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Direct molecular evidence for the degradation and mobility of black carbon in soils from ultrahigh-resolution mass spectral analysis of dissolved organic matter from a fire-impacted forest soil

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Abstract

The molecular composition of water-soluble products generated by the natural degradation of charcoal particles over a period of 100 years in a temperate forest soil has been investigated by ultrahigh resolution mass spectrometry with electrospray ionization. The detectable products are condensed aromatic ring structures extensively substituted with oxygen-containing functional groups, indicating that oxidation and dissolution of charcoal black carbon occurs on a centennial timescale. Many of the same species are also detected within the dissolved organic matter (DOM) of the forest's soil pore waters. We introduce the calculation of carbon normalized double bond equivalents (DBE/C) as a structural determinant for the empirical formulas obtained by mass spectral analysis. A threshold DBE/C value of 0.7 serves as a criterion for identifying species with condensed aromatic ring structures (CARS). A comparison with ultrahigh resolution mass spectra from previous studies shows that many of the CARS extracted directly from soil BC have the same mass (within 1 ppm) and empirical formulas as CARS detected in volcanic ash soil humic acid (HA) from Japan, and Amazonian Rio Negro DOM. The similarity of water-soluble condensed aromatics present within, and exported from fire-impacted soils of geographically and climatically disparate ecosystems indicates that the CARS reported herein are the molecular fingerprint of black carbon degradation in soils. Understanding the production mechanisms, reactivity, and fate of these molecular species should provide new insight to BC degradation and cycling. The soil charcoal particles at this site are infiltrated by filamentous microorganisms, suggesting that saprophytic fungi may be important to soil BC degradation processes.

1. Introduction

The ubiquity and abundance of black carbon (BC) in the environment have become increasingly urgent matters in geochemistry. Rapidly accumulat-

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ing evidence suggests that BC, the refractory organic products of incomplete combustion, account for a substantial portion of molecularly uncharacterized natural organic matter (NOM) (Hedges et al., 2000). The contribution of BC as a percentage of NOM-C is 15–45% in soils (Schmidt et al., 1999) and 3–15% in ocean sediments (Gustafsson and Gschwend, 1998; Masiello and Druffel, 1998). Therefore, understanding the biogeochemical cycling of this carbon pool is essential to our efforts to model global-scale carbon cycling. A number of studies show that BC is an inherently refractory component of geomedia (Skjemstad et al., 1996; Glaser et al., 1998). Still other workers conclude that BC losses from soil and sediment over time are due to chemical degradation (Czimczik et al., 2003), and suggest that factors such as regional climatic conditions and soil properties mediate the residence time of BC (Schneour, 1966; Bird et al., 1999; Glaser and Amelung, 2003).

Despite the relative recalcitrance of BC in soils, it is clear that BC is not inert. For instance, charcoal plays important roles in soil formation (Glaser and Amelung, 2003), fertility (Glaser et al., 2000), humification (Shindo et al., 1986), carbon sequestration (Kuhlbusch and Crutzen, 1996; Glaser and Amelung, 2003), and pollutant availability (Gustafsson et al., 1997). Spectroscopic evidence that natural weathering or oxidative depolymerization of soil charcoal results in the formation of humic substances has been observed for several decades (e.g., Kumada, 1983; Schnitzer and Calderoni, 1985; Shindo et al., 1986; Kramer et al., 2004). The dominant structural features generated from pyrogen humification are condensed aromatics with carboxylic acid functionality. In controlled laboratory experiments, the nitric-acid-oxidation of furnace blacks and charcoals generated similar products: condensed aromatic structures substituted with carboxylic, phenolic, and carbonyl functional groups, with molecular weights in the range ca. 400–1200 Da (Haumaier and Zech, 1995; Kamegawa et al., 2002; Trompowsky et al., 2005). The high cation exchange capacity of these types of structures is thought to be responsible for correlations between soil fertility and BC content observed in tropical terra pretta soils and native grassland soils of North America (Glaser et al., 2000; Glaser and Amelung, 2003).

As discussed by Masiello (2004), natural degradation and mineralization of soil BC is certainly occurring, despite the paucity of evidence from environmental measurements explaining degradation pathways. For instance, Czimczik et al. (2003) showed that physical removal processes such as erosion and translocation through the soil profile can not account for the loss of BC from a boreal forest soil occurring in the 250 years following a biomass burning event. In situ chemical degradation/oxidation is also invoked as an explanation for BC depletion in sedimentary deposits that have become oxic after a period of anoxia (Middleburg et al., 1999). However, degradation mechanisms and byproducts remain marginally characterized at the molecular level and their biogeochemical fate remains unclear.

As the contribution of BC to the alkali-soluble fraction (HA) of soil organic matter is being recognized, Masiello and Druffel (1998) proposed that BC cycles as dissolved organic matter (DOM), and used the radioisotope signature with sedimentation rates to estimate that dissolved BC may comprise as much as 4–22% of marine DOM. Estimates from the thermal isolation of BC in ultrafiltered DOM from the Chesapeake Bay, suggest that BC contributes roughly 4–7% of the DOC in the coastal Atlantic Ocean (Mannino and Harvey, 2004). The first molecular-level evidence of BC-like structures in DOM was observed by Kim et al. (2003a). Specifically, ultrahigh-resolution Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry was used to analyze DOM from the Rio Negro, and a blackwater stream from the New Jersey Pine Barrens, revealing the presence of hydrogen-deficient molecules that are likely to be the soluble products of BC degradation (Kim et al., 2004).

We present evidence that directly links these hydrogen-deficient molecules to the degradation of charcoal BC in the soils of a fire-impacted watershed. We use ultrahigh-resolution electrospray ionization (ESI) FT-ICR to resolve BC degradation products within the soil pore water DOM at the site, and introduce a new structural criterion for identifying the condensed aromatic ring structures (CARS) that are directly linked to BC.

2. Experimental

Soil and water samples were collected during summer 2002 at the University of Michigan Biological Station (UMBS), in Cheboygan County, Michigan, where intense logging and biomass burning during the years 1890–1920 deposited a visible layer of BC to the soils. Soils at the station are excessively-drained, medium sand under a poorly developed, acidic O horizon (~5 cm thick, pH 4.4). Soils are spodsols, specifically, Emmet medium, frigid, Typic Orthods. Development of the hard ornstein layer of organo-mineral complexes within the spodic horizon is not observed here as it is in the Wallace series of the neighboring Mackinac County. Rather, the spodic horizon is very friable, with high hydraulic conductivity and low resistance to root penetration. Soil horizons are as follows: litter, Oe, A, E, Bs, and C.

Establishment of the UMBS in 1909 provided a site where subsequent land-use has been limited to natural regrowth of a mixed-hardwood forest dominated by aspen, red pine, white pine, red maple, and red oak. Major landforms are glacial moraines and outwash planes, and the climate is characterized by long cold winters, short cool summers, with a mean annual temperature and precipitation of 6.7 °C and 79.8 cm, respectively (Vande Kopple, 2005).

Soils were sampled in an undisturbed area of the forest within two 20 × 20 m plots, each divided into quadrants. Within each quadrant, coordinates for sampling were randomly selected. In one quadrant, a trench 100 cm deep was dug for the observation and precise measurement of horizon interfaces. In each of the three remaining quadrants, a 20 × 20 cm area of the Oe and A horizons were excavated. Soil samples were sectioned by horizon (leaf litter, Oe, A), and corresponding horizons from each subsample were combined and allowed to air-dry in solvent-washed aluminum containers. Each horizon was sieved to 2 mm to remove course woody debris. Visible charcoal particles were manually isolated from the >2 mm size fractions of the Oe soil horizon using stainless steel forceps. Before utilizing the air-dried charcoal for experimentation, soil and sand were carefully dislodged from the extremities of each charcoal particle under a stream of compressed nitrogen gas. The result of this time consuming procedure was the isolation of gram quantities of native charcoal BC without thermal or chemical treatment. Particles were then imaged using a JEOL JSM-820 scanning electron microscope.

Forest soil pore waters were sampled after a rain event on 25 June, 2002 via four ceramic tension lysimeters buried at 75 cm soil depth on each corner of a 50 × 50 m plot. All four samples were combined for a total of 1.4 l water volume. Lysimeters were located atop a glacial moraine on the western border of a glacial outwash plane and the town of Pellston Michigan. Water samples were acidified to pH 2–3, stored in acid washed Nalgene™ carboys, and refrigerated until DOM was extracted using C_{18} solid-phase extraction disks (3M, Empore) as in Kim et al. (2003b). Organic matter was eluted from the C_{18} disk in a mixture of HPLC grade methanol and water (90:10 v/v). This mixture was analyzed directly by electrospray ionization mass spectrometry.

Approximately 2 g of the soil charcoal particles isolated from the O soil horizon were placed in a precombusted 4 l amber glass bottle filled with nanopure water (UHQ, Elga, Lowell, MA) adjusted to pH 4.4 with concentrated HCl. The mixture was stirred for 48 h using a Teflon coated magnetic stirrer to extract the water-soluble species. The aqueous phase was passed through a precombusted Whatman GF/F glass fiber filter (0.7 μm pore size), and the dissolved organic carbon content of the filtrate was determined using a Shimadzu TOC 5000 instrument. DOM was extracted from the filtrate as described above.

The C_{18}-extractable DOM samples were characterized using the 9.4 Tesla Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer with electrospray ionization (ESI) source housed at the National High Magnetic Field Laboratory (Tallahassee, FL). The methanol/water sample solutions were pumped at a rate of 500 nl/min through an electrospray capillary held at a −3 kV bias. All samples were electrosprayed in negative ionization mode after being made alkaline with ammonium hydroxide; the theoretical impetus being that electrostatic repulsion of deprotonated acid groups induces the folding of molecules into more compact shapes. Compact ions feel greater intermolecular repulsions per unit charge than do "unfolded" ions, thus minimizing the number of charges obtained by a molecule prior to its ejection from the droplet into the gas phase (Mann, 1989). To ensure mass accuracy, calibrant (HP tuning mix, from Agilent) was electrosprayed in tandem with sample using dual spray injection (Hannis and Muddiman, 2000). Approximately 200 transients were co-added, zero-filled once, and Hanning-apodized prior to Fourier transformation for the purpose of improving resolution and removing artifacts caused by digital sampling and truncation of the time-domain signal. Empirical formulas were calculated for the measured masses using software developed at the National High Magnetic Field Laboratory (Molecular Formula Calc v.1.0 ©NHMFL, 1998).

3. Results and discussion

ESI-FTICR mass spectrometry with dual electrospray ionization provided sufficient mass accuracy (<0.5 ppm) and resolving power (600,000 at 300 m/z) to assign unique empirical formulas to 95% of the 4000 signals in the mass spectrum of each sample, without a priori physical separation. Fig. 1 shows the ESI-FTICR mass spectrum of C_{18}-extractable charcoal leachates, and several micrographs of soil charcoal surfaces. The 100-year-old charcoal particles from UMBS soils are inhabited by microorganisms resembling filamentous fungi. Charcoals deposited by a fire just four years prior to sampling at a nearby site are not similarly colonized. Most filamentous fungi are saprophytic, degrading macromolecules to small water-soluble molecules amenable to osmotic uptake and use as an energy source (Harley and Smith, 1983). These fungi secrete strong extracellular oxidants, enzymes, and acids, capable of energetic tasks such as the oxidation and depolymerization of the refractory fossil macromolecules in coal, and the dissolution of soil minerals (Fakoussa, 1981; Catcheside and Ralph, 1999; Laborda et al., 1999). Because the soils at our sampling site are nutrient poor (Boose, 1986), it is possible that the fungal inhabitants of the charcoal are exploiting the organic matter and minerals, for which charcoal BC acts as a sorbent. For example, Andrews and Tien (1981) observed bacterial film growth on adsorbent surfaces. Although the mechanisms responsible for soil BC oxidation at this site have not been rigorously interrogated, we hypothesize that the oxidation of charcoal BC to water-soluble species is expedited by fungal co-metabolism.

The mass spectrum of these water-soluble species is shown in Fig. 1, with an expansion of the m/z axis at nominal mass 459–460 illustrating the ^{12}C and ^{13}C isotopomers at odd and even masses, respectively. The 1.003 Da spacing between corresponding isotopomers is the mass of one neutron, indicating that the ions are singly charged. The expansion of nominal mass 459 shows that the cluster of peaks at each nominal mass is bimodal. One mode is proximal to the exact nominal mass, and the second mode is observed at higher mass defect. The signals at low mass defect generally represent hydrogen-deficient and/or oxygen-rich species. In the ultra-high-resolution spectrum, each signal arises from molecular species having a single empirical formula (e.g., $C_xH_yO_z$). For the purpose of this discussion we define the term ‘species’ as a group of molecules (isomers) with the same molecular weight and empirical formula.

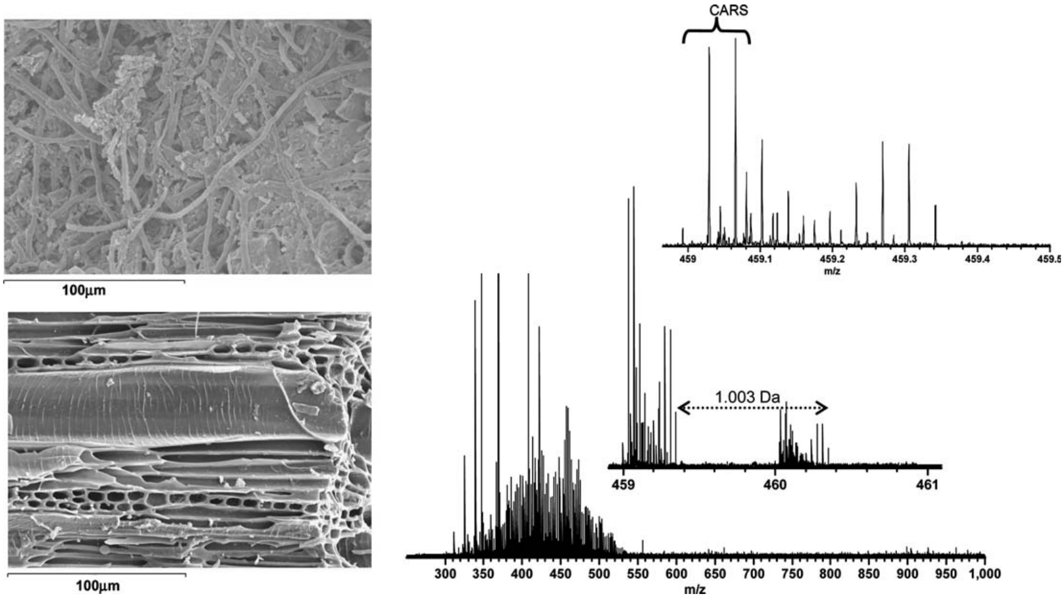

Fig. 1. Scanning electron micrographs of 100-year-old (upper left) and 4-year-old (lower left) soil charcoal particle surfaces. The optical light micrograph (upper middle) illustrates the abundance of filamentous microorganisms protruding from the edge of a 100-year-old charcoal fragment. ESI FT-ICR mass spectrum (right) of water-solubles leached from 100-year-old soil charcoal particles. The inset shows a region of nominal m/z 459, and the peaks assigned to CARS at low mass defect.

Even with knowledge of the exact mass and empirical formula for a given molecular species, structural details remain speculative. Nonetheless, Kim et al. (2003a) showed that van Krevelen analysis (Van Krevelen, 1950) can be used as a graphical tool to relate the atom ratio of a molecular species to its biopolymer class of origin. Natural biomolecules typically have H/C ratios greater than 0.8. In another study, Kim et al. (2004) pointed out that the hydrogen-deficient molecules found in DOM, with H/C ratios significantly lower than 0.8, must contain a greater number of double bonds and condensed rings than found in natural biopolymers, making it likely that these are combustion-derived, or products of BC degradation. However, without characterization of BC degradation products obtained directly from native soil BC, hydrogen-deficiency only serves as a rough indication of the presence of aromatics. This study provides direct evidence that in-situ degradation of charcoal in soils does, in fact, generate hydrogen-deficient species that are exported to DOM. Further, we introduce the concept of carbon-normalized double bond equivalents (DBE/C) as a criterion for identifying molecular species that contain condensed aromatic ring structures (CARS).

Double bond equivalents are defined as the number of double bonds and rings possessed by a single molecular species. This value is easily calculated in the absence of structural assignment, from the empirical formula using the following equation:

$$\mathrm{DBE} = 0.5 \times [((\text{number of C atoms} \times 2) + 2) - \text{number of H atoms}]. \tag{1}$$

Normalizing DBE to the number of carbon atoms within a given molecule (DBE/C) provides a measure of its aromaticity. A simple examination of a variety of model compounds shows the important point that fused-ring structures have DBE/C $\geqslant 0.7$. This value is a threshold that cannot be reached by alkenes or benzylic structures such as those found in lignin and tannin-type molecules. Even condensed tannin structures (e.g., epicatechin) have DBE/C values around 0.6, and therefore, do not meet the CARS criterion. Furthermore, a review of the literature by Lee et al. (1981) demonstrates that combustion is thought to be the predominant source of polycyclic aromatic compounds in the environment, with the exception of petrogenic contaminants. Therefore, we argue that the DBE/C $\geqslant 0.7$ criterion for the identification of CARS serves as a proxy for combustion-derived species, and their degradation products.

The DOM leached from soil charcoal particles over a period of 48 h comprised 0.2% of the total charcoal carbon. Applying the DBE/C $\geqslant 0.7$ criterion to the empirical formula assignments from the ESI FT-ICR analysis, the water-soluble portion of the soil charcoal contains 1200 species that qualify as CARS; representing 65% of the total number of peaks in the mass spectrum, and 49% of the spectral magnitude. This is most clearly demonstrated by conversion of the m/z axis of the mass spectrum to DBE/C as in Fig. 2. Comparison of the DBE/C spectrum of the soil pore DOM to the charcoal leachates highlights the condensed aromatic nature of the molecules in the latter. The origin of the remaining 35% of the molecular species in the charcoal sample with DBE/C < 0.7 are less clear. It is possible that the water-soluble species extracted from the soil charcoal contain soil organic matter that adsorbed to the charcoal during its 100-year residence in the soil, and/or degradation products of incompletely charred woody domains of the charcoal particles. The H/C and O/C ratios of the non-CARS are consistent with the humic substances found in soils and surface waters (Visser, 1983). However, a van Krevelen analysis (not shown) reveals very few contributions from the proteins and lipids that are present in the soil pore water DOM at this sampling site. Rather, the non-CARS extracted from the charcoal have atomic H/C and O/C ratios consistent with lignins and tannins, suggesting that these arise from uncharred woody domains of the charcoal.

It should be clearly stated that the relative abundance of ions generated by electrospray ionization has not been proven to accurately represent the relative abundance of molecules in a complex mixture. Electrospray preferentially ionizes molecules containing both polar and hydrophobic domains (Kebarle and Peschke, 2000). The same should be stated of the retention by the C_{18} disk. That is, the retention and elution of dissolved organic molecules by C_{18} have not been rigorously proven to supply representative sample of the entire DOM composition at the molecular level. Nevertheless, as Kujawinski et al. (2004) have pointed out, the ESI FT-ICR technique is very powerful for comparative studies of complex geochemical matrices at the molecular level of detail. In this way, Table 1 shows that humic acids from

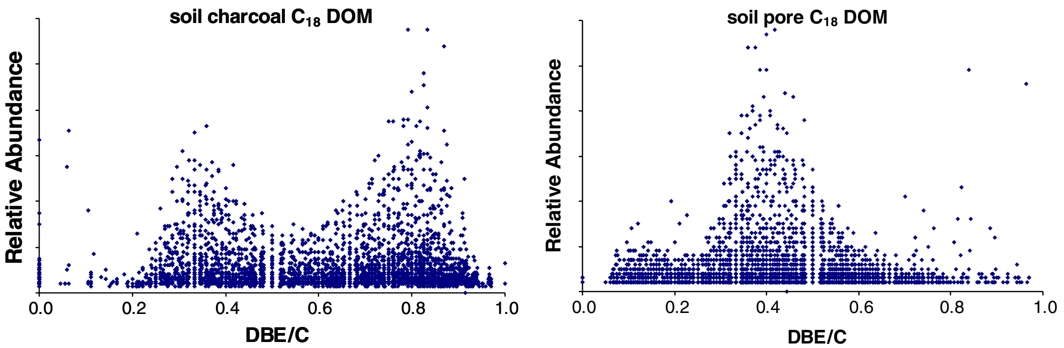

Fig. 2. DBE/C spectra of water-soluble charcoal leachates (left) and soil pore water DOM (right) showing that the charcoal is a source of condensed aromatics molecules with DBE/C values distributed around 0.8.

a volcanic ash soil of Japan (data from Kramer et al., 2004), and DOM from the Rio Negro of South America (data from Kim et al., 2004) contain many of the same condensed aromatics that leached directly from soil charcoal particles and from fire-impacted forest soil. Table 1 lists the empirical formulas of the most common CARS in order of ascending DBE/C value (increasing aromaticity). CARS tend to vary in their degree of oxidation, with those of the volcanic ash soil HA being the most highly oxidized. The observation that samples from geographically disparate fire-impacted regions of the world contain species with the same exact mass and corresponding empirical formulas as those extracted directly from soil charcoal, strongly suggests that these species are the stable molecular intermediates of terrestrial BC degradation.

The differences in the distribution of CARS among the samples compared in Table 1 are twofold; the presence or absence of certain species in one sample relative to the next, and among CARS that are present in multiple samples, there are differences in the number of oxygen atoms. The samples that are most similar to one another in both regards are the soil pore water DOM and the Rio Negro DOM. The CARS observed in these samples generally have lower DBE/C values (lower aromaticity) and lower variability in oxygen content. The number of oxygen atoms in these CARS tend toward median values (7–12 atoms). These two samples are also similar to one another in that they contain molecules that have been hydrologically exported from the soil, whereas the CARS in the HA sample are species that remained bound within the soil matrix, and were only released by extraction procedures in the laboratory. CARS in the volcanic ash soil are generally more aromatic than those in the DOM samples, and more highly oxidized. Perhaps these molecular characteristics play a causal role in their sorption to hydrophobic and ionic sites within the soil. If this is the case, then soil qualities such as clay, mineral, and organic matter content, pH, and ion exchange capacity would ultimately fractionate the CARS being exported according to their affinity for a given soil. Alternatively, the differences in CARS being exported to DOM could be attributed to localized differences in BC composition and the degradative processes at work.

Despite the fact that electrospray is a soft ionization technique that does not provide structural information through fragmentation patterns, CARS found in the dissolved phase should be constrained to a finite structural class by the physical requirement of aqueous solubility. Indeed, in the samples discussed here, CARS have low molecular weight (<550 Da), fewer than 38 carbon atoms, 1–14 oxygen atoms, three or fewer nitrogen atoms and one or fewer sulfur atoms. The hydrogen-deficiency of molecules having DBE $\geqslant 0.7$, places a limitation on the type of structure that can be assumed. Specifically, it requires structures containing multiple aromatic bridgehead carbon atoms. On the other hand, aqueous solubility requires that CARS in DOM be substituted with oxygen and nitrogen-containing functional groups to the extent that their polarity and hydrogen-bonding forces surpass the hydrophobicity of aromatic domains. Indeed, a condensed aromatic core hemmed with hydroxyl, sulfhydryl, carboxyl, carbonyl, and nitro groups are the stable aqueous products of soot and charcoal oxidation by various oxidants (e.g., O_3, HNO_3, H_2O_2, H_2O_2/NaOH, H_2SO_4 and $KMnO_4$). This is based upon structural data obtained by various authors using a

Table 1
Empirical formulas of CARS extracted directly from soil charcoal and the corresponding species found in soil pore water DOM, volcanic soil humic acid, and Rio Negro DOM

	Charcoal DOM	Soil pore DOM	Volcanic soil HA	Rio Negro DOM	DBE/C	H/C
Values of:	x	x	x	x		
$C_{20}H_{14}O_x$	6–13	9–13		7–13	0.70	0.70
$C_{23}H_{16}O_x$	5–13	6–13		6–12	0.70	0.70
$C_{27}H_{18}O_x$	4–11	5–11		7–11	0.70	0.67
$C_{30}H_{20}O_x$	5–8	8		9	0.70	0.67
$C_{17}H_{12}O_x$	7–10			9–11	0.71	0.71
$C_{21}H_{14}O_x$	5–13	8–12		7–11	0.71	0.67
$C_{24}H_{16}O_x$	4–13	7–11		7–12	0.71	0.67
$C_{28}H_{18}O_x$	4–10	7–10		8–10	0.71	0.64
$C_{18}H_{12}O_x$	6–12	9–		8–12	0.72	0.67
$C_{25}H_{16}O_x$	5–13	6–11		7–11	0.72	0.64
$C_{29}H_{18}O_x$	4–10	6,8,10		8,9	0.72	0.62
$C_{22}H_{14}O_x$	5–14	7–10		7–12	0.73	0.64
$C_{26}H_{16}O_x$	4–12	6–13		7–11	0.73	0.62
$C_{30}H_{12}O_x$	6–12		10–16		0.73	0.40
$C_{19}H_{12}O_x$	6–12	8–11		7–12	0.74	0.63
$C_{23}H_{14}O_x$	5–14	7–12		7–12	0.74	0.61
$C_{27}H_{16}O_x$	4–12	8–11		8–10	0.74	0.59
$C_{30}H_{18}O_x$	5–8			9	0.74	0.60
$C_{31}H_{18}O_x$	5–8		12		0.74	0.58
$C_{16}H_{10}O_x$	8–9			10	0.75	0.63
$C_{20}H_{12}O_x$	5–13	9–11	10	7–12	0.75	0.60
$C_{24}H_{14}O_x$	3–13	7–12	8	7–11	0.75	0.58
$C_{28}H_{16}O_x$	4–11	9–11	8,9,11	8,9	0.75	0.57
$C_{17}H_{10}O_x$	7–11		8–14	9–10	0.76	0.59
$C_{21}H_{12}O_x$	5–13	8,10,12	10	7–12	0.76	0.57
$C_{25}H_{14}O_x$	4–13	7–13	9,13	7–11	0.76	0.56
$C_{29}H_{16}O_x$	4–10	9	6–12	9	0.76	0.55
$C_{22}H_{12}O_x$	4–14	9,10,12	7,8	7–12	0.77	0.55
$C_{26}H_{14}O_x$	4–13	6–12	6	8–10	0.77	0.54
$C_{30}H_{16}O_x$	5–9		8–14		0.77	0.53
$C_{31}H_{16}O_x$	5–8		9,13,14		0.77	0.52
$C_{18}H_{10}O_x$	6–12	9	10	8–11	0.78	0.56
$C_{23}H_{12}O_x$	4–14	8–12	6–12	8–11	0.78	0.52
$C_{27}H_{14}O_x$	4–12	7–8	5,7,8	9	0.78	0.52
$C_{32}H_{16}O_x$	4		8–11,16		0.78	0.50
$C_{19}H_{10}O_x$	7–12		9–11	8–11	0.79	0.53
$C_{24}H_{12}O_x$	5–14	9–12	5–12	8–11	0.79	0.50
$C_{28}H_{14}O_x$	4–11		10	9	0.79	0.50
$C_{29}H_{14}O_x$	4–10		11,12		0.79	0.48
$C_{15}H_8O_x$	9–14		7,8		0.80	0.53
$C_{20}H_{10}O_x$	5–13	9,11	8–10	8–11	0.80	0.50
$C_{25}H_{12}O_x$	5–14	9,12	7–13	8–10	0.80	0.48
$C_{30}H_{14}O_x$	5–9		7,11–14		0.80	0.47
$C_{16}H_8O_x$	6–11		8		0.81	0.50
$C_{21}H_{10}O_x$	5–13		6–11	8–11	0.81	0.48
$C_{26}H_{12}O_x$	4–13	11	13	9	0.81	0.46
$C_{27}H_{12}O_x$	5–12		7–15		0.81	0.44
$C_{31}H_{14}O_x$	4–8		13–16		0.81	0.45
$C_{32}H_{14}O_x$	6–7		7–18		0.81	0.44
$C_{17}H_8O_x$	7–11		10		0.82	0.47
$C_{22}H_{10}O_x$	4–14		7–12	9–11	0.82	0.45
$C_{28}H_{12}O_x$	4–11		7–15		0.82	0.43
$C_{18}H_8O_x$	7–12		8,10		0.83	0.44
$C_{23}H_{10}O_x$	4–14	11	6–8	9–11	0.83	0.43
$C_{24}H_{10}O_x$	5–14		6–10	10	0.83	0.42
$C_{29}H_{12}O_x$	4–11		10–15		0.83	0.41

(*continued on next page*)

Table 1 (*continued*)

Values of:	Charcoal DOM *x*	Soil pore DOM *x*	Volcanic soil HA *x*	Rio Negro DOM *x*	DBE/C	H/C
$C_{30}H_{12}O_x$	6–9		8–16		0.83	0.40
$C_{19}H_8O_x$	6–12		9,10	11	0.84	0.42
$C_{25}H_{10}O_x$	5–13		8		0.84	0.40
$C_{31}H_{12}O_x$	5–8		12,13		0.84	0.39
$C_{20}H_8O_x$	6–13		9		0.85	0.40
$C_{26}H_{10}O_x$	4–13		8,9,12		0.85	0.38
$C_{27}H_{10}O_x$	5–12		11		0.85	0.37
$C_{21}H_8O_x$	5–14		7–9		0.86	0.38
$C_{22}H_8O_x$	5–13		7,8		0.86	0.36
$C_{28}H_{10}O_x$	5–11		7,9,12		0.86	0.36
$C_{29}H_{10}O_x$	6–10				0.86	0.34
$C_{23}H_8O_x$	6–14		8–10		0.87	0.35
$C_{30}H_{10}O_x$	6–9				0.87	0.33
$C_{31}H_{10}O_x$	8		11–13		0.87	0.32
$C_{16}H_6O_x$	9–10				0.88	0.38
$C_{24}H_8O_x$	6–14		10		0.88	0.33
$C_{25}H_8O_x$	6–13		9		0.88	0.32

Values of *x* represent the number or range of oxygen atoms attached to the given hydrocarbon backbone. ESI FT-ICR data for the volcanic ash soil humic acid and the Rio Negro DOM are taken from studies performed by Kramer et al. (2004) and Kim et al. (2004), respectively. Charcoal and soil pore water samples are those collected for this study.

range of techniques, including UV–vis spectroscopy (Kumada, 1983), FTIR spectroscopy (e.g., Akhter et al., 1984; Trompowsky et al., 2005) ^{1}H NMR spectroscopy (Decesari et al., 2002), ^{13}C NMR spectroscopy (e.g., Haumaier and Zech, 1995; Trompowsky et al., 2005), anion exchange chromatography (Decesari et al., 2002), X-ray diffraction (Kamegawa et al., 2002), and GC/MS (Matsuda and Schnitzer, 1972; Glaser et al., 1998).

Though condensed aromatic polyacids are common, readily detectable, water-soluble BC degradation products, it has been shown that mineralization (oxidation to CO_2 and H_2O) of BC must also occur in order to balance the global carbon budget (Goldberg, 1985; Dickens et al., 2004; Masiello, 2004). In fact, strong atmospheric oxidants such as ozone, and soil microbial consortia alike, have been shown to mineralize BC (Schneour, 1966; Donnet et al., 1972). Though the products of natural degradation most certainly range in extent of oxidation from small surface modifications to complete mineralization, it is everything in-between that contains the biogeochemical information regarding cycling. For instance, soil BC that experiences oxidation followed by dissolution and export to surface waters might then procede through photochemical or microbial oxidation processes. In other words, just as variations in combustion processes generate a continuum of products that we term BC, natural degradation is likely to generate a continuum of degradation products from this diverse array of starting materials. That the molecular identity of water-soluble CARS leached from soil charcoal is consistent among soil pore water, soil humic substance, and riverine samples from diverse geographical settings suggests that these molecules represent an envelope in which the combustion and degradation continua converge along the path to complete mineralization.

4. Conclusions

We report on the molecular composition of water-soluble products extracted directly from charcoal particles after 100 years of natural degradation in a forest soil. ESI FT-ICR mass spectrometry reveals condensed aromatic ring structures extensively substituted with oxygen-containing functional groups. The calculation of carbon normalized double bond equivalents from mass spectral data serves as a means to recognize BC degradation products by identifying species with condensed aromatic ring structures (CARS). A comparison to ESI FT-ICR data from previous studies shows that many of the CARS found in the charcoal leachates and soil pore water DOM at our site have the same mass (within 1 ppm) and empirical formulas as CARS detected in Japanese volcanic ash soil HA, and Rio Negro DOM. This is consistent with previous evidence suggesting that oxidation of BC in soils results in the export of BC to the DOM pool. Furthermore, the observation that the molecular identity of CARS from fire-impacted soil is consistent across geographically and climatically diverse settings, suggests that these molecules are indicators of terrestrial BC degradation. Understanding BC degradation pathways remains an important goal for future research. Our observation of an association between soil charcoal fragments and filamentous microbiota that resemble saprophytic fungi provides reason to suspect that these organisms play a role in fate of soil BC.

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