

## Organic matter composition in density fractions of Cerrado Ferralsols as revealed by CPMAS <sup>13</sup>C NMR: Influence of pastureland, cropland and integrated crop-livestock



Madalena Boeni<sup>a</sup>, Cimélio Bayer<sup>b,\*</sup>, Jeferson Dieckow<sup>c</sup>, Paulo Cesar Conceição<sup>d</sup>, Deborah Pinheiro Dick<sup>e</sup>, Heike Knicker<sup>f</sup>, Júlio Cesar Salton<sup>g</sup>, Manuel Claudio Motta Macedo<sup>h</sup>

<sup>a</sup> Fundação Estadual de Pesquisa Agropecuária, Centro de Pesquisa de Sementes, Rua Estação Experimental, S/N, CEP 98130-970, Júlio de Castilhos, RS, Brazil

<sup>b</sup> Departamento de Solos, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

<sup>c</sup> Departamento de Solos e Engenharia Agrícola, Universidade Federal do Paraná, Curitiba, PR, Brazil

<sup>d</sup> Unidade Dois Vizinhos, Universidade Tecnológica Federal do Paraná, Dois Vizinhos, PR, Brazil

<sup>e</sup> Instituto de Química, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

<sup>f</sup> Instituto de Recursos Naturales y Agrobiología, Consejo Superior de Investigaciones Científicas, Sevilla, Spain

<sup>g</sup> Embrapa Agropecuária Oeste, Dourados, MS, Brazil

<sup>h</sup> Embrapa Gado de Corte, Campo Grande, MS, Brazil

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### ABSTRACT

Integrated crop-livestock (ICL) is a promising land use system for the Brazilian Cerrado, but little is known about what this system might change in chemical composition of soil organic matter. In three long-term experiments (9–11 years old), located on Cerrado Ferralsols in Dourados, Maracaju and Campo Grande (Mato Grosso do Sul State, Brazil), we assessed the impact of continuous cropland (CC), ICL, and permanent pasture of *Brachiaria decumbens* (PP) on the C concentration and composition of the free light fraction (FLF), occluded light fraction (OLF) and heavy fraction (HF) of soil in the 0–5 cm layer. CPMAS<sup>13</sup>C NMR spectroscopy was used to determine the percentage of alkyl, O-alkyl, aromatic and carboxyl C types. In Dourados and Maracaju, PP had the highest concentrations of organic C in whole soil and physical fractions, while ICL was intermediate and CC lowest. In Campo Grande, soil organic C concentration was similar among management systems. Distribution of organic C across physical fractions was not affected by management nor by experimental site, and on average the FLF, OLF and HF contained 7%, 26% and 67% of the total storage, respectively. Signal peaks of the four main C types appeared in all CPMAS<sup>13</sup>C NMR spectra, but at different intensities. O-alkyl was the major C type (about 50%), carboxyl was the minor representative (generally less than 10%) and alkyl and aromatic C were intermediates. From FLF to OLF, the alkyl and aromatic C concentrations increased, possibly due to selective preservation of waxes, resins, cutin, suberin and lignin. The HF had greater O-alkyl and lower aromatic C concentrations than OLF, which might have been related to the accumulation of microbial carbohydrates on mineral surfaces of the HF. Along the sequence CC–ICL–PP, the most evident changes were greater of O-alkyl and lower alkyl C types, practically in all fractions and sites. In FLF and OLF, these changes were attributed to greater biomass input and less seed drill-induced disturbance of soil surface (lower decomposition of residues) in the PP and ICL. Additionally, in OLF, greater O-alkyl concentration in PP and ICL was attributed to physical protection of particulate organic matter derived from grass roots occluded inside soil aggregates. Our results suggest that PP and ICL systems increased or maintained soil organic C concentrations compared to CC, associated with a qualitative increase of the chemically labile O-alkyl C type which was possibly related to greater biomass addition and less soil disturbance.

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### 1. Introduction

In the Brazilian Cerrado, most of the *Brachiaria* pasturelands have a certain degree of degradation, while most of the annual croplands are cropped with soybean. Hence the adoption of more sustainable land use systems in this region is crucial. The

\* Corresponding author at: Av. Bento Gonçalves, 7712, CEP: 91540-000, Porto Alegre, RS, Brazil. Tel.: +55 51 33086017; fax: +55 51 33086040.

E-mail addresses: [madaboeni@hotmail.com](mailto:madaboeni@hotmail.com) (M. Boeni), [cimelio.bayer@ufrgs.br](mailto:cimelio.bayer@ufrgs.br), [00038860@ufrgs.br](mailto:00038860@ufrgs.br) (C. Bayer).

integration of perennial grass pastures with annual cropping of grains one form of integrated crop-livestock system (ICL), which can reclaim ecological interactions that make agroecosystems more efficient at cycling nutrients, preserving natural resources, improving soil quality, and enhancing biodiversity (Franzluebbers et al., 2012).

Soil organic matter is an effective indicator of soil quality (Gregorich et al., 1994) and various studies have reported improvements in soil organic matter stocks under ICL (Salton, 2005; Jantalia et al., 2006). However, information pertaining to the chemical composition of organic matter in soil physical fractions after adoption of ICL is still scarce. Soil physical fractionation based on density separation may help in defining pools with different rates of decomposition and chemical characteristics; e.g. the lighter the fraction, the lower the decomposition degree (Christensen, 1992).

Land management not only controls organic carbon stocks (Neufeldt et al., 1999; Corazza et al., 1999; Bayer et al., 2006), but also the chemical composition of soil organic matter (Golchin et al., 1995a; Helfrich et al., 2006), and consequently, its stability and function. Significant influence of land management on the composition of the light organic fraction is commonly reported, since this fraction is regarded as the most sensitive to soil management (Gregorich et al., 1989; Gregorich and Janzen, 1996; Six et al., 1998). Physical protection of occluded organic matter is enhanced by agricultural systems that improve soil structure. Mineral-associated organic matter belongs to a more stable pool and is less affected by soil management, despite differences in the amount and nature of added plant residues (Christensen, 1992; Golchin et al., 1997).

Application of solid-state cross polarization magic angle spinning (CPMAS)  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy to soils has increased our understanding of the chemical structure of soil organic matter (Golchin et al., 1995a).  $^{13}\text{C}$  NMR provides a first approximation of the relative abundance of organic C functional groups (alkyl, O-alkyl, aromatic and carboxyl), which can be used as indicators for different compounds.

To investigate the complex interactions between agricultural management practices and soil organic matter quality, CPMAS  $^{13}\text{C}$  NMR spectroscopy was used to determine the chemical composition of organic materials in soil fractions obtained by density fractionation of three Brazilian Cerrado Ferralsols exposed to different agricultural management. Our objective was to determine the effect of agricultural systems (continuous cropland; integrated crop-livestock and permanent pasture) on the chemical composition of free, occluded and heavy fractions isolated from soils.

## 2. Materials and methods

### 2.1. Study sites and soil sampling

Three long-term experiments in the Brazilian Cerrado (Dourados, Maracaju and Campo Grande, all in Mato Grosso do Sul State) were evaluated (Table 1). We selected three management systems from each experiment: continuous cropland under no-tillage (CC); integrated crop-livestock system under no-tillage (ICL), and permanent pasture of *Brachiaria decumbens* (PP). In Campo Grande, permanent pasture of *B. decumbens* plus *Stylosanthes guianensis* (PP+L) was also included. Experiments were arranged in a randomized complete block design, with three field replicates. Characteristics of the experimental sites and selected treatments are presented in Table 1.

Soil samples from the 0 to 5 cm layer were collected in April 2004 at three sampling points per plot. Samples were collected with a spatula, as undisturbed blocks of about 20 (l)  $\times$  10 (w)  $\times$  5 cm (h) that were gently broken into aggregates <9.51 mm by hand. These aggregates were air dried and stored in plastic pots. For physical

fractionation and C analysis, samples of the three sampling points were composited. We sampled and analyzed only the 0–5 cm layer, because it was considered more sensitive, quantitatively and qualitatively, to management changes under no-tillage than deeper layers (Dieckow et al., 2005); and also because spectroscopic analysis is costly.

### 2.2. Soil physical fractionation

Three physical fractions were obtained with density separation: free light fraction (FLF), which is the particulate organic matter between soil aggregates; occluded light fraction (OLF), which is the particulate organic matter inside aggregates; and heavy fraction (HF), which is associated with mineral surfaces. Briefly, about 10 g of soil aggregates were placed into a 100 ml tube containing 80 ml of sodium polytungstate solution (SPT) (Sometu, Berlin, Germany) adjusted to a density of  $2.0\text{Mg m}^{-3}$ . The tube was stopped and gently inverted five times to release the FLF. The suspension was then centrifuged at  $3630 \times g$  for 90 min and the supernatant with the floating FLF was poured into a funnel and filtered (Whatman GF-3) under vacuum. The FLF retained on the filter was washed with distilled water to remove excess SPT, dried at  $50^\circ\text{C}$  for 24 h, weighed and ground in mortar.

The SPT solution was returned to the tube containing the aggregates and the whole suspension was sonicated (Vibracel VC 750) at 411, 536 and  $809\text{J ml}^{-1}$  for Dourados, Maracaju and Campo Grande soils, respectively. The sonication energy was established previously in calibration tests to obtain a dispersion of 99% of soil particles. After sonication, the suspension was centrifuged and filtered, and the OLF was recovered, washed, dried, weighed and ground as described above.

The soil at the bottom of the tube was transferred into a 250 ml polyethylene bottle and washed with distilled water to remove excess SPT. The bottle was closed and the solution manually shaken to disperse the soil pellet and centrifuged at  $4460 \times g$  for 30 min. This procedure was repeated three times and the obtained HF in the pellet was dried to  $50^\circ\text{C}$  for 24 h, weighted and grounded in mortar.

### 2.3. Carbon analysis

Samples of the whole soil were analyzed by dry combustion (Shimadzu TOC-VCSH) for determination of total organic carbon (TOC) concentration. Similarly, the organic carbon (OC) concentration in the three physical fractions was determined. Considering TOC of whole soil and OC of density fractions, recovery of OC after fractionation was calculated and it ranged from 80% to 100%, with an average of 85%.

### 2.4. Chemical pre-treatment of physical fraction samples

Before being analyzed by CPMAS  $^{13}\text{C}$  NMR technique, samples of each physical fraction were subjected to chemical pre-treatments to improve spectra quality. The HF was pre-treated with 10% hydrofluoric acid solution (v/v) to remove paramagnetic  $\text{Fe}^{3+}$  (iron oxides), which can interfere with the spectra signal, and to concentrate OC, thus improving the signal-to-noise ratio of spectra. Based on the procedure described by Gonçalves et al. (2003), about 5 g HF sample was weighed into a 250 mL plastic bottle, 200 mL of hydrofluoric acid solution was added, and after 7 days of rotary shaking the suspension, it was centrifuged at  $3000 \times g$  for 20 min and the supernatant discarded. The treated HF sample was washed with distilled water (three times, with centrifugation) and dried at  $50^\circ\text{C}$ .

The OLF was also pre-treated with hydrofluoric acid to remove interference of residual soil particles rich in iron oxides adhering on

**Table 1**  
Characteristics of the three long-term experiments (Dourados, Maracaju and Campo Grande) used in the study.

Characteristic	Dourados	Maracaju	Campo Grande
Institution	Embrapa Agropecuária Oeste	Fundação MS	Embrapa Gado de Corte
Coordinates	22°13'16"S 54°48'20"W	21°36'52"S 55°10'06"W	20°26'34"S 54°38'47"W
Altitude	430 m	384 m	532 m
Relief	Flat to gently sloping	Flat	Flat
Soil type (IUSS) <sup>a</sup>	Ferralsol	Ferralsol	Ferralsol
Soil type (Brazilian system) <sup>b</sup>	Latossolo Vermelho distroférico típico	Latossolo Vermelho distroférico típico	Latossolo Vermelho distrófico
Soil texture	Clayey	Clayey	Sandy clay
Clay/silt/sand (g kg <sup>-1</sup> )	630/210/160	540/190/270	360/130/510
Fe <sub>o</sub> /Fe <sub>d</sub> <sup>c</sup>	0.03	0.03	0.03
Gt/(Gt + Hm) <sup>d</sup>	0.11	0.08	0.33
Gb/(Gb + Kt) <sup>e</sup>	0.04	0.22	0.47
Mean annual temperature	23.0 °C	23.0 °C	23.0 °C
Mean annual rainfall <sup>f</sup>	1635 mm	1800 mm	1527 mm
Native vegetation	Cerrado (campo cerrado)	Cerrado (campo cerrado)	Cerrado (stricto sensu)
Management before the experiment	20 years of annual cropland with conventional tillage	1 year of annual cropland with conventional tillage	20 years of permanent <i>Brachiaria</i> pasture (degraded at the end)
Experiment establishment	1995	1993	1993
Experiment duration	9 years	11 years	11 years
Plot dimensions	50 × 400 m	30 × 60 m	50 × 140 m
Treatment characteristics			
Continuous cropland (CC)	Soybean ( <i>Glycine max</i> ) or maize ( <i>Zea mays</i> ) in summer. Oat ( <i>Avena sativa</i> ), or wheat ( <i>Triticum aestivum</i> ) or radish ( <i>Raphanus sativus</i> ) in winter	Soybean in summer and oat in winter	Soybean in summer and pearl millet ( <i>Pennisetum specatum</i> ) in winter
Integrated crop-livestock (ICL)	Soybean in summer and oat in winter for 2 years, followed by 2 years of <i>Brachiaria decumbens</i> pasture	Soybean in summer and oat in winter for 2 years, followed by 2 years of <i>Brachiaria decumbens</i> pasture	Soybean in summer and pearl millet in winter for 1 year, followed by 3 years <i>Brachiaria brizantha</i> pasture
Permanent pasture (PP)	Permanent <i>Brachiaria decumbens</i> pasture	Permanent <i>Brachiaria decumbens</i> pasture	Permanent <i>Brachiaria decumbens</i> pasture.
Permanent pasture plus legume (PP + L)	No	No	Permanent pasture of <i>Brachiaria decumbens</i> plus <i>Stylosanthes guianensis</i>

<sup>a</sup> IUSS (2006).

<sup>b</sup> Embrapa (2006).

<sup>c</sup> Ratio between the concentration of iron extracted with ammonium oxalate (Fe<sub>o</sub>) and iron extracted with dithionite-citrate-bicarbonate (Fe<sub>d</sub>).

<sup>d</sup> Ratio between the concentration of Goethite (Gt) and Goethite plus Hematite (Hm).

<sup>e</sup> Ratio between the concentration of Gibbsite (Gb) and (Gibbsite plus Kaolinite (Kt)).

<sup>f</sup> Concentrated mainly in summer.

organic fragments. Approximately 0.3 g of OLF was weighed into a 40 ml plastic bottle, mixed with 20 ml of hydrofluoric acid solution (10%, v/v), horizontally shaken for 3 h and centrifuged at 3000 × g for 10 min. The supernatant was discarded and the residue resubmitted to two additional acid treatment cycles. At the end, samples were washed five times with distilled water and freeze dried.

The FLF also contained adhering residual soil particles, but less than in OLF. The FLF was pre-treated with sodium dithionite (Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>). About 0.5 g of FLF was weighed into a 40 ml polyethylene bottle and 1.50 g of sodium dithionite plus 20 ml distilled water were added. After 16 h shaking, samples were centrifuged at 3000 × g for 10 min and the supernatant discarded. Samples were washed with distilled water three times and freeze dried.

No changes in the organic matter composition of the physical fractions were expected to occur due to pre-treatments, because the C:N ratios of samples were practically the same before and after treatment (results not shown). This same result has already been evidenced elsewhere (Schmidt et al., 1997; Gonçalves et al., 2003).

### 2.5. CPMAS <sup>13</sup>C NMR spectroscopic analyses

Samples of physical fractions pre-treated with hydrofluoric acid were analyzed by solid state CPMAS <sup>13</sup>C NMR spectroscopy in a Bruker DSX 200 spectrometer. Dry powdered samples were packed in a 7 mm diameter cylindrical zirconium rotor operating at a <sup>13</sup>C resonance frequency of 50.3 MHz. The cross-polarization technique was applied during magic angle spinning of the rotor at 6.8 kHz. The experiment was performed with a contact time of 1 ms, to 90° <sup>1</sup>H pulse width of 6.6 μs and a pulse delay between 200 and 300 ms, sufficient for soil organic matter (Knicker, 2011). After Fourier

transformation, a line broadening of 100 Hz was applied on spectra. The <sup>13</sup>C chemical shift was given relative to tetramethylsilane (reference 0 ppm) and was adjusted with glycine (176.04 ppm). Total signal intensity and proportion contributed by each type of C was quantified through integration of signals in its corresponding chemical shift regions using an integration routine of the spectrometer supplied with the instrument software. Spectra obtained were subdivided into four major chemical shift regions, assignable to aliphatic or alkyl C (0–45 ppm) of lipids, fatty acids and plant aliphatic polymers; O/N-alkyl C (45–110 ppm) derived primarily from polysaccharides (cellulose and hemicelluloses), but also from proteins and side chains of lignin; aromatic or aryl C (110–160 ppm) derived from lignin and/or protein; and finally, carbonyl C (160–220 ppm) from aliphatic esters, carboxyl groups and amide carbonyls. Considering the adopted parameters, integration of peak areas in each chemical shift region enables the estimation of relative C contents expressed as percentage of the total area (Knicker, 2011).

## 3. Results and discussion

### 3.1. Carbon concentration and distribution in physical fractions

In Dourados and Maracaju, soil under PP had the highest concentrations of TOC and OC in physical fractions, while ICL had intermediate concentrations, and CC had lowest concentrations (Table 2). These result indicate the benefits of *Brachiaria* pasture at improving organic matter, either as PP or intermittently included in the rotation scheme of ICL, as compared to CC (Salton, 2005; Jantalia et al., 2006). In Campo Grande, *Stylosanthes* (PP + L) further

**Table 2**

Organic carbon concentration in whole soil (TOC) and in free light fraction (FLF), occluded light fraction (OLF) and heavy fraction (HF) of the 0–5 cm layer of Ferralsols from three sites of the Brazilian Cerrado (Dourados, Maracaju and Campo Grande), subjected to continuous crop (CC), integrated crop livestock (ICL), permanent pasture (PP) or permanent pasture plus *Stylosanthes* legume (PP+L).

Site	Soil use system	TOC (g kg <sup>-1</sup> of soil)	OC recovered <sup>a</sup> (g kg <sup>-1</sup> of soil)	FLF (g kg <sup>-1</sup> of soil)	OLF (g kg <sup>-1</sup> of soil)	HF (g kg <sup>-1</sup> of soil)
Dourados	CC	18.2 ± 0.9 <sup>c</sup>	15.3	1.0 ± 0.2	(7) <sup>b</sup> 3.9 ± 0.1	(25) 10.4 ± 0.6
	ICL	24.5 ± 0.9	19.5	1.4 ± 0.2	(7) 6.5 ± 0.5	(33) 11.6 ± 0.1
	PP	30.5 ± 2.5	24.6	2.0 ± 0.6	(8) 7.6 ± 0.4	(31) 15.0 ± 1.6
Maracaju	CC	22.4 ± 0.7	18.9	0.6 ± 0.2	(3) 4.3 ± 0.1	(23) 14.0 ± 0.4
	ICL	30.0 ± 0.6	24.2	1.1 ± 0.1	(4) 5.7 ± 0.2	(24) 17.4 ± 0.8
	PP	35.7 ± 0.2	29.4	1.9 ± 0.1	(7) 6.1 ± 0.8	(21) 21.4 ± 0.9
Campo Grande	CC	25.2 ± 1.6	21.1	3.2 ± 0.5	(15) 5.3 ± 0.5	(25) 12.6 ± 0.8
	ICL	21.9 ± 1.9	21.9	2.0 ± 0.2	(9) 6.0 ± 0.6	(27) 13.9 ± 1.3
	PP	22.4 ± 0.3	19.3	1.2 ± 0.2	(6) 4.6 ± 0.3	(24) 13.5 ± 0.5
	PP+L	27.8 ± 0.4	25.0	2.1 ± 0.2	(8) 7.4 ± 0.3	(30) 15.5 ± 0.2

<sup>a</sup> Amount of organic carbon recovered after the physical fractionation (sum of FLF, OLF and HF).

<sup>b</sup> Number in parenthesis represents the percentage of the recovered OC.

<sup>c</sup> Mean ± standard error deviation.

improved TOC concentration under PP, showing the benefits of legumes to C accumulation in pasture soils (Table 2). Contrary to that observed in Dourados and Maracaju, soil under CC unexpectedly had greater TOC than PP and ICL (Table 2). This might have been related to the fact that the winter cover crop used in CC of Campo Grande was millet (*Pennisetum glaucum*), whose phytomass yield was greater than that of oat (*Avena sativa*), used in Dourados and Maracaju (4.2–8.4 Mg DM ha<sup>-1</sup> year<sup>-1</sup> vs. 2.7–4.1 Mg DM ha<sup>-1</sup> year<sup>-1</sup>).

Distribution of OC across physical fractions was not affected by management system or by site, where in FLF, OLF and HF averaged 7%, 26% and 67% of TOC, respectively (Table 2). The HF was the largest compartment, and that was mainly because of organomineral interactions in this fraction (Kögel-Knabner et al., 2008). The OLF, although storing less C, plays very relevant roles in terms of aggregate stability, biological activity and in serving as a transitory fraction of organic matter before being ultimately stabilized in HF (Conceição et al., 2013).

### 3.2. Chemical composition of organic matter in physical fractions

NMR spectra of all physical fractions, from all management systems and experimental sites were rather similar (Fig. 1). Signal peaks of the four main C types (alkyl, O-alkyl, aromatic and carboxylic) appeared in all spectra, but at different intensities, which allowed distinction among physical fractions and management systems. O-alkyl was the major C type in all samples and represented around half of TOC (Table 3). Carboxyl was the minor representative, rarely surpassing 10%, and alkyl and aromatic C were intermediates.

From FLF to OLF, alkyl and aromatic C components increased in concentration at the expense of a decrease in O-alkyl C (Table 3). As a result, hydrophobicity index (HI) as a measure of chemical recalcitrance increased considerably in OLF. Both FLF and OLF consist of particulate organic matter derived from plant material, but the compositional difference between them is associated with the selective preservation of more recalcitrant compounds rich in alkyl (waxes, resins, cutin and suberin) and aromatic C (lignin), along with preferential degradation of O-alkyl C of polysaccharides (Baldock et al., 1992; Zech et al., 1997; Baldock and Preston, 1995) during the conversion of FLF to OLF. The OLF has usually been considered in a more advanced decomposition degree compared to FLF (Freixo et al., 2002), and entrapped in sites partially inaccessible to microbial and enzymatic attack (Christensen, 1992; Golchin et al., 1994, 1997).

Compared to OLF, the HF had greater O-alkyl and lower aromatic C concentrations, and thus lower HI (Table 3). Helfrich

et al. (2006) also found that HF contained a higher proportion of O-alkyl C than OLF. This result supports interpretations that microbial synthesized metabolites, including carbohydrates (O-alkyl), accumulate in the clay-sized fraction (mineral associated organic matter) (Guggenberger et al., 1994), where they are possibly stabilized by adsorption on mineral surfaces (Baldock et al., 1997; Golchin et al., 1997). Since aromatics, are not produced by microorganisms, but inherited from plant material, they logically have lower concentration in HF than in OLF.

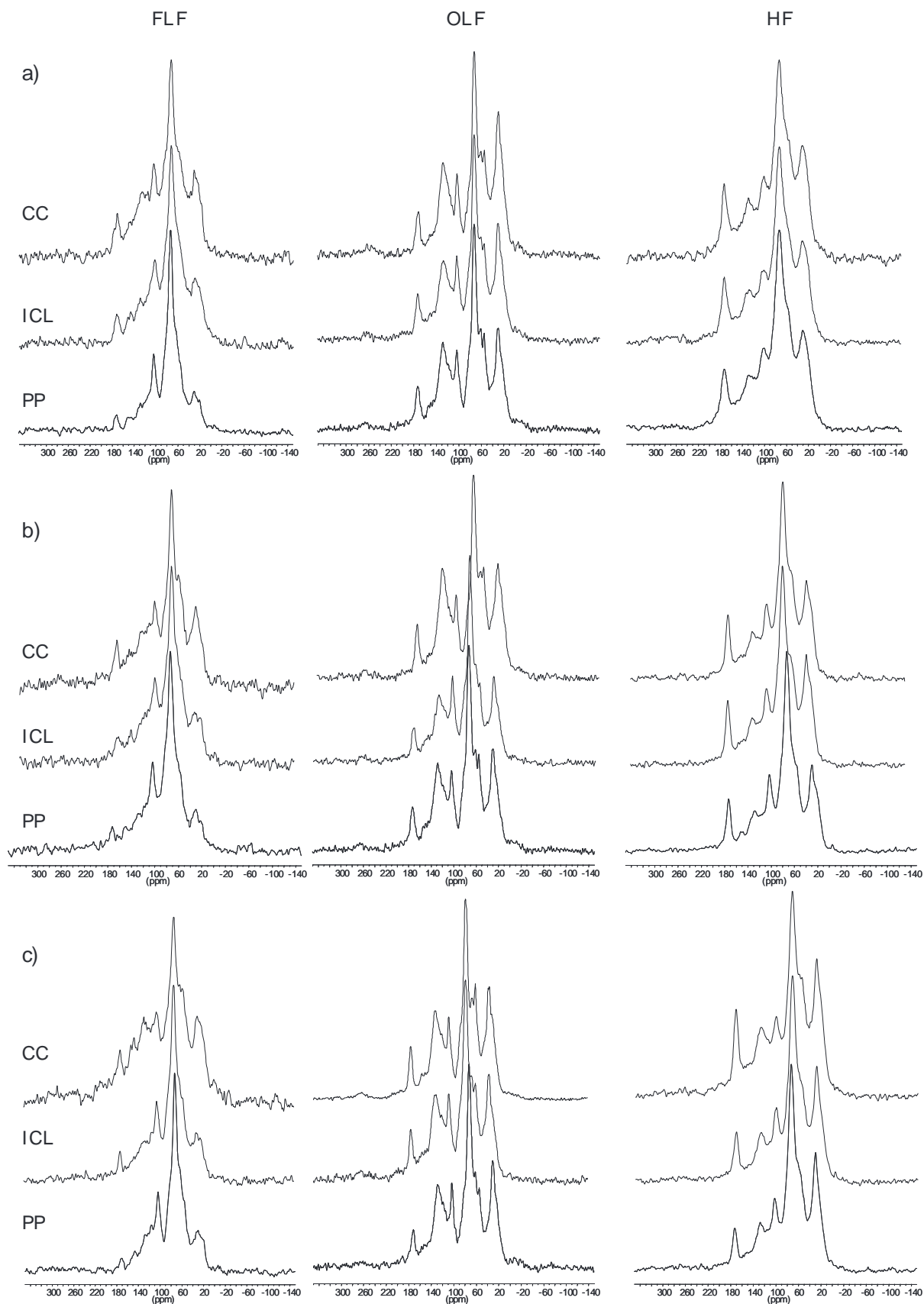
An interesting finding was that in HF, where turnover of organic matter is supposedly slower, there was large proportion of O-alkyl C, which is not as chemically recalcitrant as other C types. This suggests that chemical recalcitrance was not the major stabilization mechanism of organic matter in the HF, but possibly organomineral interaction, supporting conclusions of previous studies (Marschner et al., 2008; Kögel-Knabner et al., 2008).

### 3.3. Compositional changes due to management system

Along the sequence CC–ICL–PP, the most evident changes were greater O-alkyl and lower alkyl C types, practically in all fractions and sites (Table 3). In the FLF, considered an early stage of soil organic matter, input from greater biomass of pasture based systems than CC was logical. As more biomass is added, more FLF is enriched with plant derived carbohydrates (O-alkyl) and depleted in other C types (Kinchesh et al., 1995). Another part of these management induced changes might be attributed to greater decomposition in CC. In this system, even under no-tillage, the disturbance promoted by the seed drill in the first few centimetres of soil, at least twice a year, may have accelerated the decomposition of particulate organic matter compared to systems where the soil surface is rarely disturbed, as in PP.

The same management induced responses would be true for the OLF, which also consists of particulate organic matter that depends upon the amount of biomass added to soil. However, the extensive root system of *Brachiaria* certainly improved soil aggregation status (Tisdall and Oades, 1980, 1982; Silva and Mielniczuk, 1997; Paladini and Mielniczuk, 1991; Neufeldt et al., 1999; Salton, 2005) and, consequently, the capacity of soil to physically protect particulate organic matter. Aggregation was likely another important factor that led to higher O-alkyl concentration in the occluded fraction of PP and ICL.

The HF showed the same trend of difference among CC, ICL and PP. Besides the effect of different biomass inputs and aggregation status that affect particulate organic matter, changes in HF might also have been related to changes in microbial community across management systems. Soil under PP would have supposedly a more



**Fig. 1.** CPMAS  $^{13}\text{C}$  NMR spectra of the free light fraction (FLF), occluded light fraction (OLF) and heavy fraction (HF) of the 0–5 cm layer of Ferralsols from (a) Dourados, (b) Maracaju and (c) Campo Grande, in the Brazilian Cerrado region, subjected to continuous crop (CC), integrated crop livestock (ICL) and permanent pasture (PP).

**Table 3**

Relative contribution of carbon types in the chemical shift regions of the CPMAS  $^{13}\text{C}$  NMR spectra of the free light fraction (FLF), occluded light fraction (OLF) and heavy fraction (HF) of the 0–5 cm layer of Ferralsols from three sites of the Brazilian Cerrado (Dourados, Maracaju and Campo Grande), subjected to continuous crop (CC), integrated crop livestock (ICL), permanent pasture (PP) or permanent pasture plus *Stylosanthes* legume (PP+L).

Fraction	Soil	Agricultural system	Carboxyl C (220–160 ppm) (%)	Aromatic C (160–110 ppm) (%)	O-alkyl C (110–45 ppm) (%)	Alkyl C (45–0 ppm) (%)	HI <sup>a</sup>	
FLF	Dourados	CC	6.9 ± 0.4 <sup>b</sup>	21.7 ± 1.0	53.5 ± 1.1	17.9 ± 0.8	0.66	
		ICL	6.3 ± 0.3	18.5 ± 0.9	57.8 ± 1.1	17.4 ± 0.8	0.56	
		PP	6.2 ± 0.3	17.6 ± 0.8	62.8 ± 1.2	13.4 ± 0.6	0.45	
	Maracaju	CC	10.1 ± 1.4	22.6 ± 0.5	51.4 ± 0.5	15.9 ± 1.1	0.63	
		ICL	7.8 ± 1.1	20.2 ± 0.5	58.2 ± 0.5	13.7 ± 1.0	0.51	
		PP	7.4 ± 1.0	18.4 ± 0.4	61.9 ± 0.6	12.3 ± 1.0	0.44	
	Campo Grande	CC	12.7 ± 1.6	27.3 ± 1.4	44.1 ± 1.9	16.0 ± 1.4	0.76	
		ICL	7.8 ± 1.0	19.5 ± 1.0	58.9 ± 2.6	13.8 ± 1.2	0.50	
		PP	7.4 ± 0.9	20.5 ± 1.1	60.0 ± 2.6	12.2 ± 1.1	0.49	
			PP+L	6.5 ± 0.8	19.1 ± 1.0	58.5 ± 2.6	15.9 ± 1.4	0.54
	OLF	Dourados	CC	6.3 <sup>c</sup>	23.8	45.3	24.6	0.94
			ICL	7.9	23.3	47.8	21.0	0.80
PP			7.1	21.0	52.2	19.8	0.69	
Maracaju		CC	7.1	26.5	45.4	21.0	0.90	
		ICL	7.6	25.2	48.2	19.0	0.79	
		PP	7.3	23.0	52.3	17.3	0.68	
Campo Grande		CC	7.3	24.2	47.7	20.8	0.82	
		ICL	9.1	25.3	47.6	18.0	0.76	
		PP	8.1	25.7	47.7	18.4	0.79	
			PP+L	7.9	20.4	52.7	19.0	0.65
HF		Dourados	CC	10.6 ± 0.5	17.2 ± 0.2	50.3 ± 0.2	21.9 ± 0.4	0.64
			ICL	9.9 ± 0.4	16.7 ± 0.2	51.7 ± 0.2	21.7 ± 0.4	0.62
	PP		9.6 ± 0.4	16.4 ± 0.2	53.7 ± 0.2	20.3 ± 0.4	0.58	
	Maracaju	CC	9.9 ± 0.5	15.4 ± 0.9	53.3 ± 1.3	21.3 ± 0.4	0.58	
		ICL	9.5 ± 0.5	15.4 ± 0.9	53.6 ± 1.3	21.6 ± 0.4	0.59	
		PP	8.8 ± 0.5	14.8 ± 0.8	57.5 ± 1.4	18.9 ± 0.4	0.51	
	Campo Grande	CC	10.2 ± 1.0	17.8 ± 0.8	50.5 ± 1.8	21.5 ± 0.1	0.65	
		ICL	8.0 ± 0.7	15.1 ± 0.7	54.8 ± 2.0	22.1 ± 0.1	0.59	
		PP	7.7 ± 0.7	15.5 ± 0.7	55.0 ± 2.0	21.8 ± 0.1	0.59	
			PP+L	8.9 ± 0.8	15.7 ± 0.8	53.1 ± 1.9	22.3 ± 0.1	0.61

<sup>a</sup> Hydrophobicity index = (alkyl + aromatic)/(O-alkyl + carboxyl). According to Spaccini et al. (2002).

<sup>b</sup> Mean ± standard error deviation.

<sup>c</sup> Values of one repetition per treatment.

stabilized and adapted microbial community than under intermittent or continuous annual cropping, which could possibly affect the quality of biochemical products deposited in the HF. Differences in microbial composition between grassland and annually cropped soils were reported by Steenwerth et al. (2003). In Southern Brazil, enzymatic activities in grassland were greater than in cropland soil, suggesting distinct microbial communities between systems (Schmitz, 2003).

Chemical composition in HF was not as different as in FLF or OLF, possibly because changes in microbial community and its metabolites are not as fluctuating as the amount of plant biomass added to soil (Oades et al., 1988; Golchin et al., 1995b). Previous studies have already shown that changes in composition are more evident in particulate organic matter fractions than in mineral associated organic matter following shifts in soil management (Oades et al., 1988; Dieckow et al., 2005).

Concentration of aromatic C, was clearly greater in CC than in pasture based systems, but between PP and ICL there was no difference (Table 3). Highest concentration of aromatic C in CC was associated with lowest concentration of O-alkyl C. With lower biomass input in CC, more recalcitrant compounds like aromatics tend to predominate compared to labile ones as O-alkyl C, even in the HF. This can be referred as selective utilization of O-alkyl C (Baldock and Skjemstad, 2000). Helfrich et al. (2006) also reported increase in aromaticity of HF due to organic matter mineralization promoted by soil cultivation.

In Dourados and Maracaju, highest HI occurred in CC, lowest in PP and intermediate in ICL, which is consistent with the predominance of more recalcitrant compounds in the lower input system

of CC (Table 3). In Campo Grande, CC also had the highest HI, but no clear trend was observed between ICL and PP.

The inclusion of *Stylosanthes* in the permanent pasture of Campo Grande (PP+L) changed the composition of FLF and OLF. Compared to PP, the PP+L system had greater proportion of alkyl C in FLF (Table 3). Two possible reasons might be the high content of fatty acids derived from cutin and suberin, rich in alkyl C, of leaves and stems of *Stylosanthes*; and high content of peptide (methyl C), of *Stylosanthes*.

#### 4. Conclusions

Physical fractions of soil organic matter contained different quantities of organic C and different proportions of C types. The heavy fraction was the major compartment of soil organic C and about half of it consisted of the chemically labile O-alkyl C type. The reason why this fraction, which supposedly has the lowest turnover rate of organic matter, contained such significant amount of O-alkyl C is likely the interaction (adsorption) of organic matter on mineral surfaces, a process that minimizes microbial attack and decomposition.

The permanent pasture of *Brachiaria* and the integrated crop livestock systems had greater soil organic C concentrations compared to the continuous crop system in 2 of 3 sites evaluated. This quantitative difference was accompanied by a qualitative shift to the chemically labile O-alkyl C type, a result that was likely due to high biomass addition and less soil disturbance in pasture based systems.

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