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Molecular signature and sources of biochemical recalcitrance of organic C in Amazonian Dark Earths

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Abstract

Amazonian Dark Earths (ADE) are a unique type of soils developed through intense anthropogenic activities that transformed the original soils into Anthrosols throughout the Brazilian Amazon Basin. We conducted a comparative molecular-level investigation of soil organic C (SOC) speciation in ADE (ages between 600 and 8700 years B.P.) and adjacent soils using ultraviolet photo-oxidation coupled with ^{13}C cross polarization-magic angle spinning nuclear magnetic resonance (CP-MAS NMR), synchrotron-based Fourier transform infrared-attenuated total reflectance (Sr-FTIR-ATR) and C (1s) near edge X-ray absorption fine structure (NEXAFS) spectroscopy to obtain deeper insights into the structural chemistry and sources of refractory organic C compounds in ADE. Our results show that the functional group chemistry of SOC in ADE was considerably different from adjacent soils. The SOC in ADE was enriched with: (i) aromatic-C structures mostly from H- and C-substituted aryl-C, (ii) O-rich organic C forms from carboxylic-C, aldehyde-C, ketonic-C and quinine-C, and (iii) diverse group of refractory aliphatic-C moieties. The SOC in adjacent soils was predominantly composed of O-alkyl-C and methoxyl-C/N-alkyl-C structures and elements of labile aliphatic-C functionalities. Our study suggests that the inherent molecular structures of organic C due to selective accumulation of highly refractory aryl-C structures seems to be the key factor for the biochemical recalcitrance and stability of SOC in ADE. Anthropogenic enrichment with charred carbonaceous residues from biomass-derived black C (BC) is presumed to be the precursor of these recalcitrant polyaromatic structures. Our results also highlight the complementary role that might be played by organic C compounds composed of O-containing organic C moieties and aliphatic-C structures that persisted for millennia in these anthropic soils as additional or secondary sources of chemical recalcitrance of SOC in ADE. These organic C compounds could be the products of: (i) primary recalcitrant biomolecules from non-BC sources or (ii) secondary processes involving microbial mediated oxidative or extracellular neoformation reactions of SOC from BC and non-BC sources; and stabilized through physical inaccessibility to decomposers due to sorption onto the surface or into porous structures of BC particles, selective preservation or through intermolecular interactions involving clay and BC particles.

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1. INTRODUCTION

Amazonian Dark Earths (ADE) are a unique type of soils apparently developed between 500 and 9000 years B.P. through intense anthropogenic activities such as biomass-burning and high-intensity nutrient depositions on pre-Columbian Amerindian settlements that transformed the original soils into Fimic Anthrosols throughout the Brazilian Amazon Basin (Sombroek, 1966; Smith, 1980; Neves et al., 2003). These anthropic soils have similar texture, mineralogy, and other geochemical signatures with adjacent soils (Zech et al., 1990; Costa and Kern, 1999). However, they can be distinguished by their higher contents of ceramic and lithic artifacts and by their characteristic black color in sharp contrast with the yellowish to reddish adjacent soils. They are also anomalously enriched with P, Mg, Zn, and Mn, have higher water holding capacity, pH, cation exchange capacity (CEC), and thus sustain higher fertility compared to the intensely weathered acidic adjacent soils (Sombroek, 1966; Lehmann et al., 2001).

One of the most remarkable features of ADE, however, is their ability to accumulate large amounts of soil organic C (SOC) and its apparent stability for millennia despite the warm and humid tropical environment, which favors rapid organic matter decomposition and C loss from the soil through net flux to the atmosphere (Glaser et al., 2000, 2001). These unique attributes, coupled with their occurrence in one of the most sensitive ecosystems to global warming, make ADE an important C sink model created through anthropogenic intervention and provide an excellent opportunity to examine several hypotheses related to SOC dynamics and C sequestration in soils. However, most investigations conducted on ADE concentrated mainly on the origin and history (Sombroek, 1966; Smith, 1999; Woods and McCann, 1999), mineralogy (Costa and Kern, 1999), and soil fertility (Sombroek, 1966; Glaser et al., 2001; Lehmann et al., 2003), while little information is available concerning the structural chemistry of organic C in these anthropic soils (Zech et al., 1990; Glaser et al., 2003). Moreover, the mechanisms of SOC stabilization in ADE are unique and although both chemical recalcitrance (Zech et al., 1990) and physico-chemical protection mechanisms (Glaser et al., 2000) were broadly implicated, the actual processes are not yet well understood. The few available studies (Haumaier and Zech, 1995; Glaser et al., 1998, 2003; Schmidt et al., 1999; Skjemstad et al., 2002; Rumpel et al., 2006), however, seem to suggest that chemical resistance to biochemical degradation due to accumulation of aromatic compounds from biomass-derived black C (BC) could be the main factor controlling the stability of SOC in ADE.

Biomass-derived BC represents a suite of refractory carbonaceous residues produced by incomplete combustion of organic matter occurring along a “combustion continuum” ranging from partially charred biomass to charcoal to highly graphitized soot particles (Goldberg, 1985; Masiello and Druffel, 1998; Schmidt and Noack, 2000). A recent study by Glaser et al. (2001) estimated that up to 35% of the total SOC pool in ADE could be constituted by BC, with radiocarbon ages of up to 2000 years. However, since BC represents only part of the total SOC pool and that the structural composition of the bulk SOC accrues from both BC and non-BC constituents, the sources of biochemically recalcitrant organic C compounds and the processes involved in stabilization of the bulk SOC in ADE are still a subject of debate. Madari et al. (2004) stressed this lack of knowledge and pointed out the need for detailed investigations involving the physico-chemical and molecular properties of organic C in these model soils. Such investigations could help to identify and fingerprint the complex SOC functionalities and provide molecular-level evidence to determine whether basic compositional differences exist in the structural chemistry of SOC in ADE and adjacent soils. It may also offer concrete evidence as to whether the conferred biochemical recalcitrance of SOC in ADE is entirely attributed to: (i) enrichment of complex polyaromatic compounds from BC or complemented by (ii) selective preservation of primary recalcitrant biomolecules from plant litter and rhizodeposits, and (iii) secondary processes involving interactions of recalcitrant organic C moieties produced by microbial mediated enzymatic or extracellular neoformation reactions from BC and non-BC sources during soil organic matter (SOM) decomposition (Haumaier and Zech, 1995; Poirier et al., 2000; Lützow et al., 2006). Further impetus for such study arises from the fact that detailed structural chemistry information generated from BC-rich soils could have global level environmental importance, since its formation is often credited as a CO_2 sink by transferring fast-cycling C from the atmosphere–biosphere system into much slower cycling C forms in the geosphere (Kuhlbusch, 1998). Biomass-derived BC could also play a key role in the biogeochemical cycling of elements and due to its amphipathic nature may serve as a carrier of pollutants in soils.

In the present study, we seek to conduct molecular-level investigation of organic C speciation on 600- to 8700-year-old ADE and adjacent soils from the Brazilian Amazon Basin making use of high-energy ultraviolet photo-oxidation coupled with solid-state ^{13}C cross polarization-magic angle spinning nuclear magnetic resonance (CP-MAS NMR), synchrotron-based Fourier transform infrared-attenuated total reflectance spectroscopy (Sr-FTIR-ATR) and C (1s) near edge X-ray absorption fine structure (NEXAFS) spectroscopy techniques. Since chemical recalcitrance occurs at the molecular-level, information generated from these complimentary spectroscopic techniques is expected to provide deeper insight into the structural chemistry, as well as the nature and sources of refractory SOC compounds in ADE. This approach may help to narrow the current knowledge gap concerning the mechanisms of SOM stabilization and C sequestration in these unique anthropic soils.

2. MATERIALS AND METHODS

2.1. Site description and soil analysis

Amazonian Dark Earths and adjacent soil samples were collected in August, 2004 from four archaeological sites (Hatahara, Lago Grande, Acutuba and Dona Stella) from the central Amazon near Manaus, Brazil (3°8′S, 59°52′W;

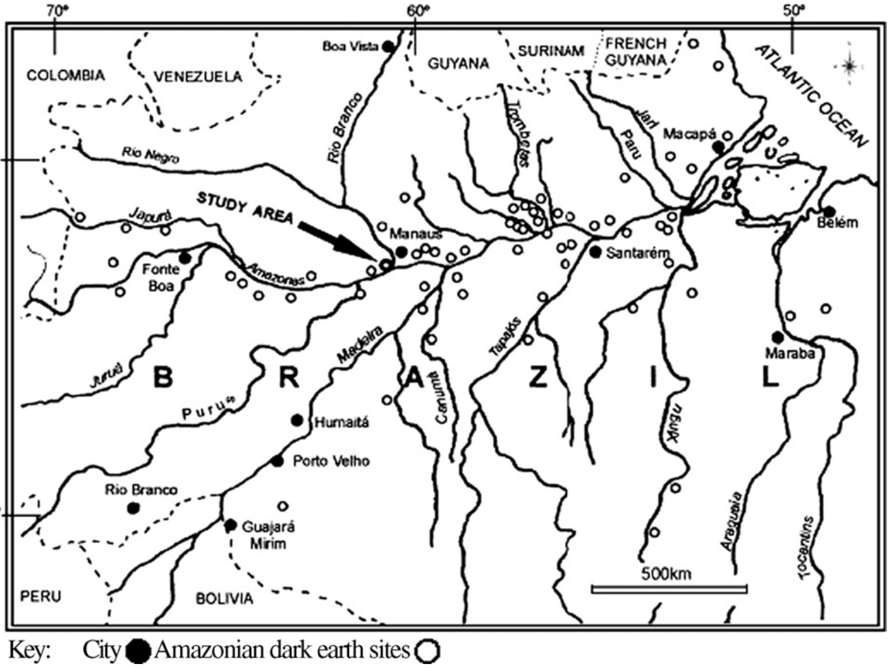

Fig. 1. Distribution of known ADE sites in the Brazilian Amazon Basin and location of the present study sites.

Fig. 1) that have been dated to span from about 600–8700 years B.P. (Table 1, Neves et al., 2003). The altitude of the sites ranges from 40 to 50 m above sea level, with a mean annual temperature of about 26.6 °C, and a mean annual precipitation of about 2400 mm. The climax vegetation of the area is represented by high closed canopy forest with large biomass and high species diversity. The most abundant species at the investigated sites are *Chrysophyllum amazonicum*, *Crepidospermum rhoifolium*, *Anacardium parvifolium*, *Ambelania acida*, *Dinizia excelsa*, *Bocageopsis multiflora*, and *Bertholletia excelsa*. The soils of the area are derived from Tertiary sediments. They are well-drained sandy to clayey textured soils developed either on Oxisols or Ultisols (Soil Survey Staff, 1999). Apart from the Lago Grande site, where ADE samples were found near the surface layer (0–16 cm), all ADE samples were collected from undisturbed buried (Hatahara 43–69 cm, Acutuba 48–83 cm, Dona Stella 190–210 cm) horizons. All samples were air-dried, visible root remnants were removed, and sieved to <2 mm before chemical analysis.

Total organic C and N concentrations were analyzed by dry combustion using Europa ANCA GSL sample combustion unit (PDZ Europa, Crewe, UK). The pH-H_2O and pH-KCl were determined in 1:2.5 soil:solution (w/v) suspension. Total P was measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Thermo Electron Corp., MA, USA) after microwave digestion with concentrated HCl, HNO_3, and HF acids according to EPA 3052 protocol. Selected site characteristics and soil chemical properties are shown in Table 1.

Table 1
Selected site characteristics and soil chemical properties of ADE and adjacent soils from central Amazon

Site	Age (years)	pH		SOC[a] ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	C/N	Total P ($g\ kg^{-1}$)
		H_2O	KCl				
Adjacent soils							
Hatahara	n.m.[b]	4.6	3.8	21.8	1.6	13.6	273
Lago Grande	n.m.	4.2	3.5	17.5	1.3	13.5	251
Acutuba	n.m.	4.7	3.9	15.4	0.8	19.3	198
Dona Stella	n.m.	3.9	2.6	10.2	0.4	25.5	51
Amazonian Dark Earths							
Hatahara	600–1000	6.4	5.5	22.0	1.0	22.0	9064
Lago Grande	900–1100	5.9	4.9	31.5	1.8	17.5	5026
Acutuba	2000–2300	5.6	4.2	15.7	1.0	15.7	777
Dona Stella	6700–8700	5.0	4.1	16.5	1.1	15.0	139

[a] SOC, soil organic carbon.
[b] n.m., not measured.

2.2. Photo-oxidation and ^{13}C nuclear magnetic resonance spectroscopy

The presence of magnetic and paramagnetic materials can cause severe interference with the NMR technique. Therefore, samples from all four sites were treated with 2% HF solution (w/v) prior to ^{13}C NMR analysis. Additionally, an ADE sample from the Lago Grande site was treated separately with high-energy ultraviolet photo-oxidation for 4 h as described by Skjemstad et al. (1996). Solid-state ^{13}C CP-MAS NMR spectra were recorded from all samples at a frequency of 50.3 MHz on a Varian Unity 200 spectrometer (Varian Inc., CA, USA) fitted with a 4.7 T wide-bore Oxford superconducting magnet. Demineralized soil samples were packed into 7 mm diameter zirconia rotors with Kel-F caps and spun at 5 kHz in a Doty Scientific MAS probe (Doty Scientific Inc., SC, USA). All spectra were obtained with 1 ms contact time and 500 ms recycle delay time to ensure complete relaxation between scans (recycle delay $> 7\ T_1H$). The spectra were plotted between -100 and 225 ppm using a Lorentzian line broadening of 50 kHz, and a 0.007 s Gaussian broadening. Chemical shift assignments were externally referenced to the methyl resonance of hexamethylbenzene (Sigma–Aldrich Corp., MO, USA) at 17.36 ppm. The relative proportion of organic C functional groups was determined by integration of the signal intensity of each spectrum over given chemical shift regions as described by Skjemstad et al. (1996). For the relative intensity distribution of the solid-state ^{13}C CP-MAS NMR, the precision was approximately 10% of the signal intensity, depending on the signal-to-noise ratio (Schmid et al., 2002).

2.3. Extraction of humic fractions

Humic fractions from ADE and adjacent soil samples were extracted with a mixture of 0.1 M NaOH and 0.4 M NaF solutions (pH = 12.4) in a 1:5 soil to extraction solution ratio (w/v) under N_2 environment. The extracts were filtered twice through 0.2 μm pore-size membrane filter (Pall Gelman Laboratory, MI, USA) to remove fine clay that may interfere with Sr-FTIR-ATR and NEXAFS measurements (Solomon et al., 2005). The extracts were transferred into dialysis tubes (MWCO 12,000–14,000 Da, Spectrum Laboratories, CA, USA), dialyzed against deionized water, and lyophilized.

2.4. Synchrotron-based fourier transform infrared spectroscopy

Synchrotron-based-FTIR-ATR spectra were recorded on the U10B beamline at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory. The beamline is equipped with a spectra tech continuum IR microscope with 32× transmission/reflection and FTIR step-scan spectrophotometer (Nicolet Magna 860, Thermo Nicolet Corp., WI, USA) fitted with a KBr beam splitter and a mercury-cadmium-telluride (MCT) detector with 500–7000 cm^{-1} frequency range and 1.0 cm^{-1} spectral resolution. Thin films of humic fractions were prepared from aqueous suspensions obtained by dispersing them in millipore water. We transferred 10 μl droplets of this suspension onto IR glass slides (Kevley Techniologies, OH, USA) and dried them at 35 °C. Sr-FTIR-ATR spectra from the samples were recorded with a $10 \times 10\ \mu m^2$ aperture size from 4000 to 650 cm^{-1} frequency range with a 4 cm^{-1} resolution. Each spectrum was composed of 256 scans co-added before Fourier transform. After background subtraction for the IR glass, we used OMNIC version 6.3 (Thermo Nicolete Corp., WI, USA) to correct the baseline, normalize and identify peak positions and calculate signal intensities of the reduced spectra (4000–800 cm^{-1}).

2.5. Near edge X-ray absorption fine structure spectroscopy

NEXAFS spectra were recorded at X-1A1 beamline of the NSLS using the scanning transmission X-ray microscopy (STXM) endstation. The essential components of the STMX are a tunable undulator inserted in the 2.8 GeV electron storage ring generating a high flux of photons at 10^7 spatially coherent photons s^{-1} in the soft X-ray region, a spherical grating monochromator with maximum spectra resolving power of 5000 lines mm^{-1}, a 160-μm Fresnel zone plate with spatial resolution of 45 nm, and a proportional counter to detect the transmitted photons. The beamline slit width was set to $45 \times 25 \times 25$ μm (Spector et al., 1998; Plaschke et al., 2004). The monochromator was calibrated using CO_2 adsorption band (290.74 eV). Thin films of humic fractions were prepared by transferring 3 μl droplets of aqueous suspensions onto 100 nm thick Si_3N_4 windows (Silson Ltd, Northampton, UK). After a high resolution STXM micrograph was taken to locate an area of uniform sample thickness, the illuminated spot was increased to 10 μm by defocusing the zoneplate. Spectra of the samples (I) were recorded from three different spots through the films and SiN_3 windows by moving the grating from 280 to 310 eV on a single spot with 0.1 eV energy step and 120 ms dwell time and averaged. Before each scan, background spectra (I_o) were collected in triplicate from sample-free regions of the Si_3N_4 windows.

For comparison, all NEXAFS spectra were baseline corrected and normalized prior to curve fitting using WinXAS version 3.1 (WinXAS Software, Hamburg, Germany) to avoid spectral dependence on C content (Stöhr, 1996). Hence, spectral properties are indicative of changes in C chemistry. Each normalized spectra was then further resolved into its individual arctangent and Gaussian curve components (G) using the nonlinear least-squares fitting routine SOLVER of Microsoft Excel. Two ionization thresholds were set for C (1s) spectra deconvolution by setting arctangent functions at 290.4 eV for aromatic/aliphatic C (Hitchcock and Ishii, 1987; Hitchcock et al., 1992) and at 292.0 eV for hydroxylated aromatic C with full width at half maximum (FWHM) of 0.4 eV to generate the continuum spectrum up to 310 eV (Schäfer et al., 2005). In order to extract semi-quantitative information from spectral deconvolution, the FWHM of Gaussian peaks was loosely constrained between 0 and 0.5 eV and six Gaussian functions representing the main 1s–π^* and Rydberg/mixed valence transitions were resolved at 284.6 ($G1$), 285.2 ($G2$), 286.6 (*G*3), 287.2 (*G*4), 288.6 (*G*5), and 289.6 (*G*6) eV. The positions of the Gaussian peaks were externally referenced using the 1s–π* and 1s–3p/σ* transitions of organic C standards (benzoquinone and benzanthracene for 284.6 eV, gallic acid and benzoic acid for 285.2 eV, vanillin for 286.6 eV, histidine and phenylalanine for 287.2, citric acid for 288.7, arabinose, rhamnose monohydrate and glucuronic acid for 289.6 eV) obtained from a chemical supplier (Sigma–Aldrich Corp., MO, USA). Furthermore, two σ* transitions (292.3, σ^1 and 293.6, σ^2) were simulated by simplified Gaussian shape function with FWHM of <1 and <2 eV, respectively (Cody et al., 1995, 1996), with an average goodness of fit $S(I_{\text{fit}} - I_{\text{measured}})^2$ of about 0.080. Details about the deconvolution procedure can be found in Scheinost et al. (2001), Schäfer et al. (2003, 2005), Scumacher (2005) and Solomon et al. (2003, 2005).

3. RESULTS AND DISCUSSIONS

3.1. Molecular signature of SOC using ^{13}C CP-MAS NMR spectroscopy

Solid-state ^{13}C CP-MAS NMR spectroscopy revealed the presence of extremely heterogeneous organic C molecular structures in ADE and adjacent soils (Fig. 2). We observed distinct peaks of alkyl-C (C–H) species between $\delta_C = 0$–50 ppm representing long-chained polymethylene $(CH_2)_n$ and short-chained or branched terminal methyl (CH_3), methylene (CH_2), and tertiary (CH) or quaternary (C) aliphatic-C structures such as those found in lipids, hemicellulose, and proteins (Skjemstad et al., 1996). Shoulders evident between $\delta_C = 50$–60 ppm were attributed to methoxyl-C ($-OCH_3$) indicative of syringyl and guaiacyl (sinapyl) units of lignin, as well as to N-alkyl-C (CH–NH) species from polypeptides (Kögel-Knabner, 2002). Signals between $\delta_C = 60$ to 95 ppm, with a peak maximum at about $\delta_C = 73$ ppm were ascribed to O-alkyl-C (CHOH, CH_2–OH, CH_2–O–) structures representing carbohydrates mainly due to C2 to C6 structures of cellulose, hemicellulose, and other polysaccharides, as well as to alcohols and ether bonded aliphatic-C structures. Peaks between $\delta_C = 95$–110 ppm represent di-O-alkyl-C structures (O–CH–O) of anomeric-C (C1) in polysaccharides and ketals. The broad signals between $\delta_C = 110$ and 150 ppm were attributed to H- and C-substituted aryl-C (C—H or C=C) mainly resonating from C in substituted phenylpropane, alkylbenzene, and other aromatic rings or to alkenic moieties conjugated with furanoid or aromatic units derived from charcoal, soot, etc (Benzing-Purdie et al., 1985). Chemical shifts between $\delta_C = 150$ and 165 ppm resulted primarily from O-aryl-C (C–O–, C–OH) groups of lignin-derived phenolic compounds such as guaiacyl, syringyl, ρ-coumaryl, and ρ-hydroxyphenyl and to a lesser extent from tannins, aromatic ethers, and N-substituted aromatic-C (Kögel-Knabner, 2002). Carbonyl-C structures resonated between $\delta_C = 165$ and 225 ppm, where signals between $\delta_C = 165$ and 185 ppm were attributed to carboxylic acid (COOH) and amide ($-CONH_2$) groups, while peaks

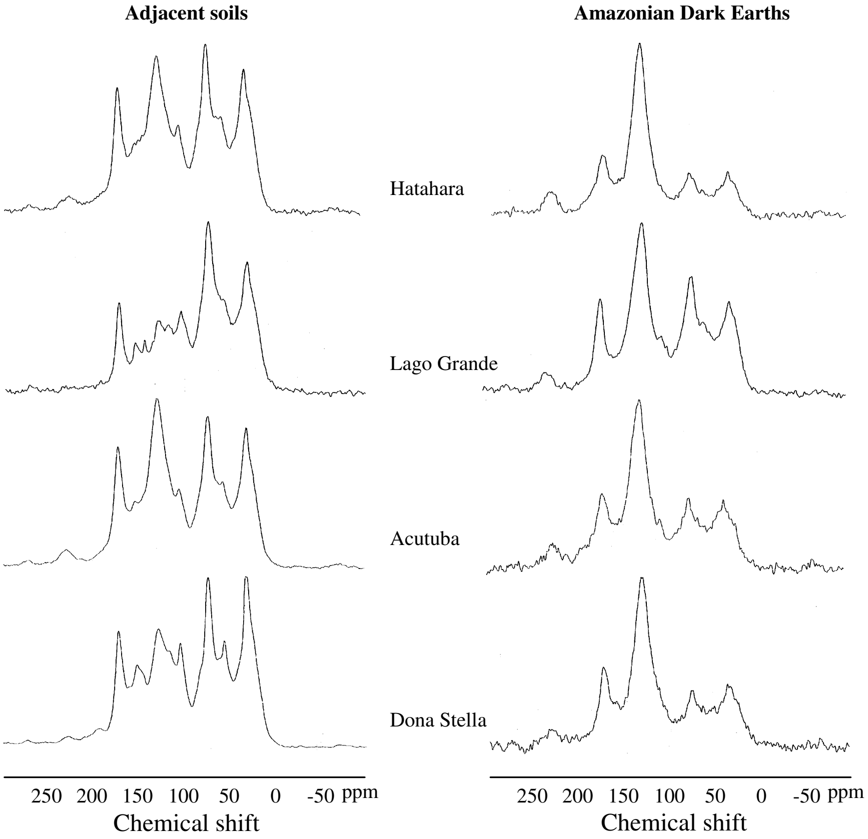

Fig. 2. Stacked ^{13}C CP-MAS NMR spectra of ADE and adjacent soils from the Brazilian Amazon Basin.

between $\delta_C = 185$ and 225 ppm were ascribed to contributions from aldehydes (HC═O), ketones, and quinones (C═O) (Skjemstad et al., 1996; González-Pérez et al., 2004; Knicker et al., 2005). The spectral features of ADE largely resemble those recorded from volcanic ash soils (Hatcher et al., 1989), charred plant materials (Haumaier and Zech, 1995; Trompowsky et al., 2005), and from soils frequently affected by fire (Skjemstad et al., 2002; Knicker et al., 2005).

The results of ^{13}C CP-MAS NMR spectroscopy (Table 2) indicate that the functional group chemistry of SOM in ADE is distinctively different from adjacent soils. Amazonian Dark Earths were largely dominated by aromatic-C structures (30.8–46.8%) compared with adjacent soils (17.6–28.1%). The differences were almost entirely due to contributions from H- and C-substituted aryl-C structures. This was confirmed by the higher degree of aromaticity of SOC [aryl-C/(alkyl-C + methoxyl-C + O-alkyl-C + di-O-alkyl-C + aryl-C + O-aryl-C + carbonyl-C)] observed in ADE (0.25–0.38) compared to adjacent soils (0.10–0.20). Skjemstad and Dalal (1987) showed that sp^2-hybridized C from condensed aromatic rings contributes to the signal around $\delta_C = 130$ ppm. The most probable sources of these condensed ring structures in soils are biomass-derived charred residues and coal-derived materials (Haumaier and Zech, 1995; Skjemstad et al., 1996; Schmidt et al., 1999).

Our results also show that the SOM in ADE (18.5–22.2%) is enriched with carbonyl-C structures compared to adjacent soils (15.4–17.8%). Carboxyl-C rich clusters were also found in humic fractions extracted from BC-rich Palaeosols (Hatcher et al., 1989) and Anthrosols (Glaser et al., 2003), as well as from charred plant materials (Haumaier and Zech, 1995; Trompowsky et al., 2005), and were partly related to partial oxidation of the polyaromatic core of BC. However, since ADE contains variety of organic substrates from different sources, these O-containing compounds could also be partly the results of SOM decomposition from non-BC sources. In fact, microorganisms are known to oxidize plant-derived phenols and polyphenols by splitting their benzene rings to form carboxylic acids (Dagley, 1967). Several types of fungus, particularly "white rot" fungus, were also shown to be able to decompose forest litter and produce oxalic acid, as well as other carboxylic acids (Blaschke, 1979). The non-BC carbonyl-C containing moieties could be stabilized in these anthropic soils through: (i) physical inaccessibility to decomposers due to sorption on the surface or into porous BC structures, (ii) inherent resistance to biochemical oxidation, or (iii) intermolecular interactions involving clay and BC particles that can considerably reduce availability and rate of degradation of these substrates due to complexation and changes in conformation (Chenu and Stotzky, 2002; Guggenberger and Kaiser, 2003; Lehmann et al., 2005). In contrast, smaller proportions of alkyl-C (10.3–13.8%), O-alkyl-C (9.9–17.3%), di-O-alkyl-C (5.3–17.0%), and methoxyl-C/N-alkyl-C (6.3–10.8%) moieties were obtained from ADE compared to adjacent soils (alkyl-C, 16.3–19.0%; O-alkyl-C, 18.0–23.7%; di-O-alkyl-C, 9.4–11.7%; methoxyl-C/N-alkyl-C, 10.3–13.3%). The O-alkyl-C to aryl-C ratio, an index often used to assess relative enrichment of labile polysac-

Table 2
Relative proportions of organic C functional groups resolved by ^{13}C CP-MAS NMR spectroscopy

Site	Alkyl (%)	Methoxyl (%)	O-alkyl (%)	Di-O-alkyl (%)	Aryl (%)	O-aryl (%)	Carbonyl (%)	Aromaticity	O-Alkyl-C/Aryl-C	Alkyl-C/O-alkyl-C
Adjacent soils										
Hatahara	16.3	11.0	19.0	10.2	17.6	8.1	17.8	0.18	1.08	0.85
Lago Grande	18.3	13.3	23.7	11.7	10.3	7.3	15.4	0.10	2.29	0.77
Acutuba	16.6	10.3	18.0	9.4	20.1	8.1	17.5	0.20	0.90	0.92
Dona Stella	19.0	12.0	18.9	11.7	13.1	9.2	16.2	0.13	1.44	1.01
Amazonian Dark Earths										
Hatahara	10.3	6.3	10.1	5.3	38.0	8.8	21.2	0.38	0.27	1.02
Lago Grande	13.8	10.8	17.3	8.7	24.9	5.9	18.5	0.25	0.69	0.80
Acutuba	12.5	8.5	12.0	8.6	27.5	8.8	22.1	0.27	0.44	1.04
Dona Stella	10.8	7.3	9.9	17.0	27.9	7.9	19.2	0.28	0.36	1.09

charides over the more recalcitrant aryl-C structures in SOM (Baldock et al., 1997), was much higher in adjacent soils (0.90–2.29) than in ADE (0.27–0.69).

Numerous studies have indicated the presence of recalcitrant aliphatic-C structures in soils (Baldock et al., 1997; Poirier et al., 2000) and have suggested that alkyl-C to O-alkyl-C ratio, herein referred to as aliphaticity, can be used to investigate the relative accumulation of these organic C moieties during the decomposition process. We found a slightly higher degree of aliphaticity from ADE at Hatahara and Acutuba sites (1.02–1.09) compared to the corresponding adjacent soils (0.85–0.92), respectively, suggesting relative enrichment of refractory aliphatic-C moieties compared to the more labile O-alkyl-C structures in ADE from the two sites. Additionally, the source of aliphatic-C in adjacent soils seems to be invariably dominated by long-chained polymethylene structures centered near 30 ppm (Fig. 2). However, the ones from ADE seem to be more diverse in nature and include not only long-chained structures but also aliphatic-C species arising from weak resonances near 15 ppm (terminal methyl-C), 20 ppm (methylene-C), and 45 ppm (tertiary-C). Our results compare favorably with the observation by Zech et al. (1990) who attributed the heterogeneous nature of aliphatic-C structures in ADE to the presence of substantial amounts of short-chained, highly branched aliphatic-C moieties. Despite large differences in age, we did not observe systematic differences in the nature and sources of recalcitrant organic C forms of ADE from the four sites.

3.2. Molecular signature of SOC following treatment with high-energy ultraviolet photo-oxidation

The structural composition of recalcitrant organic C moieties and their contribution to the bulk SOM in ADE was further investigated by subjecting an ADE sample from Lago Grande site to a high-energy ultraviolet photo-oxidation procedure (Fig. 3). This technique is designed to remove photo-oxidizable-labile C forms, and examine molecular structures of the remaining more stable SOM using NMR spectroscopy (Skjemstad et al., 1996; Schmidt et al., 1999; Baldock and Smernik, 2002). Our results show that photo-oxidation greatly reduced the proportions of alkyl-C, methoxyl-C/N-alkyl-C, O-alkyl-C, and di-O-alkyl-C structures by 25%, 55%, 54%, and 42%, respectively, an indication that these BC-rich anthropic soils might have potential to stabilize substantial amounts of labile SOC biomolecules for millennia (Fig. 4a). In contrast, the proportions of aryl-C, O-aryl-C, and carbonyl-C structures of photo-oxidation resistant SOM increased by 57%, 67%, and 24%, respectively. The degree of aromaticity and aliphaticity of the remaining SOC increased from 0.25 to 0.39 and from 0.80 to 1.31, respectively, while the O-alkyl-C to aryl-C ratio decreased substantially from 0.69 to 0.20 following photo-oxidation (Fig. 4b). The dominance of H- and C-substituted aromatic blocks following photo-oxidation was further evidence that highly resistant aryl-C structures with low degree of O-substitution seems to be the primary sources of recalcitrance in ADE. Anthropogenic enrichment by charred carbonaceous residues from

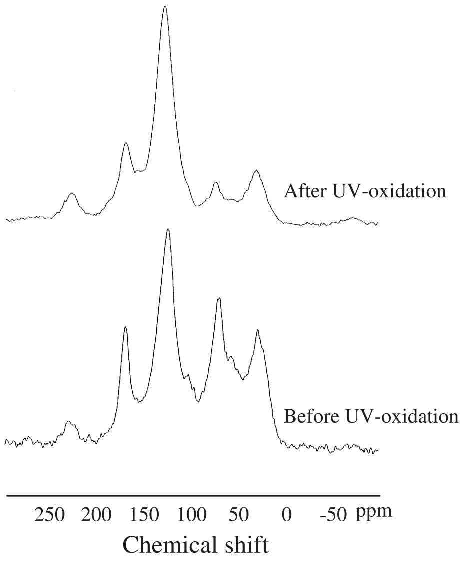

Fig. 3. Stacked ^{13}C CP-MAS NMR spectra of ADE sample from the Lago Grande site recorded before and after high-energy ultraviolet photo-oxidation.

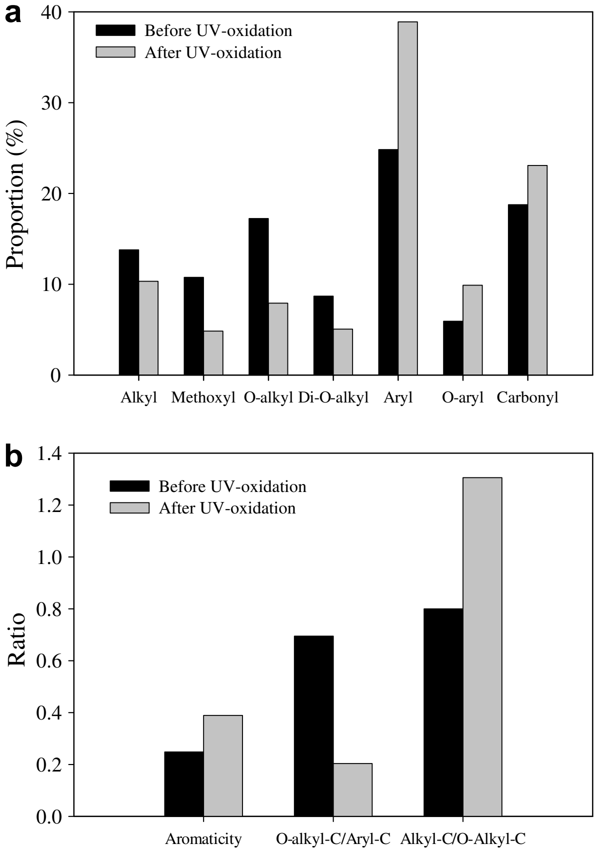

Fig. 4. Relative proportions of organic C functional groups resolved by ^{13}C CP-MAS NMR spectroscopy (a), aromaticity and aliphaticity indexes and O-alkly-C to aryl-C ratio (b) before and after treatment of ADE sample from the Lago Grande site with high-energy ultraviolet photo-oxidation.

biomass-derived BC seems to be the main precursors of these highly refractory polyaromatic structures. This conclusion concurs with the suggestions of Haumaier and Zech (1995), Skjemstad et al. (1996), Schmidt and Noack (2000), and Glaser et al. (2001). Our results also highlight the role that may be played by organic C compounds containing carbonyl-C, O-substituted aryl-C, as well as by resonances normally attributed to aliphatic-C structures that survived photo-oxidation as an additional or complementary source of chemical and structural stability of SOC in ADE. These organic C moieties could be the products of changes induced by decomposition and humification processes on SOC from BC and non-BC sources through condensation reactions or in situ conversions of mobile to rigid alkyl-C structures, as well as through reactions involving selective stabilization of refractory organic C structures from plant residues, rhizodeposits or microbial metabolites in these anthropic soils (Kögel-Knabner et al., 1992).

3.3. Molecular signature of SOC in humic fractions using Sr-FTIR-ATR spectroscopy

Synchrotron-based FTIR-ATR (Fig. 5) displayed a variety of diagnostic absorption band patterns between the frequency range of 4000 to 800 cm^{-1} representing molecular structures present in the humic fractions extracted from ADE and adjacent soils. The broad absorption bands at about 3386 cm^{-1} were assigned to O–H stretching vibrations of H-bonded hydroxyl (OH) groups of phenols and alcohols and to traces of N–H stretching from amide and amine groups (Stevenson, 1994). The weak absorption features near 2929 and 2852 cm^{-1} represent asymmetric and symmetric C–H stretching vibrational motions of aliphatic-C (CH_3 and CH_2) (Almendros et al., 2003). The intense absorption bands at about 1639 cm^{-1} were mainly attributed to aromatic-C (C=C) vibrations and to a smaller extent to C=O stretching in conjugated carbonyl-C structures such as quinones and ketonic acids (Lehmann et al., 2005). The peaks around 1384 cm^{-1} represent aliphatic-C (C–H) deformations of CH_2 or CH_3 groups (Stevenson, 1994). The absorption bands around 1261 cm^{-1} could be attributed to C–O stretching and O–H deformation of carboxylic-C (COOH) (Almendros et al., 2003). The strong signals at about 1053 cm^{-1} originated from C–O stretching vibrations of polysaccharides (Solomon et al., 2005).

Comparisons of Sr-FTIR-ATR spectra exhibited distinct differences in terms of specific spectral band intensities between ADE and adjacent soils. The most prominent

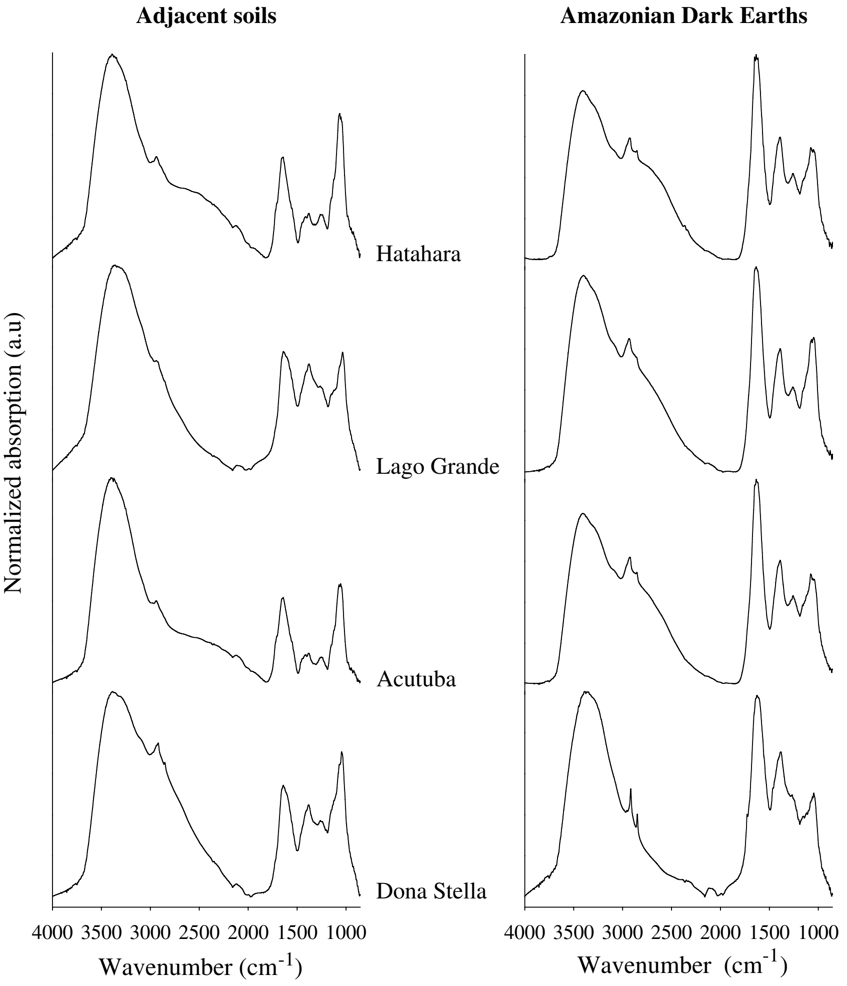

Fig. 5. Stacked Sr-FTIR-ATR spectra of humic fractions extracted from ADE and adjacent soils from the Brazilian Amazon Basin.

changes in signal intensities reflecting differences in molecular structures of organic C appeared near 1639, 2929, 2852, 1384, and 1053 cm^{-1} (Fig. 5). Amazonian Dark Earths were largely dominated by C=C vibrations of aromatic-C and C=O stretching of quinones and ketones, asymmetric, and symmetric C–H stretching and C–H deformation of aliphatic-C, as well as C–O stretching and OH deformation of carboxylic-C compared to the proportions of these organic C structures in adjacent soils (Table 3). The degree of aromaticity (0.27–0.30) and aliphaticity (1.91–3.29) were also much higher in ADE compared to the values of these indexes in adjacent soils (0.14–0.19 and 1.01–2.62, respectively). Although contributions from selective enrichment of highly carbonized primary and secondary aromatic-C components cannot be excluded, the dominance of refractory aromatic-C moieties in ADE could be primarily the product of fire-induced endothermic neoformation reactions involving dehydration and subsequent rearrangement of organic C compounds such as carbohydrates, amino acids and unsaturated alkyl chain structures (Knicker et al., 1996; Freitas et al., 1999; Almendros et al., 2003). Therefore, it is possible to suggest that fire-induced alterations could lead to transformation of originally labile organic C compounds to biochemically recalcitrant C forms that can contribute to long-term sequestration of C in soils. The abundant contributions from O-containing-C and aliphatic-C structures to the Sr-FTIR-ATR spectral features of the anthropic soils, however, were an indication that anthropogenic deposition of charred residues alone cannot be accountable for the observed structural heterogeneity and stability of SOC in ADE. This suggestion is in accordance with the fact that biochemical processes are normally credited to formation of carboxyl-containing molecular constituents and that thermal treatments have been shown to preferentially remove external O-groups from charred organic materials (Almendros et al., 1992). Similarly, high temperature has a tendency to selectively decrease the chain-length of lipids, and absorption bands representing aliphatic-C functionalities are normally absent in FTIR spectra of humic fractions extracted from charcoal (González-Pérez et al., 2004). In fact, the origin and formation pathways of these aliphatic-C moieties in stable SOM fractions remain largely unknown (Poirier et al., 2000). However, different sources varying from highly aliphatic molecular components such as cutans and suberans from higher plants (Augris et al., 1998) to microorganisms (van Bergen et al., 1997) and cross-linkage of plant and microbial components (Kögel-Knabner et al., 1992) have been considered. The higher proportion of carboxylic-C and aliphatic-C functionalities observed in ADE using Sr-FTIR-ATR spectroscopy seem, therefore, to provide further evidences that recalcitrant organic C compounds containing these functional groups could be partly the products of mechanisms predominantly involving microbial or enzymatic oxidative processes of SOM from non-BC sources.

Analogous to NMR spectroscopy, the relative proportions of signals intensities of O–H vibrations from phenols, alcohols, and amides (22.3–38.8%) and C–O stretching vibrations of polysaccharides (15.8–22.6%) recorded by Sr-FTIR-ATR spectroscopy from adjacent soils were much

Table 3
Relative proportions of the various organic C functional groups resolved by Sr-FTIR-ATR spectroscopy

Site	Phenolic (%)	Aliphatic (%)	Aromatic (%)	Aliphatic (%)	Carboxylic (%)	Polysaccharide (%)	Aromaticity	O-alkyl-C/ aromatic-C[a]	Aliphatic-C/ O-alkyl-C
Adjacent soils									
Hatahara	31.9	15.8	15.8	7.0	6.8	22.6	0.19	1.43	1.01
Lago Grande	27.6	14.8	16.0	14.3	11.4	15.9	0.19	0.99	1.83
Acutuba	38.8	15.6	16.2	5.7	4.9	18.8	0.19	1.16	1.13
Dona Stella	22.3	31.4	12.1	10.0	8.3	15.8	0.14	1.30	2.62
Amazonian Dark Earths									
Hatahara	18.3	25.1	22.0	13.3	9.4	12.0	0.28	0.54	3.21
Lago Grande	18.2	25.5	21.9	13.2	9.5	11.8	0.28	0.54	3.29
Acutuba	22.4	15.2	23.4	14.0	9.7	15.3	0.30	0.66	1.91
Dona Stella	21.6	20.1	21.3	15.3	10.8	11.0	0.27	0.52	3.23

[a] O-alkyl-C/aryl-C = polysaccharide-C to aromatic-C ratio.

higher than the proportions of these moieties from ADE (18.2–22.4%, 11.0–15.3%, respectively). We also observed higher polysaccharide-C to aromatic-C ratios in adjacent soils (0.99–1.43) compared to the values of this index in ADE (0.52–0.66, Table 3). The fact that potentially labile organic C structures, most probably from direct deposition of plant, animal and microbial residues, dominate the Sr-FTIR-ATR spectral features of adjacent soils in contrast to ADE was an indication that the sources of SOC, and consequently structural composition of the resultant SOM in ADE was considerably modified by prehistoric anthropogenic interventions stemming from pre-Columbian Amerindian settlements.

3.4. Molecular signature of SOC in humic fractions using C (1s) NEXAFS spectroscopy

Recent investigations using synchrotron-based C (1s) NEXAFS spectroscopy have shown that this technique is a powerful, noninvasive method that can be used not only to identify and fingerprint complex molecular structures but also to investigate the impact of human intervention on the composition and biogeochemical cycling of organic C in terrestrial ecosystems (Scheinost et al., 2001; Jokic et al., 2003; Schäfer et al., 2003; Solomon et al., 2005, 2007). The main 1s–π^* and Rydberg/mixed valence transitions in the fine structure regions of C K-edge spectra recorded from ADE and adjacent soils span from an energy range of 284 to 310 eV. Since fine structures in the C (1s) NEXAFS region above 290 eV transitions tend to be broad and overlap with each other (Cody et al., 1998; Schäfer et al., 2003), only the main 1s–π^* transitions were used for interpretation of the NEXAFS results. The C (1s) spectra (Fig. 6) exhibited good selectivity in specific energy regions that correspond to characteristic functional groups. This allowed us not only to fingerprint but also semi-quantitatively compare the various functional groups to gain a deeper insight into the structural chemistry of organic C in ADE and adjacent soils. The absorption bands near 284.6 eV correspond to the C 1s–π^* transition of quinone type-C (C=O) structures such as benzoquinone, protonated, and alkylated aromatic-C and heteroatom-substituted aromatics (Jokic et al., 2003; Schäfer et al., 2005; Solomon et al., 2005). The strong resonance near 285.2 eV was related to protonated and alkylated to carbonyl-substituted aryl-C (C=C) and possibly to olefinic-C (Cody et al.,

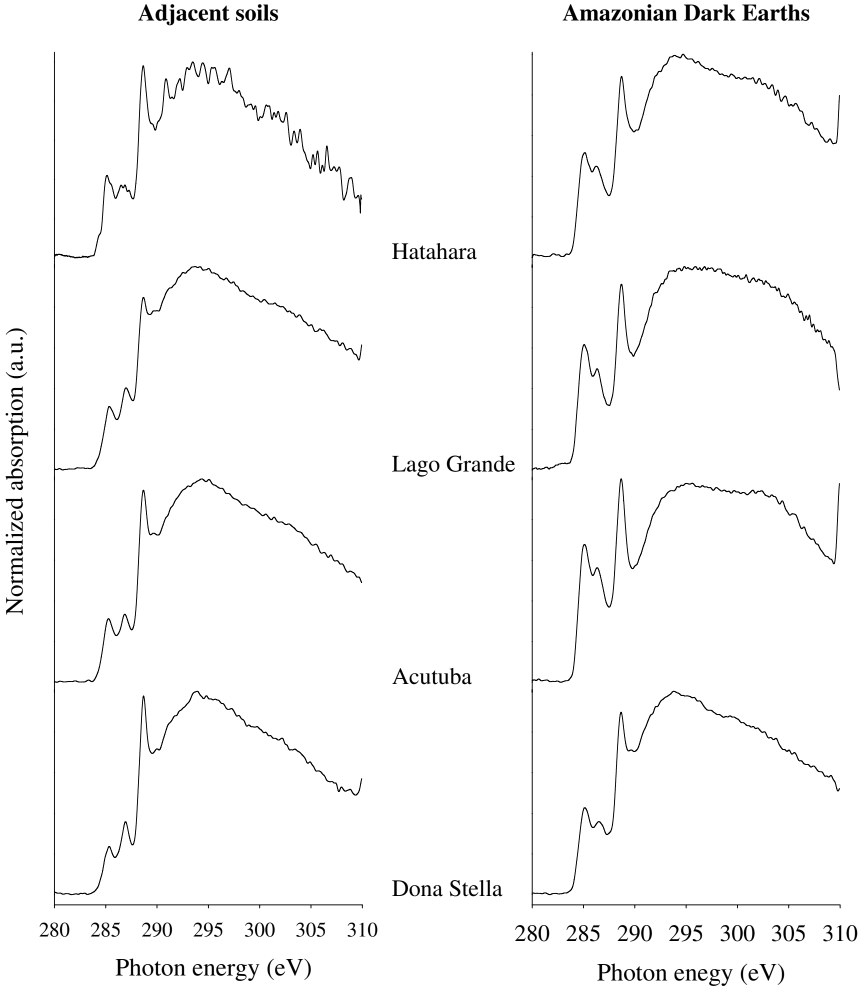

Fig. 6. Stacked C (1s) NEXAFS spectra of humic fractions extracted from ADE and adjacent soils from the Brazilian Amazon Basin.

1998; Ade and Urquhart, 2002; Lehmann et al., 2005). The sum of C moieties near 284.6 and 285.2 is used to represent total aromatic-C identified by NEXAFS in subsequent discussions throughout this paper. The absorption bands evident near 286.6 eV were primarily assigned to phenolic-C (O–H) including O-substituted aryl-C (C–O–, C–OH) indicative of lignin and possibly ketonic-C and phenyl-C attached to amide (N–H) groups (Cody et al., 1998; Rothe et al., 2000; Ade and Urquhart, 2002). The 1s–3p/σ^* transition near 287.2 eV were due to C–H aliphatic-C of CH_3, CH_2, and CH nature, while the strong absorption bands near 288.6 eV represents C 1s–π^* transition of carboxylic-C (COOH) groups (Cody et al., 1998; Braun et al., 2005). The resonances near 289.6 eV were attributed to C 1s–π^* transitions of O-alkyl-C (C–OH) moieties representing mainly polysaccharides and smaller proportions of alcohol and ether-C (Scheinost et al., 2001; Solomon et al., 2005).

From the stacked NEXAFS spectra (Fig. 6) and relative proportions of C forms obtained by spectral deconvolution (Table 4), it is apparent that C 1s–π^* transition of carboxylic-C was the most prominent form of organic C functional group representing from 36.3% to 38.8% and from 38.2% to 42.1% of the total SOC identified by NEXAFS from ADE and adjacent soils, respectively. This provides a strong indication that the SOC from BC and non-BC sources has undergone a considerable biochemical oxidation and a substantial amount of carboxylic acid containing structures were accumulated in these anthropic soils. This observation is in accordance with the results of our previous investigation using NEXAFS spectroscopy, where we reported the presence of spatially distinct highly oxidized regions on the surface of BC particles (Lehmann et al., 2005), and which led us to conclude that these features could be evidence of progressive surface oxidation of charred particles or an indication of surface adsorption and stabilization of oxidized organic C moieties from non-BC sources. Examination of NEXAFS spectral features further revealed that absorption intensities due to C 1s–π^* transition of quinone plus aryl-C moieties were much stronger in ADE (19.6–28.7%) than adjacent soils (12.2–18.7%) reflecting the larger intrinsic aromatic-C content of SOM in ADE; most probably through deposition of BC by anthropogenic means. Spectral deconvolution showed that up to 93% of these aromatic-C structures were the result of contributions from C 1s–π^* transition of protonated, alkylated to carbonyl-substituted aryl-C (C=C) moieties, and that quinone-type C species contribute only to a smaller proportion of the total aromatic-C signal (Table 4). This has been confirmed by the higher degree of aromaticity of SOC identified by C (1s) NEXAFS spectroscopy from ADE (0.20–0.29) compared to the values obtained from the corresponding adjacent soils (0.12–0.19). Additionally, higher proportions of phenolic-C (O–H) structures that are indicative of O-substituted aryl-C (C–O–, C–OH) in lignin and ketonic-C moieties and phenyl-C attached to amide (N–H) groups were obtained from ADE (14.0–17.3) than adjacent soils (9.4–10.9). On the other hand, the relative proportions of organic C species resolved from the C (1s) spectra (Table 4) indicate that adjacent soils were richer in O-alkyl-C structures (20.0–25.9%) such as those

Table 4
Relative proportions of the various organic C functional groups resolved by C (1s) NEXAFS spectroscopy

Site	Quinone (%)	Aryl-C (%)	Phenolic (%)	Aliphatic (%)	Carboxylic (%)	O-alkyl-C (%)	Aromaticity	O-alkyl-C/ aromatic-C	Aliphatic-C/ O-alkyl-C
Adjacent soils									
Hatahara	0.0	18.7	9.7	10.0	41.6	20.0	0.19	1.07	0.50
Lago Grande	0.8	14.6	10.6	11.3	38.2	24.5	0.15	1.59	0.46
Acutuba	0.0	14.6	9.4	8.0	42.1	25.9	0.15	1.77	0.31
Dona Stella	0.1	12.1	10.9	9.6	41.9	25.4	0.12	2.09	0.38
Amazonian Dark Earths									
Hatahara	3.6	20.1	17.3	7.7	36.3	14.9	0.24	0.63	0.52
Lago Grande	4.9	23.8	16.4	8.1	36.8	10.0	0.29	0.35	0.80
Acutuba	3.1	25.0	17.1	9.9	36.8	8.1	0.28	0.29	1.23
Dona Stella	1.4	18.2	14.0	7.2	37.9	21.3	0.20	1.09	0.34

found in carbohydrates or in cellulose compared to samples collected from ADE (8.1 to 21.3). This has been corroborated by the relatively higher O-alkyl-C to aromatic-C ratio from adjacent soils (1.07–2.09) than the ones from ADE (0.29–1.09). The deconvolution of the C (1s) spectra showed similar proportions of C 1s–3p/σ^* transition of aliphatic-C structures in the ADE (7.2–9.9%) and adjacent soils (8.0–11.3%). However, with the exception of Dona Stella site, a relatively higher degree of aliphaticity was found from ADE (0.52–1.23) than adjacent soils (0.31–0.50), suggesting that the bulk SOM in ADE is at an advanced stage of humification and that the more labile aliphatic components have probably been decomposed at this stage. Thus the appreciable amounts of aliphatic-C moieties observed in these anthropic soils could most probably be contributions from the more refractory aliphatic-C biopolymers of non-BC origin. These results complement our observations using ^{13}C NMR and Sr-FTIR-ATR spectroscopy of SOC from ADE.

4. CONCLUSIONS

Using integrated solid-state ^{13}C CP-MAS NMR and Sr-FTIR-ATR and C (1s) NEXAFS spectroscopy, we were able to identify the various organic C functionalities and effectively fingerprint the structural composition of SOC in ADE. The molecular-level information generated by these complementary spectroscopic techniques led us to conclude that the structural composition of SOC in ADE was markedly altered due to anthropogenic interventions and that the functional group chemistry of SOM in ADE is considerably different from adjacent soils. Amazonian Dark Earths were largely dominated by: (i) aromatic-C structures stemming from H- and C-substituted aryl-C, (ii) O-rich organic C functionalities possibly from carboxylic-C, aldehyde-C, ketonic-C, and quinine-C, and (iii) a diverse group of refractory aliphatic-C moieties. The SOC in adjacent soils was enriched with O-alkyl-C, methoxyl-C/N-alkyl-C structures, as well as with some elements of labile aliphatic-C functionalities. Our study suggests that intrinsic molecular structures of organic C due to selective accumulation of highly refractory aryl-C polymers seems to be the key factor for the biochemical recalcitrance and the conferred stability of SOC in ADE. Anthropogenic enrichment by charred carbonaceous residues from biomass-derived BC seems to be the main source of these highly refractory polyaromatic structures. The abundance of O-rich organic C structures in ADE could partly be the result of partial oxidation of the polyaromatic core of biomass-derived BC particles. However, since partial oxidation of polyaromatic structures alone cannot account for the large proportions of O-containing organic C functionalities, as well as for aliphatic-C components that persisted for millennia in these anthropic soils, our results also highlighted the complementary role that might be played by organic C compounds composed of these moieties as an additional or secondary sources of chemical recalcitrance of C in ADE. These organic C compounds could be the products of: (i) primary recalcitrant biomolecules from non-BC sources or (ii) secondary processes involving microbially mediated oxidative or extracellular neoformation reactions of organic C from non-BC and BC sources, and stabilized either through physical inaccessibility to decomposers due to sorption on the surface or into porous structures of BC particles, inherent resistance to biochemical oxidation, or intermolecular interactions involving clay and BC particles that can considerably reduce availability and rate of degradation of these substrates due to complexation and changes in conformation. The presence of large proportions of aromatic-C, carboxylic-C, and aliphatic-C structures and their persistence for millennia in these BC-rich anthropic soils is of wider ecological importance at the global level, not only from the standpoint of serving as a sink for fast-cycling C, but also from agricultural and environmental sustainability perspective since: (i) carboxylic-C and aromatic OH contents could influence CEC and the capacity of soils to form complexes with metal ions and (ii) the presence of (poly)methylene structures may promote sorption of non-ionic hydrophobic organic pollutants such as pesticides.

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