Alterations in Molecular Composition of Humic Substances from Eucalypt Plantation Soils Assessed by ^{13}C-NMR Spectroscopy

Emanuelle Mercês Barros Soares
Ivo Ribeiro Silva*
Roberto Ferreira de Novais
Soil Dep.
Federal Univ. of Viçosa
Viçosa, MG. 36570-000
Brazil

Yan-Yan Hu
Klaus Schmidt-Rohr
Dep. of Chemistry
Iowa State Univ.
Ames, IA

Land-use changes with natural vegetation removal may impact the quantity and quality of humic substances (HS), including their molecular nature. Nuclear magnetic resonance (NMR) experiments were used in the present study to evaluate the alterations in the molecular composition of fulvic (FA) and humic (HA) acids from soils under eucalypt plantations in three major biomes in Brazil: Atlantic Forest, Cerrado, and Grassland. The major NMR-identifiable components in these HS were aromatics including aromatic C-O, COO/NC = O groups, peptides, carbohydrates, lignin-derived moieties and nonpolar alkyls. In all biomes the dipolar dephasing technique indicated the presence of significant amounts of condensed aromatic C, possibly inherited from charred materials derived from natural and anthropogenic fires in the region. The nonpolar alkyl C to O-alkyl C ratio averaged 1.4 for HA and 1.1 for FA. Humic substances from eucalypt soils showed greater contribution of nonpolar alkyl groups and a smaller abundance of O-alkyl groups in comparison to the native vegetation soil. Sugarcane (*Saccharum officinarum* L.) cultivation increased HA aromatics in comparison to those from the native Atlantic Forest soil, but when sugarcane was substituted by eucalypt the aromatics decreased and O-alkyl C recovered in HA and FA. There was evidence of greater contribution of lignin-derived C for HA and FA in sites planted with *Brachiaria* spp. pastures. Except for the HA from one Cerrado soil (Itacambira), aromaticity of HA decreased following planting to eucalypt. These changes in HS molecular composition across biomes may have impact on soil organic matter processes and they should be taken into account in future studies.

Abbreviations: A/OA, alquil C/O alquil C ratio; CP, cross-polarization; CP/MAS, cross-polarization magic-angle spinning; CP/T1, cross-polarization spin-lattice relaxation time; CSA, chemical shift anisotropy; DP/MAS, direct-polarization magic-angle spinning; HA, humic acids; HS, humic substances; IHSS, International Humic Substances Society; FA, fulvic acids; NMR, nuclear magnetic resonance; NV, Native vegetation; SOM, soil organic matter; TOSS, total sideband suppression.

Humic substances represent the largest fraction of soil organic matter (SOM) and alterations in their quality will affect several soil processes ranging from nutrient to pesticide retention to aggregation and C sequestration (Stevenson, 1994). Land-use changes with natural vegetation substitution may impact not only HS stocks, but also their molecular nature, and hence their quality. Given the complexity and heterogeneity of the HS components, changes in molecular composition cannot be elucidated by routine methods such as elemental analysis.

Among several techniques available to look into HS molecular composition, nuclear magnetic resonance (NMR) spectroscopy has gained substantial attention. In the past, most NMR-based studies of SOM have focused on temperate soils

(Hatcher et al., 1981; Preston et al., 1994; Guggenberger et al., 1995; Rumpel et al., 2004; Mao et al., 2008). Only in more recent years have NMR techniques also been used to look into the effects of vegetation cover, land use change, cropping, fertilization, soil texture and depth, on SOM composition of tropical and subtropical soils in Brazil (Bayer et al., 2000; González-Pérez et al., 2004; Alcântara et al., 2004; Dick et al., 2005; Dieckow et al., 2005, 2009).

Although climate has been pointed out as an important factor regulating Brazilian SOM dynamics (Lima et al., 2006) and quality, Dieckow et al. (2009) found no clear pattern for changes in SOM composition between soils from subtropical (Grassland) and tropical (Cerrado) biomes. Some studies have shown that the SOM composition is changed when the native vegetation is replaced. For example, results of NMR experiments pointed out a recovery of the O-alkyl C, and a decrease in aromatic C in soils converted from conventional to no-tillage management in Brazil (Dieckow et al., 2009). However, the impact of distinct crop rotations and N fertilization on SOM molecular composition cannot always be observed by cross polarization magic angle spinning (CP/MAS) ^{13}CNMR spectroscopy (Dieckow et al., 2005).

Carbon-13 NMR spectroscopy has been frequently used on SOM research in the last decades, and despite the widespread use of CP/MAS, its limitations in quantitative studies have become apparent (Wilson, 1987; Preston, 1996; Mao et al., 2000). An attractive alternative to overcome the major drawbacks of CP/MAS, such as underestimation of the sp^2- to sp^3-C ratio, is direct polarization magic angle spinning (DP/MAS) (Skjemstad et al., 1999; Smernik and Oades, 2000; Mao and Schmidt-Rohr, 2004a; Knicker et al., 2005; Kaal et al., 2008). Aromaticity estimated based on routine ^{13}C-NMR either includes some alkyl C signals, namely those of O-C-O groups, or excludes some aromatics C signals, as the signals of alkyl O-C-O and aromatic carbons overlap between 90 and 120 ppm (Mao and Schmidt-Rohr, 2004a). Therefore, to obtain a quantitative aromatic fraction, overlapping signals of O-alkyl and aromatic C have to be separated, which can be achieved by using a ^{13}C chemical shift anisotropy (CSA) filter (Mao and Schmidt-Rohr, 2004b).

Large areas of planted pastures across different biomes in Brazil are being replaced by short-rotation eucalypt plantations, but the impact on SOM composition is largely unknown. Thus, in the present study, NMR approaches such as ^{13}C DP/MAS, ^{13}C CP/TOSS, CSA filtering, and spectral editing were used: (i) to identify the major components of HA and FA from soils from three major biomes in Brazil (Atlantic Forest, Grassland, and Cerrado and (ii) to evaluate the impact of short-rotation eucalypt cultivation on molecular composition of HA and FA from soils from the three distinct biomes.

MATERIALS AND METHODS
Site Description and Soil Sampling

The study involved a qualitative assessment of the humic and fulvic acids, and the alterations that occurred after eucalypt establishment, from Brazilian soils under Atlantic Forest, the savanna-like Cerrado, and Grassland. These biomes are where short-rotation eucalypt forestry is concentrated and are, respectively, the second, fifth and third largest in extent in Brazil. Within each biome, representative areas that have both commercial eucalypt plantations and adjacent primary native vegetation (NV) were selected. Two sites were selected for sampling within both Atlantic Forest and Cerrado biomes, and one site was selected in the Grassland biome. In the latter biome the planting of short-rotation eucalypt is more recent, and thus few areas with a reasonable period of eucalypt forestry were available for sampling. The characteristics of the studied areas are summarized below (Table 1).

Atlantic Forest. This biome was sampled at two sites located in Espirito Santo State, near São Mateus, Brazil. The first (Sooretama) is situated at 19°11′21.16″ S and 40°07′41.53″ W, is 36 m above sea level (m.a.s.l.), and the eucalyptus stand was 7 yr- old while the second (Montanha) is situated at 18°7′34.52″ S and 40°21′58.29″ W, is 50 m.a.s.l., and was 8 yr old. Because of the gradient in rainfall from the Brazilian Atlantic coast toward inland areas in the region studied, a more lush perennial Atlantic Forest occurs closer to the coast (Sooretama) than in the semideciduous Atlantic forest about 80 km inland in north-northwestern Espírito Santo State (seasonally dry Montanha Atlantic Forest). Since eucalypt was introduced after the sugarcane plantation at Montanha, and in Sooretama planted *Brachiaria* spp. pasture is a dominant alternative land use, soils from adjacent long-term sugarcane (21 yr) and planted pasture (27 yr) fields were also sampled.

Cerrado. Two sites were sampled in the Cerrado biome and both are located in Minas Gerais state, the state with the largest eucalypt plantation area in Brazil. The first site is located in Itacambira County and is situated at 17° 5′8.92″ S and 43°18′35.29″ W at 1048 m.a.s.l and the stand was 7 yr old. This site is on a large flat plateau, and the vegetation is dominated by shrubs with a small proportion of grassy species, locally called *senso strictu* Cerrado. The dry season spans from April to September in the Cerrado but the winters are cooler and the dry season is longer in Itacambira than at the second site in Curvelo County. The Curvelo site, at 18°44′57.00″ S and 44°26′48.32″ W at 633 m.a.s.l. and was also 7 yr old. The Cerrado in Curvelo is taller and denser and is called Cerradão. There are virtually no grasses present at the Curvelo site.

Grassland. A site was sampled in Triunfo County- Rio Grande do Sul state in subtropical (southern) Brazil, situated at 29°56′34.28″ S and 51°43′6.44″ W at 31 m.a.s.l., approximately 70 km east of the state capital, Porto Alegre. The area has been cultivated with eucalyptus for 21 yr and has never been harvested or thinned. This is unusual since the commercial stands are ordinarily harvested every 6–7 yr. Our choice of sampling was more restricted in this biome since large-scale commercial plantations were established only more recently and the impact of eucalypt cultivation is expected to be small.

At each site, commercial eucalypt stands of approximately 20 ha were chosen to be representative of plantations in each bi-

ome. Four replicated areas of 50 × 50 m were delimited randomly in each stand. Ten soil samples (0- to 10-cm layer) were taken from each area and were pooled to produce a composite sample. The same procedure was adopted for sampling soils under the adjacent pasture, sugarcane, or native vegetation. After collection, the soil samples were air dried and passed through a standard 2-mm sieve. The four replicated soil samples were further combined to yield a composite sample representative of the current land use/vegetation. That is, each composite sample we used originated from 40 independent samples. These samples were subsequently used to extract the HS for the NMR experiments.

Soil Organic Matter Chemical Fractionation

Soil humic substances fractionation was performed according to Swift (1996) with small modifications. The humic fraction was extracted, under an N_2 atmosphere, by adding 0.1 mol L^{-1} NaOH to the soil sample, yielding a final 1:2.5 soil/extractant ratio. This alkaline-extracted SOM was separated into HA and FA fractions through acidification with HCl (6 mol L^{-1}) to pH 1.0 to 2.0. The NaOH-insoluble humin fraction was reserved for future studies. The HA fraction was purified by first redissolving it in NaOH and solid KCl (to yield a 0.3 mol L^{-1} final concentration, which facilitates the removal of suspended solids). Subsequently, clay minerals in the HA were removed by treating for 2 h with a 0.5% (w/v) HCl/10% (w/v) HF solution to give a final 1:10 HA/solution ratio. This procedure was based on previous NMR work with tropical and subtropical soils (Gonçalves et al., 2003; Dieckow et al., 2009), except that the HCl/HF treatment was repeated 10 times to ensure a low enough concentration of paramagnetic ions. For all samples, the C concentration in HA was enhanced with HF treatments; the enrichment factors of C (ERC) varied from 7.3 to 11.1. Gonçalves et al. (2003) found ERC in Oxisol samples varying from 5.7 to 7.2 after eight HF (10%) treatments, and Rumpel et al. (2006) reached a 4.9 ERC to Ah horizon of a Dystric Cambisol under forest after a single 20% HF treatment. On average, across sites, the Fe content was reduced by 68% after HF treatment, with the exception of the HA fractions from Curvelo and Triunfo sites, which already presented low iron content before HF treatment, resulting in an average 13% reduction after HF treatment. The total Fe content reduction after eight HF treatments reached by Gonçalves et al. (2003) varied from 22 to 78%.

Table 1. Site and soil characteristics in the studied Atlantic Forest, Cerrado, and Grassland biomes.

Biome	Atlantic Forest		Cerrado		Grassland
Site	Sooretama	Montanha	Curvelo	Itacambira	Triunfo
Current use/ previous use	Eucalypt planted in 1969 and replanted in 2001/Atlantic Forest — Pasture: *Panicum* sp.: 1990/ *Brachiaria* spp.:1970–1990/Atlantic Forest	Eucalypt planted in 1999–2007/ Sugarcane: 1986–1999/ Pasture: 1966–1986 — Sugarcane 1986–2007/ Pasture 1966–1986	Eucalypt/Cerradão	Eucalypt/Cerrado	Eucalypt planted in 1984
Cultivation period	38 yr / 37 yr	8 yr / 41 yr	14 yr / 7 yr	14 yr / 7 yr	21 yr / 21 yr
Current age	7 yr / 27 yr	8 yr / 21 yr	7 yr / 2	7 yr / 2	21 yr
Number of rotations	5	1	2	2	–
Management	Coppiced and replanted twice	Plantation	Coppiced once	Coppiced once	Never cut
Temperature, °C	29.0 (max), 19.0 (min), 23.0 (mean)	29.3 (max), 18.6°C (min), 20.0 (mean)	30.2 (max), 16.7 (min), 22.6 (mean)	28.2 (max), 16.6 (min), 22.4 (mean)	21.8 (max), 12.6 (min), 19.0 (mean)
Rainfall, mm yr^{-1}	1500	1100	1306	1067	1365
Altitude, masl	36	50	633	1048	31
Köppen's climate	Tropical, Af	Tropical, Aw	Tropical, Aw	Tropical, Aw	Subtropical, Cfa

Soil characteristics

Biome	Atlantic Forest						Cerrado				Grassland	
Use	Atlantic Forest	Eucalypt	Pasture	Atlantic Forest	Eucalypt	Sugarcane	Cerradão	Eucalypt	Cerrado	Eucalypt	Grassland	Eucalypt
Soil C, g kg^{-1}	37.8	21.6	29.1	34.6	12.3	9.8	23.8	25.9	63.2	53.8	21.5	15.7
δ^{13}C$_{V\text{-}PDB}$	−30.5	−26.3	−17.0	−24.9	−13.1	−19.4	−23.7	−23.3	−26.4	−26.0	−16.4	−22.8
N, g kg^{-1}	8.4	7.6	8.2	7.8	7.6	7.5	8.2	7.4	9.4	8.5	7.3	7.4
Sand, %	94	88	74	90	90	91	11	12	9	15	45	46
Silt, %	1	0	2	0	0	0	12	14	10	8	16	12
Clay, %	5	12	24	10	10	9	77	74	81	77	39	42
Soil class	Ultisol						Oxisol				Alfisol	

As a result of our HF treatments, the C/Fe ratio was substantially increased for all samples after HF treatment in the present study, allowing the acquisition of better resolution spectra.

The HF-treated HA samples were transferred into dialysis tubes (molecular weight cut off 10,000 kDa) and dialyzed against ultrapure water. The desalted samples then were freeze-dried. To purify the crude FA fraction, it was passed through chromatographic columns (10 mL min^{-1}) containing a nonionic resin (DAX-8 Supelite, Supelco) to remove organic, non-humic contaminants. The FA fraction adsorbed to the resin in the column was eluted with 0.1 mol L^{-1} NaOH (4 mL min^{-1}). The eluted fraction containing the FA was desalted by passing it through H^{+}-saturated cation exchange resin (Amberlite- IR 120^{+}) at 2.5 mL min^{-1}. Then the eluate containing the purified FA was freeze-dried.

Soil and Chemical Fraction Analysis

Subsamples of the soil composites were taken for texture analysis by the pipette method (Table 1). Soil composite subsamples were also ground in an agate mortar to pass a 100 mesh (0.149 mm) sieve. The finely ground material, HA, and FA were analyzed for total C and N contents by dry combustion (PerkinElmer CHNS/O 2400) while their ^{13}C natural abundance was determined on a continuous flow isotope ratio mass spectrometer (20–20 ANCA GSL, Sercon, Crewe, UK). The ^{13}C results were expressed in a per mil basis in comparison to the PDB international standard.

Solid-State Nuclear Magnetic Resonance

All ^{13}C NMR experiments were performed using a Bruker DSX 400 spectrometer operating at a ^{13}C frequency of 100 MHz, in a double-resonance magic-angle spinning probe head. For the best sensitivity of HA and FA in CP, CP/TOSS, and CSA experiments, samples were packed in Bruker 7-mm zirconia rotors while the high-speed DP/MAS experiments were performed in Bruker 4-mm zirconia rotors. ^{13}C chemical shifts were referenced to TMS, using the COO resonance of the α-modification of glycine at 176.49 ppm as an external secondary reference.

High-speed quantitative ^{13}C DP/MAS NMR. The high-speed DP and ^{13}C NMR experiments were performed using a Bruker 4-mm double-resonance probe head at a spinning frequency of 14 kHz to provide quantitative structural information. Recycle delays were tested by the cross polarization/spin lattice relaxation time–total sideband suppression (CP/T$_1$–TOSS) technique to ensure that all C sites were fully relaxed (Mao et al., 2000). A recycle delay of five times the measured ^{13}C T$_1$ was used. Recycle delays ranged between 100 and 170 s (with one exception: 50 s), and between 288 and 400 transients were averaged. The 90° ^{13}C pulse length was 4.3 ms. High-power decoupling with γB$_1$/(2π) > 65 kHz was applied during detection of 2 ms duration. To reduce the noise in the spectra, Gaussian multiplication with σ ~ 1 ms was applied before Fourier transformation. To obtain the quantitative fraction of nonprotonated C,

dipolar dephasing of 68 ms was adapted to high-speed direct polarization MAS ^{13}C NMR according to Mao and Schmidt-Rohr (2004a). In the determination of total peak intensities, residual spinning sidebands of the aromatic-carbon signals were taken into account (Mao et al., 2000).

^{13}C CP/TOSS and ^{13}C CP/TOSS with dipolar dephasing. Qualitative composition information was obtained by ^{13}C CP/TOSS (cross polarization/total suppression of sidebands) experiments, performed at a 6.5 kHz spinning frequency, with a contact time of 1 ms and recycle delay of 0.5 s. Between 3072 and 4096 transients were averaged. The ^{1}H 90° pulse length was 4.4 ms and the ^{13}C 180° pulse length 9.1 ms. Four-pulse total suppression of sidebands (TOSS) (Dixon, 1982) was employed before detection. The corresponding subspectrum with signals of nonprotonated C and mobile groups such as rotating CH$_3$ was obtained by ^{13}C CP/TOSS combined with 40 ms dipolar dephasing.

^{13}C chemical shift anisotropy filter. The signals of anomeric C (O–C–O), which overlap with aromatic groups in the range between 115 and 94 ppm, were determined by using a CSA filter along with CP/TOSS. The aromatic-C signals were selectively suppressed by a three-pulse ^{13}C CSA filter with a CSA filter time of 70 ms (Mao and Schmidt-Rohr, 2004b). To select the signals of nonprotonated O–C–O (ketal) C, which may extend to 115 ppm, this CSA filter was combined with a dipolar dephasing time of 40 ms (Mao and Schmidt-Rohr, 2004b).

CH and CH$_2$ spectral editing. The spectrum of immobile CH$_2$ and CH C, with a small CH$_3$ contribution, comes from the difference of a CP/TOSS and a CP/TOSS spectrum after 40 ms dipolar dephasing. For CH-signal selection, a robust method based on C–H multiple-quantum coherence (Schmidt-Rohr and Mao, 2002) was used. For CH$_2$-only spectral editing, the ^{1}H–^{13}C–^{1}H three-spin coherence of CH$_2$ groups was selected (Mao and Schmidt-Rohr, 2005). Approximately 40,000 transients were averaged for each CH- and CH$_2$-only spectrum, with a 0.5-s recycle delay. The MAS frequency was 5.787 kHz for all three experiments.

Quantification of functional groups. For quantifying the solid-state ^{13}C NMR data in terms of the molecular composition of HA and FA, the spectra were divided into a dozen ranges whose areas were determined by integration using Bruker XWinNMR 3.5 software. Generally, the assignments were based on Mao et al. (2000) and spectral editing according to Mao et al. (2007) (Table 2).

RESULTS
General Soil and Fraction Characteristics

Carbon concentration in HA and FA generally increased after eucalypt cultivation, while N concentration decreased, resulting in wider C/N molar ratios of HA with FA showing larger C/N ratios than HA (Table 3). The δ^{13}C values, as expected, were less negative in sites cultivated with C4 species (sugarcane and grasses). Specifically, the ^{13}C natural abundance (δ^{13}C) values showed partial substitution of C from areas cultivated with sugarcane (-20.3 ‰ in HA, -24.9 ‰ in FA) when planted to eucalypt for 8 yr (-26.4 ‰ in HA, -28.4 ‰ in FA) at the Montanha

Table 2. Assignment of ^{13}C NMR signals in HA and FA.

Group	Technique	Range, ppm
Nonpolar alkyl	DP/MAS	0–46
OCH$_3$ groups	DP/MAS dipolar dephasing	48–65
NCH	Difference- DP/MAS and DP/MAS dipolar dephasing	48–65
Nonprotonated carbohydrate OCquaternary (OCq)	CSA-filter dipolar dephasing	65–94
Protonated carbohydrate C	Difference- CSA filter and CSA filter dipolar dephasing	65–94
Nonprotonated anomerics, O–Cq–O	CSA filter dipolar dephasing	94–115
Protonated anomerics, O–CH–O	Difference- CSA filter and CSA filter dipolar dephasing	94–115
Nonpolar nonprotonated aromatics	DP/MAS dipolar dephasing- corrected by anomeric C in the 94–115 ppm (CSA filter)	94–145
Protonated nonpolar aromatics	Difference- DP/MAS and DP/MAS dipolar dephasing corrected by contribution of anomeric C in the 94–115 ppm range (CSA filter)	94–145
Phenolics aromatic C-O	DP/MAS- after dipolar dephasing	145–161
Ketone, quinone, aldehyde C	DP/MAS	190–220
COO and NC=O	DP/MAS	161–188
CH$_2$–OH, O-CH-O and N-CH	CH and CH$_2$ spectral editing methods	

site (Atlantic Forest biome). In southern Brazil, the substitution of grassland (-15.9 ‰ in HA, -23.5 ‰ in FA) by eucalypt for 22 yr also resulted in a substantial change in the ^{13}C natural abundance in HA (-23.3 ‰ in HA, -29.0 ‰ in FA).

The differences in HA and FA elemental composition and isotopic signatures indicated that the land use and land use changes led to substantial alterations in soil humic substances, thus alterations in the molecular composition and structure of these HS were expected. Quantitative structural information was provided by fully relaxed ^{13}C DP/MAS NMR spectra. The

major NMR-identifiable components in these HS were primarily aromatics, COO groups, peptides, carbohydrates, and nonpolar alkyls. The HA and FA spectra exhibited similar molecular composition patterns despite the differences in vegetation, soils, and climate. However, differences were observed for the proportions of the groups in each sample and the distribution of the relative signal in various spectral regions among samples. The DP results showed large differences between the aromaticities of HA and FA, with the former being more aromatic than the latter in all sites (Table 4). The average HA and FA aromaticity was,

Table 3. Elemental analyses and isotopic composition of the humic substances.

Biome/Site	Use	Humic Acid					
		C	H	N	δ^{13}C, V-PDB	H/C	C/N
		——————— % ———————			‰		
Atlantic Forest Sooretama	Atlantic Forest	45.4	5.1	4.0	-28.0	1.3	13.1
	Eucalypt 38 yr	47.1	5.2	3. 6	-29.0	1.3	15.4
	Pasture	46.2	5.0	3.4	-21.1	1.3	15.6
Atlantic Forest Montanha	Atlantic Forest	45.8	4.8	4.4	-30.2	1.3	12.2
	Eucalypt 8 yr	47.8	5.1	3.8	-26.4	1.3	14.6
	Sugarcane	46.3	4.5	3.8	-20.3	1.2	14.3
Cerrado Curvelo	Cerrado	46.6	4.9	4.4	-22.1	1.3	12.4
	Eucalypt	48.5	5.5	3.7	-23.6	1.3	15.2
Cerrado Itacambira	Cerrado	45.5	4.5	4.0	-25.0	1.2	13.1
	Eucalypt	45.6	4.3	3.0	-24.3	1.1	18.0
Grassland Triunfo	Grassland	48.4	5.3	4.4	-15.9	1.3	12.9
	Eucalypt 22 yr	48.8	5.2	4.6	-23.3	1.3	12.5
		Fulvic Acid					
Atlantic Forest Sooretama	Atlantic Forest	25.0	2.3	1.3	-25.5	1.1	22.6
	Eucalypt 38 yr	26.9	2.4	1.3	-26.4	1.1	23.9
	Pasture	27.9	2.6	1.6	-19.9	1.1	20.3
Atlantic Forest Montanha	Atlantic Forest	22.2	1.9	1.8	-30.3	1.0	14.3
	Eucalypt 8 yr	27.1	2.5	1.4	-28.4	1.1	22.9
	Sugarcane	22.7	2.0	1.4	-24.9	1.1	18.8
Cerrado Curvelo	Cerrado	31.1	3.1	1.2	-26.7	1.2	29.8
	Eucalypt	29.8	2.8	1.1	-26.4	1.1	31.3
Cerrado Itacambira	Cerrado	33.2	3.2	1.2	-28.4	1.1	32.5
	Eucalypt	33.2	3.1	1.0	-25.7	1.1	38.7
Grassland Triunfo	Grassland	28.4	2.6	1.6	-23.5	1.1	20.6
	Eucalypt 22 yr	30.9	2.8	1.1	-29.0	1.1	32.5

Table 4. ¹³C DP/MAS NMR functional groups, Aromaticity and nonpolar alkyl C to O-alkyl C ratio (A/A-O) in the humic and fulvic acid samples from the Atlantic Forest, Cerrado and Grassland soils.

Functional groups				C = O	COO, NCO	Phenolic	Aromatic			O-alkyl				OCH₃ CH₃	NHC, CCH	–Nonpolar alkyl–	
					prot	nonprot	prot	nonprot	OCHO	oCqO	oCq	OCH	CH₂	CH₃	CCH		
Chemical shift range (ppm)				220–188	188–161	161–145	145–115	145–115	145–115	115–94	115–94	94–65	94–65	65–48	65–46	46–22	26–0
	Use	Ar† (%)	A/O-A‡														
									%								
Humic Acid																	
Sooretama	At For	45	1.1	3.1	18.4	8.5	9.6	17.8	1.7	1.2	2.0	10.9	1.5	9.0	12.6	5.0
	Euc	50	1.6	3.0	16.2	8.4	14.7	18.6	1.4	0.9	1.7	8.8	1.6	7.7	14.6	5.3
	Past	54	1.1	1.7	14.3	9.7	16.1	19.9	1.5	0.6	1.3	10.6	2.7	7.8	11.1	3.6
Montanha	At For	56	1.2	2.3	17.9	9.1	14.8	20.8	0.2	1.8	1.5	8.0	2.0	7.0	10.8	3.6
	Euc	51	1.6	2.1	16.2	7.4	13.5	20.8	0.4	1.7	1.2	8.7	0.7	7.9	14.8	5.1
	SC	63	1.4	2.3	17.4	9.9	18.0	22.9	0.9	0.7	1.2	6.6	1.2	6.4	8.2	4.9
Curvelo	Cer	50	1.4	2.1	17.8	7.0	12.7	19.9	1.5	0.6	1.7	9.0	0.4	8.9	13.5	4.6
	Euc	43	1.7	3.6	17.7	6.7	7.4	19.8	1.5	0.7	1.6	10.0	1.0	7.8	16.7	6.1
Itacambira	Cer	65	1.2	3.3	19.6	8.1	26.0	15.9	0.9	0.7	1.7	6.4	1.6	3.8	7.4	4.6
	Euc	68	1.2	2.7	18.8	8.3	13.2	32.1	1.0	0.7	1.3	5.9	1.5	4.0	6.8	3.9
Triunfo	Gras	53	1.2	2.7	18.0	8.8	14.2	19.3	0.8	0.8	0.2	10.5	3.3	7.3	10.5	4.2
	Euc	43	1.6	3.4	19.3	7.8	11.1	14.3	0.01	2.3	0.9	9.9	1.2	8.2	14.8	6.1
Fulvic Acid																	
Sooretama	At For	43	1.1	4.7	23	7	7.8	15.3	2.4	0.9	1.5	10.8	2.2	6.0	13.5	3.0
	Euc	38	0.9	4.1	23.9	6.2	8.4	12.9	2.6	1.9	3.4	10.7	1.0	7.5	13.6	3.8
	Past	41	0.7	4.5	24.2	7.5	8.7	13.1	2.1	2.2	2.0	12.9	3.1	7.0	10.6	2.6
Montanha	At For	51	0.9	3.5	21.7	9.3	11.0	17.5	1.3	1.2	2.1	10	1.3	7.6	10.7	2.7
	Euc	42	1.2	3.9	20.9	8.4	8.7	14.5	2.3	1.6	2.2	10	1.5	7.2	15.0	3.6
	SC	53	0.9	3.8	24.3	10.7	10.3	16.9	1.7	2.1	1.9	8.3	1.3	6.3	10.1	2.3
Curvelo	Cer	27	1.4	3.8	23.8	4.7	6.6	8.3	2.0	1.9	2.8	10.9	2.0	8.5	19.6	4.9
	Euc	40	1.4	4.6	24.0	7.8	7	13.7	1.6	1.4	2.6	8.9	1.9	5.9	15.2	5.3
Itacambira	Cer	28	1.3	3.2	24.3	4.9	4.4	10.9	1.1	3.6	6.5	7.2	1.9	7.5	18.6	5.6
	Euc	30	1.4	2.1	24.7	5.4	5.9	10.6	2.3	2.1	4.5	8.4	4.5	4.8	18.0	6.5
Triunfo	Gras	41	1.2	1.3	21.4	6.5	13.7	11.2	2.4	2.4	2.8	9.1	1.5	7.1	15.1	5.1
	Euc	38	1.3	4.7	24.4	6.8	7.2	12.8	1.8	1.9	2.4	9.4	1.2	7.3	15.5	4.9

†Ar. Aromaticity = ((115 to 161 ppm range corrected for anomerics)/(0 to 161 ppm range); At For: Atlantic Forest; Euc: Eucalypt; Past: Pasture; SC: sugarcane; Cer: Cerrado; Gras: Grassland; prot:protonated C; nonprot:nonprotonated C.
‡A/O-A = (0 to 46 ppm)/((65–115 ppm).

respectively, 54 and 38% for native vegetation, 51 and 38% for eucalypt, and 58 and 47% for other uses, i.e., pasture and sugarcane. The nonpolar alkyl C to O-alkyl C ratio (A/O-A) ratios for eucalypt and other land uses was greater for HA than for FA (Table 4), with average values of 1.4 for HA and 1.1 for FA.

Effect of Land Use Change in the Atlantic Forest Biome

At the Sooretama site, after 38 yr of eucalypt cultivation, there was an increase in HA aromatic groups (54% protonated and 5% nonprotonated), whereas there was an increase in protonated aromatics (7%) and a 16% decrease in nonprotonated FA aromatic groups in comparison to the values for the native Atlantic Forest soil (Table 4). However, the phenolic C did not change after eucalypt introduction at this site. The replacement of native forest by eucalypt resulted in 18 and 20% decreases of signals attributed to O-alkyl groups in HA and FA, respectively (Table 4). Aliphatic compounds (nonpolar alkyl- CH_3 and CH_2 groups) had a sharper peak centered at 33 ppm for HA (Fig. 1) than for FA (Fig. 2). The relative contribution of these compounds in HA decreased in the following order: eucalypt

(20%) > native forest (18%) > pasture (15%), whereas for FA, the contribution was about equal between the Atlantic Forest (16%) and the eucalypt area (17%). Planted pasture resulted in a higher contribution from compounds containing OCH_3 groups (65–46 ppm; Fig. 1 and 2), of phenolic C and nonpolar aromatic C in HA, and a greater contribution of phenolic units in FA (Table 4). The contribution of COO/NCO and C = O groups in HA decreased in the following order: Atlantic Forest > eucalyptus > pasture. There were, however, slight differences for FA; the average across all sites was 4% for C = O and 24% for COO/NCO.

At the Montanha site, the substitution of the Atlantic Forest by sugarcane resulted in 19 and 4% decreases in the intensity of O-alkyl C (115–65 ppm), respectively, in HA and FA, and increased aromaticity of HA (12%) and FA (4%)(Fig. 3, Table 4). After 8 yr of eucalypt in former sugarcane fields, the opposite pattern was observed: the aromaticity of HA and FA was reduced by 19 and 20%, respectively. In the DP/MAS and CP/TOSS spectra there were clear differences in signal intensity in the 0- to 45-ppm range; it decreased in the order: eucalypt > Atlantic Forest > sug-
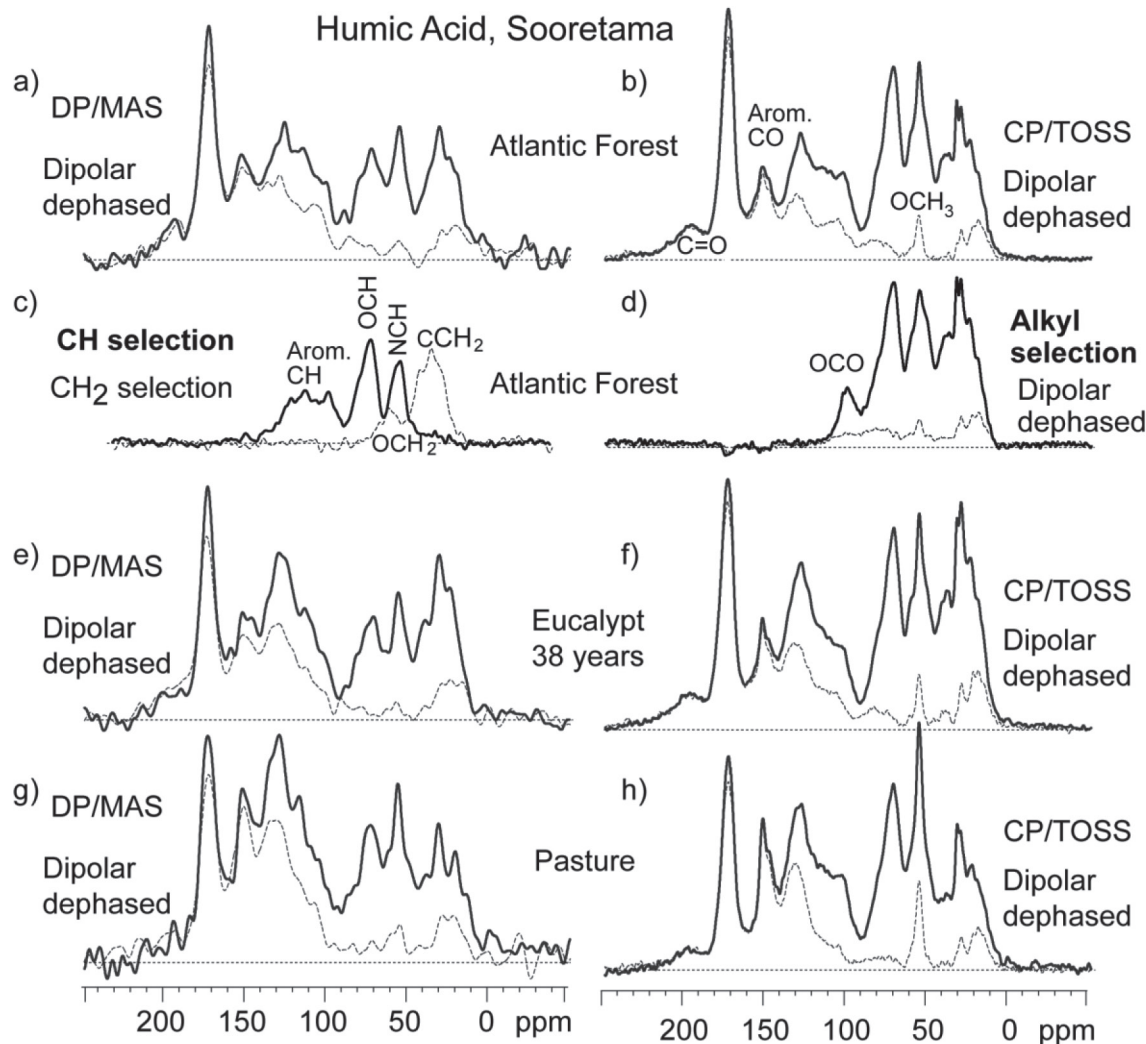

Fig. 1. DP/MAS (left column) and CP/TOSS (right column) ^{13}C NMR spectra of humic acids from soils under (a) and (b) Atlantic Forest, (e) and (f) eucalypt, and (g) and (h) pasture in Sooretama, Espirito Santo. Corresponding spectra after dipolar dephasing are shown superimposed (dashed lines). (c) and (d) Spectral editing for humic acid from soil under Atlantic Forest: (c) Spectra of CH (full line) and CH$_2$ carbons (dashed line); (d) spectra of all alkyl carbons (full line) and of nonprotonated alkyl carbons and mobile groups (dashed line).
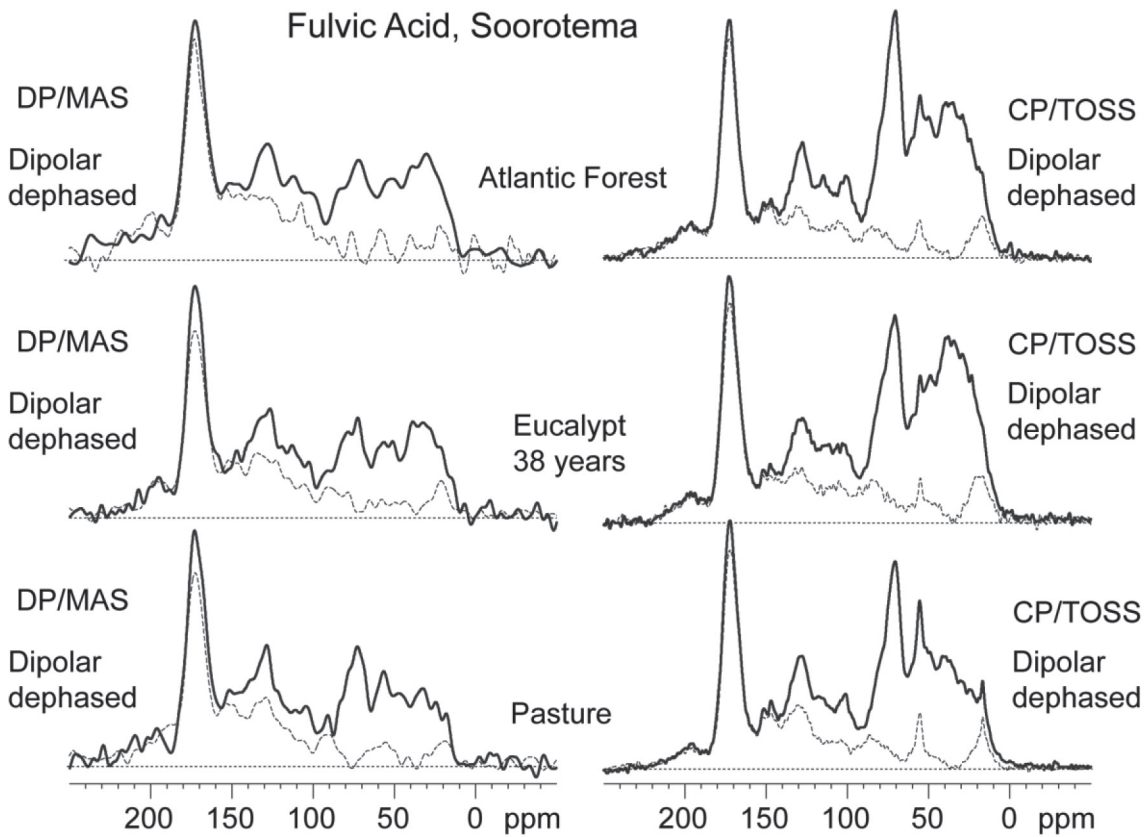
Fig. 2. DP/MAS (left column) and CP/TOSS (right column) ^{13}C NMR spectra of fulvic acid from soils under Atlantic Forest, eucalypt, and pasture in Sooretama, Espirito Santo. Corresponding spectra after dipolar dephasing are shown superimposed (dashed lines).
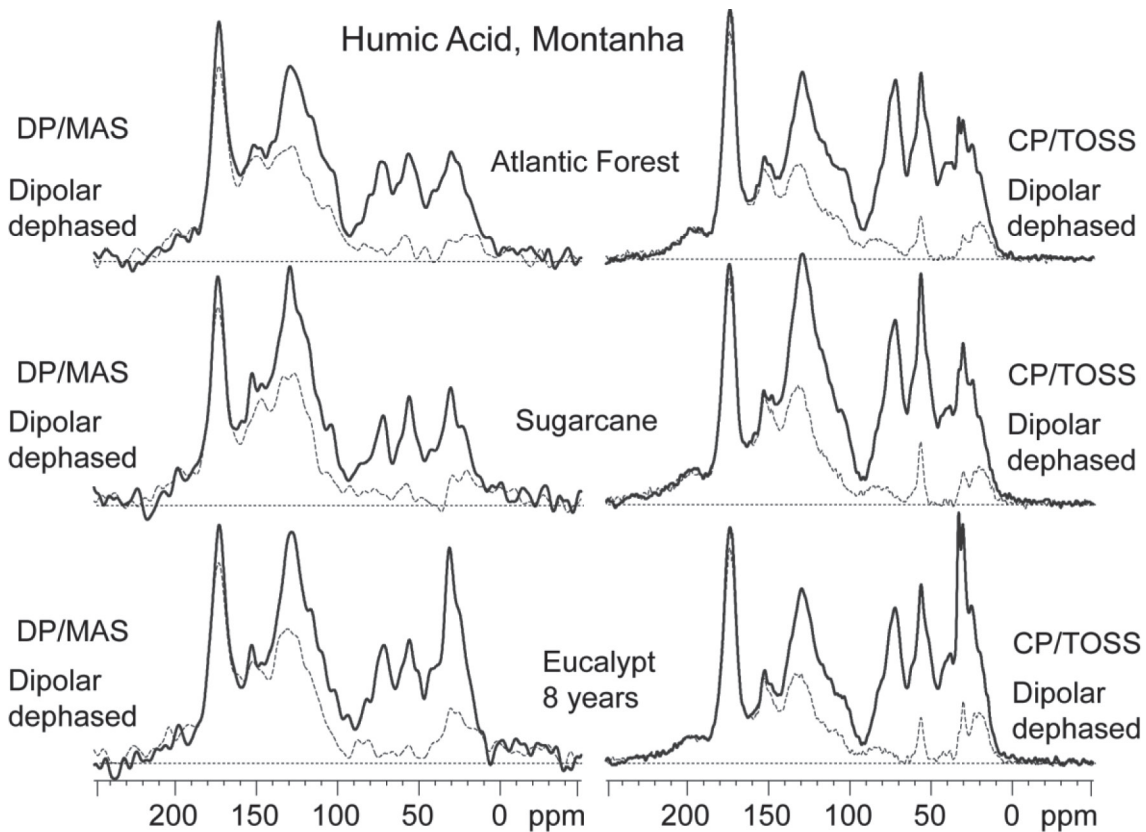

Fig. 3. DP/MAS (left column) and CP/TOSS (right column) ^{13}C NMR spectra of humic acids from soils under Atlantic Forest, eucalypt, and sugarcane in Montanha, Espirito Santo. Corresponding spectra after dipolar dephasing are shown superimposed.

arcane for both HA (Fig. 3) and FA (Table 4). The contribution of NHC/CCH groups was greater in HA from soils under eucalypt (7.9%) than in those under native forest and sugarcane (Table 4). The OCH$_3$ groups represented 0.7% of the C in the HA and 1.5% in the FA from soil cultivated with eucalypt.

Effect of Land Use Change in the Cerrado Biome

At the Curvelo site, 14 yr of eucalypt cultivation (two rotations) led to HA with lower aromaticity (50–43%), whereas the reverse was observed for FA, which became more aromatic (27–40%, Table 4). The signals corresponding to CH$_3$ groups in the 0- to 26-ppm range and to CH$_2$ groups in the 22- to 46-ppm range were more intense in HA from soil cultivated with eucalypt (23%) than in that from native Cerrado soil (18%). On the other hand, eucalypt soil FA showed a lower signal intensity (24%) of nonpolar alkyl groups than that from the Cerrado soil (20%, Table 4). Small differences between the distinct uses were observed in the Cerrado soil regarding the contribution of OCH$_3$ in HS (Fig. 4). In the FA fractions, the contribution of O-alkyl C decreased 18% after the replacement of native vegetation by eucalypt. The O-alkyl groups of the HA carbon did not change significantly after eucalypt introduction and neither did the signal intensity for either HA or FA COO/NCO.

Interestingly, eucalypt cultivation at the Itacambira site increased in contribution of aromatic carbon not bonded to O to 45% (115–145 ppm) for HA. Compared to HA from soils under other natural vegetation biomes, the HA from Cerrado soil at the Itacambira site showed the most intense peak (42%) assigned to aromatic C not bonded to O (Fig. 5). This high signal intensity for aromatic compounds in HA was also observed in the spectra after dipolar dephasing (Fig. 5). The FA aromatic group signal increased slightly from 20 to 22% after eucalypt establishment. While the aromatic signal increased, the substitution of native Cerrado vegetation by eucalypt resulted in an 8% decrease in HA and a 6% reduction in FA for the O-alkyl (Table 4), and the nonpolar alkyl C

decreased by 10% in HA. However, in FA there was no difference in nonpolar alkyl C after the replacement of the native Cerrado vegetation. The proportion of OCH$_3$ and NHC/CCH groups in HA was similar for eucalypt and Cerrado (Fig. 5). However, the OCH$_3$ group in FA from soil under eucalypt had greater signal intensity than that under Cerrado, and the opposite was observed for NHC/CCH groups (Fig. 6).

Effect of Land Use Change in the Grassland Biome

At the Triunfo site in southern Brazil, aromatic groups represented around 25% of the C in HA and 20% in FA from the eucalypt area, and 33 and 25% in HA and FA from the Grassland area. Decreases in aromaticity by 19 and 7% were observed, respectively, for HA and FA after 22 yr of continuous eucalypt cultivation (a single long rotation). Consequently, the aliphaticity increased 20 and 5%, respectively, for HA and FA in this site (Table 4). As in the other biomes, there was a 42% increase in peak intensity of nonpolar alkyl C in the HA when the native grassland vegetation was substituted by eucalypt, whereas changes in such compounds were virtually absent in the FA (Fig. 7 and 8). Nevertheless, the changes observed in the O-alkyl C showed a pattern distinct from the other biomes, with a 6% increase for HA and a 7% decrease for FA after 22 yr of cultivation in soils formerly under native Grassland. The peak centered at 55 ppm assigned to OCH$_3$ groups in HA and FA was slightly more intense in the Grassland than in eucalypt soil while the COO/NC = O groups in HA and FA increased by 7 and 14%, respectively, after planting to eucalypt.

DISCUSSION

The ^{13}C DP/MAS NMR, CP and editing experiments have been used to evaluate the molecular composition of HA from temperate soils (Mao et al., 2001, 2008). These experiments overcame a major shortcoming of the ^{13}C CP/MAS technique, namely the underestimation of sp^2–hybridized C with large CSA, such as those in aromatic rings. In the present study they enabled us to
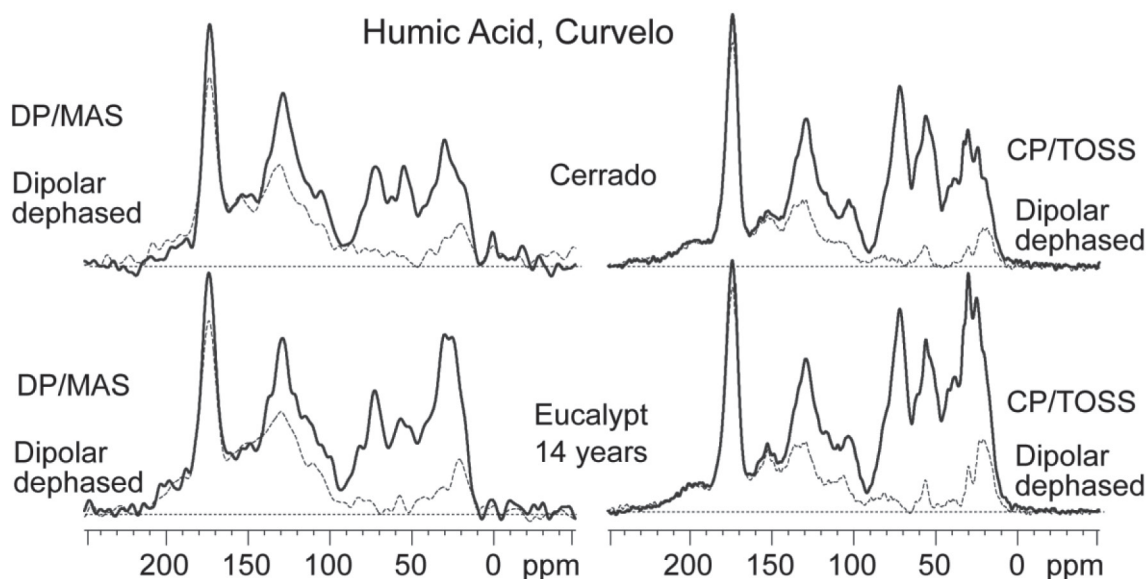

Fig. 4. DP/MAS (left column) and CP/TOSS (right column) ^{13}C NMR spectra of humic acids from soils under Cerrado and eucalypt in Curvelo, Minas Gerais. Corresponding spectra after dipolar dephasing are shown superimposed.
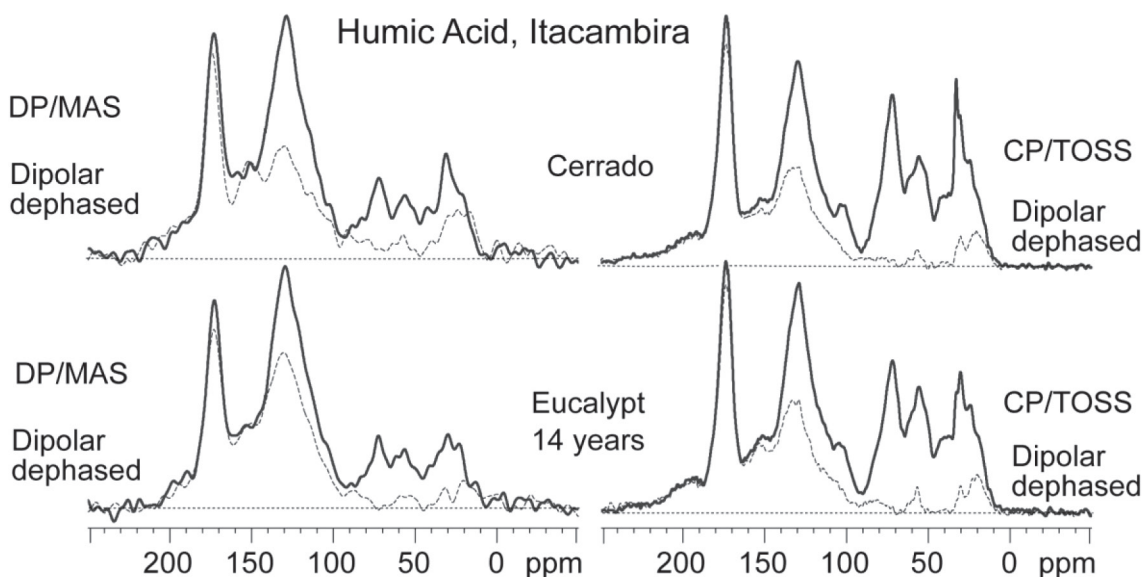
Fig. 5. DP/MAS (left column) and CP/TOSS (right column) ^{13}C NMR spectra of humic acids from soils under Cerrado and eucalypt in Itacambira, Minas Gerais. Corresponding spectra after dipolar dephasing are shown superimposed.

obtain more accurate, quantitative estimates of the aromaticity of HA and FA extracted from soils under distinct biomes in Brazil. Moreover, they allowed an estimate of the impact of introducing planted eucalypt forests on HA and FA composition. To reduce the risks of past land use effect before eucalypt planting in each biome, we sampled paired areas with the exception of Montanha site. As past use sequence and duration was not identical in each site, we must interpret such results keeping in mind such possibility. However, due to the rapid turnover rates of SOM in these tropical environments (Wilcke and Lilienfein, 2004) the features of SOM likely reflect the more recent land use. The rapid substitution of C4-derived C in SOM is evident from the lowering of δ^{13}C values for both HA and FA fractions in soils after introduction of eucalypt in the great majority of the studied regions (Table 3). The δ^{13}C values in HS from soils under eucalypt were mostly below

-26‰, which is indicative of SOM increasingly dominated by C3 (eucalypt) derived C, as observed in other tropical regions (Lima et al., 2006; Binkley et al., 2004).

Use of a composite representative soil sample with no experimental replication was necessary due to the high cost of NMR analysis and the limited equipment time. In fact, most studies of humic substances by NMR techniques have not included sample replications (Hatcher et al., 1981; Preston et al., 1994; Guggenberger et al., 1995; ; Rumpel et al., 2004; González-Pérez et al., 2008; Mao et al., 2007). Thus, in the present study the results could not be evaluated using traditional statistical tests. However, considering noise and partial peak overlap, the estimated error of the peak area fractions is $\pm -1.5\%$.

Dipolar dephasing by gated decoupling was applied to HA and FA to distinguish between protonated and nonprotonated
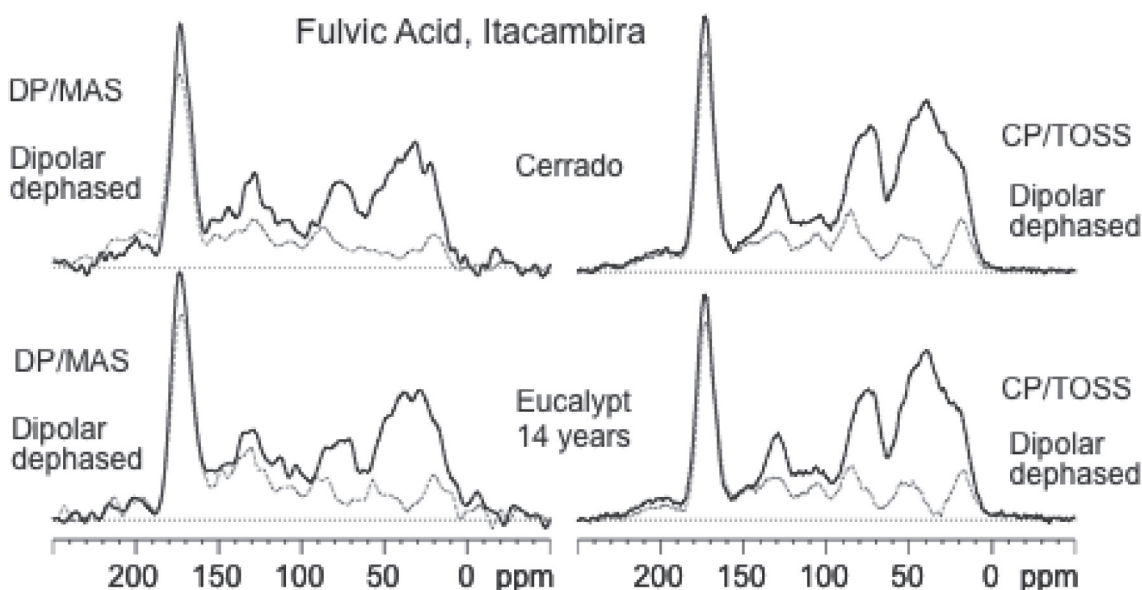

Fig. 6. DP/MAS (left column) and CP/TOSS (right column) ^{13}C NMR spectra of fulvic acids from soils under Cerrado and eucalypt in Itacambira, Minas Gerais. Corresponding spectra after dipolar dephasing are shown superimposed.
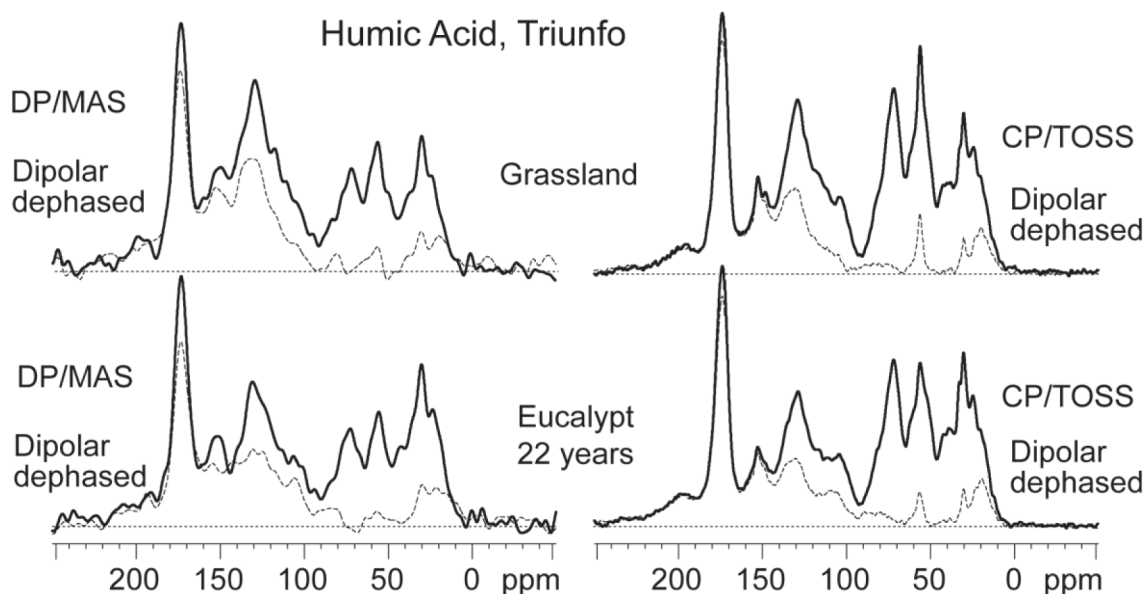
Fig. 7. DP/MAS (left column) and CP/TOSS (right column) ^{13}C NMR spectra of humic acids from soils under Grassland and eucalypt in Triunfo, Rio Grande do Sul. Corresponding spectra after dipolar dephasing are shown superimposed.

carbons. The aromatic signal remaining around 130 ppm can be explained by contributions from highly modified lignin molecules or from condensed aromatic structures such as black carbon (Mao et al., 2007; Brewer et al., 2009). Black carbon quantification was not done in our study. However, black carbon could have originated from charred material derived from burning of the native vegetation, as naturally occurs over millennia in the Cerrado region, or anthropogenic fires in the Grassland biome. Burning of harvest residues in eucalypt fields was also a common practice in the early rotations. The presence of black fragments in an optical transmission photomicrograph associated with a prominent aromatic C signal at 130 ppm in the NMR spectrum observed by Dieckow et al. (2005) was taken as evidence for the occurrence of materials derived from charcoal in SOM of a southern Brazil subtropical soil. The study performed by Glaser et al. (2000) on the

Brazilian Amazon basin, where Terra Preta soils occur in small areas, indicate that black carbon in soils can be found as particulate, unprotected, physically trapped, and as oxidatively altered forms that are complexed with minerals. The contribution of black carbon in the heavy fraction was smaller than 30% in Terra Preta soils and smaller than 20% in the sandy Oxisols but reached 56% in the clayey Oxisols. In the current study, nonprotonated C varied from 14–32% in HA and 10–18% in FA.

A decrease in HA aromaticity following eucalyptus planting, including the fraction of nonphenolic aromatic carbon, was seen at the Curvelo site (Cerrado), Triunfo site (Grassland), and in Montanha (Atlantic Forest), possibly due to an increased aliphatic contribution leading to dilution of the aromatic components. However, 38 yr of eucalypt cultivation in Sooretama (Atlantic Forest biome) did not alter the aromaticity of HA as
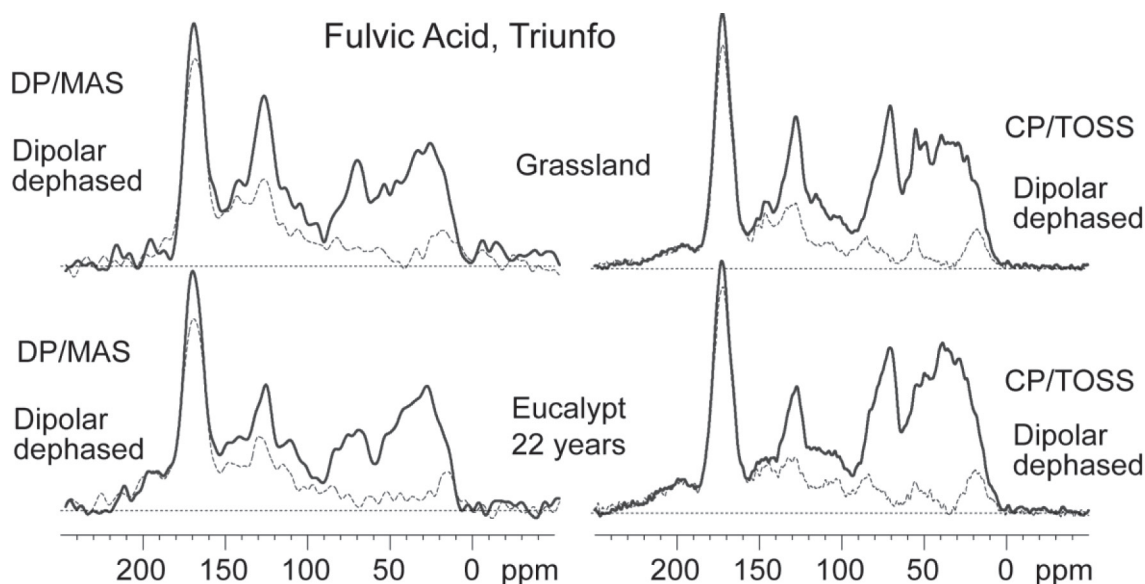

Fig. 8. DP/MAS (left column) and CP/TOSS (right column) ^{13}C NMR spectra of fulvic acids from soils under Grassland and eucalypt in Triunfo, Rio Grande do Sul. Corresponding spectra after dipolar dephasing are shown superimposed.

compared to the soils under native vegetation. The ^{13}C NMR spectra of HAs from a Brazilian Oxisol analyzed by González-Pérez et al. (2004) showed small differences in chemical composition where incorporation of plant residues was higher, but indicated that SOM formed under such conditions was less aromatic. The effect of constant accumulation of crop residues was more important in this soil than the effect of tillage system. In no-till systems, plowing is not used for the incorporation of crop residues into the soil, favoring the continuous accumulation of crop residues at the soil surface, a condition similar to what has been practiced in the last couple of rotations in eucalypt areas, where soil preparation/tillage have been minimized. Therefore, for soil microorganisms, "fresh" plant residues are always available at the soil surface, sometimes in quantities higher than their capacity to metabolize them (González-Pérez et al., 2004).

The replacement of native vegetation by eucalypt plantations increased the relative contribution of nonpolar aliphatic groups in HA from soils previously under Atlantic Forest (Sooretama and Montanha sites), Grassland, and the Cerrado (except in the Itacambira site) biomes. The same trend was observed for FA, except in the Curvelo site. Following eucalypt planting, there are large changes not only in the amount but also in the quality of the litter input to soils. The literature on NMR studies comparing eucalypt and grass species is scarce, but it is apparent that forest litter is initially richer in non-oxygenated alkyl compounds than grass species. For example, ^{13}C NMR signal in the -10 to 45 ppm region for maple litter was 21% while 6% was observed for corn litter. In the same work Australian native grass species had 4.4% and Buffel grass material presented 8% alkyl C (Nelson and Baldock, 2005). In another study with litter from several woody species, it was found that eucalyptus species were the ones with a higher abundance of compounds resonating in the 0- to 45-ppm region (Conte et al., 2010). The nonpolar alkyl source can be attributed to fatty acids (Stevenson, 1994) and poly(methylene) (Hu et al., 2000; Mao et al., 2007). The increase in those groups can be attributed to the selective preservation of compounds derived from plants, such as the non-saponifiable rigid structures of cutans and suberans and also to in situ microbial alkyl synthesis (Baldock et al., 1990, 1992). The selective preservation of aliphatic compounds (Hu et al., 2000) has been confirmed by other authors (Dieckow et al., 2005).

The periods that soils in our study have been under eucalypt plantations ranged from 8 to 38 yr. The Sooretama site has been cultivated with eucalypt for 38 yr and is in its fifth rotation. It exhibits lower contribution of polar aliphatics to HA as compared to the native Atlantic Forest vegetation. The highest contribution of those compounds was observed in the Montanha site (Atlantic Forest biome) and in southern Brazil (Grassland biome), where conditions seem to favor additional stabilization. These two sites have in common the absence of recent soil disturbance by logging operations and site preparation for new planting, and such conditions may have created conditions favorable for transferring nonpolar alkyl C compounds to soil or their selective preservation in the soil environment.

Despite the distinct conditions of soils, climate, and vegetation at Sooretama, Curvelo, and Triunfo sites, the introduction of eucalypt led to a long-term deposition and possibly incorporation of aliphatic material from above and below ground litter into HA and FA. For FA, the highest contribution of alkyl C was observed at the Montanha site. Due to the greater bioavailability of FA compared to HA, the preservation of nonpolar alkyl C in FA and also HA may have been favored in this slightly drier Atlantic Forest site in Montanha. The lower proportion of nonpolar alkyl C in HA and FA from the eucalypt soil at the Itacambira (Cerrado biome) may be a result of lower rates of litter and harvest residue decomposition and incorporation into HS because of its cooler and drier climate in winter months (Table 1). Carbon from slowly decomposing eucalypt residues accumulates to a greater extent in soils of this region as components of the light fraction. Indeed, the largest stocks of C in particulate and light fractions is found in soils of the Cerrado region, followed by those under Atlantic Forest and then those from the Grassland biome (Vergutz, 2011). Generally, at the Cerrado sites, an accumulation of alkyl structures has been favored compared to lignin structures when the native vegetation is substituted by eucalypt. Rumpel et al. (2004), evaluating SOM quality in a Cambisol under spruce forest, observed changes of the distributions of phenols and hydroxyalkanoic acids with depth that might indicate a preferential preservation of C derived from cutin/suberin compared to C derived from lignin. Alkyl C dominated the fine particle size fractions (<6.3 mm) of the Aeh horizon of the Haplic Podzol, and it was suggested by the authors that the observed compounds could be insoluble material, possibly derived from selectively preserved plant litter (Rumpel et al., 2004).

Sugarcane cultivation decreased the relative contribution of carbohydrates in HA and FA. The reduction of carbohydrate C following deforestation has been detected in previous work (Dieckow et al., 2005, 2009). Carbohydrates are the most easily degradable components of HS (Baldock et al., 1992). Their degradation is commonly attributed to microbial oxidation of the organic materials previously protected in the soil aggregates but that are destroyed by cultivation. Sugarcane fields are usually cropped annually under conventional tillage with intense site preparation for planting and after each harvest to eliminate soil compaction due to mechanized operations. Contrastingly, there was no trend for contribution of O-alkyl C (carbohydrates) in HA and FA from soils under eucalyptus compared to natural vegetation. The low oxidation and the considerable contribution of easily decomposable structures (O-alkyl C) in topsoil organic matter from South Brazil were attributed to the formation of organo-mineral complexes (Dick et al., 2005). Considering this, natural changes in clay content across soils from the Sooretama sites does not seem to be the main factor affecting HS quality because the soil with the lower clay content in the Atlantic Forest site is the one showing the higher contribution from carbohydrates.

In the present study, the contribution of other carbohydrates besides cellulose was corroborated by the CSA-filtered spectra. These showed an anomeric-carbon peak that was not

centered at the 105-ppm resonance position typical for cellulose, which is additional evidence for contribution of secondary carbohydrates (Mao et al., 2001). Non-cellulosic polysaccharides were found to be protected from mineralization by surface complexation on the clay fraction (Neufeldt et al., 2002), which could result in a relatively low degree of SOM decomposition in Ferralsols (Oxisols) in the Cerrado biome. Considering that the eucalypt rotation lengths are becoming increasingly shorter (currently around 6–7 yr), the more labile organic C components will make smaller contributions to the HS. In eucalypt stands under longer rotation periods, there was a recovery in SOM content, which was richer not only in plant-derived carbohydrates, but also microbial-derived amino sugars such as glucosamine and galactosamine (Pegoraro et al., 2011).

Lignin contributions can be indicated by the presence of peaks resonating around 150 ppm (phenolic structures and aromatic ethers) and OCH_3 signals near 55 ppm (Mao et al., 2007). The lignin contribution for HA was confirmed by the presence of both types of signals in DP/MAS spectra after dipolar dephasing. In the area cultivated with pasture in Sooretama (Atlantic Forest biome) and in the Grassland soil, the largest contributions of lignin-derived compounds were detected in HA. The pasture area has been continuously cultivated for 37 yr (10 yr with *Panicum* spp. and 27 yr with *Brachiaria* spp.). These species have a high biomass yield that results in a large input of plant material to soil, mostly as roots. It has been demonstrated that root C is more efficient than shoot C in converting to SOM and that root-derived C contributes, on average, 30% more to SOM than shoot-derived C (Rasse et al., 2005). One of the main reasons for this greater contribution of roots is their chemical recalcitrance. Roots are richer in lignin and suberin than shoots are, especially in grassy species (Rasse et al., 2005). This chemical composition slows down their decomposition, which in turn may favor the incorporation of phenolic units generated by enzymatic oxidation, and possibly of quinones generated from the breakdown of phenoxy radicals (Martinez et al., 2005) derived from lignin, into humified SOM (Stevenson, 1994; Rasse et al., 2005).

CONCLUSIONS

The major NMR-identifiable components in the HS are aromatics including aromatic C–O, COO/NC = O groups, peptides, carbohydrates, secondary carbohydrates, lignin-derived moieties, nonpolar alkyls, and contribution of nonprotonated, condensed aromatic ring structures, in the aromatic regions. The eucalypt plantations led to a greater contribution of nonpolar alkyl C groups in HA and FA in comparison to those from native vegetation soils. On average eucalypt cultivation decreases HS aromaticity when compared to HS from the native vegetation soils.

ACKNOWLEDGMENTS

REFERENCES

Alcântara, F.A., P. Buurman, A.E. Furtini Neto, N. Curi, and R. Roscoe. 2004. Conversion of grassy cerrado into riparian forest and its impact on soil organic matter dynamics in an Oxisol from southeast Brazil. Geoderma 123:305–317. doi:10.1016/j.geoderma.2004.02.014

Baldock, J.A., J.M. Oades, A.M. Vassallo, and M.A. Wilson. 1990. Solid-state CP/MAS 13C N.M.R. Analysis of bacterial and fungal cultures isolated from a soil incubated with glucose. Aust. J. Soil Res. 28:213–225. doi:10.1071/SR9900213

Baldock, J.A., J.M. Oades, A.G. Waters, X. Peng, A.M. Vassallo, and M.A. Wilson. 1992. Aspects of the chemical structure of soil organic materials as revealed by solid-state 13C NMR spectroscopy. Biogeochem. 16:1–42.

Bayer, C., L. Martin-Neto, J. Mielniczuk, and C.A. Ceretta. 2000. Effect of no-till cropping systems on soil organic matter in a sandy clay loam Acrisol from Southern Brazil monitored by electron spin resonance and nuclear magnetic resonance. Soil Tillage Res. 53:95–104. doi:10.1016/S0167-1987(99)00088-4

Binkley, D., J. Kaye, M. Barry, and M.G. Ryan. 2004. First-rotation changes in soil carbon and nitrogen in a eucalyptus plantation in Hawaii. Soil Sci. Soc. Am. J. 68:1713–1719. doi:10.2136/sssaj2004.1713

Brewer, C.E., K. Schmidt-Rohr, J.A. Satrio, and R.C. Brown. 2009. Characterization of biochar from fast pyrolysis and gasification. Environ. Prog. Sust. Energy 28:386–396. doi:10.1002/ep.10378

Conte, P., C. De Pasquale, E.H. Novotny, G. Caponetto, V.A. Laudicina, M. Ciofalo, M. Panno, E. Palazzolo, L. Badalucco, and G. Alonzo. 2010. CPMAS 13C NMR characterization of leaves and litters from the reafforestated area of Mustigarufi in Sicily (Italy). Open Magn. Reson. J. 3:89–95.

Dick, D.P., C.N. Gonçalves, R.S.D. Dalmolin, H. Knicker, E. Klamt, I. Kögel-Knabner, M.L. Simões, and L. Martin-Neto. 2005. Characteristics of soil organic matter of different Brazilian ferralsols under native vegetation as a function of soil depth. Geoderma 124:319–333. doi:10.1016/j.geoderma.2004.05.008

Dieckow, J., C. Bayer, P.C. Conceicao, J.A. Zanatta, L. Martin-Neto, D.B.M. Milori, J.C. Salton, M.M. Macedo, J. Mielniczuk, and L.C. Hernan. 2009. Land use, tillage, texture and organic matter stock and composition in tropical and subtropical Brazilian soils. Eur. J. Soil Sci. 60:240–249. doi:10.1111/j.1365-2389.2008.01101.x

Dieckow, J., J. Mielniczuk, H. Knicker, C. Bayer, D.P. Dick, and I. Kogel-Knabner. 2005. Composition of organic matter in a subtropical Acrisol as influenced by land use, cropping and N fertilization, assessed by CP/MAS 13CNMR spectroscopy. Eur. J. Soil Sci. 56:705–715.

Dixon, W.T. 1982. NMR spectra in spinning sidebands (TOSS). J. Chem. Phys. 77:1800–1809. doi:10.1063/1.444076

Glaser, B., E. Balashov, L. Haumaier, G. Guggenberger, and W. Zech. 2000. Black carbon in density fractions of anthropogenic soils of the Brazilian Amazon region. Org. Geochem. 31:669–678. doi:10.1016/S0146-6380(00)00044-9

Gonçalves, C.N., R.S.D. Dalmolin, D.P. Dick, H. Knicker, E. Klamt, and I. Kogel-Knabner. 2003. The effect of 10% HF treatment on the resolution of CP/MAS C-13 NMR spectra and on the quality of organic matter in ferralsols. Geoderma 116:373–392. doi:10.1016/S0016-7061(03)00119-8

González-Pérez, M., L. Martin-Neto, S.C. Saab, E.H. Novotny, D.B.P. Milori, V.S. Bagnato, L.A. Colnago, W.J. Melo, and H. Knicker. 2004. Characterization of humic acids from a Brazilian oxisol under different tillage systems by EPR, 13C NMR, FTIR and fluorescence spectroscopy. Geoderma 118:181–190. doi:10.1016/S0016-7061(03)00192-7

González-Pérez, M., P. Vidal-Torrado, L.A. Colnago, L. Martin Neto, L. Otero, D.B.P. Milori, and F.H. Gomes. 2008. 13C NMR and FTIR spectroscopy characterization of humic acids in spodosols under tropical rain forest in southeastern Brazil. Geoderma 146:425–433. doi:10.1016/j.geoderma.2008.06.018

Guggenberger, G., W. Zech, L. Haumaier, and B.T. Christensen. 1995. Land-use effects on the composition of organic matter in particle-size separates of

soils: II. CPMAS and solution ^{13}C NMR analysis. Eur. J. Soil Sci. 46:147–158. doi:10.1111/j.1365-2389.1995.tb01821.x

Hatcher, P.G., M. Schnitzer, L.W. Dennis, and G.E. Maciel. 1981. Aromaticity of Humic Substances in Soils. Soil Sci. Soc. Am. J. 45:1089–1094. doi:10.2136/sssaj1981.03615995004500060016x

Hu, W.-G., J. Mao, B. Xing, and K. Schmidt-Rohr. 2000. Poly(methylene) crystallites in humic substances detected by nuclear magnetic resonance. Environ. Sci. Technol. 34:530–534. doi:10.1021/es990506l

Kaal, J., S. Brodowski, J.A. Baldock, K.G.J. Nierop, and A.M. Cortizas. 2008. Characterization of aged black carbon using pyrolysis-GC/MS, thermally assisted hydrolysis and methylation (THM), direct and cross-polarization ^{13}C nuclear magnetic resonance (DP/CP NMR) and the benzenepolycarboxylic acid (BPCA) method. Org. Geochem. 39:1415–1426. doi:10.1016/j.orggeochem.2008.06.011

Knicker, H., K.U. Totsche, G. Almendros, and F.J. González-Vila. 2005. Condensation degree of burnt peat and plant residues and the reliability of solid-state VACP MAS ^{13}C NMR spectra obtained from pyrogenic humic material. Org. Geochem. 36:1359–1377. doi:10.1016/j.orggeochem.2005.06.006

Lima, A.M.N., I.R. Silva, J.C.L. Neves, R.F. Novais, N.F. Barros, E.S. Mendonça, T.J. Smyth, M.S. Moreira, and F.P. Leite. 2006. Soil organic carbon dynamics following afforestation of degraded pastures with eucalyptus in southeastern Brazil. For. Ecol. Manage. 235:219–231. doi:10.1016/j.foreco.2006.08.331

Mao, J.-D., X.W. Fang, K. Schmidt-Rohr, A.M. Carmo, L.S. Hundal, and M.L. Thompson. 2007. Molecular-scale heterogeneity of humic acid in particle-size fractions of two Iowa soils. Geoderma 140:17–29. doi:10.1016/j.geoderma.2007.03.014

Mao, J-D., W.-G. Hu, K. Schmidt-Rohr, G. Davies, E.A. Ghabbour, B. Xing, 2000. Quantitative characterization of humic substances with solid state NMR. Soil Sci. Soc. Am. J. 64:873–884. doi:10.2136/sssaj2000.643873x

Mao, J.-D., D.C. Olk, X. Fang, Z. He, and K. Schmidt-Rohr. 2008. Influence of animal manure application on the chemical structures of soil organic matter as investigated by advanced solid-state NMR and FT-IR spectroscopy. Geoderma 146:353–362. doi:10.1016/j.geoderma.2008.06.003

Mao, J.-D., and K. Schmidt-Rohr. 2004a. Accurate quantification of aromaticity and unprotonated aromatic carbon fraction in natural organic matter by ^{13}C solid state NMR. Environ. Sci. Technol. 38:2680–2684. doi:10.1021/es034770x

Mao, J.-D., and K. Schmidt-Rohr. 2004b. Separation of aromatic-carbon ^{13}C NMR signals from di-oxygenated alkyl bands by a chemical-shift-anisotropy filter. Solid State Nucl. Magn. Reson. 26:36–45. doi:10.1016/j.ssnmr.2003.09.003

Mao, J.-D., and K. Schmidt-Rohr. 2005. Methylene spectral editing in solid-state NMR by three-spin coherence selection. J. Magn. Reson. 176:1–6. doi:10.1016/j.jmr.2005.04.016

Mao, J.-D., B. Xing, and K. Schmidt-Rohr. 2001. New structural information on a humic acid from two-dimensional 1H–13C correlation solid-state nuclear magnetic resonance. Environ. Sci. Technol. 35:1928–1934. doi:10.1021/es0014988

Martinez, Á.T., M. Speranza, F.J. Ruiz-Dueñas, P. Ferreira, S. Camarero, F. Guillén, M.J. Martínez, A. Gutiérrez, and J.C. Del Río. 2005. Biodegradation of lignocellulosics: Microbial, chemical, and enzymatic aspects of the fungal attack of lignin. Int. Microbiol. 8:195–204.

Nelson, P.N., and J.A. Baldock. 2005. Estimating the molecular composition of a diverse range of natural organic materials from solid-state ^{13}C NMR and elemental analyses. Biogeochem. 72:1–34. doi:10.1007/s10533-004-0076-3

Neufeldt, H., D.V.S. Resck, and M.A. Yarza. 2002. Texture and land use effects on soil organic matter in Cerrado oxisols, Central Brazil. Geoderma 107:151–164. doi:10.1016/S0016-7061(01)00145-8

Pegoraro, R.F., I.R. Silva, R.F. Novais, N.F. de Barros, and S. Fonseca. 2011. Fenois derivados da lignina, carboidratos e aminoaçucares em serapilheira e solos cultivados com eucalipto e pastagem. Revista Árvore 35:359–370. doi:10.1590/S0100-67622011000200020

Preston, C.M. 1996. Application of NMR to soil organic matter analysis (history and prospects). Soil Sci. 161:144–166. doi:10.1097/00010694-199603000-00002

Preston, C.M., R. Hempfling, H-R. Schulten, M. Schnitzer, J.A. Trofymow, and D.E. Axelson. 1994. Characterization of organic matter in a forest soil of coastal British Columbia by NMR and pyrolysis-field ionization mass spectrometry. Plant Soil 158:69–82. doi:10.1007/BF00007919

Rasse, D.P., C. Rumpel, and M.F. Dignac. 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilization. Plant Soil 269:341–356. doi:10.1007/s11104-004-0907-y

Rumpel, C., K. Eusterhuesa, and I. Kogel-Knabner. 2004. Location and chemical composition of stabilized organic carbon in topsoil and subsoil horizons of two acid forest soils. Soil Biol. Biochem. 36:177–190. doi:10.1016/j.soilbio.2003.09.005

Rumpel, C., N. Rabia, S. Derenne, K. Quenea, K. Eusterhues, I. Kogel-Knabner, and A. Mariotti. 2006. Alteration of soil organic matter following treatment with hydroñuoric acid (HF). Org. Geochem. 37:1437–1451. doi:10.1016/j.orggeochem.2006.07.001

Schmidt-Rohr, K., and J.-D. Mao. 2002. Selective dephasing of OH and NH proton magnetization based on 1H chemical-shift anisotropy recoupling. J. Magn. Reson. 157:210–217. doi:10.1006/jmre.2002.2589

Skjemstad, J.O., J.A. Taylor, and R.J. Smernik. 1999. Estimation of charcoal (char) in soils. Commun. Soil Sci. Plant Anal. 30:2283–2298. doi:10.1080/00103629909370372

Smernik, R.J., and J.M. Oades. 2000. The use of spin counting for determining quantitation in solid-state ^{13}C NMR spectra of natural organic matter. 2. HF-treated soil fractions. Geoderma 96:159–171. doi:10.1016/S0016-7061(00)00007-0

Stevenson, F.J. 1994. Humus chemistry: Genesis, composition, reactions. John Wiley & Sons, New York.

Swift, R.S. 1996. Method for extraction of IHSS soil fulvic and humic acids. In: D.L. Sparks et al., editors, Methods of soil analysis, part 3. Chemical methods. Soil Science Society America Book Series: 5, SSSA, Madison, WI. p. 1018–1020.

Vergütz, L. 2011. Studying the global carbon cycle: Quantification and qualification of soil organic carbon and a meta-analysis on nutrient resorption efficiencies globally. Ph.D. thesis, Universidade Federal de Viçosa, Brazil.

Wilcke, W., and J. Lilienfein. 2004. Soil carbon-13 natural abundance under native and managed vegetation in Brazil. Soil Sci. Soc. Am. J. 68:827–832. doi:10.2136/sssaj2004.0827

Wilson, M.A. 1987. NMR techniques and applications in geochemistry and soil chemistry. Pergamon Press, Oxford.