Assessing humification and organic C compounds by laser-induced fluorescence and FTIR spectroscopies under conventional and no-till management in Brazilian Oxisols

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1. Introduction

Conversion of forest, savannah or grasslands to agroecosystems and their management with plow-based conventional tillage (CT) depletes soil organic carbon (SOC) concentration and pool (Guo and Gifford, 2002; Lal, 1976; Potter et al., 1998) by disrupting the macro-aggregates (Resck, 1998), and exposing the previously protected SOC to microbial processes (Elliott, 1986). The rate and magnitude of SOC depletion depend on the soil texture, mineralogy, climate, annual C input and tillage intensity. Changes in the SOC pool generally occur in fractions comprised of polysaccharides, water soluble SOC, and to a lesser extent the stable or mineral-associated fractions. In contrast, no-till (NT) systems enhance soil aggregate stability (Madari et al., 2005), reduce the rate of decomposition of soil organic matter (SOM), and improve the physical protection of SOM within stable
aggregates (Six et al., 2000). At the process level, the stabilization of SOC in aggregates is the principal mechanism of long-term sequestration of C in SOM, because the degree and stability of aggregates protect SOM from enzymatic and microbial attacks, and reduce the rate of mineralization in temperate (Balesdent et al., 2000; Six et al., 1999) and sub-tropical environments (Bayer et al., 2006). While quantitative estimates of changes in SOC have been widely reported for contrasting conditions, qualitative changes upon conversion of native vegetation (NV) to CT and NT systems have not been widely studied for sub-tropical and tropical Brazilian environments. Yet, this research is essential for a better understanding of the SOM dynamics, and to predict changes in SOC storage among contrasting land uses and management.

Milori et al. (2006) reported a good applicability of Laser-Induced Fluorescence Spectroscopy (LIFS) for the characterization of SOM, and for the assessment of its humification degree in the bulk soil samples collected at different depths in three long-term experiments in a tropical agro-ecoregion in Brazil. Milori and colleagues reported that LIFS can be used as a rapid non-destructive tool for analyzing SOM, and reported a high correlation between the data from LIFS with those from conventional fluorescence spectroscopic analysis of the humic acids extracted from the same soil samples. LIFS has been widely used in conjunction with other analytical techniques (i.e., Electron Paramagnetic Resonance — EPR and/or cross polarization magic angle spinning 13C Nuclear Magnetic Resonance — CPMAS/NMR). Several studies reported a close relationship among humification degree obtained by LIFS and semiquinone-type-free radical levels determined by EPR (Segnini et al., 2010), and also with the degree of aromaticity determined by 13C NMR spectra (González-Pérez et al., 2007). These relationships were observed in contrasting conditions such as Brazilian Oxisols and Wetlands from the high Andes. Other studies, including those by Milori et al. (2006) and Dieckow et al. (2009), indicated an increase in the aromatic components of SOM upon conversion of NV to CT and, as an opposite trend when CT is converted to a NT-based cropping system. In addition, Fourier Transform Infra-Red spectroscopy (FTIR) has been widely used for the characterization of organic compounds (Barancikova et al., 1997; Stevenson, 1994) to study the vibrational characteristics of their structural chemical bonds. Ellerbrock et al. (1999) and Kaiser et al. (2007) analyzed the changes in functional groups of humic acids with FTIR under different land uses and management systems in temperate conditions. Several researchers focused their analyses on specific organic compounds (i.e., C — H, C = C — C) which reflect SOM characteristics such as its cation exchange capacity (Celi et al., 1997), soil wettability (Capriel et al., 1995), and organo-mineral interactions (Lehmann et al., 2007). In addition, partial least square models have been applied to FTIR data to predict the concentration of functional groups (Ludwig et al., 2008). SOC pools (Bornemann et al., 2010), decomposition status of SOM on bulk samples from different horizons (Haberhauer et al., 2000), soil aggregation (Madari et al., 2006), and as a tool for assessing soil quality (Artz et al., 2008). Therefore, more emphasis needs to be placed on the qualitative changes in functional organic C groups, the stabilization mechanism, and the contributions of land use to SOC sequestration. In a previous study, Tivet et al. (2013) showed that the difference in SOC stock among land uses (i.e., native vegetation, plow-based tillage and NT cropping systems) is largely attributed to storage in the 8—19 mm aggregate size class, indicating that high biomass-C inputs under NT rebuilt the largest macroaggregates, which are crucial for the stabilization of SOC. The experience gained in the Cerrado region of Brazil by Séguy et al. (1998) was essential to build cropping sequences with diverse and high biomass-C inputs managed under NT. These NT systems are based on the insertion of supplementary biomass-generating crops, which are intercropped before or after the commercial crops or in relay, enhancing ecosystemic functions (Séguy et al., 2006). The present study is based on the hypothesis that the LIFS and FTIR analytical techniques can identify the effects of land use and cropping systems in contrasting climates on: (i) the humification degree of SOM, and (ii) the relative composition and changes in functional organic C groups throughout the soil profile and on aggregate size fractions. Thus, the specific objective of this study was to assess changes in humification and in organic C groups using laser-induced fluorescence and FTIR spectroscopies under native vegetation and two contrasting tillage systems.

2. Materials and methods

2.1. Location of study sites and experimental design

The experimental design and land uses management have been described in previous manuscripts (Sá et al., 2013; Tivet et al., 2013). Briefly, field experiments were conducted (i) at the experimental station of the Instituto Agronômico do Paraná (PG site, sub-tropical condition), and (ii) at the experimental station of Fundação Rio Verde (LRV site, tropical condition).

2.1.1. PG site

The experiment, established in 1981, comprises three tillage treatments: (i) conventional tillage (CT) such as a plow tillage after summer harvest and another after winter harvest and two narrow diskings; (ii) minimum tillage (MT) comprising of one chisel plow and one narrow disk ing; and (iii) continuous no-till (NT) without any soil disturbance. In the present study, only CT and NT were sampled and analyzed. The dimensions of each plot were 100 × 100 m for the NT treatment, and 50 × 140 m for the CT and MT. The cropping system for all tillage treatments comprised a three year crop sequence with two crops per year with soybean (Glycine max, L. Merril) in six summers and maize in four summers in the last ten years alternating with oats (Avena strigosa Schreb), wheat (Triticum aestivum L) and vetch (Vicia sativa) in winter. In close proximity to the experimental plots, a NV plot was selected as a base line for comparison. Six sub-plots were delimited in the cropped fields and under NV for soil sampling.

2.1.2. LRV site

The experiment was established in 2001, and among the seven tillage treatments (Sá et al., 2013), five were selected for this study: (i) soybean alternating with cotton under conventional plow-based tillage designed CT; (ii) NT1 — soybean in summer followed by maize (Zea mays L.) + ruzi grass (Brachiaria ruzizensis) as second crop; (iii) NT4 — soybean in summer followed by finger millet + Crotalaria spectabilis or sunflower (Helianthus annuus) + B. ruzizensis as second crop; (iv) NT5 — soybean in summer followed by sorghum (Sorghum bicolor) + B. ruzizensis as second crop; and (v) NT6 — soybean in summer followed by millet (Pennisetum glaucum) or maize + B. ruzizensis as second crop. These NT systems are based on extensive work by Séguy et al. (1998) in the Cerrado region. These authors have largely reported the strong efficiencies of NT built on high biomass-C input and with a large biodiversity to recover the original soil fertility and to ensure farming systems sustainability (Séguy et al., 2006). The dimension of each plot was 216 × 42 m for the NT treatments with three sub-plots (72 × 42 m), and 216 × 252 m for the CT treatment with four sub-plots (216 × 62 m). An adjacent NV Cerrado was included to represent the original vegetation and the undisturbed soil conditions as the baseline, and six sub-plots were delimited for soil sampling.

2.2. Soil sampling

Soil samples were collected from seven depths: 0–5, 5–10, 10–20, 20–40, 40–60, 60–80, and 80–100 cm. Four sampling points were selected for each replicate, and samples were composited. The native vegetation at each location was used as base line. The bulk samples
were oven-dried at 40 °C, gently ground, sieved through a 2 mm sieve, and homogenized.

2.3. Water-stable aggregate

The method is described in detail in Tivet et al. (2013). Briefly, aggregate size classes were obtained by wet sieving procedure (Kemper and Rosenau, 1986). Each soil sample was passed through a 19 mm mesh sieve, and clods > 19 mm were softly broken along their natural cleavage planes (Castro Filho et al., 2002). Samples were stored in polystyrene boxes to prevent moisture loss and excessive drying. A nest of seven sieves (8, 4, 2, 1, 0.5, 0.25, and 0.053 mm) was used, and the following classes were obtained: 0.053–0.25, 0.25–0.5, 0.5–1, 1–2, 2–4, 4–8, and 8–19 mm. At the end of the cycle, the stable aggregates from each sieve were back-washed off the sieves, oven dried (40 °C), and weighed to calculate the proportion of micro- and macroaggregate.

2.4. Total soil organic carbon concentration

Sub-samples of the 2-mm sieved bulk soil, and of each aggregate size class, were finely ground before analysis for organic C by dry combustion (Nelson and Sommers, 1996) with an elemental CN analyzer (TruSpec CN, LECO, St. Joseph, USA).

2.5. Spectroscopic analysis

2.5.1. Laser-Induced Fluorescence Spectroscopy (LIFS)

Fluorescence spectra were recorded using equipment developed by the Embrapa Agricultural Instrumentation Center in the Lasers and Optics Laboratory. Excitation is mediated by a continuous wave laser at 405 nm with 20 mW power. An optical bundle collects the emitted light and directs it to a mini-spectrometer. The resolution of the system was around 10 nm for all acquisition ranges (420–800 nm). Data acquisition software was developed to open a shutter, excite the sample, register the fluorescence signal, and close the shutter again. The software also controls the laser power and spectrometer parameters such as integration time and number of averages for each measurement. Three replicates were recorded for each sample and averaged. The humification index ($H_{flu}$) was determined by the procedure described by Milori et al. (2006) ($H_{flu} = FCA/TOC$, i.e., the $H_{flu}$ is equal to the ratio of the fluorescence curve area (FCA) to the TOC concentration of the samples).

2.5.2. Fourier-transform infrared spectroscopy

FTIR absorbance spectra were recorded using a Nicolet IR 4700 FTIR spectrophotometer over the range 4000–400 cm$^{-1}$. All individual FTIR spectra were composed of 32 scans with a resolution of 2 cm$^{-1}$, and corrected against the spectrum of KBr pellet in ambient conditions as background. Prior to analysis, sub-samples of bulk soil and of each aggregate size class were finely milled, using a hammer mill. Powdered samples of 3 mg were mixed with 200 mg of KBr, finely ground in an agate mortar, and pressed into a pellet that was used in transmission FTIR analyses. Spectra were automatically corrected for absorption of atmospheric water and CO$_2$, and the automatic baseline correction was applied. In order to determine relative changes in the most important peaks (i.e., 3696, 3621, 3526, 3440, 2922, 2852, 1632, 1404, 1030, 914, 798, 750, 672, 538, 470 cm$^{-1}$) and for comparison of the spectra, $H_{flu}$ values were compared using the Least Significant Differences (LSD) at the 5% probability level (Webster, 2007). Relative absorbance data from the corrected peaks of FTIR spectra, TOC and $H_{flu}$ were used for principal components analysis (PCA) and discriminant analysis of principal components (DA). The PCA was used to select and reduce the

![Fig. 1. Total soil organic carbon concentration (g kg$^{-1}$) and humification index ($H_{flu} = $ fluorescence curve area/TOC, insert) managed under either no-till (NT) or conventional tillage (CT), and under the neighboring native vegetation (NV), at the PG and LRV sites. NT1, NT4, NT5, and NT6: no-till systems with contrasted biomass-C input at the LRV site (Sá et al., 2013). P values ≤ 0.05 are given (*P ≤ 0.05, **P < 0.01, ***P < 0.001).]
variables by identifying the most important components. The objective of variable reduction is to make data analysis more manageable and straightforward. Application of PCA in the present study reduced sixteen FTIR variables, plus TOC and $H_{IR}$, to three principal components, and yet was able to capture 61% and 71% of the original variance at the LRV and PG sites, respectively. Only principal components explaining > 10% of the total variance were retained.

The reduction increased the sample size to variable ratio and made subsequent analysis and interpretation easier. A discriminant analysis of PCA was carried-out to differentiate the land uses along with the relative absorbance of the main FTIR peaks (clay minerals and organic compounds), TOC and $H_{IR}$. The avn and ade4 packages of R software (R Development Core Team, 2006) were used in this study. SigmaPlot 12.0 was used for graphic representation.

3. Results and discussion

3.1. Soil organic concentration in bulk soil samples and aggregate size classes

Extensive comparisons of SOC concentrations and stocks between tillage treatments and NV are given in Sá et al. (2013). At the PG site, a significant difference in SOC concentrations was observed among all the tillage treatments and NV in the 0–10 cm layer (Fig. 1). Conversion of NV to CT depleted SOC by 27.1 g kg$^{-1}$ in the 0–10 cm layer ($P < 0.01$) over 42-yr. In contrast, a gain of 18.4 g C kg$^{-1}$ ($P < 0.01$) occurred in NT in comparison with CT over 29-yr. At the LRV site, the same trend was observed with a distinct distribution pattern of the concentration of SOC among land uses. However, a significant difference in the SOC concentration among land uses was observed only in the 0–5 cm layer. SOC losses occurred under CT, and its concentration in 0–5 cm depth decreased from 38.3 g kg$^{-1}$ under NV to 18.3 g kg$^{-1}$ under CT over the 23-yr period, when SOC ranged from 19.7 to 25.2 g kg$^{-1}$ under NT management.

At the PG site, a decline of ~27% in SOC stock over 42 years since the conversion of NV (92.0 Mg ha$^{-1}$) into cultivated field and use of CT (67.4 Mg ha$^{-1}$) was observed in 0–20 cm depth. In contrast, the soil under NT contained 17.0 Mg ha$^{-1}$ more SOC than under CT. At the LRV site, the difference in SOC stock between CT and the Cerrado NV was 14.2 Mg ha$^{-1}$ in 0–20 cm depth. No significant differences were observed between SOC stocks under the Cerrado NV and three NT systems (i.e., NT1, NT5 and NT6) with a predominance of grasses during the dry season. On average, the difference ($P < 0.05$) in SOC stock at a depth of 20-cm between NT1, NT5, NT6 and CT ranged from 6.9 to 10.4 Mg C ha$^{-1}$.

The SOC concentration in each aggregate size class under NV was significantly higher than that observed under cropped fields (CT and NT) in the 0–5 and 5–10 cm layers at the PG site, but mainly in the 0–5 cm layer at the LRV site (Fig. 3). At the PG site, the SOC concentration in the 0–5 cm layer under CT was significantly less than that under NT in all aggregate size classes. The positive effect of NT is attributed to the absence of soil disturbance and to leaving crop residues on the soil surface, which enhance soil aggregation and the incorporation of labile organic compounds in aggregates. At the LRV site, the SOC concentration in the 0–5 cm layer was higher under NT systems (mean of NT1, NT4, NT5 and NT6) than under CT except that in the microaggregates (Fig. 3). The difference between CT and NT at both locations was largely due to SOC storage in large macroaggregates (Tivet et al., 2013).

3.2. Fluorescence emission spectra and humification index assessed by fluorescence spectroscopy

3.2.1. In bulk soil samples

The fluorescence emission spectra of bulk soils of NV at PG and LRV sites are shown in Fig. 2. A distinct shape and wavelength of the maximum fluorescence were observed at the two locations. The fluorescence spectra of the tropical Typic Haplustox (LRV site) had a smooth and regular shape with: (a) distinct shoulders at 462 and 492 nm in the surface layers but which were less marked in subsoil layers; (b) a more intense excitation peak at intermediate wavelength (530 nm) for 0–10 cm depth and less intense peaks at longer wavelengths; and (c) one clearly defined excitation peak at 530 nm plus a plateau between 540 and 560 nm in the subsoil layers (40–100 cm). In contrast, the fluorescence spectra of the subtropical Rhodic Hapludox (PG site) had a fragmented shape probably due to more complex interactions between organic compounds and minerals. Distinct peaks were observed with: (a) a distinct shoulder

Fig. 2. Fluorescence emission spectra of native vegetation soils at the PG and LRV sites for a depth of 0 to 100 cm.

Fig. 3. Land use effect on soil organic C concentration (○ SOC mean ± std. error, g C in aggregate fraction kg$^{-1}$ soil in the aggregate fraction) and humification index (● $H_{IR}$, mean ± std. error, au.) among aggregate size classes in the 0–5 and 5–10 cm layers at the PG (a) and LRV (b) sites. NV: native vegetation; CT: conventional tillage; NT: no-till. At the LRV site, SOC and $H_{IR}$ were calculated as the means of no-till systems (NT1, NT4, NT5, and NT6).
at 462 nm in the soil surface layers; (b) more intense excitation peaks at intermediate wavelength (494 and 528 nm) for 0–5 cm depth and less intense peaks at longer wavelengths; and (c) an intense excitation peak at 572 nm for 5 to 100 cm depth. This shift toward longer wavelengths at the PG site might reflect a higher concentration of condensed aromatic rings, a high degree of conjugation, which bear carbonyl and carboxyl groups (electron-withdrawing substituents) and organo-mineral linkages. The emission intensity increased sharply with an increase in soil depth at the LRV site while the TOC concentration decreased in the C-depleted subsoil layers (Fig. 1). At depth, a larger proportion of aromatic C, conjugated macromolecules, was observed at the LRV site with similar emission spectra from 40 to 100 cm. In contrast, the variation in emission intensity with soil depth was of lower amplitude at the PG site. At both locations, a shift of the fluorescence peak from 530 nm to 560 nm (LRV) or 572 nm (PG) was observed with an increase in soil depth, suggesting the presence of molecular components with a high level of aromatic polycondensation.

A strong vertical distribution of $H_{flu}$ with depth (Fig. 1, inserts) was observed at both locations ($P < 0.001$). $H_{flu}$ increased from the surface to the sub-soil layers, which is consistent with the deposition of labile SOM from plant residues on the soil surface resulting in a less aromatic and humified compounds. In the sub-soil, however, the decomposition of humic substances continues thus increasing the degree of aromaticity and complexation. $H_{flu}$ was more than 5 times greater in the 40 to 100 cm layer under Cerrado NV than in the forest soil at the PG site. The difference in $H_{flu}$ between the sites is a function of the TOC concentration, the morphology and chemical structure of SOM, the chemical and physical nature of the mineral fraction, and the architecture of the matrix (Baldock and Sjögrim, 2000). High clay content, and Al and Fe-sesquioxides at the PG site, which enhance the potential adsorptive and protective capacities of the mineral fractions, might be the main reason for the lower $H_{flu}$ under sub-tropical conditions, where larger physical protection mechanisms operate, in contrast with the highly weathered soils of the tropics. In addition, the higher mineral content at the PG site may also contribute to the saturation of the complexation sites of the SOM, and thus partially extinguish the fluorescence signal (Merdy et al., 2009).

At PG, the $H_{flu}$ was significantly higher under CT (Fig. 1) than under NT in 0–10 cm depth ($P < 0.001$), whereas no significant difference was observed among land use and management systems for 10 to 60 cm depth. This result indicates that most of the labile moieties in surface layers under CT have been oxidized and that additional protection mechanisms are unable to protect these fractions. In contrast, lower $H_{flu}$ under NV and NT soils suggests that physical protection in aggregates protects the most labile fractions of SOC. Yet, the same humification gradient was observed in NT and NV soils for 0 to 60 cm depth, while CT homogenized $H_{flu}$ in the 0–20 cm depth. Favoretto et al. (2008) and Martins et al. (2011) reported a similar trend at the PG site and observed that the $H_{flu}$ of humic acids was significantly lower under NT than under CT in 0–20 cm layer. Using fluorescence spectroscopy and electron pin resonance, Bayer et al. (2002) reported that the lowest concentration of stable semiquinone-type free

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**Fig. 4.** Fourier transform infrared spectra of macroaggregate (8–19 mm) samples of the native vegetation (NV) at the PG and LRV sites. Characteristic FTIR bands of the major organic and inorganic compounds are indicated. The inserts represent magnified sections of the spectrum for aliphatic symmetric and asymmetric CH$_2$ (left, aliphatic-C$_{2}$), and aromatic-C plus aliphatic-C$_{1}$ (right). Values between parentheses represent additional peaks observed under Cerrado NV soil at the LRV site.
radicals of humic acid samples was observed under NT in a typical Paleudeult in Southern Brazil for the 0–25 cm layer. At the LRV site, contrasting patterns with soil depths (Fig. 1) were observed between NV and cropped fields, and higher H_LIF was recorded under NV than in cultivated soils in the 60 to 100 cm layer. However, the H_LIF index was significantly higher under CT in 0–5 cm depth, when compared with Cerrado NV and NT systems (except NT4). Several studies, including those by González-Pérez et al. (2007) and Dieckow et al. (2009), have reported similar results for soils of sub-tropical and tropical regions of Brazil when comparing CT and NT systems. A low humification degree under NT was attributed to higher input of metabolizable organic compounds, a lower turnover time of the labile pool of SOM, a lower oxidation rate (Bayer et al., 2006), a higher C stabilization by incorporation within microaggregates and interactions with surface minerals (Six et al., 2004; Solinas et al., 1996).

3.2.2. Aggregate size classes

No significant differences in H_LIF were observed among aggregate size classes at the PG site under NV and NT (Fig. 3a), but a significant effect was observed under CT. At the PG site, and considering both soil layers (0–5 and 5–10 cm), the H_LIF index across all aggregate size classes was 2.4 and 1.5 times higher under CT (loss of labile moieties) than those under NV and NT, respectively. Lower H_LIF under NT suggests that spatial inaccessibility by aggregates plays a major role in enhancing SOC accumulation rather than selective preservation by these peaks were not classified as functional C groups, but were categorized as aromatic inorganic peaks.

3.3. Fourier-transform infra-red spectroscopy

3.3.1. General observations of FTIR spectra

The FTIR spectra of the native vegetation at the PG and LRV sites displayed a number of characteristic absorbance peaks (Fig. 4). In general, the spectra at both locations were characterized by the same peak pattern except in the 3400–3700 cm⁻¹ and 1200–900 cm⁻¹ regions, which refer mainly to inorganic components of clay minerals and quartz. The predominant clay minerals in the sub-tropical Rhodic Hapludox are gibbsite, kaolinite, halloysite, and hematite. The Typic Hapludox of the Cerrado biome has kaolinite, gibbsite, hematite, and goethite as principal constituents of the mineral matrix. Thus, well-resolved peaks were identified at both locations. The assignment of the main IR absorption bands at both locations is listed in Table 1. Additional well resolved peaks of clay minerals and quartz were observed in the Cerrado soil at 1006 (Si–O–Si in-plane stretching modes), 1092 (quartz and O–H stretch of kaolinite or halloysite) and 3646 cm⁻¹ (O–H stretch on kaolinite surface). Bands in the regions of 1632 and 1404 cm⁻¹ represented coupled vibrations of complex organic compounds. It was consequently not possible to group them in a specific functional group. The peak at 1632 cm⁻¹ was termed ‘aromatic-C’, the peak at 1404 cm⁻¹ ‘aliphatic-C’ and the cumulative bands at 2852 and 2922 cm⁻¹ ‘aliphatic-C’2. The band from 3300 to 3700 cm⁻¹ due to hydroxyl stretching vibrations associated with clay minerals is also found in alcohols and phenols (Ellerbrock et al., 1999). The same characteristic is observed between 1030 and 1080 cm⁻¹ with the superposition of Si–O–Si of quartz, Si–O stretching of clay and C–O stretching of polysaccharides. It is due to this superposition that these peaks were not classified as functional C groups, but were categorized as aromatic inorganic peaks.

3.3.2. In bulk soil samples

At the PG site, the relative absorbance of aliphatic-C and aromatic-C decreased significantly (P < 0.05) from the surface to C-depleted subsoil layers under NV and NT (Fig. 5). In contrast, a significant effect of depth under CT was observed only in the aliphatic-C group (P < 0.01), with decreasing values along the soil profile, while aromatic-C intensity remained similar from the surface to the subsoil layers. Significant differences were observed in aliphatic-C and in aromatic-C among land uses. In the 0–5 cm layer, higher aliphatic-C values (P < 0.01) were observed under NV and NT, while higher peak values (P < 0.001) were recorded under CT from 20–40 to 80–100 cm depths. In contrast, aromatic-C was significantly higher under NV (except in 10–20 and 20–40 cm depths) followed by NT and CT along the soil profile. Peaks of asymmetric and symmetric CH2 stretch, consisting of aliphatic-C2 were recorded under NT in the surface soil (0–20 cm layer), and none of these peaks were clearly identified in a

<table>
<thead>
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<th>Correspondence with molecular vibrations in specific compounds</th>
<th>Reference</th>
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<tr>
<td>3646–3696</td>
<td>OH stretching of inner-surface and outer hydroxyl groups of kaolinite</td>
<td>Brinatti et al. (2010), Castellano et al. (2010)</td>
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<td>3621</td>
<td>OH stretching of the inner hydroxyl group of the layer of kaolinite, gibbsite</td>
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<td>3394–3440–3526</td>
<td>In-plane and out-of-plane OH stretching of gibbsite</td>
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<td>2922</td>
<td>Aliphatic asymmetric C–H stretch</td>
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<td>2852</td>
<td>Aliphatic symmetric C–H stretch</td>
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<td>Aliphatic C=C deformation of CH2 and CH3 bending, C=O deformation of COOH, COO− symmetric stretch</td>
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<td>1092</td>
<td>Si–O stretching mode in clay minerals and Si–O–Si of quartz</td>
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<td>1030</td>
<td>Si–O–Si in-plane stretching mode of kaolinite, OH vibrations of gibbsite, and C–O stretching of polysaccharides</td>
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<td>Si–O–Si in-plane stretching modes of kaolinite</td>
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<td>OH bending modes of the inner hydroxyl groups of clay minerals (kaolinite, gibbsite)</td>
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<td>Si–O–Al translation of kaolinite, halloysite and Al–OH of gibbsite</td>
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<td>OH stretching of gibbsite and Si–O–Si stretch of quartz</td>
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<td>538</td>
<td>Vibrations of the octahedra involving Al3+ ions, Si–O–Si deformations should also participate.</td>
<td>Castellano et al. (2010)</td>
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spectrum of any soil under NV and only in the 0–5 cm layer under CT (data not shown).

At the LRV site, the relative absorbance of aliphatic-C1 ($P < 0.001$) and aromatic-C ($P = 0.03$) decreased significantly from the surface to the C-depleted subsoil layers under NV (Fig. 5). Aliphatic-C1 was significantly affected by depth under NT1 ($P < 0.001$) and NT5 ($P < 0.001$), while aliphatic-C1 and aromatic-C were significantly affected under NT4 ($P < 0.024$) and NT6 ($P < 0.001$). Lower values of aliphatic-C1 along the soil profile were also observed ($P < 0.001$) under CT, and aromatic-C was not altered with depth. The variability of aliphatic-C2 did not allow identification of significant trends along the soil profile (data not shown). Significant changes were observed in C groups among land uses. The main differences ($P < 0.05$) were observed in aliphatic-C1 (0–20 cm) with higher values recorded under NV and NT1. Significant changes in aromatic-C were also observed in the surface layers (0–20 cm) with higher absorbance

**Fig. 5.** Relative Fourier Transform Infra-Red (FTIR) absorption intensity of aliphatic-C1 and aromatic-C groups from bulk soil samples (0–100 cm layer) at the PG and LRV sites. $P$ values $< 0.05$ are given (* $P \leq 0.05$, ** $P < 0.01$, *** $P < 0.001$). NV: native vegetation; CT: conventional tillage; NT: no-till.
recorded under NV. In addition, high values of aromatic-C (20–40, 40–60 and 80–100 cm depths) were recorded under CT, when compared to NT systems.

3.3.3. Within aggregate size classes

At the PG site, the relative absorbance of functional groups was almost constant among classes (Fig. 6a, b). At the LRV site, under the Cerrado vegetation, significant differences were observed in the aliphatic symmetric CH₂ group (Fig. 6d, P < 0.001) among aggregate size classes, with higher absorbance recorded in the microaggregate size class (0.053–0.25 mm). Under CT, higher absorbance of aromatic-C group (P < 0.05, Fig. 6c) was observed for 0.25–0.5 mm size class, and lower values for large macroparticulate size classes (8–19 and 4–8 mm). Significant changes in aromatic-C and aliphatic-C₂ in NT1 (P < 0.05) and aliphatic-C₁ in NT6 (P < 0.05) were observed between aggregate size classes, but it was difficult to identify a specific trend among aggregate fractions (Fig. 6c, d).

3.3.4. Effects of land use on aggregate size classes

At the PG site, a significant effect (P < 0.05) of land use was observed in aromatic-C and aliphatic-C₁ in all aggregate size classes (Fig. 6a, b). The relative absorbance of aliphatic-C₁ and aromatic-C in NT was similar to that under NV. In contrast, lower absorbance (P < 0.05) was observed for aromatic-C and for aliphatic-C₁ in soil under CT than under NV and NT. Changes in aliphatic-C₂ group were observed in each fraction, except for 4–8 mm and 1–2 mm classes. A lower intensity of aliphatic-C₂ was observed under CT than under NT; with intermediary values under NV (Fig. 6b).

At the LRV site, higher values of aromatic-C and aliphatic-C₁ were observed in macro- and microaggregate size classes under Cerrado NV, and for the NT cropping systems with high biomass-C input (i.e., NT1, NT6 and NT5). In contrast, relatively lower values were observed under CT and NT4. Due to a marked variability the relative

### Table 2

Loading of principal components of discriminant analysis.

<table>
<thead>
<tr>
<th>Measurements</th>
<th>PG principal components</th>
<th>LRV principal components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC1</td>
<td>PC2</td>
</tr>
<tr>
<td>471</td>
<td>−0.23 0.16  −0.77 0.09</td>
<td></td>
</tr>
<tr>
<td>540</td>
<td>0.02 0.31  −0.49 0.30</td>
<td></td>
</tr>
<tr>
<td>684</td>
<td>0.14 0.08  −0.44 0.53</td>
<td></td>
</tr>
<tr>
<td>749</td>
<td>0.39 0.14  0.31 0.03</td>
<td></td>
</tr>
<tr>
<td>795</td>
<td>−0.04 0.07  0.00 0.23</td>
<td></td>
</tr>
<tr>
<td>914</td>
<td>−0.50 0.05  0.55 0.23</td>
<td></td>
</tr>
<tr>
<td>1032</td>
<td>0.16 0.46  0.54 0.51</td>
<td></td>
</tr>
<tr>
<td>Aliphatic-C₁ (1404 cm⁻¹)</td>
<td>−0.71 0.34  0.07 0.38</td>
<td></td>
</tr>
<tr>
<td>Aliphatic-C (1632 cm⁻¹)</td>
<td>−0.73 0.31  0.03 0.67</td>
<td></td>
</tr>
<tr>
<td>Aliphatic-C₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aliphatic symmetric stretch (2852 cm⁻¹)</td>
<td>−0.21 0.57  −0.09 0.14</td>
<td></td>
</tr>
<tr>
<td>Aliphatic asymmetric stretch (2922 cm⁻¹)</td>
<td>−0.42 0.55  −0.11 0.06</td>
<td></td>
</tr>
<tr>
<td>3396</td>
<td>0.24 0.06  0.00 0.45</td>
<td></td>
</tr>
<tr>
<td>3444</td>
<td>0.64 0.06  0.95 0.20</td>
<td></td>
</tr>
<tr>
<td>3526</td>
<td>0.47 0.25  0.93 0.09</td>
<td></td>
</tr>
<tr>
<td>3622</td>
<td>0.10 0.11  0.45 0.30</td>
<td></td>
</tr>
<tr>
<td>3699</td>
<td>−0.34 0.05  −0.37 0.37</td>
<td></td>
</tr>
<tr>
<td>TOC</td>
<td>−0.04 0.01  0.55 0.22</td>
<td></td>
</tr>
<tr>
<td>Hₑₑₑₑ</td>
<td>0.95 0.19  0.00 0.33</td>
<td></td>
</tr>
</tbody>
</table>

* Only variables with significant loadings (correlation coefficients > 0.45) were considered.
absorbance of aliphatic-C$_2$ was similar among all land uses, except for NT4 which had a higher peak intensity.

On average, absorbance of aromatic-C and aliphatic-C under NT than under CT was higher at both locations, which may have a positive impact on soil aggregation, through organo-mineral interactions, and because aliphatic-C acts as a binding agent between microaggregates. This function is essential for the stabilization of SOC within aggregates, and for long-term sequestration.

PCA was conducted using the data sets of aggregate size classes from both the PG and LRV sites. The PCA was followed by a discriminant analysis of principal components retained on the PCA, and loading values are shown as correlation coefficients in Table 2. Land uses and management practices were clearly separated into three groups (Fig. 7) at each location. At the PG site, the X-axis separates most of the inorganic peaks, TOC, aromatic-C, and aliphatic-C$_1$, and H$_{LIFS}$, and on the other additional inorganic peaks (538 and 1030 cm$^{-1}$) and aliphatic-C$_2$. Few inorganic peaks (i.e., 914, 1030, 3440, and 3526 cm$^{-1}$) were distinguished (loading value $>$ 0.45). TOC, aromatic-C and aliphatic-C$_1$ were negatively correlated with H$_{LIFS}$, but aromatic-C and aliphatic-C$_2$ were negatively correlated with O–H stretching vibrations of clay minerals (i.e., 3440, 3526, 3621 cm$^{-1}$), but aromatic-C and aliphatic-C$_2$ were negatively correlated with O–H stretching vibration of kaolinite at 3696 cm$^{-1}$ and gibbsite at 672 cm$^{-1}$. Lehmann et al. (2007) observed the same correlation between O–H stretching of clay minerals at 3620 cm$^{-1}$ and both aliphatic and aromatic C. Analysis of discriminant functions showed that the soils at both locations were clustered into three groups corresponding to the three main land-use and management practices, indicating that soils under NV, NT, and CT differed strongly in terms of the composition of organic compounds, and interactions between inorganic and organic compounds.

4. Conclusions

The data presented here support the conclusion that LIFS and FTIR are reliable methods to assess changes upon conversion to agricultural land use managed under conventional plow-base tillage — CT and no-till systems — NT. The degree of aromaticity was relatively higher in the top soil layers under CT than under NT and under native vegetation (NV), indicating that physical protection by aggregation was not sufficient to protect the most labile SOM fraction in CT. In addition, a general depletion of functional C groups was observed under CT. On average, a higher concentration of TOC in the whole soil sample, and in aggregate size classes, as well as greater intensity of functional C groups were observed under NT cropping systems. In addition, lower H$_{LIFS}$ under NT suggests that spatial inaccessibility by aggregates plays a major role in enhancing SOC accumulation rather than the selective preservation by aromaticity of SOM. These processes are essential to promote long-term carbon sequestration in soils. LIFS and FTIR are thus rapid, efficient, and precise techniques for analyzing the degree of SOC humification, functional C groups, and hence the efficiency of NT cropping systems to promote long-term carbon sequestration in soils.

Acknowledgments

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