



A laser-induced fluorescence spectroscopic study of organic matter in a Brazilian Oxisol under different tillage systems

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Abstract

Laser-induced fluorescence (LIF) spectroscopy has been proposed as new method for determining the degree of humification of organic matter (OM) in whole soils. It can be also used to analyze the OM in whole soils containing large amounts of paramagnetic materials, and which are neither feasible to Electron Paramagnetic Resonance (EPR) nor to ¹³C Nuclear Magnetic Resonance (NMR) spectroscopy. In the present study, LIF spectroscopy was used to investigate the OM in a Brazilian Oxisol containing high concentration of Fe⁺³. Soil samples were collected from two areas under conventional tillage (CT), two areas under no-till management (NT) and from a non-cultivated (NC) area under natural vegetation. The results of LIF spectroscopic analysis of the top layer (0–5 cm) of whole soils showed a less aromatic OM in the non-cultivated than in the cultivated soils. This is consistent with data corresponding to HA samples extracted from the same soils and analyzed by EPR, NMR and conventional fluorescence spectroscopy. The OM of whole soils at 5–10 and 10–20 cm depth was also characterized by LIF spectroscopy.

Analysis of samples of NT and NC soils showed a higher OM aromatic content at depth. This is a consequence of the accumulation of plant residues at the soil surface in quantities that are too large for microorganisms to metabolize fully, thus, resulting in less aromatic or less humified humic substances. In deeper soil layers, the input of residues was lower and further decomposition of humic substances by microorganisms continued, and the aromaticity and degree of humification increased with soil depth. This data indicates that the gradient of humification of OM in the NT soil was similar to those observed in natural soils. Nevertheless, the degree of humification of the OM in the soils under no-till management varied less than that corresponding to non-cultivated soils. This may be because the former have been managed under these practices for only 5 years, in contrast to the continuous humification process occurring in the natural soils. On the other hand, LIF spectroscopic analysis of the CT soils showed less pronounced changes or no change in the degree of humification with depth. This indicates that the ploughing and harrowing involved in CT lead to homogenization of the soil and thereby also of the degree of humification of OM throughout the profile.

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1. Introduction

It's well known that in tropical regions the intensive use of soils under conventional tillage (CT) practices results in rapid

turnover of OM. To mitigate this problem, the no-till (NT) system has been increasingly used in the last years to aid in soil conservation, from 100 ha cultivated in 1972 to order of 21.86 million hectares in 2004 (FEBRAPDP, 2006). Studies carried out with a sandy clay loam Acrisol in southern Brazil, to which large amounts of residues were added and no-till (NT) was applied, showed an increase in soil carbon and total nitrogen contents (Bayer et al., 2000). In addition to the agricultural benefits, the possibility of reduction in CO₂ emission due to C retention in the soil under NT and addition

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of large amounts of crop residues may help to mitigate the greenhouse effect (Bayer et al., 2002).

We have studied the effects of different tillage systems on the chemical composition of purified humic acids from Oxisols, by means of spectroscopic techniques (Bayer et al., 2000; González-Pérez et al., 2004), such as Electron Paramagnetic Resonance (EPR), ^{13}C Nuclear Magnetic Resonance (NMR), Fourier Transform Infrared Spectroscopy (FTIR) and conventional fluorescence spectroscopy, which allow possible insights into the chemical composition of humic substances (HS) (Schulten and Schnitzer, 1993). The techniques also enable determination of parameters that describe the degree of humification of soils, such as the degree of aromaticity, as revealed by solid-state ^{13}C NMR (Stevenson, 1994), and the semiquinone-type free radical concentration, as determined by EPR (Schnitzer and Levesque, 1979).

However, NMR and EPR are not suitable for studying the OM in whole Oxisols, which are found in all Brazilian States and which in some regions constitute most of the agricultural soils. The high levels of iron oxides and clays confer characteristics to these soils that make them highly suitable for agricultural use, but at the same time, interfere in the use of NMR and EPR, because they cause a reduction in the relaxation times and the resolution of the spectra (Skjemstad et al., 1994; Novotny and Martin-Neto, 2002).

Recently Milori et al. (2006) showed that laser induced fluorescence (LIF) spectroscopy can be used as a fast and non-destructive method for analyzing organic matter of whole soils and for studying OM associated with minerals in the soil matrix.

The technique and instrumentation are similar to those corresponding to conventional fluorescence spectroscopy, already used to analyze purified soil OM fractions. The major difference is in the irradiation source. In LIF spectroscopy the soil samples are irradiated with 400 mW of monochromatic light at 351 nm, produced by an argon laser. The use of a high power source improves the sensitivity, allowing application of the technique in whole soil samples.

Laser induced fluorescence spectroscopy has been used to study the degree of humification of SOM in whole soil samples collected from different depths in three long-term field experiments with two soils from the Brazilian Cerrado region (Milori et al., 2006). The results show a high correlation with those obtained by conventional fluorescence spectroscopic analysis of the humic acids extracted from the same soil samples.

The evaluation of OM quality or degree of humification with depth has been less researched than the variation in total OM, probably because of the need for more sophisticated techniques such as EPR, NMR and fluorescence spectroscopy.

In the present study we show that LIF spectroscopy is a simpler method of studying the quality of soil OM than the techniques cited above. It is a sensitive technique that allows rapid analysis of the OM of whole Oxisol samples without any prior chemical treatment. The technique can also be used where it is not possible to obtain reliable EPR and NMR spectra because of interference from iron oxide, minerals and other paramagnetic ions in soils such as the Brazilian Oxisols under study, which contain large amounts of Fe^{+3} .

In the present study, LIF spectroscopy was used to evaluate soil OM in the topsoil of whole Brazilian Oxisols under different tillage practices (NT, CT and NC). The LIF results were compared with results obtained by EPR, ^{13}C NMR and conventional fluorescence spectroscopic analysis of purified humic acids samples extracted from the same Oxisols. Moreover, whole soil samples from different depths were also characterized by LIF spectroscopy.

2. Materials and methods

2.1. Site descriptions

The study involved a field experiment located in an area with geographic coordinates $21^{\circ}15'22''$ S, $48^{\circ}15'18''$ W and 610 m altitude, in the city of Jaboticabal, State of Sao Paulo, Brazil. The climate in this subtropical region is characterized by a mean annual temperature of 22°C , humid summers and dry winters.

2.2. Experimental design and treatments

The field experiment evaluated different tillage systems over the course of 5 years. The experimental design included a randomized block with split-plots for tillage with 80 m^2 and cropping systems (sub-plots), and four field replicates. The following treatments were selected for study: non-cultivated soil under natural vegetation (NC), conventional tillage/maize–bare fallow (CT1), no-till/maize–bare fallow (NT1), conventional tillage/maize rotation with soybean–bare fallow (CT2) and no-till/maize rotation with soybean–bare fallow (NT2). Some soil characteristics are shown in Table 1.

2.3. Soil sampling

Soil samples were collected from an Oxisol (Latossolo Roxo) containing 53% clay, 24% silt, 23% sand and 3.6% OM.

Table 1

Selected chemical characteristics of whole Oxisol samples (Latossolo Roxo) from conventional tillage (CT), no-till (NT) and non-cultivated (NC) areas at different depths 0–5 cm, 5–10 cm and 10–20 cm

Management	Soil layer (cm)	Fe g kg^{-1}	pH H_2O	pH CaCl_2	OM g kg^{-1}	CTC $\text{mmol}_\text{c}\text{kg}^{-1}$	Clay %
NC	0–5	15	7.0	6.7	86	190	53.9
	5–10	14	6.9	6.5	49	145	58.3
	10–20	12	6.7	6.1	28	102	62.3
CT1	0–5	10	7.0	6.3	25	83	53.4
	5–10	9	7.0	6.4	25	94	51.2
	10–20	13	6.7	6.2	27	69	55.5
NT1	0–5	7	7.3	6.8	32	117	48.5
	5–10	11	6.9	5.7	19	74	63.1
	10–20	14	6.5	5.6	18	68	55.7
CT2	0–5	8	7.2	6.5	27	99	52.1
	5–10	10	6.9	6.3	24	84	52.8
	10–20	13	6.5	5.9	19	70	55.7
NT2	0–5	6	7.5	6.8	28	114	53.1
	5–10	12	6.9	6.2	22	82	60.3
	10–20	13	6.7	5.9	18	62	61.8

T1: maize–bare fallow. T2: maize rotation with soybean–bare fallow.

Table 2

Comparison between degree of humification of HA (0–5 cm) obtained by different spectroscopic techniques: ^{13}C NMR (degree of aromaticity), EPR (semiquinone-type free radical level) and fluorescence (area of emission spectra with maximum of intensity around 520 nm, excitation wavelength at 436 nm)

Technique Sample	^{13}C NMR Degree of aromaticity (%)	EPR no. spins/g HA (10^{17})	Fluorescence (a.u.)
NC	23.86	1.02	0.31
CT1	30.36	2.21	0.91
NT1	30.47	2.27	0.97
CT2	30.37	2.46	1.0
NT2	30.14	2.56	0.97

NC: non-cultivated area. CT: conventional tillage. NT: no-till T1: maize–bare fallow. T2: maize rotation with soybean–bare fallow. Source: González-Pérez et al. (2004).

The soil was collected after crop harvesting, from three depths: 0–5 cm, 5–10 cm and 10–20 cm, from a field experiment in the 5th year. For each treatment 12 samples were mixed to form a homogenized soil sample. All samples were then air-dried, crushed and sieved through a 2 mm-seive.

2.4. Humic acids: Extraction, purification and spectroscopic data

The humic acids were extracted from the topsoil (0–5 cm) samples, with 0.5 mol L^{-1} NaOH, precipitated with 6 mol L^{-1} HCl (pH 2.0) and purified by successive repetition of the extraction procedure. The HAs obtained were then dialyzed by Spectrapor membrane (size exclusion limit, 6000–8000 Da) and finally freeze-dried.

Details of spectroscopic characterization by EPR, ^{13}C NMR, FTIR and fluorescence spectroscopy have been published in González-Pérez et al. (2004). Comparison of the degree of humification obtained by different spectroscopic techniques: ^{13}C NMR (degree of aromaticity), EPR (semiquinone-type free radical level) and fluorescence (area of emission spectra with maximum of intensity around 520 nm, excitation wavelength at 436 nm) is shown in Table 2.

2.5. Laser Induced Fluorescence (LIF) spectroscopy

Laser induced fluorescence spectroscopy was carried out with pellets from whole soil prepared with 0.5 g of dry samples. The spectra were recorded in an experimental set up (Milorí et al., 2006) using an argon-ion laser tuned at 351 nm to excite soil fluorescence, with 400 mW power. The resolution of the set up was approximately 4 nm. The degree of humification was estimated as the ratio between the area of emission spectrum and the percentage of carbon (H_{LIF}). The percentage of carbon was determined by the Walkley–Black method (Nelson and Sommers, 1996). To confirm whether the LIF signal originated from the organic matter fluorescence, 2 g of non-cultivated soil were calcinated at 700°C for 2 h and then measured.

3. Results and discussion

Some of the chemical characteristics of the studied soils at different depths are shown in Table 1. The NC soil contained 2

to 3 times more OM than the cultivated soils. The OM content decreased with depth for all treatments (except CT1), as reported by several authors.

The LIF emission spectrum of whole NC soil shows a single broad band from 350 nm to 650 nm (Fig. 1), similar to those reported elsewhere for HA fluorescence spectra (Senesi et al., 1991; Zsolnay et al., 1999; Milorí et al., 2002). The spectrum of the calcinated sample (Fig. 1) did not show any signal, demonstrating that LIF emission of soils can be associated with OM, as reported by Milorí et al. (2006).

The 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). Milorí et al. (2006) proposed the application of fluorescence spectroscopy to investigate organic matter in whole soil samples. The LIF emission spectra measure carbon in more complex or rigid structures, such as aromatic and quinone groups in whole soil samples. However, this is not the total organic carbon. When fluorescence is excited at near ultraviolet or blue wavelengths, information is provided about more humified structures (Milorí et al., 2002).

Milorí et al. (2006) have shown that the ratio between the normalized area of total fluorescence emission spectrum and the percentage of carbon (H_{LIF} ratio), can be used as a parameter for evaluating the degree of humification of soil organic matter, i.e., to provide information about the humification process.

The H_{LIF} ratios for all treatments are shown in Fig. 2. The H_{LIF} ratio in the NC topsoil was lower than in the CT and NT soils. The less aromatic OM from NC soil (0–5 cm) has been reported elsewhere and is related to less humified OM from partially decomposed residues (Bayer et al., 2000). There was no difference in H_{LIF} among the CT and NT tillage systems at topsoil considering the error bar (Fig. 2).

The H_{LIF} ratio for the whole topsoils is well correlated with the measurements made in extracted humic acids from the same samples. Good correlations were obtained between the H_{LIF} ratio and semiquinone-type radicals, as determined by EPR ($R=0.94$) and between the H_{LIF} ratio and the degree of

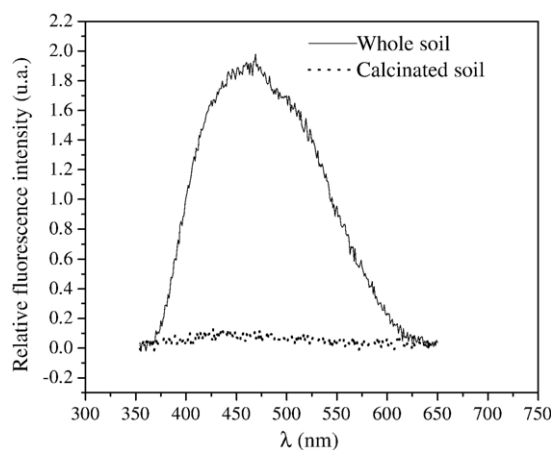


Fig. 1. LIF emission spectra of whole (non-cultivated treatment) and calcinated soil samples.

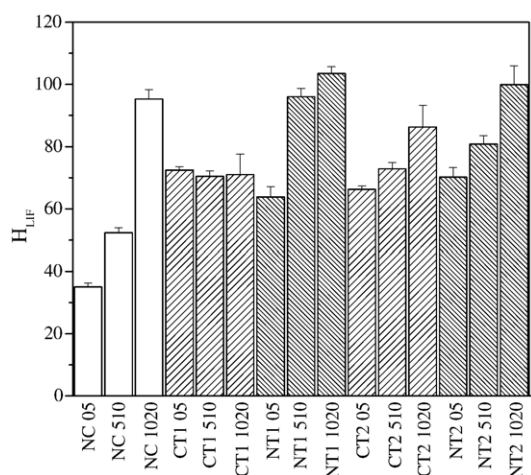


Fig. 2. LIF results, determined as H_{LIF} for whole soils under conventional tillage (CT1, CT2), no-till (NT1, NT2) and non-cultivated (NC) treatments, and at different depths 0–5 cm, 5–10 cm and 10–20 cm.

aromaticity, as determined by NMR ($R=0.97$) (Table 2 and Fig. 3). The correlation between H_{LIF} ratio and intensity of fluorescence was good ($R=0.95$) (Table 2). Good correlations were also obtained between the aromatic C content of humic acids, as measured by NMR and the degree of humification, as measured by other spectroscopic techniques, such as EPR and conventional fluorescence (González-Pérez et al., 2004). The high correlations with the results obtained by more conventional techniques confirm the potential of LIF spectroscopy in OM studies, and the usefulness of the H_{LIF} ratio in determining the degree of humification.

In the first phase of residue decomposition, the process is dominated by the rapid mineralization of labile components and in the second phase it is dominated by the accumulation of refractory molecules. Thus, some constituents of litter, such as polysaccharides and proteins are easily decomposable, although lignin, polyphenols and some aliphatic compounds are relatively resistant to microbial breakdown and accumulate in soils (Zech et al., 1997). The quantification of aromatic compounds therefore remains a good indicator of the degree of humification.

The degree of humification (H_{LIF}) of the OM in whole soils with depth is shown in Fig. 2. The H_{LIF} ratio for the NC soil was lower in the 0–5 cm and 5–10 cm horizons than for the same horizons in the NT and CT soils. The ratio indicates a lower degree of humification in the NC soil than in other soils. This is because plant residues accumulate at the soil surface in quantities that overwhelm the capacity of microorganisms to metabolize them, resulting in a less aromatic or less humified humic substances. In other horizons, the input of residues is lower and further decomposition of humic substances by microorganisms continues, increasing the degree of aromaticity and humification.

The NT soils showed a tendency for the degree of humification to increase with depth in the same way as in the NC soil. These data show that OM humification gradients in the NT soils were similar to those observed in natural areas. Nevertheless, the OM in the NT soils showed a narrower range

of values of humification values than in the NC soil. These practices have only been carried out during the past 5 years, which is a relatively short time, in contrast with the continuous humification process that takes place in natural areas.

The LIF measurements of the soils from conventional tillage system show no change in the degree of humification of CT1 with depth (Fig. 2). However, CT2 varied in a similar way to NC and NT soils, with a minor alteration in degree of humification. Soil OM losses of CT1 may be associated with the intensive degradation of SOM due to the intensive soil preparation by ploughing and harrowing, which favour mineralization of organic matter. The soil becomes homogenized and thus the degree of humification of OM is also homogenized throughout the profiles. The lack of metabolizable organic compounds must also be taken into account. The non incorporation of fresh available residues containing compounds necessary for the metabolism of microorganisms (Bayer et al., 2002; González-Pérez et al., 2004) leads them to decompose the organic matter already existing in the soil more thoroughly.

The results of the present study show minor changes in the chemical composition of soil OM as a consequence of tillage practices. They demonstrate that CT practices do not maintain a gradient of humification with soil depth as in the NC soils. The gradient of humification of OM in the NT soils was similar to that observed in NC soils, but with a low variation in humification values. The NT practice thus appears to favour soil conservation to a greater degree than CT; however, after 5 years, this treatment had not succeeded in restoring the original levels of OM observed in NC areas. In NC areas, humification is a continuous process and gradients are achieved after a long time.

By comparing conventional tillage treatments, the small effect of crop rotation on LIF results can be observed. It appears that the quantity of plant residues added in this treatment is not great enough to reverse the effect of the decreased OM content caused by conventional tillage practices. The effect of intensive

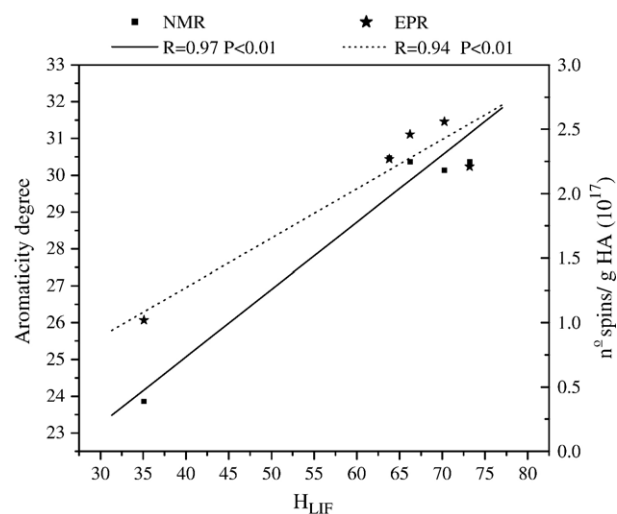


Fig. 3. Relationship among LIF results (H_{LIF}) and semiquinone-type-free radical levels, as determined by EPR, and also with the degree of aromaticity, as determined by VACP/MAS ^{13}C NMR spectra.

ploughing on the decomposition process of organic matter in this clayey soil in tropical conditions probably cannot be compensated only by crop rotation.

Studies in which the quality and quantity of soil organic matter were characterized have demonstrated that a long time is needed to reverse the effect of decreasing OM contents and homogenization throughout the profile caused by conventional tillage practices (Bayer et al., 2000).

For an Oxisol with high clay content (~53%), where the physical protection of soil organic matter hinders strong structural alterations, even conventional tillage practices cannot destroy the clay-Fe-OM complex. Soil matrix and minerals protect natural organic materials in such a way that even after UV photo-oxidation they were still present in the soil OM fraction. Encapsulation into the hydrophobic paraffinic network of some biopolymers and incorporation of OM within microaggregates may also act as mechanisms of OM protection (Baldock and Skjemstad, 2000; Knicker and Skjemstad, 2000). The results of the present study revealed small changes in the degree of humification of OM as a consequence of different tillage practices (conventional and no-till), after 5 years from the beginning of the field experiment.

4. Conclusions

LIF spectroscopy is a promising technique for OM studies with whole Oxisol samples. It is sensitive and allows rapid analysis of samples without any prior chemical treatment. LIF can also be used when it is not possible to obtain reliable EPR and NMR spectra because of interference from iron oxide, minerals and other paramagnetic ions.

The high correlation of the results obtained by LIF spectroscopy and those obtained by other techniques such as EPR, NMR and conventional fluorescence spectroscopy, indicates its potential for use in OM studies and the usefulness of the H_{LIF} ratio for determining the degree of humification. The H_{LIF} ratio can be used to discriminate, in whole soils, the variation in degree of humification at depth for most treatments, showing the effect of constant accumulation of plant residues in the topsoil.

The gradient of humification degree of OM in the NT soil was similar to those observed in natural soils, although the variations among depths are small. The CT soils showed less pronounced changes or no change in the degree of humification with depth. This indicates that the ploughing and harrowing involved in CT lead to homogenization of the soil and thereby also of the degree of humification of OM throughout the profile. The NT management seems to reproduce conditions similar to the naturals because it causes a low environmental impact on soils.

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