1 **Molecular properties of ultrafiltered dissolved organic matter and dissolved black**
2 **carbon in headwater streams as determined by pyrolysis-GC-MS**

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Abstract

This study aimed to assess the molecular properties of dissolved organic matter (DOM) and dissolved black carbon (DBC) using analytical pyrolysis (Py-GC-MS). The sample set was comprised of ultrafiltered DOM (UDOM) from North American headwater streams associated with Long Term Ecological Research network sites. Pyrolysis products for each UDOM sample were categorized as being sourced from non-pyrogenic sources and DBC. Major non-pyrogenic components of the headwater stream UDOM were comprised of phenolic compounds derived from lignin and chitin markers from microbial biomass, and their relative contributions indicated differences in organic matter dynamics of these ecosystems. The DBC pyrolyzates included benzene, PAHs and benzonitriles, which accounted for 12.5 ± 4.5 % of total quantified peak area (TPQA), and decreased in the order Alaskan boreal forest (19 %), Alaskan tundra (17 %), Appalachian deciduous forest (11 %), Colorado alpine tundra (9 %), Puerto Rican mountainous tropical rainforest (9 %) and Kansas tallgrass prairie (7 %). Pyrolysis products were compared to DBC content as determined by the benzenepolycarboxylic acid (BPCA) method. Although Py-GC-MS has quantitative limitations, this technique can detect weakly condensed and other DBC structures which fall outside of the BPCA analytical window.

Keywords: dissolved organic matter, black carbon, pyrolysis-GC-MS

1. Introduction

Extensive connectivity exists between rivers and the catchments they drain. As such, the dissolved organic matter (DOM) composition in fluvial systems can serve as an indicator of regional climate, land use and/or vegetation cover [1]. This is especially true for headwater streams, where DOM quality reflects local sources of organic matter (OM) and disturbances (e.g. fire) within the watershed [2,3]. The bioavailability and photolability of DOM is largely controlled by its chemical character, therefore knowing the composition of DOM aids in the prediction of its reactivity and ultimate environmental fate. Among the knowledge gaps regarding DOM dynamics is the lack of a profound understanding of the importance and nature of pyrogenic C mobilization and export in the dissolved phase as a fraction of the DOM pool. Black carbon (BC), a collective term for organic residues of incomplete combustion including charcoal and soot, has a significant role in the global C cycle [4]. With the occurrence of wildfires, burning of fossil fuels and fast-rising use of BC into cultivated soils as “biochar” [5,6], there is an urgent need for a deeper understanding of BC stability and fate. Previously considered to be a refractory component of OM, BC is actually recognized to be reactive in aquatic environments where it can be translocated to the aqueous phase as dissolved BC (DBC; [7]) and participate in reactive processing [8,9].

The ubiquity and abundance of BC in the environment has long been underestimated mainly because of the analytical challenges posed by the condensed aromatic structures which characterize it [10]). One of the commonly-employed methods of BC and DBC determination is the benzene polycarboxylic acid (BPCA) method

[11,12,13]. The BPCA method does not involve the creation of artifact BC and is therefore capable of detecting small amounts of BC [14]. The BPCA method yields oxidation products consisting of benzene rings with three (B3CA), four (B4CA), five (B5CA) and six (B6CA) carboxylic acid groups which form upon chemical degradation of polycondensed aromatic structures [15,16]. Greater relative proportions of B6CA to total measured BPCAs reflect a more polycondensed aromatic structure, whereas greater relative proportions of B3CA and B4CA reflect a less polycondensed aromatic structure [16]. Therefore, BPCA patterns are useful in estimating the degree of polycondensation of BC. However, they do not provide detailed information on specific molecular properties or sources of BC. The BPCA method has been employed to quantify DBC in a variety of aquatic systems [7,17,18], however the efficiency of the BPCA method (i.e. the C yield) remains largely undetermined. Ziolkowski et al. [16] established a C recovery of 20-25 % for several reference PAHs. Furthermore, it is possible that the chemical heterogeneity of DBC (e.g., the inclusion of O-containing functional groups, such as hydroxyl/ether and carboxyl/ester, and heterocyclic N and O moieties) may further compromise the BPCA yield.

Analytical pyrolysis (Py-GC-MS) [19] is a technique for the rapid molecular characterization of soil OM (SOM) and DOM ([20]. Recent studies have successfully applied Py-GC-MS to decipher the molecular properties of BC, including wildfire residues such as charcoal [21-23]. Pyrolysis-GC-MS has quantitative limitations, as only estimations of relative proportions of pyrolysis products can be obtained. In addition, BC is known to contain a non-pyrolyzable fraction, and therefore, pyrolysis products are unlikely to represent the entire BC pool. However, Py-GC-MS has been shown to provide

valuable information on source materials and degree of thermochemical alteration of BC [22,24,25]. The pyrolysis products which indicate the presence of BC include monocyclic and polycyclic aromatic hydrocarbons (MAHs/PAHs) [26,27], N- and O-containing aromatic compounds such as benzonitriles [21,28] and (di)benzofurans [29,30]. The degree of polycondensation of BC is reflected by the abundance of alkyl-cross bridges between aromatic moieties, and indicated by the ratios of benzene-to-toluene (B/T) and naphthalene-to-methylnaphthalenes (N/C₁N) [22,23,31]. However, these parameters have not been applied for the characterization of DBC in DOM isolates. Here, we applied Py-GC-MS to ultrafiltered DOM (UDOM) obtained from headwater streams across a climatic gradient in order to further probe the molecular properties of DOM, aiming to clarify unresolved observations from [32] and paying special attention to the molecular properties of DBC.

2. Materials and Methods

2.1 Study sites

Samples were collected from headwater streams from six Long Term Ecological Research (LTER) study sites across a range of climates and land cover including biomes such as tundra (ARC), boreal forest (BNZ), alpine forest (NWT), grassland prairie (KNZ), deciduous forest (CWT) and tropical rainforest (LUQ; see map in [32]). The watershed characteristics for each site are briefly described here, however details regarding vegetation, climate, rainfall and soil type can be found in Jaffe et al. [32].

102 ARC is located in the foothills of the Alaska North Slope in an area underlain
103 with continuous permafrost. ARC is described as a Tussock tundra environment with a
104 cold climate. Sample collection for ARC was from the Kuparuk River, whose watershed
105 vegetation is characterized by sedges and mosses. BNZ is located in the boreal forests of
106 central Alaska, with a continental climate characterized by warm summers and cold
107 winters. Sample collection for BNZ was at Caribou Creek, in the zone of discontinuous
108 permafrost, where watershed vegetation primarily consists of hardwoods, black spruce,
109 alder and mosses. NWT is primarily located in the alpine tundra of the Colorado Rocky
110 Mountains. The NWT water sample was collected from Como Creek which drains a sub-
111 alpine watershed dominated by mixed coniferous forests. The hydrology of Como Creek
112 is controlled by snowmelt and most of its catchment is below the treeline. Site KNZ is
113 located at the Konza Prairie Biological Station, a preserve of native tallgrass prairie in the
114 Flint Hills of northeastern Kansas. The KNZ sample was collected from Kings Creek,
115 whose watershed is dominated by C4 grasses and larger stream channels are bordered by
116 a riparian forest containing primarily oak and hackberry. Sub-watersheds of the Konza
117 Prairie are burned at frequencies ranging from annually to every 20 years. CWT is
118 located in the southern Appalachian Mountains of North Carolina at the Coweeta
119 Hydrological Laboratory. The climate is marine humid temperate and the vegetation is
120 primarily deciduous forest characterized by mixed hardwood stands. The CWT sample
121 was collected from Henson Creek, which drains a watershed containing coppice
122 regrowth, poplar cove and mature hardwoods. The LUQ site is an experimental forest
123 located in the mountainous tropical rainforest in northeastern Puerto Rico. The LUQ

water sample was collected from the Rio Mameyes, which drains the El Yunque rainforest. Regional soils are clayey, highly leached and weathered.

2.2 Sample preparation

The UDOM samples were obtained by freeze-drying preconcentrated, ultrafiltered stream waters as described in detail by Jaffe et al. [32]. Briefly, approximately 50–100 L of surface water was collected in 25 L pre-cleaned low density polyethylene bottles. Samples were kept on ice in the dark and transported to the laboratory within 48 hrs after collection. Samples were then filtered through pre-combusted GF/F glass fiber filters (nominal pore size, 0.7 μm ; Whatman International Ltd., Maidstone, England), followed by a polypropylene membrane cassette (nominal pore size, 0.22 μm) using a Pellicon 2 Mini tangential flow ultrafiltration system (Millipore Co., Billerica, MA, USA). The filtered waters were then concentrated using the above mentioned ultrafiltration system equipped with a nominal 1 kDa molecular weight cut-off regenerated cellulose membrane [33]. During ultrafiltration the samples were cooled in an ice water bath. The remaining salts were eliminated by triple diafiltration with 1 L of Milli-Q water to the concentrated sample (<100 mL). Concentrated UDOM samples were then freeze-dried and powdered with an agate mortar and pestle.

2.3 Analytical pyrolysis (Py-GC-MS)

For conventional analytical pyrolysis (Py-GC-MS), approximately 0.5 mg of UDOM powder was inserted into quartz tubes with quartz wool at both ends and pyrolyzed using a CDS Pyroprobe 5250 for 10 s at 750 $^{\circ}\text{C}$ (10 $^{\circ}\text{C ms}^{-1}$ heating rate) with

both the valve oven and the transfer line held isothermal at 325 °C. The pyrolysis temperature of 750 °C is considered suitable for detection and characterization of pyrogenic organic matter such as the DBC [34]. Pyrolysis products were separated on a 6890N gas chromatograph (Agilent Technologies) operating in 1:10 split mode, using He carrier gas (1 ml min⁻¹) and a HP-5MS polysiloxane-based column. The oven was initially at 60 °C, held for 1 minute, heated to 325 °C at 20 °C min⁻¹ and held four minutes isothermal. The detector was an Agilent 5975 mass spectrometer operating in 70 eV electron ionization (m/z 50–500). Quantification of pyrolysis products was based on the peak area of dominant fragment ions m/z . The relative proportions of each pyrolysis product were calculated as the percentage of the sum of all peak areas (total quantified peak area, TQPA).

Compounds producing a very dominant m/z 58 fragment and M^+ m/z 241 or 269, were identified as *N,N*-dimethyltetradecanamine and -hexadecanamine, respectively. In addition, two peaks with dominant ions m/z 134, 91 and traces of M^+ 317 and 345 probably correspond to *N*-methyl-*N*-benzyltetradecanamine and -benzylhexadecanamine, respectively. These dimethyl- and benzyl-substituted alkylamines were detected in all samples (9 ± 6 % of TQPA) but most abundant in the KNZ sample (19 % of TQPA). As far as we know these compounds have never been detected in SOM or DOM pyrolyzates and even though it cannot be discarded that they represent an amine-bridged alkylaromatic constituent of the UDOM, they may also originate from analytical artefacts (such as a myristyl dimethyl amine oxide surfactant). Therefore, these alkylamines were eliminated from the quantification dataset and the relative proportions of the other compounds recalculated to 100 % of TQPA. Diisooctyl phthalate is not included in the

analysis because it originates from plasticizers used in the laboratory or sampling material.

3. Results and Discussion

Table 1 summarizes relevant information on the composition of the ultrafiltered DOM samples from previous studies [17,32].

3.1 Identification of pyrolysis products and product-precursor relationships

Pyrolysis-GC-MS was employed for the molecular characterization of UDOM samples which represented a wide range of headwater streams with diverse climate and vegetation. The pyrolysis chromatograms (pyrograms) for each of the UDOM samples (ARC, BNZ, CWT, KNZ, LUQ, NWT) are presented in Figure 1. Peaks in the pyrolysis chromatograms correspond to identified pyrolysis products listed in Table 2 which have been categorized by compound class and probable source. The relative abundance of pyrolysis products (% TQPA) were then summed according to class (MAHs, carbohydrate products, phenols, N-compounds, PAHs, methoxyphenols, alkylamides, benzofurans, fatty acids) in order to simplify the comparison of UDOM composition among samples as determined by Py-GC-MS. The relative distributions of pyrolysis product classes for each UDOM sample are listed in Table 3.

Monocyclic aromatic hydrocarbons (MAHs) accounted for an average of 33 ± 11 % of TQPA across samples (Table 3). The most abundant pyrolysis product among all samples was toluene, which has no specific origin and has been frequently reported to be a principal pyrolysis product of DOM [20, and references therein] and many potential OM precursor materials including proteinaceous biomass, chitin, lignin, tannins and BC (e.g. [26]). If present in sufficient proportions, benzene is more frequently associated with BC [21]. The same can be argued for the C₂-benzenes (ethylbenzene and dimethylbenzenes) and C₃-benzenes. Although lignin and analytical artifact pathways can be assigned as potential sources for these compounds, Kracht [35] reported that the presence of these MAHs in DOM pyrolyzates was indicative of BC. In this study, all MAHs except for toluene and one of the C₂-benzenes were significantly correlated to the sum of PAHs ($r^2 > 0.78$, $P < 0.005$; see below), providing support that these MAHs were generated primarily from DBC.

Carbohydrate markers accounted for an average of 23 ± 9 % of TQPA (Table 3). The most abundant carbohydrate pyrolysis products were furaldehydes, furans and cyclopentenones. Pyrolysis products that represent more intact carbohydrate structures include pyrans, dianhydrosugars and 1,6-anhydrosugars [36]. These products were found to be less abundant among our UDOM sample set (not shown). Such depletions have been observed in earlier studies where DOM pyrolyzates are compared to SOM pyrolyzates [37]. Within the context of the present study, the 1,6-anhydrosugar “levoglucosan” requires some additional attention. This compound has been used as a marker of low temperature combustion products in environmental media, including DOM, and has been linked to fire regime intensity in soils and sediments [38-40].

Nonetheless, the link between levoglucosan and combustion products is only valid if it occurs freely in the sample (i.e. detected by GC-MS of organic solvent extracts) and not in the case of Py-GC-MS. In Py-GC-MS, the pyrolysis process (akin to the impact of wildfire) breaks the interglucosidic bonds in cellulose thereby releasing levoglucosan. Freely-occurring levoglucosan can be evaporated during pyrolysis, but its yield is typically overshadowed by the production from structural polysaccharides. In the present study, however, levoglucosan had only a minor contribution (0–1.6 % of TQPA), which implies that a mixed source consisting of polysaccharides and combustion residues cannot be ruled out. Either way, the lack of larger proportions of levoglucosan or other anhydrosugars suggests that the carbohydrate pyrolysis products originate predominantly from degraded and/or microbial polysaccharides, not intact structural plant polysaccharides. Finally, the deoxyhexose compound (0.3–3.5 % of TQPA), which is a relatively uncommon carbohydrate product in OM pyrolyzates, has been linked with microbial carbohydrates specifically [41] and is therefore a useful indicator of microbial DOM.

Compounds with N in their structure (excluding long-chain *n*-alkylamides, discussed below) accounted for 15 ± 3 % of TQPA (Table 3). Pyrrols and pyridines of unspecific N-containing precursor were abundant in all samples. Acetamide was found to be strongly enriched in the pyrolyzate of the KNZ sample (6.5 % compared to 1.5 ± 0.5 % of TQPA in all other samples). Acetamide is a marker of chitin-derived organic matter, which originates from fungal cell walls or arthropod exoskeleta [42]. As such, chitin products are an indication of intense organic matter mineralization conditions. The detection of 3-acetamido-4-pyrone is also generated from chitin-derived DOM [42]. The

large proportions of benzonitriles (benzonitrile and methylbenzonitriles), especially when unambiguous proteinaceous products such as benzene propanitrile are absent, are associated with N-containing structures in the DBC [28,34]. This is in agreement with the significant correlation between benzonitriles and PAHs for this sample set ($r^2=0.67$; $P<0.05$). Finally, the detection of indole and diketodipyrrole pyrolysis products are indicative of relatively intact proteinaceous precursors in DOM [43], not of chitin or DBC.

Phenols accounted for an average of 16 ± 3 % of TQPA among the UDOM samples (Table 3). Phenols are major pyrolysis products of degraded lignin and proteinaceous biomass and have been previously reported as being abundant in DOM pyrolysates [41]. Our suite of UDOM samples yielded phenol, methylphenols and C₂-phenols, which includes 4-vinylphenol, a pyrolysis product of *p*-coumaric acid (a non-lignin phenolic compound in vascular plants; [44]). High phenol yields, relative to methoxyphenol yields, in DOM have been reported previously and are considered to be indicative of degradation products of lignin-derived DOM in aquatic ecosystems [35,45,46].

The UDOM samples yielded PAHs (5 ± 1 % of TQPA) (Table 3), including indenenes, naphthalenes, biphenyl, fluorene, phenanthrene and anthracene, which have previously been identified as major products of BC [27]. Non-pyrogenic sources of PAHs such as plant resins and OM from coal deposits (mostly coalified plant resins) may interfere in BC identification by Py-GC-MS [47]. However, resin- or coal-derived constituents produce a PAH fingerprint typically dominated by poly-alkyl naphthalenes and phenanthrenes [48], with significant proportions of iso-alkyl substitutions, whereas

pyrogenic BC-derived PAHs lack iso-alkyl groups and have large proportions of unsubstituted or mono- and di-alkyl-substituted PAHs [47]. As such, the PAH fingerprint of the UDOM samples analyzed here strongly resembled that of BC. Another potential interference of PAHs in pyrolysis fingerprints may be non-pyrogenic humified OM [49]. Such PAHs are produced upon cyclization and aromatization of methylene chain-based OM, and produce concomitant series of long-chain linear *n*-alkylbenzenes and *n*-alkylnaphthalenes [49]. As demonstrated by the low abundance of *n*-alkanoic acids and virtual absence of *n*-alkane/*n*-alkene doublets and long-chain *n*-alkylbenzenes, PAH formation from humified OM was negligible. This attests to the fact that this artificial PAH formation pathway requires catalysis by reactive minerals [50], which were thoroughly eliminated by the UDOM isolation procedure. It is concluded that the PAHs must originate from DBC.

Among the UDOM samples, 2-methoxyphenols (guaiacols) and 2,6-dimethoxyphenols (syringols) comprised an average of 4 ± 4 % of TQPA (Table 3). Guaiacols and syringols are pyrolysis products of lignin in vascular plants, whereas compounds with 4-vinyl-substitution originate predominantly from lignin-like cinnamic acids (4-vinylguaiacol from ferulic acid and 4-vinylsyringol from sinapic acid) (e.g. [44]). Therefore, the methoxyphenols act as markers of plant-derived polyphenols, mainly lignin.

Finally, minor pyrolysis product groups included (i) benzofurans (1.4 ± 0.6 % of TQPA; Table 3), which originate from BC [30] and/or microbial sources [51], (ii) *n*-alkylamides ($C_{16:0}$, $C_{16:1}$ and $C_{18:0}$), which may originate from algae cell walls [52] or reactions between fatty acids and ammonia that may occur naturally [53] in soils,

sediments or streamwaters, or perhaps during analytical pyrolysis [54] (1.4 ± 0.8 % of TQPA) and (iii) *n*-alkanoic acids (C_{16} - and C_{18} -fatty acid), from unspecific lipids (0.5 ± 0.2 % of TQPA).

There are strong similarities between the UDOM pyrolyzate compositions of the present study and earlier DOM characterization studies by Py-GC-MS. Large proportions of MAHs, phenols, furans/furaldehydes, cyclopentenones and N-containing compounds, had been demonstrated for a variety of DOM pyrolyzates, including peat deposits [55,56], bog lake water [57,58], reservoir lake water [45], soil percolates [35], water-soluble SOM [41], river DOM [59,60], Mississippi River plume DOM [61]) and other samples of aquatic DOM [20]. We were unable to find unequivocal evidence of autochthonous DOM from planktonic primary production (such as pristenes and phytadienes from chlorophyll's phytol component) in the headwater stream UDOM. In fact, all detected compounds could be traced back to SOM components, with the possible exception of the ambiguous *n*-alkylamides. This is not surprising as headwater DOM is typically comprised of terrestrial inputs whereas lower order streams tend to exhibit higher proportions of autochthonous ones [62].

In order to facilitate a more comprehensive comparison between headwater UDOM in the current study and previously assessed DOM samples, the pyrolysis products were categorized as being associated with one of three main sources: microbial, plant polyphenol and BC (Table 2). Microbially-sourced pyrolysis products include all carbohydrate (except levoglucosan) and N-containing products (except benzonitriles, indole and diketodipyrrole). Plant polyphenolic pyrolysis products include lignin markers, such as methoxyphenols. Although the other phenolic products (phenol and

alkylphenols) may indicate non-vascular plant origins, their abundance was significantly correlated with lignin-derived methoxyphenols ($r^2=0.73$; $P<0.05$) suggesting that most phenols originated from lignin and were categorized as plant polyphenolic in origin. Products classified as microbial and plant polyphenols represent pyrolyzates generated from non-pyrogenic sources of DOM. DBC pyrolysis products included benzene, PAHs and benzonitriles. Note that even though White et al. [59] did not discuss the potential BC origin of these compounds detected in their DOM sample from BNZ, the pyrolysis fingerprint they obtained is suggestive of abundant BC as well. Ambiguous pyrolysis products which can originate from various sources (C_1 - C_3 -benzenes, levoglucosan, benzofurans, alkylamides, fatty acids, indole and diketodipyrrole) accounted for approximately one-third of TQPA (35 ± 6 % of TQPA) and were not assigned to the above-described biomass sources. Since the biomass sources of these pyrolysis products are unknown, they will not be discussed in the following sections.

3.1.1 Pyrolysis products from non-pyrogenic sources of DOM

The results presented here suggested that part of the pyrolysis products which indicate microbial DOM (32 ± 7 % of TQPA; Fig. 2a) can be traced back to chitin (acetamide and 3-acetamido-4-pyrone markers), an *N*-acetylglucosamine polymer [42]. Chitin-derived pyrolyzates were most abundant in the KNZ sample compared to other UDOM samples within the data set. The value of acetamide as a marker of microbial activity in DOM samples was postulated by Kracht [35], who presented unequivocal evidence of its microbial origin using compound-specific isotope analysis of pyrolyzates.

Templier et al. [63] have also associated acetamide in riverine DOM to chitin from microbial cell walls. The sum of microbial products was strongly correlated to acetamide ($r^2=0.94$; $P<0.005$; Fig. 2b), and to the unidentified deoxyhexose (not shown; $r^2=0.82$; $P<0.005$), which is also supposed to be a microbial carbohydrate marker. The sum of microbial pyrolysis products accounted for 46 % of TQPA in KNZ and 30 ± 3 % of TQPA in the other samples (Fig. 2a), indicating that the tallgrass prairie has a relatively large proportion of microbial DOM inputs, which is suggestive of fast OM cycling in its watershed. Microbial pyrolysis products were found to be negatively correlated with molar C/N ratio ($r^2=0.81$; $P<0.01$; Fig. 2c) and positively correlated with total hydrolysable amino acids ($r^2=0.72$; $P<0.05$; Fig. 2d). Low C/N ratios for KNZ have been attributed to high autochthonous (algae) production [32,64], however links to microbial DOM, and chitin in particular, suggest that terrestrial microbes (fungi) or perhaps aquatic invertebrates may also be important sources of N. In fact, Pisani et al. [64] suggested that while zooplankton is rarely reported in these prairie streams, invertebrates such as isopods are commonly found and may contribute to these molecular features.

Pyrolysis products of recognizable plant polyphenols included methoxyphenols and phenols which contributed to 20 ± 8 % of TQPA and decreased from 32 to 12 % in the order LUQ > CWT > NWT > ARC > KNZ > BNZ (Fig. 3a). Trends in the relative contribution of plant polyphenols can be influenced by several underlying mechanisms, such as the relative proportions of terrestrial-to-aquatic sources (allochthonous vs. autochthonous), differences in the polyphenol composition of source vegetation and the degree of preservation of plant-derived organic materials. The syringol-to-guaiacol (S/G) ratio for ARC (0.17) and NWT (0.12) were lower than for BNZ, CWT, KNZ and LUQ

(0.26–0.35; Fig. 3b). This might reflect the predominantly coniferous forest vegetation at NWT and the abundant sedges at ARC, which metabolize little or no syringyl lignin, contrary to the hardwood forest vegetation that is abundant in the surroundings of the other streams. The ratios of C₃-guaiacols to total guaiacols (excluding 4-vinylguaiacol; C₃G/G) and C₃-syringols to total syringols (C₃S/S) are indicators of the degree of lignin degradation [65]. These ratios for the Alaskan samples (ARC and BNZ) were zero (Fig. 3c/e), which may be associated with a more evolved, degraded C source. Nonetheless, fire also eliminates preferentially C₃-side chains, which may have also resulted in a decline in these ratios [22]. The sum of plant polyphenols was found to be significantly correlated with C₃G/G ($r^2=0.89$; $P<0.005$, not shown) and C₃S/S ($r^2=0.92$; $P<0.005$, not shown), which suggests that the proportion of plant-derived polyphenols in the DOM is controlled by the intensity of its degradation, and not by the proportions of different DOM sources, which is in line with observations by Neff et al. [46] for DOM pyrolyzates in Siberian rivers and streams.

3.1.2 Pyrolysis products from DBC

Interestingly, despite belonging to diverse ecosystems, all samples contained significant amounts of DBC, as determined by both Py-GC-MS (Fig. 4a) and the BPCA method [17,32]. Both methods suggested that the samples from Alaska (ARC and BNZ) contained the highest proportions of DBC as determined by Py-GC-MS. While pyrogenic residues from vegetation fires are the most likely source of the BC, the contribution of BC in boreal areas in general is still matter of debate [66], as it has been argued that it

might also originate from old OM sources in permafrost thaw and river bank erosion, rather than recent forest fires [67].

The DBC content indicated by the BPCA method, when normalized to DOC content, was found to be linearly correlated to the sum of predominantly BC-derived pyrolysis products ($r^2=0.59$; $P<0.05$; Fig. 4b). However, the % DBC from the BPCA method were much smaller (1.5 ± 1.2 % of DOC) than the sum of predominantly BC-derived pyrolysis products (13 ± 5 % of TQPA). In order to understand this discrepancy we have to look at the theoretical background of both methods and their relation to differences in the degree of polycondensation of DBC molecules.

Pyrolysis-GC-MS is known to underestimate the more polycondensed fraction of BC, because the hybridized C-C bonds are stable under pyrolysis conditions at 750 °C. This is reflected by the low signal intensities of pyrolysis chromatograms of high-temperature charcoals (produced at >550 °C) and soot [22]. The proportion of DBC-derived pyrolysis products (Fig. 4a) was found to be significantly correlated to the concentrations of B3CA ($r^2=0.84$, $P<0.01$) and B4CA ($r^2=0.71$, $P<0.05$), but was not significantly correlated to B5CA ($r^2=0.49$, $P>0.05$) or B6CA ($r^2=0.32$, $P>0.05$; Fig. 4c-f). Significant correlations between B3CA and B4CA and DBC pyrolysis products is in agreement with the preferential detection of smaller fused aromatic ring structures by Py-GC-MS. The benzene to toluene (B/T) and naphthalene to methylnaphthalene (N/C₁N) ratios have been used as indications of the degree of polycondensation [23,34], as they reflect the abundance of alkyl cross-bridges between aromatic groups. This B/T ratio was significantly correlated with the % DBC from the BPCA method ($r^2=0.82$; $P<0.005$), but this does not represent differences in DBC chemistry, but rather the balance between

DBC and non-pyrogenic OM, as the UDOM samples in the current study appeared to contain toluene generated from microbial biomass sources. Therefore the B/T ratio is unsuitable for this particular sample set (it is only suitable for pure BC samples). Here, the N/C₁N ratio seems more reliable, as it is limited to the detection of polyaromatic groups and is therefore more specific for BC. The N/C₁N ratio shows values increasing from 0.6 (weakly condensed BC) to 1.2 (moderately condensed BC) in the order KNZ < CWT < NWT < LUQ < BNZ < ARC (not shown). The N/C₁N ratio was not significantly correlated to the % B6CA or other parameters of the degree of polycondensation such as B_μCA (average number of carboxylic groups; [68]). A larger sample pool would be necessary to determine the compatibility of BPCA patterns and Py-GC-MS patterns of naphthalenes.

The BPCA method is known to efficiently convert large fused aromatic ring structures in e.g. soot, fullerenes, carbon lampblack or activated charcoal, into B6CA (70–100 % of total BPCA; [13,69,70]). However, as fused ring size decreases, the proportions of B5CA, B4CA and B3CA increase because the relative proportion of C atoms at the edges of aromatic clusters, which do not carboxylate, increases. The % B6CA of total BPCAs for DBC in the present data set averaged only 8 ± 4 % (Table 1), which suggests a dominance of relatively small fused aromatic ring size for DBC among these UDOM samples [68]. In fact, a comparison between the % B6CA of the DBC with BPCA distributions of solid wood charcoal produced in laboratory ovens between 200 and 1200 °C [71] would suggest that the fused aromatic ring size of DBC would be equivalent to wood charcoal formed at only 200–250 °C. The thermal alteration of OM at such low temperatures does not induce significant aromatization let alone generate

abundance of polycondensed aromatic structures [72]. These results suggest that the small size of the fused aromatic rings in the DBC is not controlled by formation temperature, but by a combination of (1) BC degradation and translocation to the dissolved phase upon ageing in the soil environment [73] and (2) photo-reactive processing of DBC in the aquatic environment [8,9]. This line of argument is similar to that of Hockaday et al. [74] who analyzed DBC in soil solutions by the BPCA method and of [75] who suggested that relatively low BPCA yields for DBC measured in forested tundra streams may be due to the presence of “small and oxidatively degraded” aromatic clusters indicated by additional characterization via spectroscopic methods. The relative importance of depolymerization in the soil environment and photodegradation upon exposure to sunlight in surface waters, to the depolyaromatization (aromatic ring opening, not necessarily depolymerization) of fused aromatic ring moieties, remains unknown.

In summary, the discrepancy between the two methods can be largely explained by the bias towards small-sized aromatic ring structures by Py-GC-MS and towards large-sized polycondensed structures by the BPCA method. The small average fused aromatic ring size of DBC implies that the estimate given by Py-GC-MS is probably more representative of the proportion of the UDOM that originates from DBC.

4. Conclusions

The pyrolysis fingerprints of UDOM samples from headwater streams across North America were composed primarily of DBC (MAHs, PAHs, benzonitriles), microbial biomass (most carbohydrates and N-containing compounds), and (degraded) lignin (phenols and methoxyphenols). The DBC composition was mainly composed of small fused aromatic ring clusters and abundant heteroatomic groups, which may explain the large difference in the estimation of DBC content between Py-GC-MS and the BPCA method. In aquatic environments, there is an urgent need for accurate measurements of the proportion and chemical diversity along the combustion continuum of DBC to understand its importance in the global C cycle. Although the BPCA method may be one of the more reliable methods for polycondensed aromatic DBC recognition and quantification, it may however, not be as useful in assessing the contribution of smaller or non-condensed pyrogenic structures. Py-GC-MS may help to fill in some of the gaps for characterizing DBC structures which fall outside the BPCA detection window.

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Table 1. Origin and properties of the ultrafiltered dissolved organic matter (DOM) samples analyzed in this study. DOC= dissolved organic carbon; BPCA = benzene polycarboxylic acid, B_nCA = benzene polycarboxylic acids with *n* carboxylic acid groups.

Site	ARC	BNZ	CWT	KNZ	LUQ	NWT
Biogeographical region	Alaskan tundra	Alaskan taiga	Deciduous forest	Great Plains grassland	Tropical rain forest	Sub-alpine mixed forest
Sampling date	07/29/06	07/30/06	09/19/06	07/11/06	03/02/07	08/25/06
DOC (mg/L)	4.01	2.82	1.85	0.77	2.11	1.86
DOM filtration recovery (% DOC)	35.5	47.6	68.7	31.3	32.0	46.2
DOM C/N (molar ratio)	32.8	46.3	38.5	17.7	28.4	41.2
Amino acid C (%)	2.1	1.1	2.3	4.7	4.0	1.9
Total BPCA (% of C)	2.9	2.3	0.9	1.5	1.6	0.8
B3CA (% of total BPCA)	13.12	17.64	42.89	9.26	20.60	33.58
B4CA (% of total BPCA)	54.28	54.38	30.91	37.86	55.27	49.97
B5CA (% of total BPCA)	28.87	24.59	11.41	44.22	16.78	8.63
B6CA (% of total BPCA)	3.73	3.39	14.79	8.66	7.34	7.82
B3CA (% of C)	0.45	0.44	0.19	0.09	0.22	0.27
B4CA (% of C)	1.87	1.34	0.14	0.36	0.58	0.39
B5CA (% of C)	0.99	0.61	0.05	0.42	0.18	0.07
B6CA (% of C)	0.13	0.08	0.07	0.08	0.08	0.06
B4CA/B3CA (-)	4.14	3.08	0.72	4.09	2.68	1.49

Table 2. Pyrolysis product list, with retention time (RT), ion fragments used for quantification (m/z), compound classes (MAH/PAH = monocyclic/polycyclic aromatic hydrocarbon) and predominant sources (DBC = dissolved Black C).

Label	pyrolysis product	RT (min)	m/z	Class	Source
1	benzene	2.082	78	MAH	DBC
2	<i>N</i> -methylpyrrole	2.357	80+81	N-compound	Microbial
3	pyridine	2.454	79	N-compound	Microbial
4	pyrrole	2.466	67	N-compound	Microbial
5	toluene	2.54	91+92	MAH	
6	acetamide	2.605	59	N-compound	Microbial
7	C ₁ -pyridine	2.821	93+66	N-compound	Microbial
8	C ₁ -pyrrole	2.952	80+81	N-compound	Microbial
9	3/2-furaldehyde	2.964	95+96	Carbohydrate	Microbial
10	2-methylfuran	2.992	82+53	Carbohydrate	Microbial
11	C ₁ -pyrrole	3.015	80+81	N-compound	Microbial
12	C ₁ -pyridine	3.152	93+66	N-compound	Microbial
13	C ₂ -benzene	3.158	91+106	MAH	
14	C ₂ -benzene	3.215	91+106	MAH	
15	C ₂ -benzene	3.349	91+106	MAH	
16	C ₁ -cyclopenten-1-one	3.467	67+96	Carbohydrate	Microbial
17	2-acetylfuran	3.56	95+110	Carbohydrate	Microbial
18	C ₃ -benzene	3.89	120	MAH	
19	5-methyl-2-furaldehyde	3.925	110+109	Carbohydrate	Microbial
20	C ₁ -cyclopenten-1-one	3.954	96+67	Carbohydrate	Microbial
21	phenol	4.045	94+66	Phenol	Polyphenol
22	C ₃ -benzene	4.097	120	MAH	
23	benzonitrile	4.154	103+76	N-compound	DBC
24	4-hydroxy-5,6-dihydro-(2H)-pyran-2-one	4.211	114+58	Carbohydrate	Microbial
25	benzofuran	4.257	118+89	Benzofuran	
26	2-hydroxy-3-methyl-2-cyclopenten-1-one	4.451	112+55	Carbohydrate	Microbial
27	2,3-dimethylcyclopent-2-en-1-one	4.549	67+110	Carbohydrate	Microbial
28	dianhydorrhamnose	4.56	113+128	Carbohydrate	Microbial
29	indene	4.663	115+116	PAH	DBC
30	C ₁ -phenol	4.714	107+108	Phenol	Polyphenol
31	4-methylphenol	4.88	107+108	Phenol	Polyphenol
32	guaiacol	4.955	109+124	Methoxyphenol	Polyphenol
33	C ₁ -benzofuran	5.075	132+131	Benzofuran	
34	C ₁ -benzofuran	5.115	132+131	Benzofuran	
35	5-hydroxymethyl-2-furancarboxaldehyde	5.167	126	Carbohydrate	Microbial
36	C ₂ -phenol	5.355	107+122	Phenol	Polyphenol
37	C ₁ -benzonitrile	5.441	117+116	N-compound	DBC
38	C ₂ -phenol	5.447	107+122	Phenol	Polyphenol
39	C ₁ -indene	5.493	130+115	PAH	DBC
40	C ₁ -indene	5.538	130+115	PAH	DBC
41	4-ethylphenol	5.59	107+122	Phenol	Polyphenol
42	unidentified deoxyhexose	5.619	69+100	Carbohydrate	Microbial
43	naphthalene	5.79	128	PAH	DBC
44	4-vinylphenol	5.91	120+91	Phenol	Polyphenol
45	indole	6.534	117+90	N-compound	Microbial

46	C ₁ -naphthalene	6.603	142+115	PAH	DBC
47	4-vinylguaiacol	6.694	150+135	Methoxyphenol	Polyphenol
48	C ₁ -naphthalene	6.729	142+115	PAH	DBC
49	3-acetamido-4-pyrone	6.912	111+153	N-compound	Microbial
50	syringol	6.923	154+139	Methoxyphenol	Polyphenol
51	C _{3:1} -guaiacol	6.929	164+149	Methoxyphenol	Polyphenol
52	biphenyl	7.175	154+153	PAH	DBC
53	C ₂ -naphthalene	7.278	156+141	PAH	DBC
54	C _{3:1} -guaiacol	7.278	164+149	Methoxyphenol	Polyphenol
55	4-formylguaiacol	7.287	151+152	Methoxyphenol	Polyphenol
56	C ₂ -naphthalene	7.369	156+141	PAH	DBC
57	C ₂ -naphthalene	7.41	156+141	PAH	DBC
58	C _{3:1} -guaiacol (<i>trans</i>)	7.553	164+149	Methoxyphenol	Polyphenol
59	4-acetylguaiacol	7.845	151+166	Methoxyphenol	Polyphenol
60	dibenzofuran	8.062	168+139	Benz	
61	4-vinylsyringol	8.274	180+165	Methoxyphenol	Polyphenol
62	levoglucosan	8.274	60+73	Carbohydrate	
63	fluorene	8.525	166+165	PAH	DBC
64	C _{3:1} -syringol	8.777	194+179	Methoxyphenol	Polyphenol
65	4-formylsyringol	8.897	181+182	Methoxyphenol	Polyphenol
66	C ₁₄ -C ₂ -amine*	9.006	58	Alkylamine*	
67	C _{3:1} -syringol (<i>trans</i>)	9.063	194+179	Methoxyphenol	Polyphenol
68	diketodipyrrole	9.206	186+93	N-compound	
69	4-acetylsyringol	9.298	181+196	Methoxyphenol	Polyphenol
70	phenanthrene	9.693	178	PAH	DBC
71	anthracene	9.75	178	PAH	DBC
72	C ₁₆ -C ₂ amine*	10.065	58	Alkylamine*	
73	C _{16:0} fatty acid	10.345	60+73	Fatty acid	
74	C _{18:0} fatty acid	11.295	60+73	Fatty acid	
75	C _{16:0} amide	11.426	59+72	Alkylamide	
76	C ₁₄ -C ₁ -benzylamine*	12.05	134+91	Alkylamine*	
77	C _{18:1} amide	12.227	59+72	Alkylamide	
78	C _{18:0} amide	12.319	59+72	Alkylamide	
79	C ₁₆ -C ₁ -benzylamine*	12.891	134+91	Alkylamine*	

684 *Alkylamines **might** correspond to contamination (Section 2.3)

Table 3. Pyrolysis product groups and relative proportions (% of total quantified peak area, TQPA), of the ultrafiltered DOM samples analyzed. MAHs = monocyclic aromatic hydrocarbons; PAHs = polycyclic aromatic hydrocarbons.

	ARC	BNZ	CWT	KNZ	LUQ	NWT
MAHs	40.9	48.8	34.8	20.0	23.3	31.5
Carbohydrate products	19.1	15.1	16.6	39.7	23.2	24.3
Phenols	15.9	11.2	18.4	11.3	21.7	17.2
N-compounds	15.1	13.5	16.7	20.3	14.3	13.5
PAHs	5.7	6.6	5.5	3.8	4.9	3.4
Methoxyphenols	0.8	0.8	5.0	2.2	10.4	4.8
Alkylamides	0.8	2.1	0.8	1.6	0.7	2.6
Benzofurans	1.5	1.7	2.0	0.5	1.0	1.8
Fatty acids	0.3	0.3	0.4	0.7	0.4	0.8

Figure captions

Figure 1: Total ion current pyrolysis chromatograms of the DOM samples analyzed. Peak labels correspond to pyrolysis products numbers in Table 2. *=contamination, red labels correspond to alkylamine derivatives (possibly contamination, see text).

Figure 2: (a) Sum of microbial products from microbial DOM. (b) Microbial products plotted against acetamide. (c) Microbial products plotted against molar C/N ratio. (d) Microbial products plotted against hydrolysable amino acid C.

Figure 3: (a) Sum of pyrolysis products from plant polyphenols (lignin). (b) Syringyl to guaiacyl ratio. (c) C₃-guaiacols to total guaiacols ratio. (d) C₃-guaiacols to total guaiacols ratio plotted against BPCA yield. (e) C₃-syringols to total syringols ratio. (f) C₃-syringols to total syringols ratio plotted against BPCA yield.

Figure 4: (a) Sum of pyrolysis products from Black C. (b) Black C pyrolysis products plotted against total BPCA (benzene polycarboxylic acids) from the BPCA method. (c) Black C pyrolysis products plotted against B3CA from BPCA method. (d) Black C pyrolysis products plotted against B4CA from BPCA method. (e) Black C pyrolysis products plotted against B5CA from BPCA method. (f) Black C pyrolysis products plotted against B6CA from BPCA method.

Figure 1

Figure 2

Figure 3

Figure 4