

Comparison of dialysis and solid-phase extraction for isolation and concentration of dissolved organic matter prior to Fourier transform ion cyclotron resonance mass spectrometry

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Abstract We compare two methods, solid-phase extraction (SPE) and dialysis, commonly used for extraction and concentration of dissolved organic matter (DOM) prior to molecular characterization by electrospray ionization (ESI) and ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry. Spectra of DOM samples from Minnesota and Sweden peatlands that were extracted with styrene divinyl benzene polymer SPE sorbents included ions with formulas that had higher oxygen to carbon (O/C) ratios than spectra of DOM from the same samples after de-salting by dialysis. The SPE method was not very effective in extracting several major classes of DOM compounds that had high ESI efficiencies, including carboxylic acids and organo-sulfur compounds, and that out-competed other less-functionalized compounds (e.g., carbohydrates) for charge in the ESI source. The large abundance of carboxylic acids in the dialysis-extracted DOM, likely the result of in situ microbial production, makes it difficult to see other (mainly hydrophilic) compounds with high O/C ratios. Our results indicate that, while dialysis is generally preferable for the isolation of DOM, for samples with high microbial inputs,

the use of both isolation methods is recommended for a more accurate molecular representation.

Keywords Solid-phase extraction · Dialysis · Peatlands · Dissolved organic matter · Ultrahigh-resolution mass spectrometry of DOM

Introduction

Dissolved organic matter (DOM) is a complex heterogeneous mixture of organic compounds that play a fundamental role in both terrestrial and aquatic environments through a number of physical, chemical, and biological processes [1, 2]. Organic matter in peatlands, including both solid (SOM) and dissolved (DOM) fractions, represents a large pool of terrestrial organic carbon and may therefore exert a large influence on the global carbon cycle [3].

Peatlands store a large amount of organic carbon (C) due to depleted oxygen in water-saturated soils and a climate that results in low decomposition relative to production [4]. The amount of organic C that is stored in northern peatlands has been estimated at between 250 and 450 pg of carbon, which is nearly half the amount of carbon stored in the atmosphere as CO₂ [3]. Associated with this sequestered carbon in peatlands is the emission of microbial respiration products such as methane and carbon dioxide to the atmosphere [5, 6]. Concerns have been raised whether these ecosystems will continue to be sinks of atmospheric C or if increasing global temperatures will turn them from net carbon sequestering environments to carbon emitters because of increased microbial decomposition of SOM and/or DOM. As DOM is a reactive intermediate in organic carbon decomposition and emission, determining its

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molecular composition is an important aspect of understanding the pivotal role it plays in carbon cycling.

The origin, function, and fate of DOM in the carbon cycle are, however, still only partially understood. One reason for this lack of knowledge about DOM is the analytical challenge in resolving such complex molecular mixtures. The limitations in DOM analysis are the result of its high complexity, low concentration of individual components, and generally high polarity of solutes. Through isolation of DOM, highly concentrated organic samples with low salt content can be obtained, and advanced analytical measurements such as ^1H and ^{13}C NMR spectrometry and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) can then be carried out on these concentrates. However, one significant challenge with any such isolation procedure is in obtaining a representative fraction of the DOM pool to be analyzed [7].

Electrospray ionization (ESI) is the most widely used ionization method for DOM prior to FT-ICR MS analysis. ESI creates positively or negatively charged ions over a wide mass range ($10 < m/z < 3,000$) and can be easily coupled with ultrahigh-resolution mass spectrometry [8]. ESI preferentially ionizes polar compounds with functional groups such as amines ($-\text{NH}_2$) and carboxylic acids ($-\text{COOH}$) to produce largely intact gaseous ions at room temperature and atmospheric pressure [9].

ESI requires relatively concentrated samples with low salt content [10]. Solid-phase extraction (SPE) is one of the most widely used techniques to concentrate and de-salt DOM from aquatic systems. XAD resins were the first materials used for SPE [11]. For example, SPE with XAD resins was used to concentrate and de-salt phytoplankton-derived DOM, retaining up to 65% of the organic material [12]. Another method for isolating organic compounds from aqueous solutions for subsequent analysis is adsorption onto C_{18} SPE cartridges [13]. Dittmar et al. developed a very practical and robust extraction method that is able to extract >60% of coastal and >40% of deep-sea DOM with a styrene divinyl benzene polymer (PPL)-based sorbent [14]. The PPL method is applicable for extracting nonpolar to highly polar substances from large volumes of water without major instrumentation and can be performed under difficult field conditions without electric power.

More recently, ultrafiltration using low-molecular-weight membranes (nominal molecular weight cutoff (MWCO) of 1,000 Da) has become a widely accepted method for the extraction of DOM. This material is now commonly referred to as high-molecular-weight DOM (HMW-DOM), or colloidal DOM. Depending upon the environment being sampled, the HMW-DOM fraction can range from 20% to 60% of the total DOM pool [15].

A previous study compared ultrafiltration with C_{18} solid-phase extraction to isolate DOM [16]. That work

demonstrated that C_{18} -extracted DOM and ultrafiltered HMW-DOM differ markedly in their chemical composition. The ultrafiltered HMW-DOM was enriched in (degraded) polysaccharides along with amino sugars when compared with the C_{18} -extracted DOM. The C_{18} -extracted DOM appeared enriched in aromatic compounds, probably from lignin and/or aromatic amino acids in proteins. Sleighter et al. [17] compared the molecular composition of a C_{18} extract of riverine water to the organic composition of whole, unfractionated water by FT-ICR mass spectrometry. They observed that that C_{18} extraction is selective in that it eliminates two major series of compounds, the aliphatic amines/amides, and tannin-like compounds, both of which were found not to adsorb to the C_{18} material.

Various forms of dialysis have also been used to isolate DOM. Koprivnjak et al. [18] used a combination of reverse osmosis and electro-dialysis (RO/ED) to isolate DOM from marine waters with an average yield of 75% for the 16 samples they studied. Their system, while exhibiting higher yields than SPE methods, requires a complicated and expensive device and is still somewhat selective, resulting in an isolate that is relatively rich in fatty acids and low in unsaturated organic compounds. Simple dialysis followed by freeze-drying is also commonly used to isolate and concentrate DOM samples before FT-ICR MS analysis. Like the RO/ED method, the combination of dialysis and freeze-drying should in principle recover more DOM from a water sample than SPE, since all molecules larger than the nominal MWCO of the dialysis membrane used should be retained.

The objective of this study was to provide for the first time a comparison of dialysis and PPL solid-phase extraction as DOM isolation methods for peatland porewater samples. Isolates from both methods were characterized by ultrahigh resolution ESI FT-ICR MS. A 200-Da MWCO dialysis membrane was used, since this is the lower molecular weight range that can be observed by FT-ICR MS.

Experimental

Sampling

Three porewater samples were collected from peatlands in northern Minnesota and northern Sweden. The northern Minnesota sample was collected in 2008 from the Sturgeon River fen site (SR). The SR fen site is located northeast of the Red Lake II complex above the Lost River site where different fen vegetation types could be found, making it a rich fen site. The northern Sweden samples were collected in 2010 from Stordalen Mire, a discontinuous permafrost peatland located 10 km southeast of the Abisko Scientific Research Station (ANS). This site is characterized by a mixture of *palsa* (permafrost hummock), bog, and fen

habitats. The samples used in this study were collected from two bog sites (Bog 1 and SOS) dominated by *Sphagnum* mosses. Immediately after collection, samples were filtered in field (Minnesota samples through 0.7 μm Whatman Nuclepore QTEC membrane filter cartridges and Sweden samples through 0.7 μm Whatman GF/F glass microfiber filters), and their pH was measured using a portable pH meter. Samples were then stored frozen in the dark ($-20\text{ }^{\circ}\text{C}$), until analysis. The dissolved organic carbon concentration of the different porewater samples was measured by high-temperature catalytic oxidation by a Shimadzu Total Organic Carbon analyzer equipped with a non-dispersive infrared detector. Triplicate measurements were done for each sample, and the coefficient of variance was always $<2\%$.

DOM extraction and sample preparation

One set of samples from each site was extracted by the SPE method described by Dittmar et al. [14]. The SPE cartridges (PPL, 500 mg, Varian Mega Bond Elut, Varian Inc., Palo Alto, CA, USA) were rinsed with one cartridge volume (6 mL) of methanol (p.a.) immediately before use. For DOM adsorption, 30 mL of each of the porewater samples was acidified with hydrochloric acid (p.a.) to pH 2 and pumped through the SPE cartridge, at a flow rate of $<50\text{ mL/min}$. Before elution of DOM with methanol, the cartridges were rinsed with at least two cartridge volumes of 0.01 M HCl for complete removal of salts. Sorbents were then dried under a stream of N_2 and DOM then eluted with 6 mL (one cartridge volume) of methanol (Merck Lichrosolv) at a flow rate of $<10\text{ mL/min}$. Eluted DOM samples were then stored in the dark until analysis.

The second set of samples was desalted with 200 Da MWCO SPECTRA dialysis membranes. Prior to use, the membranes were soaked in water for $\sim 30\text{ min}$ to remove the sodium azide preservative. Approximately 7 mL from each sample was dialyzed in the 200 Da, 10 mm dialysis membranes for 24 h in Milli Q water (water purified with a Millipore Milli Q to purity greater than 18 MW/cm). Milli Q water was changed twice every 2 h four times and then left overnight.

The dialyzed DOM samples were then concentrated by freeze drying. A solution containing the freeze-dried or SPE DOM in methanol was then prepared for each of the samples. The final concentration of all samples was $\sim 500\text{ mg CL}^{-1}$.

ESI FT-ICR MS

A custom-built FT-ICR mass spectrometer with a 9.4-Tesla (9.4-T) superconducting magnet located at the National High Magnetic Field Laboratory was used for all mass spectral measurements. A home-built ESI source was used to generate negatively charged molecular ions. Prepared samples were introduced to the ESI source through a syringe pump at a flow

rate of $0.5\text{ }\mu\text{L min}^{-1}$ equipped with a 50- μm i.d. fused silica tube. Experimental conditions were as follows: needle voltage, -2.5 kV ; tube lens, -340 V ; and heated metal capillary operated at 8 W. These parameters were chosen based on previous humic acid mass analyses for optimal DOM MS characterization. Two hundred individual scans were summed. The mass difference between the measured mass and that calculated from the assigned elemental composition was typically $<0.5\text{ ppm}$, and the resolving power was $>800,000$ at $m/z\ 499$. Spectra were calibrated by two internal homologous series that included formulas separated by 14 Da (i.e. one $-\text{CH}_2$ group), and the mass accuracy was calculated to be $<1\text{ ppm}$ for singly charged ions ranging across the mass spectral distribution (300–900 Da). A Modular ICR Data Acquisition System created at the NHMFL was used to calculate all possible molecular formulas within a $\pm 1\text{ ppm}$ error range. Molecular formulas were assigned based on the following criteria: signal-to-noise ratio must be $>6\sigma$, formulas must be a part of a homologous series, must have ^{13}C peak confirmation, and must have a mass error $<1\text{ ppm}$, taking into consideration the presence of C, H, O, S, and N. All observed ions were singly charged as confirmed by the 1.0034 Da spacing found between isotopic forms of the same molecule (between $^{12}\text{C}_n$ and $^{12}\text{C}_{n-1}^{13}\text{C}_1$).

Analysis of FT-ICR MS data

Ultrahigh-resolution mass spectrometry of DOM typically produces several thousand unique elemental formulas between 200 and 1,000 Da from a single spectrum. An efficient data analysis method is therefore required to interpret the large data sets from complex mass spectra generated by FT-ICR MS. One method for characterizing and visualizing large DOM data sets is the van Krevelen (vK) diagram [19]. The vK diagram plots the molar hydrogen to carbon (H/C) ratios on the y -axis and the molar oxygen to carbon (O/C) ratios on the x -axis and is utilized to assist in clustering molecules according to their functional group compositions. The diagram displays one data point for every molecular formula assigned in the mass spectrum. Chemical classes of biomolecules commonly found in DOM have characteristic H/C and O/C ratios and therefore cluster within specific regions of the diagram [19]. The vK diagrams not only identify the source material of DOM but also allow identification of differences in the molecular composition by overlaying plots. Alternatively, molecular formula data can be sorted into categories such as carbon number, heteroatom class, and double-bond equivalents (DBE; number of rings plus double bonds) and double-bond equivalents minus oxygen (DBE-O). DBE is a measure of unsaturation (e.g., a fully saturated hydrocarbon has a DBE equal to zero). Each additional ring or double bond results in a loss of two hydrogen atoms [20]. DBE-O is obtained from the molecular

formula by subtracting the number of oxygens in the formula from the DBE. Assuming that oxygen in DOM exists mainly as double-bond oxygen in carboxyl groups, DBE-O provides information about the degree of unsaturation or ring closure for only the backbone carbon structure [21].

All assigned formulas were also grouped according to elemental composition into three sub-classes, CHO, CHON, and CHOS. The total number of formulas in each class were then summed and normalized by the total number of assignable formulas to produce a relative abundance (as percent) for the three classes.

Multivariate analysis

Combinational cluster analysis with group average method was performed using the Unscrambler software. The Bray–Curtis dissimilarity measure was used to calculate the distance matrix, and the abundances (as percent) of the different chemical classes present in each sample were used as the data input.

Results

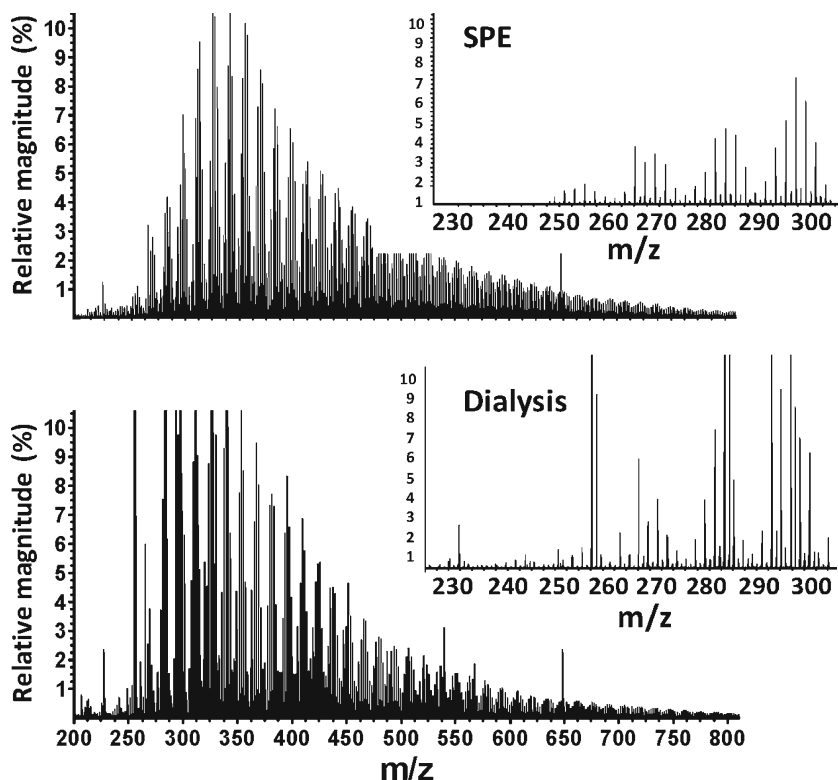
FT-ICR MS of SR fen DOM

ESI FT-ICR mass spectra of the SPE- and dialysis-extracted SR fen DOM are included in Fig. 1. Both spectra exhibit a pseudo Gaussian distribution with m/z values ranging from

200 to 800. The most abundant signals in the insets of the m/z range of 225–300 in the dialysis-extracted DOM mass spectrum are assigned to carboxylic (fatty) acids (saturated and unsaturated). Fatty acids do not appear as abundant in the solid-phase extracted DOM (SPE-DOM). The mass scale expanded segments insets of Fig. 2a, b emphasize the difference between the two samples in a narrow mass window (0.3 Da), a difference that is noted at nearly every odd nominal mass throughout the mass spectra. Between m/z 283 and 283.3, 19 assignable molecular formulas are observed for dialysis-extracted DOM but only 13 for SPE-DOM (Table 1) with ten molecular formulas appearing in both samples (i.e., “matching formulas”). Peaks at low mass defect in the SPE-DOM spectrum are not observed in the dialysis-extracted DOM spectrum. Low-mass-defect species are indicative of compounds with high oxygen content and/or low hydrogen content (i.e., unsaturation) [19]. This observation is more pronounced at high m/z values (Fig. 2b and Table 2), where 37 peaks were observed in the SPE-DOM spectrum at a nominal mass of 581 and only 30 in the dialyzed DOM spectrum, with 23 molecular formulas observed in both. An abundance of low-mass-defect peaks were again observed in the SPE-extracted DOM spectrum but not in the dialysis-extracted DOM spectrum (Table 2).

To extend these observations beyond the limited 0.3 m/z mass ranges evaluated in Fig. 2 and Table 2, peaks observed in SPE- and dialysis-extracted DOM spectra were evaluated for chemical formula matches of neutral molecules over the

Fig. 1 FT-ICR mass spectra of SR fen DOM. *Top*: SPE-extracted DOM; *bottom*: Dialyzed DOM. The *insets* are the mass-expanded regions of the m/z range 225–300. Relative magnitudes represent peak intensities normalized to most intense peak in the spectrum and have been truncated at 10% so that fine structure in each spectrum is visible



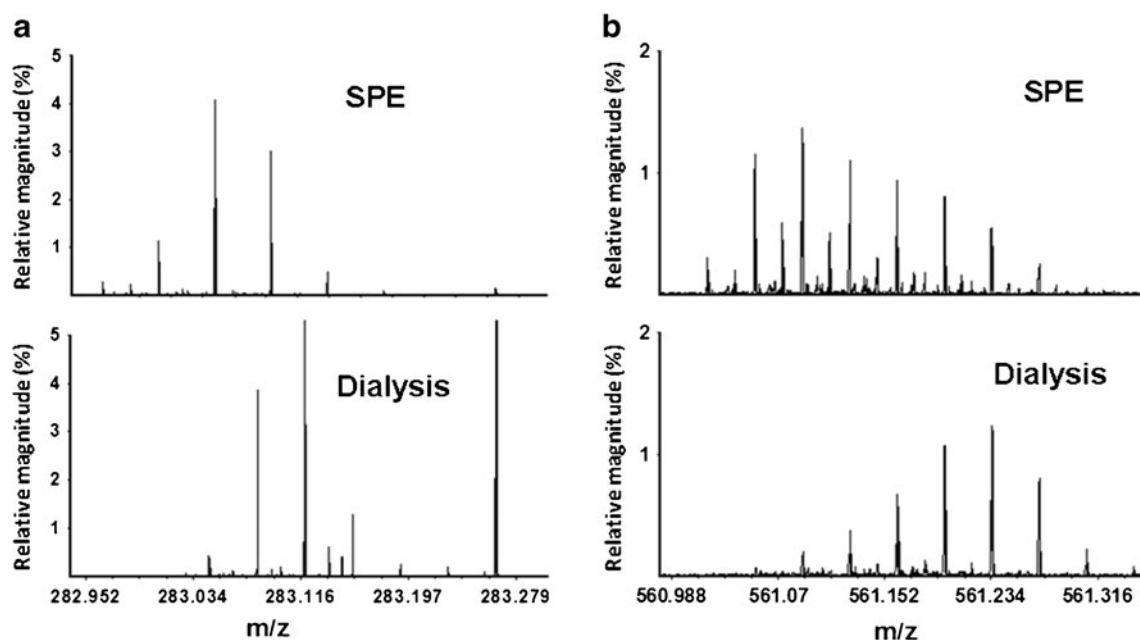


Fig. 2 Zoomed 0.3 m/z segments for **a** nominal m/z 283 and **b** nominal m/z 561. *Top*: SPE-extracted DOM; *bottom*: dialyzed DOM

entire mass range of observed ions. For the SR fen DOM sample, 11,102 elemental compositions were identified in both data sets, while 1,282 formulas were uniquely observed in dialysis-extracted DOM, and 4,362 were unique to SPE-extracted DOM. Additional information about the selectivities of SPE and dialysis can be obtained from three-dimensional vK diagrams, where the atomic ratios of O/C and H/C for each formula observed are plotted with their corresponding relative abundances [19].

Figure 3a and b includes vK diagrams of the dialysis-extracted and SPE-extracted DOM, respectively. Figure 3c and d are vK of the molecular formulas unique to dialyzed-

Table 1 Chemical formulas derived from ions observed in SR fen DOM spectrum at m/z 283 (Fig. 2a)

Method	Formula	Method	Formula
a/b	$\text{C}_{12}\text{H}_{11}\text{O}_6\text{S}_1$	a	$\text{C}_{15}\text{H}_{11}\text{O}_4\text{N}_2$
a/b	$\text{C}_{12}\text{H}_{11}\text{O}_8$	a	$\text{C}_{13}\text{H}_{15}\text{O}_7$
a/b	$\text{C}_{11}\text{H}_{11}\text{O}_7\text{N}_2$	a	$\text{C}_{17}\text{H}_{15}\text{O}_4$
a/b	$\text{C}_{16}\text{H}_{11}\text{O}_5$	a	$\text{C}_{16}\text{H}_{15}\text{O}_3\text{N}_2$
a/b	$\text{C}_{13}\text{H}_{15}\text{O}_5\text{S}_1$	a	$\text{C}_{13}\text{H}_{19}\text{O}_5\text{N}_2$
a/b	$\text{C}_{12}\text{H}_{15}\text{O}_6\text{N}_2$	a	$\text{C}_{15}\text{H}_{23}\text{O}_3\text{S}_1$
a/b	$\text{C}_{14}\text{H}_{19}\text{O}_4\text{S}_1$	a	$\text{C}_{17}\text{H}_{31}\text{O}_3$
a/b	$\text{C}_{14}\text{H}_{19}\text{O}_6$	a	$\text{C}_{18}\text{H}_{35}\text{O}_2$
a/b	$\text{C}_{15}\text{H}_{23}\text{O}_5$	b	$\text{C}_{11}\text{H}_7\text{O}_9$
a/b	$\text{C}_{16}\text{H}_{27}\text{O}_4$	b	$\text{C}_{15}\text{H}_7\text{O}_6$
a	$\text{C}_9\text{H}_{15}\text{O}_{10}$	b	$\text{C}_{20}\text{H}_{11}\text{O}_2$

^a Ions detected in dialyzed DOM

^b Those found in SPE-extracted DOM

and SPE-extracted DOM, respectively. There was a considerable overlap in the elemental ratios of formulas observed in the two samples, with around ~66% matching molecular formulas between the two samples, i.e., 66% of the formulas found in the dialyzed sample were also found in the SPE-extracted sample. The first major difference in the two vK plots of Fig. 3c and d is the abundance of formulas that appear at high O/C ratios (0.4–0.95) in the SPE-extracted DOM. These data correspond to the low-mass-defect species that are only present in the SPE-extracted DOM (Fig. 2), and they plot mainly in the tannin region. In general, the relative abundance of tannins in SPE-extracted DOM was 20% compared with 10% in dialysis-extracted DOM (Table 3). Another difference is the number of peaks observed in the fatty-acid-like region ($\text{H/C} > 1.6$ and $\text{O/C} < 0.2$) of the dialysis-extracted DOM sample and their absence in the SPE-extracted DOM sample. The relative abundance of fatty-acid-like compounds was 0.3% in SPE-extracted DOM compared with 2.7% in dialysis-extracted DOM. Furthermore, there is an abundance of peaks with $0.4 < \text{H/C} < 1.2$ and $0.2 < \text{O/C} < 0.4$ in the dialysis-extracted DOM and not in the SPE-extracted DOM. Those compounds fall in the condensed aromatic region of the vK diagram and are mainly organo-sulfur compounds with low oxygen numbers. In general, aromatic sulfur compounds constituted 1.4% of the total molecular formulas observed in SPE-extracted DOM compared with 3.1% in dialysis-extracted DOM (Table 3). It should also be noted that the majority of the unique formulas observed in both SPE- and dialysis-extracted DOM were of relatively low abundance. The relative abundance of the matching molecular formulas

Table 2 Chemical formulas derived from ions observed in SR fen DOM spectrum at m/z 561 (Fig. 2b)

Method	Formula	Method	Formula	Method	Formula
a/b	C ₂₄ H ₁₇ O ₁₆	a/b	C ₃₅ H ₂₉ O ₇	b	C ₂₃ H ₁₃ O ₁₇
a/b	C ₂₈ H ₁₇ O ₁₃	a/b	C ₂₈ H ₃₃ O ₁₂	b	C ₂₀ H ₁₇ O ₁₇ S ₁
a/b	C ₂₁ H ₂₁ O ₈	a/b	C ₂₅ H ₃₇ O ₁₂ S ₁	b	C ₂₇ H ₁₃ O ₁₄
a/b	C ₂₅ H ₂₁ O ₁₅	a/b	C ₂₇ H ₃₃ O ₁₁ N ₂	b	C ₂₄ H ₁₇ O ₁₄ S ₁
a/b	C ₂₄ H ₂₁ O ₁₄ N ₂	a/b	C ₂₅ H ₃₇ O ₁₄	b	C ₂₀ H ₁₇ O ₁₉
a/b	C ₂₉ H ₂₁ O ₁₂	a/b	C ₂₉ H ₃₇ O ₁₁	b	C ₂₁ H ₂₁ O ₁₆ S ₁
a/b	C ₂₂ H ₂₅ O ₁₇	a/b	C ₂₆ H ₄₁ O ₁₃	b	C ₂₃ H ₁₇ O ₁₅ N ₂
a/b	C ₂₆ H ₂₅ O ₁₄	a/b	C ₃₀ H ₄₁ O ₁₀	b	C ₁₄ H ₂₅ O ₂₃
a/b	C ₂₅ H ₂₅ O ₁₃ N ₂	a/b	C ₃₁ H ₄₅ O ₉	b	C ₂₂ H ₂₅ O ₁₅ S ₁
a/b	C ₃₀ H ₂₅ O ₁₁	a	C ₃₁ H ₂₉ O ₁₀	b	C ₃₃ H ₂₁ O ₉
a/b	C ₂₃ H ₂₉ O ₁₆	a	C ₃₆ H ₃₃ O ₄ S ₁	b	C ₂₃ H ₂₉ O ₁₄ S ₁
a/b	C ₂₇ H ₂₉ O ₁₃	a	C ₃₂ H ₃₃ O ₉	b	C ₃₄ H ₂₅ O ₈
a/b	C ₂₄ H ₃₃ O ₁₃ S ₁	a	C ₃₂ H ₄₉ O ₈	b	C ₃₆ H ₃₃ O ₆
a/b	C ₂₆ H ₂₉ O ₁₂ N ₂	a	C ₃₃ H ₅₃ O ₇		
a/b	C ₂₄ H ₃₃ O ₁₅	a	C ₃₆ H ₆₅ O ₄		

^aIons detected in dialyzed DOM

^bThose found in SPE-extracted DOM

varied considerably between SPE- and dialysis-extracted DOM. Matching molecular formulas with high O/C ratios ($O/C > 0.5$) had high relative abundances in SPE-extracted DOM compared with the relative abundances of compounds with similar elemental ratios in dialysis-

extracted DOM. Conversely, compounds with lower O/C ratios ($O/C < 0.5$) had relatively higher abundances in dialysis-extracted DOM compared with SPE-extracted DOM. The majority of these formulas contain sulfur and have low oxygen numbers.

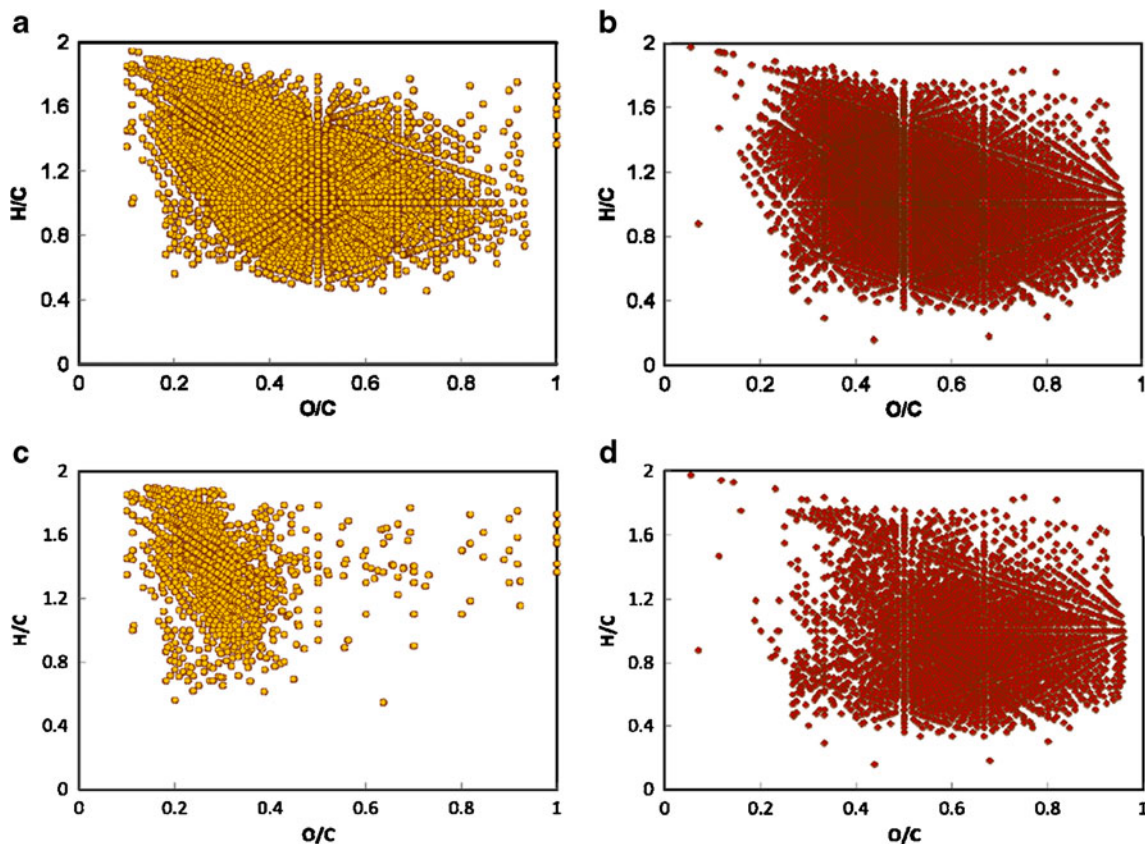


Fig. 3 van Krevelen diagrams for **a** all formulas observed in SR fen dialysis-extracted DOM, **b** all formulas observed in SR fen SPE-extracted DOM, **c** formulas observed only in dialyzed DOM, and **d** formulas observed only in SPE-extracted DOM

Table 3 Relative abundances of formulas from various chemical classes

Sample	No. formulas	CHO (% <i>m</i>)	CHON (% <i>m</i>)	CHOS (% <i>m</i>)	Aromatics (% <i>n</i>)	Tannin-like (% <i>n</i>)	Lignin-like (% <i>n</i>)	Lipid-like (% <i>n</i>)	Protein-like (% <i>n</i>)	Others (% <i>n</i>)
SR fen (SPE)	9,907	84.5	13.1	1.9	1.4	20.0	47.8	0.3	0.8	29.7
SR fen (dialysis)	6,832	73.8	6.1	20.2	3.1	10.2	57.5	2.7	0.9	25.6
SOS (SPE)	8,093	93.1	5.1	1.8	2.3	19.7	41.4	1.1	0.5	28.2
SOS (dialysis)	5,039	90.6	3.8	5.9	10.7	13.3	34.2	1.7	0.7	33.4
Bog 1 (SPE)	9,187	90.4	8	1.4	1.9	19.6	41.6	0.2	0.5	36.2
Bog 1 (dialysis)	6,788	85.3	5.9	8.8	10.9	11.1	43.7	1.9	0.9	31.7

Abundances of CHO, CHON, and CHOS formulas are reported as the magnitude-weighted percentage (% *m*). Other classes representative of specific types of molecules based on van Krevelen analyses (e.g., aromatics) are reported as the number averaged percentage (% *n*)

Comparisons based on elemental compositions of assigned formulas (Table 3) yield additional information about the differences in the dialysis- and SPE-extracted DOM. The CHO class is the most abundant in both samples. CHO compounds in SPE-DOM have more oxygen atoms compared with dialysis-extracted DOM, with compounds ranging from O₂ to O₂₅, while dialysis-extracted DOM contained CHO compounds ranging from O₂ to O₂₁ (data not shown). A *t* test performed on the relative abundance of compounds with low oxygen number (for example, O₂), as well as of compounds with high oxygen number (for example, O₁₉) in dialysis- and SPE-extracted DOM yielded a significance level of 0.05, suggesting that there is a statistically significant difference in the extraction selectivities of the two methods.

Similar trends were observed for CHOS₁ compounds, with oxygen content ranging from O₄ to O₁₇ for SPE-DOM and O₃ to O₁₂ for dialysis-DOM (data not shown). SPE also appears to selectively extract CHON₁ and CHON₂ compounds, regardless of oxygen number, compared with dialysis. Trends in the oxygen content of organic nitrogen compounds paralleled those for CHO and CHOS compounds. SPE-DOM contained CHON₁ compounds ranging from O₅ to O₂₂ and CHON₂ compounds ranging from O₅ to O₁₉, whereas dialysis-extracted DOM contained CHON₁ compounds ranging from O₃ to O₁₈ and CHON₂ compounds ranging from O₅ to O₁₅. Again, these results were statistically significant at the 95% confidence level.

DBE and DBE-O plots of SPE-DOM and dialysis-extracted DOM are included in Fig. 4. SPE-DOM exhibits higher abundances of high-DBE compounds than dialyzed DOM. For SPE-extracted DOM, the distribution peaks at 8 whereas, for the dialyzed DOM, the distribution peaks at 7, with an exceptionally high relative abundance for samples with low DBE (i.e., DBE=1 and DBE=4). Compounds with DBE=1 in the dialyzed DOM are assigned primarily to the saturated C₁₆ and C₁₈ fatty acids. Fatty acids have very high ionization efficiencies that result in large peak magnitudes that are not necessarily proportional to their actual

concentrations. Peaks at *m/z* 297.15289, 311.16854, 325.18418, and 339.19979 are assigned to a homologous series of O₃S₁ compounds with DBE=4 that are abundant and ionized efficiently in dialysis-extracted DOM.

The DBE-O distributions are inverted relative to DBE distributions, with greater abundances of low and negative DBE-O compounds in SPE-DOM, suggesting more highly polar, oxygen-rich compounds in SPE-DOM relative to

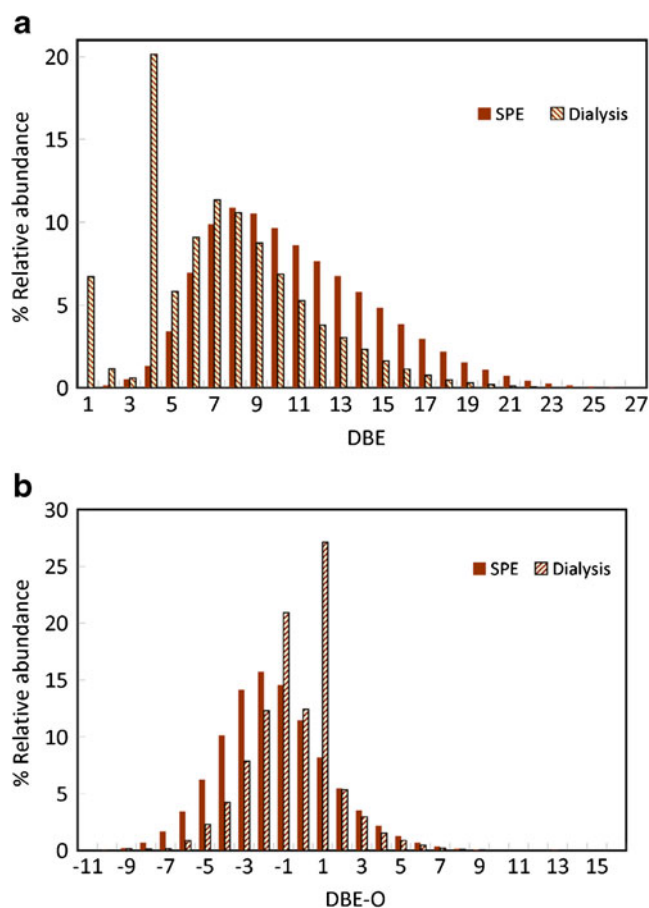


Fig. 4 Relative abundances as a function of **a** DBE and **b** DBE-O values for SPE-extracted and dialyzed DOM

dialysis-extracted DOM. Comparison of the DBE and DBE-O distributions also suggests that the high level of unsaturation in SPE-DOM compounds is due to molecules rich in carboxylic acid functional groups.

FT-ICR-MS of Bog 1 and SOS DOM

Molecular formula analysis of SPE-DOM and dialysis-extracted DOM data from the Bog 1 and SOS sites in Stordalen Mire, Sweden, were obtained in exactly the way as for SR fen DOM from Minnesota peatlands. The results from both the Bog 1 and SOS samples largely confirm findings from the SR fen experiments. There was again a 66% match of molecular formulas between SPE- and dialysis-extracted Bog 1 DOM and 61% match of molecular formulas between the SPE- and dialysis-extracted SOS DOM.

van Krevelen diagrams for all observed ions detected in both dialysis- and SPE-extracted Bog 1 DOM are included in Fig. 5a and b, respectively, ions observed only in the dialyzed DOM in Fig. 5c, and ions observed only in the SPE-extracted DOM in Fig. 5d. ν K diagrams for the SOS sample were quite similar (data not shown). As with SR fen DOM, SPE-extracted Bog 1 unique formulas occurred at

high O/C elemental ratios (Fig. 5d) whereas dialysis-extracted DOM showed an abundance of fatty-acid-like compounds (Fig. 5c). The relative abundances of tannins were higher in the SPE-extracted DOM of both SOS (19%) and Bog 1 (19%) DOM compared with the dialysis extracted DOM (10% in SOS and 13% in Bog1). Conversely, the relative abundances of fatty-acid-like compounds were higher in the dialysis-extracted DOM compared with the SPE-extracted DOM (Table 3).

An interesting observation is the number of formulas with low O/C and H/C elemental ratios (aromatic region of the ν K diagram) in the dialysis-extracted DOM of the Bog 1 and SOS samples and not in the SPE-extracted DOM. Those formulas were assigned to aromatic sulfur compounds with low oxygen number and were less abundant in the dialysis-extracted SR fen DOM. Those compounds represented 10.7% and 10.9% of the total molecular formulas observed in dialysis-extracted SOS and Bog 1 DOM, respectively, compared with 2.3% and 1.9% in SPE-extracted SOS and Bog 1 DOM, respectively. These observations suggest a difference in the composition of peatland DOM from Minnesota and Sweden, or possibly a difference in DOM between bog and fen sites.

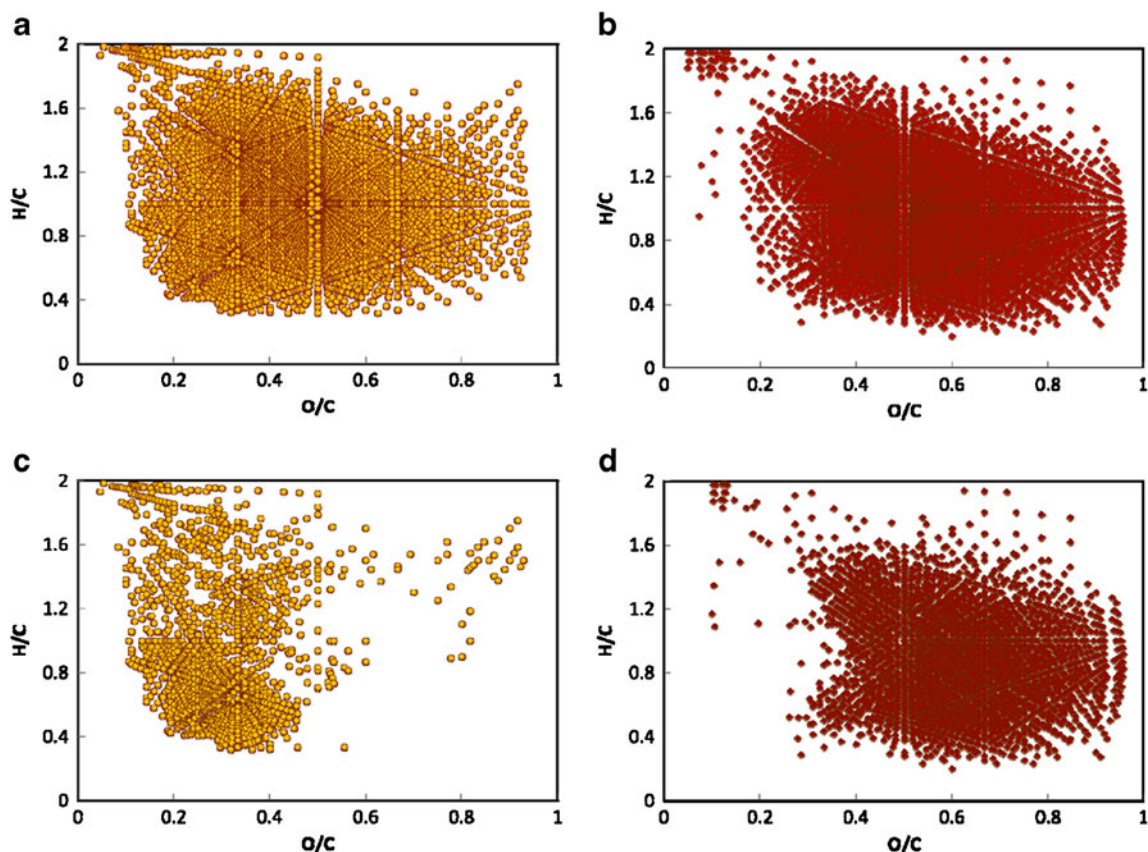


Fig. 5 van Krevelen diagrams for **a** all formulas observed in Bog dialysis-extracted DOM, **b** all formulas observed in Bog 1 SPE-extracted DOM, **c** formulas observed only in dialyzed DOM, and **d** formulas observed only in SPE-extracted DOM

Figure 6a, b, and c shows the vK plots of the total sulfur-containing compounds unique to the dialysis-extracted DOM for Bog 1, SOS, and SR fen samples, respectively, overlain with the sulfur-containing compounds unique to the SPE-extracted DOM for Bog 1, SOS, and SR fen. Sulfur-containing compounds appear to exist in three different forms in peatland DOM. One form is polar molecules, probably sulfates with the sulfate groups attached to aliphatic rather than aromatic core structures. Those compounds are observed in SPE-extracted DOM and not in dialysis-extracted DOM, with $0.4 < O/C < 0.8$. A second form includes hydrophobic molecules with $0.1 < O/C < 0.4$ and $H/C > 1.2$. The third form is sulfur as a part of core aromatic structures, with $H/C < 1.2$ and $0.1 < O/C < 0.4$.

Heteroatom (N and S) and CHO class abundances for SPE and dialysis-extracted DOM for Bog 1 and SOS are included in Table 3. The CHO class is the most abundant in both samples. Again, SPE appears to selectively extract CHO and $CHOS_1$ compounds with more oxygen atoms compared with dialysis. SPE-extracted SOS DOM contained CHO compounds ranging from O_2 to O_{25} , and $CHOS_1$ compounds ranging from O_3 to O_{12} . Conversely,

dialysis-extracted SOS DOM contained CHO compounds ranging from O_2 to O_{20} , and $CHOS_1$ compounds ranging from O_3 to O_{10} . Compared with dialysis, SPE appears to more efficiently extract $CHON_1$ and $CHON_2$ compounds, regardless of their oxygen number. SPE-extracted DOM contained $CHON_1$ compounds ranging from O_5 to O_{20} and $CHON_2$ compounds ranging from O_7 to O_{16} , whereas dialysis-extracted DOM contained $CHON_1$ compounds ranging from O_4 to O_{15} and $CHON_2$ compounds ranging from O_5 to O_9 . Similar results were obtained for Bog 1 DOM. These data taken together suggest that the majority of N-containing compounds exist as polar molecules. As with SR fen, the difference in the heteroatom (N and S) and CHO class abundances for SPE- and dialysis-extracted DOM statistically was significant at the 95% confidence level.

Cluster analysis

We used classification via cluster analysis as described by Minor et al. [22] to characterize the similarity among DOM samples. Our cluster analysis, however, was based on the

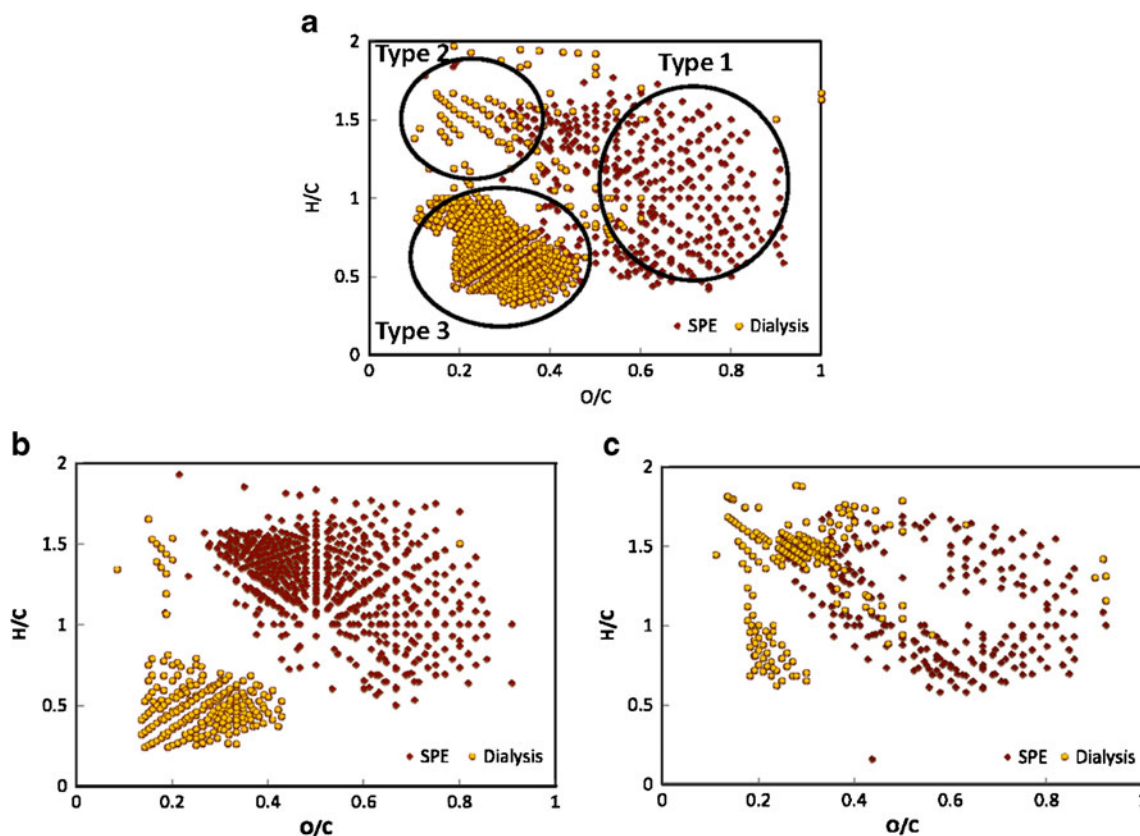


Fig. 6 van Krevelen diagrams for sulfur-containing compounds unique to dialysis-extracted DOM and SPE-extracted DOM. **a** Bog 1, **b** SOS, and **c** SR fen. *Type 1* molecular formulas are present only in SPE-extracted DOM having high O/C ratios and high H/C ratios. *Type*

2 are molecular formulas specific to dialysis-extracted DOM having low O/C and high H/C ratios. *Type 3* are molecular formulas that are specific to dialysis-extracted DOM having low O/C and low H/C ratios

relative abundances of the different chemical classes as defined by van Krevelen formula reductions rather than peak presence versus absence data. Two observations can be made from the data in Fig. 7. First, the dialysis-extracted samples from the three different sites did not group with their respective SPE-extracted samples, suggesting that SPE and dialysis extract different compound classes with different function groups, regardless of sample origin. Nevertheless, the two bog DOM samples (SOS and Bog1) did group together, regardless of sample isolation technique, and were distinct from the fen DOM sample. These observations reinforce previous data that suggest DOM in fens and bogs are compositionally different [23], and either isolation technique captures such differences, in spite of the variability in the classes of compounds the isolation techniques yield.

Discussion

After comparing SPE with hydrophobic PPL resins and dialysis as DOM isolation methods, we suggest that hydrophobic SPE appears to be selective in that it discriminates against three major series of compounds. One group is fatty acids with high H/C and low O/C elemental ratios that are strongly adsorbed to the PPL cartridges because they are highly hydrophobic. The other classes of molecules that adhere strongly to PPL are sulfur-containing compounds having low O/C elemental ratios ($0.1 < O/C < 0.3$). The low oxygen numbers associated with molecules responsible for these peaks likely amplifies hydrophobicity, leading to strong hydrophobic interactions with the PPL polymer. These strong interactions of both fatty acids and low-oxygen organic-S compounds cannot be overcome by methanol which is used to elute DOM in the last step in solid-phase extraction. We can also suggest that, based on these results, organic sulfur exists in different forms in peatland

DOM. Moreover, there appears to be compositional differences between DOM in bogs and fens.

The abundance of formulas in the tannin-like region of SPE-extracted DOM suggests that PPL cartridges efficiently isolate the hydrophilic portion of DOM. Tannins are known to be polyphenolic compounds that contain a variety of linkages and central cores [24]. Moreover, a big portion of these compounds appear to be N-containing compounds, suggesting that majority of nitrogen exists in one form and as polar molecules.

Tannin-like compounds, a majority of N-containing compounds, and S-containing compounds with high oxygen number are absent in dialysis-extracted DOM. Two reasons might be responsible for these observations. The fatty acids (which have very high ionization efficiencies) in the dialysis-extracted samples limit the range of compounds that can be ionized and observed in the mass spectra and make it difficult to see other (mainly hydrophilic) compounds. In addition, we recently demonstrated that acidification can affect the molecular composition of DOM [25]. Compositional information obtained by ultrahigh-resolution FT-ICR mass spectrometry identified a shift towards highly oxygenated compounds with high nominal mass upon acidification that was also sample-dependent. Since SPE involves acidifying the DOM sample to pH=2 prior to extraction, we believe that some of the compounds that were unique to SPE-extracted DOM and absent in dialysis-extracted DOM are due to acid-catalyzed reactions that occurred in the sample upon acidification.

Conclusions

We have demonstrated that SPE using PPL cartridges can selectively isolate DOM from peatland porewaters, eliminating certain groups of compounds. Dialysis is a more comprehensive approach to isolate DOM and desalt it, provided that the samples do not have high fatty acid content. The presence of high levels of fatty acids, which have very high ionization efficiencies, in the dialysis-extracted samples will limit the range of compounds that will appear in mass spectra, making it difficult to see other (mainly hydrophilic) compounds with high O/C ratios. For samples with a high microbial input of fatty acids, we recommend the use of both isolation methods for a more accurate representation of DOM.

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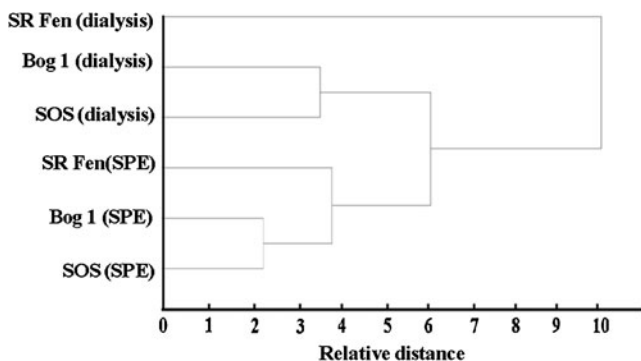


Fig. 7 Cluster diagram based on abundances of number-averaged chemical classes summarized in Table 3. The *x*-axis represents the dissimilarity in samples

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