



# Hydrogen-deficient molecules in natural riverine water samples—evidence for the existence of black carbon in DOM

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## Abstract

Understanding the role of black carbon (BC) in the natural environment is very important because of the potential impacts of black carbon on human health, global carbon cycling, and pollutant transport. There has been circumstantial evidence for the existence of black carbon molecules in dissolved organic matter (DOM); however, direct evidence has been lacking. In this paper, the presence of black carbon-like material in DOM is demonstrated for the first time. Electrospray ionization (ESI) with ultra-high resolution mass spectrometric analyses of DOM samples reveals the existence of hydrogen-deficient molecules. The van Krevelen analysis of the ultra-high resolution mass spectra clearly differentiates black carbon-like material from the complex assemblage of other, relatively hydrogen-rich natural molecules and indicates that the hydrogen-deficient molecules in the DOM are from black carbon-like material. The observation of black carbon molecules in DOM suggests that BC may be degraded and included in the active carbon pools of the earth.

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## 1. Introduction

Black carbon (BC) is produced by the incomplete combustion of a variety of contemporary and fossil fuels. The exact molecular-level structure of black carbon is largely unknown. However, it is well

known that BC is composed of multitudes of condensed aromatic rings that explain BC's richness in carbon and depletion of hydrogen (Goldberg, 1985). There has been escalating interest in BC recently because of its role in processes related to climate change (Jacobson, 2001), air pollution (Dickhut et al., 2000), interactions with hydrophobic contaminants (Gustafsson et al., 1997), and global carbon cycling (Crutzen and Andreae, 1990; Kuhlbusch, 1998; Masiello and Druffel, 1998; Mitra et al., 2002). The formation of BC diverts carbon and

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oxygen that would otherwise cycle through plant and animal biomass, into compounds that are refractory, and therefore poorly mineralized by microbes, and are thought to persist for centuries to millennia (Goldberg, 1985). In this sense, BC is regarded as a carbon sink in the global carbon cycle. The strength of this sink depends upon how readily BC is oxidized. A recent study by Czimeczik et al. (2003) suggests that some removal mechanism must be in place to explain depletions in inventories derived from forest fires in a boreal forest in Siberia. Laboratory studies suggest that soot, a type of BC, can be oxidized to soluble species in the presence of ozone (Chughtai et al., 1991). Thus, it seems likely that the view that BC is a long-term sink for carbon is not entirely substantiated.

Numerous studies have shown that BC is distributed in various environmental compartments. For example, BC has been found in oceanic and lacustrine sediments (Masiello and Druffel, 1998; Middelburg et al., 1999; Muri et al., 2002), soil organic matter (Glaser et al., 1998), particulate matter in rivers (Mitra et al., 2002), and atmospheric particulates (Crutzen and Andreae, 1990). However, even though it has been suggested that BC may be present in rivers (Suman et al., 1997) as dissolved organic matter (DOM) for 2400–13,900 years prior to deposition in oceanic sediments (Masiello and Druffel, 1998), there is no direct evidence of the existence of BC in DOM other than the studies by Mannino and Harvey (in press). One might not expect BC to exist in DOM because it is normally a black solid composed of a network of high molecular weight condensed aromatic ring systems whose aqueous solubility is low or nonexistent. Most studies (Dickhut et al., 2000; Jacobson, 2001; Mitra et al., 2002) have focused on particulate BC. However, it was recently reported that oxidation of soot produced water-soluble organic compounds (Decesari et al., 2002; Kamegawa et al., 2002). Thus, we expect BC, which is oxidized in the environment via either microbial or photochemical mechanisms, could yield products that contribute to DOM.

There must be some mechanism for cycling of BC from the refractory carbon pool to the active carbon pool, or the oceans would be inundated by BC (Goldberg, 1985). Annually, 0.050–0.260 Gt of C ( $Gt=1 \times 10^{15}$  g) in the form of BC is produced by

biomass burning (Kuhlbusch and Crutzen, 1995) and 0.012–0.024 Gt is produced by fossil fuel combustion (Penner et al., 1993). If we consider an estimate of the size of the active pool of carbon (4000 G t) that cycles through the biosphere annually (Hedges et al., 1997) and assume that no degradation of BC occurs, one can estimate that it would require less than 80,000 years to convert the entire pool of actively cycling carbon to BC. Conversion of BC to DOM, followed by eventual mineralization, could provide the mechanism for removal of BC over geologic time. Therefore, if BC is shown to exist in DOM, then the role of DOM in the cycling of BC needs to be considered in models of BC cycling that currently assume that it remains in the particulate phase.

A major difficulty in measuring BC is that there is no generally accepted methodology, especially as it might apply to DOM. Carbon measurements following thermal oxidation, acid digestion, or combinations of both methods are the most commonly used analytical techniques (Haumaier and Zech, 1995; Gustafsson et al., 1997; Muri et al., 2002). However, the methods are operational and do not provide molecular level identifications. We introduce electrospray ionization (ESI) coupled to ultra-high resolution mass spectrometry (MS) as a new method for characterization of individual BC-derived molecules. ESI is a technique where a solution is nebulized and simultaneously subjected to high voltage (typically several thousand volts). Ionization occurs as the aerosol formed devolatilizes in the spray chamber. The ions are transferred to a mass spectrometer for mass determinations. ESI MS has become an important technique for characterization of natural organic matter (Hatcher et al., 2001). There are numerous types of MS analyzers available for coupling to an ESI source. Among them is the ultra-high resolution MS represented by the Fourier Transform Ion Cyclotron MS that has been successfully employed to study polar organic matter (Brown and Rice, 2000; Kujawinski et al., 2002; Stenson et al., 2002, 2003). Specifically, ultra-high resolution mass spectrometry has been successfully applied to characterize a fulvic acid sample extracted from the Suwannee River, revealing elemental compositions of each ionizable molecule in the samples (Stenson et al., 2003).

## 2. Materials and methods

### 2.1. Samples and preparation

A water sample was collected from McDonalds branch located in the New Jersey Pine Barrens (USA). Water was filtered through a three-stage glass fiber cartridge system (75, 25, and 0.3  $\mu\text{m}$ ), and acidified to pH 2–3. DOM was extracted by a  $\text{C}_{18}$  solid phase extraction disk and approximately 60% of the DOC was recovered. Detailed information about the extraction and recovery procedures is provided by Kim et al. (2003b). McDonalds branch is an Atlantic white cedar bog that coalesces into a first-order stream and eventually flows to the Delaware River. This black-water system has relatively high dissolved organic carbon (DOC) concentrations (averaging 16–18 mg/L). McDonalds branch has been a U.S. Geological Survey hydrologic station since 1953 and the subject of numerous scientific studies (Lord et al., 1990; Johnsson and Barringer, 1993; Maurice et al., 2002). This fire-adapted ecosystem experienced at least eight wild fires between 1850 and the early 1900s, and the entire area was burned repeatedly in 1922 (McCormick, 1955). More recently, a wildfire in 1963 burned the northern half of the McDonalds branch watershed, and prescribed burns of low intensity were performed in the 1980s and 1990s (A. Windisch, personal communication).

A DOM sample (<0.2  $\mu\text{m}$  pore size) was isolated from the Rio Negro branch of the Amazon River, by tangential-flow ultrafiltration with a 1000 Da molecular weight cutoff membrane (Benner and Hedges, 1993). About 88% of the DOC was recovered by ultrafiltration. The freeze-dried sample was dissolved in 70% HPLC grade methanol (Fisher Scientific, Itasca, IL) and 30% nanopure water prepared from a water purification unit (UHQ, ELGA, Lowell, MA). It was then prepared as described by Kim et al. (2003b) for ESI MS.

The Rio Negro, a black water tributary of the Amazon River, is one of the three largest tributaries of the Amazon River and accounts for 30% of the annual discharge ( $5.1 \times 10^{12}$  m<sup>3</sup>/year) of the Amazon River to the ocean (Ertel et al., 1986; Mounier et al., 1999). The Amazon River is the largest river in the world and accounts for 20% of annual fresh

water discharge into the ocean. Water samples from the Rio Negro are also rich in DOC, with a concentration of 8.7 mg/L at the time of sampling (Benner and Hedges, 1993). The rain forest in the Amazon basin is largest in the world and there have been frequent and extensive natural or man-made fires in this area during the last 2000 years (Carcaillet et al., 2002). Accordingly, there are numerous reports of BC in the Amazon Basin (Echalar et al., 1998; Yamasoe et al., 2000; Carcaillet et al., 2002).

### 2.2. Instrumentation

Samples in methanol–water were analyzed by a 9.4-T Fourier transform ion cyclotron resonance (FT ICR) mass spectrometer at the National High Magnetic Field Laboratory (Tallahassee, FL, USA). A drop or two of 30%  $\text{NH}_4\text{OH}$  solution was spiked into both samples, now in methanol–water mixtures, to induce a negative charge on humic acid components during the electrospray ionization process. Samples were introduced by a syringe pump through a micro-electrospray ionization source (Emmett et al., 1998) at a rate of 350 nL/min. All the samples were analyzed in negative ionization mode with a needle voltage of –1.8 kV. Ions were stored in an octapole ion trap for 45 s before being transferred to a Penning trap (Senko et al., 1997). Approximately 200 time domain signals were added for a time period of approximately 5 h. The summed FID signal was zero-filled once and Hanning apodized before being processed by magnitude computation mode Fourier transformation.

### 2.3. Mass calibration

Obtaining exact mass to charge values ( $m/z$ ) is a critical step to being able to assign elemental compositions of observed peaks in mass spectra. To obtain exact and highly resolved  $m/z$  values, experiments were performed in two steps. In the first step, an internal calibrant (polyethylene glycol solution of 600 Da average molecular weight purchased from Sigma) was used as an internal calibration mass standard. Dual-spray injection (Hannis and Muddiman, 2000) was employed to inject standard molecules into the FT-ICR cell along with the sample. The peaks in the resultant spectra

were calibrated by reference to the exact  $m/z$  of internal calibrants. In the second step, only the sample was analyzed by FT-ICR. The spectra obtained in this step are free from peak contributions of the calibrant material. The spectra were first calibrated by use of an external calibrant material (G2421A electrospray “tuning mix” from Agilent) and second by the exact  $m/z$  numbers of major sample peaks on the spectra obtained in the first step.

### 3. Results and discussion

#### 3.1. ESI MS results

Both DOM samples were analyzed by electrospray ionization coupled to the 9.4-T FT-ICR mass spectrometer, and the obtained spectra are presented in Figs. 1 and 2. Exact mass numbers of peaks obtained from the spectra are used to calculate elemental compositions for each of the peaks, and some of

these are listed in Table 1. The calculations were performed according to procedures described in a previous article (Kim et al., 2003a). The expanded mass regions of selected areas of the spectra are also displayed in Fig. 1. It is apparent from the spectra that there are peaks located at  $m/z$  values close to the nominal mass and others that are further removed from nominal mass. Considering the mass defects (mass defect from nominal mass) of elements like carbon (12.00000 amu), hydrogen (1.007825 amu), and oxygen (15.994915 amu) and the fact that DOM is primarily composed of C, H, and O, the molecules that correspond to the peaks near nominal masses must have a small number of hydrogen atoms and/or a relatively large number of oxygen atoms. We considered the possible presence of  $N$ -containing molecules, but generally these did not account for significant numbers of peaks because the  $N$  contents of these samples are low and  $N$  usually gives peaks at even nominal masses (if an odd number of  $N$  atoms are present) that were not considered in the calculations below.

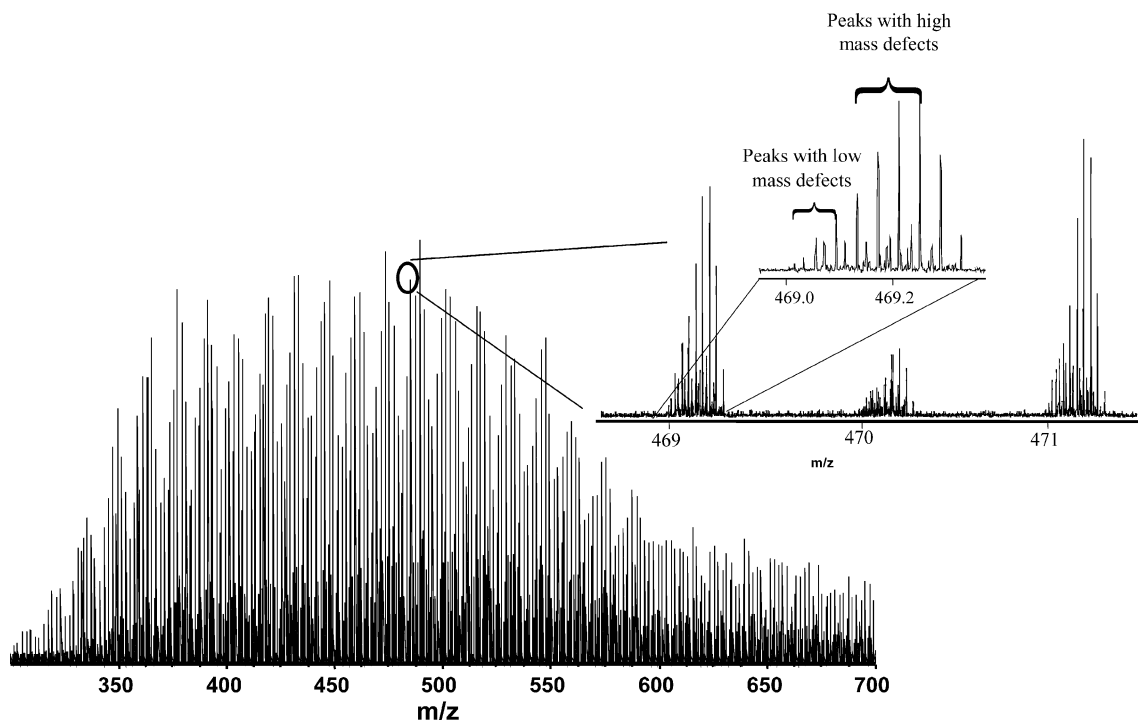


Fig. 1. Ultra-high resolution mass spectrum of DOM extracted from McDonalds branch and expanded views of the indicated regions.

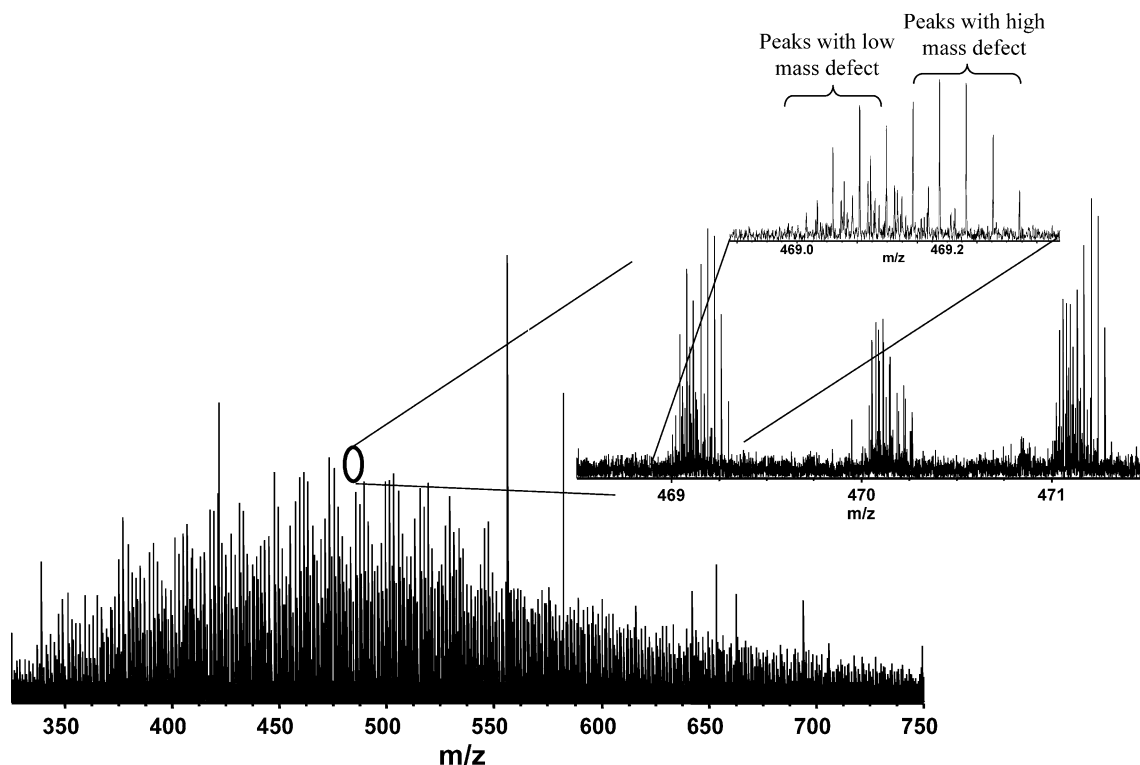


Fig. 2. Ultra-high resolution mass spectrum of DOM extracted from the Rio Negro water sample and expanded views of the indicated regions.

The sum of the numbers of rings and double bonds for a given molecular formula can be calculated as double bond equivalents (DBE) using the following equation (Badertscher et al., 2001):

$$\text{DBE} = 1 + \frac{1}{2} \left( \sum_i n_i (v_i - 2) \right) \quad (1)$$

where  $n$  represents number of atoms for an element  $i$ , and  $v_i$  is formal valence of the element. DBE values of selected peaks in the McDonalds branch mass spectrum are calculated and tabulated along with their unique elemental compositions (Table 1). The molecules that show an exact mass near the nominal mass have high DBE values indicating that the molecules are composed of numerous multiple bonds and/or rings. One of the molecules with a DBE value of 19 was selected ( $\text{C}_{27}\text{H}_{18}\text{O}_8$ ) and a possible chemical structure of the molecule was constructed (Fig. 3). Although multiple isomeric structures could be assigned to this elemental formula, it is clear that the structures drawn with 19 multiple bonds and/or rings

most likely contain a highly condensed ring system, as does the one shown. Numerous peaks with high DBE values can be observed throughout the entire mass range of the spectrum. This indicates the existence of many highly condensed and oxygenated compounds whose structures are related to suggested structures of degraded BC molecules (Kamegawa et al., 2002).

### 3.2. The van Krevelen analysis

The van Krevelen plot, a diagram constructed using the molar ratio of hydrogen to carbon (H/C ratio) as the ordinate and the molar oxygen to carbon ratio (O/C ratio) as the abscissa, has been used extensively to study the evolution of coals or oil samples. Major biogeochemical classes of compounds (such as lignin compounds, lipids, carbohydrates, etc.) have their own characteristic H/C or O/C ratios. As a result, each class of compounds plots in a specific location on the diagram (Kim et al., 2003a). The van Krevelen plot can be used to study complex mass spectra, as was shown by Kim et al. (2003a).

Table 1

List of elemental compositions,  $m/z$  values, and double bond equivalence for peaks identified at a nominal mass of 469 in the spectrum of McDonalds branch DOM

Proposed molecular formula	Observed value	DBE
$C_{25}H_{10}O_{10}$	469.02018	21
$C_{22}H_{14}O_{12}$	469.04118	16
$C_{26}H_{14}O_{12}$	469.05646	20
$C_{23}H_{18}O_{11}$	469.07763	15
$C_{27}H_{18}O_8$	469.09288	19
$C_{24}H_{22}O_{10}$	469.11401	14
$C_{28}H_{22}O_7$	469.1293	18
$C_{25}H_{26}O_9$	469.15042	13
$C_{29}H_{26}O_6$	469.16576	17
$C_{22}H_{30}O_{11}$	469.17151	8
$C_{26}H_{30}O_8$	469.18681	12
$C_{30}H_{30}O_8$	469.20201	16
$C_{23}H_{34}O_{10}$	469.20789	7
$C_{27}H_{34}O_7$	469.22316	11
$C_{31}H_{34}O_4$	469.23838	15
$C_{24}H_{38}O_9$	469.24423	6
$C_{28}H_{38}O_6$	469.25949	10
$C_{29}H_{42}O_5$	469.29584	9

Complicated mass spectra of natural organic matter can be visualized in a way that allows for qualitative analyses of major classes of compounds that comprise ultra-high resolution spectra, based on the locations of the peaks in the van Krevelen diagram.

The van Krevelen diagrams for the ultra-high resolution mass spectral data for the two DOM samples are presented in Fig. 4a and b. For McDonalds branch DOM, peaks span the region with molar oxygen to carbon (O/C) ratios between 0.1 and 0.6 and molar hydrogen to carbon (H/C) ratios between 0.4 and 1.6. The most intense region of the

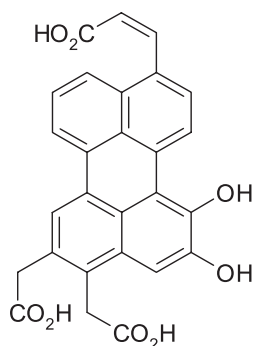


Fig. 3. A possible chemical structure for one peak ( $C_{27}H_{18}O_8$ ) in the mass spectrum of McDonalds branch DOM.

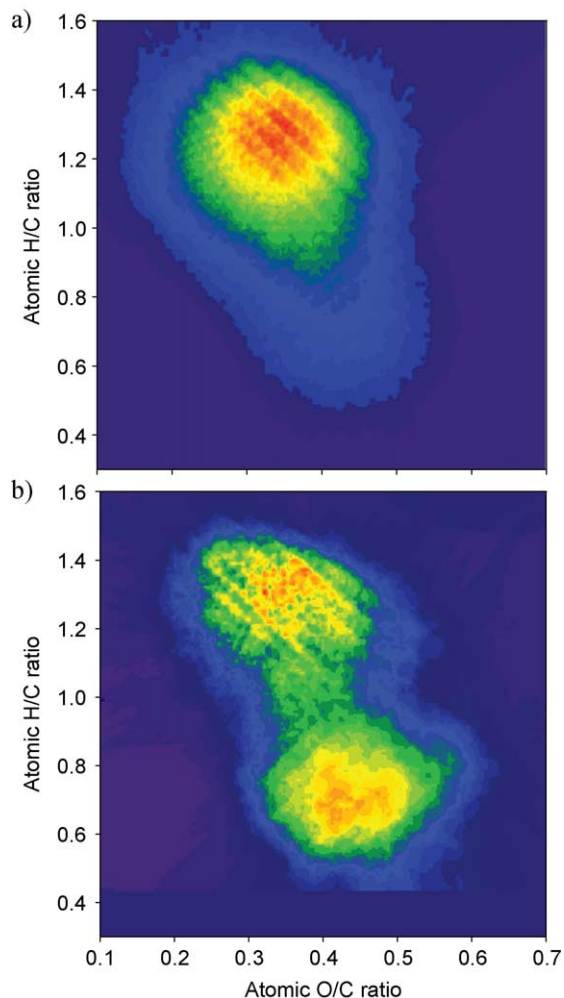


Fig. 4. The 3D van Krevelen diagrams constructed from ultra-high resolution data of (a) McDonalds branch and (b) Rio Negro DOM. Relative intensities of the signal increase in the order purple, blue, green, yellow, and red.

McDonalds branch DOM data (in Fig. 4a) is located at O/C ratios 0.25–0.5 and H/C ratios 1–1.5. This region coincides well with what might be expected from lignin-type molecules (Kim et al., 2003a). In the case of the Rio Negro DOM, the distribution of intensities (Fig. 4b) is bimodal with one of the regions plotting in a location similar to that of McDonalds branch DOC. However, there is another region of intensity located at O/C ratios 0.3–0.6 and H/C ratios 0.5–0.8. This region corresponds to molecules that are deficient in hydrogen (low H/C ratio).

Most naturally occurring molecules typically have H/C ratios that are greater than 1. Cellulose-type molecules, lipids, waxes, and plant cuticles are known to have H/C ratios of about 2 and lignin-type molecules and tannins have H/C ratios around 1 (Humphreys and Chapple, 2002), indicating that these are not the source of the hydrogen-deficient molecules in our samples. The H/C and O/C ratios of BC have been measured. Cope (1979) reported that natural chars (one form of BC) had O/C ratios ranging from 0.1 to 0.67 and H/C ratios ranging from 0.27 to 1.3. Baldock and Smernik (2002), in their study of thermally altered wood samples, reported that charred wood samples (heated to temperatures of 200–350 °C) had O/C ratios of 0.32–0.61 and H/C ratios of 0.46–1.02. The elemental ratios of the hydrogen-deficient molecules in our DOM samples fall neatly in the envelope of values observed for BC, and we propose that BC is the source of these molecules.

It appears in Fig. 4 that BC is more significant in the Rio Negro sample than it is in the McDonalds branch DOM. However, it is important to point out that the two samples were obtained by two different methods (C<sub>18</sub> extraction vs. ultrafiltration) and the differences observed could be due to the isolation method. Consequently, we cannot compare the absolute amounts of BC-derived molecules in the two samples to evaluate differences that may be due to input amounts. Furthermore, peak intensities for BC-derived molecules may not be similar to those from other non-BC molecules, mainly due to differences in ionization efficiencies during electrospray ionization. However, we are working towards establishing a method that would allow this type of quantitative representation for BC.

#### 4. Conclusions

The ultra-high resolution mass spectrometric approach used in this study identified hydrogen-deficient molecules in samples of riverine DOM isolated by both C<sub>18</sub> solid-phase extraction and by tangential flow ultrafiltration. These molecules are probably derived from black carbon that has been oxidized and rendered soluble. To our knowledge, this is the first direct identification of such molecules in riverine DOM, although many have postulated

their existence (Masiello and Druffel, 1998; Mitra et al., 2002). The aerosol deposition of small BC particles can be an important source of BC in riverine DOM as suggested by Masiello and Druffel (1998). However, given the fact that BC is known to be one of the major components of soil organic carbon (Glaser et al., 1998; Gelinas et al., 2001) and that carbon from soil humic substances constitutes a major portion of riverine DOC (e.g., 60% of the riverine DOC in Amazon River Basin; Ertel et al., 1986), it is reasonable to expect that soil leachate, that includes BC, would be an important source of riverine BC.

As discussed earlier in this paper, there must be some mechanism for cycling of BC from the refractory carbon pool to the active carbon pool. The current finding of condensed and oxidized BC among DOM molecules suggests that some part of BC may be degraded in the environment and may participate in the global cycling of carbon. BC most likely exists as solid particles in the soil initially, and the mechanisms leading to its incorporation in riverine DOM remain uncertain. It is known that addition of hydroxyl groups to aromatic rings and subsequent oxidation are the major steps in the process of photodegradation (Fasnacht and Blough, 2002) or biological (Tschech, 1989; Willmann and Fakoussa, 1997) degradation of aromatic compounds. The added polar functional groups would increase the solubility of the aromatic compounds in water and could lead to the solubilization of BC degradation products. In fact, Decesari et al. (2002) reported that oxidation of BC produced water soluble organic compounds.

Given the current understanding of BC degradation, it is reasonable to expect that degradation would take place slowly. BC is known to be persistent in nature even for geological time scales because of its refractory nature. For example, Bird and Cali (1998) used BC as a tracer of Earth's fire history from approximately 1 million years ago. Therefore, if the BC molecules observed in this study were produced by oxidative degradation of BC, then the process would be slow and the resulting BC molecules in DOM would be composed of old (<sup>14</sup>C depleted) carbon. Bulk radiocarbon ages of DOC and dissolved humic substances in the Amazon River indicate mostly young carbon, but this does not preclude a

small but old fraction of black carbon (Hedges et al., 1986; Raymond and Bauer, 2001). Also, some of the BC molecules may be younger than anticipated, especially if they derive from recent burning events in the Amazon Basin or oxidative processes are more rapid in the tropical climate in the region. Raymond and Bauer (2001) studied the biodegradation of riverine DOM and found that the younger components of DOM were selectively degraded compared to the older components. If black carbon in DOM is resistant to degradation, as might be expected based on its structure, and its age is older than modern carbon from DOM produced by recently degraded plants, then old riverine BC will likely survive degradation processes in rivers and eventually flow into the ocean. The fate of BC and riverine DOM in the coastal ocean is unknown; but if it survives photooxidation and biodegradation processes, it could be transported into the deep ocean. Bauer and Druffel (1998) concluded that DOC input from ocean margins to the open ocean is much greater than organic carbon input from the surface ocean. Therefore, they suggested that ocean margins might be one of the important sources of the old apparent age of organic carbon observed in the deep ocean. These combined processes may explain the existence of old carbon found in deep oceanic DOC (Williams and Druffel, 1987).

It is premature to use the FT-ICR MS data in a quantitative fashion because it is well known that ESI ionization efficiencies vary significantly for different types of compounds. However, if we assume an efficiency of 1 for all peaks, we can estimate that 50% of the peak intensities are attributable to BC. The annual discharge of DOC from the Rio Negro is 8.1 Tg/year (Ritchie et al., 1990). From the above data, we can estimate that approximately 4.0 Tg/year of dissolved BC is being supplied to the ocean from the Rio Negro alone. If one assumes that the Rio Negro DOC is representative of Amazon DOC in which the annual flux of DOC is about 36 Tg/year, then approximately 18 Tg/year of BC enters the ocean as DOC from the Amazon system.

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## References

- Badertscher, M., Bischofberger, K., Munk, M.E., Pretsch, E., 2001. A novel formalism to characterize the degree of unsaturation of organic molecules. *J. Chem. Inf. Comput. Sci.* 41 (4), 889–893.
- Baldock, J.A., Smernik, R.J., 2002. Chemical composition and bioavailability of thermally altered *Pinus resinosa* (red pine) wood. *Org. Geochem.* 33 (9), 1093–1109.
- Bauer, J.E., Druffel, E.R.M., 1998. Ocean margins as a significant source of organic matter to the deep open ocean. *Nature* 392 (6675), 482–485.
- Benner, R., Hedges, J.I., 1993. A test of the accuracy of freshwater DOC measurements by high-temperature catalytic oxidation and UV-promoted persulfate oxidation. *Mar. Chem.* 41, 161–166.
- Bird, M.I., Cali, J.A., 1998. A million-year record of fire in sub-Saharan Africa. *Nature* 394 (6695), 767–769.
- Brown, T.L., Rice, J.A., 2000. Effect of experimental parameters on the ESI FT-ICR mass spectrum of fulvic acid. *Anal. Chem.* 72 (2), 384–390.
- Carcaillet, C., Almquist, H., Asnong, H., Bradshaw, R.H.W., Carrion, J.S., Gaillard, M.J., Gajewski, K., Haas, J.N., Haberle, S.G., Hadorn, P., Muller, S.D., Richard, P.J.H., Richo, I., Rosch, M., Goni, M.F.S., von Stedingk, H., Stevenson, A.C., Talon, B., Tardy, C., Tinner, W., Tryterud, E., Wick, L., Willis, K.J., 2002. Holocene biomass burning and global dynamics of the carbon cycle. *Chemosphere* 49 (8), 845–863.
- Chughtai, A.R., Jassim, J.A., Peterson, J.H., Stedman, D.H., Smith, D.M., 1991. Spectroscopic and solubility characteristics of oxidized soots. *Aerosol Sci. Technol.* 15, 112–126.
- Cope, M.J., 1979. Physical and chemical properties of coalified and charcoaled phytoclasts from some British Mesozoic sediments: an organic geochemical approach to paleobotany. Manuscript Presented at 9th International Meeting on Organic Geochemistry.
- Crutzen, P.J., Andreae, M.O., 1990. Biomass burning in the tropics—impact on atmospheric chemistry and biogeochemical cycles. *Science* 250 (4988), 1669–1678.
- Czimczik, C.I., Preston, C.M., Schmidt, M.W.I., Schulze, E.-D., 2003. How surface fire in Siberian Scots pine forests affects soil organic carbon in the forest floor: stock, molecular structure, and conversion to black carbon (charcoal). *Glob. Biogeochem. Cycles* 17 (1), 1020.
- Decesari, S., Facchini, M.C., Matta, E., Mircea, M., Fuzzi, S., Chughtai, A.R., Smith, D.M., 2002. Water soluble organic compounds formed by oxidation of soot. *Atmos. Environ.* 36 (11), 1827–1832.

- Dickhut, R.M., Canuel, E.A., Gustafson, K.E., Liu, K., Arzayus, K.M., Walker, S.E., Edgcombe, G., Gaylor, M.O., Macdonald, E.H., 2000. Automotive sources of carcinogenic polycyclic aromatic hydrocarbons associated with particulate matter in the Chesapeake Bay region. *Environ. Sci. Technol.* 34 (21), 4635–4640.
- Echalar, F., Artaxo, P., Martins, J.V., Yamasoe, M., Gerab, F., Maenhaut, W., Holben, B., 1998. Long-term monitoring of atmospheric aerosols in the Amazon Basin: source identification and apportionment. *J. Geophys. Res., D: Atmos.* 103 (D24), 31849–31864.
- Emmett, M.R., White, F.M., Hendrickson, C.L., Shi, S.D.H., Marshall, A.G., 1998. Application of micro-electrospray liquid chromatography techniques to FT-ICR MS to enable high-sensitivity biological analysis. *J. Am. Soc. Mass Spectrom.* 9 (4), 333–340.
- Ertel, J.R., Hedges, J.I., Devol, A.H., Richey, J.E., Ribeiro, M.D.G., 1986. Dissolved humic substances of the Amazon River system. *Limnol. Oceanogr.* 31 (4), 739–754.
- Fasnacht, M.P., Blough, N.V., 2002. Aqueous photodegradation of polycyclic aromatic hydrocarbons. *Environ. Sci. Technol.* 36 (20), 4364–4369.
- Gelinas, Y., Prentice, K.M., Baldock, J.A., Hedges, J.I., 2001. An improved thermal oxidation method for the quantification of soot/graphitic black carbon in sediments and soils. *Environ. Sci. Technol.* 35 (17), 3519–3525.
- Glaser, B., Haumaier, L., Guggenberger, G., Zech, W., 1998. Black carbon in soils: the use of benzenecarboxylic acids as specific markers. *Org. Geochem.* 29 (4), 811–819.
- Goldberg, E.D., 1985. *Black Carbon in the Environment*. John Wiley & Sons, New York, 198 pp.
- Gustafsson, O., Haghseta, F., Chan, C., MacFarlane, J., Gschwend, P.M., 1997. Quantification of the dilute sedimentary soot phase: implications for PAH speciation and bioavailability. *Environ. Sci. Technol.* 31 (1), 203–209.
- Hannis, J.C., Muddiman, D.C., 2000. A dual electrospray ionization source combined with hexapole accumulation to achieve high mass accuracy of biopolymers in Fourier transform ion cyclotron resonance mass spectrometry. *J. Am. Soc. Mass Spectrom.* 11 (10), 876–883.
- Hatcher, P.G., Dria, K.J., Kim, S., Frazier, S.W., 2001. Modern analytical studies of humic substances. *Soil Sci.* 166 (11), 770–794.
- Haumaier, L., Zech, W., 1995. Black carbon-possible source of highly aromatic components of soil humic acids. *Org. Geochem.* 23 (3), 191–196.
- Hedges, J.I., Ertel, J.R., Quay, P.D., Grootes, P.M., Richey, J.E., Devol, A.H., Farwell, G.W., Schmidt, F.W., Salati, E., 1986. Organic carbon-14 in the Amazon river system. *Science* 231, 1129–1131.
- Hedges, J.I., Keil, R.G., Benner, R., 1997. What happens to terrestrial organic matter in the ocean? *Org. Geochem.* 27 (5–6), 195–212.
- Humphreys, J.M., Chapple, C., 2002. Rewriting the lignin roadmap. *Curr. Opin. Plant Biol.* 5 (3), 224–229.
- Jacobson, M.Z., 2001. Strong radiative heating due to the mixing state of black carbon in atmospheric aerosols. *Nature* 409 (6821), 695–697.
- Johnsson, P.A., Barringer, J.L., 1993. Water quality and hydro-geochemical processes in McDonalds branch Basin, New Jersey Pinelands, 1984–1988. *U.S. Geol. Surv. Rep.*, 91–4081, 1–111.
- Kamegawa, K., Nishikubo, K., Kodama, M., Adachi, Y., Yoshida, H., 2002. Oxidative degradation of carbon blacks with nitric acid—II: formation of water-soluble polynuclear aromatic compounds. *Carbon* 40 (9), 1447–1455.
- Kim, S., Kramer, R.W., Hatcher, P.G., 2003a. Graphical method for analysis of ultrahigh-resolution broadband mass spectra of natural organic matter—the van Krevelen diagram. *Anal. Chem.* 75 (20), 5336–5344.
- Kim, S., Simpson, A., Kujawinski, E.B., Freitas, M.A., Hatcher, P.G., 2003b. High resolution electrospray ionization mass spectrometry and 2D solution NMR for the analysis of DOM extracted by C<sub>18</sub> solid phase disk. *Org. Geochem.* 34 (9), 1325–1335.
- Kuhlbusch, T.A.J., 1998. Black carbon and the carbon cycle. *Science* 280 (5371), 1903–1904.
- Kuhlbusch, T.A.J., Crutzen, P.J., 1995. Toward a global estimate of black carbon in residues of vegetation fires representing a sink of atmospheric CO<sub>2</sub> and a source of O<sub>2</sub>. *Glob. Biogeochem. Cycles* 9 (4), 491–501.
- Kujawinski, E.B., Hatcher, P.G., Freitas, M.A., 2002. High-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) of humic and fulvic acids: improvements and comparisons. *Anal. Chem.* 74 (2), 413–419.
- Lord, D.G., Barringer, J.L., Johnsson, P.A., Schuster, P.F., Walker, R.L., Fairchild, J.E., Sroka, B.N., Jacobson, E., 1990. Hydro-geochemical data from an acidic deposition study at McDonalds branch Basin in the New Jersey Pinelands, 1983–1986. *U.S. Geol. Surv. Rep.*, 88–500, 1–124.
- Mannino, A., Harvey, H.R., 2004. Black carbon in estuarine and coastal dissolved organic matter. *Limnol. Oceanogr.* (in press).
- Masiello, C.A., Druffel, E.R.M., 1998. Black carbon in deep-sea sediments. *Science* 280 (5371), 1911–1913.
- Maurice, P.A., Pullin, M.J., Cabaniss, S.E., Zhou, Q., Namjesnik-Dejanovic, K., Aiken, G.R., 2002. A comparison of surface water natural organic matter in raw filtered water samples. XAD, and reverse osmosis isolates. *Water Res.* 36, 2357–2371.
- McCormick, J.S., 1955. A vegetation inventory of two watersheds in the New Jersey Pine Barrens. PhD thesis, Rutgers University, 125 pp.
- Middelburg, J.J., Nieuwenhuize, J., van Breugel, P., 1999. Black carbon in marine sediments. *Mar. Chem.* 65 (3–4), 245–252.
- Mitra, S., Bianchi, T.S., McKee, B.A., Sutula, M., 2002. Black carbon from the Mississippi River: quantities, sources, and potential implications for the global carbon cycle. *Environ. Sci. Technol.* 36 (11), 2296–2302.
- Mounier, S., Braucher, R., Benaim, J.Y., 1999. Differentiation of organic matter's properties of the Rio Negro Basin by crossflow ultra-filtration and UV-spectrofluorescence. *Water Res.* 33 (10), 2363–2373.
- Muri, G., Cermelj, B., Faganelj, J., Brancelj, A., 2002. Black carbon in Slovenian alpine lacustrine sediments. *Chemosphere* 46 (8), 1225–1234.
- Penner, J.E., Eddleman, H., Novakov, T., 1993. Towards the development of a global inventory for black carbon emissions. *Atmos. Environ., A Gen. Topics* 27 (8), 1277–1295.

- Raymond, P.A., Bauer, J.E., 2001. Riverine export of aged terrestrial organic matter to the North Atlantic Ocean. *Nature* 409 (6819), 497–500.
- Ritchie, J.E., Hedges, J.I., Devol, A.H., Quay, P.D., Victoria, R., Martinelli, L., Forsberg, B.R., 1990. Biogeochemistry of carbon in the Amazon River. *Limnol. Oceanogr.* 35 (2), 352–371.
- Senko, M.W., Hendrickson, C.L., Emmett, M.R., Shi, S.D.H., Marshall, A.G., 1997. External accumulation of ions for enhanced electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *J. Am. Soc. Mass Spectrom.* 8 (9), 970–976.
- Stenson, A.C., Landing, W.M., Marshall, A.G., Cooper, W.T., 2002. Ionization and fragmentation of humic substances in electrospray ionization Fourier transform-ion cyclotron resonance mass spectrometry. *Anal. Chem.* 74 (17), 4397–4409.
- Stenson, A.C., Marshall, A.G., Cooper, W.T., 2003. Exact masses and chemical formulas of individual Suwannee River fulvic acids from ultrahigh resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectra. *Anal. Chem.* 75 (6), 1275–1284.
- Suman, D.O., Kuhlbusch, T.A.J., Lim, B., 1997. Marine sediments: a reservoir for black carbon and their use as spatial and temporal records of combustion. In: Clarke, J.S., Cachier, H., Goldammer, J.G., Stocks, B.J. (Eds.), *Sediment Records of Biomass Burning and Global Change*. Springer-Verlag, Berlin, pp. 271–293.
- Tschech, A., 1989. Der anaerobe Abbau von aromatischen Verbindungen. *Forum Mikrobiol.* 12, 251–264.
- Williams, P.M., Druffel, E.R.M., 1987. Radiocarbon in dissolved organic-matter in the Central North Pacific-Ocean. *Nature* 330 (6145), 246–248.
- Willmann, G., Fakoussa, R.M., 1997. Biological bleaching of water-soluble coal macromolecules by a basidiomycete strain. *Appl. Microbiol. Biotechnol.* 47 (2), 95–101.
- Yamasoe, M.A., Artaxo, P., Miguel, A.H., Allen, A.G., 2000. Chemical composition of aerosol particles from direct emissions of vegetation fires in the Amazon Basin: water-soluble species and trace elements. *Atmos. Environ.* 34 (10), 1641–1653.