4.08 <sup>a</sup>	48.95 <sup>b</sup>	4.58 <sup>c</sup>	3.49 <sup>a</sup>	42.98 <sup>c</sup>	1.10 <sup>b</sup>	0.70 <sup>ns</sup>	0.60 <sup>b</sup>	16.40 <sup>c</sup>
Y10	2.75 <sup>b</sup>	47.42 <sup>d</sup>	3.72 <sup>e</sup>	3.19 <sup>b</sup>	45.67 <sup>a</sup>	0.90 <sup>c</sup>	0.70 <sup>ns</sup>	0.80 <sup>a</sup>	17.30 <sup>a</sup>
Y20	2.68 <sup>b</sup>	49.22 <sup>a</sup>	4.30 <sup>d</sup>	3.38 <sup>a</sup>	43.10 <sup>c</sup>	1.00 <sup>c</sup>	0.70 <sup>ns</sup>	0.60 <sup>b</sup>	17.00 <sup>b</sup>
Median	2.68	48.98	4.44	3.43	43.04	1.05	0.70	0.60	16.70
Error	0.83	0.41	0.31	0.07	0.74	0.08	0.02	0.06	0.22
Cerrado	4.23 <sup>a</sup>	47.74 <sup>c</sup>	4.49 <sup>c</sup>	3.40 <sup>a</sup>	44.37 <sup>b</sup>	1.10 <sup>b</sup>	0.70 <sup>ns</sup>	0.60 <sup>b</sup>	16.40 <sup>c</sup>
Pasture	4.12 <sup>a</sup>	47.37 <sup>d</sup>	4.88 <sup>b</sup>	3.45 <sup>a</sup>	44.30 <sup>b</sup>	1.20 <sup>a</sup>	0.70 <sup>ns</sup>	0.60 <sup>b</sup>	16.00 <sup>d</sup>
0.05–0.10 m									
Y1	2.11 <sup>b</sup>	52.15 <sup>a</sup>	4.32 <sup>c</sup>	3.82 <sup>a</sup>	39.71 <sup>e</sup>	1.00 <sup>b</sup>	0.60 <sup>ns</sup>	0.60 <sup>ns</sup>	15.90 <sup>d</sup>
Y5	4.15 <sup>a</sup>	51.81 <sup>b</sup>	4.54 <sup>b</sup>	3.76 <sup>a</sup>	39.89 <sup>d</sup>	1.10 <sup>a</sup>	0.60 <sup>ns</sup>	0.50 <sup>ns</sup>	16.10 <sup>c</sup>
Y10	2.52 <sup>b</sup>	52.11 <sup>a</sup>	4.07 <sup>d</sup>	3.54 <sup>b</sup>	40.28 <sup>c</sup>	0.90 <sup>b</sup>	0.60 <sup>ns</sup>	0.60 <sup>ns</sup>	17.20 <sup>a</sup>
Y20	2.70 <sup>b</sup>	51.29 <sup>c</sup>	4.24 <sup>c</sup>	3.56 <sup>b</sup>	40.91 <sup>b</sup>	1.00 <sup>b</sup>	0.60 <sup>ns</sup>	0.60 <sup>ns</sup>	16.80 <sup>b</sup>
Median	2.77	51.96	4.28	3.66	40.08	1.00	0.60	0.60	16.40
Error	0.76	0.19	0.09	0.07	0.26	0.04	0.00	0.06	0.30
Cerrado	4.18 <sup>a</sup>	51.31 <sup>c</sup>	4.63 <sup>b</sup>	3.75 <sup>a</sup>	40.31 <sup>c</sup>	1.10 <sup>a</sup>	0.60 <sup>ns</sup>	0.50 <sup>ns</sup>	16.00 <sup>d</sup>
Pasture	3.65 <sup>a</sup>	47.49 <sup>d</sup>	4.81 <sup>a</sup>	3.55 <sup>b</sup>	44.15 <sup>a</sup>	1.20 <sup>a</sup>	0.70 <sup>ns</sup>	0.60 <sup>ns</sup>	15.60 <sup>e</sup>
0.10–0.20 m									
Y1	2.26 <sup>b</sup>	50.86 <sup>b</sup>	4.74 <sup>b</sup>	3.79 <sup>b</sup>	40.61 <sup>d</sup>	1.10 <sup>b</sup>	0.60 <sup>b</sup>	0.50 <sup>ns</sup>	15.70 <sup>d</sup>
Y5	2.78 <sup>b</sup>	51.11 <sup>a</sup>	4.76 <sup>b</sup>	3.79 <sup>b</sup>	40.34 <sup>e</sup>	1.10 <sup>b</sup>	0.60 <sup>b</sup>	0.50 <sup>ns</sup>	15.70 <sup>d</sup>
Y10	2.08 <sup>b</sup>	50.76 <sup>c</sup>	3.97 <sup>e</sup>	3.34 <sup>c</sup>	41.93 <sup>c</sup>	0.90 <sup>c</sup>	0.60 <sup>b</sup>	0.70 <sup>ns</sup>	17.70 <sup>a</sup>
Y20	1.57 <sup>b</sup>	49.42 <sup>c</sup>	4.39 <sup>d</sup>	3.43 <sup>c</sup>	42.76 <sup>b</sup>	1.10 <sup>b</sup>	0.60 <sup>b</sup>	0.60 <sup>ns</sup>	16.80 <sup>b</sup>
Median	2.12	50.53	4.56	3.61	41.27	1.10	0.60	0.55	16.25
Error	0.43	0.37	0.18	0.11	0.56	0.05	0.00	0.04	0.48
Cerrado	2.00 <sup>b</sup>	50.68 <sup>b</sup>	4.59 <sup>c</sup>	4.09 <sup>a</sup>	40.64 <sup>d</sup>	1.10 <sup>b</sup>	0.60 <sup>b</sup>	0.60 <sup>ns</sup>	14.50 <sup>e</sup>
Pasture	4.08 <sup>a</sup>	41.38 <sup>d</sup>	5.25 <sup>a</sup>	3.00 <sup>d</sup>	50.37 <sup>a</sup>	1.50 <sup>a</sup>	0.90 <sup>a</sup>	0.60 <sup>ns</sup>	16.10 <sup>c</sup>
0.20–0.30 m									
Y1	1.69 <sup>b</sup>	51.06 <sup>b</sup>	4.44 <sup>c</sup>	3.86 <sup>c</sup>	40.64 <sup>c</sup>	1.00 <sup>b</sup>	0.60 <sup>a</sup>	0.60 <sup>a</sup>	15.40 <sup>c</sup>
Y5	1.66 <sup>b</sup>	49.45 <sup>d</sup>	4.70 <sup>b</sup>	4.08 <sup>b</sup>	41.77 <sup>b</sup>	1.10 <sup>a</sup>	0.60 <sup>a</sup>	0.60 <sup>a</sup>	14.10 <sup>e</sup>
Y10	1.62 <sup>b</sup>	50.70 <sup>c</sup>	3.95 <sup>a</sup>	3.45 <sup>d</sup>	41.90 <sup>b</sup>	0.90 <sup>b</sup>	0.60 <sup>a</sup>	0.70 <sup>a</sup>	17.10 <sup>a</sup>
Y20	1.96 <sup>b</sup>	46.62 <sup>e</sup>	4.23 <sup>d</sup>	3.44 <sup>d</sup>	45.71 <sup>a</sup>	1.10 <sup>a</sup>	0.70 <sup>a</sup>	0.70 <sup>a</sup>	15.80 <sup>b</sup>
Median	1.72	50.07	4.33	3.65	41.83	1.05	0.60	0.65	15.60
Error	0.13	1.00	0.15	0.15	1.10	0.04	0.02	0.02	0.61
Cerrado	1.79 <sup>b</sup>	58.46 <sup>a</sup>	5.18 <sup>a</sup>	4.84 <sup>a</sup>	31.52 <sup>d</sup>	1.10 <sup>a</sup>	0.40 <sup>b</sup>	0.40 <sup>b</sup>	14.10 <sup>e</sup>
Pasture	3.03 <sup>a</sup>	50.41 <sup>c</sup>	5.14 <sup>a</sup>	3.92 <sup>c</sup>	40.53 <sup>c</sup>	1.20 <sup>a</sup>	0.60 <sup>a</sup>	0.50 <sup>b</sup>	15.00 <sup>d</sup>

Y1, Y5, Y10 and Y20: 1, 5, 10 and 20 years under sugarcane-burning management. Soil from natural Cerrado and Pasture areas was sampled as a reference.

Bartlett's test; the results were analyzed in a completely randomized design with 6 areas (1-, 5-, 10- and 20-year old sugarcane crop, Cerrado vegetation and forage grass), and 6 repetitions for each parameter were evaluated up to a depth of 0.3 m. The *F*-test (ANOVA), followed by Scott-Knott for multiple comparisons, was applied using the SAEG 9.0 (*Sistema de Análises Estatísticas e Genéticas*—UFV) statistical package. The error probability was set at 0.05.

The Statgraphics® Centurion XVI package (StatPoint Technologies, Inc.) was used to perform the Principal Component Analyses (PCA) using data on the humic acid elemental composition. The Unscrambler® X 10.3 package (Camo Software AS Inc., Oslo, Norway) was used to perform Principal Component Analysis (PCA) using data on the FTIR and <sup>13</sup>C NMR spectra.

For <sup>13</sup>C NMR spectrometrical data, the integrated regions of each carbon type were loaded as a function of carbon structure (0–45 ppm for the aliphatic groups, 45–60 ppm for the N-aliphatic and methoxyl, 60–110 ppm for the O-aliphatic, 110–140 ppm for the C-aromatic, 140–160 ppm for the O-aromatic, 160–185 ppm for the carboxyl and 185–230 ppm for the carbonyl). The regions assumed non-integrated were assigned a value of zero. The data were transformed using the equation  $\sqrt{(n+1)}$ , where *n* is the integration value obtained in the spectra and then loaded in the computational package to produce a 24 × 10 matrix (scores:integration region/loading: type of carbon structure). The matrix was then analyzed by PCA (maximum of 7 PCs) using the cross validation method and NIPALS algorithm (non-linear iterative partial least squares). The number of aromatic and aliphatic structures was calculated (in percent) based on their location in the PCA graphs produced in Bi-plot (loadings:scores).

For FTIR, a 16 × 1915 (scores:loading) matrix was created using only the spectra of the HA samples of soil managed with sugarcane straw burning. After loading and normalization, FTIR data were subjected to PCA with a maximum number of 7 principal components (PC) and using the cross validation methods and NIPALS algorithm (non-linear iterative partial least squares).

### 3. Results and discussion

#### 3.1. Elemental composition of humic acids

The elemental composition of humic acids free of moisture and ash and their atomic ratios are shown in Table 1. The carbon proportion in HA, except in the Y20 area (at 0.20–0.30 m), showed a small variation as a function of burning management time in the four evaluated soil layers. However, the carbon content was lower at 0.0–0.05 m than in the deeper layers. When comparing the mean

HA carbon content in the burned areas at the different depths to the respective controls (Cerrado and Pasture grass), a slight increase in depth was observed at a depth of 0.0–0.05 m. In the 0.05–0.10 m and 0.10–0.20 m layers, the mean carbon content was similar to that of HA in the Cerrado area, but in the deepest layer, it was similar to that quantified in the Pasture grass area.

The hydrogen content was specific for each HA and did not change with the burning management time at the different soil layers. However, the mean HA nitrogen content in the top layer (0.0–0.05 m) was smaller than in the other depths.

Regarding TOC, a decrease was observed for soil carbon where the fire was applied with respect to both the Cerrado and Pasture soils. Interestingly, soils with a burning application for five years had the lowest losses of TOC. Other studies have shown that the application of burning causes TOC losses in soil (Chen and Shrestha, 2012; Badía-Villas et al., 2014).

The principal component analysis (PCA) confirmed the findings regarding the elemental composition (Fig. 2). With a total explained variability of 61.34%, the PCA plot showed HA separation into two groups in component PC-1. The region of positive PC-1 values grouped HA from the surface layers (0.0–0.05 and 0.05–0.10 m), whereas HA from deeper layers were positioned in the region of negative values. The PCA showed that the top soil HA is more associated with parameters from elemental composition but not HA from the deepest layers.

The PCA confirmed that the highest changes occurred in the most superficial layers. In particular, HA in the 0.0–0.05 m layer showed a close relationship with carbon, oxygen, the C/N ratio and nitrogen levels. In the 0.05–0.10 m layer, HA showed a narrow relationship with hydrogen content and the O/C and the H/C ratio.

The results indicate that at 0.0–0.05 m, sugarcane burning decreased the mean HA carbon and nitrogen levels and increased the HA oxygen, thereby increasing the O/C and C/N ratio.

The reported HA elemental composition and atomic ratio were obtained under specific management conditions and are similar to those reported in other studies (Fontana et al., 2010; Assis 2008; Crespo 2011). Although studies on this issue are scarce, changes in HA composition as a function of sugarcane burning have been observed in other conditions. For instance, González-Pérez et al. (2004) report that burning events caused carbon loss by over 50% in Humic Cambisol (Inceptsol) up to a depth of 0.10 m, and carbon loss was also detected in North American soils subjected to burning practices for 10 years. Moreover, Yustiawati et al. (2014) found that the H/C ratio in HA from burned soil is lower than in HA from unburned soil. Despite these findings, the effects reported in other studies were not detected in the present investigation.

#### 3.2. UV-vis spectroscopy

The UV-vis spectra of the HA samples decreased with an increase in wavelength (Fig. 3). In soil samples from sugarcane areas, the highest absorption was detected in the 300–600 nm range and a shoulder was observed at 350–400 nm. However, this pattern was not observed in HA from Cerrado and Pasture areas. The identified pattern of the UV-vis spectra may be associated with the elemental composition (Fig. 3) and the *E*<sub>4</sub>/*E*<sub>6</sub> ratio of HA at the different layers and management systems (Table 2). According to Schnitzer (1985), the increase in light absorption in the UV-vis spectra is related to the increase in aromatic condensation of humic substances, C content and molecular mass. Compared to HA from Cerrado and Pasture areas, the HA from areas with straw burning showed higher light absorption as a result of the aromatic nature of carbon and as indicated by the *E*<sub>4</sub>/*E*<sub>6</sub> ratio (Table 2).

HA isolated from soil under burned sugarcane for 1, 5 and 20 years (Y1, Y5, Y10 and Y20) at 0.0–0.05 m showed increased absorbance because of the increase in the higher levels of aromatic

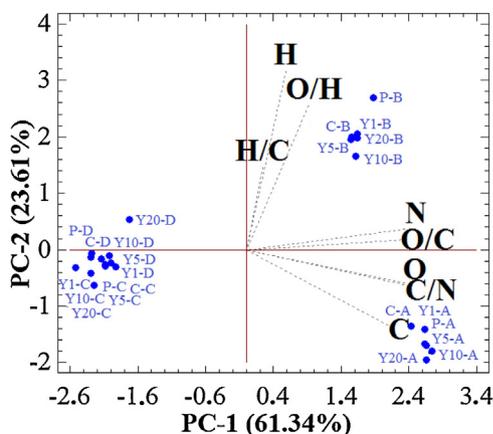
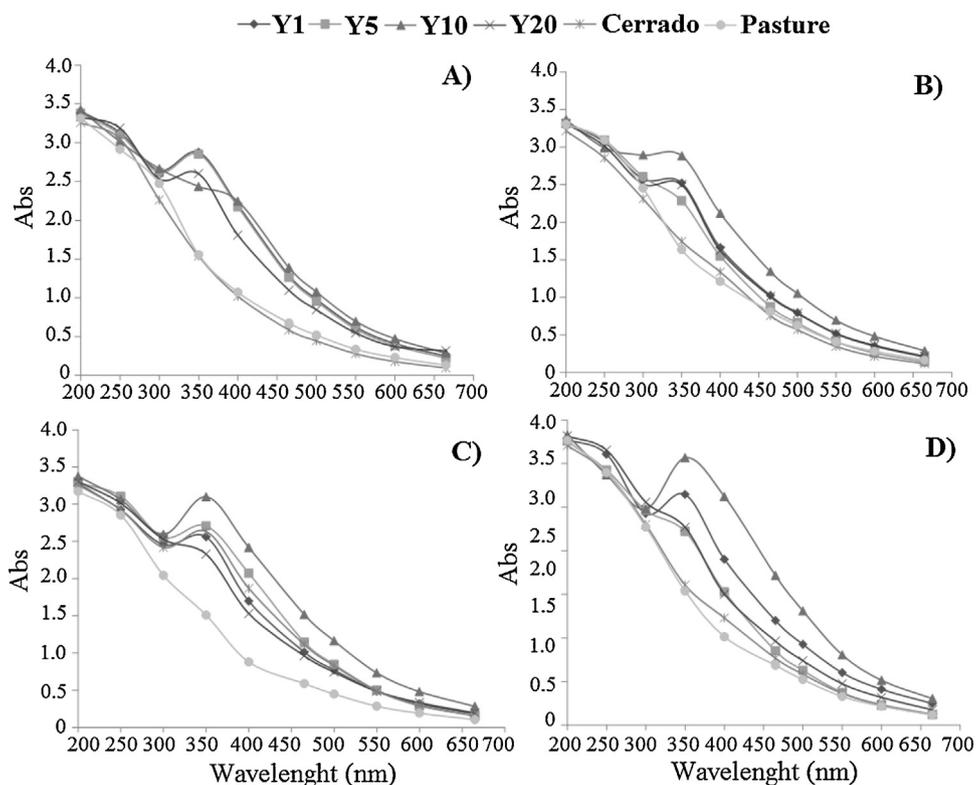


Fig. 2. PCA plot for elemental composition of the humic acids.



**Fig. 3.** UV-visible spectra of humic acids extracted from soil under a pre-harvest sugarcane straw burning chronosequence in a Cerrado area in Goiás. Soil from natural Cerrado and Pasture areas was sampled as reference. (A) 0–0.05 m; (B) 0.05–0.10 m; (C) 0.10–0.20 m; (D) 0.20–0.30 m.

hydrocarbons ( $<E_4/E_6$ ). The higher absorbance of HA isolated from Y1, Y5 and Y10 at 0.05–0.10 m was a consequence of C incorporation, whereas in HA isolated from Y20, this pattern is explained by the increase in the content of aromatic hydrocarbons ( $<E_4/E_6$ ). At 0.10–0.20 m, the increase in absorbance by HA from Y1, Y5, Y10 and Y20 results from an increase in C content. At 0.20–0.30 m, the highest absorbance is related to the dominance of aromatic C in HA from sugarcane areas.

In general, the more pronounced differences in the  $E_4/E_6$  ratio of HA from burned (Y1, Y5, Y10 and Y20) and control (Cerrado and Pasture grass) areas were found in the 0.0–0.05 m layer. Combined with the spectral behavior of HA from the different samples, this result is in accordance with the elemental analysis that showed a lower H/C ratio in HA from burned soil, which is related to the higher aromaticity of this humic fraction in the top soil.

Similar to the present study, others have applied the UV-vis technique to investigate the  $E_4/E_6$  ratio in SOM. In HA sampled in the Amazonian region, Cunha (2005) found an  $E_4/E_6$  ratio of 4.0 in soil from crops and forests affected by anthropogenic factors and a ratio of 6.0 in forest areas without anthropogenic impact. Fontana

et al. (2010) also found a higher  $E_4/E_6$  ratio in forests compared to sugarcane areas submitted to burning practices for 50 years and Pasture areas. In a Pinus forest, Tinoco et al. (2006) observed a lower  $E_4/E_6$  ratio in soil in burned areas because the fire promotes molecular condensation, which results in lower  $E_4/E_6$  ratios. In addition, Crespo (2011) showed that the  $E_4/E_6$  ratio is higher in the topsoil of burned areas (0.0–0.02 m).

### 3.3. Fourier transform infrared spectroscopy (FTIR)

Fig. 4 shows the FTIR spectra of HA from burned and reference areas organized according to soil depth. The FTIR spectra indicate the occurrence of the same functional groups, irrespective of the sampling site. The spectral bands did not indicate visible differences associated with specific functional groups for any of the studied conditions.

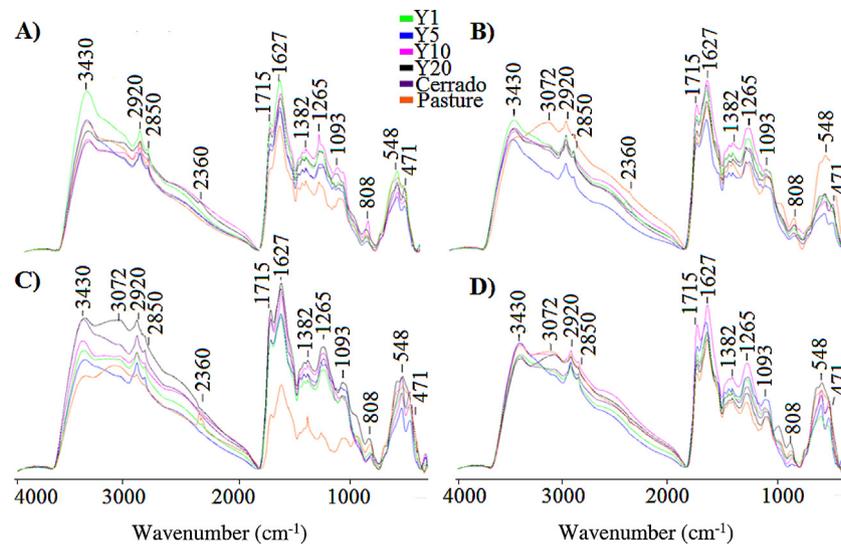
However, the spectral range was similar to that reported in a number of studies (Dick et al., 2008; Czycza, 2009; Dias et al., 2009; Dobbss et al., 2009; Fontana et al., 2010; Crespo, 2011). Wide bands, typical of HA spectra, were observed in the 3440–3380  $\text{cm}^{-1}$  range and correspond to O–H stretching of the phenolic groups, the contribution of aliphatic OH groups and NH from several functional groups (Vergnoux et al., 2011). The 3000–2800  $\text{cm}^{-1}$  range, which indicates the level of humic substance saturation, exhibits two bands: one at 2925  $\text{cm}^{-1}$  and the other at 2855  $\text{cm}^{-1}$ . These bands correspond to the asymmetrical and symmetrical C–H stretch of  $\text{CH}_2$  and  $\text{CH}_3$ , respectively (Baes and Bloom, 1989; Niemeyer et al., 1992). A signal at 2360  $\text{cm}^{-1}$  was observed in the Y20 samples collected at 0.0–0.05 m, Y1 and Pasture at 0.05–0.10 m and at higher intensity in the Pasture at 0.10–0.20 m. Narimoto (2006) attributed this band to the vibration of the carboxyl group (COOH). In the 1720–1600  $\text{cm}^{-1}$  range, two other bands were identified and corresponded to the C=O stretch of carboxyl, aldehyde and ketone groups, and the other band (ca. 1627  $\text{cm}^{-1}$ ) represented several

**Table 2**

The  $E_4/E_6$  ratio of humic acids extracted from soil under chronosequence of sugarcane straw burning, Cerrado and pasture areas in Goiás.

Systems	Depth (m)			
	0.0–0.05	0.05–0.10	0.10–0.20	0.20–0.30
Y1	5.24	5.06	5.56	4.83
Y5	5.71	6.28	7.30	7.16
Y10	4.87	4.69	5.36	5.66
Y20	3.47	4.73	4.93	5.47
Cerrado	6.09	6.55	7.13	6.10
Pasture	5.15	4.98	5.62	5.81

Y1, Y5, Y10 and Y20: 1, 5, 10 and 20 years under sugarcane-burning management. Soil from natural Cerrado and Pasture areas was sampled as a reference.



**Fig. 4.** Infrared spectra (FTIR) of humic acids extracted from soil under a pre-harvest sugarcane straw burning chronosequence in a Cerrado area in Goiás. Soil from natural Cerrado and Pasture areas was sampled as a reference. (A) 0–0.05 m; (B) 0.05–0.10 m; (C) 0.10–0.20 m; (D) 0.20–0.30 m.

processes including the C=C stretch of aromatic rings and the C=O stretch of amides (band I) and quinones.

–1000  $\text{cm}^{-1}$  range is frequently marked by vibrations, such as the C–O stretch of alcohols, acids, phenols and esters and the O–H deformation of alcohols and carboxyl groups, and the bands they produce allow interpretations of these complex regions (Vergnoux et al., 2011). However, some observations should be made. The bands in the 1460–1370  $\text{cm}^{-1}$  range are attributed to C–H deformation of aliphatic chains, O–H deformation, C–O stretching of phenol groups and O–C–O stretching of COO<sup>-</sup> groups. The absorbance in the 1270–1120  $\text{cm}^{-1}$  range corresponds to the C–O stretching of several oxygen groups.

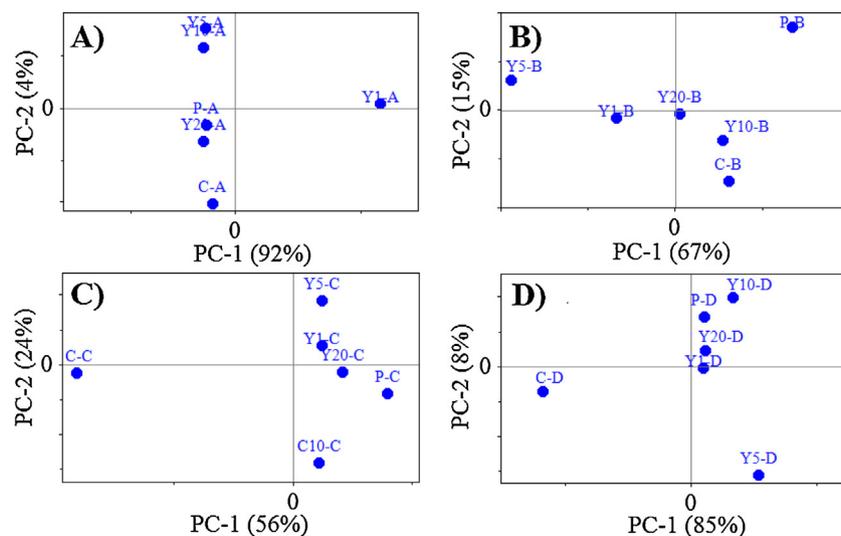
The band in the 1047–1044  $\text{cm}^{-1}$  range, in turn, is associated with polysaccharide C–O stretching or molecules with similar chemical structure. The higher intensity of these bands in an FTIR spectrum of HA may be related to cellulose decomposition (Giovanela et al., 2010).

The band at approximately 1090  $\text{cm}^{-1}$  was found in all of the areas evaluated up to 0.30 m and is related to the C–O stretch from aliphatic alcohols, ether linkages (C–O–C) and C–O from

polysaccharides. This band was also detected by Narimoto (2006) in HA spectra from *Argissolo Amarelo* (Ultisol) with different levels of sewage and explained by C–O stretching of polysaccharides and impurities such as silicates (Si–O) in HA. In the 900–700  $\text{cm}^{-1}$  region, HA absorbance may be attributed to C–H deformation in aromatic rings. Another band observed in the 500  $\text{cm}^{-1}$  region of all of the spectra evaluated is attributed to mineral impurities in the HAs.

For a detailed analysis of FTIR data, the spectra were separated according to depth (for burned and reference areas) and analyzed using PCA (Fig. 5). The treatment of FTIR spectra identified differences between HA from each sample. In the 0.0–0.05 m layer (Fig. 5A), the PCA (96% of the explained variance) detected in PC-1 and PC-2 showed a differentiation between HA from Y1-A and HA from the other areas; the Y5-10 and Y10-A grouping showed a close relation with Y20-A, C-A and P-A. This distribution indicates that the greatest differences between the HA spectra were obtained with burning management for 1, 5 and 10 years.

According to the PCA of data from the depth of 0.05–0.10 m (Fig. 5B) (82% of the explained variance), Y1-B and Y5-B was



**Fig. 5.** PCA of FTIR spectra analyzed separately by depths. Y1, Y5, Y10 and Y20: 1, 5, 10 and 20 years under sugarcane-burning management, respectively; C stands for Cerrado and P for pasture areas. (A) 0–0.05 m; (B) 0.05–0.10 m; (C) 0.10–0.20 m; (D) 0.20–0.30 m; -A, -B, -C and -D: 0–0.05, 0.05–0.10, 0.10–0.20 and 0.2–0.3 m, respectively.

separated from the other samples, which indicated differences in the HA spectral characteristics. For data of 0.10–0.20 m (Fig. 5C), the PCA (80% of explained variance) indicated no differences among HA from the burned areas (i.e., between these areas) and that of the Pasture area. This indicates that at this depth, FTIR spectra did not indicate changes in functional groups caused by burning. At the depth of 0.20–0.30 m, the PCA showed the same distribution previously reported (Fig. 5D) (93% of the explained variance).

The greater changes in FTIR spectra were detected in the most superficial layers (0.0–0.05 m and 0.05–0.10 m), and after 20 years of burning management, the HA in the top soil was different from HA in the reference areas. The chemometric treatment of the FTIR spectra therefore allowed confirmation of the results of the elemental composition analysis. The main changes in the content of the elements and atomic ratios were detected in the two most superficial layers.

### 3.4. Carbon 13 nuclear magnetic resonance spectroscopy ( $^{13}\text{C}$ NMR)

Table 3 shows the frequency of the functional groups associated with the bands representing the aliphatic (0–45 ppm), N-aliphatic and methoxyl (45–60 ppm), O-aliphatic (60–110 ppm), C-aromatic (110–140 ppm), O N-aromatic (140–160 ppm), carboxyl (160–185 ppm) and carbonyl groups (185–230 ppm). The signatures of the HA spectra are similar to those reported for other areas (Canellas et al., 2007; Dick et al., 2008; Spaccini and Piccolo, 2009; Crespo, 2011; Aguiar et al., 2012). The main signals were associated with aliphatic carbon at 22, 28 and 30 ppm, which can be attributed to end-methyl groups, aliphatic methylenes and long alkyl chains of varied origin (such as fatty acids, fats, cutin and other aliphatic biopolymers), respectively (Cunha, 2005). Lower-

intensity signals (56 ppm) attributed to C-methoxyl overlap the N-C-alkyl signal, which exhibits a chemical shift between 46 and 67 ppm (Preston, 1996). These signals likely result from the incorporation of lignin and similar components into the soil (Pérez et al., 2004; Cunha, 2005). The signal of some samples is observed in the 72–75 ppm region, which indicates the overlapping resonance of carbons (C2, C3 and C5) in the pyranose structure of cellulose and hemicellulose (Aguiar et al., 2012) or may correspond to carbon atoms of the polysaccharide rings (Giovanela et al., 2010). Different signals in the 65–110 ppm region are attributed to monomeric units of oligosaccharide and polysaccharide chains of plant tissues (Spaccini and Piccolo, 2009). A resonance in the 130 ppm region is associated with the occurrence of aromatic and unsaturated carbons, whereas resonance at 152 ppm is related to carbons bonded to phenols and other aromatic compounds that contain C, O and S. Considering the methoxyl (55 ppm) and phenolic (152 ppm) signals, lignins are assumed to be the predominant aromatic structure (Xiaoli et al., 2007). At approximately 176 ppm, the spectra showed signals with similar intensity, indicating a high content of functional carboxyl groups, amide carbon and ester of vegetal and microbial origin. Fire likely increases the proportion of aromatic compounds in humic substances of the superficial soil layers (Baldock and Smernik, 2002; Knicker, 2007). This pattern was detected in the present study at 0.0–0.05 m, where the contribution of aromatic compounds increased with burning management up to a period of 10 years. At 0.05–0.10 m, an inverse pattern was observed with a higher contribution of aromatic components, but in the other layers (0.10–0.20 and 0.20–0.30), the aliphatic components contributed more. The reference areas showed higher homogeneity in signal distribution with C-alkyl and O-alkyl content 70% higher in the Cerrado and 50% higher in the Pasture areas than in

**Table 3**  
Distribution (%) of carbon compounds, aliphaticity index (Aliph) and aromaticity index (Arom) of humic acids.

Area	Chemical shift (ppm)								Aliph	Arom
	0–45 $C_{\text{Alk-H,R}}$	46–60 $C_{\text{Alk-O,N}}$	60–90 $C_{\text{Alk-O}}$	90–110 $C_{\text{Alk-di-O}}$	110–140 $C_{\text{Ar-H,R}}$	140–160 $C_{\text{Ar-O,N}}$	160–185 $C_{\text{COO/NCO (H,R)}}$	185–230 $C_{\text{CO}}$		
0.0–0.05 m										
Y1	33.6	9.3			6.5		29.0	21.5	86.8	13.2
Y5	14.5	10.9	11.8		19.1	1.8	41.8		64.1	35.9
Y10	4.6	3.7	6.5		34.3		50.9		30.2	69.8
Y20	10.3	6.5	2.8		14.0		53.3	13.1	58.3	41.7
Cerrado	18.0	1.8	6.3	1.8	13.5		51.4	7.2	67.4	32.6
Pasture	30.4	5.9	11.8		10.8	4.9	34.3	2.0	75.4	24.6
0.05–0.10 m										
Y1	7.4	2.5	13.1		30.3	0.8	44.3	1.6	42.4	57.6
Y5	9.4	8.5	15.1		25.5	1.9	39.6		54.7	45.3
Y10	3.2	1.6		5.6	21.4	1.6	58.7	7.9	31.0	69.0
Y20	22.4	5.1		11.2	9.2	2.0	43.9	6.1	77.6	22.4
Cerrado	20.9	2.6		0.0	7.0		67.0	2.6	77.1	22.9
Pasture	15.8		21.8	5.9	12.9	1.0	29.7	12.9	75.9	24.1
0.10–0.20 m										
Y1	18.5	4.6	1.9	1.9	29.6		38.9	4.6	47.5	52.5
Y5	34.7	9.2	1.0		18.4		34.7	2.0	71.0	29.0
Y10	27.1	11.8	14.1		1.2		35.3	10.6	97.8	2.2
Y20	13.4	7.2	25.8	5.2	20.6		26.8	1.0	71.4	28.6
Cerrado	33.0	1.9	13.2	4.7	5.7		41.5		90.3	9.7
Pasture	22.6	0.9	18.3		36.5		21.7		53.3	46.7
0.20–0.30 m										
Y1	11.8	2.0	12.7	0.0	47.1	2.0	24.5		35.1	64.9
Y5	38.5	3.4	32.5	6.8		1.7	17.1		97.9	2.1
Y10	35.0	5.1	4.3				53.8	1.7	100.0	0.0
Y20	10.3	10.3	3.4				58.6		58.3	41.7
Cerrado	17.1	1.9	17.1	3.8	17.1	1.9	41.0		67.7	32.3
Pasture	12.9		12.3	8.0	16.6		49.1	1.2	66.7	33.3

Y1, Y5, Y10 and Y20: 1, 5, 10 and 20 years under sugarcane-burning management, respectively.

areas under sugarcane straw burning. This result indicates that organic matter in the reference areas is less humified than in areas frequently managed with burning (Table 3).

The pattern described by Crespo (2011) differs from the results presented in the present study and shows that HA in burned soil, especially in the most affected layer (0.0–0.02 m), is more similar to the most humified organic matter than unburned soil samples, which supports the theory that humic acids become more aromatic with decomposition.

In the 0.0–0.05 m layer, which is most susceptible to fire, the content of the carboxyl groups increased, especially in the Y20 area. The advance in the humification process increases the content of carboxyl aromatic groups, whereas O-aromatic and methoxyl groups decreased because they are components of lignin, which declines with SOM transformations (Leinweber et al., 1993).

Canellas et al. (2007) reported that the main effect of sugarcane straw preservation on SOM is the significant increase in C levels, which is attributed to aromatic compounds and/or alkenes that show a chemical shift between 110 and 150 ppm. They report that in the 0.0–0.10 and 0.10–0.20 m layers, C content increased up to four times compared to the burned sugarcane areas. They also found that a lower content of C atoms was attributed to carboxyl groups in crude sugarcane areas (a chemical shift between 165 and 200 ppm including the absorption of ketones, esters and amides).

The increase in C-alkyl content in the decomposed matter can be attributed to the selective preservation of the most recalcitrant materials, which resist biological activity (Theng et al., 1992; Canellas et al., 2007). The presence of carbohydrate C may indicate microbial degradation of other C sources or the occurrence of aromatic biopolymers such as lignin. The results obtained by FTIR spectroscopy and  $^{13}\text{C}$  NMR show that sugarcane management with straw burning practices affects the structural properties of the HA fraction of SOM and thereby affects C humification. As indicated by FTIR spectroscopy, fire affected the shape of some bands of HA spectra, which is related to the chemical environment of the respective functional groups. This was corroborated because  $^{13}\text{C}$  NMR showed that the most affected C structures were  $\text{C}_{\text{Alq-di-O}}$  (90–110 ppm),  $\text{C}_{\text{Ar-O}}$  (140–160 ppm) and  $\text{C}_{\text{C=O}}$  (180–230 ppm).

### 3.5. Statistical treatment of spectral data obtained by FTIR and $^{13}\text{C}$ NMR

The PCA plots grouped  $^{13}\text{C}$  NMR spectra of HA according to the integration of each region (Fig. 6). Two components explained 78% of spectral variance (PC-1: 59%; PC-2: 19%). Likewise, in the analysis of the functional groups (Table 3), the PCA confirmed that  $\text{C}_{\text{Alq-H,R}}$  (0–45 ppm) and  $\text{C}_{\text{Ar-H,R}}$  (110–140 ppm), the structures that determine aromatic and aliphatic properties, respectively, were

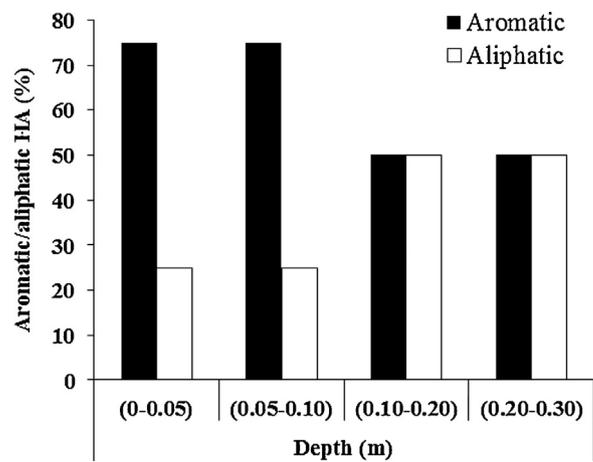


Fig. 7. Frequency (%) of aromatic and aliphatic structures in humic acids (HA) from different soil layers, calculated according to their distribution in PCA plots of  $^{13}\text{C}$  NMR spectra.

most affected by sugarcane straw burning. A total of 58% of HAs showed aromatic properties, and 41% showed aliphatic characteristics (Fig. 6). Neither burning management time nor soil depth affected group formation.

The PCA plot permitted detection of the amount of HAs with higher aromaticity in relation to the depth evaluated (Fig. 7). The highest content of HAs with aromatic characteristics was found in the most superficial layers (0.0–0.05 m and 0.05–0.10 m), which confirmed that the effects of fire on HA structure are more severe in the top soil layers. This occurs because aromatic structures are more resistant to soil heating by sugarcane straw burning. Irrespective of the length of time of the burning practices, from 0.10 m to the deeper layers, the impact of fire on the aromatic-aliphatic properties of HAs seems to be lower.

The spectra obtained using FTIR were also subjected to PCA (Fig. 8), which allowed a better understanding of the burning effects on HA structure. The PCA graph shows that two groups explain 97% of the data variance (PC-1: 95%; PC-2: 2%). The separation between spectra from 0.0–0.05 m HA and HA from other layers is clear, irrespective of the length of time under burning management. As previously discussed, the differences in the bands and intensity of the FTIR spectra are related to the chemical environment and functional groups.

The significant difference between HAs at 0.0–0.05 m and in the other layers reinforces the strong effects of fire on HA structure and properties (especially aromaticity) at this depth, irrespective of the length of time burning practices had been adopted.

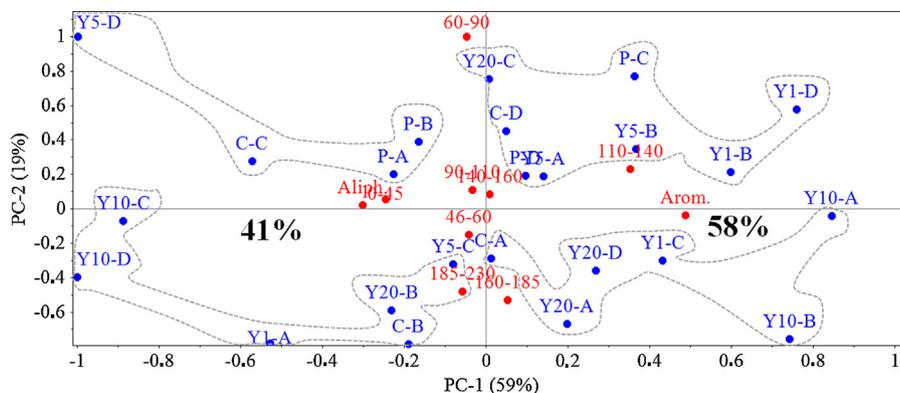
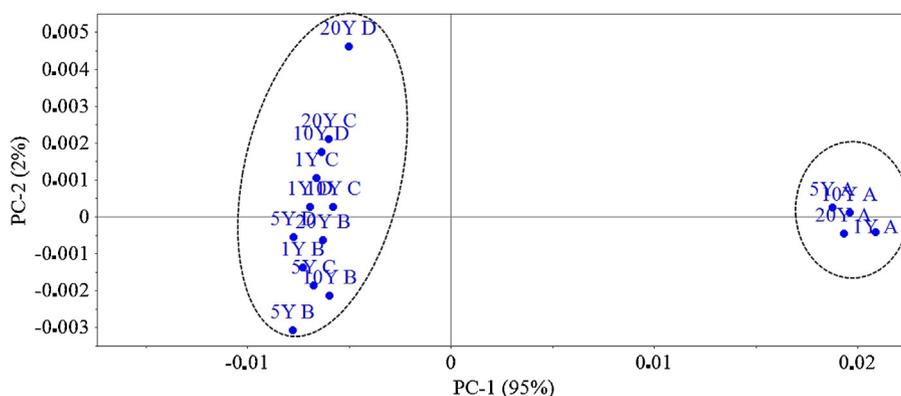


Fig. 6. PCA plot of  $^{13}\text{C}$ -NMR spectra in the integration regions. Y1, Y5, Y10 and Y20: 1, 5, 10 and 20 years under sugarcane-burning management, respectively; C stands for Cerrado and P for pasture areas. (A) 0–0.05 m; (B) 0.05–0.10 m; (C) 0.10–0.20 m; (D) 0.20–0.30 m; -A, -B, -C and -D: 0–0.05, 0.05–0.10, 0.10–0.20 and 0.2–0.3 m, respectively.



**Fig. 8.** PCA from FTIR spectra. Y1, Y5, Y10 and Y20: 1, 5, 10 and 20 years under sugarcane-burning management, respectively; C stands for Cerrado and P for pasture areas. (A) 0–0.05 m; (B) 0.05–0.10 m; (C) 0.10–0.20 m; (D) 0.20–0.30 m; -A, -B, -C and -D: 0–0.05, 0.05–0.10, 0.10–0.20 and 0.2–0.3 m, respectively.

#### 4. Conclusions

Sugarcane crop management with pre-harvest straw burning can affect both the chemical composition and structural properties of soil HA, especially in the most superficial layers. At 0.0–0.05 m, HA composition is characterized by decreased mean levels of C and N and enrichment of O. Aromaticity is the structural property that indicates the effects of fire on HAs, and although this property increased in burned areas in the 0.0–0.05 and 0.05–0.10 m layers, the highest high-intensity HA content was observed in the most superficial layer (0–0.05 m), irrespective of the length of time of the burning practices.

The present study is important in assessing the impacts of pre-harvest sugarcane straw burning management on humified SOM because it combines spectrometric and chemometric techniques, which represent a study procedure that can be used as a methodological basis to monitor phenomena such as soil burning management.

#### Acknowledgements

We are grateful for the support provided by CPGA-CS/UFRRJ, PROEX, CNPq, CAPES, the Federal Institute of Goiás—Campus Rio Verde, the Power Station and the University of Coruña.

#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.agee.2015.09.022>.

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