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Characterization by Fluorescence of Organic Matter from Oxisols under Sewage Sludge Applications

Larissa Macedo dos Santos
Embrapa Agricultural Instrumentation
P.O. Box 741
13560-970, São Carlos-SP, Brazil
and
Univ. of São Paulo
Institute of Chemistry of São Carlos
P.O. Box. 369
13560-970, São Carlos-SP, Brazil

Débora Marcondes Bastos Pereira Milori
Marcelo Luiz Simões
Wilson Tadeu Lopes da Silva
Embrapa Agricultural Instrumentation
P.O. Box 741
13560-970, São Carlos-SP, Brazil

Edenir Rodrigues Pereira-Filho
Federal Univ. of São Carlos
Dep. of Chemistry
P.O. Box. 676
13565-905, São Carlos-SP, Brazil

Wanderley José de Melo
Univ. of the State of São Paulo
Dep. of Technology
Road Prof. Paulo Donato Castellane, Km 5
14884-900, Jaboticabal-SP, Brazil

Ladislau Martin-Neto*
Embrapa Agricultural Instrumentation
P.O. Box 741
13560-970, São Carlos-SP, Brazil

Sewage sludge from wastewater treatment contains organic matter and plant nutrients that can play an important role in agricultural production and the maintenance of soil fertility. The present study has aimed to evaluate the degree of humification following sewage sludge application of soil organic matter by laser-induced fluorescence and humic acids using ultraviolet-visible fluorescence, and including comparison with Fourier-transform infrared spectroscopy and elemental analysis. Sewage sludge applications to the soil caused a decrease in the degree of humification of the soil organic matter and humic acids for both a Typic Eutrorthox (clayey) soil and a Typic Haplorthox (sandy) soil of around 14 and 27%, respectively. This effect is probably due to incorporation of newly formed humic substances from the sewage sludge into the characteristics of less humified material, and to the indigenous soil humic substances. The minor alterations observed in the clay soil probably occurred due to both the greater mineral association, which better stabilized the indigenous soil organic matter, and the higher microbial activity in this soil, which accelerated sewage sludge mineralization. Sewage sludge applications increased the C content for the clay and sandy soils by 7.4 and 15.4 g kg^{-1}, respectively, suggesting a positive effect on these two soils.

Abbreviations: FTIR, Fourier-transform infrared; HA, humic acid; LIF, laser-induced fluorescence; SOM, soil organic matter; TOC, total organic carbon.

Soil organic matter (SOM) is universally recognized to be among the most important factors responsible for soil fertility, crop production, and land protection from contamination, degradation, erosion, and desertification (Senesi et al., 2007; Soane, 1990). The intensive cropping of SOM-rich soils with consequent SOM depletion, the necessity to cultivate and enhance crop production of intrinsically SOM-poor soils, and the need to protect soils from degradation and erosion have urged a series of efforts to restore or improve the SOM content and its functions. As a result, recycling organic residues, byproducts, wastes, and effluents, such as sewage sludge, has become a very popular and efficient agricultural practice (De Bertoldi and Zucconi, 1987; Senesi, 1989; MacCarthy et al., 1990).

The relevance of recycling sewage sludge for agricultural purposes is due to its high concentration of organic matter and plant nutrients such as N, P, and Ca (Hue, 1988), which can benefit both the soil and the plants (Melo and Marques, 2000). This aspect is more relevant in several tropical regions, and particularly in Brazil, where there is accelerated organic matter decomposition and a predominance of soils with 1:1 clay minerals, mainly kaolinite, with a low cation exchange capacity (Denef et al., 2002; Six et al., 2002).

Control of the qualitative and quantitative changes that occur in SOM under sewage sludge applications is of crucial importance to ensure the environmentally safe and agronomically efficient use of sludge as a soil amendment (Bertoncini et al., 2005). The qualitative and quantitative changes that take place in the SOM due

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*Corresponding author (martin@cnpdia.embrapa.br).

to sewage sludge application have been investigated using chemical and spectroscopic techniques (Adani and Tambone, 2005; Brunetti et al., 2007; González-Pérez et al., 2006a,b; Harrison et al., 2006; Polak et al., 2005; Rivero et al., 1998; Rovira and Vallejo, 2002; Senesi et al., 2007). In general, these works have observed considerable changes in the SOM from amended soil. Adani and Tambone (2005) observed that the humic acid (HA) composition of a soil treated with sludge had developed an HA composition closer to that of the HA–sludge as a result of the enrichment of recalcitrant fractions contained in the sludge. Brunetti et al. (2007) suggested that proteinaceous, S-containing, and aliphatic structures contained in HA-like fractions of organic amendments were partially incorporated into the native soil HA. Rivero et al. (1998) showed that the additions of compost to soil increased the quality of SOM by increasing the quantity of HAs with more functional groups and aromaticity. The present research has demonstrated that the addition of compost increased not only the quantity but also the quality of the SOM, thus improving the soil quality and productivity.

Other spectroscopies have been used in soil HA analysis in areas with sewage sludge applications including the well established Fourier-transform infrared (FTIR) spectroscopy for detecting functional groups (Amir et al., 2004; Brunetti et al., 2007; Jouraiphy et al., 2005; Pajaczkowska et al., 2003; Polak et al., 2007, 2005; Senesi et al., 2007). In Brunetti et al. (2007) and Senesi et al. (2007), the FTIR spectra of HA-like fractions extracted from sewage sludge were characterized by relatively more intense absorptions of aliphatic, amide, and polysaccharide structures, and less intense absorptions of carbonyl and carboxyl groups than native soil HAs.

Canellas et al. (2001), using ultraviolet and visible light spectroscopy, infrared spectroscopy, and solid-state ^{13}C nuclear magnetic resonance spectroscopy, determined potential changes in the distribution of humified fractions of the organic matter together with the structural features of the HAs in samples from the uppermost horizon of two soils (an Ultisol and an Oxisol) under applications of sewage sludge (80 Mg ha^{-1}). In that work, Canellas et al. (2001) observed that the HAs studied underwent structural changes, mainly a decrease in the long alkyl chain content along with an increase in the C content related to polysaccharides incorporated into the HAs.

García-Gil et al. (2004) showed the effects of time after sludge amendment on the properties of HAs from amended soil and on some soil microbiological and biochemical parameters, including basal respiration, microbial biomass C, the metabolic quotient, and enzyme activities involved in oxidation processes and nutrient cycling, in soil samples collected 9 and 36 mo after the sewage sludge application (40 Mg ha^{-1}). In that experiment, the elemental, functional, FTIR, and biochemical data indicated that the use of sewage sludge in a degraded Mediterranean agroecosystem resulted in a number of short-term modifications of the compositional, structural, functional, and reactive properties of the amended-soil HAs and soil microbiological activities (García-Gil et al., 2004).

Bayer et al. (2002), González-Pérez et al. (2007), Kalbitz et al. (1999), Milori et al. (2002, 2006), and Zsolnay et al. (1999) have shown the potential of ultraviolet (UV)-visible fluorescence and laser-induced fluorescence (LIF) spectroscopy to evaluate the humification degree of organic materials in solutions of humic substances and whole soil samples, respectively. Their theoretical basis lies in the assumption that a shift in the maximum fluorescence intensity from shorter to longer wavelengths can be attributed to an increase of aromatic group condensation or an increase in conjugation in these molecules (Kalbitz et al., 1999); therefore, organic substances that are more transformed or humified have a higher fluorescence intensity signal at a lower wavelength and it becomes possible to associate this signal alteration with chemical alterations in organic compounds and to follow reactions in soil and water environments. Also, these more humified materials generally have higher chemical stability, increasing the residence time of organic matter in the environment and consequently improving the soil structure and fertility (mainly those aspects associated with the cation exchange capacity) (Schnitzer and Khan, 1972, p. 192–197).

Bertoncini et al. (2005) showed that unidimensional fluorescence spectroscopy in the emission, excitation, and synchronous-scan modes and total luminescence spectroscopy are sensitive techniques for characterization and differentiation of HAs extracted from two Brazilian Oxisols amended with biosolids.

Milori et al. (2006) and González-Pérez et al. (2007) showed the applicability of LIF spectroscopy for characterizing the SOM and for assessing its humification in bulk samples of soils subjected to different management systems, assuming that the concentration of rigid conjugated systems increases with humification. Fluorescence signals are emitted by rigid conjugated systems in individual molecules or structures (probably aromatics) bearing substituents such as carbonyl and carboxyl groups (Senesi et al., 1991; McGarry and Baker, 2000). The LIF emission spectra measure C in more complex or rigid structures, such as aromatic and quinone groups in whole soil samples. So when fluorescence is excited at near-ultraviolet or blue wavelengths, information is provided about more humified structures (Milori et al., 2002). Based on these assumptions, Milori et al. (2006) proposed a humification index based on LIF spectroscopy, and compared it with the humification indexes proposed by Zsolnay et al. (1999), Kalbitz et al. (1999), and Milori et al. (2002). Milori et al. (2006) observed that there was a close correlation between the humification degree obtained by LIF (H_{LIF}) of the whole soil samples and the humification indexes A_4/A_1 (Zsolnay et al., 1999), I_{465}/I_{399} (Kalbitz et al., 1999), and A_{465} (Milori et al., 2002) of the corresponding HA samples studied.

Also, data obtained with LIF methodology using whole soil samples were comparable with electron paramagnetic resonance evaluations through detections of semiquinone-type free radicals of HAs in the same soil sites, supporting the hypothesis that LIF can measure the humification degree of SOM (Milori et al., 2006). According to Milori et al. (2006), this technique can be innovative in SOM studies because the sample can be analyzed

in bulk, avoiding possible interference by physical and chemical fractionations or treatments. Until now, however, few studies have been conducted using LIF spectroscopy applied to SOM research and it is opportune to increase the use of this tool.

The present study aimed to evaluate the changes in the humification degree of SOM by LIF, and of HAs using UV-visible fluorescence, FTIR, and elemental analysis for comparison between two tropical soils with and without seven consecutive years of sewage sludge application. The information obtained from this study is important to understand some possible specific modifications in the characteristics of SOM and its humic fractions in the tropical soils under sewage sludge applications in a long-term experiment. There are few long-term experimental fields in tropical areas, including those with sewage sludge applications, and there is a lack of a more detailed and mechanistic research related to SOM dynamic and reactivity.

MATERIALS AND METHODS

Soil Samples

Soil samples have been collected from two tropical soils cultivated with *Zea mays* L. in the first 6 yr and with *Crotalaria juncea* L. in the seventh: a clay soil (61 g kg^{-1} clay, 17 g kg^{-1} silt, 22 g kg^{-1} sand) classified as a Typic Eutrorthox, and a sandy soil (36 g kg^{-1} clay, 4 g kg^{-1} silt, 60 g kg^{-1} sand) classified as a Typic Haplorthox soil. They were sampled from the 0- to 10-, 10- to 20-, 20- to 40-, and 40- to 60-cm depths in a 7-yr field experiment that began in the agricultural season of 1997 to 1998 to evaluate sewage sludge applications. The experimental area is located in Jaboticabal, São Paulo State, Brazil (21°15′22″ S, 48°15′18″ W, and 610-m altitude). The climate is Cwa (subtropical, mesothermic, with a hot and humid summer and a cold and dry winter; the average annual temperature is 22°C and the average annual rainfall is about 1400 mm), according to Köppen climatic classification (Rolim et al., 2007). Before the installation of the field experiment, the area was plowed and lime was applied to increase the base saturation to 70% (2.5 Mg ha^{-1}). It was incorporated into the soil by gradation. In the first year of experimentation, the treatments were a control (without sewage sludge or mineral fertilization) and three rates of sewage sludge (2.5, 5.0, and 10.0 Mg ha^{-1}, dry basis) with five replications. The 5.0 Mg ha^{-1} rate was selected to supply the N required by *Zea mays*, supposing that one third of the N present in the sewage sludge would be available to the plants. From the second year on, the control plots were fertilized with mineral fertilizers according to the soil chemical analysis and the recommendations of van Raij et al. (1997) From the fourth year on, the 2.5 Mg ha^{-1} rate was replaced by 20.0 Mg ha^{-1} to increase the content of heavy metals in the soil. Consequently, in the seventh year of experimentation, the accumulated rates of sewage sludge in the treatments were 0, 35.0, 70.0, and 87.5 Mg ha^{-1}, dry basis. Sewage sludge was applied annually to the soil surface and incorporated to 0 to 10 cm by gradation. The mineral fertilizers and seeds were applied in the furrows. Cultural wastes and seeds were managed by herbicides in that growing season.

Sewage Sludge

Sewage sludge was obtained from the Barueri sewage treatment plant located in the metropolitan region of São Paulo City, São Paulo State, Brazil, and operated by the Basic Sanitation Company of the State of São Paulo. Two sewage sludge samples were collected for analysis before application to the soils. The samples were dried and the HA-like fraction was extracted according to the International Humic Substances Society (IHSS) methodology (Swift, 1996).

Chemical Fractionation and Purification

The HA and the HA-like fractions were extracted from the soil and sewage sludge samples, respectively, according to the IHSS methodology (Swift, 1996). Briefly, the method includes extraction with 0.1 mol L^{-1} NaOH, using a sample/solvent ratio of 1:10. After centrifugation, the HA was separated from the supernatant by precipitation with 6 mol L^{-1} HCl added to the extract until a pH of 2.0 was reached. The precipitated HA was separated by centrifugation and purified by dialysis using a Spectra/Por membrane (size exclusion limit 6000–8000 Da, Spectrum Labs, Rancho Dominguez, CA) and was finally freeze-dried.

Total Organic Carbon

The total organic C (TOC) contents of the soil samples were determined by a TOC analyzer (Model TOC-V CPH, Shimadzu Corp., Kyoto, Japan) coupled to a SSM-5000 (Shimadzu Corp.).

Ultraviolet–Visible Fluorescence Spectroscopy

Fluorescence spectra were recorded using a PerkinElmer LS-50B luminescence spectrophotometer (PerkinElmer Corp., Waltham, MA). Two replicates were recorded for each sample. The HA samples were brought to a concentration of 20 mg L^{-1} at pH 8 by dissolving them in a solution of 0.05 mol L^{-1} $NaHCO_3$ (Milori et al., 2002). Fluorescence spectra in the emission and synchronous scan mode were then recorded. The emission spectra were measured with excitation at 240 and 465 nm (Milori et al., 2002; Zsolnay et al., 1999). The synchronous scan was measured with $\Delta\lambda = 55$ nm, adapted from the procedure proposed by Kalbitz et al. (1999).

Laser-Induced Fluorescence Spectroscopy

The experiments were conducted on compressed pellets prepared with 0.5 g of dry soil, 1 cm in diameter and 2 mm thick, which were then inserted into a homemade apparatus to run LIF spectroscopy measurements (Milori et al., 2006). The samples were excited with 465.8-nm blue radiation by Ar laser equipment (Coherent Innova 90-6, Coherent Inc., Santa Clara, CA) with an exit power of 400 mW. The spectral resolution was adjusted to 4 nm. Four replicates were recorded for each sample.

Humification Degree

The degree of humification of the HA samples was calculated according to three different procedures. The degree of humification according to the procedure proposed by Zsolnay et al. (1999) was calculated as the ratio between the area of the last quarter (A_4, 560–640 nm) and the area of the first quarter (A_1, 320–400 nm) of the emission spectrum. This index is called A_4/A_1. The degree of humification according to the procedure proposed by Milori et al. (2002) is the area of the fluorescence spectrum obtained by excitation of the sample at the blue wavelengths, and is referred to as A_{465}. The degree of humification according to the procedure proposed by Kalbitz et al. (1999) was calculated as the ratio

between the fluorescence intensities at 460 and 399 nm (I_{460}/I_{399}) in synchronous-scan excitation mode spectra. The degree of humification (H_{LIF}) of the SOM was calculated through the ratio between the LIF emission spectrum area and TOC (Milori et al., 2006).

Fourier-Transform Infrared Spectroscopy

The FTIR spectra were acquired using a PerkinElmer Spectrum 1000 spectrometer. The experiments were performed using compressed pellets prepared using 1 mg of sample and 100 mg of KBr according to the methodology proposed by Stevenson (1994).

Elemental Composition (Carbon, Nitrogen, Hydrogen, and Oxygen)

The elemental contents of C, N, and H of the HA were determined using a CE Instrument EA 1110 (Carlo Erba, Milan, Italy). The O content was calculated by difference. The C/N and H/C atomic ratios were calculated by the ratio between the C and N contents and the ratio between the H and C contents, respectively.

Statistics Analysis

The results obtained by fluorescence procedures were evaluated by paired *t*-test ($P < 0.05$, 95% confidence level) (Christian, 1994).

RESULTS AND DISCUSSION

The fluorescence emission spectra of the HA extracted from the Typic Haplorthox soil are presented for the unamended control (Fig. 1a) and the sewage sludge application treatment (Fig. 1b) for depths of 0 to 10 and 10 to 20 cm. Results from lower depths are not presented because there was no clear effect of the sewage sludge treatment (data not shown), probably because the sewage sludge was incorporated only into the uppermost 10 cm. Figure 1c shows the fluorescence emission spectrum of the HA-like fraction that was extracted from the sewage sludge.

The wavelength of maximum fluorescence varied from 498 to 502 nm for the HA extracted from the Typic Haplorthox soil; for the HA-like fraction, the wavelength of maximum fluorescence was observed around 340 nm. These results are consistent with Bertoncini et al. (2005), who noted the maximum emission of the HA-like fraction at shorter wavelengths than the HA extracted from Brazilian Oxisols. The emission spectra of the HA extracted from the soil treated with sewage sludge showed a fluorescence shoulder around 340 nm and the appearance of a new fluorescence band at 650 nm compared with the HA extracted from the control soil. These spectra showed a decrease in the fluorescence intensity as a consequence of the sewage sludge application, suggesting the presence of molecular components having a relatively small molecular size with a low level of aromatic polycondensation and a low degree of humification in the HA extracted from the soil treated with sewage sludge (González-Pérez et al., 2006a,b; Senesi et al., 1991, 2007).

Senesi et al. (2007) also found that, compared with native soil HAs, the fluorescence spectra of HA-like fractions generally show: (a) a maximum emission at a much shorter wavelength; (b) more intense excitation peaks at short and intermediate wavelengths and less intense bands at longer wavelengths; and (c) several synchronous-scans bands and shoulders with a relative intensity decreasing with increasing wavelength. Also according to Senesi and Brunetti (1996), these results indicate that the HA-like fraction of organic amendments is generally characterized by a higher aliphatic character and molecular heterogeneity, lower amounts of O-containing and acidic functional groups, and lower degrees of aromatic polycondensation and humification.

The results from the Typic Eutrorthox soil showed smaller changes in the HAs of the unamended control than the HAs from the sewage-sludge-amended soil (data not shown)

Fluorescence of the whole SOM using LIF spectroscopy is shown for the control soil and the sludge-treated soil for the 0- to 10-cm depth of the Typic Eutrorthox (Fig. 2a) and Typic Haplorthox (Fig. 2b). The maximum fluorescence intensity was about 530 nm, and its magnitude decreased with the application of sewage sludge in the Typic Haplorthox (sandy) soil (Fig. 2b) but not in the finer textured Typic Eutrorthox (Fig. 2a).

The TOC content for both soils showed more significant alterations due to sewage sludge application in the Typic Haplorthox (sandy soil) than in the Typic Eutrorthox (clayey soil). The increase in the C content of the Typic Haplorthox after sludge applications was about 13% in the 0- to 10- and 10- to 20-cm depths, while the corresponding increase in the Typic Eutrorthox was only about 4% (Table 1). In tropical regions, decomposition of plant materials and organic materials is faster than in temperate regions, so it is difficult to cause substantial increases in SOM contents within only a few years (Chander et al., 1997; Goyal et al., 1992; Kaur et al., 2005; Six et al., 2002). With conventional tillage in Brazil, there is normally a decrease in SOM content due to the oxidation of organic

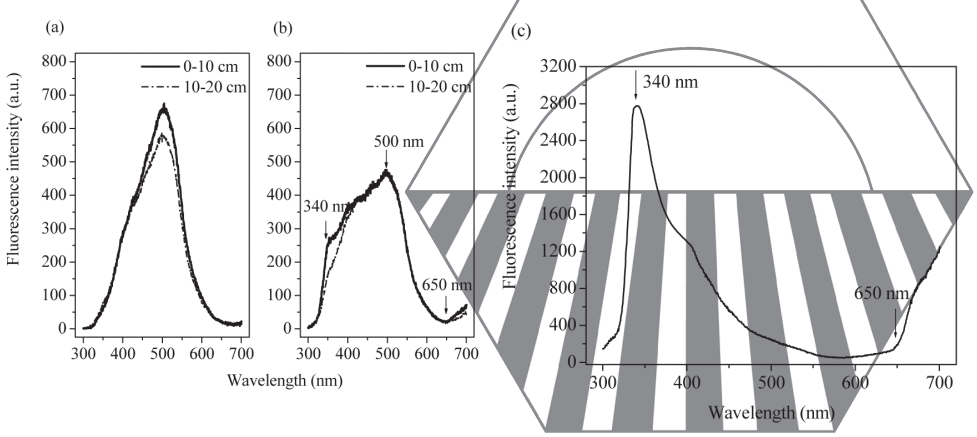

Fig. 1. Fluorescence emission spectra (excitation wavelength at 240 nm) of the humic acids and humic-acid-like material extracted from (a) the control treatment and (b) the sewage sludge application treatment of Typic Haplorthox soil samples obtained from the 0- to 10- and 10- to 20-cm depths, and (c) the sewage sludge.

matter, and the soil becomes a source (Bayer et al., 2006). The main alternative to this situation has been the use of no-till or direct drilling that in several situations showed an increase of SOM content, and consequently indicated soil C sequestration besides several other soil benefits (Bayer et al., 2004, 2006; Diekow et al., 2005). In these experiments with sewage sludge applications, the detection of any increase in SOM content is meaningful to soil conservation, fertility (Melo et al., 1994), and mitigation of greenhouse gas emissions to the atmosphere.

The results obtained through fluorescence and C content for the two soils are consistent with Bertoncini et al. (2005) and Senesi et al. (2007), who observed that, in general, sewage sludge applications affect the HAs and fulvic acids of a sandy soil more than those of a clay soil. According to Baldock and Skjemstad (2000), the mineralogy, surface charge characteristics, and precipitation of amorphous Fe and Al oxides on clay mineral surfaces give clay minerals a capacity to adsorb organic matter. For Bertoncini et al. (2005) and Senesi et al. (2007), however, this behavior is probably due to protective effects exerted by the clay minerals (with the formation of organo-mineral associations) in the soil against disturbances caused by sewage sludge application. A possible effect is the generally higher microbial activity in a clay soil than a sandy soil (Smith and Paul, 1990). This is reinforced under tropical conditions where, in general, Oxisols have a good infiltration rate, and in sandy soils this conditions is even more evident, reducing surface soil humidity, and temperatures are very high, limiting the growth of soil biota (Six et al., 2002; Tian, 1998), probably resulting in slowed decomposition of organic residues and permitting slower turnover of organic residues and their decomposition products in the soil.

The smaller changes observed for the fluorescence intensity and C content of the HAs extracted from the clay soil compared with the HAs extracted from the sandy soil are probably due to a higher organo-mineral association in this soil. Greater stability of clay-bound SOM than bulk SOM has been previously noted by Mahieu et al. (1999).

In line with these studies, for an Oxisol under different treatments—conventional tillage with maize–bare fallow, conventional tillage with a maize rotation with soybean–bare fallow, no-till with maize–bare fallow, no-till with a maize rotation with soybean–bare fallow, no-till with maize–cajanus, and no cultivation under natural vegetation—with high clay content (~53%), where the organo-mineral association hinders strong structural alterations, even conventional tillage practices could not destroy the clay–Fe–organic matter complex (González-Pérez et al., 2004).

The results obtained for the degree of humification are presented in Fig. 3 and 4. The indexes were obtained by the procedures of Zsolnay et al. (1999), Milori et al. (2002, 2006), Kalbitz et al. (1999).

The index obtained according to the procedure proposed by Zsolnay et al. (1999) showed a decrease in the degree of humification of HAs extracted from soils treated with sewage sludge compared with the degree of humification of the HAs extracted from control soils (Fig. 3a and 3b) (Zsolnay et al., 1999). Similar results were obtained according to the procedures proposed by Milori et al. (2002, 2006) for the Typic Haplorthox (Fig. 3d and 4b).

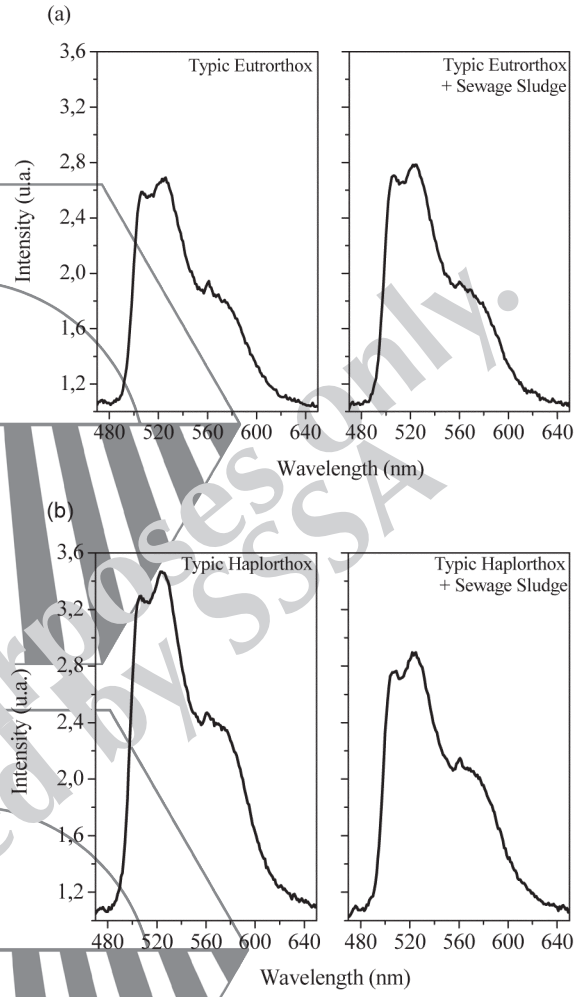

Fig. 2. Laser-induced fluorescence emission spectra (excitation wavelength in 458 nm) of control and sewage sludge application treatments of (a) a Typic Eutrorthox and (b) a Typic Haplorthox.

The decrease observed in the degree of humification of the HAs extracted from the Typic Haplorthox soil was more significant than that observed for the degree of humification of the HAs extracted from the Typic Eutrorthox soil (Fig. 3 and

Table 1. Carbon contents of the Typic Eutrorthox and Typic Haplorthox soils control and under sewage sludge application, after 7 yr.

Soil	Depth	Total organic C	
		Control	Sewage sludge application
	cm	g kg^{-1}	
Typic Eutrorthox	0–10	1.84 ± 0.03†	1.97 ± 0.02
	10–20	1.80 ± 0.01	1.82 ± 0.01
	20–40	1.36 ± 0.02	1.59 ± 0.04
	40–60	1.03 ± 0.02	1.11 ± 0.02
Typic Haplorthox	0–10	1.31 ± 0.03	1.48 ± 0.09
	10–20	1.14 ± 0.03	1.30 ± 0.01
	20–40	0.95 ± 0.04	1.17 ± 0.04
	40–60	0.73 ± 0.01	0.90 ± 0.03

† Mean ± standard error (n = 2) for each sample.

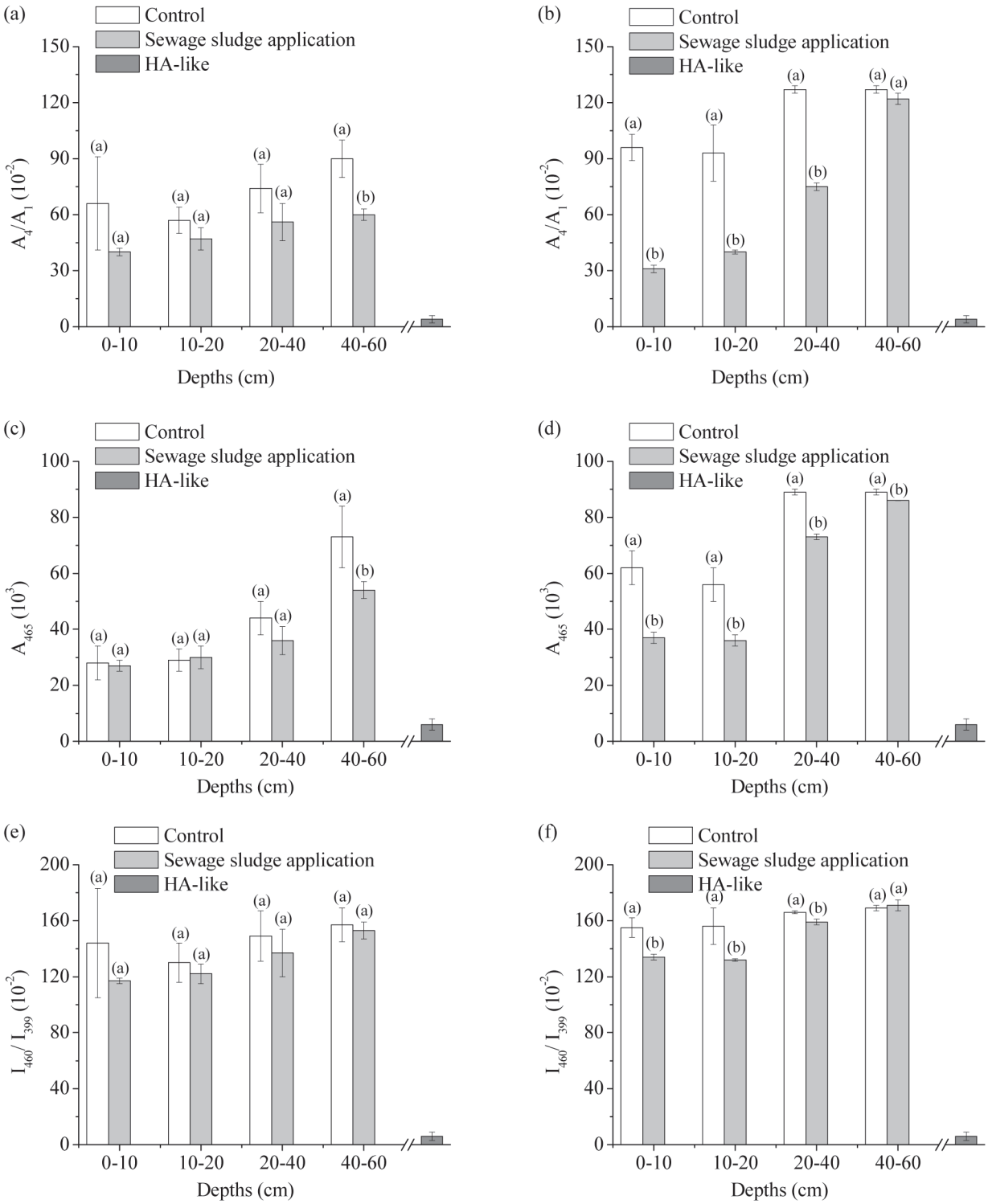

Fig. 3. Humification degree, obtained from fluorescence, for the humic acids (HAs) extracted from the control and sewage sludge application treatments of: (a) the Typic Eutrorthox and (b) the Typic Haplorthox, determined according to the procedure proposed by Zsolnay et al. (1999); (c) the Typic Eutrorthox and (d) the Typic Haplorthox, determined according to the procedure proposed by Milori et al. (2002); and (e) the Typic Eutrorthox and (f) the Typic Haplorthox, determined according to the procedure proposed by Kalbitz et al. (1999). Error bars represent the standard errors of the mean (*n* = 2) for each sample.

4). This behavior is consistent with the results obtained for the TOC (Table 1) and fluorescence intensity (Fig. 1 and 2).

The degree of humification obtained according to the procedure proposed by Zsolnay et al. (1999), Milori et al. (2002), and Kalbitz et al. (1999) for the HA-like fraction was smaller than the degree of humification obtained for the HAs extracted from the soils (Fig. 3). These results confirm the low degree of humification of the HA-like fraction compared with the HAs extracted from the soils.

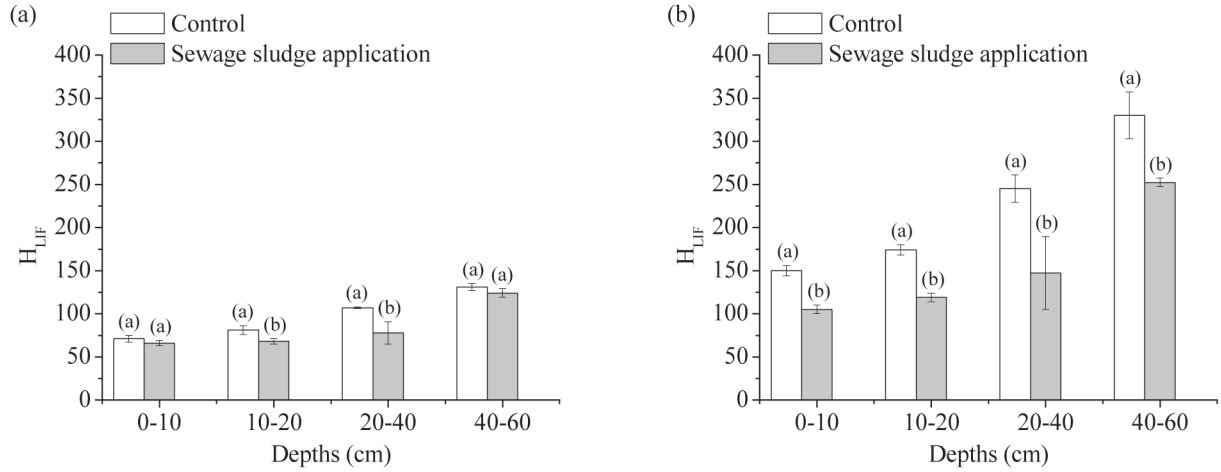

Fig. 4. Humification degree obtained by laser-induced fluorescence (H_{LIF}) determined according to the procedure proposed by Milori et al. (2006), for the control and sewage sludge application treatments of (a) the Typic Eutrorthox and (b) the Typic Haplorthox. Error bars represent the standard errors of the mean (n = 2) for each sample.

In conclusion, different groups of the HA-like fraction of amendments can be partially incorporated into the native HA, which will modify its composition, structure, and chemistry to varying extents. This is an important result that supports the fundamental objective of partially recycling humified organic waste material as a beneficial soil amendment (Senesi et al., 2007). These results also indicate the possibility of chemical alterations in the more stable SOM constituents, the humic substances, in short periods, reinforcing more recent concepts about the structure of humic substances as an association of small molecules held together due to weak bonding forces, such as H and hydrophobic bonding (Diallo et al., 2003; Piccolo, 2001, 2002; Simpson, 2002; Simpson et al., 2002; Sutton et al., 2005). Taking the structure of the humic substances as an association of small molecules, it is easier to understand alterations in the original soil humic substances due to the incorporation of recently transformed humic-substances-like molecules due to sewage sludge decomposition.

Milori et al. (2006) have shown that the ratio between the normalized area of the total fluorescence emission spectrum and the percentage of TOC (H_{LIF}) can be used as a parameter for evaluating the degree of humification of SOM. Its significance lies in the fact that it is performed on the whole soil, without any chemical or physical treatment and consequently avoiding any possible experimental artifacts, permitting analysis of SOM that retains its field composition.

The results obtained by H_{LIF} (Fig. 4) showed an increase in the degree of humification with increasing depth for all treatments. On the other hand, the H_{LIF} of the Typic Eutrorthox and Typic Haplorthox soils became lower after sewage sludge applications, an effect that may be attributed to the incorporation of less humified residues (González-Pérez et al., 2006a). A similar behavior was also observed through the use of electron paramagnetic resonance (EPR) by González-Pérez et al. (2006a).

The H_{LIF} of the Typic Eutrorthox and Typic Haplorthox control soils were lower in the 0- to 10- and 10- to 20-cm depths than in the deeper samples, although for the Typic Eutrorthox and Typic Haplorthox soils after sewage sludge amendment, the H_{LIF} was higher only at 40 to 60 cm. These results indicate a lower degree of humification in the soil surface. This is probably because plant residues are continuously added and incorporated into the surface soil layer (as deep as 20 cm) and there is not enough time for humification to progress fully, resulting in less aromatic or less humified humic substances. In deeper horizons, the input of residues is lower and further humification continues, increasing the degree of aromaticity and the degree of humification (González-Pérez et al., 2006a).

These LIF results are a novel contribution because few SOM studies have used the technique. Our results expand the database of LIF analyses. They also show that data obtained using this new tool with whole soil samples are in agreement with those obtained by soil humic substances analysis from measurements with more frequently used spectroscopic analyses such as UV-visible fluorescence, FTIR, and EPR. The value of LIF observations is due not only to the fact that they are consistent with the humic substances analysis but also to the fact that they represent a qualitative evaluation of SOM in a field situation, which until now had been unavailable.

The values of the degree of humification of the soil samples obtained by fluorescence procedures (Zsolnay et al., 1999; Milori et al., 2002, 2006; Kalbitz et al., 1999) were evaluated by the paired t-test (Christian, 1994). These studies aimed to determine whether the observed changes in the degree of humification of the soils after sewage sludge applications are statistically significant.

The results obtained according to the procedure proposed by Zsolnay et al. (1999) showed that the observed changes in the degree of humification of the organic matter in the soils with sewage sludge applications are different at the 95% confidence level. Similar results were obtained only for the Typic Haplorthox soil according to the procedures proposed by Milori et al. (2002, 2006). The results obtained for the Typic Eutrorthox by the procedures proposed by Milori et al. (2002) and Kalbitz et al. (1999) , however, did not show a significant decrease in

the degree of humification with sewage sludge application. These results show that the fluorescence spectroscopy methodologies (Kalbitz et al., 1999; Milori et al., 2002, 2006; Zsolnay et al., 1999) were sensitive for evaluating the changes in the degree of humification of the SOM and HAs from two tropical soils with and without sewage sludge applications after 7 consecutive yr.

To compare them with the results obtained by fluorescence spectroscopy, the FTIR spectra of the HAs and the HA-like fraction samples are presented in Fig. 5. These spectra are typical of HA samples, extensively available in the literature (Brunetti et al., 2007; Polak et al., 2005; Senesi et al., 2007). The main difference between the HA-like fraction and the soil HAs is the more pronounced band at 2924 to 2930 cm^{-1} for the HA-like fraction, associated with the presence of CH vibrations of aliphatic groups in the HA-like fraction spectrum compared with other soil HAs. By comparing HA spectra from soils with and without sewage sludge addition, it is also possible to suggest a higher intensity of aliphatic groups, indicated by a 2924 to 2930 cm^{-1} band, in the samples originated from treatments with sewage sludge addition. The interpretation is that the incorporation of decomposed material from sewage sludge residues provides changes in the original characteristics of the indigenous soil HAs such as the increase of aliphatic compounds. Considering that the UV-visible fluorescence and LIF data indicated a decrease in the degree of humification, which is mainly related to aromatic moieties, the HAs from the soils that were amended with sewage sludge appeared to have received an incorporation of aliphatic groups, which decreased their relative aromaticity. The FTIR data obtained in this study are also consistent with other previous studies (Brunetti et al., 2007; Polak et al., 2005; Senesi et al., 2007), where the HAs extracted from soil and the HA-like fractions presented structural and functional differences. In accord with Brunetti et al. (2007), the FTIR spectra of HA-like materials are generally characterized by relatively more intense absorptions of aliphatic, amine, and polysaccharide structures and less intense absorptions of carbonyl and carboxyl groups than those of native soil HA.

To compare the results obtained through fluorescence, the elemental compositions of the HAs and the HA-like fraction are presented in Table 2. The C content of HAs extracted from soils ranged from 37.2 to 48.6 g kg^{-1}, compared with 50.5 g kg^{-1} for the HA-like fraction. The O content ranged from about 3.1 to 4.9 g kg^{-1}, compared with 8.6 g kg^{-1} for the HA-like fraction. The H content ranged from 3.4 to 5.0 g kg^{-1}, compared with 7.0 g kg^{-1} for the HA-like fraction. The C/N ratio ranged from about 11.0 to 15.1 for the HA extracted from the soils and it was 6.9 for the HA-like fraction; the H/C ratio ranged from about 0.9 to 1.3 for the HA extracted from the soils and it was 1.7 for the HA-like fraction. These results reinforce the difference in composition between the HAs extracted from soils and sewage sludge, where C, N, and H contents are higher for the HA-like fraction compared with the HA extracted from the soils, and the O content is lower for the HA-like fraction than the HA extracted from the soils, as also observed by other researchers (Stevenson, 1994; Gonzalez-Vila et al., 1992). From comparisons of elemental analysis between HAs extracted from soils with and without the addition of sewage sludge, however, there were very few differences. A slight increase was detected in the H content in the sludge-amended treatment areas, which is consistent with the HA-like fraction characteristic (Table 2). This observation is also consistent with the literature (Yonebayashi and Hattori, 1988). This small change in H content is probably the reason for a slight increase in the H/C atomic ratios in soils treated with sewage sludge, or alternatively it could be consistent with a lower degree of aromatic condensation (Bravard and Righi, 1991).

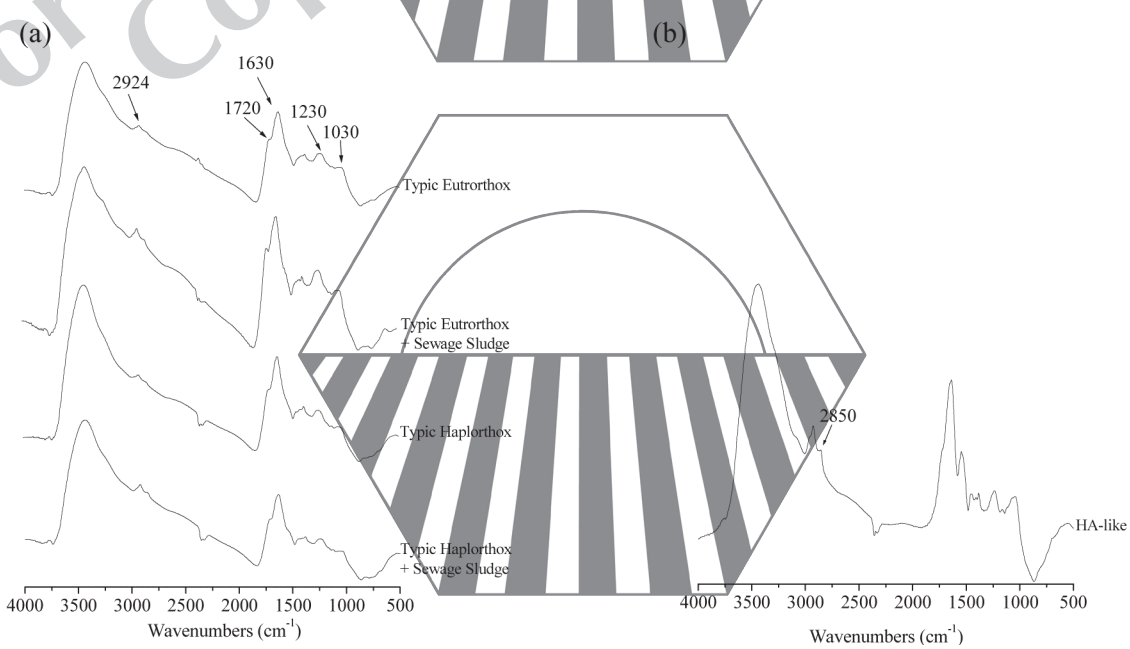

Fig. 5. Fourier-transform infrared spectra of (a) the humic acids extracted from the control and sewage sludge application treatments of the Typic Eutrorthox and Typic Haplorthox soils at the 10- to 20-cm depth, and the (b) humic acid (HA)-like fraction extracted from the sewage sludge.

From a qualitative point of view, the fact that in soils with sewage sludge additions the SOM and humic substances generally have a lower degree of humification than untreated soils indicated that the residues of the sewage sludge were chemically different from the native SOM, and their incorporation in the soil altered the overall characteristics of the indigenous organic matter. This fact has a positive aspect in that part of the sewage sludge material was decomposed and transformed into humic substances that were incorporated into the soil as stable compounds instead of being completely mineralized by microorganisms or being incorporated into the soil only as small or single organic molecules that are chemically less stable.

This is a very complex issue that needs further research to be completely answered. For example, what are the relationships between qualitative changes in the humic substances and the overall soil properties? The necessity of new research is also due to the fact that the degree of humification concept was established with the consideration that humic substances are formed by macromolecules with very high chemical stability (Schnitzer and Khan, 1972 p. 192–197; Stevenson, 1994). Now, however, the concept of macromolecule to humic substances has been seriously questioned (Piccolo, 2001, 2002; Simpson, 2002; Simpson et al., 2002; Sutton and Sposito, 2005) and consequently our understanding about their dynamic and reactivity in different environments must be reconsidered in the light of new findings. In the present stage of knowledge, it is considered beneficial to soils to have increased the SOM content, including incorporation of newly formed humic substances into the indigenous SOM, in spite of the decrease in the degree of humification of HAs in areas under sewage sludge treatment. It will also be important to maintain this long-term experiment and to realize continuous and more complete evaluations in the future for a better understanding of the fate of sewage sludge and consequent alterations in these Oxisols.

CONCLUSIONS

The sewage sludge incorporated into the soils has induced modifications to various extents in the composition, structure, and chemistry of the SOM and its specific component HAs. The sewage sludge applications to soils for seven consecutive years caused a decrease in the degree of humification of the SOM and HAs for the Typic Eutrorthox (clay soil) and Typic Haplorthox (sandy soil) soils of around 14 and 27%, respectively. This effect is probably due to incorporation of less humified C compounds derived from sewage sludge into the native SOM. Smaller changes were observed in the SOM and HAs from the Typic Eutrorthox soil (clay soil) due to a higher organo-mineral association, which gives greater chemical stability to indigenous SOM and its HA fraction, and also probably due to greater microbial activity with sewage sludge mineralization in this soil.

The main alterations were observed in the SOM and HAs from the Typic Haplorthox soil (sandy soil), probably due to a lower clay mineral concentration, allowing greater changes in the intrinsic humic substances structure, as well as a probable lower microbial activity, allowing a slower decomposition rate of the sewage sludge with the greater SOM increase.

The results obtained by paired *t*-test confirmed, at the 95% confidence level, a decrease in the degree of humification of the organic matter after sewage sludge applications, which was observed through fluorescence procedures.

Our results indicated apparent positive effects of sewage addition in both Oxisols studied in this 7-yr experiment, with an increase in SOM and incorporation of new humic substances to the indigenous soil humic substances, especially in the sandy soil. The decrease in the degree of humification of the HAs of the treated soils and possible agronomic and environmental implications must be better explored in the future. The present difficulty can be partly attributed to recent findings about the molecular structure of humic substances and their implications in organic matter dynamics and reactivity in different environments, including soils.

Table 2. Carbon, N, H, and O contents and C/N and H/C ratios of the humic acids extracted from the Typic Eutrorthox and Typic Haplorthox soils control and sewage sludge application treatments after 7 yr.

Humic acid source	Treatment	Depth	C	N	H	O	C/N	H/C
		cm	g kg^{-1}					
Typic Eutrorthox	control	0–10	47.7	4.2	4.4	40.8	13.2	1.1
		10–20	48.6	4.5	4.4	40.3	12.7	1.1
		20–40	47.1	4.8	4.6	39.7	11.5	1.2
		40–60	46.7	5.0	4.6	40.7	11.0	1.2
	sewage sludge application	0–10	45.5	4.3	4.8	38.4	12.2	1.3
		10–20	44.4	4.3	4.7	36.4	12.2	1.3
		20–40	45.1	4.4	4.7	38.3	11.9	1.3
		40–60	47.4	4.9	5.0	39.3	11.3	1.3
Typic Haplorthox	control	0–10	43.9	3.9	3.4	38.6	13.2	0.9
		10–20	44.8	4.0	4.3	37.0	13.1	1.2
		20–40	39.5	3.5	4.0	34.7	13.0	1.2
		40–60	37.2	3.1	3.6	42.6	13.9	1.2
	sewage sludge application	0–10	43.2	4.2	4.4	43.1	12.1	1.2
		10–20	43.3	4.0	4.8	33.9	12.5	1.3
		20–40	41.1	3.8	4.4	34.2	12.6	1.3
		40–60	41.0	3.2	3.5	43.6	15.1	1.0
Sewage sludge	–	–	50.5	8.6	7.0	33.1	6.9	1.7

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