

MOLECULAR BIOGEOCHEMISTRY OF
DISSOLVED ORGANIC MATTER IN THE
PERMAFROST-INFLUENCED LENA DELTA
AND THE COASTAL LAPTEV SEA

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Ivan Dubinikov

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Abstract

Dissolved organic matter (DOM) is a ubiquitous complex mixture of organic compounds in aquatic systems. DOM is an important contributor to the global carbon cycle and is involved in a variety of biogeochemical processes where it serves as a carbon and energy source for biota, controls levels of oxygen, nitrogen and phosphorus, and mediates the availability of dissolved nutrients and metals. The Arctic is experiencing ongoing dramatic climatic changes. Climate change drives permafrost degradation. The global inventory of permafrost carbon encompasses twice as much carbon as currently stored in the atmosphere and represents half of the global belowground soil carbon. The mobilization and degradation of this carbon pool would have critical implications for microbial processes, primary production and carbon cycling in the Arctic. A substantial amount (18-26 Tg C year⁻¹) of terrestrial DOM is transported along the fluvial continuum from land to the Arctic Ocean. The water and dissolved organic carbon (DOC) discharge into the Arctic Ocean are expected to change in future due to increasing temperatures and subsequent permafrost degradation. The ultra-complex mixture of organic compounds in DOM is a proxy of ecosystem metabolism and represents an imprint of past and ongoing biogeochemical processes. Recent progress in molecular analytics of DOM and data processing tools has allowed the characterization of thousands of molecular components and their response to changing environmental parameters in the natural systems.

The main goal of this thesis is the molecular characterization of DOM composition, reactivity and properties in the permafrost-influenced Lena Delta and coastal Laptev Sea, Siberia. The systematics of the molecular DOM composition with respect to source of natural waters, reactivity during the Lena River - Laptev Sea transition, mobilization, mineralization and turnover of permafrost-derived DOM were studied. Ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) and statistical analyses were performed on DOM samples obtained in various expeditions to the Lena Delta and Laptev Sea.

Most DOM analyses require pre-concentration and purification for high resolution and sensitivity

analyses. The variety of commonly accepted DOM isolation and purification methods (three non-ionic sorbents: XAD-8 resin, PPL and C18 sorbents, and one anion exchanging resin – diethylaminoethyl (DEAE) -cellulose, their sorption selectivity, representativeness and influence on DOM molecular composition were studied. The DOM isolates obtained with a use of PPL and C18 cartridges were characterized with higher content of aliphatic compounds as compared to XAD-8 and DEAE-isolates. The DEAE-isolate was characterized with the highest oxygen content indicating enrichment with oxygenated DOM compounds. Differences between DOM extracts generally reflected the differences in extraction mechanisms and have to be considered in future studies.

Studies on molecular systematics revealed that DOM from various natural waters of the Lena Delta (the Lena River and its channels, permafrost creeks, lakes and the bay) can be discriminated based on molecular information obtained by FT-ICR MS. Source-specific DOM molecular markers and their relative contribution to DOM of different origin were identified. Along the river-sea transition where sharp physico-chemical gradients of temperature, salinity, pH, density and DOC concentration occurred, reactive and non-reactive DOM molecular components were characterized. 27% of all identified DOM components exhibited a strong conservative behavior in the river-sea transition, 32% were moderately affected by estuarine processes, and 41% were actively involved. Highly conservative components can find further applications as tracers of terrigenous DOM and for estimation of its persistence in the oceans. Reactive components are future targets in the exploration of estuarine processes on the molecular level.

Permafrost-derived DOM was a good substrate for microbial metabolism. Microbial incubation experiments of permafrost-derived DOM showed mineralization rates of ~44% of DOC over 18 days of incubation whereas photodegradation only led to minor DOC decomposition rates. The distinct differences in the chemical nature of labile and relatively recalcitrant components indicated that the chemical composition is an important factor for DOM bioavailability. Additionally, continuous and reproducible molecular changes of DOM mobilized from permafrost deposits along the terrestrial-aquatic transition were recorded in the Lena Delta. These regional scale observations supported microbial

regulation of DOM molecular composition on the terrestrial-aquatic interface. The studies on fluxes of DOM from ice-complex outflows showed that the majority of DOM mineralization (during permafrost thaw) occurs without long-distance transport. Thus, contribution of microbial DOM metabolism to CO₂ outgassing in the permafrost outflows and creeks is highly relevant in the Arctic.

Molecular DOM approaches as a toolbox of analysis of biogeochemical and environmental properties of DOM will be an important research direction in aquatic sciences. The combined efforts of multiple scientific disciplines such as molecular chemistry, biochemistry, microbiology, hydrology and biogeochemistry are required to resolve complex unanswered questions in DOM research. These questions include: (1) genesis and evolutionary development of DOM in natural systems, (2) persistence of DOM in natural waters, (3) characterization of processes, which regulate DOM properties and composition, (4) feedback of the DOC pool to the changing climate, both on land and in the ocean and (5) characterization of turnover of terrigenous DOM in the global oceans.

Zusammenfassung

Gelöstes organisches Material (DOM) ist eine, im aquatischen Milieu allgegenwärtige, komplexe Mischung organischer Verbindungen. DOM leistet einen wichtigen Beitrag zum globalen Kohlenstoffkreislauf und ist in viele verschiedene biogeochemische Prozesse involviert. So dient es z.B. als Kohlenstoff- und Energiequelle für Lebewesen, beeinflusst den Sauerstoff-, Stickstoff- und Phosphorgehalt und reguliert die Verfügbarkeit von gelösten Nährstoffen und Metallen. Die Arktis erfährt fortlaufend dramatische Klimaveränderungen, die zum Abbau von Permafrost beitragen. Der globale Anteil von Kohlenstoff in Permafrost umfasst zurzeit doppelt so viel Kohlenstoff wie in der Atmosphäre vorhanden ist und macht die Hälfte des globalen Kohlenstoffes im Boden aus. Die Mobilisierung und der Abbau dieses Kohlenstoffreservoirs hätten kritische Auswirkungen auf mikrobielle Prozesse, Primärproduktion und den Kohlenstoffkreislauf in der Arktis. Ein erheblicher Anteil des terrestrischen DOM ($18-26 \text{ Tg C Jahr}^{-1}$) wird kontinuierlich vom Land in den arktischen Ozean transportiert. Der Zufluss von Wasser und gelöstem organischen Kohlenstoff (DOC) in den arktischen Ozean wird sich in der Zukunft voraussichtlich aufgrund steigender Temperaturen und der daraus resultierenden Schmelze des Permafrostes ändern. Die hochkomplexe Mischung organischer Verbindungen in DOM ist ein Proxy für den Metabolismus des Ökosystems und spiegelt die Einflüsse vergangener und fortlaufender biogeochemischer Prozesse wieder. Jüngste Fortschritte in der molekularen DOM Analytik und der Datenverarbeitung ermöglichten die Analyse tausender molekularer Bestandteile und ihrer Reaktionen auf sich ändernde Umweltbedingungen.

Das vorrangige Ziel dieser Arbeit ist die molekulare Charakterisierung der DOM Zusammensetzung, Reaktivität und Eigenschaften in dem von Permafrost beeinflussten Lena Delta und der küstennahen Laptewsee in Sibirien. Unter Berücksichtigung des Wasserursprungs, der Reaktivität innerhalb des Überganges des Lena Flusses zur Laptewsee, der Mobilisierung, Mineralisierung und des Umsatzes des aus Permafrost stammendem DOM, wurde die Systematik der molekularen DOM Zusammensetzung untersucht. DOM Proben von verschiedenen Expeditionen in das Lena Delta und der Laptewsee wurden

mit Ultra-hochauflösender Fourier Transformation Ionenzyklotronresonanz Massenspektrometrie (FT-ICR MS) und statistischen Analysemethoden untersucht.

Die meisten hochauflösenden und empfindlichen Analysemethoden erfordern eine vorherige Anreicherung und Aufreinigung des DOM. Eine Vielzahl der gemeinhin akzeptierten Methoden zur DOM Anreicherung und Aufbereitung (drei nicht-ionische Sorptionsmaterialien: XAD-8 Harz, PPL und C18, und ein Anionen Austausch Harz – Diethylaminoethyl (DEAE) -Cellulose), ihre Sorptions-Selektivität, Repräsentativität und ihr Einfluss auf die molekulare DOM Zusammensetzung wurden untersucht. DOM, welches mithilfe von PPL und C18 Kartuschen isoliert wurde, zeichnete sich durch einen höheren Anteil an aliphatischen Verbindungen aus als durch XAD-8 und DEAE isoliertes DOM. Das DEAE-Isolat wies den höchsten Sauerstoffgehalt auf, was auf eine Anreicherung von sauerstoffhaltigen DOM Verbindungen hindeutet. Die Unterschiede in den DOM Extrakten spiegeln im Allgemeinen die Unterschiede der Extraktionsmechanismen wider und müssen in zukünftigen Studien berücksichtigt werden.

Untersuchungen der molekularen Systematik machen deutlich, dass DOM aus unterschiedlichen natürlichen Wasserquellen des Lena Deltas (der Fluss Lena und seine Kanäle, Permafrost-Ästuar, Seen und die Bucht) mithilfe der FT-ICR MS anhand der molekularen Information unterschieden werden kann. Quellenspezifische molekulare DOM Marker und ihr Beitrag zu DOM unterschiedlichen Ursprungs wurden identifiziert. Entlang des Überganges vom Fluss zum Meer traten starke physikalisch-chemische Temperatur-, Salinitäts-, pH-, Dichte- und DOC Konzentrationsgradienten auf. Reaktive und unreaktive molekulare DOM Komponenten wurden charakterisiert. Wir haben gezeigt, dass 27% aller identifizierter DOM Komponenten ein stark konservatives Verhalten im Übergang vom Fluss zum Meer aufwiesen, 32% wurden mäßig von ästuarinen Prozessen beeinflusst und 41% waren aktiv involviert. Stark konservative Komponenten können außerdem als DOM Tracer und zur Beurteilung ihrer Beständigkeit in den Ozeanen dienen. Reaktive Komponenten sind zukünftige Ziele der Erforschung ästuariner Prozesse auf molekularer Ebene.

DOM, welches aus Permafrost stammt, war ein gutes Substrat für mikrobielle Stoffwechselreaktionen. Mikrobielle Inkubationsexperimente mit DOM aus Permafrost ergaben DOC Mineralisierungsraten von ~44% über einen Zeitraum von 18 Tagen, wohingegen Photoabbau nur zu geringen Abbauraten führte. Die deutlichen Unterschiede der chemischen Natur zwischen labilen und relativ abbauresistenten Verbindungen wiesen darauf hin, dass die chemische Zusammensetzung ein wichtiger Faktor für die Bioverfügbarkeit von DOM ist. In DOM, welches aus Permafrost-Ablagerungen entlang des terrestrisch-aquatischen Überganges im Lena Delta stammt, wurden außerdem fortlaufende und reproduzierbare molekulare Änderungen des DOM belegt. Diese regionalen Beobachtungen unterstützen die mikrobielle Regulierung der molekularen DOM Zusammensetzung an der terrestrisch-aquatischen Grenzfläche. Studien an DOM Proben aus Abläufen von Eisvorkommen haben gezeigt, dass ein Großteil der DOM Mineralisierung (während der Permafrost Schmelze) auch ohne den DOM Transport über eine längere Strecke auftritt. Deshalb ist der Beitrag des mikrobiellen DOM Metabolismus zur CO₂ Ausgasung in Permafrost-Ausflüssen und Ästuaren in der Arktis von maßgeblicher Bedeutung.

Der Methodenpool molekularer DOM Forschungsansätze wird zukünftig ein wichtiger Bestandteil für die Analyse biogeochemischer und ökologischer DOM Eigenschaften sein. Das gemeinsame Bestreben vieler wissenschaftlicher Fachrichtungen, wie der molekularen Chemie, Biochemie, Mikrobiologie, Hydrologie und Biogeochemie, ist nötig um die komplexen ungelösten Fragen der DOM Forschung zu beantworten. Diese Fragen beinhalten: (1) Entstehung und evolutionäre Entwicklung von DOM in der Natur, (2) Beständigkeit von DOM in natürlichen Gewässern, (3) Charakterisierung der Prozesse, welche die Eigenschaften und Zusammensetzung von DOM regulieren, (4) Feedback des DOC zu Klimaveränderungen an Land und im Ozean und (5) Bestimmung des Umsatzes von terrigenem DOM in den Weltmeeren.

Abbreviation list

AA	amino acids
AGC	automatic gain control
AI	aromaticity index
APPI	atmospheric pressure photo ionization
BC	black carbon
CA	cluster analysis
CDOM	colored (chromophoric) dissolved organic matter
CI	chemical ionization
COSY	correlation spectroscopy
CRAM	carboxyl-rich alicyclic molecules
DBE	double bond equivalents
DCAA	dissolved combined amino acids
DEAE-cellulose	diethylaminoethyl cellulose
DFAA	dissolved free amino acids
DIN	dissolved inorganic nitrogen
DMSO	dimethyl sulfoxide
DOC	dissolved organic carbon
DOM	dissolved organic matter
DON	dissolved organic nitrogen
DOP	dissolved organic phosphorus
DOS	dissolved organic sulfur
EEM	excitation-emission matrix
ESAS	East-Siberian arctic shelf
ESI	electrospray ionization
FID	free induction decay
FT-ICR	Fourier transform ion cyclotron resonance
FTIR	Fourier transform infrared (spectroscopy)
HCA	hierarchical cluster analysis
HMQC	heteronuclear multiple quantum correlation
HMW	high molecular weight
HPLC	high-performance liquid chromatography
HS	humic substances
HSQC	heteronuclear single quantum correlation
ISA	indicator species analysis
KMD	Kendrick mass defect
MAP	mean annual precipitation
MAT	mean annual temperature
MDLT	material derived from linear terpenoids
MDS	multidimensional scaling
MS	mass spectrometry
MW	molecular weight

NESS	North-East science station
NMDS	non-linear multidimensional scaling
NMR	nuclear magnetic resonance
NOM	natural organic matter
OC	organic carbon
OM	organic matter
ON	organic nitrogen
OPA	ortho-phthaldehyde
PARAFAC	parallel factor analysis
PC	principal component
PCA	principal component analysis
POC	particulate organic carbon
POM	particulate organic matter
RMS	root mean squared
SD	standard deviation
SEC	size exclusion chromatography
SI	supplementary information
SOM	soil organic matter
SPE	solid phase extraction
TDN	total dissolved nitrogen
THAA	total hydrolyzable amino acids
TN	total nitrogen
TOC	total organic carbon
TOCSY	total correlation spectroscopy
TP	total phosphorus
uHRMS	ultrahigh resolution mass spectrometry
UPLC	ultra-performance liquid chromatography
WRT	water residence time

1. Introduction

Dissolved organic matter in natural waters

Non-living organic matter (OM) in aquatic systems exists in form of dissolved molecules, colloids, and particles. This differentiation is dynamic in natural systems because OM can be converted readily between these forms by dissolution, precipitation, sorption and desorption, aggregation and disaggregation (Perdue and Ritchie, 2003). DOM is a broad classification of compounds of different origin and composition, which exist in form of aqueous solutions within aquatic systems both in freshwater and marine environments. In general, DOM is a result of decomposition processes of living OM such as plants, animals, plankton, bacteria, and viruses. Studies on DOM are an important part of the biogeochemistry, aquatic sciences, oceanography, limnology, hydrology, and soil science.

The “dissolved fraction” of OM is usually operationally defined by filtration with specific pore sizes. The size limit, which differentiates DOM from particulate organic matter (POM), is arbitrary, but the most accepted filtration size limit is 0.45 μm . It should be noted, that in many DOM studies, 0.2 μm or 0.7 μm cutoffs were used for filtration (Dawson et al., 2004; Hope et al., 1997; Pelegri et al., 1999). Also, filtration can cause artifacts through partial adsorption, desorption or cavitation (Zlonay, 2003). DOM samples can be obtained from different environments including atmosphere, hydrosphere, sediments and soils which strongly differ in their organic matrix (structured for sediments and soils and unstructured for hydrosphere) and water content (saturated for hydrosphere and sediments and unsaturated for soils). The most common representation of DOM concentrations in natural waters is DOC concentration. DOC concentration is usually quantified via high temperature catalytic oxidation to CO_2 (Sugimura and Suzuki, 1988). Typical open ocean DOC concentrations in surface waters range from 45 $\mu\text{M C}$ to 80 $\mu\text{M C}$. In the coastal zones DOC concentrations can reach 200 $\mu\text{M C}$ (Vlahos et al., 2002). The concentration of 34-48 $\mu\text{M C}$ is characteristic for deep ocean waters (Hansell and Carlson, 1998). For rivers it is about 580 μM , 60 $\mu\text{M C}$ for groundwater, and 180-1000 $\mu\text{M C}$ for lakes. In marshes and bogs the DOC concentration is

about 1420 $\mu\text{M C}$ and 2750 $\mu\text{M C}$ accordingly (Thurman, 1985).

DOM plays a major role for biogeochemical cycles in the ocean and on land. The marine DOC pool alone contains $662 \pm 32 \text{ Pg C}$ (Hansell et al., 2012) and is comparable to the atmospheric CO_2 carbon pool ($\sim 800 \text{ Pg C}$) and the carbon stored in living biomass on land and in the water: $600\text{-}1000 \text{ Pg C}$ (Falkowski et al., 2000). Even minor changes in the DOM pool will impact global biogeochemical cycles. DOM is an active fraction of soil organic carbon pool, playing an important role in the carbon cycling of terrestrial ecosystems. DOM participates in soil carbon sequestration and greenhouse gas emission (McCarl et al., 2007) and provides a direct link between the terrestrial and aquatic ecosystem (Cole et al., 2007).

Environmental roles of dissolved organic matter

Due to global abundance and reactivity, DOM is involved in a wide variety of biogeochemical processes. In aquatic food webs DOM mediates the availability of dissolved nutrients and metals, modifies optical properties of the water (Findlay and Sinsabaugh, 2003). DOM serves as a carbon and energy source for biota, controls levels of dissolved oxygen, nitrogen, phosphorus, sulfur, trace metals, and acidity (Leenheer and Croué, 2003). Considering biogeochemical importance of DOM, it is also involved and affected by the global climate change triggered processes. With respect to global change, DOM could play a role in soils and permafrost, if it enabled carbon to be removed from the metabolically active surface soils (Guggenberger and Kaiser, 2003; Kalbitz et al., 2003). And even a minor increase of DOM concentrations in marine environments could bind enormous amounts of atmospheric CO_2 , even though it is difficult to envision as to how this could be anthropogenically induced (Zlonay, 2003).

In marine and coastal environments DOM is a subject to phyto- and zooplankton mediated processes (Benner and Opsahl, 2001), microbial alteration (Santos et al., 2014), photochemical degradation and mineralization (Helms et al., 2013; Santos et al., 2014), abiotic sorption and desorption processes (McCallister et al., 2006), and particle formation and deposition. DOM plays a crucial role in the “microbial carbon pump” in the oceans (Jiao et al., 2011). Generally, most of the DOM produced in

surface waters, such as labile proteins, carbohydrates, and lipids, is mineralized by microbial activity, and the remaining material is transformed into semi-labile and finally refractory DOM.

Analytical challenges

DOM concentration, composition, and chemistry are highly variable and depend on the temperature, ionic strength, pH, major cation composition of the water and sample filtration, isolation and purification (Zlonay, 2003). Due to the diversity of sources, formation and turnover mechanisms, DOM is a complex mixture of organic compounds and major fraction of it is still uncharacterized (Mead et al., 2013; Nebbioso and Piccolo, 2013; Sipler and Seitzinger, 2008). DOM extraction and purification methods affect its composition and properties (Dittmar et al., 2008; Perminova et al., 2014; Serkiz and Perdue, 1990). Components of complex mixture of DOM cannot be separated completely. Only building blocks of biomolecules such as amino acids (Yamashita and Tanoue, 2003a), lignin phenols (Kaiser and Benner, 2012), lipids (Karlsson et al., 2011; Vonk et al., 2008) and few other biomolecules within DOM pool can be characterized. However, the large fraction of DOM is still uncharacterized (Hedges et al., 2000). That is why non-targeted molecular tools found a broad range of applications in DOM research: from optical methods (Nebbioso and Piccolo, 2013; Zhou et al., 2013) to high magnetic field mass spectrometry (e.g. FT-ICR MS (Hertkorn et al., 2008; Koch et al., 2014) and nuclear magnetic resonance (NMR) spectroscopy (Lam and Simpson, 2008; Simpson et al., 2011; Simpson et al., 2004).

Recent progress in the molecular analytics of DOM has allowed not only characterization of thousands of molecular components but detection of their response to changing environmental parameters in the natural systems. Advanced analytical instrumentation and developments in the molecular data processing approaches tremendously expanded our understanding of the complex chemistry of DOM (Hertkorn et al., 2008; Hertkorn et al., 2012; Kellerman et al., 2014).

2. Methods for the chemical characterization of dissolved organic matter

Due to heterogeneity and complexity of DOM, its chemical analysis is challenging. DOM can be analyzed in filtered original water or after purification and/or pre-concentration (Fig. 1). The analytical strategy may result in fractionation of DOM and in analytical artifacts induced by the applied procedures. There is still no generally accepted methodology of water sample filtration (usually water passed through 0.45 μ m filter; Perdue and Ritchie, 2003). The specific choice of the filter material and membrane pore size depends on the aims of the analysis, e.g. for high sensitivity chemical analyses the combustion or advanced cleaning of the filter is needed, for some studies bacteria-free samples are required. Many studies use glass microfiber filters (GF/F, 0.7 μ m pore size) due to the possibility to pre-combust these filters for cleaning, good flow parameters and variety of available sizes. However, some bacteria and viruses are smaller than 0.7 μ m and in case of GF/F filtration microbial component will be included into the DOM. Alternatively, membranes with smaller pore sizes (0.1-0.2 μ m) effectively exclude bacteria but have many limitations in flow parameters, filter resource and often contaminate DOM samples with membrane material.

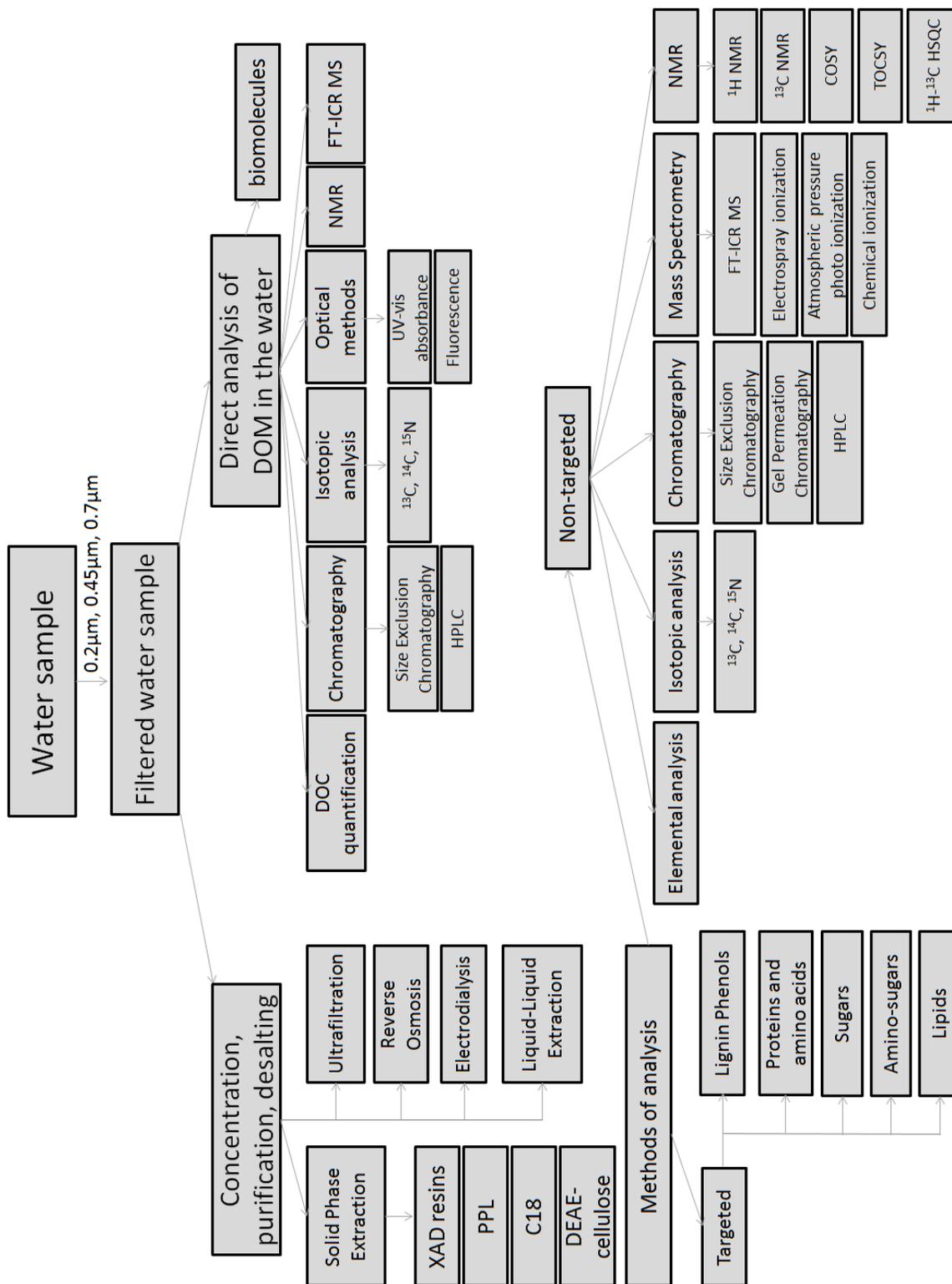


Fig. 1. Strategies in the chemical analyses of DOM.

2.1. Quantification of dissolved organic carbon and nitrogen

After water filtration some analytical methods can be applied directly to DOM in filtered water. DOM can be quantified by determining the concentration of DOC, dissolved organic nitrogen (DON), or, in rare cases, dissolved organic phosphorus (DOP) and sulfur (DOS) (Cutter et al., 2004). Usually, DOC and total dissolved nitrogen (TDN) in natural water are characterized by high temperature catalytic oxidation to CO₂ and N₂ (Sugimura and Suzuki, 1988). DON is quantified by subtracting dissolved inorganic nitrogen (DIN) (in form of NO₃⁻, NO₂⁻, and NH₄⁺) from the TDN concentration (Kattner and Becker, 1991) or using Kjeldahl digestion after inorganic nitrogen removal (Doval et al., 1997). Usually routine DOC/TDN analysis requires about 7 ml of water, but in case of direct injection to the total organic carbon (TOC) analyzer about 200 µl of filtered water would be sufficient which is highly important for low concentration and low volume water samples (Lechtenfeld et al., 2011; Stubbins and Dittmar, 2012).

2.2. Optical methods

Optical methods such as UV-vis absorbance, fluorescence and more specifically – fluorescence excitation-emission matrix (EEM) spectroscopy found numerous applications in studies on DOM, because they can be applied on original sample water, and are simple and relatively cheap. These methods can be utilized directly in the field or onboard of research vessels using portable detectors (with limited selection of wavelengths) or even using full-size instruments with high sensitivity and resolution.

Optically measurable component of DOM is called colored or chromophoric dissolved organic matter (CDOM). CDOM absorbs short wavelength light ranging from blue to ultraviolet. CDOM is responsible for yellow, green or brownish color of natural waters (Hoge et al., 1995). UV-vis absorbance spectra have numerous derivatives which found extensive applications in the environmental research on DOM. Absorbance ratios are characteristic for DOM quality: a₂₅₀:a₃₆₅ absorbance ratio (ratio of CDOM absorbance at 250 nm and 365 nm, also called E₂:E₃) can be applied for analysis of changes in relative size of DOM molecules (Peuravuori and Pihlaja, 1997). With increasing molecular size, the E₂:E₃ ratio decrease because of stronger light absorption of high-molecular-weight (HMW) DOM. There are other

absorbance ratios, e.g. $a_{465}:a_{665}$ ($E_4:E_6$) which is inversely related to CDOM aromaticity (Piccolo et al., 1992; Summers et al., 1987). The $E_4:E_6$ ratio can also be related to molecular size, O/C and C/N atomic ratios, carboxyl content, total acidity and level of DOM humification (Chen et al., 1977; Senesi et al., 1989). $SUVA_{254}$ (Specific UV absorbance on 254 nm wavelength) values can be determined by dividing the UV absorbance measured at the 254 nm wavelength by DOC (in mg L^{-1}) concentration in the water sample. $SUVA_{254}$ parameter is demonstrative for DOM aromaticity as determined by relation of this parameter with the data from ^{13}C NMR spectroscopy (Weishaar et al., 2003). Spectral slopes can be calculated using non-linear fit of exponential functions to the absorbance spectra in the wavelength ranges of 275-295 nm, 290-350 nm and 350-400 nm (Helms et al., 2008; Hernes et al., 2008). These slopes can provide insights into the characteristics such as chemistry, source, transformations and diagenesis of CDOM in natural waters (Brown, 1977; Vähätalo and Wetzel, 2004).

The fluorescent properties of DOM are often studied in order to infer DOM characteristics in aquatic environments, including its source, quantity, composition, and behavior in natural waters (Chari et al., 2013; Yamashita and Tanoue, 2003b). In general, fluorescence provides information on the existing fluorophores which present in DOM. Often, the fluorescence measured across a range of excitation-emission wavelengths, with three dimensional surface plots as a result. Key peaks in the excitation-emission responses are associated with different sources and components of organic matter in water (e.g. protein-like autochthonous organic material, terrestrial humic and fulvic acids; Coble, 1996; Coble, 2007; Goldman et al., 2012; Stedmon and Markager, 2005; Stedmon et al., 2003). Recent applications of parallel factor analysis (PARAFAC; Stedmon and Bro, 2008), a three-way method which originates from psychometrics (Carroll and Chang, 1970) found many applications in DOM research, particularly in discrimination of different organic components within DOM (Murphy et al., 2008; Stedmon and Markager, 2005; Zhang et al., 2009).

Few specific organic compounds, such as amino acids, monosugars, lignin phenols, low molecular weight acids, ketones, and aldehydes can be measured directly in natural waters. Other examples of the

direct non-targeted chemical characterization of DOM include: (i) direct gel chromatographic characterization with online quantification of DOC (Huber et al., 2011; Huber and Frimmel, 1994); (ii) ^1H NMR analysis of original water samples, which is extremely challenging due to presence of salts, low concentration of DOM, need in water suppression techniques and long signal accumulation times (Lam and Simpson, 2008); (iii) direct electrospray ionization (ESI) FT-ICR MS can be applied to unaltered freshwater samples as well (Sleighter et al., 2009).

2.3. DOM purification and concentration methods

Apart from the methods mentioned above, the chemical DOM characterization requires pre-concentration and purification to avoid interference of inorganic ions in high sensitivity analyses (Fig. 1). A variety of methods can be applied here: solid phase extraction (SPE) methods, ultrafiltration, nanofiltration, reverse osmosis, electrodialysis, or liquid-liquid extraction. Currently two major approaches are common within the DOM research field: filtration/membrane -based methods and solid phase extraction based methods.

Sorbent-based techniques

In the last decades different sorbents have been utilized to isolate DOM from natural waters: sorption on activated charcoal (Kerr and Quinn, 1980), hydrophobic bonded-phase silica sorbents (Durant et al., 1994) and Amberlite XAD resins (Stuermer and Harvey, 1974). Standard protocols for the solid phase extraction of humic substances were developed for the XAD resins. With these protocols two operational fractions of DOM can be isolated: a) hydrophobic organic acids (humic and fulvic acids) that sorb on XAD-2 and XAD-8 resins (Leenheer and Huffman, 1976; Mantoura and Riley, 1975; Thurman and Malcolm, 1981); b) hydrophilic organic acids isolated using XAD-4 resin (Aiken et al., 1992; Malcolm and Maccarthy, 1992). Preparation and purification of functionalized solid phases for DOM extraction are time consuming, and during extraction DOM experiences strong shifts in pH, salinity and polarity. Moreover, the XAD extracts require cation-exchange procedures after alkaline elution from resins. XAD

resins and activated charcoal have to be carefully cleaned by Soxhlet extraction before use. In case of extraction with sorbents such as C18 and PPL (Fig. 2), the solid phase must be activated by polar organic solvents (e.g. methanol or acetonitrile) and then rinsed with aqueous solutions.

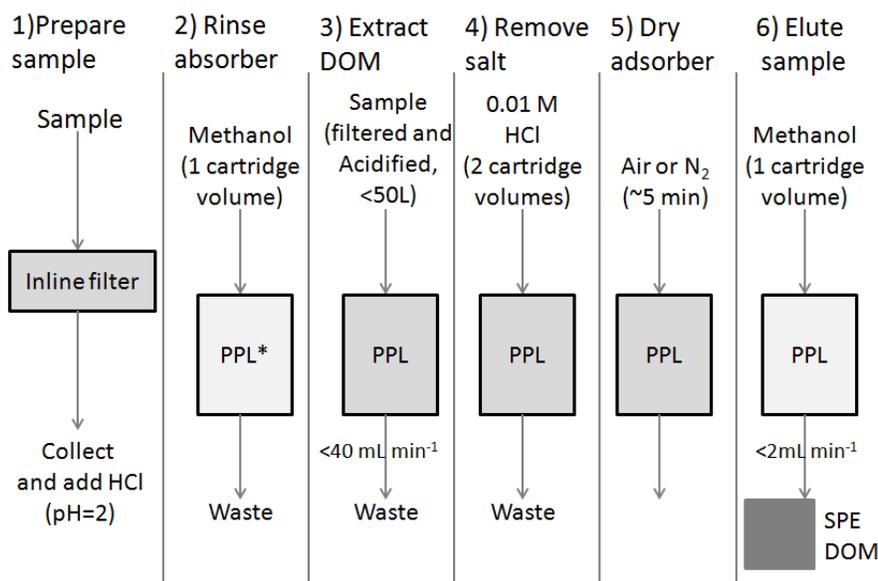


Fig. 2. Scheme for the isolation of the SPE-DOM from water. Adapted from Dittmar and Koch (2008) with permission of John Wiley & Sons.

PPL based extraction was recently suggested by Dittmar and Koch (2008) and currently becomes one of the analytical standards in the DOM extraction both in marine and fresh waters. Polystyrene based PPL sorbent concentrates a wide range of hydrophobic and hydrophilic components of DOM with distinct characteristics which are, however, not completely representative for the whole DOM. To maximize sample recovery, filtered water samples are acidified with hydrochloric acid to pH 2. At lower pH protonated carboxylic acids and phenols are reducing their hydrophilic properties (Fig. 2). Compared to C18, PPL shows a higher affinity to hydrophobic DOM components (Dittmar et al., 2008; Perminova et al., 2014). Major advantages of the cartridge-based PPL and C18 extraction is higher purity of the resulting DOM samples, ease in use and absence of aggressive reagents in this technique. Typical extraction efficiency for this method is about ~65% for DOM in freshwaters and ~45% for marine waters.

Alternatively, DEAE–cellulose has been shown to extract DOM by an anion exchange mechanism with carboxylic groups (Tuschall Jr et al., 1985). DEAE-cellulose extraction allows isolation of about 80% of the organic material in freshwater systems. However, this method is not suitable for saline waters due to inorganic ion competition on the anion exchange resin and requires cation-exchange after alkaline elution of DOM from the resin. Several studies compared different extraction methods and showed that extraction method is highly influential on resulting DOM quality and composition (Perminova et al., 2014; Santos et al., 2010; Tfaily et al., 2012).

Ultrafiltration

Ultrafiltration separates DOM based on its larger hydrodynamic diameter of organic molecules compared to inorganic ions. This technique, which can be applied to seawater and freshwater, is based on a hydrostatic pressure across a semi permeable membrane with very small pores (1-15 nm in diameter). Salts, water and organic matter with small hydrodynamic diameter passes through the membrane and high molecular weight (HMW) organic matter is retained and concentrated. Used in ultrafiltration membrane cut offs are highly variable and start from 1 kDa (Guo et al., 2009; Simjouw et al., 2005). Further, the DOM sample can be purified with the ultra-pure water until the required degree of “desalination” is reached. Ultrafiltration concentrates hydrophilic and hydrophobic DOM and the recovery rates (about ~30%; Walker et al., 2011) and chemical characteristics of the isolates are dependent on the membrane material and selected pore size.

Reverse osmosis and electrodialysis

The application of combined reverse osmosis and electrodialysis allows DOM sample concentration and desalting by an altering series of positive and negative ion-exchange membranes under the influence of an electric potential (Gurtler et al., 2008; Koprivnjak et al., 2009; Vetter et al., 2007). Anions pass through positively charged ion-exchange membranes toward the anode, while cations pass through negatively charged ion-exchange membranes toward the cathode. The resulting sample with lower

salinity is reduced in volume by reverse osmosis, and then desalted a second time in a final electro dialysis phase. Reverse osmosis and electro dialysis allow efficient extraction of a broad range of organic compounds. DOC recoveries of more than 60% can be reached using combined reverse osmosis and electro dialysis (Vetter et al., 2007). Reverse osmosis and electro dialysis can be used independently.

2.4. Analytical strategies in DOM research

There are two major groups of methods in DOM research: targeted and non-targeted. Targeted methods are focused on the analysis of DOM organic mixture with focus on specific structurally well-defined organic compounds. Usually these organic compounds are called biomarkers. Targeted methods commonly used in organic geochemistry and are focused on the analysis of the evolution of organic compounds from the moment of their formation with further studies of the subsequent changes in their composition and distribution in natural environments. Non-targeted methods characterize either bulk parameters of DOM or detect the analytical signal (or superposition of signals) from multiple molecular components of DOM mixture.

2.5. Targeted approaches: biomolecules in DOM

Many biomolecules (lipids, proteins, sugars, lignin, nucleic acids and etc.) are found in biota. Microbially metabolized compounds like lipids, proteins and sugars can provide some insights into the level of DOM processing and heterotrophic activity in natural waters, but they are less useful as chemical tracers. Recent studies demonstrated that average riverine DOC includes ~1-3% of neutral sugars, ~1-2% of amino acids (AA), ~3% of carboxylic acids, ~0.02% of phenols (Findlay and Sinsabaugh, 2003). Together amino acids and sugars contributes ~2-5% of DOC in the rivers, ~14-44% of DOC in clear lakes and ~8% of DOC in humic lakes (Benner and Opsahl, 2001). Therefore, biomolecules significantly contribute to DOM carbon pool, but large fraction of dissolved organic matter is uncharacterized.

Proteins and amino acids

Proteins are a significant fraction of carbon in living organisms and significantly contributes to OC and organic nitrogen (ON) in aquatic organisms, e.g. microbes or microalgae (Geider and La Roche, 2002). Significant amount of proteins and their degradation products are transformed to non-living organic matter, one part of which is DOM. Some methods allow direct characterization and quantification of proteins (e.g. proteomics; Powell et al., 2005; Schulze et al., 2005). Currently, the detection of free and hydrolyzed amino acids in nanomolar concentrations is possible even in the complex organic matrices like DOM (Davis et al., 2009; Yamashita and Tanoue, 2004). Modern methods of quantification of AA's are based on the derivatization of free or hydrolyzed AA's by ortho-phthaldehyde (OPA) with primary amines in basic aqueous solutions to form fluorescent, hydrophobic products that can be retained and separated by the high-performance liquid chromatography (HPLC; Lindroth and Mopper, 1979; Mopper and Lindroth, 1982). Dissolved free amino acids (DFAA), total hydrolysable amino acids (THAA), and dissolved combined amino acids (DCAA=THAA-DFAA) are operationally classified based on applied procedures. DFAA – measured by direct reaction with OPA, THAA – results of hydrolysis procedure which transforms proteins, peptides and glycoproteins to the monomeric amino acids (Lee and Bada, 1975; Lee and Bada, 1977). The hydrolysis and derivatization of proteins and peptides has some limitations: tryptophan is destroyed during acid hydrolysis; proline is not derivatized by OPA because it does not have a primary amino group. Analysis of single amino acids and their stereochemistry significantly contributed to the understanding of the cycling of DON in natural waters (Davis et al., 2009). THAA in natural waters contribute about 2-3% of DOC in the rivers and 3-13% of DOC in eutrophic lakes (Thurman, 1985).

Lignin and lignin phenols

Lignin is an abundant three-dimensional polymer biomolecule which is formed exclusively in vascular plants and is relatively recalcitrant (Lebo et al., 2000). The phenolic monomers from which

lignin is formed are covalently linked by various combinations of carbon–carbon and carbon–oxygen bonds involving the aromatic rings of the phenols and their three-carbon side chains. Lignin phenols are excellent tracers of terrigenous DOM transported to the oceans via riverine runoff (Opsahl and Benner, 1997) and are the proxy of vegetation types on land (Page et al., 2001). Lignin phenols can be obtained by oxidation of lignin in alkaline CuO. Hydrolyzed phenols are usually converted into trimethylsilyl derivatives, which are then separated using gas chromatography (Benner and Opsahl, 2001; Hedges and Parker, 1976). Underivatized phenols can also be separated using HPLC and detected spectrophotometrically (Lobbés et al., 2000; Lobbés et al., 1999).

Sugars

Free and combined sugars constitute a large fraction of the biomass of most organisms – up to 60% dry weight for some algae. Carbohydrates can be released from living organisms as a result of excretion, inefficient grazing, death and lysis of cells. Carbohydrates are one of the major components of the identifiable fraction of DOM (~3%; Henrichs and Williams, 1985). Similarly to amino acids, sugars can be quantified as total hydrolysable sugars in unmodified water samples or in concentrated seawater samples (Mopper et al., 1992). Hydrolysis is usually carried out with concentrated H₂SO₄ followed by neutralization with BaCO₃. In solution, sugars exist as a dynamic mixture of the open-chain form and anomeric pairs of pyranose or furanose rings (Amon and Benner, 2003; Medeiros and Simoneit, 2007; Mopper et al., 1992). Total hydrolysable sugars usually accounted for 5-10% of DOC in rivers.

Lipids

Lipids are actively involved in the metabolism of aquatic organisms and can also be found in DOM. Microalgae synthesize many unusual compounds, such as long-chain alkenones, alkenoates and alkenes, long-chain alkyl diols, highly branched isoprenoid alkenes and distinctive sterols and unsaturated fatty acids, thus enabling inputs of microalgae-derived organic matter to be easily recognized. Many lipid compounds can be used as biomarkers for characterization of sources of OM in marine and freshwater

systems (Rethemeyer et al., 2010; Vonk et al., 2008). The input of terrestrial organic matter to marine environments can be recognized from higher plant lipids, such as long-chain alcohols, alkanes and fatty acids, and C₂₉ sterols. Bacteria synthesize a diverse range of compounds, such as branched fatty acids, hopanoids and isoprenoids, many of which are particularly stable, for instance those that contain an ether bond. Qualitative assignments of organic matter sources are thus reasonably straightforward, although even now lipids can be found for which no source is known (Volkman, 2006). With different extraction techniques different lipid compounds can be extracted and analyzed including: hydrocarbons, straight chain alkanes (Volkman et al., 1997), branched acyclic alkanes, n-alkenes, highly branched isoprenoid alkenes, and fatty acids (Volkman, 2006). The chemistry and biogeochemistry of lipids is complex but informative in terms of analysis of geological processes and the analysis of food webs (Graeve et al., 1997; Lee et al., 2006). The volatile and nonvolatile fatty acids together may account for ~6% of DOC in freshwaters, more polar low-molecular-weight carboxylic acids are less abundant (Thurman, 1985).

2.6. Non-targeted approaches

Non-targeted studies focus on the bulk DOM and on the simultaneous detection of multiple molecular components. The analytical challenge arises from the superposition of analytical signals from multiple molecules in DOM, which can be derived from simple biochemicals (amino acids, simple sugars, fatty acids), complex biopolymers (proteins, polysaccharides, lignin), but also from very complex degradation products of unknown origin that are currently not fully characterized like humic substances (HS) (Ghabbour and Davies, 2001) or black carbon (Dittmar, 2008).

In the last decades, non-targeted molecular tools such as high-magnetic field NMR and FT-ICR MS found numerous applications in DOM research and in the water chemistry. Partially improved DOM purification methods allowed application of these methods which are characterized with high sensitivity and resolution. The recent developments in analytical instrumentation allowed detection and characterization of single organic compounds and in-depth characterization of homogenous mixtures. However, due to the complexity and heterogeneity of DOM, its molecular characterization remains

challenging and do not cover the whole complexity of DOM. High sensitivity and high resolution methods in combination with modern data processing tools allows in-depth characterization of DOM and analysis of its properties in natural environments.

The analysis of bulk DOM as a “substance” includes carbon isotopes (^{13}C , ^{14}C) analyses (Druffel et al., 1992; McIntosh et al., 2015), elemental analysis (Benner et al., 1992). ^{13}C is a good proxy for determining sources and pathways of transformations of organic and inorganic carbon (Zurbrugg et al., 2013). These methods do not give detailed information on DOM, but are quite useful for the analysis of changes in the composition during biogeochemical processing of DOM. Optical methods, Fourier transform infrared spectroscopy (FTIR; Abdulla et al., 2010), NMR and MS give informative results, but in case of non-targeted research they are extremely difficult for interpretation. High-resolution MS and high magnetic field NMR and new approaches in data processing significantly broadened and deepen our understanding of DOM composition, behavior and its biogeochemical fate and roles. However, applications of high-magnetic field methods in the whole analysis of DOM as a complex mixture are still under development.

Nuclear magnetic resonance spectroscopy

A wide variety of NMR methods is used in the environmental research and in advanced chemical characterization of DOM (Gelinas et al., 2001; Simpson et al., 2011). Both solution and solid state NMR found many applications (Abdulla et al., 2010; Gelinas et al., 2001; Paytan et al., 2003; Sannigrahi et al., 2005). Molecular structural information and information on DOM functionalities can be obtained from mono, two- and three- dimensional NMR spectra (Kaiser et al., 2003; Simpson, 2001; Simpson et al., 2003). Solution state NMR provides detailed information on the structure and distribution of functional groups even for very complex organic mixtures. However, the high resolution data is available only for extractable fraction of organic matter (Fan et al., 2000; Hertkorn et al., 2006; Hertkorn et al., 2002; Lam et al., 2007). Three most commonly used solvents in DOM analytics include deuterated DMSO- d_6 , methanol (CD_3OD) and water (D_2O , usually in mixture with NaOD). Fig. 3 demonstrates two

characteristic ^1H NMR spectra of DOM measured in different solvents with highlighted major structural moieties.

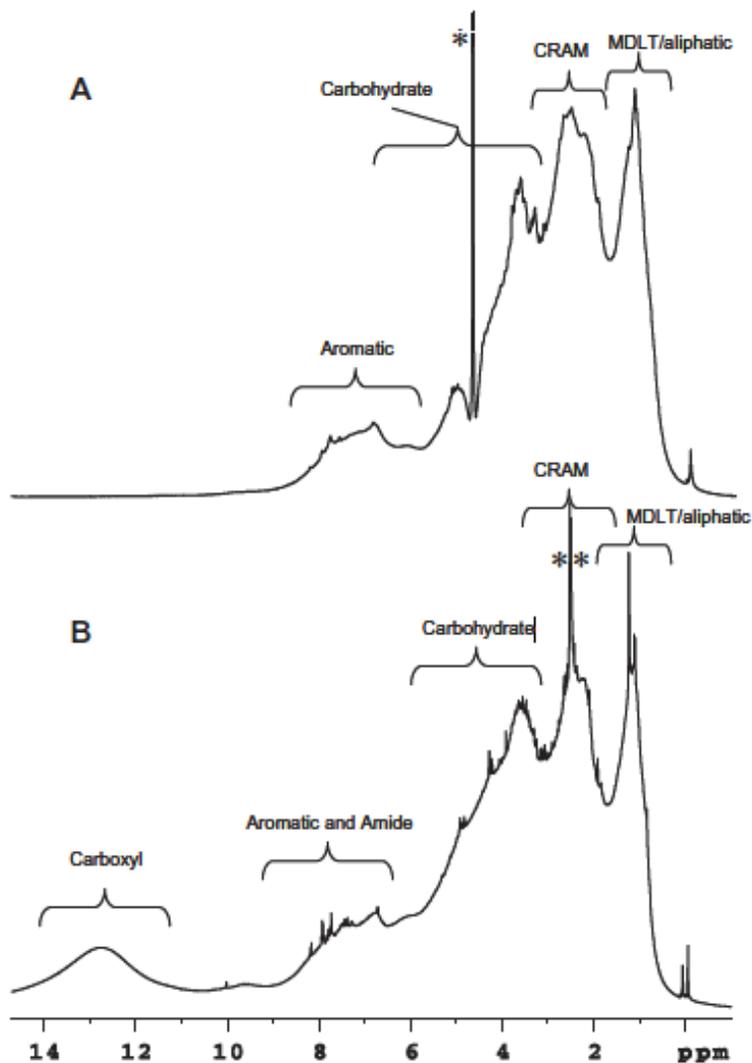


Fig. 3. Comparison of ^1H NMR spectra at 500 Mhz of Suwannee River DOM in A) $-\text{D}_2\text{O}/\text{NaOD}$ and B) $-\text{DMSO}-d_6$. Peaks of residual protons for HOD and DMSO are starred. Specific structural features including carboxyl-rich alicyclic molecules (CRAM) and material derived from linear terpenoids (MDLT) are indicated. Adapted from Simpson et al. (2011) with permission of Elsevier.

Correlation spectroscopy (COSY) and total correlation spectroscopy (TOCSY) are a very powerful tools for experimental characterization of complex mixtures (Simpson, 2001; Simpson et al., 2002).

Heteronuclear correlation NMR experiments are one of the most informative in DOM research. The most popular and applicable heteronuclear correlation NMR techniques are heteronuclear multiple quantum coherence (HMQC) and heteronuclear single quantum correlation (HSQC; Otting and Wüthrich, 1988; Ruiz-Cabello et al., 1992; Vuister et al., 1991).

In summary, the application of multiple NMR techniques allow in-depth characterization of different functional groups and compound classes including: MDLT, CRAM, carbohydrates, aromatics and phenols, amide functionalities in peptides, carboxylic groups, anomeric protons from carbohydrates, α -protons in peptides, variety of aliphatic functionalities and etc. (Hertkorn and Kettrup, 2005). However, clear structural analysis of single DOM molecular components is still limited due to overlapping of signals from various components of organic mixture. NMR data on DOM is successfully applied for classification of DOM according to its origin (Thomsen et al., 2002), performance in analytical methods (Francioso et al., 2003; Ussiri and Johnson, 2003), and reactivity towards organic (Kulikova and Perminova, 2002; Perminova et al., 2001) and inorganic materials (Balcke et al., 2002).

Fourier transform ion cyclotron resonance mass spectrometry

Over the last decades the number of applications of FT-ICR MS in DOM chemistry significantly increased. FT-ICR MS reveals extraordinary details of the complexity of the DOM composition (Dittmar and Koch, 2006; Fievre et al., 1997; Kim et al., 2004; Sleighter and Hatcher, 2007). In some cases FT-ICR MS analyses were complemented with NMR data to obtain structural information which is very limited in FT-ICR MS (Hertkorn et al., 2012; Kim et al., 2003b). FT-ICR MS methodology, principles, data treatment and interpretation approaches are covered in detail in the following chapter. DOM can be analyzed mass spectrometrically in different ways: (i) hyphenation with HPLC (Dittmar et al., 2007; Navalon et al., 2010; Sandron et al., 2014), (ii) direct injection of sample extracts (Hertkorn et al., 2008; Kim et al., 2003a; Kujawinski and Behn, 2006; Sleighter and Hatcher, 2008), or (iii) chromatographic fractions (Koch et al., 2008; Liu et al., 2011). All of these approaches usually require preliminary concentration and purification of DOM (see Chapter 2.3).

Basics of the method, methodological features of FT-ICR MS analysis

FT-ICR MS determines the mass-to-charge ratio (m/z) of ions based on their cyclotron frequency in a fixed magnetic field (Marshall and Hendrickson, 2002). The ions are trapped in a Penning trap (a magnetic field with electric trapping plates) where they are excited (at their resonant cyclotron frequencies) to a larger cyclotron radius by an oscillating electric field orthogonal to the magnetic field. After the excitation field is removed, the ions are rotating on their cyclotron frequency at phase. These ions induce a charge (detected as an image current) on a pair of electrodes as the packets of ions pass close to them. The resulting signal is called free induction decay (FID), transient of interogram that consists of a superposition of sine waves. The useful signal is extracted from this data by performing a Fourier transform to give a mass spectrum (Marshall and Hendrickson, 2002). The relationship between the cyclotron frequency and the m/z is given by: $f = qB/2\pi m$, where f = cyclotron frequency, q = ion charge, B = magnetic field strength and m = ion mass. Or in form of angular frequency: $\omega_c = qB/m$ where ω_c is the angular cyclotron frequency which is related to frequency as $f = \omega/2\pi$ (Fig. 4).

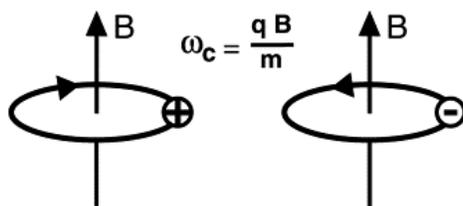


Fig. 4. Ion cyclotron motion. Ions rotate in a plane perpendicular to the direction of a spatially uniform magnetic field, B . Note that positive and negative ions orbit in opposite senses. Reproduced from Marshall and Hendrickson (2002) with permission of Elsevier.

Usually, it is possible to observe 10-40 peaks at each nominal mass in the DOM FT-ICR mass spectra (Fig. 5). Two general approaches of spectra acquisition are possible: data acquisition either in narrow or broadband mode scan mode, the latter of which is more common in DOM research because it is less time

consuming. Within the m/z dynamic range of 200 to 2000 m/z thousands of molecular ions are usually detected.

All of the above ionization methods are considered to be soft, meaning that ionizable compounds are not destroyed (not fragmented) within the ionization process so that predominantly intact molecular ions are observed in the mass spectrum. ESI is the most common ionization method in DOM research. During ESI, the liquid sample is sprayed through the needle, and the high voltage difference between the spray needle and metal inlet induces a charge on the sprayed droplets. The charged droplet diminishes in size as the solvent is evaporated (as result of heat and drying gas), concentrating the charges held on the droplet. As a result, the droplet bursts into many smaller droplets that can be completely desolvated, leaving only charged analyte ions in the gas phase for further induction into mass spectrometer. ESI can be operated either in positive or in negative ionization mode, depending on the aim of the analysis. ESI is a competitive process, with a preferred ionization of highly polar compounds including inorganic ions. This is why removal of inorganic ions is extremely important. Since FT-ICR MS is highly sensitive, it is common that contaminants derived from solvents and the solid phase extraction procedure can be detected. Therefore, blank controls are important to discriminate DOM from contaminant signals.

Functional groups that will readily lose a proton (alcohols, carboxylic acids, cyanides, peptides, nitric- and sulfonic- acids, and phosphates) are covered in negative ion mode whereas basic functional groups that can easily gain a proton (i.e., amines, amides, peptides, and thiols) are preferably detected in positive ion mode. Changing the pH of the sample solution (slightly basic for ESI negative and slightly acidic for ESI positive mode) can increase the ionization efficiencies.

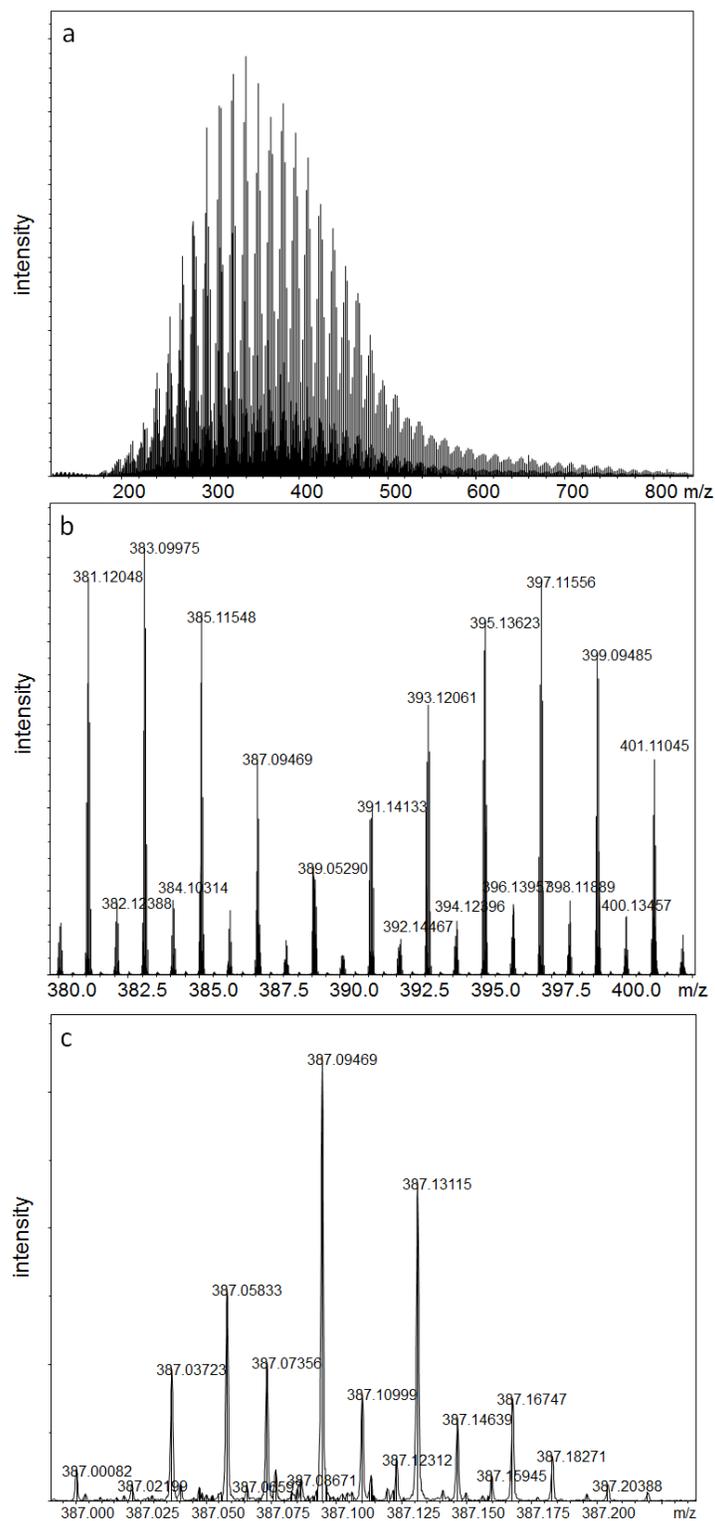


Fig. 5. FT-ICR MS spectra of Lena River DOM. *a* – mass spectrum in the 100-850 m/z range, *b* – 380-400 m/z, *c* – 387 m/z nominal mass.

Major advantages of FT-ICR MS are the high mass resolution and mass accuracy (difference between the measured and theoretical mass). High field FT-ICR mass spectrometers typically deliver mass resolutions of >300000 (at 400 Da) and mass accuracies of <0.5 ppm, which allows to resolve thousands of mass peaks in DOM samples and the assignment of their respective molecular formulas (Koch et al., 2005; Lechtenfeld et al., 2014). Depending on ionization and ion charge determination, molecular formulas can be assigned to these masses. Charges can be determined using isotopic peaks, if a compound is singly charged, then the ^{13}C isotope peak will be observed at 1.0034 mass units higher than the ^{12}C (monoisotopic) peak, which is the mass difference between the ^{12}C and ^{13}C . Doubly charged peaks have isotopes that appear at 0.5017 mass units higher, but these peaks are rarely detected in the DOM mass spectra (Kim et al., 2003b; Kujawinski, 2002; Stenson et al., 2002). The simplest way of molecular formula assignment is to solve a Diophantine equation. A Diophantine equation is an equation in which the coefficients and solutions are required to be integers. The general form of the linear Diophantine equation is: $a_1x_1+a_2x_2+\dots+a_kx_k=d$, where the a_1 , a_2 , a_k are integer values. In case of the formula assignment procedure x_1 , x_2 and x_k are the accurate masses of isotopes (C, H, O, N, S and P) and a_1 , a_2 and a_k are corresponding coefficients in the molecular formulas, d is the highly accurate mass of the neutral molecule. Fundamentals of the formula assignment procedure of DOM are covered in Koch et al. (2007). The formula assignment is crucial in DOM research since it is affecting further data evaluation and interpretation. Bulk DOM is primarily composed of C, H, O and N, with minor contributions of P and S. In addition the most abundant isotopologues (^{13}C and ^{34}S) can be used in the formula assignment procedure. Usually, with increasing mass the number of possible solutions of the Diophantine equation increases and one of the problems is the determination of correct formula among the many theoretically possible solutions (Fig. 6).

Since thousands of molecular peaks are detected in the DOM mass spectra, the assignment procedure has to be automatized. Several rules and thresholds can be applied for the exclusion of false assigned molecular formulas (Hughey et al., 2001; Kim et al., 2003b; Koch et al., 2005; Kujawinski et al., 2004).

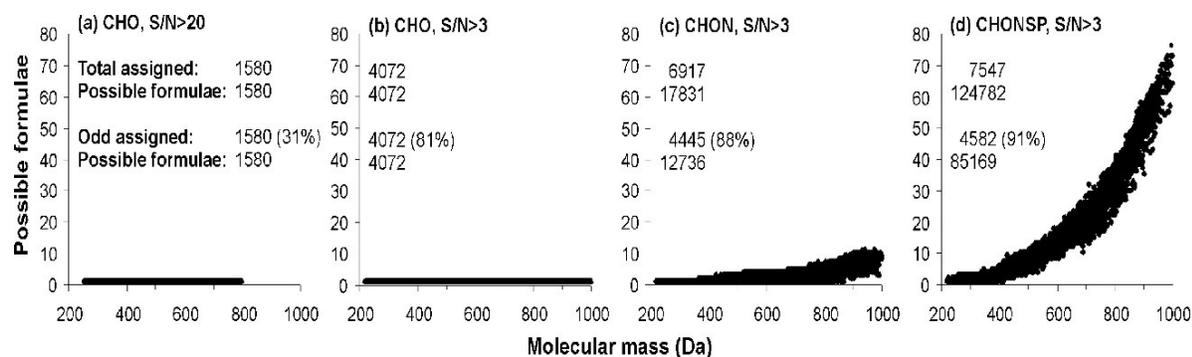


Fig. 6. Suwannee River fulvic acid standard (SRFA II, International Humic Substances Society). ESI, negative mode: number of possible molecular formulas for each detected ion with odd (5029 total peaks with $S/N > 3$ in the spectrum) and even (3400 total peaks with $S/N > 3$) nominal m/z . Number of total and odd peaks with at least 1 molecular formula assignment (total assigned, odd assigned) and the respective sum of all possible assignments (possible formulas) are presented. Four different assumptions regarding the number of elements and intensities were used for formula determination: (a) $C_{0-\infty}H_{0-\infty}O_{0-\infty}$, $S/N > 20$; (b) $C_{0-\infty}H_{0-\infty}O_{0-\infty}$; (c) $C_{0-\infty}H_{0-\infty}O_{0-\infty}N_{0-30}$; $C_{0-\infty}H_{0-\infty}O_{0-\infty}N_{0-30}S_{0-2}P_{0-2}$ (all $S/N > 3$). Common assumptions in all four scenarios: mass accuracy < 1 ppm, double bond equivalent for uncharged formula must be an integer value, conformance with nitrogen rule, $H \leq 2C + 2 + N$, molecular $O/C \leq 1$, $N/C \leq 1$, $H/C > 0.3$. Assuming that every molecule contains at least one C and one H atom is generally very useful to rule out some false positives. Here, this conservative assumption was already covered by $H/C > 0.3$. Quantitative validation of even m/z ions are not included, because ^{13}C compounds that contribute substantially to even m/z peaks are not considered. Reproduced from Koch et al. (2007) with permission of American Chemical Society.

The “nitrogen rule” can be applied to identify potential nitrogen in the formula because neutral molecules containing an odd number of ^{14}N atoms always exhibit an odd nominal mass. Also, the DOM mass spectra usually exhibit regularity in peak distributions which appear from homologous (or pseudo-homologous) series. The most common differences include CH_2 , CH_4-O , and H_2 . Additionally, molecular formula assignments can be validated according to the presence of the corresponding ion with one ^{13}C

isotope. The ratio of $^{13}\text{C}/^{12}\text{C}$ allows an estimation of the carbon number in the molecule since the abundance of ^{13}C in natural samples is well known (Koch et al., 2007).

After molecular assignment the data can be visualized and interpreted using several tools. Although molecular formulas (stoichiometry) do not yield structural information, some structural elements can be extracted by calculating e.g. the carbon oxidation state, the amount and density of double bonds (double bond equivalents, DBE), the aromaticity index (AI; Koch and Dittmar, 2006). DBE is used to calculate the number of double bonds and/ or rings in an organic molecule based on the number of C, H and N atoms per molecule ($DBE = C - H/2 + N/2 + I$). For instance, if DBE equals 1, the molecule contains one ring or one double bond. The most popular method of visualization of molecular information obtained from FT-ICR MS is the van Krevelen diagram. First suggested in 50's for classification of coals (based on elemental stoichiometry) it found many applications in FT-ICR MS (Schmidt et al., 2009; van Krevelen, 1950), (Fig. 7). Signal intensity, molecular mass or presence of heteroatoms can be added as a third dimension to this diagram.

Another visualization method frequently used for DOM characterization is the Kendrick mass defect (KMD) plot (Kendrick, 1963). This analysis converts m/z values to Kendrick mass values by multiplying the m/z by the ratio of the nominal mass CH_2 group (14.00000) to the exact mass of CH_2 group (14.01565):

$$\text{Kendrick Mass (KM)} = m/z \text{ value} * (14.00000/14.01565);$$

$$\text{Kendrick Mass Defect (KMD)} = \text{KM} - \text{nominal KM};$$

While CH_2 is the most commonly used group, other functional groups can be utilized (e.g. CH_2O , COO , O , H_2O , H_2 , etc.) (Sleighter and Hatcher, 2007). Originally, KMD was utilized for assigning molecular formulas, by establishing homologues CH_2 series that could be expanded from low m/z to high m/z (Stenson et al., 2003). KMD in some cases is used for formula assignments for peaks with high m/z (Grinhut et al., 2010). Peaks at low m/z can more easily be assigned with a molecular formula because

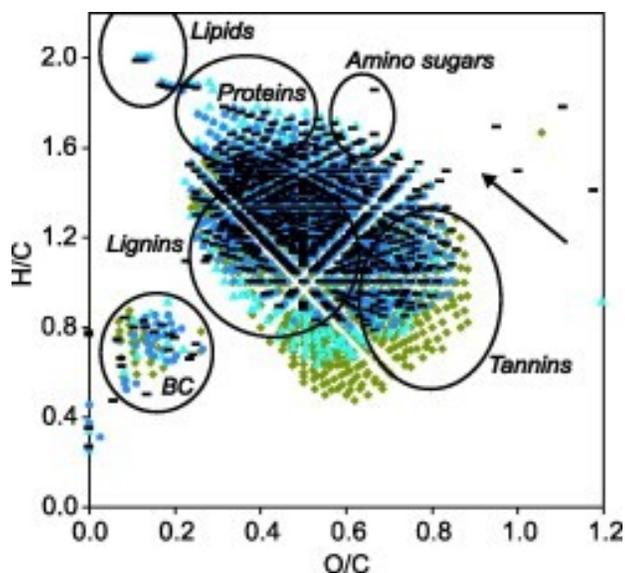


Fig. 7. Van Krevelen diagram of CHO-formulas in DOM from the Galicia–Minho shelf. A shift in direction of the arrow is observed to higher H/C and lower O/C ratios from the Douro River (green rhombs) to the central mudbelt (GeoB 11002, light blue triangles) down to the outer shelf (GeoB 11006, blue circles) and the continental slope (GeoB 11033, black dashes). Black circles correspond to general compound classes (adapted from Kim et al. (2003) and Sleighter & Hatcher (2008)) and are indicated for reference, not necessarily implying the presence of these compounds in the DOM samples. Adapted from Schmidt et al. (2009) with permission of Elsevier.

fewer formulas exist within the selected error limit (usually 0.5 ppm). Generally, only 1 molecular formula exists within this error for peaks less than 500 m/z, but beyond this value, multiple formulas are possible. Once molecular formulas are unambiguously assigned to peaks of low m/z, peaks at high m/z values that have multiple formula choices can be related to formulas assigned at lower mass by assuming that they belong to a CH₂ homologous series. If one of the formulas belongs to a homologous series, then it is very likely the correct formula and the others can be eliminated. This approach to formula assignment is called “formula extension” (Kujawinski and Behn, 2006; Grinhut et al., 2010).

Statistical methods in interpretation of molecular information

Correlation analyses and multivariate statistics are critical and widely used in DOM research to elucidate its sources (Sleighter et al., 2010), composition (Abdulla et al., 2013), environmental roles, behavior and transformations (Kellerman et al., 2014; Lechtenfeld et al., 2014). For example, the aging of DOM was assessed using correlation analysis. In brief, MS signal intensities were correlated with the radiocarbon age of organic carbon, and correlations were utilized for modeling of degradation state of organic matter and to identify recalcitrant and reactive compounds within the DOM pool in the Atlantic and Southern Ocean (Flerus et al., 2012; Lechtenfeld et al., 2014). The application of multidimensional statistics significantly facilitates interpretation of the large FT-ICR MS data sets. For instance, (Kujawinski et al., 2009) applied hierarchical cluster analysis (HCA), nonmetric multidimensional scaling (MDS) and indicator species analysis (ISA) to identify molecular markers for photo degradation of DOM and microbial metabolism.

Sleighter et al. (2010) successfully applied HCA and principal component analysis (PCA) to measure relationships between numerous samples of marine DOM (Fig. 8). The information from a PCA analysis demonstrated that DOM from various sources can be discriminated based on molecular information, and source-specific molecular components can be identified. Statistical tools can either be applied directly to the identified molecular formulas or to the data in compressed form, e.g. in form of calculated weighted averages O/C_{wa} , H/C_{wa} , C/N_{wa} , or DBE_{wa} . Magnitude weighted parameters X_{wa} can be calculated according to $X_{wa} = \sum_{i=1}^n X_i * M_i / \sum_{i=1}^n M_i$ where X is e.g. O/C , H/C and C/N , i is the individual molecular formula and M is the relative mass peak intensity (Sleighter et al., 2010). It was demonstrated on the example of the Yenisey River, where ten magnitude weighted parameters including O/C_{wa} , H/C_{wa} , N/C_{wa} , DBE_{wa} , DBE/C_{wa} , DBE/O_{wa} , $DBE-O_{wa}$, C_{wa} , MW_{wa} and AI_{wa} were related to latitude and pH driven changes in DOM molecular composition (Roth et al., 2013).

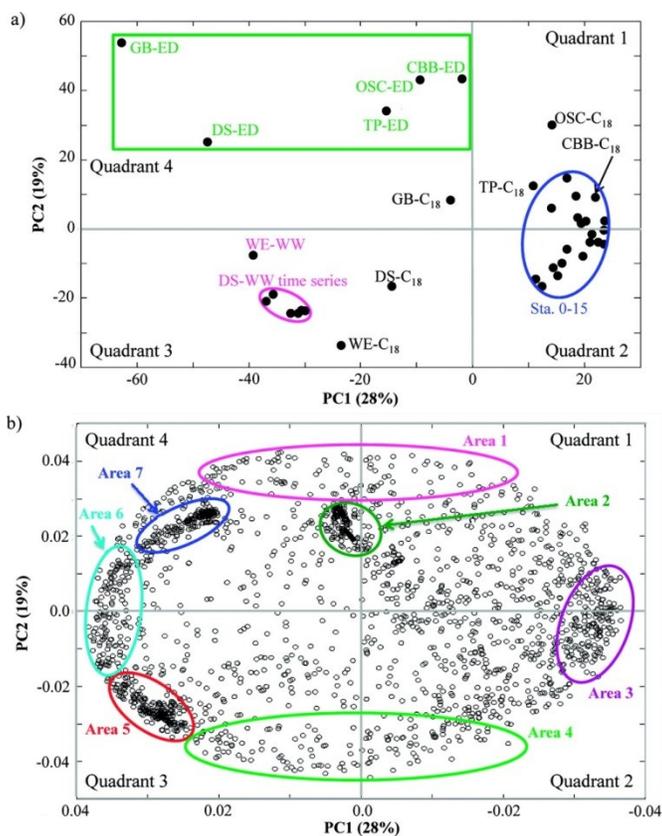


Fig. 8. Statistical plots using the relative magnitudes of the selected 2143 molecular formulas. (a) Biplot of the scores from the principal component analysis and (b) Biplot of the loadings from the principal component analysis. Adapted from Sleighter et al. (2010) with permission of American Chemical Society.

In the arctic lakes of Sweden the biodiversity approaches for characterization of DOM were applied. It was shown that in lakes, as hot spots of carbon dynamics, DOM is actively influenced by the environmental parameters. In 120 lakes, the DOM chemodiversity and composition was driven by the DOC and nutrient concentrations. Terrestrially derived DOM was selectively lost with increasing water residence times (WRT); warmer temperatures stimulated more active production of the nitrogen-containing compounds. Therefore, it was shown that DOM is shaped by the precipitation, water residence times and temperature. With respect to statistical methods, the nonlinear multidimensional scaling (NMDS) was applied; ordination to the results of nonlinear multidimensional scaling allowed characterization of the influence of environmental parameters on the DOM molecular composition (Fig. 9; Kellerman et al., 2014). Also, with the help of multivariate statistics, geochemical and biogeochemical

approaches allowed characterization of the bioreactivity of DOM in the headwater stream (Sleighter et al., 2014) and the microbial regulation of DOM molecular composition in natural waters (Koch et al., 2014).

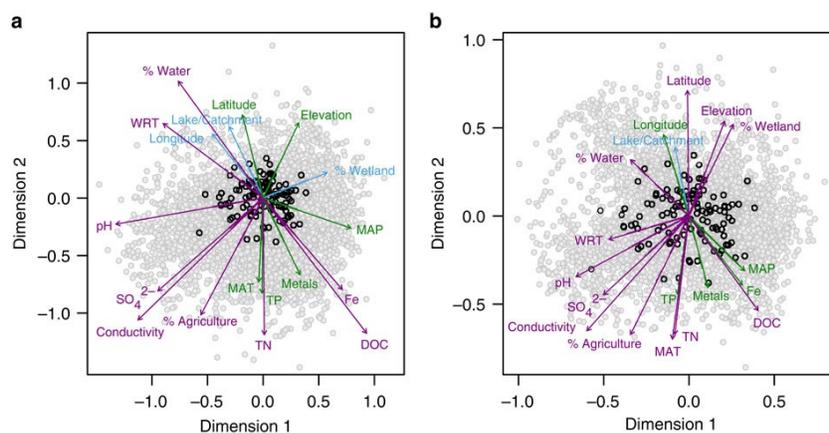


Fig. 9. Multivariate analysis of molecular data and drivers using non-metric multidimensional scaling. Ordinations are based on either Bray–Curtis (**a**, stress=0.0966), which utilizes relative compound abundances, or Jaccard distances (**b**, stress=0.1143), which utilizes presence/absence information. Landscape, climate and in-lake chemistry variables were fit to the ordination. Grey-shaded circles are DOM compounds, whereas black circles indicate the site. Variables with a significance level of <0.05 (light blue), <0.01 (green) and <0.001 (purple) are shown. DOC, dissolved organic carbon; mean annual precipitation (MAP); mean annual temperature (MAT); total nitrogen (TN); total phosphorus (TP); water residence time (WRT). Adapted from Kellerman et al. (2014) with permission of Nature Publishing Group.

Another approach which can significantly assist in the interpretation of molecular information of DOM is the 2D correlation spectroscopy (Abdulla and Hatcher, 2014). The 2D correlation spectroscopy is one of the chemometric methods which have a great potential in characterization of subtle changes in complex mixture under the specific external perturbation. One of the most contrasting perturbations in DOM chemistry is the perturbation of DOM along the freshwater – saline transition, which can be easily parametrized with salinity. The original approach (Noda and Ozaki, 2004) has been widely applied to understand changes and behavior of different chemical mixtures (Abdulla and Hatcher, 2014; Abdulla et

al., 2013; Sleighter et al., 2014). Moreover, 2D correlation spectroscopy can also be applied to other analytical methods used in DOM research and even in form of two-dimensional heterocorrelations (e.g. MS vs. NMR).

Molecular analysis of DOM is extremely important for the understanding of how DOM molecules are organized and related. Detailed information on molecular composition and properties would enable recognition of biomarkers present in specific geographical areas. However, due to its heterogeneity and the low concentration of single components in DOM, the characterization and quantification of single compounds is extremely challenging on molecular level. The opportunities for DOM characterization either derive from more generalized approaches (e.g. concentration of organic carbon, isotopic composition, and optical properties of water) or in the methods with a limited analytical windows. The highest level of structural information and concentration of DOM molecular components can be reached only for well-defined biomarkers which do not represent the whole complexity of DOM organic mixture. Therefore, even though high magnetic field analytical methods give massive information on DOM molecular composition and functionalities which found many applications in environmental studies on DOM, this information is still fragmented and incomplete. Applied methodologies require further development and optimization in instrumentation and data interpretation procedures.

3. The Arctic, permafrost, arctic rivers and organic matter

3.1. Climate change in the Arctic

The Arctic together with the Antarctic Peninsula experienced the greatest regional warming on Earth in the recent decades. Average annual temperatures have risen about 2 to 3 °C since the 1950s and by up to 4 °C in winter (Przybylak, 2007). The warming has been largest over the land areas (Chapman and Walsh, 1993). The warming has resulted in extensive melting of glaciers (Sapiano et al., 1998), thawing of permafrost (Osterkamp and Romanovsky, 1999), and reduction of sea ice in the Arctic Ocean (Kwok et al., 2009; Rothrock et al., 1999; Vinnikov et al., 1999). The latest proposed climate change scenarios for the Arctic suggest dramatic temperature increase in the nearest future. Certainly a substantial temperature increase will affect biogeochemical cycles in the region and will cause shifts in the carbon cycle (Porcal et al., 2009), because of changes in sinks and sources of carbon (Wu, 2012). Due to the vulnerability of the Arctic, the increased concentrations of greenhouse gases in the atmosphere, very likely, would have larger effects than anywhere else on the globe. Global coupled physical atmosphere-land-ocean models were applied for the prediction of possible future climate change scenarios. According to predicted changes in greenhouse gas concentrations the resulting changes in temperature, precipitation, and seasonality were projected. Toward the end of the century, the global mean temperature increase is projected to 2.5-3.5 °C for different scenarios with predictions up to 7 °C temperature increase in the Arctic (Berner et al., 2005). The changes in snow, ice and permafrost are not only sensitive indicators of the climate change but also play a crucial role in shaping the arctic environment. Changes in these components of the system will affect the environment, biota, ecosystems, and humans.

3.2. Permafrost

Permafrost is defined as ground (soil or rock with included ice or organic material) that remains at or below 0°C for at least two consecutive years. Permafrost is widely distributed in the northern hemisphere and usually classified into several types based on its geographic distribution (Fig. 10).



Fig. 10. Distribution of continuous, discontinuous, sporadic and isolated permafrost in the northern hemisphere (from International Permafrost Association). Adapted from Schuur et al. (2008) with permission of International Permafrost Association .

Permafrost can be continuous (underlying 90-100% of landscape), discontinuous (50-90%), sporadic (0-50%) and isolated. Permafrost occupies ~22% of the exposed land surface area in the Northern Hemisphere (Zhang et al., 1999) and also occurs on the continental shelf of the Arctic Ocean and in the mountains. Permafrost depth and thickness are highly variable and typically range from 350 m to 650 m (up to ~1500 m in unglaciated areas of Siberia) in zones with continuous permafrost and <50 m in the zones with discontinuous permafrost (Anisimov and Reneva, 2006; French, 2000). The active layer is the

top layer of soil that thaws during the summer and freezes again in autumn or winter. It experiences many cycles of freezing and thawing over the years. The thickness of the active layer ranges from a few tens of centimeters to more than 2 m. Under the active layer there is the transition zone, the ice rich layer that separates the active layer from the stable permafrost (Shur et al., 2005). Active layer is important for the roots of the plants, hydrology of the surface layers, organic matter in the soil and microbial activity. Numerous factors affect the distribution of the permafrost including topography, hydrology, vegetation, snow cover, and properties of subsurface material.

Ice Complexes (Yedoma)

Vast areas of northern Siberia are underlain by the late Pleistocene ice-rich permafrost deposits of the ice complexes (also called Yedoma). These syngenetically frozen, fine grained deposits contain large amounts of ground ice in the form of segregated ice and large ice wedges. Ice complex deposits are characterized with high content of ice (50-90 volume %) and organic carbon (~2 wt%). Due to the considerable ice content, these formations are extremely sensitive to climate warming and prone to degradation (Schirrmeister et al., 2012). They contain considerable amounts of fossil organic carbon that could become accessible due to permafrost thaw, degradation and mobilization (Khvorostyanov et al., 2008; Schirrmeister et al., 2011). Examples of active degradation of ice complex deposits of the Muostakh Island are shown in Fig. 11. The degradation of these ice-rich deposits already occurred due to global warming during transition from late Pleistocene to Holocene; therefore it is not an exclusively modern phenomenon (Romanivskii et al., 2004). Previous studies estimated that >75% of the ice complex areas have been degraded during the Holocene (Grosse et al., 2005; Grosse et al., 2006; Veremeeva and Gubin, 2009). However, there are no detailed knowledge and understanding about the evolution of different types of degradational landforms and their interactions.



Fig. 11. Permafrost degradation and exposures of the ice complex deposits (Muostakh Island). Photos by I. Dubinenkov.

Permafrost carbon pool

The permafrost is highly relevant in the context of the changing climate due to the amounts of organic carbon stored in it. The organic-rich permafrost represents one of the largest and vulnerable sources of organic carbon (Engelhaupt, 2008). Most of the carbon stored in permafrost is a result of photosynthesis and the carbon content is typically higher in the surface layers of the permafrost. Cryoturbation processes (results of freeze-thaw cycles) can cause mixing of sediments and affect the distribution of carbon in the

sediments (Schirrmeister et al., 2002; Zimov et al., 2006). Current estimated carbon pools stored in permafrost are 191 Pg C for the 0-30 cm depth, 496 Pg C for the 0-100 cm depth, and 1024 Pg C for the 0-300 cm depth. Carbon pools in layers deeper than 300 cm were estimated to be 407 Pg in Yedoma deposits and 241 Pg in deltaic deposits. The total permafrost contains ~1700 Pg of organic carbon which is ~50% of the estimated global belowground organic carbon pool (Tarnocai et al., 2009). Thus, mobilization, degradation and mineralization of this massive carbon pool is of great importance with respect to regional and global carbon cycling and ecosystem health.

Permafrost degradation

As a product of a cold climate, permafrost is extremely sensitive to climate change. Warming of air temperatures lead to permafrost thaw and degradation (Demchenko et al., 2006; Hinkel and Nelson, 2003) and the degradation includes thickening of the active layer, talik formation (Wellman et al., 2013), thermokarst development (Jorgenson et al., 2006), expansion or creation of a thaw lakes, lateral permafrost thawing and northward migration of the southern permafrost boundary (Zhang et al., 2005), coastal erosion and thermo abrasion of the coasts (Lantuit et al., 2012; Lantuit et al., 2013). All of these mechanisms mobilize previously frozen material and carbon from permafrost deposits. On average, coastal erosion rates in the Arctic are 0.5 m year⁻¹ with a cumulative transport to adjacent coastal waters of ~ 46.5 Tg of organic carbon per year (Lantuit et al., 2013). Thaw of the ice-rich permafrost, melting of massive ice wedges results in surface subsidence and characteristic landforms such as thermokarst lakes and thermokarst basins (alasses) (van Everdingen, 1998). These changes are accompanied by modifications in surface hydrology, disturbance of vegetation, mobilization of previously frozen pools of fossil organic carbon with their further release to the atmosphere (Grosse et al., 2011; Osterkamp et al., 2000; Zimov et al., 1997). Another common type of permafrost degradation is thermal erosion (van Everdingen, 1998). This process also interacts with other landscape factors and processes, for example by forming thermo-erosional valleys that affect surface runoff systems, increase nutrient and sediment

delivery to rivers, lakes, and the sea (Bowden et al., 2012; Marsh and Neumann, 2001; Toniolo et al., 2009).

Decomposition of permafrost organic matter

Under a warming climate, release of carbon from permafrost to the atmosphere will occur primarily through accelerated microbial decomposition (Davidson and Janssens, 2006; Mackelprang et al., 2011; Vonk et al., 2013a). Previously conserved carbon can be introduced to aquatic systems in particulate or dissolved form and will significantly affect hydrochemistry and carbon cycling in creeks, lakes, rivers and in the Arctic Ocean (Frey and McClelland, 2009; Vonk et al., 2013b). During thaw, previously conserved OM may become accessible for microbial degradation and result in greenhouse gas emission (Osterkamp, 2007; Prater et al., 2007). Bacteria which have survived in the frozen permafrost deposits activates and grows upon permafrost thaw. Metagenomics previously demonstrated rapid shifts in microbial, phylogenetic and functional gene abundance and pathways during the transition from a frozen to a thawed permafrost state (Mackelprang et al., 2011). Moreover, accumulated methane in permafrost is released during thaw and subsequently partly consumed by methanotrophic bacteria. DOM in the permafrost outflows is a subject to photodegradation and severe mineralization processes (Cory et al., 2014; Knoblauch et al., 2013; Vonk et al., 2013a). Microbial DOC decomposition rates of 13-62% in aquatic environments suggest rapid substantial mineralization and turnover of permafrost-emitted organic matter (Spencer et al., 2015; Vonk et al., 2013a). DOC is actively processed in the small streams and permafrost creeks resulting in high CO₂ outgassing rates (Butman and Raymond, 2011; Denfeld et al., 2013). These streams are the hot spots of mineralization and turnover of mobilized permafrost OM.

Degrading permafrost can release enough carbon dioxide and methane to influence global climate. The permafrost carbon feedback is the amplification of surface warming due to CO₂ and CH₄ emissions from thawing permafrost. Model estimated the exposure of 11.6 Pg (6.8 cm increase in the active layer thickness) of carbon in thawed soil organic matter (SOM) with consequent cumulative net source of 3.7

Pg C released to the atmosphere from 1970-2006. Enhanced decomposition from the newly exposed SOM accounts for the release of both CO₂ (4.0 Pg C) and CH₄ (0.03 Pg C), but is partially compensated by CO₂ uptake (0.3 Pg C) associated with enhanced net primary production (Hayes et al., 2014). The CO₂ and CH₄ outgassing are mostly result of biological processes in the thawed and mobilized permafrost carbon.

However, the rate and form of permafrost carbon release on the continental and landscape level is not studied well. To understand how permafrost carbon is mobilized and transported to the atmosphere and hydrosphere it is necessary to understand processes of permafrost OM mobilization and mineralization.

3.3. Arctic rivers

The permafrost degradation influences the hydrology, biogeochemistry and carbon cycling of the arctic rivers and the Arctic Ocean. The Arctic Ocean is relatively small (~1% of the World's Ocean by volume) but receives ~11% of the world river runoff (Hayes et al., 2014; Shiklomanov et al., 2000). The majority of this runoff is contributed by the largest arctic rivers: Ob, Yenisey, Lena, and Kolyma in Siberia, and Yukon and Mackenzie in North America (Fig. 12).



Fig. 12. Watersheds of the six largest arctic rivers. Adapted from Holmes et al. (2012) with permission of Springer.

Cumulatively, these rivers discharge the majority of the continental fresh water to the Arctic Ocean ($\sim 2350 \text{ km}^3 \text{ year}^{-1}$), the most freshwater dominated of all Earth's oceans (Holmes et al., 2012). Factors such as changing snow cover, precipitation regimes and surface temperatures directly and indirectly impact watersheds as well (Saito et al., 2013). In the period from 1936 until 1999, an increase of the annual discharge of about $2.5 \text{ km}^2 \text{ year}^{-1}$ of the six largest Eurasian arctic rivers (Yenisey, Ob, Lena, Kolyma, Pechora and Severnaya Dvina) was recorded (McClelland et al., 2004). The reason for this trend, however, remains unclear. It cannot be completely explained by dams, permafrost thaw, or increases in fires. Construction of dams affected seasonality of discharge. Thawing of permafrost and increased fires may have contributed to the long-term changes in discharge, but neither can be considered a major driver. Potentially increasing northward transport of moisture as a result of global warming remains the most viable explanation for the observed increases in Eurasian arctic river discharge (McClelland et al., 2004). Together with increased water discharge, significant amounts of biogeochemical constituents (e.g. DOC, POC, nutrients, metals) are delivered to the coastal seas of the Arctic Ocean, to the shelf and further to the Arctic Ocean, which potentially will affect the biogeochemistry of the coastal Arctic Ocean. However, the fate of terrestrial and permafrost-derived DOM in the coastal waters of the Arctic Ocean is ambiguous (Holmes et al., 2012; Le Fouest et al., 2013).

DOC and POC discharge and its changes

Changes in the drainage basin of the Arctic Ocean influence the continental flux of water, organic and inorganic constituents. The DOC concentration in the arctic rivers are generally high ($\sim 500\text{-}1000 \text{ }\mu\text{M C}$). The cumulative arctic rivers DOC and POC discharges are $\sim 18\text{-}26 \text{ Tg C year}^{-1}$ and $\sim 4\text{-}6 \text{ Tg C year}^{-1}$, respectively. Thus, the majority of OC is discharged by the rivers in form of DOM. In contrast to OC, the concentration of nutrients are among the lowest worldwide, with $0\text{-}20 \text{ }\mu\text{M DIN}$, $0\text{-}0.8 \text{ }\mu\text{M}$ of phosphorus, and $0.5\text{-}110 \text{ }\mu\text{M}$ silicate (Dittmar and Kattner, 2003). The majority of nitrogen and phosphorus is transported in form of organic compounds to the shelf of the Arctic Ocean.

Waters of the Arctic Ocean are stratified due to the large freshwater input from the arctic rivers. Riverine input carries relatively refractory soil-derived humic materials from soils. Contrary, marine autochthonous OM is labile and actively turned over in the ocean. The majority of the particulate organic matter is deposited in the estuaries and coastal regions. Consequently, the riverine DOM imprints significant terrestrial signatures in the Arctic Ocean. High concentrations of DOC and terrestrial DOM are characteristic for polar surface waters (Benner et al., 2004; Opsahl et al., 1999). In contrast to marine DOM, terrestrial DOM has elevated C/N ratios, depleted stable carbon isotope ratios, and higher contributions of aromatic components (Benner et al., 1992; Hedges, 1992). Studies on the reactivity of DOM demonstrates that DOC behaves relatively conservatively along the salinity gradient in the river-coastal sea transition (for Lena River - Laptev Sea, Ob and Yenisey rivers - Kara Sea, Fig. 13). Previous studies in the estuaries of the Ob, Yenisey and Lena rivers showed conservative DOC mixing along the salinity gradients (Cauwet and Sidorov, 1996; Dittmar and Kattner, 2003; Kattner et al., 1999; Köhler et al., 2003). Conservative mixing implies a quantitative transition of DOC from the river to the sea but compositional and chemical changes are usually not taken into account. However, it is well known that during the river-sea transition, DOM experiences phyto- and zooplankton mediated modification processes (Benner and Opsahl, 2001), microbial alteration (Santos et al., 2014), photochemical degradation and mineralization (Helms et al., 2013; Santos et al., 2014), abiotic sorption and desorption processes (McCallister et al., 2006), particle formation and deposition. The stratified surface waters of the Arctic Ocean share many characteristics of the estuary. The distributions of dissolved and particulate matter are spatially and temporally heterogeneous in polar surface waters (Amon and Benner, 2003; Guay et al., 1999; Wheeler et al., 1997). High concentrations of DOC and biomarkers of terrigenous DOM (e.g. lignin phenols) are common in polar surface waters (Benner et al., 2004; Opsahl et al., 1999). Residence times for surface waters in the Arctic are relatively short and variable, ranging from a few to ~20 years (Bauch et al., 1995; Schlosser et al., 1994).

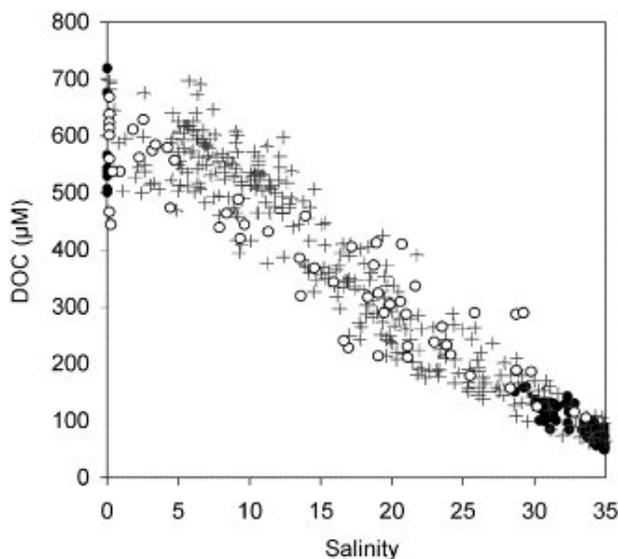


Fig. 13. DOC concentrations versus salinity in the eastern Arctic Ocean. Lena River/ Laptev Sea (Cauwet and Sidorov, 1996): white dots, (Kattner et al., 1999): black dots). Ob and Yenisey rivers/Kara Sea ((Köhler et al., 2003): crosses). Adapted from Dittmar and Kattner (2003) with permission of Elsevier.

The Lena River and coastal Laptev Sea

The Lena River is one of the Arctic largest. Estimates for the annual discharge of DOC range between 4.1-4.9 Tg C year⁻¹ (Dittmar and Kattner, 2003; Opsahl et al., 1999) and 5.6-5.8 Tg C year⁻¹ (Holmes et al., 2012; Raymond et al., 2007). The annual discharge of the Lena River POC was estimated to be 0.38 Tg C year⁻¹ (Semiletov et al., 2011) which is less than one tenth of the contribution of DOC emphasizing the importance of DOM to the TOC discharge. Late summer runoff of the Lena River is dominated by DOM input from organic-rich soils, ice complexes, and the mires of northern Asia. Hydrologic processes mobilize organic carbon stored in the permafrost soils and carry it downstream (Guo et al., 2007). The Lena River delta is the second largest in the world (after the Mississippi River delta) with a territory about 32,000 km² with developed system of channels and islands. Are and Reimnitz (2000) suggested that the unique structure, shape and relief of the Lena Delta setting it apart from other arctic deltas, are caused by tectonics. Formation of the modern Lena Delta began during middle Holocene inside of the archipelago created by the vertical tectonic block movements (Are and Reimnitz, 2000). Permafrost underlie about

78-93% of the Lena River watershed with continuous permafrost extending south to 50°N (Zhang et al., 1999). Therefore, the influence of the permafrost and permafrost degradation processes in the Lena River and in the Lena Delta are highly important.

The Lena River flows into the Laptev Sea, which is a part of the East-Siberian arctic shelf (ESAS, ~20% of the total area of the Arctic Ocean). A distinctive feature of the Arctic Ocean is its shallow low-salinity shelves, which are particularly extensive on the Eurasian side. The arctic shelf seas play a key role in a land-sea interaction processes and transformations of terrestrially exported OM. The fate of terrestrial DOC in the marine environments is still a matter of debate. Some have argued that riverine DOC in high-latitude areas is refractory and behaves conservatively in the estuaries and shelves of the Arctic Ocean based on DOC-salinity relationships, with little influence on biological cycles and the net ocean-atmosphere exchange of CO₂ (Amon and Meon, 2004; Dittmar and Kattner, 2003; Polyak, 2004). Microbial incubation experiments showed low or absent of decomposition of the estuarine DOM (Amon and Meon, 2004). High concentrations of lignin phenols and other recalcitrant organic compounds supports conservative behavior of river-derived DOM in the Arctic Ocean (Amon and Benner, 2003; Lobbes et al., 2000). Contrary, some researchers suggested the presence of a significant labile fraction in high-latitude DOM which is broken down via microbial respiration or photochemical oxidation (Cooper et al., 2005; Holmes et al., 2008; van Dongen et al., 2008). Observations of substantial CO₂ emission to the atmosphere above coastal regions in the sub-Arctic (Algesten et al., 2006) and in the ESAS (Anderson et al., 2009; Pipko et al., 2002; Semiletov et al., 2007) further support the view of substantial degradation of DOC (the main form of terrestrial OC delivered by rivers). Also, it was recently found that DOC in the arctic spring flood waters has a large labile fraction, as opposed to DOC in more commonly sampled late summer base flow (Alling et al., 2010; Holmes et al., 2008).

Thus, the characterization of DOM role, turnover, and mineralization is particularly important within the arctic region with its dramatic changes. Recent progress in molecular analytics allowed a deeper characterization of this complex mixture of organic compounds (Chapter 2.6). Similar to metabolites in

human blood, molecular components of DOM can be illustrative for past, ongoing and potential future biogeochemical processes.

4. Research questions and hypotheses

4.1. Major research questions

The incomplete understanding of the molecular diversity of DOM leads to two groups of questions: (i) Analytical questions that address the advanced chemical and physical characterization of DOM properties on a bulk and molecular levels; and (ii) biogeochemical questions which aim at an improved understanding of the biogeochemical and environmental role of DOM. For the Arctic, the understanding of changes in the cycling of DOM and its feedback to the changing climate is particularly crucial: transport and fate of terrigenous and permafrost-derived organic matter in the large Arctic rivers and in the Arctic Ocean; characterization of persistence and traceability of permafrost-derived organic matter in the Arctic Ocean; analysis of mobilization, emission and turnover of permafrost-derived organic matter;

Advanced chemical characterization of DOM

The separation and characterization of multiple components of DOM is the analytical challenge (Chapters 2.4 to 2.6). High sensitivity chemical analytics usually require high sample purity. From one point, the DOM isolation procedures and extraction protocols are driven by the research community. The popular DOM extraction methods become more and more popular due to growing number of publications where commonly accepted protocols have been used. However, continuous studies on DOM isolation selectivity's, optimization of methodologies and understanding of artifacts introduced by the isolation procedure are of great interest and importance. Certainly, current DOM isolation methods require increased DOM recoveries and an assessment of fractionations caused by the isolation procedures. Many studies were done and will be done using different protocols, therefore comparison of various DOM extraction methodologies have to be provided. New concepts are needed to obtain the most representative fraction of DOM.

An important issue in DOM chemistry is the expansion of the “analytical window” of detectable DOM components. None of the modern analytical techniques allow overall chemical characterization of DOM. Modern methods (e.g. UPLC, 2D NMR and FT-ICR MS) and their combination significantly expanded the “analytical window” and increased the resolution to a molecular level. However, they require expensive instrumentation, long data acquisition times, sophisticated methodologies and data processing/interpretation. Despite fundamental new analytical insights, the comprehensive determination of the molecular composition of DOM, its molecular weight, structure, and functionalities are still unresolved.

Understanding of biogeochemistry of DOM

The large-scale global studies on organic carbon in the ocean and on land in the last decades were mostly driven by the interest in the climate change and carbon cycle. The Arctic as a hot spot of climate change deserves special attention, particularly in the context of the susceptibility of the carbon cycle to the climatic changes in this region (Chapter 3.1). Mechanisms and rates of biogeochemical processing of permafrost-derived OM and its biogeochemical relevance have to be studied (Chapter 3.2). Special attention has to be focused on the large arctic rivers and the coastal Arctic Ocean. Therefore, characterization of mobilization pathways, mineralization mechanisms and turnover of permafrost-derived OM will deepen the knowledge of the impact of permafrost carbon to the carbon cycling in the changing Arctic. The question of the fate of terrigenous DOM in the Arctic Ocean is highly relevant and the river-sea transition is a hot spot of biogeochemical processes involving DOM. In general, the ultra-complex mixture of organic compounds in DOM is a proxy of ecosystem metabolism and represents an imprint of past and ongoing biogeochemical processes.

4.2. Hypotheses

As already discussed in the previous chapters there are multifaceted key questions in DOM research. This thesis addressed following hypotheses:

-
- Hypothesis 1: Isolation and purification procedures substantially affect the molecular composition of DOM.

A set of sorbents commonly used in aquatic sciences (XAD-8, PPL, C18 and DEAE-cellulose) was studied with respect to performance for the isolation of Arctic freshwater DOM. The extraction efficiency of different sorbents, usability in the field and compositional characteristics of extractable DOM were addressed (Chapter 5.1, manuscript 1).

- Hypotheses 2: The molecular composition of DOM changes systematically with the water sources in the Lena Delta. The existence of a systematic composition of DOM from different waters offers a great potential for future source characterization. We aimed at an identification of source-specific molecular indicators of DOM and their relative contribution to the Lena River, permafrost meltwater creek, lake and coastal DOM samples (Chapter 5.2, manuscript 2).

- Hypothesis 3: DOM is subject to substantial transformation along the Lena River - Laptev Sea transition.

The conservative mixing properties of DOC in the Lena River – coastal Laptev Sea transition were questioned. We aimed at an identification of the reactivity of DOM components in the Lena River - Laptev Sea transition and at a quantification of active/non-active DOM molecular formulas (Chapter 5.3, manuscript 3).

- Hypothesis 4: Microbial activity regulates concentration and molecular composition of DOM in permafrost outflows within the Lena Delta.

Our goal was to determine molecular alterations that are a result of microbial degradation. Further we related the results to the observed regional-scale patterns of DOM in the terrestrial-aquatic transition. We hypothesized that the dominant fate of terrigenous DOM released from permafrost is either complete oxidation to CO₂ or rapid partial transformation with further export to the coastal ocean in a relatively recalcitrant form. The importance of

DOM mobilized from permafrost to the Lena River was estimated in a set of hydrological studies on fluxes of OM from permafrost outcrops to adjacent riverine or coastal waters (Chapter 5.4, manuscript 4).

All these hypotheses were primarily tested using non-targeted high resolution and sensitive organic analytics such as ^1H NMR and FT-ICR MS. Routine statistical tools (e.g. correlation analysis, t-tests and simple regression models) and tools of multivariate statistics (e.g. cluster analysis, principal component analysis, variety of multidimensional scaling techniques and analysis of indicator species) were also applied. The observed molecular patterns were related to organic carbon fluxes in the permafrost-influenced systems.

5. Cumulative Manuscripts

Chapters 5.1 to 5.4 represent reprints of independent original research papers that have been published (2), submitted (1) or prepared for publication (1) in international peer-reviewed journals (Table 1). The content is unchanged and the style adapted to the general format. The references are included in the complete list at the end of the thesis. Supplementary information for each manuscript is available in the end of this thesis. Original reprints of the published manuscripts can be found as addendum to this thesis.

Table 1. Overview of publications presented in thesis.

Publication	Chapter
Irina V. Perminova, <u>Ivan V. Dubinenkov</u> , Alexey S. Kononikhin, Andrey I. Konstantinov, Alexander Ya. Zhrebker, Mantsa A. Andzhushev, Vasiliy A. Lebedev, Ekaterina Bulygina, Robert Max Holmes, Yury I. Kostyukevich, Igor A. Popov, and Eugene N. Nikolaev. <i>Molecular mapping of sorbent selectivities with respect to isolation of arctic dissolved organic matter as measured by Fourier transform mass spectrometry</i> , Environmental Science and Technology, 2014, 48: 7461-7468.	5.1
<u>Ivan V. Dubinenkov</u> , Ruth Flerus, Philippe Schmitt-Kopplin, Gerhard Kattner, Boris P. Koch. <i>Origin-specific molecular signatures of dissolved organic matter in the Lena Delta</i> , Biogeochemistry, 2015, 123: 1-14.	5.2
<u>Ivan V. Dubinenkov</u> , Ruth Flerus, Philippe Schmitt-Kopplin, Mourad Harir, Gerhard Kattner, Boris P. Koch. <i>Molecular reactivity of dissolved organic matter along the Lena River - Laptev Sea transition</i> , submitted to Marine Chemistry.	5.3
<u>Ivan V. Dubinenkov</u> , Anne Morgenstern, Ruth Flerus, Philippe Schmitt-Kopplin, Antonina Chetverova, Gerhard Kattner, Irina Fedorova, Olga Bobrova, Boris P. Koch. <i>Mobilization, mineralization and turnover of permafrost-derived dissolved organic matter</i> , prepared for publication to PLOS ONE.	5.4

Author's contributions

As result of the multidisciplinary character of the investigations provided in this thesis, several co-authors contributed to four manuscripts with their specific expertise. In manuscript 1, the author of this thesis sampled and extracted DOM from waters of the Kolyma River. He characterized samples by elemental analysis, ¹H NMR and FT-ICR MS, interpreted results and equally contributed to the

preparation of the manuscript with Irina V. Perminova. Manuscript 2 is based on existing samples and chemical analysis provided by Ruth Flerus. The author of the thesis provided the synthesis of results, their interpretation and prepared the manuscript. In manuscript 3, he used existing samples provided by Ruth Flerus and analyzed them using FT-ICR MS. He compiled the data, carried out data synthesis and interpretation and prepared the manuscript and integrated contributions by the coauthors. Finally, he suggested the idea of manuscript 4, took the samples in a field campaign and provided the synthesis of the results, their interpretation, and wrote the article with contributions by the coauthors.

5.1. Manuscript 1

“Molecular mapping of sorbent selectivities with respect to isolation of dissolved organic matter as measured by Fourier transform mass spectrometry”

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Abstract

The objectives of this study were to identify molecular features characteristic to arctic DOM from the Kolyma River basin and to elucidate structural imprints induced by a choice of the sorption technique. To achieve this goal, DOM was isolated from the Kolyma River basin with a use of three non-ionic sorbents: Amberlite XAD-8 resin, PPL- and C18 - SPE cartridges, and one anion exchanging resin – diethylaminoethyl (DEAE) -cellulose. The structural studies were conducted with a use of electrospray ionization Fourier Transform Ion Cyclotron Resonance (ESI FT-ICR) mass spectrometry and liquid state ^1H NMR spectroscopy. The DOM isolates obtained with a use of PPL and C18 cartridges were characterized with higher content of aliphatic compounds as compared to XAD-8 and DEAE-isolates. In total, for all arctic DOM isolates we observed predominance of hydrogen saturated compounds with high H/C values of identified formulas from FT-ICR MS data. ^1H NMR spectroscopy studies have confirmed this trend and revealed high contribution of alkyl-chain protons into the spectral density of the arctic DOM reaching 43% for PPL isolates.

Introduction

Identification of molecular features of dissolved organic matter (DOM) of permafrost origin discharged by the arctic rivers into the ocean is a subject of intense research due to growing contribution of dissolved organic carbon (DOC) released by permafrost thaw into the global carbon cycle (Mann et al., 2012). The major focus of those studies is a search of molecular markers which would be characteristic of the DOM originating from the arctic systems (Amon and Meon, 2004; Cooke et al., 2009). The main challenge here is molecular complexity of freshwater DOM represented by extremely heterogeneous ensemble of natural organic constituents which include lipids, terpenoids, lignin-like-structures, polyphenols, carbohydrates, and proteinaceous structures (Rachold et al., 1996). As a result, Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR MS) and ^1H NMR spectroscopy with their unprecedented resolution capacity became the methods of choice in investigating molecular features of DOM (Cook, 2004; Hertkorn et al., 2006; Hertkorn et al., 2012; Kim et al., 2003a; Koch et al., 2007;

Koch et al., 2005; Lam et al., 2007; Simpson et al., 1997; Simpson et al., 2011; Wu et al., 2004). However, application of these high resolution magnetic resonance techniques is very demanding with respect to the sample preparation procedures, in particular, to a choice of appropriate extraction protocol (Peuravuori et al., 2005; Santos et al., 2010; Tfaily et al., 2012).

Recently, solid phase extraction (SPE) on prepacked cartridges (e.g., C18 or PPL) has been proposed for DOM isolation from marine waters as an alternative to the long-standing practice of column extraction using Amberlite XAD resins (Dittmar et al., 2008). The major advantages of this new technique as compared to XAD-8 technique are much higher purity of the DOM isolates and higher extraction efficiency (Aiken et al., 1985; Dittmar et al., 2008; Ivanova et al., 1986). As a result, the SPE-isolates are much better suited to the purposes of ESI FT-ICR MS studies which attracted substantial interest of the DOM researchers to this extraction technique (Tfaily et al., 2012). However, comparative studies on molecular compositions and structures of the DOM isolates obtained with a use of XAD-resins and SPE-cartridges are very scarce. At the same time, they are of ultimate importance for reaching unbiased conclusions on structures and properties of DOM from the largely unknown sources such as runoffs of thawing permafrost discharged into the arctic rivers.

This motivated us to conduct a study on molecular variations within the same source of arctic DOM induced by a choice of the isolation technique. As a source of arctic DOM we used the Kolyma River and its tributary – the Panteleikha River, which are located in northeastern Siberia. The Kolyma River has Earth's largest watershed that is completely underlain by continuous permafrost (Strauss et al., 2012). The permafrost here contains very high amount of organic carbon ($14 \pm 8 \text{ kg m}^{-3}$) which gives rise to mud springs in the summer with concentration of organic carbon achieving 60 mg L^{-1} (Kawahigashi et al., 2004). For DOM isolation we have used three non-ionic polymeric sorbents: Amberlite XAD-8 resin, PPL- and C18- SPE cartridges which extract protonated (hydrophobicized) species of DOM after acidification, and one alternative sorbent – a weak anion exchanging resin (diethylaminoethyl (DEAE)-cellulose) which extracts negatively charged DOM compartments. To enable comparative study of

molecular features of the DOM isolates, we applied ESI FT-ICR mass spectrometry and ^1H NMR spectroscopy.

Materials and Methods

Site description and sampling

The DOM sampling took place during the July 2011 Polaris Project expedition to the Kolyma River basin (www.thepolarisproject.org). The study site was located near the North-East Science Station of the Russian Academy of Science (NESS), near Cherskiy, Sakha Republic, Russia (Fig. M1-1).

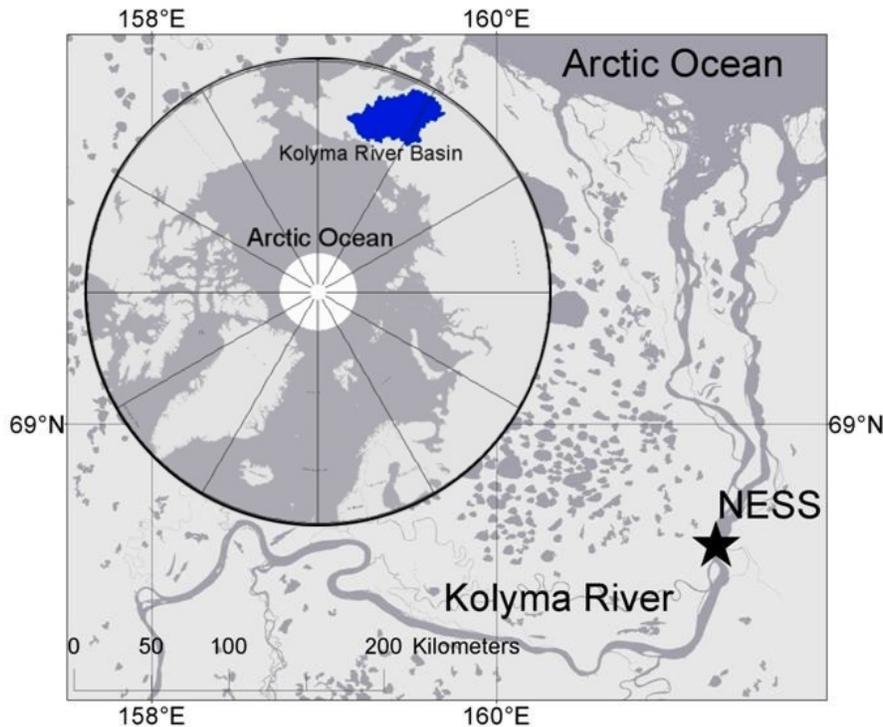


Fig. M1-1. Location of the North-East Science Station of Russian Academy of Sciences (NESS) near the Kolyma and Panteleikha rivers, Sakha Republic, Russia (designated with a black star).

Water was sampled two times from the main stem of the Kolyma River at 68.74858 N, 161.27721 E, and once from its tributary - the Panteleikha River - at 68.731769 N, 161.27721 E. The water sample size

used for DOM isolation was 40 L. The water samples were placed into 20 L LDPE containers and transported to the NESS laboratory.

Materials and chemicals

In-line filters (0.45 μm) (AquaPrep 600, Gelman Sciences) were used for water filtration. DEAE cellulose (Whatman, fiber), Amberlite XAD-8 resin (Rohm and Haas Ltd.), Varian Bond Elut PPL cartridges (Mega Pack, 6 g, 60 ml), and Varian Bond Elut C18 cartridges (Mega Pack, 6 g, 60 ml) were used for isolation of DOM. Amberlite 120R in H-form (Rohm and Haas Ltd.) was used for desalting alkaline extracts. Concentrated HCl was used for samples acidification, 0.1 M NaOH was used for DOM elution from resins. HPLC-grade methanol was used for DOM elution from cartridges. XAD-8 resin and DEAE cellulose sorbents were pre-cleaned according to manufacturers recommendations to eliminate sorbent bleeding which could otherwise contaminate the isolated DOM samples.

Water samples preparation

The sampled water was filtered through 0.45 μm in-line filters using peristaltic pump for removing particulates. It was further acidified with HCl until pH 2 in case of PPL, C18, and XAD-8, or used as it is, in case of DEAE-cellulose.

DOM extraction using PPL and C18 cartridges

DOM isolation with a use of BondElut cartridges was performed as described by Dittmar et al. (2008). Prior to use, the cartridges were activated by rinsing with one volume of methanol and washed with two volumes of acidified MilliQ water. The acidified water sample (40 L) was discharged through the cartridge with a flow rate of 40 mL min^{-1} . Then, the DOM-loaded cartridge was switched off the pump, dried, and stored in the dark at 2°C. All loaded cartridges were further transported to the lab facilities at the Lomonosov Moscow State University, Russia. The cartridge was dried with nitrogen and then eluted with 30 mL of methanol at a flow rate of 2 mL min^{-1} . The obtained extracts were rotor-

evaporated to dryness and stored at -20°C in the dark. The corresponding samples were assigned as PPL-RK for the Kolyma River sample and PPL-RP for the Panteleikha River sample (PPL-cartridge isolate) and C18-RK, C18-RP (C18-cartridge isolate).

XAD isolation technique

The standard protocol for DOM isolation from natural waters was used (Aiken et al., 1985). In brief, the filtered water sample (40 L) was acidified to pH 2 and discharged through the column (2x20 cm) packed with 60 g of XAD-8 resin. The loaded XAD-8 resin was stored in the dark at 2°C and transported to the lab facilities of the Lomonosov MSU. The resin was eluted with 0.1 M NaOH. The obtained alkaline extract was immediately passed through the cation-exchanging resin in H-form. The desalted samples were dried in the vacuum oven at 40°C, and stored in the darkness at -20°C. The samples were designated as XAD-RK (Kolyma River) and XAD-RP (Panteleikha River).

DEAE isolation technique

DOM extraction with DEAE-cellulose was performed according to the method outlined by Ivanova et al. (Ivanova et al., 1986). The pretreated DEAE-cellulose (60 g on dry weight basis) was packed into 2.5x40 cm column. The sample of filtered water (40 L) was discharged through the column at a flow rate of 10 mL·min⁻¹. Elution and desalting procedures were as described for XAD-8 resin. The isolation was conducted only for the Kolyma River water. The corresponding sample was designated as DEAE-RK.

Sorbent-blanks preparation for FT-ICR MS measurements

The sorbent blanks for FT-ICR MS measurements were prepared by reproducing DOM desorption protocols on the unloaded sorbents. Methanol was used for rinsing PPL and C18 cartridges, while 0.1 M NaOH was used in case of XAD-8 and DEAE-cellulose. The methanol-eluates from PPL and C18 cartridges were further evaporated to dryness. In case of the alkali eluates, they were first desalted using

cation-exchanging resin, and then evaporated to dryness. The obtained residues were added with 1 mL methanol prior to FT-ICR MS analysis.

Elemental analysis

All solid DOM samples were analyzed for CHN compositions using elemental analyzer (Vario EL, Germany). Ash content was determined manually. The content of oxygen was calculated as a difference after correction for the content of ash: $O\% = 100\% - C\% - H\% - N\%$.

Dissolved organic carbon measurement

DOC concentrations for filtered acidified (pH 2) water samples were determined as non-purgeable total organic carbon by high temperature combustion (680°C) on a Shimadzu TOC-VCPN analyzer equipped with a Pt catalyst (Shimadzu Scientific Instruments) which was located at the NESS station. Samples were purged for 5 min immediately prior to analysis with the same ultra-high purity oxygen that was used as a carrier gas in the TOC analyzer. Standards (potassium hydrogen phthalate) were analyzed immediately prior to the analysis of samples. Water for the standards was prepared by deionization and distillation. All samples were analysed in triplicate. Standard deviations (SD) for DOC concentrations within the range from 0 to 20 mg L⁻¹ did not exceed 2%. Initial DOC concentrations in water samples from the Kolyma and Panteleikha rivers were 6.5±0.1 and 9.7± 0.2 mg L⁻¹, respectively (± stands for SD (n=3)).

Extraction efficiency determination

Extraction efficiencies were determined on the basis of a decrease in the DOC content in the initial and processed water samples of 40 L. For this calculation, we determined DOC concentration in filtered water sample immediately before and after it was passed through the sorbent. An amount of intercepted DOC was calculated as a difference in DOC concentrations of initial and processed water samples

multiplied with a volume of the sample (40 L). Ratio of the intercepted amount of DOC to its initial content in the bulk water sample was used as an estimate of the extraction efficiency of the sorbent.

Electrospray ionization FT-ICR MS analysis

A commercial LTQ FT Ultra mass spectrometer with a 7 Tesla superconducting magnet equipped with Ion Max Electrospray Ion source (Thermo Electron Corp., Bremen, Germany) located at the Institute of Biochemical Physics of RAS was used for all mass spectral measurements. An electrospray ionization (ESI) source was used to generate negative charged molecular ions. Experimental conditions were as follows: needle voltage - 2.9 kV, no sheath and auxiliary gas flow, tube lens voltage - 160V, heated capillary temperature 200 °C.

The solid DOM samples were dissolved in methanol immediately before FT-ICR MS analysis, to minimize formation of methyl esters. The sample concentration was 0.5 g DOM L⁻¹ (or 0.2 g DOC L⁻¹). Full scan MS spectra (m/z 200-900) were acquired in the FT-ICR with resolution R=400k at m/z 400. The automatic gain control (AGC) target was set to 1×10⁶ corresponding to the number of ions accumulated in the linear ion trap and transferred to the ICR cell. The average FT-ICR mass spectrum was a sum of 500 consecutive scans. FT-ICR data were obtained as processed mass spectra with an associated peak list using Qual Browser version 2.0 (Thermo Electron Corporation, Bremen, Germany).

The formula assignments on the basis of the FT-ICR MS data were made using the lab-made “Transhumus” software designed by A. Grigoriev, which is based on previously described total mass difference statistics algorithm (Kunenkov et al., 2009). The isotopes ¹²C (maximum number of atoms for molecular formula computation: 50), ¹H (100), ¹⁶O (30), ¹⁴N (2), and ¹³C (1) were included in the calculations. Error threshold in formula assignments was set to ±0.5 ppm, intensity threshold was 1%. In this work we therefore focused on the most abundant ions by “intensity”-excluding peaks belonging to potential contaminations. For all ions the mass accuracy (measured as the root mean squared (rms) errors for the given mass) was below 2 ppm in the mass range from 250 to 650 m/z. The corresponding

histogram of rms versus m/z covering 250 – 650 Da with data every 100 Da is given in Fig. M1-S1 in the Supporting Information. We also provide an estimate for percentage of assigned molecular peaks within selected mass ranges (Fig. M1-S1). It did not vary substantially from low to high molecular weight peaks. That is why full range of ion masses was used for further data treatment. ^{13}C peaks were used for quality assurance and removed from the final dataset. The blanks were measured for each sorbent yielding the sorbent-specific peaks of individual compounds, which did not demonstrate broad distributions characteristic of DOM patterns. We removed these sorbent-specific peaks from the peak lists of the corresponding DOM isolates before the formula assignment.

^1H NMR spectroscopy

^1H NMR spectra were acquired using a Bruker Avance 400 NMR spectrometer operating at 400 MHz proton frequency. Sample preparation was conducted as described elsewhere (Hertkorn et al., 2002). In brief, a weight of 15 mg of solid DOM sample was placed into NMR tube, attached to vacuum line and dried for 6 hours at 45°C for complete water removal. The dry sample was then dissolved in 0.7 mL of anhydrous DMSO- d_6 (Merck, Germany 99,95 isotopic purity) under vacuum conditions. The spectra were acquired in a 5 mm tube using 90 excitation pulses ($90(^1\text{H}) = 9 \mu\text{s}$ relaxation delay, 150 scans). Fourier transformation, phase correction and integration were performed using ACD-labs software Version 10 (Advanced Chemistry Development, Canada). As a reference for proton assignments, a signal of residual protons of DMSO- d_6 located at 2.5 ppm was used. The integration of spectral density was conducted using the following assignments (ppm): 0.5-2.05 – protons of alkyl chains (CH_n – protons), 3.2-2.05 – protons of alkyl chains located in α -position to electro-negative functional group or aromatic ring (α - CH_n), 3.2-6 – “heteroaliphatic” protons attached to O or N substituted C atom or to the corresponding heteroatom ($\text{CH}_n\text{-O(N)H}$), 6-11.5 - aromatic protons ($\text{C}_{\text{Ar}}\text{-OH} + \text{C}_{\text{Ar}}\text{-H}$), 11.5-15.0 – protons of carboxyl groups COO-H .

Results and Discussion

Isolation of the arctic DOM using different sorbents

To achieve the goals of this study, we isolated DOM from the Kolyma River and its tributary – the Panteleikha River using three non-ionic adsorbents. In addition, an anion exchanging resin was used for isolation of DOM from the Kolyma River. The selected sorbents could be put into the following ascending order with regard to their polarity: C18 < PPL \approx XAD-8 \ll DEAE. The extraction efficiency of the sorbents studied and the elemental compositions of the corresponding DOM isolates are summarized in Table M1-1.

Table M1-1. Extraction efficiency of the sorbents studied and elemental compositions of the isolated DOM samples from the Kolyma River (RK) and Panteleikha River (RP).

DOM sample	Extraction efficiency* %	Content of element, % mass on ash-free basis				Atomic ratio	
		C	H	N	O**	H/C	C/N
C18-RK	67 \pm 5	52.2	5.50	1.03	41.27	1.3	51
PPL-RK	68 \pm 5	50.9	5.22	1.07	42.81	1.2	48
XAD-8-RK	50 \pm 3	49.0	4.49	0.95	45.56	1.1	56
DEAE-RK	82 \pm 7	41.1	4.21	1.09	53.60	1.2	37
C18-RP	65 \pm 5	52.1	5.54	1.40	40.96	1.3	43
PPL-RP	67 \pm 6	52.8	5.93	1.28	40.01	1.3	48
XAD8-RP	47 \pm 3	43.0	4.08	0.81	61.9	1.1	62

*Extraction efficiency was determined as a difference between concentrations of DOC at the inlet and outlet of the column and corrected to the sample volume treated. **Oxygen was calculated by mass balance as a difference ($O\% = 100\% - C\% - H\% - N\%$).

It can be seen that DEAE-cellulose with its extraction efficiency of 82 % had the highest affinity for various DOM compartments which corroborates well the previous findings (Peuravuori et al., 2005). The PPL and C18 cartridges had comparable extraction efficiency on the level of 67-68% which is in line with the reported data (Dittmar et al., 2008) . The lowest extraction efficiency had XAD-8 resin (50%) which is on the high end of extraction efficiencies observed for most unpolluted uncolored waters (30-50%) (Thurman, 1985). We have to stress here that the above values of extraction efficiencies were calculated as a difference in DOC concentrations of the acidified water sample before and after it was passed through the cartridge. Hence, they do not account for possible irreversible sorption of DOM

compartments on the cartridge, and the real DOM recoveries might be smaller. The C18 samples were characterized with the highest H/C values indicative of the maximum content of aliphatic saturated structures in this sample. This was to expect from the highly hydrophobic nature of the corresponding sorbent. The lowest H/C values were observed for XAD-8 samples, which might reflect preferred sorption of aromatic DOM constituents. Among the non-ionic sorbents, PPL and C18 -isolates were characterized with the highest content of nitrogen. The DEAE-isolate was characterized with the highest oxygen content indicating enrichment with oxygenated compounds. It can be concluded that a use of three non-ionic sorbents yielded the DOM samples with much more similar elemental compositions as compared to the DEAE-cellulose isolate.

FT-ICR mass spectrometry of the arctic DOM isolated with different sorbents

All DOM isolates were analyzed using FT-ICR mass spectrometry under identical conditions to enable appropriate comparison of the data obtained. The corresponding mass spectra are shown in Fig. M1-S2 in Supporting Information. They were characterized with maximum peak density located between 400 and 600 m/z similar to those reported for other aquatic DOM (Hertkorn et al., 2008; Koch et al., 2005; Sleighter and Hatcher, 2007). The obtained mass spectra were processed by assigning molecular formulas ($C_xH_yO_zN_n$) to individual peaks, which were plotted in the corresponding van Krevelen diagrams.

Fig. M1-2 shows van Krevelen diagrams with relative peak magnitude intensities for DOM samples isolated from the Kolyma River using the four sorbents studied. The most remarkable differences in peak magnitude distribution were characteristic of the sample isolated with DEAE-cellulose. It was characterized with the highest abundance of formulas intrinsic to most oxidized, tannin-like components. C18-RK, PPL-RK, XAD-8-RK samples showed similar intensity distributions with predominance of aliphatic components in the observed molecular formulas. XAD-8-RK sample was characterized with the highest abundance of unsaturated molecular components located in the bottom-left corner of van

Krevelen diagram. At the same time, C18 sample was dominated with hydrogen-saturated components as compared to other samples.

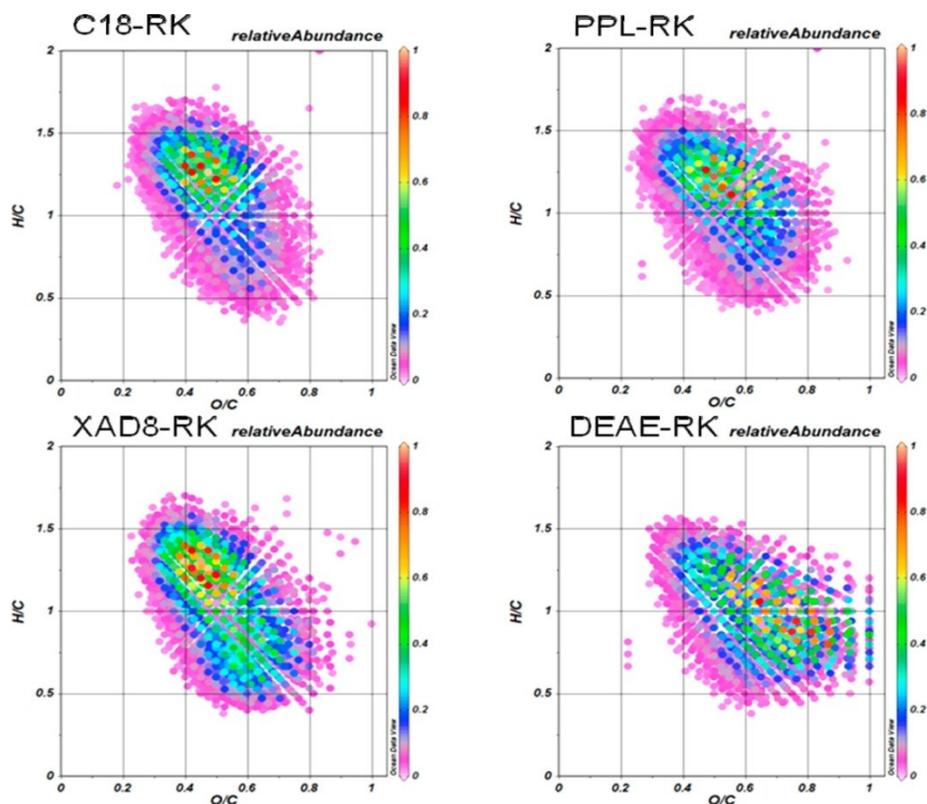


Fig. M1-2. Van Krevelen diagrams for the Kolyma River DOM obtained from the assigned formulas (CHON) in each sample: a) C18-RK, b) PPL-RK, c) XAD-8-RK, and d) DEAE-RK. Color codes correspond to relative peak abundance in FT-ICR Mass Spectra. Diagrams were plotted using Ocean Data View software.

It was of importance to compare the above trends observed for the samples isolated from the Kolyma River with those for the Panteleikha River. Their similarity for two independent sample sets would be indicative of reproducibility of the data obtained. The corresponding comparison was conducted for three non-ionic sorbents which were applied for isolation of DOM from the Panteleikha River. Fig. M1-3 shows the corresponding van Krevelen diagrams.

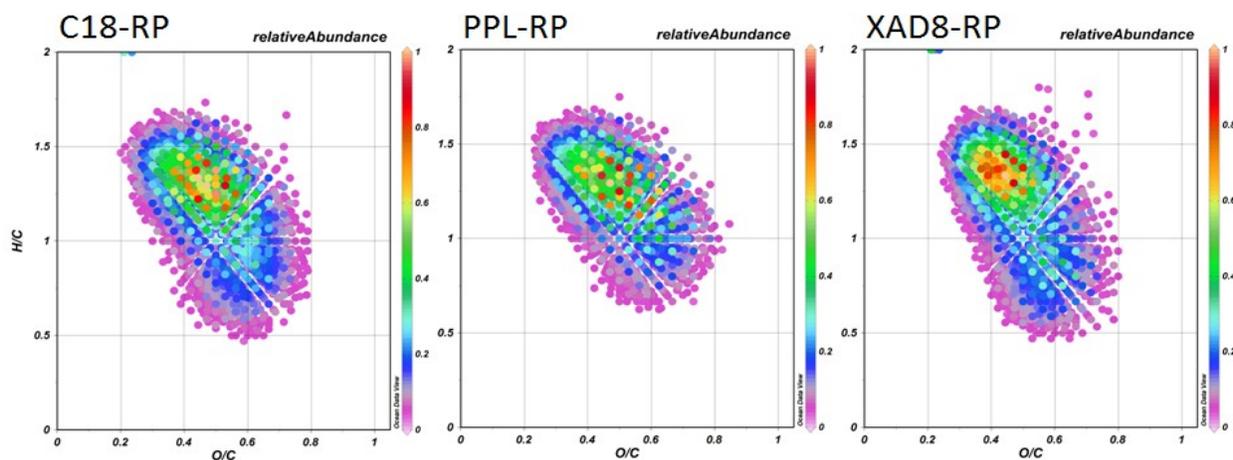


Fig. M1-3. Van Krevelen diagrams for the Panteleikha River DOM obtained from the assigned formulas (CHON) in each sample: a) C18-RP, b) PPL-RP, c) XAD-8-RP. Color codes correspond to relative peak abundance in FT-ICR Mass Spectra. Diagrams were plotted using Ocean Data View software.

Visual inspection of the VK diagrams for DOM from the Panteleikha River allows us to conclude about substantial similarity of these molecular trends to those observed for the Kolyma River. Again, we could see the largest contribution of saturated components for C18-RP sample, the highest abundance of unsaturated components was seen within XAD-8-RP sample, and the densest population within the nitrogenous components was observed for PPL-RP.

Upon examining van Krevelen diagrams for the seven DOM samples from the Kolyma River basin studied, a conclusion can be made on prevalingly aliphatic character of this DOM reflected in higher density of hydrogen-saturated compounds with $H/C > 1.0$ as compared with the corresponding data on DOM from temperate or tropical regions (Charkin et al., 2011; Tfaily et al., 2012). To illustrate an impact of sorption selectivities on molecular compositions of the DOM isolates, we show in Fig. M1-4 locations of unique identifications which were present only in the DOM isolates obtained with a use of the given sorbent, and absent in all other isolates. Unique identifications together with common identifications are also shown in Fig. M1-S3 and Fig. M1-S4 in the SI.

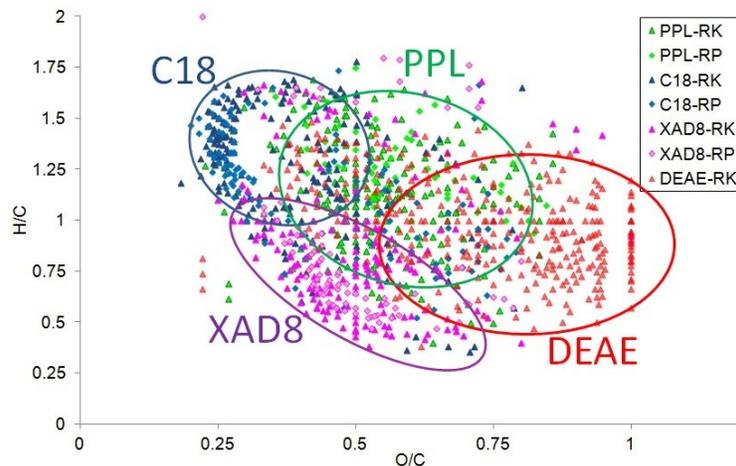


Fig. M1-4. Molecular mapping of sorbent selectivities with respect to DOM compartments isolated from the rivers Kolyma and Panteleikha for C18- and PPL-SPE cartridges, XAD-8 resin and DEAE-cellulose. Blue dots designate unique CHON-compositions in the C18-RK and C18-RP samples, green dots – in the PPL-RK and PPL-RP samples, purple dots – in the XAD-8-RK and XAD-8-RP samples, and red dots – in the DEAE-RK sample.

The patterns in van Krevelen diagrams followed rather consistently the trends expected from the sorption mechanisms specific to the sorbent used (Fig. M1-4). A use of the most non-polar C18 SPE cartridge lead to much more abundant pools of the saturated aliphatic compartments located in the range of lipids and terpenoids (here and below we refer to mapping scheme of the van Krevelen diagram conducted by Kujawinski and Behn and Hockaday et al. with respect to major molecular precursors of humic substances) (Hockaday et al., 2009; Kujawinski and Behn, 2006). At the same time, a use of more polar PPL-cartridge induced enhancement of the DOM isolates with protein-derived moieties (upper central part of the diagram) and carboxyl-rich alicyclic moieties (CRAM; Hertkorn et al., 2006) with H/C values ranging from 1.0 to 1.6 and O/C values from 0.25 to 0.6. XAD-8 isolates were characterized with much richer pool of more condensed aromatics, which is characteristic of flavonoids or higher molecular weight lignins with prevailing H/C values from 0.5 to 1.0 and O/C values from 0.3 to 0.6. DEAE-RK sample had the most specific character designated with rich pool of highly oxidized structures present in

polyhydroxyphenols as well as in oxyhydroxyaromatic acids, and carbohydrates, characteristic of hydrolyzable tannins. This is in line with anion exchanging properties of this sorbent.

The obtained results allow us to conclude that care should be exercised to avoid misinterpretation upon relating molecular features of DOM isolated with a use of different extraction technique to the particular source of its origin. For example, in case of SPE and XAD-8 it can be expected that a use of the former will lead to the more aliphatic-rich DOM isolate as compared to the latter regardless of the DOM source. To support this statement, the more structure-specific studies on the DOM isolates were conducted using ^1H NMR spectroscopy.

^1H NMR spectroscopy of the arctic DOM isolated with different sorbents

^1H NMR spectra acquired for the samples from the Kolyma River are shown in Fig. M1-5, and from the Panteleikha River - in Fig. M1-S5 in the SI. All spectra were characterized with well resolved and intense resonances in the range of aliphatic protons dominated by methylene protons of alkyl chains. Quantitative assessment of proton distributions is given in Table M1_2. It is also visualized for the samples from the Kolyma River as pie diagrams shown in Fig. M1-5.

For both arctic rivers studied, the DOM samples isolated with a use of non-ionic sorbents (XAD-8, PPL and C18) were rather similar in the proton distributions, while the DEAE-RK sample was very different from all others (Fig. M1-5). The major difference was a lesser amount of alkyl-chain protons and higher contribution of the carbohydrate- and aromatic protons. These features corroborate well the findings of FT-ICR MS on enrichment of this sample with highly oxidized polar compounds which could be assigned to hydrolyzable tannins containing both carbohydrates and gallic acids in their structures. At the same time, all other samples were characterized with very high content of aliphatic alkane protons varying from 35 to 43 %. These alkyl-chain fragments could belong to side chains of proteins as well as to linear terpenoid compounds (Hertkorn et al., 2006; Lam et al., 2007; Metje and Frenzel, 2007).

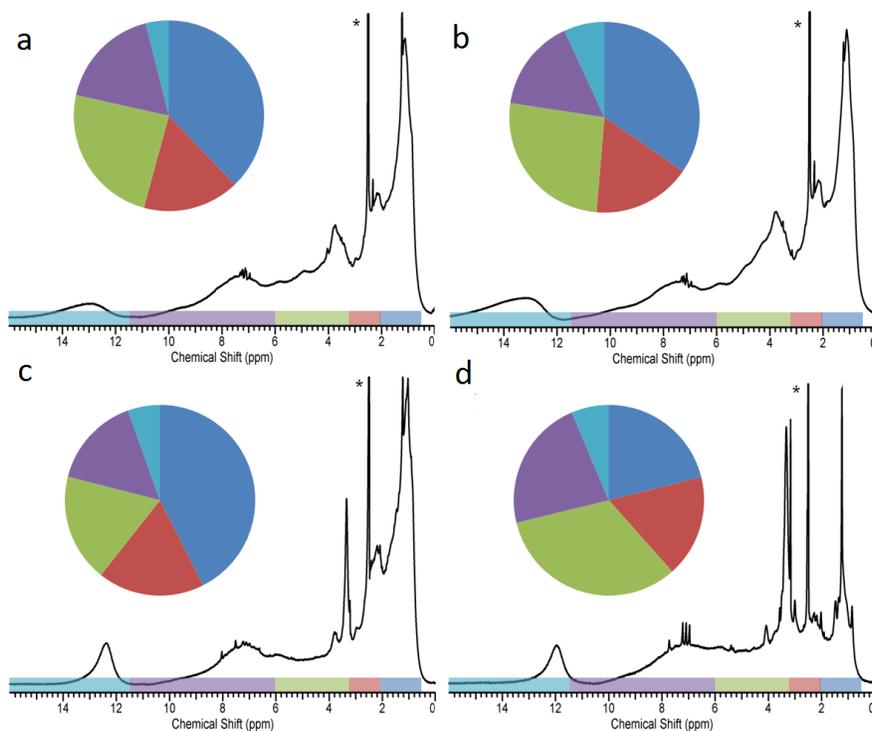


Fig. M1-5. ^1H NMR spectra of the Kolyma River DOM isolated by four different sorbents: a - C18, b - PPL, c - XAD -8, d - DEAE-cellulose. Starred peak designates residual protons of DMSO. The pie diagrams represent proton distributions in each sample where dark blue represents CH_n protons, red - $\alpha\text{-CH}_n$ protons, green - $\text{CH}_n\text{O(N)H}$ -protons, violet - $\text{C}_{Ar}\text{H(OH)}$ protons, light blue - COOH protons.

Table M1_2. Distribution of integral spectral density in ^1H NMR spectra acquired on DOM from the rivers Kolyma and Panteleikha isolated with a use of the four different sorbents.

DOM Sample	CH_n 0.5-2.05 ppm	$\alpha\text{-CH}_n$ 2.05-3.2 ppm	$\text{CH}_n\text{O(N)H}$ 3.2-6.0 ppm	$\text{C}_{Ar}\text{H(OH)}$ 6-11.5 ppm	COOH 11.5-16 ppm
C18-RK	38	16	24	18	4
PPL-RK	35	17	26	16	7
XAD8-RK	43	18	18	16	5
DEAE-RK	21	17	33	22	6
C18-RP	42	18	26	8	6
PPL-RP	41	20	24	8	6
XAD8-RP	37	24	28	7	5

A lack of higher content of aromatic protons which could be expected from multiple unsaturated species which are uniquely present in XAD-8-sample (Fig. M1-4) can be explained by “invisibility” of

condensed or highly substituted aromatic structures for ^1H NMR spectroscopy due to a lack of protons on those aromatic carbons.

In general, comparison of all ^1H NMR spectra acquired for DOM from the Kolyma River and the Panteleikha River allows us to conclude that the most profound structural feature intrinsic to all samples studied is extremely high content of alkyl-chain protons located in the region from 0.5 to 2.05 ppm (up to 43%). This might be related to the dominance of poorly degraded, hydrogen-rich structures in the pool of the arctic DOM in the Kolyma River basin DOM. This was to expect from the uniqueness of the main source of its origin – least degraded ancient organic carbon on Earth preserved in the Yedoma permafrost deposited in the Kolyma River lowland (Strauss et al., 2012). This corroborates well the recent findings on high bioavailability and molecular characterization of permafrost DOM (Frey and Smith, 2005; Mann et al., 2012; Neff et al., 2006).

Conclusions

Summarizing results of our investigations we would recommend a use of SPE-technique (in particular, PPL sorbent) for DOM extraction from natural waters. It yields rather representative pool of DOM components, and it is much easier to handle experimentally as compared to XAD-8 technique. Particular advantage of this technique for the arctic region is higher affinity of PPL cartridge to dominating aliphatic compartments as compared to XAD-8 resin. On the other side, if conclusions on specific structural features of the DOM formed under arctic conditions are sought, the SPE-isolates should be compared only to the corresponding SPE isolates: they cannot be directly compared to the pool of data obtained previously for the XAD-8 DOM isolates.

Acknowledgements

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Supporting Information Available

The results of quality assurance of formula assignments are obtained on the example of XAD-8-RK sample and given in Fig. M1-S1. ESI FT-ICR mass spectra are shown in Fig. M1-S2. 2D van Krevelen diagrams for the Kolyma and Panteleikha rivers with common and unique identifications are shown in Fig. M1-S3 and Fig. M1-S4. ¹H NMR spectra of the Panteleikha River DOM are shown in Fig. M1-S5.

5.2. *Manuscript 2*

“Origin-specific molecular signatures of dissolved organic matter in the Lena Delta”

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Abstract

Large arctic rivers discharge significant amounts of dissolved organic matter (DOM) into the Arctic Ocean. We sampled natural waters of the Lena River, the Buor-Khaya Bay (Laptev Sea), permafrost melt water creeks, ice complex melt water creeks and a lake. The goal of this study was to characterize the molecular DOM composition with respect to different water bodies within the Lena Delta. We aimed at an identification of source-specific DOM molecular markers and their relative contribution to DOM of different origin. The molecular characterization was performed for solid-phase extracted DOM by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). Average dissolved organic carbon (DOC) concentrations in the original samples were $490 \pm 75 \mu\text{mol C L}^{-1}$ for riverine and bay samples and $399 \pm 115 \mu\text{mol C L}^{-1}$ for permafrost melt water creeks. Average TDN concentrations were elevated in the permafrost melt waters ($19.7 \pm 7.1 \mu\text{mol N L}^{-1}$) in comparison to the river and the bay (both $13.2 \pm 2.6 \mu\text{mol N L}^{-1}$). FT-ICR MS and statistical tools demonstrated that the origin of DOM in the Lena Delta was systematically reflected in its molecular composition. Magnitude weighted parameters calculated from MS data (O/C_{wa} , H/C_{wa} , C/N_{wa}) highlighted preliminary sample discrimination. The highest H/C_{wa} of 1.315 was found for DOM in melt water creeks in comparison to 1.281 for river and 1.230 for the bay samples. In the bay samples we observed a higher fraction of oxygen-rich components which was reflected in an O/C_{wa} ratio of 0.445 in comparison to 0.425 and 0.427 in the river and creeks, respectively. From the southernmost location to the bay we observed a relative depletion of nitrogenous molecular markers and an enrichment of oxidized DOM components. The highest contribution of nitrogenous components was indicative for creeks and reflected in a C/N_{wa} of 104 in comparison to 143 and 176 in the river and bay, respectively. These observations were studied on a molecular formula level using principal component and indicator value analyses. The results showed systematic differences with respect to water origin and constitute an important basis for a better mechanistic understanding of DOM transformations in the changing arctic rivers.

Introduction

The large rivers discharge huge amounts of freshwater into the Arctic Ocean. Arctic rivers drain vast areas characterized by variable vegetation and soil conditions (Bhatt et al., 2010; Lantuit et al., 2009) which are important sources of dissolved organic carbon (DOC) in the Arctic Ocean. Dissolved organic matter (DOM) is involved in a wide range of biogeochemical processes in the arctic river watersheds (Yenisei, Ob, Lena, Indigirka, Kolyma, Mackenzie and Yukon) (Roehm et al., 2009; Wickland et al., 2012) and in the Arctic Ocean (Amon and Meon, 2004; Dittmar and Kattner, 2003). It supports microbial growth, affects primary production and therefore plays an important role in carbon and nutrient cycling (Le Fouest et al., 2013).

The water (McClelland et al., 2004; Peterson et al., 2002) and DOC discharge (Frey and McClelland, 2009; Semiletov et al., 2011) into the Arctic Ocean are expected to change in future due to increasing temperatures and subsequent permafrost thawing and degradation (Zhao-ping et al., 2010). The largest and most vulnerable source of organic matter is represented by the organic-rich permafrost (Engelhaupt, 2008). The estimated amount of organic carbon accumulated in permafrost is ~1700 Pg of carbon (Tarnocai et al., 2009). The degradation and mobilization of this carbon pool would have critical implications for microbial processes, primary production and carbon cycling in the Arctic and the Arctic Ocean basin interior (Frey and McClelland, 2009; Schuur et al., 2008). DOM represents the most mobile part in these soils and is therefore of particular importance when studying organic carbon fluxes in changing permafrost environments. DOM is a highly complex mixture of organic compounds with different chemical properties (Thurman, 1985). In the context of global element cycles it remains an unresolved issue which fractions of DOM are bioavailable or recalcitrant (Frimmel et al., 2002; Jiao et al., 2011) and it is one of the most important challenges in the field of DOM research to develop a better mechanistic understanding of the processes, which determine the wide range of residence times (Flerus et al., 2012; Lechtenfeld et al., 2014).

The Lena River is one of the largest rivers in the Arctic, and permafrost underlies 78-93% of the

watershed with continuous permafrost extending south to 50°N (Zhang et al., 1999). Estimates for the annual discharge of DOC range between 4.1-4.9 Tg C year⁻¹ (Dittmar and Kattner, 2003; Opsahl et al., 1999) and 5.6-5.8 Tg C year⁻¹ (Holmes et al., 2012; Raymond et al., 2007). The annual discharge of the Lena River particulate organic carbon (POC) was estimated to be 0.38 Tg C year⁻¹ (Semiletov et al., 2011) which is less than one tenth of the contribution of DOC emphasizing the importance of DOM to the total organic carbon discharge. Late summer runoff of the Lena River is dominated by DOM input from organic-rich soils, ice complexes, and the mires of northern Asia. Hydrologic processes mobilize organic carbon stored in the permafrost soils and carry it downstream (Guo et al., 2007).

The majority of existing studies, which addressed carbon fluxes, sources and transformation of organic matter in the Arctic, used bulk DOC, isotopic signatures and targeted biomarker approaches. Several studies used isotopic and biomarker approaches to investigate DOM and particulate organic matter fluxes in the Buor-Khaya Bay of the Laptev Sea (Karlsson et al., 2011) and other arctic regions (Cooke et al., 2009; Vonk et al., 2008; Yunker et al., 1995). Carbon isotope and lipid biomarker studies in the Buor-Khaya Bay demonstrated that a large terrestrial input from the Lena River leaves a significant imprint on the surface water POC composition (Karlsson et al., 2011). The young fluvial/alluvial POC pool is relatively buoyant, organic carbon rich and bioavailable, which is consistent with clear spatial trends of rapidly degrading POC throughout the Buor-Khaya Bay surface water (Karlsson et al., 2011). Most of the annual discharge of lignin (an unambiguous terrestrial tracer) occurs during the two months of spring freshet with extremely high lignin concentrations and a lignin phenol composition indicative of fresh vegetation from boreal forests. At low flow conditions DOM was dominated by older, soil, peat and wetland-derived DOC (Doxaran et al., 2012). Despite several studies on DOM molecular markers in the Lena River (Doxaran et al., 2012; Kraberg et al., 2013; Lara et al., 1998), there is still a gap in our understanding of regional biogeochemical processes involving DOM from different sources (i.e. riverine, soil and permafrost).

In the permafrost influenced Kolyma River the composition and age of DOM was analyzed during

different seasons using radiocarbon analysis (Neff et al., 2006). The bulk DOM in winter and spring is modern with a high concentration of lignin. At the end of summer and in autumn, the age of DOM becomes significantly older indicating that the DOM generation changed from surface to deep soils or other terrestrial sources of old and more refractory DOM. The Lena River and Kolyma River may have similar mechanisms and trends of organic matter release. However, integral DOM signals such as isotopic composition or radiocarbon age and more specific signals from other molecular markers have to be combined with new analytical tools and markers on a molecular level for a better understanding of the Lena River Delta ecosystem processes. Identification of new groups of biomarkers will also broaden our current knowledge of changing processes in the arctic rivers caused by climate change.

There is still a gap of knowledge of the quality of DOM in arctic rivers which is an important factor to assess its bioavailability and degradability. Untargeted analytics such as Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) allows to cover the complexity of the molecular pattern of DOM and to identify molecular markers and their transformation pathways in the pool of DOM molecules (Stubbins et al., 2010). Molecular markers, which demonstrate not only the most dramatic but also individual changes, may also represent major biogeochemical processes. FT-ICR MS already demonstrated its applicability in a number of studies on DOM molecular compositions (Hertkorn et al., 2008; Nebbioso and Piccolo, 2013; Schmidt et al., 2011). These studies take into account thousands of molecular components of DOM. Further data analysis allows understanding what presence/absence (or changes in relative biomarker contribution) of different markers reflect on the biogeochemical level. Previous studies demonstrated that the application of statistical tools simplifies data interpretation (Koch et al., 2008; Kujawinski et al., 2009; Sleighter et al., 2010). Recent FT-ICR MS studies on arctic DOM observed latitude and pH driven trends in the molecular composition of DOM in the Yenisey River (Roth et al., 2013). The authors showed that the climatic gradients along the latitude (from south to north) were related to a higher abundance of low molecular weight components, nitrogen-containing components and an increase in the degree of unsaturation of riverine DOM. For boreal lakes of Sweden it was

demonstrated that parameters such as precipitation, water residence time and mean annual temperature are reflected in the DOM composition and its chemodiversity (Kellerman et al., 2014). In arctic fjords, FT-ICR MS indicated the rapid turnover of DOM (Osterholz et al., 2014).

The aims of this research were to quantify DOC and TDN concentrations in different waters of the Lena Delta and to identify source-specific molecular markers of DOM and their relative contribution to the Lena River, melt water creeks and the Buor-Khaya Bay DOM samples for an advanced understanding of DOM compositional changes in the permafrost dominated riverine region. The broad molecular view derived by FT-ICR MS is expected to expand our current knowledge about the processes involving DOM in the Lena Delta.

Materials and Methods

The field sampling campaign took place in August 2009 in the Lena Delta. The highest mean air temperatures in this region occur in July (10.1°C) and August (8.5°C) (Boike et al., 2012). Several DOM sources were sampled including Lena River channels, permafrost melt water creeks (including ice complex creeks), a permafrost lake and the Buor-Khaya Bay, Laptev Sea (Fig. M2-1). The majority of melt water creeks and lakes were sampled on the Samoylov Island. Creeks and lakes were connected and represented different parts of the Samoylov Island system. Ice complex melt water creeks derived from the ice complex on the Bykovsky Peninsula. All samples except the bay were fresh waters. In the bay the range of salinity varied from 0 to 2.

Water was sampled from different depths using a Niskin water sampler or pre-cleaned glass bottles for creek sampling. Samples were filtered through 0.7 µm GF/F filters (Whatman, pre-combusted, 4 h, 450°C). DOC and total dissolved nitrogen (TDN) concentrations were measured using high temperature catalytic oxidation (TOC-VCPN, Shimadzu). Water samples were acidified in the auto sampler to remove inorganic carbon and analyzed directly.

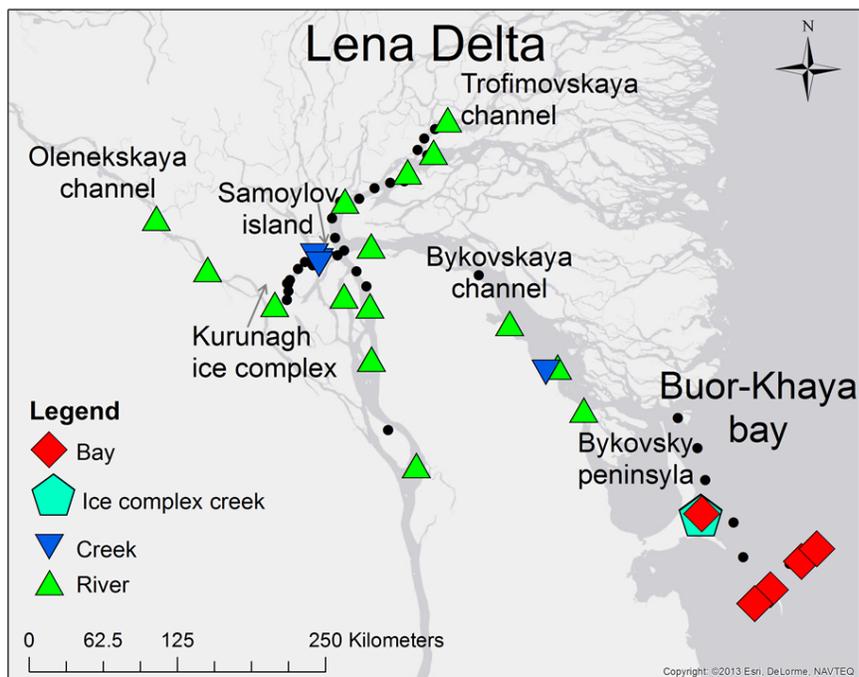


Fig. M2-1. Lena Delta ($73^{\circ}0'N$, $127^{\circ}0'E$) and sampling stations of the 2009 campaign. Riverine samples (triangles), bay samples (diamonds), permafrost creek samples (upside down triangles), ice complex melt water creeks (pentagons) and water samples used only for DOC and TDN analyses (dots).

DOM samples were concentrated directly in the field using pre-packed solid phase extraction (SPE) cartridges (PPL, Varian). SPE was applied for enrichment and purification (i.e. removal of inorganic constituents) using PPL resin (Varian). After extraction, the cartridges were dried with nitrogen and stored at $-20^{\circ}C$. Back in the lab, the cartridges were eluted with methanol (Lichrossolv, Merck). The DOC content of SPE-DOM was determined by evaporation of $50\ \mu l$ methanol extract and re-dissolved in ultra-pure water. Details of the method are described in (Dittmar et al., 2008). The extraction efficiency varied from 30% to 60%.

Fourier transform ion cyclotron resonance mass spectrometry

Ultra-high resolution mass spectra for 31 Lena Delta DOM samples were acquired in one batch on a Bruker Apex 12 Qe FT-ICR MS equipped with a 12T superconducting magnet and an APOLLO II

electrospray source. SPE-DOM methanol extracts were adjusted to $\sim 200 \mu\text{mol C L}^{-1}$ DOC concentrations by dilution with methanol/water (1:1). Samples were analyzed with electrospray ionization (ESI) in negative ionization mode. The average FT-ICR mass spectrum was composed of 512 consecutive scans. The spectra were recalibrated internally by characteristic DOM ions ($[\text{C}_{16}\text{H}_{19}\text{O}_8]^-$ - 339.10854 m/z; $[\text{C}_{17}\text{H}_{21}\text{O}_9]^-$ - 369.11911 m/z; $[\text{C}_{19}\text{H}_{23}\text{O}_{10}]^-$ - 411.12967 m/z; $[\text{C}_{21}\text{H}_{25}\text{O}_{12}]^-$ - 469.13515 m/z; $[\text{C}_{24}\text{H}_{29}\text{O}_{14}]^-$ - 541.15628 m/z; $[\text{C}_{28}\text{H}_{35}\text{O}_{14}]^-$ - 595.20323 m/z; $[\text{C}_{28}\text{H}_{35}\text{O}_{15}]^-$ - 611.19814 m/z; $[\text{C}_{31}\text{H}_{39}\text{O}_{15}]^-$ - 651.22944 m/z; $[\text{C}_{33}\text{H}_{41}\text{O}_{16}]^-$ - 693.24001 m/z). All ions were singly charged.

FT-ICR MS data was evaluated in the mass range of 200-500 m/z. Molecular formulas were assigned for peaks with a signal to noise ratio of more than 4 and a mass accuracy window of $\pm 0.5\text{ppm}$ (Koch et al., 2007; Koch et al., 2005) and included following isotopes: ^{12}C (0- ∞), ^{13}C (0-1), ^1H (0- ∞), ^{16}O (0- ∞), ^{14}N (0-2), ^{32}S (0-1). The nitrogen rule was applied and thresholds for elemental ratios were: $\text{O}/\text{C} \leq 1.2$, $\text{H}/\text{C} \leq 2\text{C} + 2 + \text{N}$ (Koch et al., 2005). ^{13}C isotopes were used for quality control (verification of the parent ion) but removed from the final dataset. FT-ICR MS provided exact masses of singly charged ions which allowed calculations of corresponding molecular formulas. It is important to note, that each molecular formula can represent a number of isomers (Hertkorn et al., 2008). In this article, for the ease of readability, we generally refer to molecular formulas as "compounds", "components", "markers" or "constituents". We assigned $\sim 55\%$ of peaks within the chosen thresholds. And number of assigned formulas (excluding ^{13}C isotopes) varied from 2737 to 3601.

FT-ICR MS data exploration and analysis

The peak magnitudes were normalized to the sum of intensities of all identified molecular formulas of the molecular mass region. Peak magnitude weighted-average elemental ratios $\text{O}/\text{C}_{\text{wa}}$, $\text{H}/\text{C}_{\text{wa}}$, $\text{C}/\text{N}_{\text{wa}}$, double bond equivalents (DBE) and mass defects were calculated from the assigned formulas. Magnitude weighted parameters X_{wa} were calculated according to $X_{\text{wa}} = \sum_{i=1}^n X_i * M_i / \sum_{i=1}^n M_i$ where X is e.g. O/C, H/C and C/N, i is the individual molecular formula and M is the relative mass peak intensity (Sleighter et

al., 2010). We prepared a data matrix for samples with molecular formulas as parameters where the peak magnitude corresponded to a parameter value. This data matrix was used for hierarchical clustering, principal component analysis (PCA) and indicator value analysis. For the statistical data processing, we only considered formulas, which were observed at least in 5 samples. By this, we avoided differences between samples, which resulted from discrete signal to noise threshold and FT-ICR MS signal fluctuation and tried to avoid false formula assignments. Molecular components revealed by FT-ICR MS were visualized using van Krevelen diagrams as ratios of elements: O/C and H/C (van Krevelen, 1950).

Cluster analysis was based on Bray-Curtis similarity (Bray and Curtis, 1957) and performed using commercial software (PRIMER v.6; PRIMER-E Ltd, UK). For principal component analysis (PCA, “Chemometrics” package, R) we used a similar approach as described in (Sleighter et al., 2010).

Indicator value analysis was performed according to a previous approach (Dufrene and Legendre, 1997). We used molecular peaks (assigned formulas) as species and the relative intensity of peaks as species abundance. We renormalized peak intensities in each sample to a lowest intensity and rounded obtained values to integer values. By this, we mimic species counts and minimum “specie” value in each sample was 1 compare (Kellerman et al., 2014). For the indicator value analysis we used R (“labdsv” package).

Results

Dissolved organic carbon and total dissolved nitrogen

The average measured DOC concentrations were $492 \pm 79 \mu\text{mol C L}^{-1}$ for river water, $484 \pm 55 \mu\text{mol C L}^{-1}$ for bay samples and $399 \pm 115 \mu\text{mol C L}^{-1}$ permafrost melt water creeks. Ice complex influenced samples from the Olenekskaya channel west of the Kurunagh showed higher DOC concentrations ($657 \pm 8 \mu\text{mol C L}^{-1}$) compared to the riverine samples. It is, however, difficult to test the statistical significance because of the small number of Olenekskaya channel samples ($n=4$) compared to riverine samples ($n=51$). The average TDN concentrations were $13.1 \pm 2.8 \mu\text{mol N L}^{-1}$ for river, $13.7 \pm 1.7 \mu\text{mol N L}^{-1}$ for bay

samples, and $19.7 \pm 7.1 \mu\text{mol N L}^{-1}$ for permafrost melt waters. DOC and TDN in ice complex melt water creeks were highly variable but also showed the highest concentrations of the whole Lena Delta system reaching average values of $3910 \pm 1780 \mu\text{mol C L}^{-1}$ and $220 \pm 138 \mu\text{mol N L}^{-1}$, respectively. DOC/DON molar elemental ratios of the SPE-extracts, which do not contain inorganic nitrogen, were 63 ± 17 for riverine samples, 56 ± 14 for bay samples, 35 ± 11 for permafrost melt waters and 33 ± 4 for ice complex samples (complete DOC and TDN data available online: <http://dx.doi.org/10.1594/PANGAEA.831765>; average DOC and TDN values available in supplementary information (SI) in Table M2-S1 and Fig. M2-S1).

Molecular characterization of Lena Delta DOM

FT-ICR MS characterization resulted in typical monomodal peak magnitude distributions for all DOM spectra (Fig. M2-2).

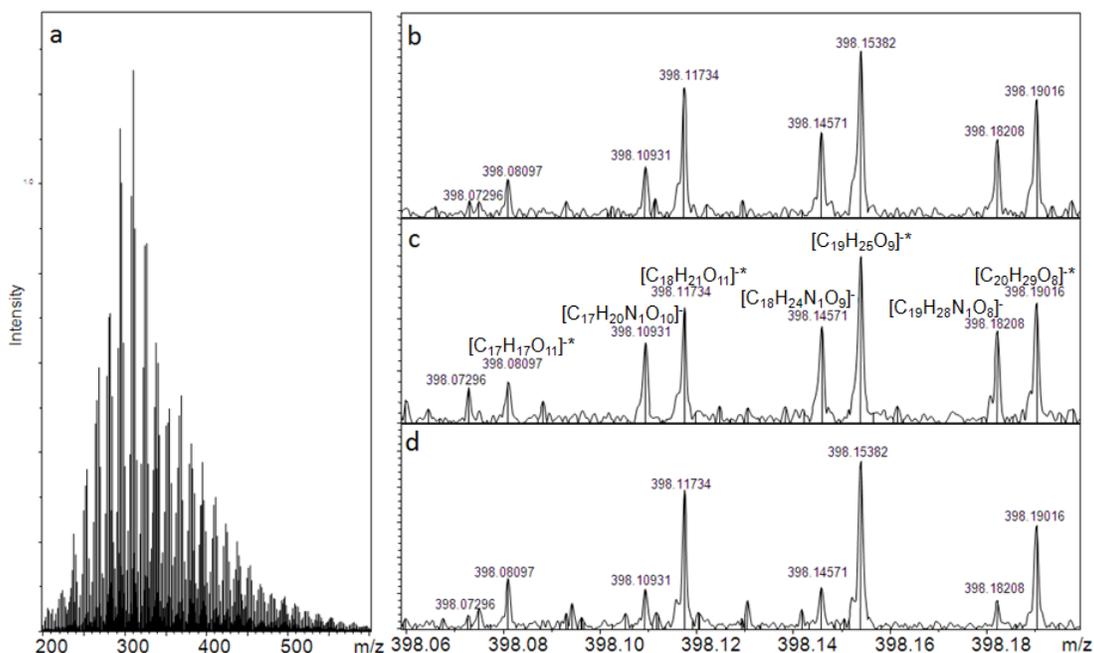


Fig. M2-2. Characteristic ESI negative FT ICR mass spectrum for Lena Delta DOM with (a) a mass range of 200-600 m/z, (b) mass spectrum at nominal mass 398 for a typical Lena river sample, (c) melt water creek sample, and (d) Buor-Khaya Bay sample. “*” indicates the presence of one ¹³C isotope in the assigned formula.

The typical peak distribution spanned a mass range from 200 (lower limit of the mass detector) to 600 m/z (Fig. M2-2). We identified, on average, 3100 formulas (excluding ^{13}C isotopologues) in each sample (detailed information in Table M2-SI2). The molecular patterns on single nominal masses revealed pronounced molecular differences between samples from the Buor-Khaya Bay, melt water creeks and the Lena River (Fig. M2-2b-d). Particular differences were found in the relative peak magnitude of nitrogen containing mass peaks (e.g. $[\text{C}_{17}\text{H}_{20}\text{N}_1\text{O}_{10}]^-$, $[\text{C}_{18}\text{H}_{24}\text{N}_1\text{O}_9]^-$, $[\text{C}_{19}\text{H}_{28}\text{N}_1\text{O}_8]^-$). The highest relative magnitudes of nitrogenous peaks were characteristic for the permafrost melt water creek samples, the lowest for the Buor-Khaya Bay samples.

Molecular differences between samples were reflected in their peak magnitude weighted average elemental ratios. Compared to the weighted average H/C and O/C ratios for riverine samples (1.281 ± 0.013 and 0.425 ± 0.006 , respectively), Buor-Khaya Bay samples showed lower weighted average H/C ratios ranging from 1.217 to 1.241 (mean 1.230 ± 0.009), and higher O/C ratios of 0.445 ± 0.002 . For the permafrost melt water creek/lake DOM, the O/C and H/C ratios were 0.427 ± 0.007 and 1.315 ± 0.013 , respectively. DOM in ice complex melt water creeks was more oxidized than in the other creek samples. Weighted average O/C and H/C ratios in the ice complex DOM samples were 0.445 ± 0.007 and 1.268 ± 0.024 , respectively. Highest magnitude weighted average C/N ratios were observed for the bay samples (C/N: 176 ± 23) followed by the riverine (C/N: 143 ± 17) and creek/lake samples (C/N: 104 ± 15). Detailed information on weighted average elemental ratios available in Table M2-SI3 and Fig. M2-SI2.

Molecular fingerprints

Hierarchical cluster analysis yielded four major clusters which clearly corresponded to the sample origin (Fig. M2-3). The Lena River, Buor-Khaya Bay, permafrost creeks (including the lake sample) and ice complex creeks. Samples derived from the Bykovsky Peninsula melt water creeks (ice complex samples, 76 and 77) were most dissimilar from all other samples and formed a separate cluster. Samples

from the same station in the river but from different depths (samples 14/15 and 12/13) showed a highly similar molecular composition (Bray-Curtis similarity > 95).

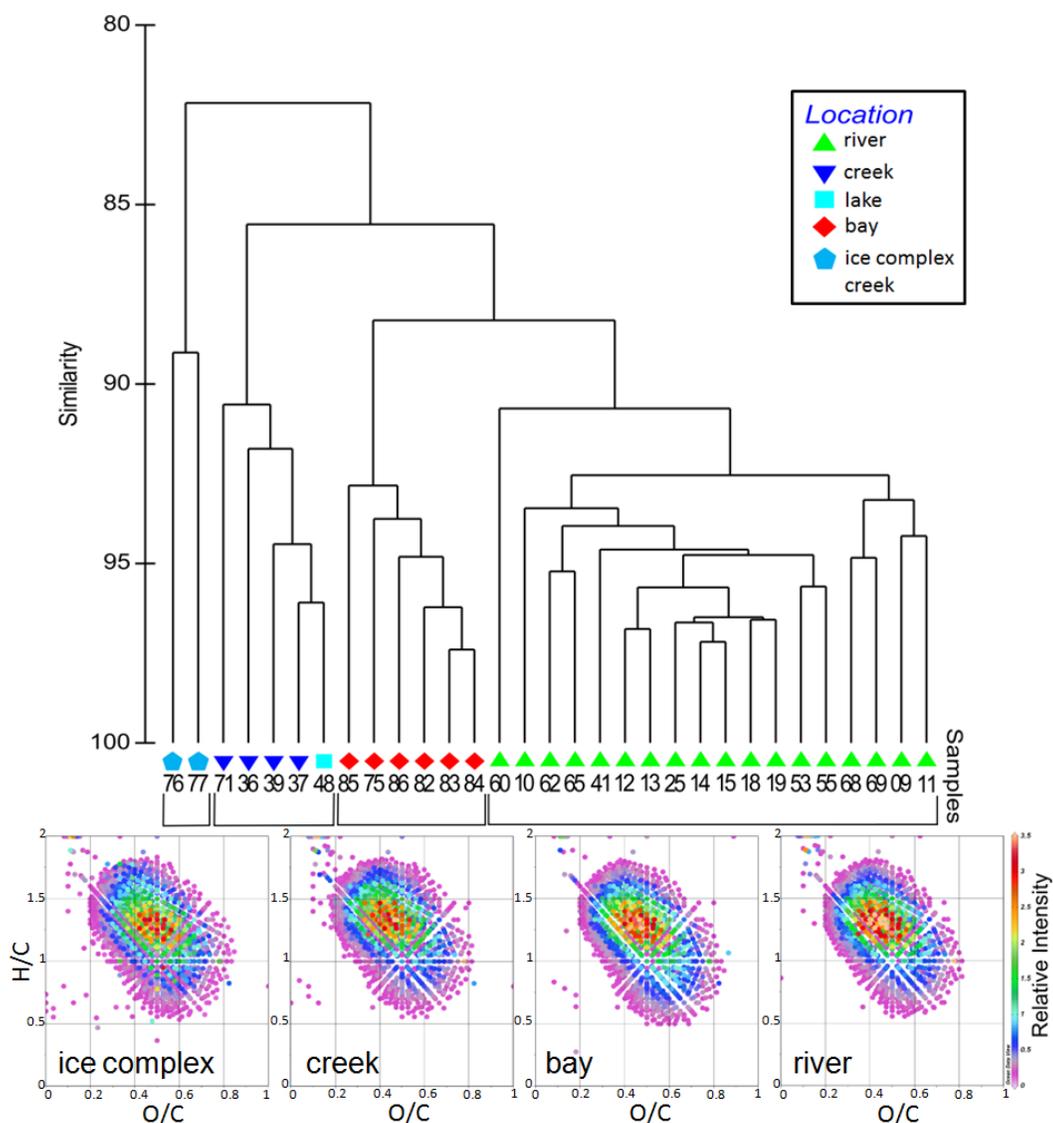


Fig. M2-3. Hierarchical cluster analysis (Bray-Curtis Similarity) and van Krevelen diagrams for “average samples”. Molecular differences were observed between (a) ice complex melt water creeks (pentagons, $n=2$), creek (triangles, $n=4$) plus lake samples (square, $n=1$), Buor-Khaya Bay samples (diamonds, $n=6$) and Lena River samples (green triangles, $n=18$). For each sample type, all identified molecular formulas containing C, H, O and N are represented by their molecular H/C and O/C ratio (van Krevelen diagram). The color code represents the average relative peak magnitude.

Although samples showed a high degree of similarity (van Krevelen diagrams, Fig. M2-3), it was still possible to differentiate sources based on the molecular formula information. To elucidate the molecular differences we calculated the average relative peak magnitude for each sample type identified by the cluster analysis. The average results for each formula containing C, H, O and N were presented as their elemental H/C and O/C ratios in the van Krevelen diagrams (Fig. M2-3). For the determination of those formulas which were responsible for the sample variability in the cluster analysis, we applied principal component analysis (PCA) for the same data matrix (Fig. M2-4).

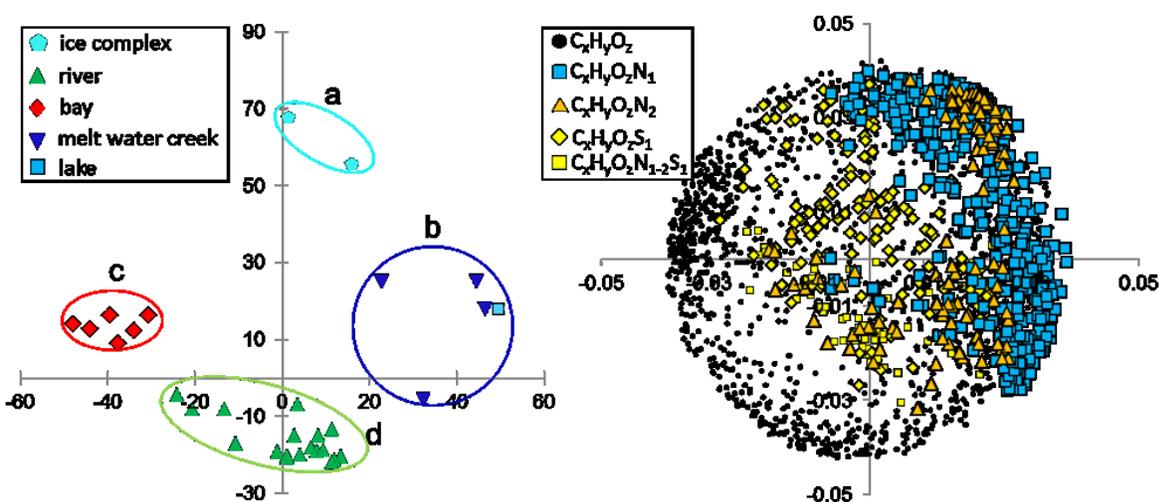


Fig. M2-4. Principal component analysis. PC1 and PC2 explained 56.7% of the variance in the biplot (left panel): (a) ice complex creek DOM samples (pentagons), (b) permafrost creeks and lake DOM samples (upside down triangles and square), (c) Buor-Khaya Bay samples (diamonds), (d) Lena River DOM samples (triangles). The PCA loadings highlighted the molecular formulas with respect to nitrogen and sulfur content (right panel): no nitrogen and sulfur (circles), one nitrogen atom (squares), two nitrogen atoms (triangles), one sulfur (light diamonds), sulfur and nitrogen (light squares).

The PCA highlighted the molecular differences of the various sample types. In total, 56.7% of variance could be explained by the first two principal components (PC, Fig. M2-4). PC1 and PC2 explained 31.5% and 25.2% of the variance, respectively. Since PC3 explained only 9%, only the first

two principal components were used for the data interpretation. Bour-Khaya Bay and permafrost melt water creeks were primarily separated on the PC1 axis whereas riverine samples showed exclusively negative PC2 values. The only exception which also showed negative PC2 values was the creek sample (sample 36) which was collected near the research station on Samoylov Island.

To identify specific molecular DOM markers for each source of DOM, the molecular formulas with the highest absolute PCA loadings for PC1 and PC2 were analyzed (Fig. M2-4). The combined loadings were irregularly distributed; some regions had a higher and others a lower density of data points. The majority of $C_xH_yO_z$ identifications showed negative loadings of PC1 whereas nitrogenous compounds ($C_xH_yO_zN_1$ and $C_xH_yO_zN_2$) had positive PC1 loadings. Therefore, $C_xH_yO_zN_{1-2}$ had a higher contribution to sample variance as compared to $C_xH_yO_z$. Sulfur containing compounds did not contribute considerably to the sample variability. Additionally, negative PC1 loadings were characteristic for identifications with the highest molecular masses. For the identification and generalization of molecular drivers responsible for the sample groupings and to understand the nature of molecular differences and transformations of DOM the 500 highest positive/negative eigenvalues for each principal component were used and plotted in van Krevelen diagrams (Fig. M2-5).

The Buor-Khaya Bay samples (negative PC1 values, Fig. M2-4) showed a predominance of oxygen-rich components and nitrogenous compounds were almost absent in formulas which showed negative PC1 loadings (Fig. M2-5b). Formulas with high positive PC1 loadings contained many N-containing formulas (Fig. M2-4 and Fig. M2-5a). The clear separation of compounds with positive and negative PC1 loadings in the van Krevelen diagram particularly reflected the differences in chemical compositions and properties and the relation to sample origin.

High PC2 values were characteristic for bay and creek water samples with the highest values for ice complex creek samples. The $C_xH_yO_z$ identifications with the highest PC2 loadings had high O/C ratios, but were more hydrogen saturated compared to the lowest PC1 loadings.

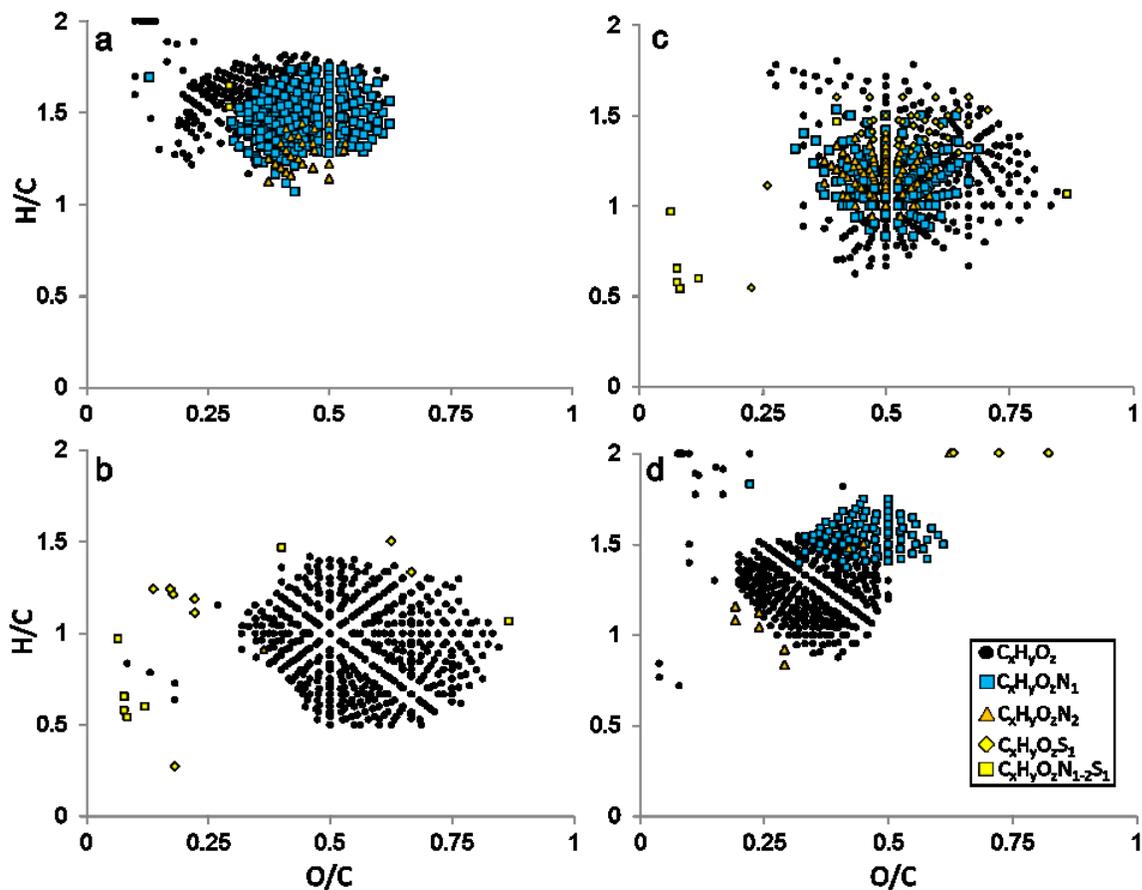


Fig. M2-5. Van Krevelen plots for the 500 highest positive/negative PC loadings:(a) 500 positive eigenvalues for PC1, (b) 500 negative for PC1, (c) 500 positive PC2, (d) 500 negative for PC2. $C_xH_yO_z$ molecular formulas (circles) were distinguished from $C_xH_yO_zN_1$ (squares), $C_xH_yO_zN_2$ identifications (triangles), $C_xH_yO_zS_1$ (light diamonds), sulfur and $C_xH_yO_zN_{1-2}S_1$ (light squares).

There was also a core of nitrogenous compounds with the “center of mass” at $O/C \sim 0.55$ and $H/C \sim 1.2$ (Fig. M2-5c). The contribution of molecular formulas with low PC2 scores to the bay samples variability was minor and formulas with high positive PC2 loadings did not explain variance in the bay samples. Molecular components with negative PC2 loadings (Fig. M2-5d) mostly represent riverine-dominant DOM molecular formulas. We have visualized molecular transformation trends on the upstream-downstream transect. This information with applied procedures is available in the supplementary materials.

Indicator peaks supported the sample groups (permafrost melt water creeks, ice complex creeks, river and bay samples) assigned (Dufrene and Legendre, 1997) in the cluster analysis (Fig. M2-6). Using only significant indicator values ($p < 0.05$; Fig. M2-6), bay samples had high O/C ratios and a relatively high degree of unsaturation in comparison to ice complex, creek and river DOM samples. Creek DOM samples had highly aliphatic indicator molecular markers. Riverine indicator markers took an intermediate position between the bay and creek DOM samples (Fig. M2-6). It is difficult to judge representativeness of the indicators of ice complex creek DOM due to the low number of samples ($n=2$). Using the available data, riverine indicators (Fig. M2-6) match well with the PCA loadings (negative PC2 values; Fig. M2-5d). Therefore, river-dominant DOM compounds exposed a relative depletion of oxygen (O/C ranged from 0.25 to 0.5) and high H/C ratios (ranging from 1 to 1.55; (Fig. M2-6, Fig. M2-SI4,5 and Table M2-SI4)).

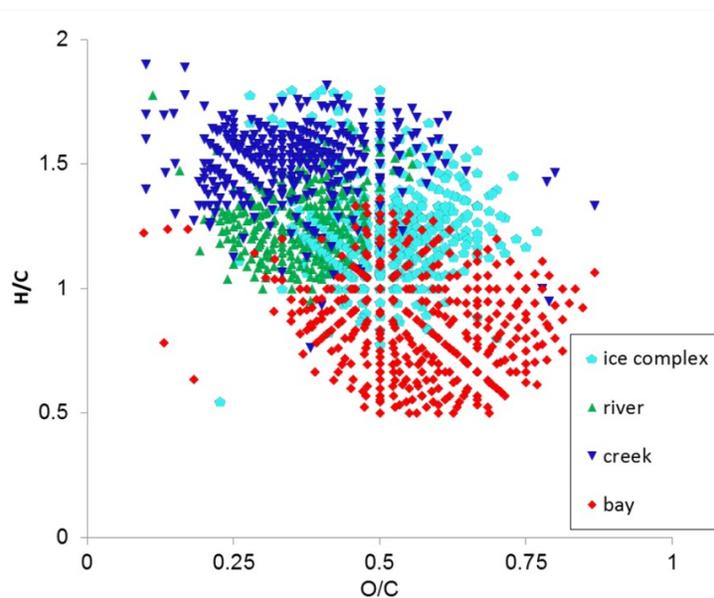


Fig. M2-6. Van Krevelen diagrams with indicator peaks determined by indicator value analysis (indicator values of Dufrière and Legendre).

DOM classification

Based on the information derived from the PCA, the individual formulas were examined in more detail. The series of formulas which solely contained C, H, and O (Fig. M2-7a) revealed that compounds with higher numbers of oxygen clearly prevailed in the bay samples. This observation fit well with the prevalent oxygenated formulas in the bay samples (Fig. M2-5b). The classes O₁-O₆ of the bay samples had the lowest number of identifications compared with the riverine and creek samples. However, in the compound classes with >5 oxygen atoms, the bay samples showed the highest number of oxygen rich identifications (Fig. M2-7).

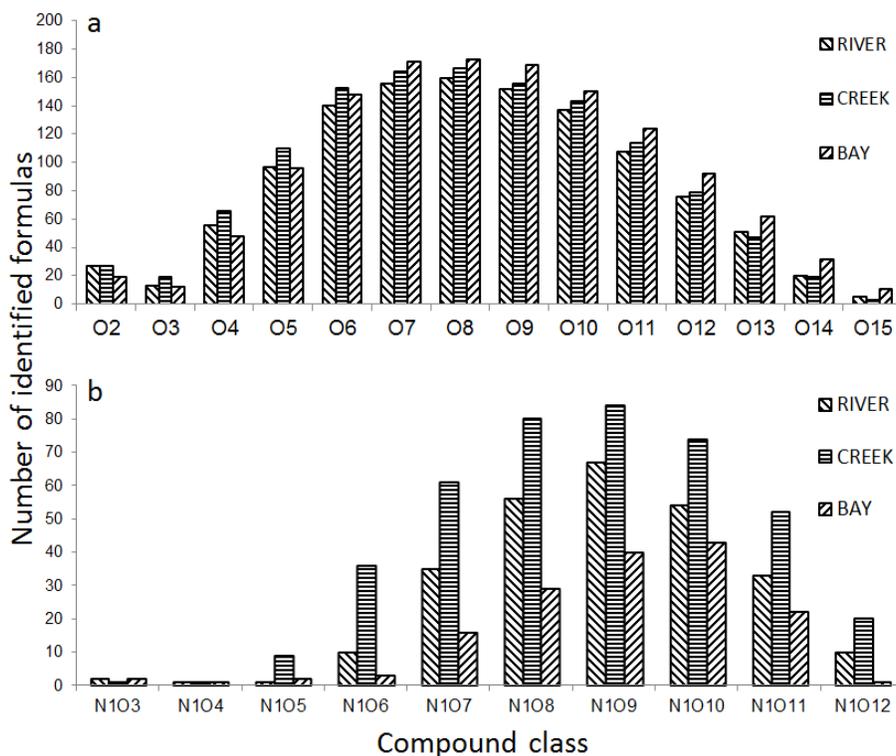


Fig. M2-7. (a) Classification of $C_xH_yO_z$ identified molecular formulas based on the number of oxygen atoms per identification. (b) Classification of $C_xH_yO_zN_1$ identified molecular formulas based on the number of oxygen atoms per identification.

Compounds containing one nitrogen atom were predominantly detected in river and creek samples (Fig. M2-7b). The lowest abundance of this compound class was observed for the bay samples. A similar trend was found for compounds containing two nitrogen atoms. These results agreed well with the PCA loadings.

Discussion

Little is known about the role, dynamics and transformation of DOM in the arctic ecosystem in particular with regard to permafrost, its thawing and predicted changes due to global warming. The average DOC concentration of about 500 $\mu\text{mol C L}^{-1}$ and TDN concentration of 13 $\mu\text{mol N L}^{-1}$ in the Lena River in the summer 2009 were similar to values presented in previous studies (Lara et al., 1998; Lobbes et al., 2000). DON concentrations in the summer months are the highest within the seasons (Holmes et al., 2012), potentially because of the high number of very different DOM sources with less degraded material. Highest DOC concentrations are characteristic for freshet period. Elevated DON values in late summer represent a source shift of organic matter in the river, likely because of increasing contribution of permafrost organic matter. Freshet periods are characterized by a large contribution of organic matter from surface layers of soils (Finlay et al., 2006). In late summer, the contribution of surface soil layer DOM decreases and the contribution of deeper soil horizons increases. An increase of soil derived organic matter in the permafrost influenced Kolyma River was inferred from the changes in the radiocarbon age of DOC in late summer (Neff et al., 2006). Since it was demonstrated that organic matter released by permafrost degradation could be more labile (Woods et al., 2011), this organic matter will affect regional microbial activity and primary production in the Lena River and the inner Laptev Sea. Decomposition of about 34 % of DOC was characteristic for Yedoma (ice complex) melt water streams and of 17% to 33% for river- creek water mixtures (Vonk et al., 2013a). We did not observe that DOC concentrations increased continuously on the way from the most upstream to the downstream sample. Thus, deltaic permafrost emitted DOM obviously did not affect riverine DOC concentration probably due to high mineralization rates and dilution by the huge river water volume.

DOC and TDN values for ice complex melt waters were extremely high. Several melt water creeks with very high DOC concentrations drain the massive ice complex on Kurunagh Island and discharge into the Olenekskaya channel. These DOM pulses probably caused the elevated DOC concentration in the channel ($660 \mu\text{mol C L}^{-1}$). Since the Olenekskaya channel discharges only ~6.5% as compared to the total Lena River water (Fedorova et al., 2015) ice complex derived DOC contributes only very little to river DOC in the entire Lena Delta. The few ice complexes are sporadically distributed (Are and Reimnitz, 2000). They are syngenetically frozen, fine-grained deposits and are built of large amounts of ground ice and massive ice wedges. The ice complexes also contain considerable amounts of fossil organic carbon that might become accessible due to permafrost thawing (Khvorostyanov et al., 2008; Schirrmeister et al., 2011) but it is unknown if this material is labile or refractory.

DOC and TDN concentrations in permafrost melt waters were highly variable and ~10 times lower than in the ice complex derived water. In contrast to the river and bay, the DOC concentrations of the melt water creeks were more variable and were slightly lower in DOC and higher in TDN. Permafrost melt waters are exposed to more environmental factors in comparison with the river. The influence of vegetation type, intense exposure to the light, different contributions of active organic matter layers and nutrients are more pronounced (Rodionov et al., 2007). Also, mixing processes, which are intense in the river, might be less important in the creeks resulting in a higher DOC and TDN variability.

Molar C/N ratios of DOM and SPE-DOM were different but the trends were similar. Inorganic nitrogenous nutrients are almost negligible in summer (Holmes et al., 2012; Lobbes et al., 2000) and thus DON is only slightly lower than TDN determined in our study. SPE-DOM contains only traces of inorganic material, and therefore TDN concentrations were equivalent to DON. The C/N ratios did not allow discrimination of riverine and bay samples, although ratios were slightly lower in the bay samples which however were still mostly dominated by the Lena River water with almost no salinity. The high C/N ratios are typical for the arctic rivers (e.g., Lobbes et al. 2000) decreasing by a factor of two in the open Laptev Sea (Kattner et al. 1999). DOM of riverine and bay samples were reduced in DON compared

to the creeks. The C/N ratios of this ice complex runoff (~33) reflect a high contribution of relatively fresh material released by the thawing process. This is further evidence for the lability and degradability of organic matter deposited in permafrost. The DOM may also originate from thawing of long-term frozen particulate material. In contrast, the particulate material in the Lena River is mostly allochthonous, soil-derived organic matter accumulated along the enormous length of the river. Autochthonous production is low because of the nutrient limitation and the high turbidity.

The obvious and interesting changes in the relative nitrogen content of the samples were reflected in the FT-ICR MS analyses. This supports the observations derived from bulk DOC and TDN measurements that during the transport of DOM from the thawing permafrost into the melt water creeks and the river and finally the Buor-Khaya Bay a continuous decline of nitrogen containing molecules occurred (Fig. M2-4 and 6). The N-containing formulas in Fig. M2-5a (those enriched in the permafrost creeks and lake DOM) are mostly aliphatic (based on their H/C ratios) and could likely be related to peptide-like moieties (based on their O/C ratio). Although FT-ICR MS is a semi-quantitative method, it clearly demonstrated the relative depletion of nitrogenous compounds in the bay samples. The O/C_{wa} , H/C_{wa} and C/N_{wa} ratios exposed the general enrichment of creek DOM samples by nitrogenous components and vice versa in the bay samples, which were more oxidized. The clearly higher C/N ratios calculated from the weighted average FT-ICR MS data than from the bulk data are due to the strict intensity threshold used in FT-ICR MS data processing, which excludes the low intensity nitrogenous mass peaks. Nevertheless, the trend was similar to the bulk measurements. Hierarchical clustering of the FT-ICR MS data showed that the origin of DOM was systematically reflected in its molecular composition also beyond the contribution of nitrogen containing compounds. Principal component analysis also highlighted the importance of nitrogenous and oxidized components for the molecular variance of DOM in the creek, river and Buor-Khaya Bay samples. Even the different types of creeks could be distinguished: permafrost melt water creeks differed from organic-rich ice complex melt water creeks. Obviously, the type of permafrost also affected the composition of released DOM.

On a smaller spatial scale, as studied on Samoylov Island (samples 48, 36, 37, 39), the composition of all DOM samples was very similar. Even the molecular composition of the lake sample matched with the creek samples. Creeks on Samoylov Island drain the lakes and therefore, lake and creek DOM samples have the same origin that is the active layer of permafrost and the local vegetation. The similarity of the one single lake sample and the creeks suggests that the prevailing molecular pattern from the lake island remained largely unaltered in the creek run-off on its short way (<1000 m) to the Lena River.

The molecular changes from river to bay might be partially explained by precipitation/flocculation of less-hydrophilic molecules although there was no pronounced salinity gradient in the bay (maximum salinity of 2). This observation opposed previous findings. For example, compositional differences of DOM presented for marine and mangrove systems (Koch et al., 2005) and for a river-ocean transect in the Chesapeake Bay (Sleighter and Hatcher, 2008) showed that DOM tends to become more aliphatic and depleted in oxygen-rich molecules from inshore to offshore (Sleighter and Hatcher, 2008). However, the bay samples in our study were still largely influenced by the riverine DOM and therefore did not represent marine waters. Also, it was shown that the phytoplankton communities are different in the Lena Delta and Buor-Khaya Bay due to the increasing amounts of seawater mixed to the riverine freshwater (Kraberg et al., 2013). These observations might be more important for molecular differences between river and bay samples than physico-chemical processes due to the slight salinity gradient. Open ocean studies have shown that the molecular composition of DOM differs between biological provinces (Bauer and Bianchi, 2011). Also, previously it was demonstrated that DOM photo-degradation resulted in production of highly oxidized organic molecules, similar to molecular components dominated in the bay samples of our study (Gonsior et al., 2013). Since summers in the Arctic are characterized with intense 24h sun activity, the observable molecular characteristics of the bay samples could also be a result of riverine DOM photo-degradation.

Characterized source-specific and source-dominant molecular markers will allow in future estimation of contribution of different sources into DOM which integrates signals from various sources (i.e. riverine

and estuarial DOM). Since Lena River is affected by the climate change driven factors (discharge increase, permafrost degradation, changes in vegetation) and permafrost-derived DOM believed to be labile, quantification of its contribution to riverine DOM and analysis of its further fate is important.

Conclusions

Bulk DOM parameters matched with the DOM molecular fingerprints, especially with focus on the nitrogenous fraction. FT-ICR MS was successfully employed as a tool for non-targeted biogeochemical studies of DOM in the Lena Delta. DOM samples were clearly differentiated according to source. DOM systematic molecular characteristics with respect to water origin potentially allow understanding of processes and a transformation involving DOM. Origin-systematic molecular composition is originating from different mechanisms of DOM formation. Therefore, our study supports the identification of processes and environmental parameters responsible for molecular DOM fingerprints. Special attention needs to be addressed to the quantification of DON fluxes in the region.

The compositional changes could present a major direction of DOM mineralization and transformation processes in the deltaic system of the Lena River. Since DOM is an important player in the carbon cycle the advanced understanding of DOM mineralization which results in CO₂ emission could provide additional knowledge on dynamics of changes in DOM. Understanding of these dynamics will allow predictions and record changes in carbon cycling. Climate change can potentially cause an increase of the active permafrost layer in the Arctic which will increasingly contribute to the amount of soil-derived organic matter in the rivers (Gustafsson et al., 2011). Since we demonstrated significant differences of the DOM molecular composition of melt water creeks and the Lena River, the growing contribution by permafrost DOM might also change the riverine biogeochemistry in the Lena Delta and the Laptev Sea with consequence on the ecosystem.

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5.3. *Manuscript 3*

“Molecular reactivity of dissolved organic matter along the Lena River-Laptev Sea transition”

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Abstract

Terrestrial dissolved organic matter (DOM) has a large impact on the marine carbon cycle but its reactivity in estuaries is poorly understood. Here we aimed at the characterization of active and inactive DOM components along the river-sea transition. We studied four transects in the coastal Laptev Sea with strong physico-chemical gradients of salinity, temperature, pH, nutrients, and dissolved organic carbon (DOC). DOC showed conservative mixing. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) was applied for the molecular analysis of solid-phase extracted DOM. With increasing salinity the molecular complexity increased and the magnitude weighted elemental ratios changed linearly from 0.55 to 0.52 for oxygen/carbon, from 1.09 to 1.15 for hydrogen/carbon, from 235 to 105 for carbon/nitrogen and 1092/711 for carbon/sulfur. When using peak magnitude-salinity correlation as a measure of the reactivity of DOM we could show that - 27% of all identified DOM components exhibited a strong conservative behavior, 32% were moderately affected by estuarine processes, and 41% were actively involved. We called it *pseudo-conservative* mixing of DOM. Components with negative correlation coefficients with salinity were mainly represented by aromatic compounds. Components with positive correlations were mainly represented by the highly unsaturated and phenolic compounds and aliphatic compounds.

Introduction

Rivers and estuaries are links between land and ocean. Terrestrial organic matter exported to the seas annually contributes about 0.4×10^{15} g of dissolved (DOC) and particulate organic carbon (POC) to the world oceans and hence has a large impact on the carbon cycle (Baumgartner and Reichel, 1975). DOM plays an important role in the regulation of aquatic ecosystems. It is, for example, of significant importance in aquatic food webs, mediates the availability of dissolved nutrients and metals, and modifies optical properties of the water (Findlay and Sinsabaugh, 2003). Dissolved organic matter (DOM) is involved in a wide variety of bio-chemo-physical processes during the river-sea transition (Bauer and Bianchi, 2011).

Studies on estuarine processes in the Arctic gain a high priority due to the changing hydrology of the large rivers (McClelland et al., 2004) and permafrost degradation processes within their watersheds caused by climate warming (Stocker et al., 2013). The discharge of the Eurasian arctic rivers have increased significantly during the last decades and accounted for about $\sim 2.5 \text{ km}^3 \text{ year}^{-1}$ for Yenisey, Ob, Lena, Kolyma, Pechora and Severnaya Dvina combined (McClelland et al., 2004). One of the contributors to the changing hydrology and biogeochemistry is permafrost degradation (permafrost thaw, active layer deepening, and thermokarst processes) (Frey and McClelland, 2009). The increasing discharge will potentially affect the biogeochemistry of the coastal seas in the Arctic (Le Fouest et al., 2013). In addition, factors such as changing snow cover, precipitation and surface temperature directly and indirectly impact watersheds and hence the coastal regions (Saito et al., 2013). Permafrost degradation processes influence not only the hydrology of the large arctic rivers but also the cycles of elements, e.g. carbon release (Natali et al., 2014) with further mineralization of its organic components in the creeks, rivers and coastal seas (Cory et al., 2014). The fate of terrestrial and permafrost-derived DOM in the coastal waters of the Arctic Ocean is ambiguous (Holmes et al., 2012).

It is still unclear how much of terrestrial components persist in the ocean because of the analytical limitations in the detection of low concentrations of terrestrial biomarkers (Cooke et al., 2009; Tesi et al., 2014). The Arctic Ocean is relatively small ($\sim 1\%$ of the World Ocean by volume) but receives 11% of the world's river runoff (Shiklomanov et al., 2000) mainly from Ob, Yenisey, Lena, Yukon, and McKenzie. Therefore, the riverine DOM imprints significant terrestrial signatures in the Arctic Ocean. High concentrations of DOC and terrestrial DOM are characteristic for polar surface waters (Benner et al., 2004; Opsahl et al., 1999). In contrast to marine DOM, terrestrial DOM has elevated C/N ratios, depleted stable carbon isotope ratios, and higher contributions of aromatic components (Benner et al., 1992; Hedges, 1992).

Previous studies in the estuaries of the Ob, Yenisey and Lena rivers showed conservative DOC mixing along the salinity gradients (Cauwet and Sidorov, 1996; Dittmar and Kattner, 2003; Kattner et al.,

1999; Köhler et al., 2003). Conservative mixing implies a dilution of DOC from the river to the sea but compositional and chemical changes are usually not taken into account. However, during the river-sea transition, DOM experiences phyto- and zooplankton mediated processes (Benner and Opsahl, 2001), microbial alteration (Santos et al., 2014), photochemical degradation and mineralization (Helms et al., 2013; Santos et al., 2014), abiotic sorption and desorption processes (McCallister et al., 2006), particle formation and deposition. All of these processes affect the DOC concentration and the DOM molecular composition. Understanding the qualitative changes of DOM in spite of a quantitative conservative mixing is required to elucidate this contrariness.

Assessing the contribution of different processes to DOM transformation and mineralization is challenging and limited due to its extreme molecular diversity and low concentration of individual components (Hertkorn et al., 2008). A possible solution lays in a complex and multilateral approach, i.e. using non-targeted molecular analytics such as ultrahigh resolution mass spectrometry (uHRMS) (Hertkorn et al., 2008), NMR (Lam and Simpson, 2008; Simpson et al., 2011) and excitation emission matrix fluorescence spectroscopy (Nebbioso and Piccolo, 2013; Zhou et al., 2013).

Applications of FT-ICR MS as an uHRMS significantly broaden our view on the molecular complexity and chemistry of DOM. We applied FT-ICR MS in combination with exploratory and descriptive statistics to unravel biogeochemical and microbiological processes impacting on DOM (Shabarova et al., 2014; Singer et al., 2010). DOM molecular chemodiversity and composition in boreal lakes of Sweden are regulated by the mean annual precipitation, water residence times, and mean annual temperature (Kellerman et al., 2014). In the Yenisey River, the DOM molecular composition was driven by latitude and pH (Roth et al., 2013). Previously we have shown that DOM molecular composition has origin-specific and systematic properties with respect to its source in the Lena Delta and Buor Khaya Bay (Laptev Sea) (Dubinenkov et al., 2015). In the study of Sleighter et al. (2008), the authors observed significant differences in the DOM molecular composition along a river-ocean transect of the Chesapeake Bay. There, DOM tended to be more aliphatic and contained lower abundances of oxygen-rich molecules

towards the ocean. Molecular transformation and degradation of DOM were also studied in the Atlantic and Southern Ocean (Lechtenfeld et al., 2014). Therefore, FT-ICR MS is an appropriate tool for region-scale analysis of DOM molecular transformations under the influence of different environmental drivers. FT-ICR MS is also applicable to small-scale analysis of processes. Recent studies gave some insights in the microbial formation (Koch et al., 2014) and photo-reactivity of DOM (Gonsior et al., 2013; Gonsior et al., 2011).

In our study, we questioned conservative mixing properties of DOM in the Lena River - coastal Laptev Sea transition, analyzed DOC concentrations along salinity/temperature gradients and revealed dynamics of the DOM composition. Molecular characterization was done using FT-ICR MS with its ultra-high mass resolution and exceptional sensitivity. We aimed at an identification of DOM components with conservative behavior in the Lena River estuary and components which are actively involved in the estuarine processes (non-conservative markers). This approach allowed estimates of active/non-active DOM molecular formulas in the river-sea transition zone and their relative contribution to the total DOM molecular pool.

Materials and Methods

Study area

The field sampling campaign took place in the end of July 2010 as part of the “Lena Delta 2010” expedition. We collected samples in the Lena Delta (73°0’N, 127°0’E, Fig. M3-1) and the Lena River – Laptev Sea transition zone along the transects T1-T4 (71°25’N, 130°49’E, Fig. M3-1). Sampling in the Lena Delta included the main stream of the Lena River and the beginning of the Trofimovskaya, Olenekskaya and Bykovskaya channels. DOC, TDN and DON concentrations of the riverine sample were averaged as typical characteristics for riverine DOM as well as for simulation of the “average” FT-ICR mass spectra. A complete station overview (station name, date, time, coordinates, salinity, temperature, DOC, TDN) is available online, PANGAEA: <http://doi.pangaea.de/10.1594/PANGAEA.842220>

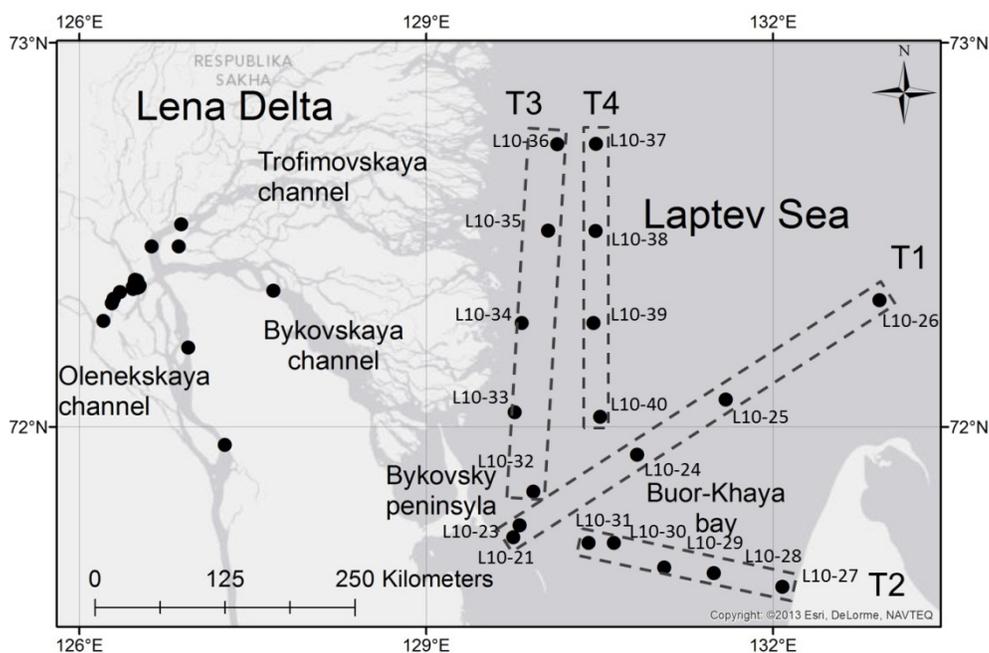


Fig. M3-1. Sampling stations and transects of the Lena Delta 2010 sampling campaign.

Sampling and solid-phase extraction

Subsurface sampling depths were determined based on CTD (conductivity, temperature, depth) measurements (Sea and Sun Technology GmbH), and water was sampled using a Niskin water sampler or pre-cleaned glass bottles. For DOC analysis and DOM extraction, samples were filtered through 0.7 μm GF/F filters (Whatman, pre-combusted, 4 h, 450°C) and adjusted with suprapure HCl (Merck) to pH 2. DOC samples were stored frozen in the dark until analysis. Nutrient samples were also filtered through GF/F filters and stored frozen in pre-cleaned HDPE bottles until analysis in the home lab. DOM samples were concentrated and desalted in the field using pre-packed solid-phase extraction (SPE) cartridges (1 g, PPL, Varian). Definitely some characteristics of DOM can arise exclusively from the extraction procedure (Perminova et al., 2014), we used PPL extraction due to large number of applications of this technique. After extraction of about 1 L of water, the cartridges were dried with nitrogen and eluted with methanol (5 ml, Lichrosolv, Merck). The DOC concentration and extraction efficiency of SPE-DOM was

determined by evaporation of 50 μ l methanol extract, re-dissolved in ultrapure water (Dittmar et al., 2008). The DOC extraction efficiency was $\sim 70 \pm 10\%$. DOC in blank extracts did not exceed the measurement error of DOC analysis.

DOC, DON and nutrient analyses

DOC and total dissolved nitrogen (TDN) concentrations were measured using high temperature catalytic oxidation (TOC-VCPN, Shimadzu). Water samples were acidified in the autosampler to remove inorganic carbon. DON concentrations were calculated as difference of TDN and inorganic nitrogen (NO_x). Nutrient samples (nitrate, nitrite, phosphate, silicate) were analyzed using an autoanalyzer (Evolution III, Alliance instruments) with a method described elsewhere (Kattner and Becker, 1991). Additionally, we used available DOC datasets for the coastal Laptev Sea region from 1999, 2001 (Romankevich and Vetrov, 2001) and our dataset from “Lena 2013” expedition (PANGAEA: <http://doi.pangaea.de/10.1594/PANGAEA.842221>).

FT-ICR MS analysis and data evaluation

Ultra-high resolution mass spectra for 21 Lena River and 22 coastal Laptev Sea DOM samples were acquired in one batch on a Bruker Apex 12 Qe FT-ICR MS equipped with a 12 T superconducting magnet and an APOLLO II electrospray source. We used a randomized sample order. SPE-DOM methanol extracts were adjusted to the same DOC concentrations of the sample (500 $\mu\text{M C}$) by dilution with methanol and water (1:1 MeOH/ H_2O). Samples were analyzed with electrospray ionization (ESI) in negative ionization mode. FT-ICR MS data were evaluated in the mass range of 200-600 m/z. Details on spectra accumulation, calibration and formula assignments can be found elsewhere (Dubinenkov et al., 2015; Koch et al., 2007; Koch et al., 2005). It is important to note, that each molecular formula can represent a number of isomers (Hertkorn et al., 2008). In this article for appropriate readability, we generally refer to assigned molecular formulas as "compounds", "components", "markers" or "constituents". The peak magnitudes were normalized to the peak with the highest intensity. The lower

intensity threshold was 0.3% of the base-peak intensity. The peak magnitude weighted-average elemental ratios O/C_{wa} , H/C_{wa} , C/N_{wa} , double bond equivalents (DBE), DBE-O and mass defects were calculated from the assigned formulas (Dubinenkov et al., 2015). Molecular formulas revealed by FT-ICR MS were visualized using van Krevelen diagrams as ratios of elements O/C and H/C (van Krevelen, 1950). Only 22 coastal samples were used for multivariate analysis presented in the manuscript. Here wanted to study a response of DOM molecular composition to changing environmental parameters during river-sea transition. Cluster analysis is based on the Bray-Curtis similarity (Bray and Curtis, 1957) and was performed using commercial software (PRIMER v.6; PRIMER-E Ltd, UK). Non-linear multidimensional scaling (NMDS) was calculated using relative magnitudes of assigned molecular formulas (R, software package vegan (Oksanen et al., 2013)). Temperature, salinity, DOC concentration, DON concentration, phosphate and NO_x concentrations were fit to each ordination, with p-values calculated over 999 permutations.

Results and Discussion

Hydrography

Our main focus was on the river-sea transition zone of the Buor-Khaya Bay and the coastal Laptev Sea. The depths along the transects varied from 2.8 m for the shallowest coastal transect 3 to 19 m for the deeper transects 1, 2 and 4. The transects were divided in two groups: the near shore relatively shallow transect 3 with clear dominance of freshwater originating from the Bykovskaya and Trofimovskaya channels of the Lena Delta and the transects 1, 2, 4 which had a clear marine water influence at depth (Fig. M3-2). The near-shore stations of transects 1 and 2 were also dominated by freshwater. We generally observed a strong stratification of the water column with the thermocline between 5 and 10 m. The transition was often very sharp with a decrease of $\sim 7^\circ\text{C}$ in temperature and an increase of 8 in salinity. For near-bottom water (~ 20 m depth), we observed temperatures of about 0°C . This situation is the typical signature of the warm riverine outflow and the cold polar marine water masses. The surface water of the coastal transects 3 and 4 remained fresh and warm whereas transects 1 and 2 showed an

increasing salinity towards north and north-east. A clear mixing of riverine and marine waters occurred in the outer Buor-Khaya Bay (Kraberg et al., 2013). Our results fit well with previously published data for the same time period (for additional details on the hydrography refer to Kraberg et al. 2013). The mean concentration of NO_x ($\text{NO}_2^- + \text{NO}_3^-$) in the Lena River channels was $1.55 \pm 0.65 \mu\text{M}$ and $0.08 \pm 0.04 \mu\text{M}$ for phosphate. The saline waters (salinity >20) of the Buor-Khaya Bay contained clearly higher concentrations of NO_x ($6.62 \pm 1.69 \mu\text{M}$) and phosphate ($0.35 \pm 0.10 \mu\text{M}$) which is in accordance with data published before (Holmes et al., 2012).

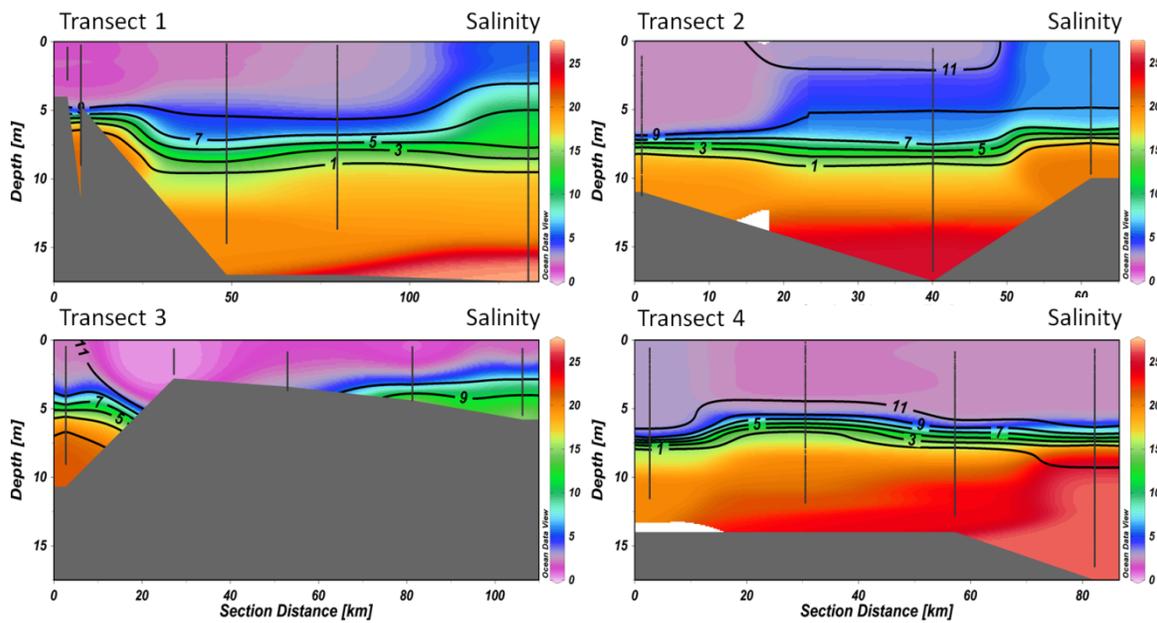


Fig. M3-2. Salinity (color) and temperature (isolines) profiles for the coastal transects 1-4. Distances are from the Buor-Khaya Bay to the north and east.

Dissolved organic matter in the Lena River estuary

The average DOC and DON concentrations in the Lena River channels were $653 \pm 38 \mu\text{M C}$ and $13 \pm 6 \mu\text{M N}$ ($n=14$). These DOC values were about 30% higher compared with previously published data (Lara et al., 1998; Lobbes et al., 2000) and our own results from 2009 (Dubinenkov et al., 2015). These differences are due to seasonal variations since our sampling was carried out end of July, whereas the 2009 campaign took place end of August. July is characterized by a larger water discharge of about 19% of the annual discharge compared with about 13% in August. The freshet discharge peaks in June, typical for arctic rivers (Holmes et al., 2000), and thus the DOC discharge is highest at the same time (Manizza et al., 2009). In the Kolyma River, for example, the spring freshet mobilizes organic matter from the soil surface layer which results in higher river DOC concentrations (Finlay et al., 2006) being similarly higher in July than in August (Mann et al., 2012). The average DOC/DON ratio in the Lena River was 55 ± 28 ($n=14$) comparable to published values (Holmes et al., 2012; Kattner et al., 1999; Lara et al., 1998). These high C/N ratios are characteristic for terrestrial DOM and reflect the dominance of terrestrially-derived (allochthonous) material in the riverine DOM and a relatively high biodegradability of DON.

The near-shore low salinity samples of the Laptev Sea transects had comparable high DOC concentrations as the riverine samples reflecting the outflow of riverine water along the coast to the north and east. The average DOC and DON concentrations for the freshwater dominated waters of the Buor-Khaya Bay (0-2 salinity) were $618 \pm 7 \mu\text{M C}$ and $18 \pm 6 \mu\text{M N}$, being slightly lower than values for the Lena River channels. This can be explained by partial decomposition of riverine DOM by biomineralisation (Obenosterer and Benner, 2004; Vonk et al., 2013a) or photodegradation (Gonsior et al., 2014; Gonsior et al., 2013), by flocculation processes (Benner and Opsahl, 2001) or by small but appreciable contribution of marine waters. The C/N ratio of 43 ± 19 was similar to the riverine waters.

On average, our most saline samples contained $327 \pm 40 \mu\text{M DOC}$ and $10.5 \pm 6 \mu\text{M DON}$. The lowest DOC concentrations in the coastal Laptev Sea ($258 \mu\text{M C}$) were measured in the sample with the highest

salinity (26.5) representing the most marine dominated water (Table M3-1). These DOC concentrations for saline waters are typical for this area (Alling et al., 2010; Cauwet and Sidorov, 1996; Romankevich and Vetrov, 2001). The bottom layer of the coastal Laptev Sea is characterized by even lower DOC concentrations of about 130 $\mu\text{M C}$ (Alling et al., 2010) but not as low as in the open Arctic Ocean (Benner et al., 2005). Thus, in our study we sampled only coastal marine waters with an insignificant influence of open oceanic waters. The DON concentration in the most saline sample was 7 $\mu\text{M N}$ resulting in a DOC/DON ratio of 36. This shows an enrichment of DON compared with that of the Lena River DOM. The DOC/DON ratios further decrease towards central Laptev Sea, where the marine end member has 93 $\mu\text{M DOC}$ and 6 $\mu\text{M DON}$ (C/N of 15.5) (Kattner et al., 1999). Thus, for more offshore samples the contribution of DON relative to DOC typically increases in DOM.

Whereas the DOC concentration in the coastal Laptev Sea and Buor-Khaya Bay decreased linearly with increasing salinity (Fig. M3-3), the distribution of DON was not that systematic, but tended also to decrease with increasing salinity (Fig. M3-S1 in the Supporting Information). In Fig. M3-3 several data sets of DOC concentrations were plotted versus salinity to generalize the DOC behavior in the Lena River – Laptev Sea transition. DOC behaved conservatively in the estuary with large but non-specific (with respect to DOC sources and sinks) deviations from the conservative mixing line. Therefore, in approximation, the majority of riverine DOC passes almost quantitatively the mixing zone, but whether qualitative transformations of DOM occur is still unclear. Conservative behavior can demonstrate relative stability (recalcitrance) of riverine DOC with respect to the changing environment, or compensational processes of DOC sources and sinks occur which means that DOC can be removed and generated in the estuary (Bauer and Bianchi, 2011). Therefore, we postulate a pseudo-conservative behavior of DOC in the Lena estuary. By the term “*pseudo-conservative*” we mean that although DOC exhibits quantitatively a conservative mixing in the river-sea transition, DOM is actively involved in various bio-chemo-physical processes along this transition and experiences transformation. However, even non-reactive OC constituents may display non-conservative mixing plots due to timescale differences in river

concentrations of the constituent relative to estuarine mixing (Loder and Reichard, 1981; Officer and Lynch, 1981). Therefore, combination of multiyear data may lead to misinterpretations in DOC mixing properties. It is also known that in some cases of conservative DOC behavior some DOM components (e.g. humic acids, amino acids) are markedly non-conservative (Fox, 1981).

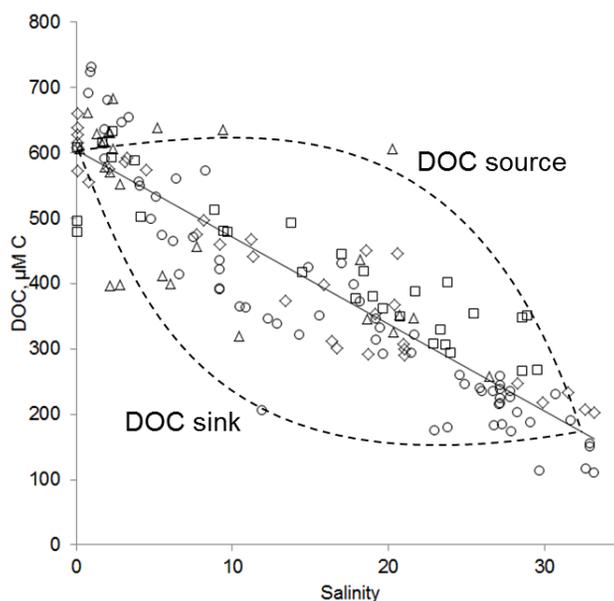


Fig. M3-3. DOC concentrations versus salinity in the coastal and open Laptev Sea. Lena 2013 dataset (circles), Lena 2010 (triangles), Spasiba 1 (squares) and Spasiba 2 (diamonds); (Romankevich and Vetrov, 2001) Linear correlation: $r=0.897$, $p<0.001$.

Many autochthonous and allochthonous processes affect DOC concentration and composition in the complex river-sea transition systems. Allochthonous sources are terrestrial soil-derived organic matter, and autochthonous DOM and DON include organic matter which originates from phytoplankton production and/or is released by micro- and macrozooplankton, prokaryotes and viruses (Bronk, 2002). In the Lena River channels and river-sea mixing zone the community structure of phyto- and microzooplankton clearly differed between freshwater (Kraberg et al., 2013), non-stratified riverine

waters and the coastal stratified waters certainly imprinting the DOM concentration and composition which is also reported for other regions (Osterholz et al., 2014; Zhang et al., 2014).

Other large arctic rivers also show conservative DOC behavior in the estuaries. In Ob and Yenisey rivers DOC and DON behave conservatively along the salinity gradient, and riverine DOM showed predominantly refractory characteristics (Köhler et al., 2003). In addition, laboratory experiments showed only minor DOM losses due to flocculation processes and bacterial consumption (Köhler et al., 2003). However, Alling et al. 2010 demonstrated that DOC exhibits non-conservative behavior across the coastal Laptev and East-Siberian seas (Alling et al., 2010), but these observations were characteristic for more offshore locations with much longer residence times of water masses.

DOM molecular markers

Thousands of ions within each DOM sample were detected by FT-ICR MS. Table M3-1 gives an overview of salinity and the magnitude weighted elemental compositions of the estuarine and coastal samples, complemented with the averaged data of the riverine samples. The complete FT-ICR MS data set for the Lena Delta DOM samples is available in Table M3-S1.

The number of assigned formulas for riverine samples was lower (4364 ± 140) than that of the estuarine samples (4763 ± 222). The number of assigned formulas was more variable in the estuary as compared to the river. The highest number of molecular formulas was determined for estuarine sample L10_79 (5254) and the lowest for river sample L10_39 (4126). The peak distribution of the FT-ICR mass spectra for all DOM samples was monomodal without clear visual differences. Since we applied normalization by the highest peak, the relative peak magnitude was not affected by the number of peaks in each spectrum. This approach facilitated correlation analyses and data interpretation.

Table M3-1. Salinity, number of assigned formulas and magnitude-weighted averaged parameters including m/z_{wa} , O/C_{wa} , H/C_{wa} , C/N_{wa} , C/S_{wa} , DBE_{wa} , and $DBE-O_{wa}$.

Sample	Salinity	Number of formulas	m/z_{wa}	O/C_{wa}	H/C_{wa}	C/N_{wa}	C/S_{wa}	DBE_{wa}	$DBE-O_{wa}$
L10_72	0.2	4497	410.5	0.54	1.09	217	1140	9.61	-0.59
L10_74	0.7	4380	399.6	0.52	1.13	234	1217	9.15	-0.5
L10_76	1.3	4455	407.9	0.54	1.1	214	1041	9.45	-0.63
L10_73	1.6	4513	404.3	0.53	1.12	224	1078	9.31	-0.55
L10_52	1.8	4768	404.2	0.54	1.09	209	1088	9.49	-0.52
L10_54	1.9	4805	401.9	0.54	1.09	204	990	9.41	-0.57
L10_57	2.1	4651	396.7	0.53	1.12	216	1141	9.15	-0.51
L10_71	2.1	4831	402.1	0.54	1.09	203	1019	9.41	-0.6
L10_82	2.1	4621	396.6	0.53	1.12	215	1177	9.18	-0.48
L10_75	2.2	4536	407.9	0.53	1.11	218	1036	9.43	-0.58
L10_78	2.4	4871	405.0	0.54	1.09	204	1017	9.49	-0.56
L10_58	2.8	4850	400.9	0.54	1.1	199	1005	9.33	-0.55
L10_55	4.1	4737	400.8	0.53	1.11	205	1111	9.29	-0.56
L10_63	6.1	4827	401.6	0.54	1.11	177	849	9.28	-0.64
L10_59	7.7	4787	402.7	0.53	1.12	182	909	9.26	-0.62
L10_77	9.4	4525	401.1	0.53	1.13	195	1037	9.16	-0.64
L10_62	10.4	4924	401.1	0.53	1.13	154	768	9.12	-0.74
L10_56	18.2	4967	400.8	0.53	1.13	139	729	9.09	-0.73
L10_60	18.6	5076	403.7	0.53	1.13	137	672	9.16	-0.68
L10_83	20.2	4952	400.2	0.53	1.14	138	786	9.04	-0.67
L10_53	20.7	4969	397.1	0.52	1.15	131	777	8.91	-0.7
L10_79	26.5	5254	405.4	0.53	1.15	104	571	9.03	-0.82
L10_R*	-	4364±140	403±7	0.52±0.02	1.12±0.03	223±15	1088±120	9.27±0.3	0.53±0.11

* calculated exclusively for the riverine DOM samples: L10_30, L10_39, L10_42, L10_43, L10_48, L10_49, and L10_50)

The magnitude weighted average m/z for the estuarine samples was 403 ± 4 , the highest m/z was 410.5 for sample L10_72, and the lowest average m/z of 396.6 was determined for sample L10_82. For the riverine DOM samples m/z_{wa} was 403 ± 7 . Thus, the calculated molecular masses were similar in the whole dataset with a slightly higher variance for riverine DOM. We categorized the samples into two groups based on salinity: freshwater DOM in low salinity waters (salinity of 0-2; <5% of marine water contribution) and more saline water DOM (salinity >20; >50% of marine water contribution). The

strongest differences were found for the C/N_{wa} ratios, which were 217 ± 11 for freshwater DOM versus 124 ± 17 for saline water DOM. The differences in C/N ratios for bulk DOM and C/N_{wa} calculated from molecular data could result from DON loss during extraction procedure and from selective ionization in the electrospray. The relative contribution of nitrogenous peaks to the total intensity was two times higher for marine DOM than for freshwater DOM. All other parameters (except O/C ratio) also showed clear differences. The means (fresh/saline) were for O/C_{wa} $0.534\pm 0.009/0.525\pm 0.003$, for H/C_{wa} $1.10\pm 0.02/1.14\pm 0.01$, for C/S_{wa} $1092\pm 79/711\pm 122$, and for DBE_{wa} $9.4\pm 0.2/9.0\pm 0.1$.

The number of assigned formulas correlated well with salinity ($r=0.77$, $p<0.0001$) representing the increasing contribution of marine DOM to the riverine DOM molecular pool, hence, increasing the molecular complexity along the river-sea transition similar to results from Chesapeake Bay (Sleighter and Hatcher, 2008). This growing number of molecular components may also result from contributions of estuarine processes. Weighted average parameters correlated well with salinity: during the river-sea transition of DOM we observed a linear decrease in C/N_{wa} ($r=-0.96$, $p<0.00001$), C/S_{wa} ($r=-0.89$, $p<0.00001$), DBE_{wa} ($r=-0.76$, $p<0.0001$) and an increase in H/C_{wa} ($r=0.81$, $p<0.00001$) reflecting the diverse chemical nature of marine and riverine DOM (Fig. M3-S2). The marine DOM contribution can be characterized by a higher content of aliphatic (high H/C ratio) components and components with the heteroatoms, nitrogen and sulfur responsible for the higher structural diversity of the offshore DOM.

Non-linear multidimensional scaling demonstrated that mixing of riverine and marine water masses contributed to most of the changes in the molecular DOM composition (Fig. M3-4a). Salinity, temperature, DOC, and phosphate are strongly linked with the water mass mixing. Only DON and NO_x showed different directions of the influence on the molecular composition. The hierarchical clustering reflected a high molecular similarity ($S>90$, Bray-Curtis) between all samples, but also the relation to salinity. Similarly in study of Kujawinski et al. 2009, marine and riverine DOM were discriminated using non-metric multidimensional scaling with further extraction of source-indicator molecular markers on the river-sea transect (Kujawinski et al., 2009). The influence of the changes in salinity on the molecular

composition is certainly accompanied by many other biogeochemical parameters such as phytoplankton community, bacterial activity, flocculation, and precipitation. However, salinity can still be applied for a simplified parameterization and description of the river-sea transition and mixing of marine/riverine DOM. Since DOC showed a conservative behavior, we can relate DOM transformations to changing salinity in the river-sea transition.

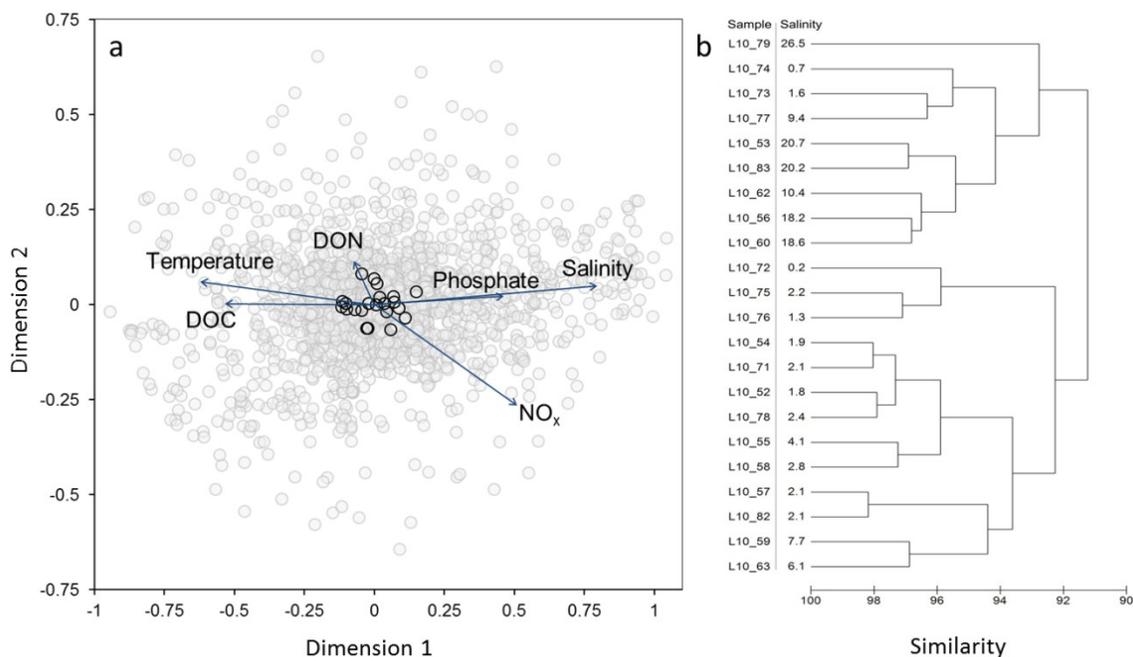


Fig. M3-4. Molecular information for DOM in the Lena River estuary as revealed by FT-ICR MS: **a** - Multivariate analysis of molecular data and drivers using non-metric multidimensional scaling. Ordinations are based on Bray-Curtis similarity with respect to relative peak magnitudes (stress = 0.0809). Salinity, temperature, phosphate, NO_x , DON and DOC were fit to the ordination. Grey circles are DOM compounds, black circles are DOM samples. **b** - Hierarchical tree based on the Bray-Curtis similarity.

Molecular components and reactivity of DOM

We analyzed 5165 molecular peaks and their correlation properties with respect to salinity in water

samples (Table M3-2). 3047 peaks (59%) correlated significantly with salinity ($p < 0.05$), 1753 molecular formulas (34%) had highly significant correlations ($p < 0.001$) and 2218 (41%) molecular formulas showed no correlation with salinity. About 57% of the total summed intensity was characteristic for the molecular peaks with the highly significant correlations.

Table M3-2. Significant and non-significant correlations and correlation coefficients for compounds from compound classes with salinity.

Significance (p)	<0.001	<0.001	<0.05	<0.05	>0.05
Correlation coeff. (r)	$r \geq 0.7$	$r \leq -0.7$	$0.2 > r > 0.7$	$-0.7 < r < -0.2$	-
Number of formulas	881	529	584	1053	2118
$C_xH_yO_z$	49	525	85	977	1072
$C_xH_yO_zN_1$	446	1	272	53	618
$C_xH_yO_zN_2$	220	0	81	7	185
$C_xH_yO_zS_1$	147	3	135	16	236
$C_xH_yO_zN_1S_1$	19	0	11	0	7

We estimated the reactivity of DOM components in the river-sea transition by the analysis of the strength and significance of the Pearson's correlation. We considered correlations with $|r| \geq 0.7$ as strong, with $0.2 < |r| < 0.7$ as moderate to weak and $|r| < 0.2$ as negligible or absence of correlation. Within the 1753 molecular formulas with highly significant correlations (Table M3-2) we observed 1410 molecular formulas with a strong positive (881 formulas) and a strong negative (529 formulas) correlation. Thus, 80% of the formulas with highly significant correlations had a strong correlation coefficient (predictive behavior). Within the group of molecular formulas with significant correlations ($p < 0.05$) 1637 molecular components had moderate to weak correlation coefficients: 584 being positive and 1053 negative. Table M3-2 illustrates the distribution of the different compound classes and their correlation properties. Compounds with heteroatoms represented 94% (832 of 881) of the total pool of DOM components with highly significant positive correlations with salinity. In contrast, more than 99% of the compounds with negative correlations were $C_xH_yO_z$. For the significantly correlating compounds the tendency was similar.

Within the compounds with no correlation there were 51% of $C_xH_yO_z$ formulas and 49% of formulas with heteroatoms. For majority of sulfur-containing formulas positive correlations were characteristic with significant fraction of components without correlations.

We interpreted the correlation of DOM compounds with salinity as a measure of DOM reactivity along the river-sea transition. Following this assumption, highly significant negative and positive correlations with a strong coefficient should be characteristic for molecular components of DOM with predictive and conservative behavior in the river-sea transition. 41% must be involved in the estuarine processes. In this approach, positive correlating peaks can be interpreted as DOM components which appear to increase in their relative contribution with increasing influence of marine DOM. Further, negative correlations reflect a “dilution” of terrestrial DOM with marine DOM. Additionally we demonstrated that there was no selectivity based on the peak magnitude in mass spectra by studying the dependence of the average peak intensity and correlation coefficient (Fig. M3-S3).

The molecular formulas allow some chemical interpretations of the conservative and reactive DOM components based on elemental stoichiometry (Fig. M3-5). H/C ratios of >1 were characteristic for the majority of markers with positive salinity correlations, H/C ratios of <1 for molecular components with negative correlations. The O/C ratio varied from 0.25 to 0.8 for highly significant correlations. For negative highly significant correlations (salinity-peak magnitude) less oxidized and more unsaturated compositions were characteristic in comparison with positively correlating molecular components (Fig. M3-5a).

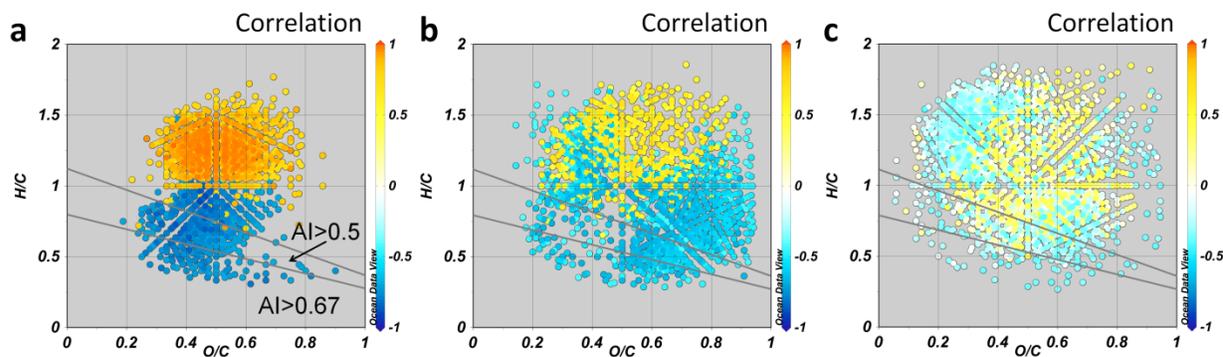


Fig. M3-5. Van Krevelen diagrams with color indication of the peak intensity-salinity correlation coefficient and aromaticity indexes: a – strong, highly significant correlations ($|r| \geq 0.7$, $p < 0.001$), b – weak and moderate significant correlations ($0.2 < |r| < 0.7$, $p < 0.05$), c – no correlation.

Based on the stoichiometry derived from the molecular formulas, we chemically characterized molecular components with different correlations (Kellerman et al., 2014; Koch and Dittmar, 2006; Šantl-Temkiv et al., 2013). Aromaticity index (Koch and Dittmar, 2006) (AI) in combination with elements ratios (e.g. O/C and H/C) for identified formulas allows virtual classifications of identified molecular formulas. For instance, molecular components with strong negative correlation coefficients can be potentially represented by polycyclic aromatics ($AI > 0.67$) and vascular plant-derived polyphenols ($0.67 \geq AI \geq 0.5$). These observations fit well with the terrestrial origin of riverine DOM. Molecular components with strong positive correlations were mainly represented by unsaturated ($AI \leq 0.5$ and $H/C < 1.5$) and aliphatic compounds ($2.0 \geq H/C \geq 1.5$) typical for a relative enrichment of marine DOM (Sleighter and Hatcher, 2008). Therefore, molecular formulas with a higher degree of aromaticity and unsaturation exposed negative correlations with salinity during the river-sea transition. This is also reflected in the changes of the magnitude weighted parameters (O/C_{wa} , H/C_{wa} and DBE_{wa}). Assessed in our study marine and terrestrial DOM molecular components (Fig. M3-5a) are not in good relation with the indicator markers revealed before on the Delaware River – Sargasso Sea transect (Kujawinski et al., 2009) but in a very good relation with the study on the freshwater influence in the German Bight of the North Sea (Koester, 2013). Formulas, which did not correlate with salinity, could be demonstrative for

components involved in estuarine processes, showing a more reactive behavior. A large fraction of molecular formulas with moderate to weak negative correlation coefficients had a high oxidation state: $O/C > 0.5$ and $H/C < 1.5$. These components could be involved, for example, in photodegradation processes. Those molecular formulas, which did not correlate with salinity, were widely distributed in the van Krevelen diagram (Fig. M3-5c). The region around $H/C > 1$ and $O/C < 0.4$, where the peak-magnitudes of the molecular formulas were not correlated with salinity, likely represents labile permafrost-derived compounds which were subject to estuarine bio-mineralization and transformation processes. Characterized in our study reactive components of DOM in the river-sea transition partly fit observed molecular signatures of degradable fraction of permafrost DOM molecular pool (Spencer et al., 2015).

To support our arguments regarding dilution and removal of aromatic compounds along the river-sea transition we considered slopes and intercepts for modeled linear equations (Fig. 6).

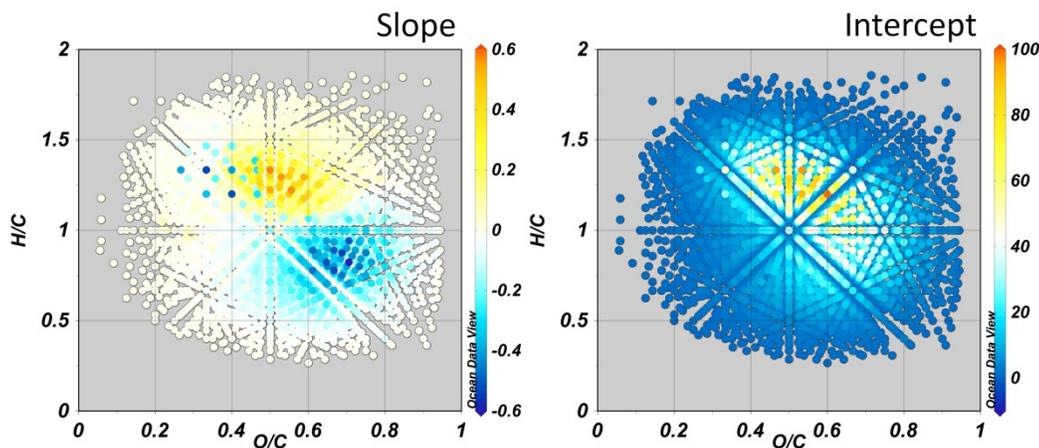


Fig. M3-6. Van Krevelen diagrams with slopes and intercepts of modelled linear equations of peak magnitude – salinity dependence.

Definitely the slopes are interconnected with correlation coefficients, but slopes also demonstrate the “strength of response” of peak intensity in mass spectra to salinity in the water sample. Positive slopes were characteristic for nitrogenous components in the region of $1 < H/C < 1.5$ and $0.4 < O/C < 1.7$ and

negative slopes for oxygen-rich formulas: $0.5 < \text{H/C} < 1.1$ and $0.5 < \text{O/C} < 0.81$. The intercepts of the linear equations exposed large values (>40% of relative intensity) for a large group of diverse compounds and in general just reflected peak intensities plot (Fig. M3-S4). The bubble plot for additional slopes visualization is available on Fig. M3-S6. Analytical window did not allow detection of unsaturated DOM molecular components in study of Kujawinski et al. 2009, but characterized in our study hydrogen-saturated terrigenous DOM components fit well (Fig. M3-5,6 and Fig. M3-S6) previous observations (Kujawinski et al., 2009). Slopes of linear equations exposed systematic properties with respect to DBE. Molecular components with DBE from 7 to 9 had positive slopes and DBE of 10-12 had negative slopes. Therefore, unsaturated components (high DBE values) had mainly negative slopes and intensities of these signals decreased along the river-sea transition. Additionally, positive aromaticity indices were characteristic for molecular formulas with negative slopes.

Conclusions

In this study we showed that DOC exhibited conservative behavior in the Lena River - Laptev Sea transition and passed the mixing zone almost quantitatively, but, simultaneously, thousands of DOM molecular components showed active involvement to the transitional processes; about 40% of them were highly affected by processes, which occurred in the river-sea transition zone. Thus, transformations of DOM in the river-sea transition are rather qualitative than quantitative. We call this the “*pseudo-conservative*” behavior of DOM. Highly conservative components can find further applications as tracers for molecular DOM components of terrestrial origin and for estimating their persistence in the oceans. Reactive components are the subjects of future research in the context of dominant DOM turnover and mineralization mechanisms and their contribution to the regional carbon cycle. These compounds are potential targets in exploration of estuarine processes on the molecular level. Interpretation of detailed DOM compositional changes as result of estuarine processes is the aim of future research, particularly in studies on fate of terrigenous DOM in the Arctic Ocean.

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5.4. Manuscript 4

“Mobilization, mineralization and turnover of permafrost-derived organic matter”

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Abstract

The global inventory of carbon stored in permafrost soils encompasses twice as much carbon as currently stored in the atmosphere and represents half of the global belowground soil carbon. Permafrost experiences substantial climate change driven degradation processes and permafrost-derived dissolved organic matter (DOM) influences increasingly the biogeochemical cycle of carbon in the Arctic. The ultra-complex mixture of organic compounds of DOM is a proxy of ecosystem metabolism and represents an imprint of past and ongoing biogeochemical processes. Recent progress in molecular analytics of DOM has allowed the characterization of thousands of molecular components and their response to changing environmental parameters in the natural systems. Here we present a synthesis of quantification of dissolved organic carbon (DOC) mobilized from permafrost. We experimentally simulated changes in DOM molecular richness and composition at the terrestrial-aquatic interface. Microbial alteration explained, at least in part, initial changes of DOM throughout its evolution and transport from terrestrial to aquatic environments. Extreme bioavailability of permafrost organic carbon and rapid transformation of DOM suggest that this ancient organic carbon is highly reactive upon fluvial release.

Introduction

A substantial amount of terrestrial dissolved organic matter (DOM) is transported along the fluvial continuum from soils to the coastal seas and ocean (Regnier et al., 2013). Climate change causes the release and mobilization of vast amounts of soil organic carbon in the permafrost-dominated watersheds of the arctic rivers (Guo et al., 2007). This organic matter is subject to transformation and mineralization processes and further transport to the river and coastal Arctic Ocean (Holmes et al., 2012).

Permafrost is widely distributed in the northern hemisphere and covers ~24% of the exposed land surface area (Zhang et al., 1999). Approximately 34% of the world's coastlines are permafrost affected arctic coasts (Lantuit et al., 2013). Permafrost represents one of the largest pools of belowground organic carbon with an estimated global amount of ~ 1300 Pg (uncertainty range ~1100 to 1500 Pg) (Hugelius et al. 2014), with 211 + 160/-153 Gt of carbon in the ice complexes (also called Yedoma) (Strauss et al.,

2013). Permafrost degradation and thaw processes, such as active layer deepening, thermokarst, and thermal erosion, activate previously conserved carbon and move significant quantities of land carbon into the ocean and atmosphere providing positive feedback to climate warming (Grosse et al., 2011; Schuur et al., 2008). Permafrost currently underlies significant areas of the watersheds of the large arctic rivers; e.g., for the Lena River it is ~75-85% of the watershed. Permafrost degradation processes influence the hydrology and the cycles of elements of the large arctic rivers, e.g. by carbon release (McClelland et al., 2004; Natali et al., 2014), and finally the Arctic Ocean which is relatively small (~1% of the World Ocean by volume) but receives 11% of the world river runoff (Shiklomanov et al., 2000). Thus, significant increases in water discharge of the Eurasian arctic rivers (McClelland et al., 2004) will affect the biogeochemical cycles of the Arctic Ocean.

Permafrost thaw results in the mobilization of substantial quantities of terrestrial organic matter. This organic material can be transferred to the rivers and coastal seas with sediments by river bank and coastal erosion, or via outflows which drain permafrost areas and outcrops. The degradation of ice complex deposits is of particular interest, because these late Pleistocene deposits are widely distributed in the arctic lowlands and are characterized by high contents of ice (65-90 volume %) and organic carbon (1.2-4.8 weight %). This makes them highly vulnerable to thaw and carbon mobilization (Schirrmeister et al., 2012; Schirrmeister et al., 2011; Zimov et al., 2006) (Fig. M4-1). Furthermore, bacteria which have survived in the frozen ice complex deposits activates and grows upon permafrost thaw (Mackelprang et al., 2011). Dissolved organic carbon (DOC) decomposition rates of 13-62% in aquatic environments suggest rapid substantial mineralization and turnover of permafrost-emitted organic matter (Spencer et al., 2015; Vonk et al., 2013a). DOC is actively processed in small streams and permafrost creeks (Butman and Raymond, 2011; Denfeld et al., 2013) resulting in high CO₂ outgassing rates. These streams are hot spots of turnover and mineralization of mobilized permafrost DOM. The fate of DOC released by permafrost to inland waters is controlled by its coupled photochemical and biological oxidation (Cory et al., 2014).

However, there are still many uncertainties in the mobilization rates of permafrost organic matter and its mineralization potential. The mechanistic relationship between DOM degradability and its molecular composition is far from being understood. Our understanding of the complex chemistry of DOM has been tremendously expanded by advanced analytical facilities and developments in the molecular data processing (Hertkorn et al., 2008; Hertkorn et al., 2012; Kellerman et al., 2014). The ultrahigh-resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) allows characterization of thousands of molecular components in the complex mixture of DOM. Molecular formulas can be assigned for ions because of the extraordinary mass accuracy of FT-ICR MS (Koch and Dittmar, 2006; Koch et al., 2005; Stenson et al., 2003). The stoichiometry of identified compounds provides chemical insights on, e.g., oxidation state of carbon, amount of double bond equivalents per molecule, and content of heteroatoms. This information about DOM can be related to environmental parameters for biogeochemical interpretations (Flerus et al., 2012). FT-ICR MS data were used in DOM studies on age models, molecular transformations, insights of recalcitrance (Lechtenfeld et al., 2014), microbial processing (Koch et al., 2014; Santos et al., 2014; Shabarova et al., 2014), and light mediated processes (Gonsior et al., 2014; Gonsior et al., 2013).

We hypothesized that the dominant fate of terrestrial DOM released from permafrost is either the complete mineralization to CO₂ or the substantial microbial alteration with further export of DOM in relatively recalcitrant form to the coastal ocean. Here we assessed the bio- and photo-availability of permafrost-derived organic matter to reveal its relevance for carbon cycling in the Lena Delta. The molecular alterations of DOM transformations were assessed from experimental approaches on microbial degradation. We determined molecular components, which control DOM degradability and related obtained results with the regional-scale molecular patterns of DOM in the terrestrial-aquatic transition.

Materials and Methods

Study areas and sampling

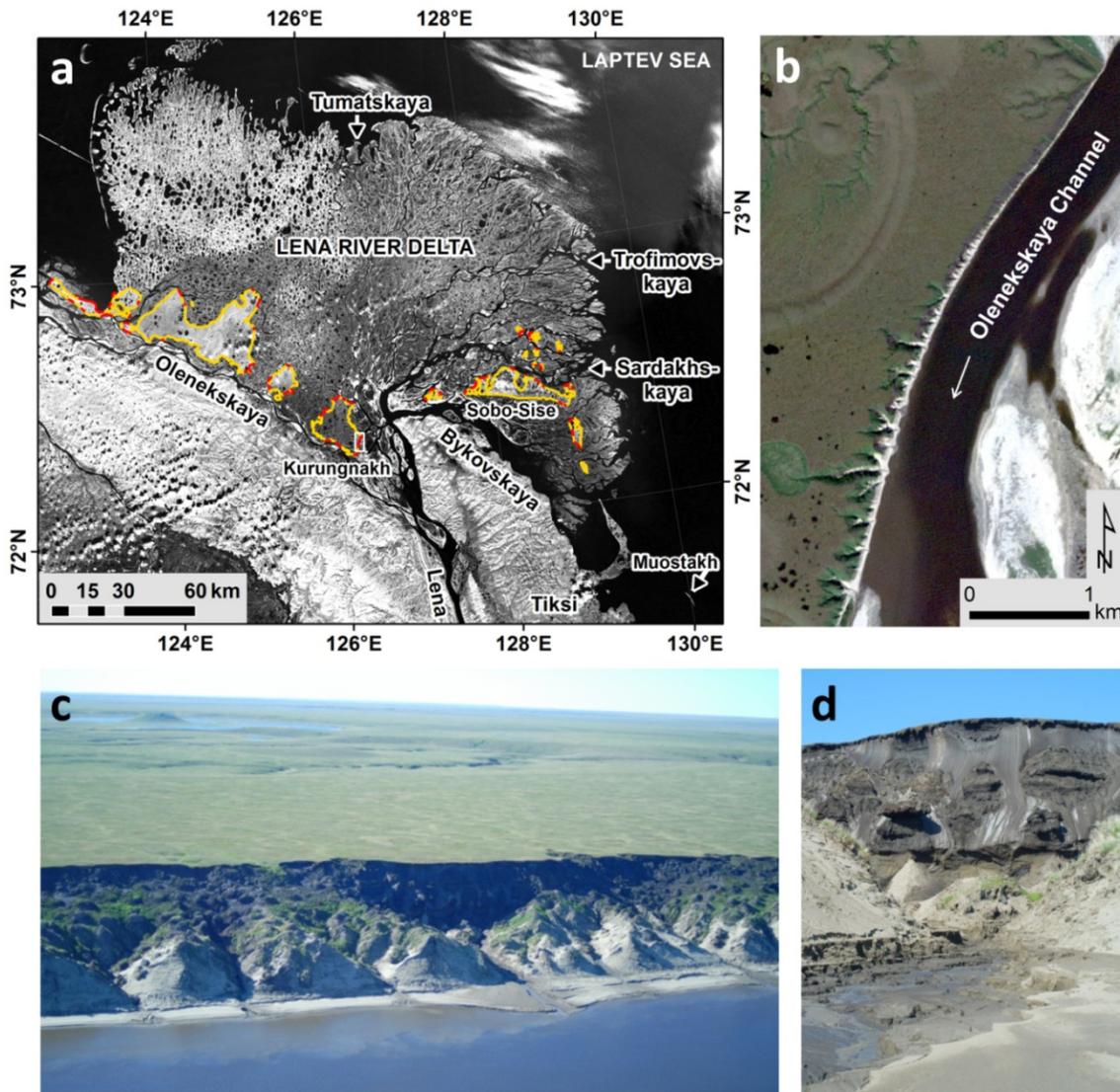


Fig. M4-1. **a** – Location of the study sites and distribution of ice complex deposits in the Lena Delta. Yellow outline: ice complex extent, red outline: direct contact of ice complex with delta channels or coastal waters, white rectangle: extent of **b**. (Landsat-7 ETM+ mosaic: GeoCover™ 2000, band 2) **b** – Extent of the key study location Kurungnakh Island: ice complex bluff with outflows eroded by the Olenekskaya Channel. (RapidEye, 4 August 2010, RGB 3-2-1) **c**, **d** – Examples of the ice complex exposures and outflows of Kurungnakh Island (photos by A. Morgenstern).

The field sampling campaigns took place in late summer of 2009, 2010, 2012, 2013 and 2014 as part of the Russian-German expedition program “Lena”. Samples were collected in the Lena Delta and in the coastal Laptev Sea. Water was sampled with pre-cleaned glass bottles. Routine water samples were filtered through 0.7 µm GF/F (Whatman, pre-combusted at 450 °C for 4 hr).

Hydrological measurements

Water discharges from the ice complex were estimated for two sites in the Lena Delta: a) the key study site on Kurungnakh Island and b) two sections of eroding ice complex cliffs at the northern coastline of Sobo-Sise Island. Discharge of all outflows that flowed from the actively degrading ice complex cliff into the delta channels was measured using the volumetric method and calculated as $Q = V \times t$, where Q is water discharge [L/sec]; V is water volume in beaker [L]; t is time of beaker filling [sec]. All methods used for water discharges measurements have been described in detail (Fedorova et al., 2015).

DOM degradation experiments

To assess bioavailable DOC, photo-degradable DOC and accompanying degradation processes DOM molecular changes, incubation experiments were performed. Prior to incubation experiments, permafrost outflow water was filtered through sterile 0.2 µm cellulose acetate filters to remove bacteria. Lena River water was filtered through 2.7 µm GF/C filters to remove phytoplankton but allow bacteria to stay in the water. For dark bacterial incubation 0.2 µm filtered permafrost outflow water was inoculated with 2.7 µm filtered Lena river surface water (19:1, 5 L in total). The mixture was poured into 500 mL glass bottles and incubated for 5, 11 and 18 days in triplicates at 20°C in darkness. Water, 0.2 µm filtered, used as control sample. For DOM photo-degradation experiments 500 mL of 0.2 µm filtered permafrost outflow water was filled into 1 L quartz glass bottles. The bottles were placed outside and were directly exposed to the sunlight. Five bottles were prepared for duplicate sampling on days 5 and 18, and a single sampling on day 11. Bacterial cell numbers of microbial degradation samples and of the control samples were analyzed by flow cytometry (FACS Calibur, Becton Dickinson). Before analysis fluorescent latex beads

(diameter 0.94 μm , Polysciences) were added to the diluted samples (1:8, sample/NaCl-solution) as an internal standard. SybrGreen (Invitrogen) was added to stain nucleic acids. TruCount beads (Becton Dickinson) were used for daily instrument calibration and calculation of the absolute volume (Gasol and del Giorgio, 2000).

DOC, TDN, solid phase extraction

DOC and TDN concentrations were measured using high temperature catalytic oxidation (TOC-VSPN, Shimadzu). DOM samples were concentrated and purified using solid phase extraction method on PPL sorbent (Dittmar et al., 2008). The DOC extraction efficiency was ~65%.

FT-ICR MS analysis, data evaluation and statistical methods

Ultra-high resolution mass spectra for DOM samples were acquired on Bruker Apex 12 Qe FT-ICR MS equipped with a 12 T superconducting magnet and an APOLLO II electrospray source. Sample order was randomized. SPE-DOM methanol extracts were adjusted to the same DOC concentrations (500 μM) by dilution with methanol/ water (1:1 MeOH/H₂O). Samples were analyzed with electrospray ionization (ESI) in negative ionization mode. FT-ICR MS data were evaluated in the mass range of 200-600 m/z. Details on spectra accumulation, calibration and formula assignments can be found elsewhere (Dubinenkov et al., 2015; Koch et al., 2007; Koch et al., 2005). It is important to note, that each molecular formula can represent a number of isomers (Hertkorn et al., 2008). In this article for appropriate readability, we generally refer to assigned molecular formulas as "compounds", "components", "markers" or "constituents". The peak magnitude weighted-average elemental ratios $\text{O}/\text{C}_{\text{wa}}$, $\text{H}/\text{C}_{\text{wa}}$, $\text{C}/\text{N}_{\text{wa}}$, double bond equivalents (DBE), DBE-O and mass defects were calculated from the assigned formulas (Dubinenkov et al., 2015). Multidimensional scaling (MDS) was based on the Bray-Curtis similarity (Bray and Curtis, 1957) and were performed using commercial software (PRIMER v.6; PRIMER-E Ltd, UK). All molecular changes in incubation experiments were matched with control sample. Indicator value analysis was performed according to a previous approach (Dufrene and Legendre

1997). We used molecular peaks (assigned formulas) as species and the relative intensity of peaks as species abundance. We re-normalized peak intensities in each sample to a lowest intensity and rounded obtained values to integer values. By this, we mimic species counts and minimum “specie” value in each sample was 1 compare (Kellerman et al. 2014). For the indicator value analysis we used “labdsv” package R software. Chemical richness and its estimations (Ciao 1 and ACE) were done in a similar way using “vegan” package of R.

Results and Discussion

Two key locations were studied that are representative for eroding ice complexes at the terrestrial-aquatic interface: the eastern shore of Kurungnakh Island (72°20' N, 126°17' E) with the adjacent Olenekskaya Channel (Lena Delta) and Muostakh Island (71°35' N, 130°00' E) located in the Buor-Khaya Bay (coastal Laptev Sea; Fig. M4-1a). The distance between the two locations is ~180 km. The islands of Kurungnakh and Muostakh are well studied in terms of ice complex characteristics and degradation (Morgenstern et al., 2011; Schirrmeister et al., 2011; Vonk et al., 2012). Both locations are characterized by intense mobilization of permafrost deposits (and consequently permafrost organic matter) via riverine/coastal erosion. The permafrost thaw and estimated coastal retreat rates are 2.9 m year⁻¹ and 1.8±1.3 m year⁻¹ for Kurungnakh and Muostakh, respectively (Günther et al., 2013).

Mineralization rates of permafrost-derived DOM

Extremely high and variable DOC and total dissolved nitrogen (TDN) concentrations (3890±2560 µM C (47±31 mg C L⁻¹), 266±161 µM N (3.7±2.3 mg N L⁻¹, n=35) were found for mud streams and permafrost outflows formed by the mobilization of thawed permafrost deposits. This high variability is caused by temperature and precipitation which control DOM leaching and dilution in the permafrost outflows (Guo et al., 2007). Since DOM in the permafrost outflows is subject to severe mineralization processes (Knoblauch et al., 2013; Vonk et al., 2013a) microbial and photo-degradation experiments were performed. The initial DOC concentration in both experiments was 10,700±33 µM C (129±0.4 mg C L⁻¹;

Fig. M4-2a). 18 days of dark microbial incubation resulted in a decrease to $6050 \pm 144 \mu\text{M C}$ ($72.6 \pm 1.7 \text{ mg C L}^{-1}$). Within the 18 days, the bacterial cell abundance increased by $1.3 \pm 0.4 \cdot 10^6 \text{ cells mL}^{-1} \text{ d}^{-1}$ (to $2.4 \pm 0.7 \cdot 10^7 \text{ cells}$) and $\sim 44\%$ of the DOC was mineralized ($260 \mu\text{M C d}^{-1}$ ($3.1 \text{ mg C L}^{-1} \text{ d}^{-1}$)). TDN decreased from $574 \pm 14 \mu\text{M N}$ to $428 \pm 58 \mu\text{M N}$. The C/N ratio decreased from 18.7 to 14.1 suggesting higher consumption rates of organic carbon compared to organic nitrogen.

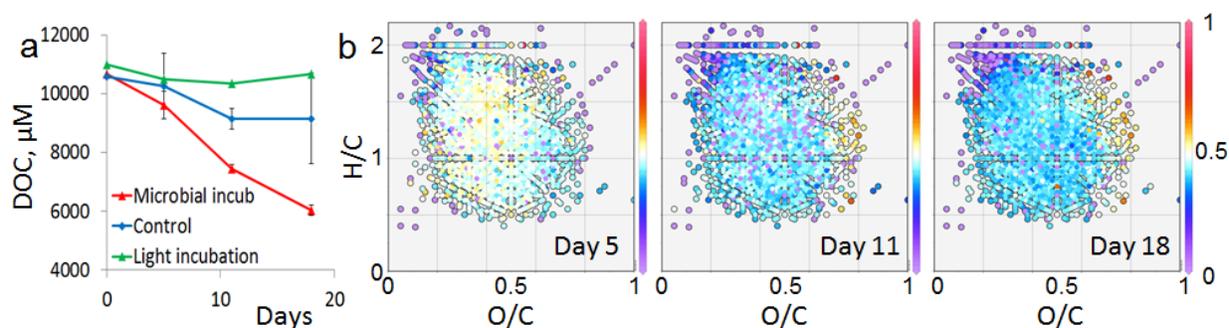


Fig. M4-2. DOC concentration and DOM molecular composition changes in bio-mineralization. **a** – DOC concentrations during the permafrost DOC bacterial and photo-incubation experiments; **b** – van Krevelen diagrams indicating DOM molecular transformations resulting from bacterial incubation. All formulas are represented by the color which reflects the ratio of the peak magnitude in the sample versus the respective magnitude in the controls ($\text{Sample} / (\text{Sample} + \text{Ref})$) (Koch et al., 2014).

The DOC decomposition during the photo-incubation experiment was only small and not significantly different from the control samples, suggesting only a minor contribution of direct photo-degradation to the permafrost-derived DOC mineralization. However, we have not assessed photo-stimulated bacterial respiration and DOC degradation rates in our experiments. There are evidences that photo-stimulation can alter the quality of terrestrial DOM and stimulate bacterial respiration (Judd et al., 2007; Tranvik and Bertilsson, 2001). Cory et al. (2014) showed that rates of photo-chemical oxidation exceed rates of microbial respiration of DOC in arctic streams and lakes and therefore significantly contributes to the carbon cycling in arctic surface waters (Cory et al., 2014). Our microbial mineralization rates were similar to published before confirming high biolability of ice complex-emitted DOM

(Balcarczyk et al., 2009; Vonk et al., 2013a). These decomposition rates fit well with the fact that small streams in permafrost areas are substantial sources of CO₂ to the atmosphere (Butman and Raymond, 2011; Denfeld et al., 2013). Therefore, the contribution of DOM microbial metabolism to CO₂ outgassing in the permafrost outflows and creeks is significant for the regional carbon cycling. Microbial metabolism not only mineralizes DOC, but will also change the DOM composition to a more recalcitrant state because labile components are removed, and more stable products of metabolism and turnover are generated.

Molecular prospective of permafrost-derived DOM microbial degradation

Molecular DOM transformations during the incubation experiments were recorded by FT-ICR MS and visualized with van Krevelen diagrams (Fig. M4-2b). Thousands of molecular components were detected in non-degraded and degraded DOM samples from the permafrost outflows. The bacterial degradation of DOM reduced not only the DOC concentration, but also the chemical richness and diversity of DOM: the microbial incubation resulted in a considerable compression of the molecular space with ~18% (from 4768 to 3907 elemental formulas) decrease of the chemical richness. Previously Kellerman et al. (2014) have demonstrated that the chemo-diversity of DOM is driven by numerous environmental factors (water residence time, precipitation, and temperature) on the regional scale level in arctic lakes. The contribution of microbial metabolism is relevant for the turnover of DOM in natural waters (Koch et al., 2014).

On the basis of our microbial degradation experiments we observed a bio-signature in the permafrost-derived DOM, a signature which has the material with a “fresh” and bio-labile character within the old organic matter from the ancient permafrost deposits (Fig. M4-2b, Fig. M4-S1-3). DOM in ice complex outflows is considered to be as old as >20,000 years (Spencer et al., 2015). This fresh and bio-labile material determines probably the DOC concentration changes and molecular transformations and may be supported by a rapid and dynamic response of bacterial communities released from the permafrost during

the thaw processes (Mackelprang et al., 2011). Hence, the introduction of reactive permafrost organic carbon could have critical implications for regional carbon cycling. The changes in the composition of the permafrost DOM is shown by the FT-ICR MS data: magnitude weighted elemental ratios changed during our microbial incubation experiment from 0.416 and 1.44 to 0.450 ± 0.006 and 1.35 ± 0.01 for O/C_{wa} and H/C_{wa} , respectively; the average DBE (degree of unsaturation of a molecular formula) increased from 5.52 to 6.26 ± 0.07 . All of these molecular changes are a result of oxidation of permafrost-derived DOM and a preferential removal of hydrogen-saturated components. Similar to the changes of the bulk DOC/DON ratio, the average C/N ratio decreased from 140 to 110 ± 8 indicating the increased relative contribution of nitrogen-containing moieties in degraded DOM. In contrast, the average C/S_{wa} ratio did not show considerable changes during the bacterial incubation.

The incubation experiments highlighted labile and relatively recalcitrant DOM components (Fig. M4-2b). Highly bio-labile components (>80% signal intensity decrease during incubation) were characterized by highly saturated elemental stoichiometry and represented ~9% of the total DOM molecular pool. The fraction, which persisted the 20-day incubation (<20% intensity decrease) represented ~13% of total number of identified molecular formulas. The majority of molecular transformations occurred after day 11 with minor changes after the 11th day of incubation. Permafrost-derived DOM is a good substrate for microbial metabolism ranging from the 9% of very labile DOM via a continuum of compounds with undefined bioavailability to resistant molecules. During the microbial processing the bio-signature vanished. Observed DOM quantitative and molecular tendencies are typical for DOM processing in the terrestrial-aquatic interface in permafrost organic matter mobilization processes.

Regional molecular transformation of DOM during terrestrial-aquatic transition

Continuous and reproducible molecular changes for DOM mobilized from permafrost deposits along the terrestrial-aquatic transition were recorded at our two key locations in the Lena Delta region with exceptional spatial resolution (Fig. M4-1). The DOC concentration in the coastal waters of the Laptev Sea

around Muostakh Island was $603 \pm 40 \mu\text{M C}$ (TDN $17 \pm 13 \mu\text{M N}$), in the Olenekskaya Channel at Kurungnakh Island it was $646 \pm 81 \mu\text{M C}$ (TDN $14 \pm 2 \mu\text{M N}$). The DOC concentration in permafrost outflows significantly differed from DOC in adjacent riverine and coastal waters.

Exploratory statistics (MDS, Fig. M4-S4) based on the molecular DOM composition revealed two distinct groups of samples: riverine/coastal DOM and DOM from permafrost outflows. DOM from permafrost outflows showed significantly (t-test, $p < 0.001$) higher chemical richness in comparison with riverine DOM: 5267 ± 410 vs. 4569 ± 310 elemental formulas (Fig. M4-S5). Chemical richness in the riverine DOM was $\sim 13\%$ lower in comparison to permafrost DOM. Comparable to our incubation experiment, ice complex outflow samples were characterized by a higher chemical richness due to the removal of the most labile and reactive components along the terrestrial-aquatic transition. O/C_{wa} , H/C_{wa} , C/N_{wa} and C/S_{wa} for riverine and permafrost DOM varied from 0.518 ± 0.05 to 0.507 ± 0.10 , from 1.11 ± 0.02 to 1.16 ± 0.04 , from 181 ± 7 to 76 ± 13 , from 668 ± 66 to 392 ± 120 , respectively. These ratios are typical for the higher relative contribution of hydrogen saturated and heteroatom moieties in permafrost-derived DOM compared to the riverine DOM.

The analysis of riverine and permafrost DOM molecular indicators (Dubinenkov et al., 2015; Dufrene and Legendre, 1997; Kujawinski et al., 2009) revealed group-specific molecular components (Fig. M4-3a,b). The riverine group of samples is characterized by the clear dominance of oxidized molecular indicators, predominantly $C_xH_yO_z$. Contrary, for DOM from permafrost outflows a significant contribution of heteroatomic moieties (mostly $C_xH_yO_zN_1$ and $C_xH_yO_zN_2$) and highly aliphatic compounds was observed.

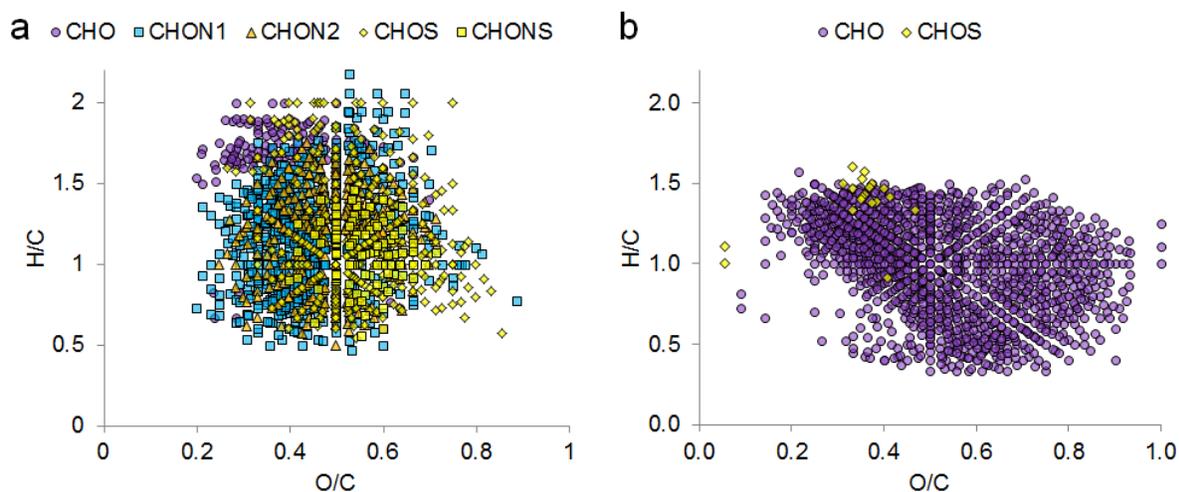


Fig. M4-3. *Molecular indicators. Van Krevelen diagrams of significant ($p < 0.005$) molecular indicators for: a – ice complex DOM, b – riverine DOM*

Riverine DOM molecular indicators matched well with semi-recalcitrant DOM components obtained from incubation experiments. A large fraction of permafrost DOM indicators matched well with the biodegradable DOM components revealed from the microbial incubation experiment. This fact suggests a significant role of microbial regulation in the molecular composition of DOM on the regional scale. The high number of samples and the broad regional coverage of our observations lead to these previously uncharacterized molecular patterns and changes making them representative for terrestrial-aquatic transitions. Thus, we experimentally and observationally assessed the molecular fraction of permafrost-derived DOM, which experiences rapid/active turnover and mineralization. This fraction actively contributes to the organic carbon cycling with a high potential of transformation to greenhouse gases.

Mobilization of permafrost DOM Role of permafrost-derived DOM in the Siberian Arctic

To assess the magnitude of DOM contribution from degrading ice complexes to the river and coastal waters, we measured and up-scaled water discharge and DOC concentrations. In addition to our 4.8 km long study section on Kurungnakh Island in the central part of the delta, a 4.8 km long study section on

Sobo-Sise Island in the eastern part of the delta was also studied for water discharge of ice complex outflows in 2014 (Fig. M4-1a). In sum, these two sections are representative for 14.7% (126.7 km) of the boundary of ice complex deposits in the Lena Delta (with total estimated length of 859.2 km) that are situated in direct contact with the delta channels or coastal waters. Here, at both study sections the ice complex is actively eroded by the delta channels, which results in the formation of steep cliffs with deep thermo-erosional ravines and ice complex outflows (Fig. M4-1c,d). The cumulative averaged water discharge of these ice complex sections for July and August of 2013 and 2014 ranged from $1.8 \text{ L s}^{-1} \text{ km}^{-1}$ to $16.2 \text{ L s}^{-1} \text{ km}^{-1}$, with an average of $9.6 \text{ L s}^{-1} \text{ km}^{-1}$. Similar fluxes were reported for the well-studied ice complex degradation area of Dyvanniy Yar at the Kolyma River, for instance $2.5\text{-}10 \text{ L s}^{-1} \text{ km}^{-1}$ (3.6 km section) in July 2010 (Vonk et al., 2013a). Assuming that this averaged value of water discharge is representative for the whole length of direct ice complex-channel contact region in the Lena Delta (red outlines in Fig. M4-1a), we calculated a total estimated direct discharge of 1216 L s^{-1} from ice complex outflows to the delta channels during the summer period. This is only a very small contribution to the large discharge of the Lena River itself with $33,467 \pm 9407 \times 10^3 \text{ L s}^{-1}$ ($n=154$, data for July and August 1935-2011). Hence, the discharge of the outflows of the ice complexes affected the shoreline in the Lena Delta was about 28,000 times lower than the Lena River discharge, which value vary depending on year and data. Here we considered only direct mechanisms of organic matter transport from ice complex deposits to adjacent waters of the Lena River. Certainly, there is a number of indirect organic matter transport mechanisms from permafrost deposits to the river with longer mobilization times and consequent degradation of organic matter.

Thermal erosion at coastlines and riverbanks is the most active material transport mechanism in permafrost degradation areas. The dominant mobilization mechanism on Muostakh Island is the coastal erosion with direct sediment transfer to coastal waters in frozen form. Total organic carbon (TOC) in the ice complex deposits of Muostakh Island at different depths ranged from 0.34 wt% to 14.1 wt%, $2.33 \pm 2.82 \text{ wt\%}$ (dry weight). The highest TOC content was found in peat layers (14.1 wt%). The majority

of this carbon is stored and mobilized in particulate form (particulate organic carbon, POC). DOC in thawed waters from permafrost deposits represented $1.0 \pm 0.9\%$ of the total TOC pool. Cold water extraction of organic matter was performed as a simulation of the coastal processes. It yielded additional 45% DOC. The estimated carbon flux arising from erosional processes at the arctic coast is 5.84-46.54 Tg C year⁻¹ (Lantuit et al., 2013), which is comparable to riverine carbon transport (18-26 Tg DOC year⁻¹ and 4-6 Tg year⁻¹ POC for all arctic rivers; 4.1-5.8 Tg DOC year⁻¹ (Dittmar and Kattner, 2003; Holmes et al., 2012) and ~ 0.38 Tg POC year⁻¹ (Semiletov et al., 2011) for the Lena River). The carbon flux load estimated from the whole ice complex in the delta to the river is about $0.3 \pm 0.2 \times 10^3$ Tg per summer season (July-August). In percentage, the carbon flux from ice complexes is 0.03-0.04 % of the riverine carbon transport during the summer period considering that 18% of annual carbon flux is transported during summer. Certainly, many processes further mobilize organic carbon from particulate to dissolved material, but DOM transported natively in dissolved form represents only a minor fraction of the mobilized permafrost organic matter. Additionally, 3.64 ± 0.34 molar % (1.04% to 6.33%) of permafrost-derived DOC existed in form of hydrolysable amino acids with high potential to biomineralization (unpublished data).

Conclusions

In summary, the total contribution of ice complex outflows to the riverine dissolved organic carbon at eroding river banks and coastal bluffs is minor with regard to the total water discharge of the large Siberian rivers. However, it is locally important, particularly due to high concentrations of biogeochemical constituents in the ice complex outflows. In contrast to these areas representing the direct terrestrial-aquatic interface with highly effective material transport from thawing permafrost, vast permafrost territories are only indirectly or over longer distances connected to rivers and coastal waters. POC as the dominant form of mobilized carbon in coastal erosion indicates the extreme importance of organic matter mobilization and degradation from particulate to dissolved form. Thus, the majority of DOM mobilization and mineralization (during permafrost thaw) occurs without long-distance transport.

This results in intense CO₂ outgassing not only from permafrost outflows, but from local creeks, lakes, wetlands and thawed permafrost. Local small-scale processes involving DOM during terrestrial-aquatic transition has a higher value for the CO₂ carbon cycle compared to organic matter transported to the rivers and coastal waters. However, processes on the shores and coasts result in a much faster feedback and their potential influence is more relevant in context of hydrology, hydrochemistry and biogeochemistry of the large arctic rivers and coastal Arctic Ocean.

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6. *Synthesis and Conclusions*

- Summarizing results of the investigation on DOM isolation procedures we would recommend a use of PPL sorbents which usually supplied in form of pre-packed SPE cartridges of different volume. The volume of the cartridge (the mass of sorbent) can be varied according to needs of researcher and needed amount of extracted material. This method is applicable for fresh and saline waters, yields in high DOC recovery (usually ~45-65%) and results in relatively representative and inorganic-free fraction of DOM. It is easy to handle experimentally in the field or onboard of research vessel. The extraction procedure does not require aggressive reagents like sodium hydroxide in case of XAD-8 extraction. PPL solid phase extraction technique doesn't require complicated and time consuming careful Soxhlet cleaning of the sorbent like in case of XAD-8 resin. Differences between DOM extracts generally reflected the difference in extraction mechanisms and have to be considered in future studies. DEAE method showed the highest DOM extraction efficiency of 82%, the lowest efficiency of ~50% was characteristic for XAD-8 resin. The C18 extracted samples were characterized with the highest affinity to aliphatic saturated structures. This was to expect from the highly hydrophobic nature of the corresponding sorbent. XAD-8 showed a higher affinity to aromatic DOM constituents. Among the non-ionic sorbents, PPL and C18 - isolates were characterized with the highest content of nitrogen in the extracted DOM. The DEAE-isolate was characterized with the highest oxygen content indicating enrichment with oxygenated compounds. It can be concluded that a use of three non-ionic sorbents yielded the DOM samples with much more similar elemental compositions as compared to the DEAE-cellulose isolate. The comparison of various extraction methods also highlighted the problem of the incomparability of DOM samples extracted using different protocols.
- Summarizing the study on DOM molecular composition in different waters of the Lena Delta it can be concluded that DOM samples have clear molecular systematics with respect to the

type of natural waters: Lena River and its channels, lake, permafrost meltwater creeks, permafrost outflows and coastal waters of the Laptev Sea. Molecular systematics allowed identification of source-specific DOM molecular markers and their relative contribution to DOM of different origin. The relative contribution of organic nitrogen was highest in the permafrost-derived samples. Bulk DOM parameters matched well with the molecular DOM fingerprints, especially with focus on the nitrogenous fraction. Molecular diversity in DOM from different origins can be explained by regulation of different environmental processes. Characterized source-specific and source-dominant molecular markers will allow in future estimation of contribution of different sources into DOM which integrates signals from various sources (e.g. riverine and estuarial DOM). Since we demonstrated significant differences of the DOM molecular composition of melt water creeks and the Lena River, the growing contribution of permafrost-derived DOM might also change the riverine biogeochemistry in the Lena Delta and the Laptev Sea with consequences on the ecosystem.

- Summarizing the study on DOM reactivity in the river-sea transition we showed that DOC exhibited a conservative behavior in the Lena River - Laptev Sea transition and passed the mixing zone almost quantitatively, but, simultaneously, thousands of DOM molecular components showed active involvement to the transitional processes. We observed strong physico-chemical gradients of salinity, temperature, pH, nutrients, and DOC on studied transects in the coastal Laptev Sea. The correlation of salinity and molecular peak intensity were successfully applied for the estimation of DOM reactivity in the river-sea transition. About 40% of DOM molecular pool was highly affected by processes, which occurred in the river-sea transition zone. 27% of all identified DOM molecular components exhibited strongly conservative behavior in the river-sea transition. We called this the “pseudo-conservative” behavior of DOM. Highly conservative components can find further applications as tracers for DOM components of terrestrial origin and for estimation of their persistence in the oceans. Reactive components are the subjects of future research in the

context of dominant DOM turnover and mineralization mechanisms and their contribution to the regional carbon cycle. These compounds are potential targets for the exploration of estuarine processes on the molecular level. The interpretation of detailed DOM compositional changes as result of estuarine processes is the aim of further studies.

- The study on mobilization, mineralization and turnover of permafrost-derived DOM highlighted several aspects with high relevance for the permafrost carbon cycle. Extremely high and variable DOC and TDN concentrations were found for mud streams and permafrost outflows formed by mobilization of thawed permafrost deposits. DOC concentration in permafrost outflows significantly differed from DOC in adjacent riverine and coastal waters. Within 18 days of dark microbial incubation ~44% of the DOC was mineralized. Photo incubation resulted in a relatively low DOC decomposition rate which was comparable with the control samples, suggesting minor contribution of direct photo degradation in permafrost-derived DOC mineralization. Observed decomposition rates fit well with the fact that small streams in permafrost areas are substantial sources of CO₂ to the atmosphere. Bacterial metabolism regulated not only DOC concentration, but chemical richness and molecular diversity of the permafrost-derived DOM: microbial incubation resulted in a considerable compression of the molecular space with ~18% decrease of chemical richness. Hence reintroduction of reactive permafrost carbon could have critical implications for regional carbon cycling. The diverse chemical nature of labile and relatively recalcitrant components indicates that chemical composition drives availability of molecules for microbial metabolism. All observed quantitative and molecular tendencies are illustrative for DOM processing on the terrestrial-aquatic interface in permafrost OM mobilization processes. Continuous and reproducible molecular changes for DOM mobilized from permafrost deposits along the terrestrial-aquatic transition were recorded. This fact suggested a significant role of microbial regulation in the molecular composition of DOM in the Lena Delta. Thus, we experimentally and observationally assessed the fraction of DOM which

actively contributes to carbon cycling with a high potential of transformation to greenhouse gases. In summary, the total contribution of ice complex outflows to the riverine OC at eroding river banks and coastal bluffs was minor. However, it is locally influential, particularly due to high concentrations of biogeochemical constituents in ice complex outflows. Thus, the majority of DOM mobilization and mineralization during permafrost thaw occurs without long-distance transport. Processes on the shores and coasts result in much faster feedback and their potential influence is more relevant in context of hydrology, hydrochemistry and biogeochemistry of the large arctic rivers and coastal Arctic Ocean.

The non-targeted mass-spectrometric characterization of DOM is not a routine tool in DOM research but the number of applications is continuously growing. In the last decade it found many applications not only in pure analytical chemistry of DOM but in characterization of biogeochemical properties and environmental roles of DOM in natural waters. It is important to note, that non-targeted approaches in DOM chemistry are still highly sophisticated, not unified and under development. Different research groups use diverse analytical protocols, which sometimes are not completely transparent and reproducible. Therefore, future methodologies of chemical analysis and data treatment/interpretation procedures have to be unified for an improved development of the field. FT-ICR MS is a semi-quantitative method in organic analytics of DOM and usually peaks are treated only based on their presence/absence. Recent studies showed informativity of peak intensities in mass spectra of DOM, they can be used for data mining in large datasets, but do not reflect concentrations of single molecular components.

In this thesis it was demonstrated that non-targeted analytics can be applied for studies on DOM biogeochemistry in the changing systems like the Arctic. The studies showed that although FT-ICR MS and NMR have many limitations, they can significantly deepen our understanding of DOM roles and behavior in natural waters. Therefore, in case of DOM analysis we are dealing with the puzzle which

cannot be assembled completely, but available pieces of the puzzle could be already sufficient to guess the silhouette of the big picture.

7. Outlook

One of the limiting factors in the analysis of DOM is the lack of structural molecular information. Without the knowledge on structure and concentrations of chemical compounds which constitute DOM, it is impossible to accurately determine its environmental and biogeochemical role in the natural system.

As it was demonstrated in Chapters 1, 2.6 and Chapters 5.1 to 5.4 the high sensitivity and high resolution organic analytics (e.g. MS and NMR) usually require pure DOM samples (mostly separation of inorganic ions is needed, especially in the case of marine waters). Although in this thesis (Chapter 5.1) and in a number of previous publications (Aiken et al., 1992; Serkiz and Perdue, 1990; Tfaily et al., 2012) the question of DOM isolation was addressed, further developments of DOM purification techniques are required. To achieve extraction of the most representative fraction of DOM and minimize DOM alteration during extraction and purification procedures it is necessary to avoid aggressive reagents, to work with the well-characterized sorbents and materials, and to establish effective dialogue of scientists with the sorbent and membrane manufacturers. I see a good perspective for the application of reverse osmosis – electro dialysis techniques which allow high DOM recovery rates and do not require pretreatment of natural waters with the aggressive reagents (Gurtler et al., 2008; Serkiz and Perdue, 1990; Vetter et al., 2007). One of the major disadvantages of this method is the time of the extraction procedure, which is extremely important in case of ship cruises and field laboratories in remote areas.

As it was demonstrated in Chapters 1, 2.6 and 5.1-5.4 ultra-high resolution mass spectrometric characterization of DOM allows the detection of thousands of DOM components. The high resolution is, undoubtedly, a great advantage of this technique regarding the amount of information which can be obtained from one sample. However, the molecular information is not quantitative and do not represent the entire chemical space and characteristics of DOM due to the technical limitations in ionization and detection of compounds. For including molecular specific data in models on organic carbon balance, the relative abundances of molecular compartments with different structural features are of particular importance. Applications of quantitative ^1H NMR, ^{13}C NMR and some multidimensional NMR

techniques would significantly simplify the characterization of DOM quality, transformation and roles in natural systems. Recently, a promising approach of direct ^1H NMR analysis of freshwaters was suggested (Lam and Simpson, 2008). It is important to note that like in MS characterization, NMR techniques usually require pre-concentration and purification of DOM sample for high quality of obtained analytical data.

One-dimensional NMR spectroscopy deserves specific consideration. One-dimensional ^{13}C and ^1H NMR spectra provide near quantitative data on the distribution of carbon and hydrogen, respectively, among major structural moieties within the molecular ensemble of DOM when appropriate conditions are used for their acquisition (Schmitt-Kopplin et al., 2010; Simpson et al., 2011). In case of the ^{13}C nucleus, these conditions include an appropriate time delay between the impulses to assure full relaxation of carbon atoms with different chemical environments (Kovalevskii et al., 2000). For proton measurements, specific care should be exercised with respect to the quantification of labile and exchangeable protons present in high quantities in the molecular ensembles of DOM and humic substances (Hertkorn and Kettrup, 2005; Kovalevskii et al., 2000). ^1H NMR spectroscopy is much more sensitive as compared to ^{13}C NMR, which makes it usually a method of choice for investigation of DOM in routine analyses. Application of modern data processing and exploration tools significantly deepen and extended the applicability and informational content of FT-ICR MS in DOM research (Kellerman et al., 2014; Lechtenfeld et al., 2014). Examples of statistical NMR data mining and exploration in characterization of environmental functions of DOM are still rare. Therefore, methods of multivariate statistics can be successfully applied to the NMR data on DOM structure without additional optimization of these methods; they can be directly ported from the FT-ICR MS studies. This will allow an in-depth characterization of the response of DOM functional groups (as characterized in ^1H NMR and ^{13}C NMR) to the environmental parameters, biological activity and reactivity in natural fresh and marine waters. Several examples of correlations of non-targeted MS with non-targeted NMR data of DOM already

showed high potential of this approach (2D correlation spectroscopy; Abdulla and Hatcher, 2014; Abdulla et al., 2013) but further method development and studies on its applicability are needed.

Several questions of current interest exist in studies on DOM in the Arctic, particularly in characterization of response of permafrost OM pool to the changing climate in the Arctic and on feedback of ecosystems to permafrost degradation (Schuur et al., 2008; Zhao-ping et al., 2010). In Chapter 5.4 and in a number of previous publications (Spencer et al., 2015; Vonk et al., 2013a) it was shown that permafrost-derived DOM has a high potential to mineralization and actively contributes to microbial metabolism. Although some estimation on mobilization of permafrost-derived OM was done (Chapter 5.4), this question still requires clarification in large scale models of carbon dynamics in the Arctic. It is known, that the majority of permafrost carbon is mobilized in particulate form (Lantuit et al., 2013, Chapter 5.4). Therefore particular attention has to be addressed to solid – aqueous phase transition upon permafrost thaw and degradation. A series of experiments on unaltered but thawed permafrost have to highlight mobilization potential and major drivers of this mobilization with respect to OM release and mineralization. I suppose that transition of OM from the particulate to the dissolved phase is controlled mostly by the microbial activity, since it was demonstrated that physical water extraction of OM from solid permafrost sediments contributes only marginally to the carbon budget. Microbial activity and dramatic shifts in microbial communities in thawed permafrost are supported by observations of Mackelprang et al. (2011). Ideally, a series of experiments on thawed permafrost would provide very valuable knowledge on carbon dynamics upon permafrost thaw. This would include the characterization of the response of microbial communities over incubation of thawed permafrost, mobilization and emission of DOM over incubation of sediments, CO₂ and CH₄ outgassing and continuous control of radiocarbon age of outgassed CO₂. These incubations of thawed permafrost can last from three to four months simulating the duration of summer in the Arctic. The radiocarbon age characterization will allow answering the question on extreme biodegradability of the ancient permafrost in comparison with the modern one. Preliminary experiments on radiocarbon characterization were provided before by Spencer et

al. (2015) and showed preferable decomposition of the ancient fraction of mobilized from permafrost OM.

All listed questions and methodological approaches logically follow the Chapters 1 to 5 and clearly demonstrate potential research directions for future studies on DOM with the emphasis on the carbon dynamics in the Arctic.

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Supplementary Information

Supplementary Information to Manuscript 1

“Molecular mapping of sorbent selectivities with respect to isolation of dissolved organic matter as measured by Fourier transform mass spectrometry”

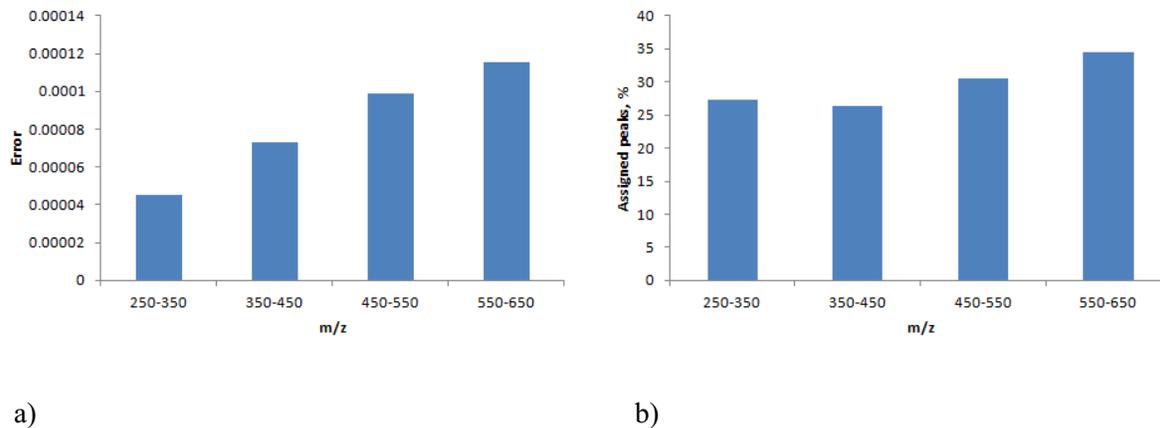


Fig. M1-S1. Quality assurance of formula assignments (on the example of XAD-8-RK sample):
(a) Root mean squared (rms) errors for assigned molecular formulas as a function of mass;
(b) Percentage of assigned molecular peaks within selected mass range.

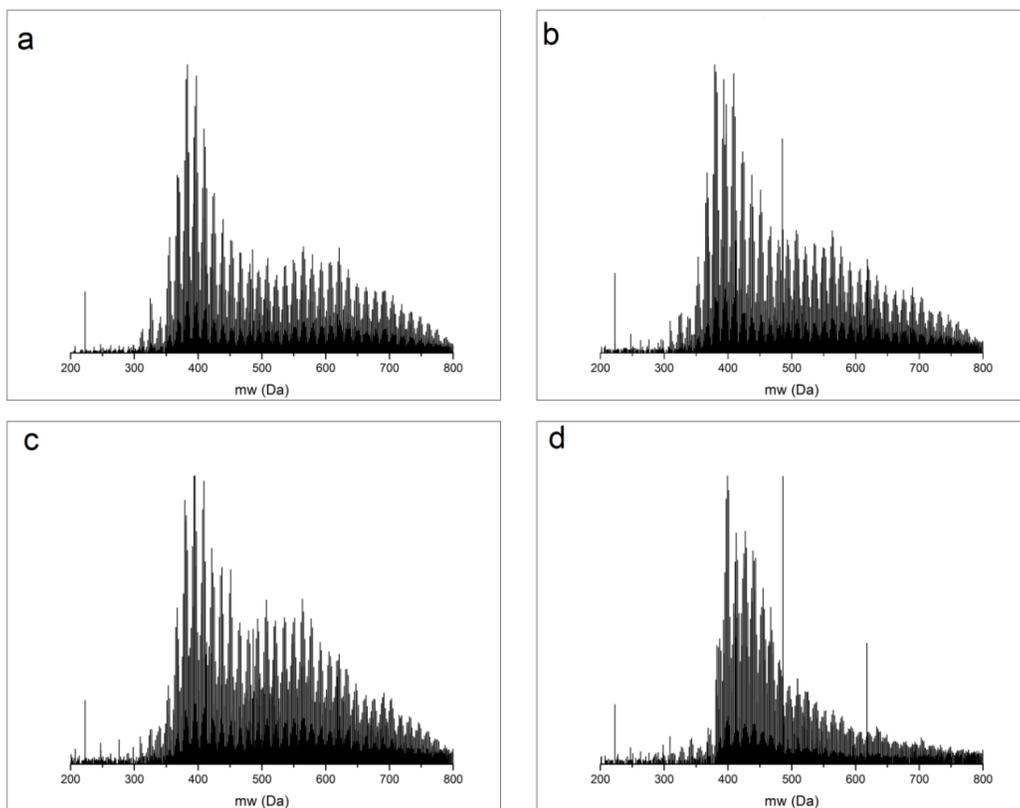


Fig. M1-S2. ESI FTICR mass spectra of the DOM samples isolated from the Kolyma River using different sorbents: (a) C18-RK, (b) PPL-RK, (c) XAD-8-RK, (d) DEAE-RK . Solvent – methanol, concentration 0.5 g/L, electrode potential (EP) 2.9 kV.

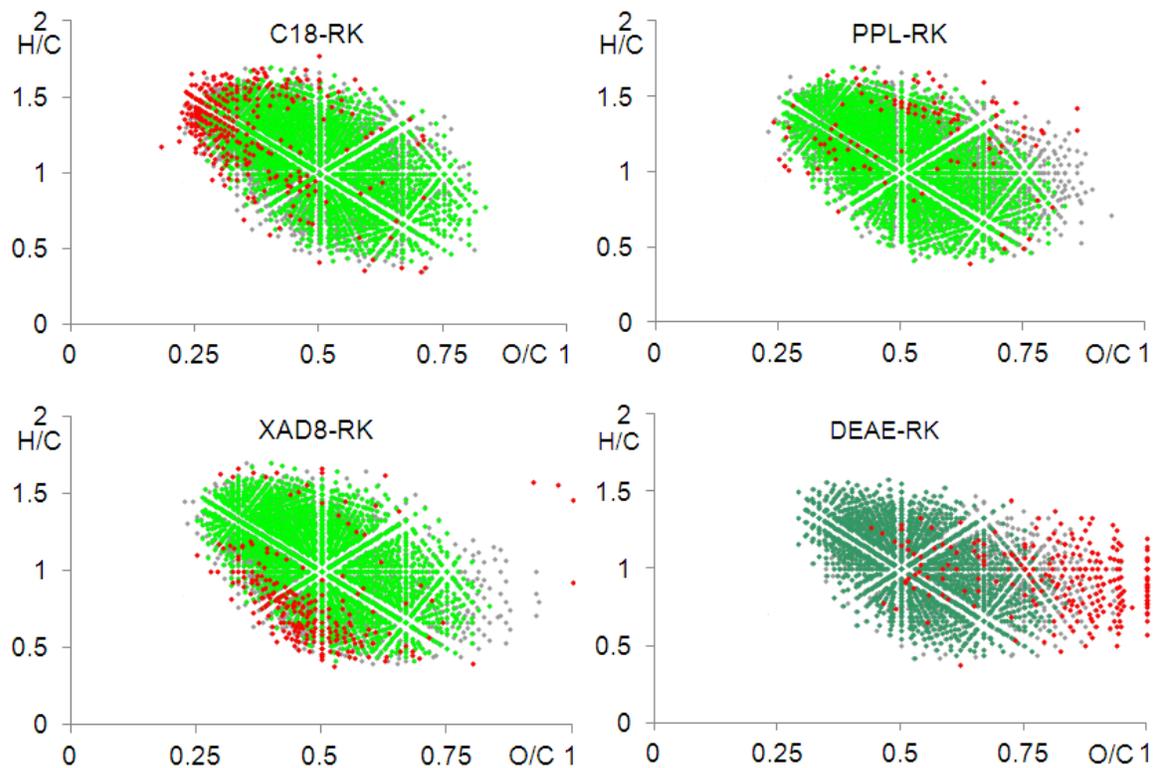


Fig. M1-S3. Van Krevelen diagrams for the Kolyma River DOM obtained from the assigned formulas (CHO-only) in each sample: a) C18-RK, b) PPL-RK, c) XAD-8-RK, and d) DEAE-RK. Light green dots in a-c represent common identifications for the three non-ionic sorbents studied, dark green dots in d represent common identifications for all four sorbents studied, red dots - unique compositions for each sorbent, grey dots – residual identifications.

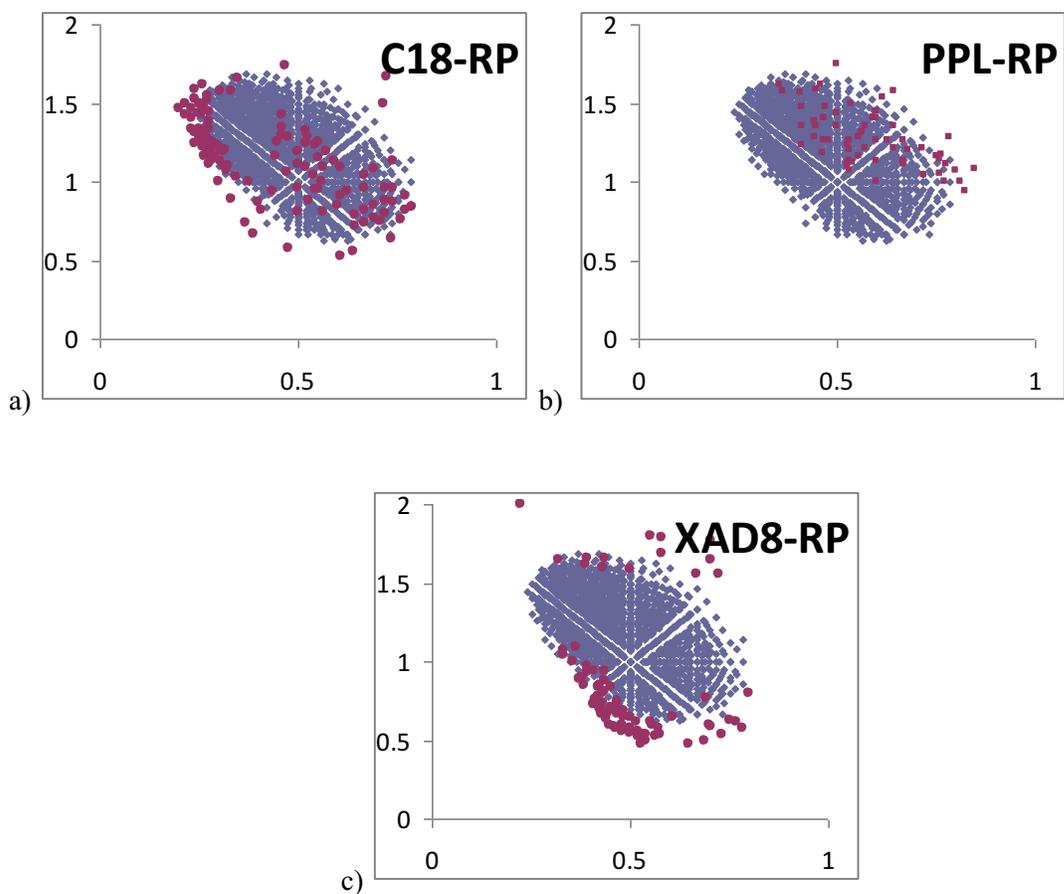
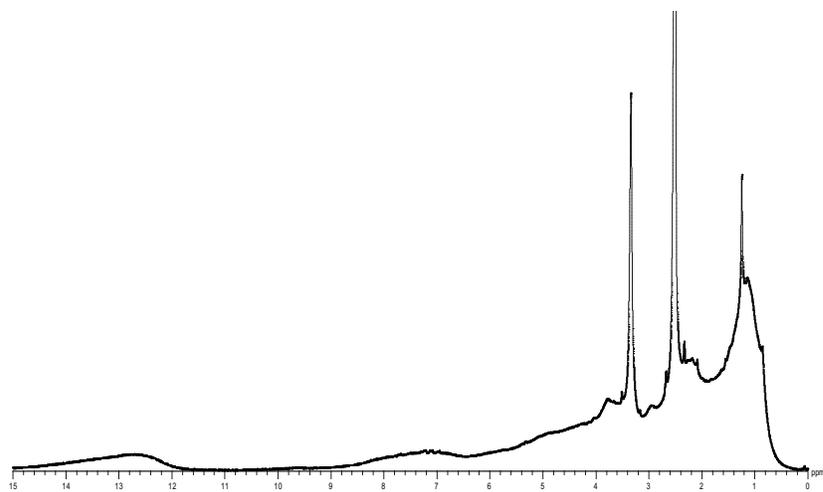
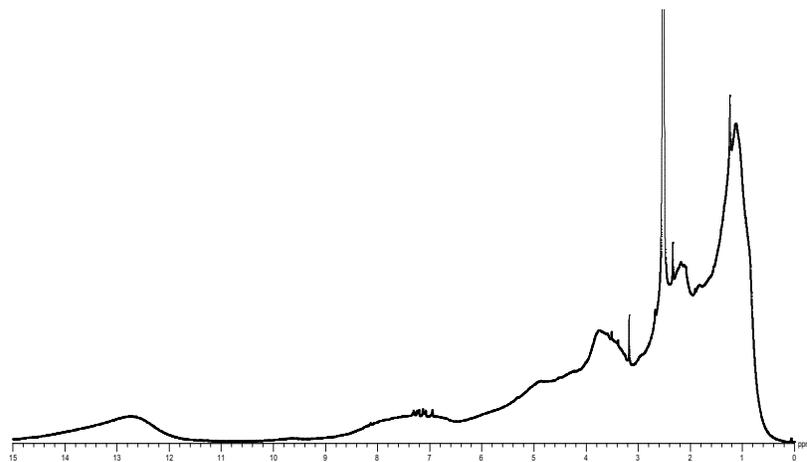


Fig. M1-S4. Van Krevelen diagrams for the Panteleikha River DOM obtained from the assigned formulas (CHON) in each sample: a) C18-RP, b) PPL-RP, c) XAD-8-RP. Blue dots represent common identifications for the three non-ionic sorbents studied, red dots - unique compositions for each sorbent.



a)



b)

Fig. M1-S5. ¹H NMR spectra of the Panteleikha River DOM isolated by two different sorbents:
a -PPL-RP , b – XAD-8-RP.

Supplementary Information to Manuscript 2

Origin-specific molecular signatures of dissolved organic matter in the Lena Delta

Table M2-SII. Calculated mean and standard deviations of DOC ($\mu\text{mol C L}^{-1}$) and TDN ($\mu\text{mol N L}^{-1}$) concentrations for river, bay, creek and ice complex groups of samples.

Sample Type	bay	ice complex	creek	river
# of sample	13	8	6	51
Mean DOC	484	3913	399	492
Stdev DOC	55	1780	115	76
Mean TDN	13.7	220.3	19.7	13.1
Stdev TDN	1.7	137.7	7.1	2.8

Table M2-SI2. Summary of molecular FT-ICR-MS analyses. Number of identified formulas, the intensity-weighted average molecular weight (m/z_{wa}), double bond equivalents (DBE_{wa}), elemental compositions and elemental ratios of the Lena Delta DOM sample extracts.

Sample №	Sample type	Identified formulas	m/z_{wa}	DBE_{wa}	C_{wa}	O_{wa}	N_{wa}	O/C_{wa}	H/C_{wa}	C/N_{wa}
82	bay	3098	392.72	8.45	19.206	8.533	0.102	0.444	1.230	187.390
83	bay	3070	393.95	8.60	19.285	8.566	0.095	0.444	1.217	202.310
84	bay	3181	393.66	8.56	19.276	8.553	0.105	0.444	1.221	183.210
85	bay	3249	390.08	8.33	19.073	8.457	0.114	0.443	1.237	167.030
86	bay	3325	393.23	8.33	19.130	8.572	0.140	0.448	1.241	136.280
75	bay	2737	394.48	8.45	19.281	8.585	0.106	0.445	1.233	181.300
9	river	3186	390.17	8.20	19.224	8.351	0.108	0.434	1.257	178.230
10	river	3318	387.69	7.93	19.230	8.124	0.139	0.422	1.287	138.580
11	river	3264	389.79	8.20	19.280	8.251	0.136	0.428	1.260	141.750
12	river	3073	389.75	7.97	19.410	8.099	0.142	0.417	1.289	136.490
13	river	3088	389.45	7.92	19.413	8.067	0.144	0.416	1.294	134.560
14	river	2981	391.67	8.04	19.427	8.214	0.139	0.423	1.282	139.800
15	river	3012	391.18	7.99	19.397	8.200	0.144	0.423	1.287	135.090
18	river	2917	393.19	8.13	19.491	8.262	0.136	0.424	1.276	143.360
19	river	2988	392.39	8.06	19.395	8.282	0.144	0.427	1.280	134.690
25	river	2944	392.49	7.96	19.464	8.213	0.143	0.422	1.292	136.440
41	river	2558	391.62	8.11	19.445	8.215	0.121	0.423	1.275	160.300
53	river	3161	393.01	8.05	19.501	8.221	0.142	0.422	1.284	137.530
55	river	3158	393.51	7.96	19.570	8.172	0.135	0.418	1.296	144.960
60	river	2590	390.49	7.83	19.155	8.305	0.182	0.434	1.296	105.400
62	river	2948	391.44	8.00	19.379	8.235	0.142	0.425	1.284	136.490
65	river	2691	387.10	7.80	19.062	8.213	0.140	0.431	1.294	136.610
68	river	2721	393.63	8.23	19.513	8.292	0.120	0.425	1.265	162.150
69	river	2749	393.26	8.26	19.370	8.415	0.109	0.434	1.256	177.530
36	creek	3209	386.12	7.68	19.190	8.015	0.158	0.418	1.312	121.600
37	creek	3508	381.68	7.50	18.763	8.036	0.206	0.428	1.318	91.060
39	creek	3174	382.60	7.58	18.861	8.024	0.203	0.425	1.313	92.830
71	creek	3230	383.54	7.66	18.764	8.208	0.156	0.437	1.298	120.640
48	lake	3507	382.36	7.36	18.819	8.017	0.198	0.426	1.334	95.120
76	creek	3661	386.60	8.06	18.562	8.534	0.215	0.460	1.250	86.480
77	creek	3520	386.09	7.78	18.708	8.413	0.170	0.450	1.285	109.870

Table M2-SI3. Peak-magnitude weighted average elemental ratios (O/C, H/C and C/N) and standard deviations for river, creek, ice complex and bay samples.

Sample type	Bay	Ice complex	Creek	River
mean O/C	0.445	0.455	0.427	0.425
stdev O/C	0.002	0.007	0.007	0.006
mean H/C	1.230	1.268	1.315	1.281
stdev H/C	0.009	0.024	0.013	0.013
mean C/N	176.253	98.175	104.250	143.331
stdev C/N	22.626	16.539	15.471	17.060

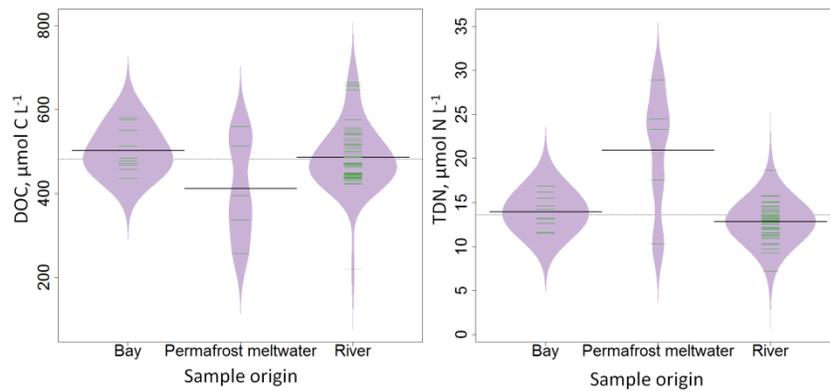


Fig. M2-SI1. DOC ($\mu\text{mol C L}^{-1}$) and total dissolved nitrogen (TDN) ($\mu\text{mol N L}^{-1}$) concentrations for the Buor-Khaya Bay ($n=13$ samples), permafrost melt waters ($n=6$) and Lena River and its channels ($n=51$). In the bean plots single data values (short green lines), median values (black lines), and the distribution of concentrations (grey-shaded areas) are displayed.

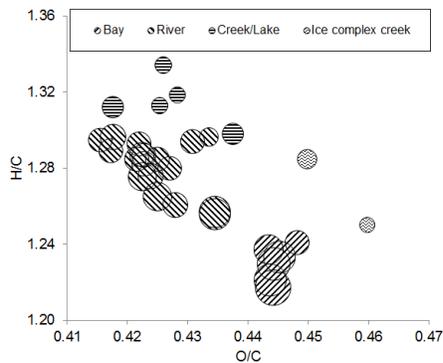


Fig. M2-SI2. Van Krevelen diagram for weighted average oxygen to carbon and hydrogen to carbon ratios calculated from FT-ICR mass spectra. Size of the bubbles indicates the value of the C/N ratio.

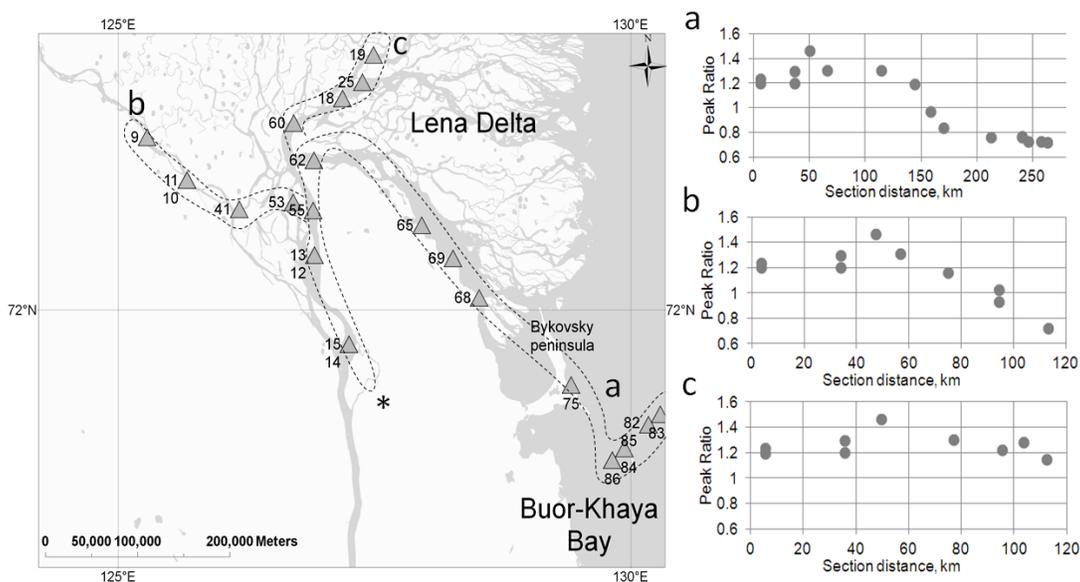


Fig. M2-SI3. Changes of peak magnitude ratios along the Lena River transects: the sum of the peak magnitudes for the 500 molecular formulas with the highest PC1 loadings was calculated and divided by the sum of peak magnitudes for 500 formulas with the lowest PC1 loadings. Upstream-downstream transects: (a) Bykovskaya channel (the main stem channel), (b) Olenekskaya channel, (c) Trofimovskaya channel, (*) starting point for all transects.

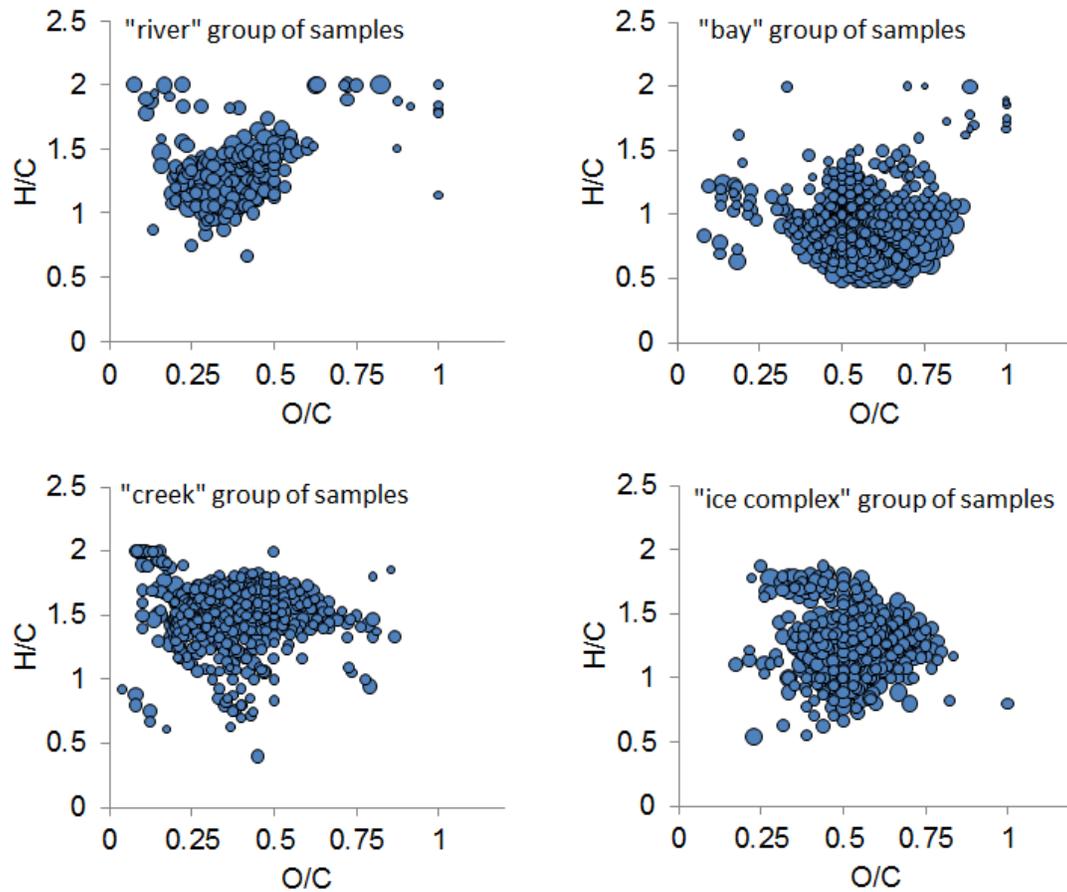


Fig. M2-SI4. Van Krevelen diagrams for indicator values for riverine, bay, creek and ice complex groups of samples. Size refers to corresponding indicator values.

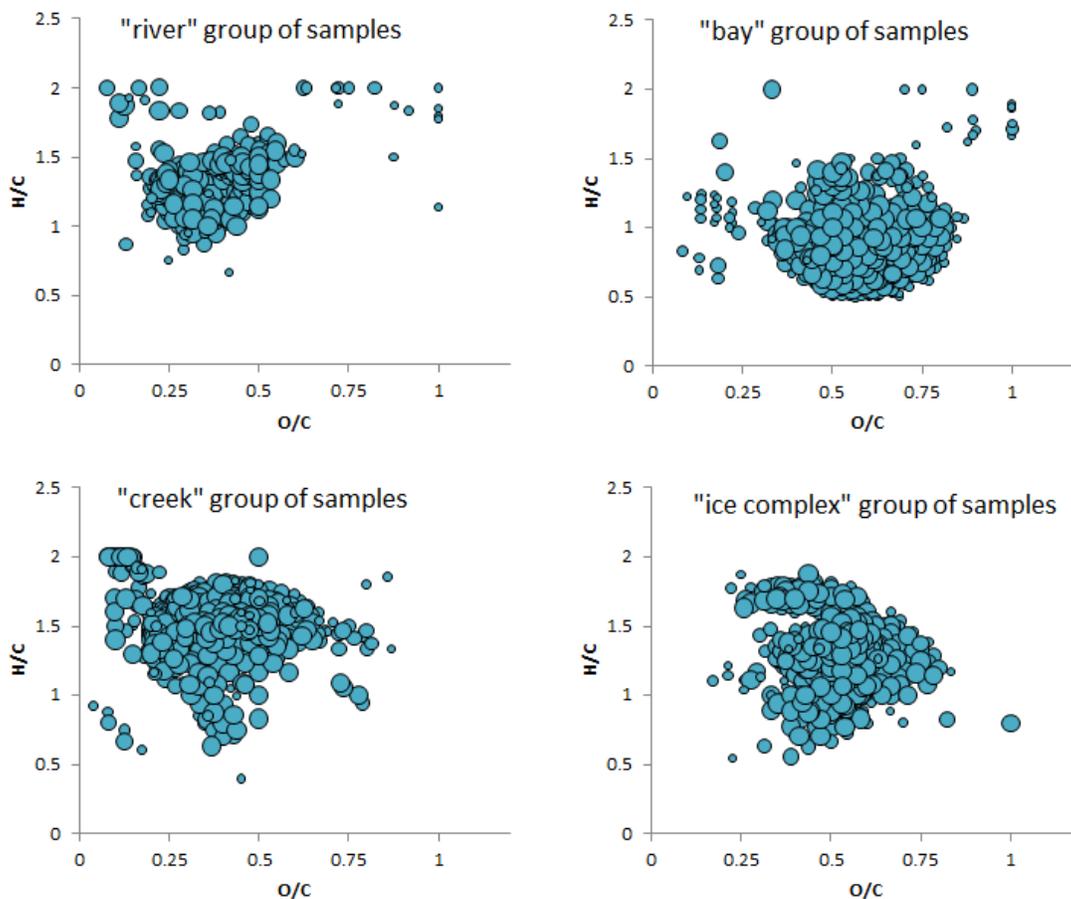


Fig. M2-SI5. Van Krevelen diagrams for indicator values for riverine, bay, creek and ice complex groups of samples. Size refers to frequency of a peak appearance.

Table M2-SI4. Significant indicator values, *p* values and frequencies for river, creek, bay and ice complex groups of samples. (Due to the large size of the table it is not included to the supplementary information. The table is available online: http://static-content.springer.com/esm/art%3A10.1007%2Fs10533-014-0049-0/MediaObjects/10533_2014_49_MOESM1_ESM.docx)

Supplementary Information to Manuscript 3

“Molecular reactivity of dissolved organic matter along the Lena River-Laptev Sea transition”

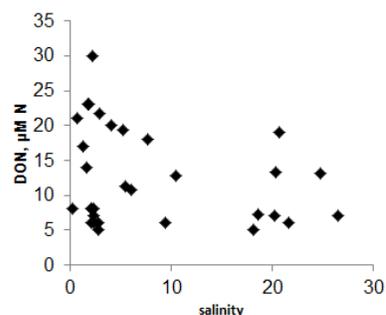


Fig. M3-S1. DON concentrations with respect to salinity in the coastal zone of the Laptev Sea and in the Laptev Sea.

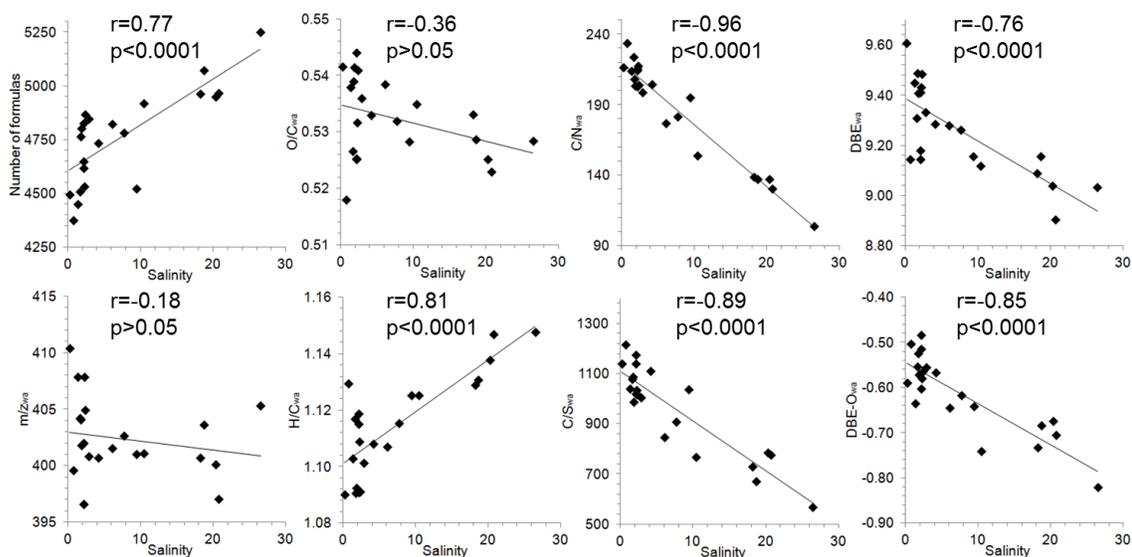


Fig. M3-S2. Number of formulas, m/z_{wa} , O/C_{wa} , H/C_{wa} , C/N_{wa} , C/S_{wa} ratios, DBE_{wa} and $DBE-O_{wa}$ for estuarine samples vs salinity. “wa” stands for weighted average, which were calculated from FT-ICR MS data. Only coastal samples are plotted here. r is the correlation coefficient and p is a significance level of salinity-weighted average parameter correlation.

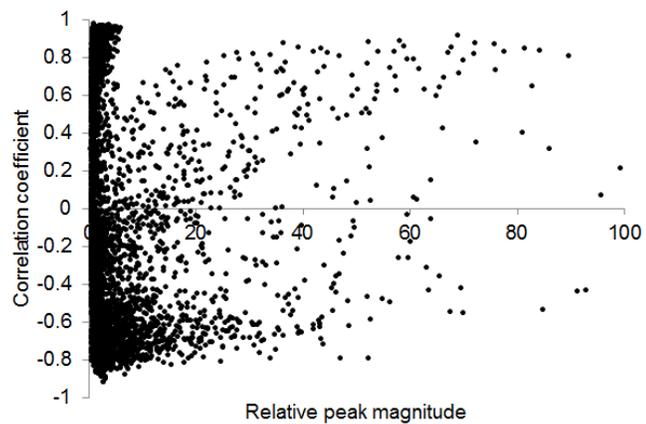


Fig. M3-S3. Dependence of the correlation coefficient (single peak magnitude vs salinity) for the relative magnitude of each mass peak in the spectra.

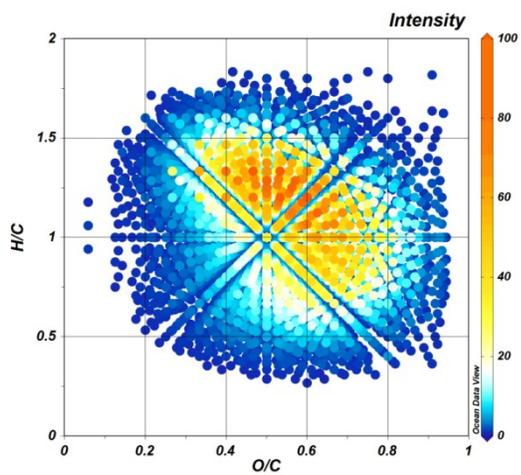


Fig. M3-S4. Van Krevelen Diagram with color indication of relative peak magnitude for Sample 52.

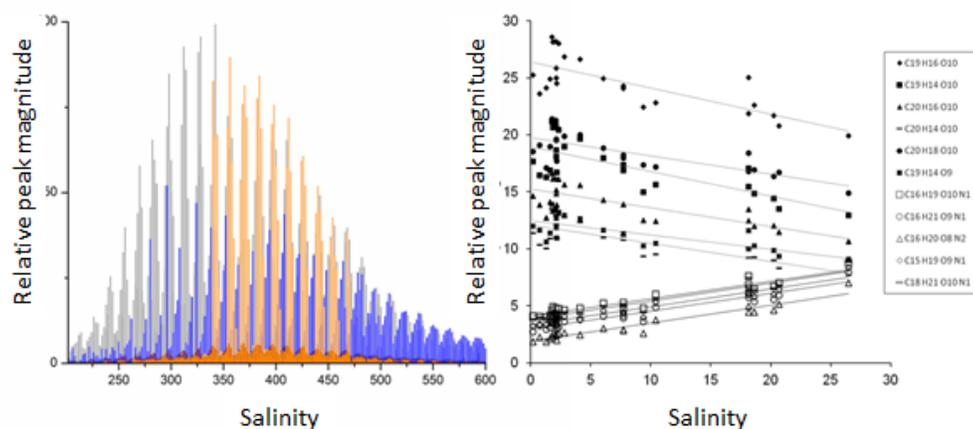


Fig. M3-S5. (a) Characteristic FT-ICR mass spectra of the Lena estuary DOM. Color indicates relation of peak magnitudes with respect to salinity in the estuary: orange - highly significant positive correlation, blue - highly significant negative correlation ($p < 0.001$), gray - weak and non-significant correlation. **(b)** Selected molecular formulas exhibiting a highly significant positive or negative correlation ($p < 0.001$) between relative peak magnitude and salinity

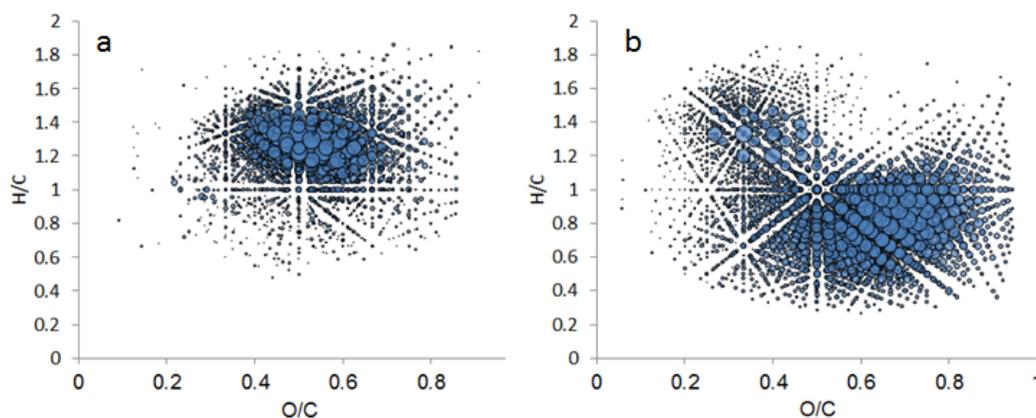


Fig. M3-S6. Van Krevelen diagrams with slopes of modelled linear equations of peak magnitude – salinity dependence, bubble size corresponds to the absolute value of the slope (higher the value – larger the bubble). **(a)** – positive slopes. **(b)** – negative slopes

Table M3-S1. Number of formulas and magnitude-weighted parameters including m/z_{wa} , C_{wa} , O_{wa} , N_{wa} , H/C_{wa} , O/C_{wa} , N/C_{wa} , DBE_{wa} , AI_{wa} for DOM samples from the Lena Delta, Lena River and river-sea transitional zone.

Sample	Number of formulas	m/z_{wa}	DBE_{wa}	C_{wa}	O_{wa}	N_{wa}	O/C_{wa}	H/C_{wa}	C/N_{wa}	C/S_{wa}
L10_001	5516	381	8.44	17.4	9.3	0.2	0.53	1.16	85.88	395.78
L10_002	6107	396	9.11	18.1	9.7	0.23	0.53	1.12	79.85	487.49
L10_003	5260	388	8.65	17.8	9.4	0.2	0.53	1.15	88.52	593.82
L10_018	4971	396	8.93	18.1	9.7	0.19	0.54	1.13	96.42	619.81
L10_028	6050	387	8.28	18.3	8.9	0.22	0.48	1.22	83.38	485.85
L10_030	4343	405	9.33	18.7	9.9	0.09	0.53	1.11	215.39	962.8
L10_031	5831	395	9.11	18.0	9.8	0.22	0.55	1.11	81.14	557.58
L10_032	5894	398	9.5	18.3	9.8	0.2	0.53	1.08	91.48	730.57
L10_033	5422	394	8.85	17.9	9.7	0.21	0.54	1.14	85.15	567.18
L10_034	5386	380	8.37	17.9	8.8	0.21	0.49	1.19	86.69	817.58
L10_035	5759	387	8.69	17.7	9.5	0.21	0.54	1.14	82.74	431.07
L10_036	5848	392	9.25	18.2	9.5	0.22	0.52	1.1	83.74	1359.74
L10_037	5800	398	9.52	18.1	10.0	0.21	0.55	1.07	87.53	597.37
L10_038	5720	391	8.95	17.9	9.6	0.22	0.54	1.12	80.42	553.79
L10_039	4126	391	8.83	18.4	9.3	0.08	0.5	1.16	238.32	1306.14
L10_042	4430	403	9.34	18.7	9.9	0.08	0.53	1.11	222.23	1079.55
L10_043_1	4189	389	8.63	18.8	8.8	0.07	0.47	1.19	251.53	1220.85
L10_043_2	4231	395	8.87	18.8	9.2	0.08	0.49	1.17	247.36	1187.41
L10_048	4514	410	9.59	18.8	10.2	0.09	0.54	1.09	206.03	990.35
L10_049	4439	408	9.43	18.8	10.1	0.09	0.54	1.11	211.82	1030.02
L10_050	4462	409	9.52	18.8	10.1	0.09	0.54	1.1	217.66	1059.25
L10_051	4440	409	9.53	18.8	10.1	0.08	0.54	1.1	224.39	1106.35
L10_052	4768	404	9.49	18.6	10.0	0.09	0.54	1.09	208.61	1088.2
L10_053	4969	397	8.91	18.4	9.6	0.14	0.52	1.15	130.6	777.25
L10_054	4805	402	9.41	18.4	10.0	0.09	0.54	1.09	203.52	989.69
L10_055	4737	401	9.29	18.5	9.9	0.09	0.53	1.11	204.71	1110.6
L10_056	4967	401	9.09	18.4	9.8	0.13	0.53	1.13	139	729.36
L10_057	4651	397	9.15	18.4	9.7	0.09	0.53	1.12	215.51	1141.41
L10_058	4850	401	9.33	18.5	9.9	0.09	0.54	1.1	199.12	1005.24
L10_059	4787	403	9.26	18.6	9.9	0.1	0.53	1.12	181.6	908.74
L10_060	5076	404	9.16	18.6	9.8	0.14	0.53	1.13	137.32	672.39
L10_062	4924	401	9.12	18.4	9.9	0.12	0.53	1.13	154.48	768.16
L10_063	4827	402	9.28	18.4	9.9	0.1	0.54	1.11	176.9	849.14
L10_071	4831	402	9.41	18.4	10.0	0.09	0.54	1.09	203.16	1019.36
L10_072	4497	410	9.61	18.8	10.2	0.09	0.54	1.09	216.87	1140.13
L10_073	4513	404	9.31	18.7	9.9	0.08	0.53	1.12	223.79	1078.22
L10_074	4380	400	9.15	18.6	9.7	0.08	0.52	1.13	233.92	1217.03
L10_075	4536	408	9.43	18.8	10.0	0.09	0.53	1.11	217.8	1035.56
L10_076	4455	408	9.45	18.8	10.1	0.09	0.54	1.1	214.32	1041.19
L10_077	4525	401	9.16	18.5	9.8	0.09	0.53	1.13	195.3	1036.87
L10_078	4871	405	9.49	18.6	10.1	0.09	0.54	1.09	204.45	1017.49
L10_079	5254	405	9.03	18.6	9.9	0.18	0.53	1.15	104.47	570.74
L10_082	4621	397	9.18	18.4	9.7	0.09	0.53	1.12	214.75	1177.24
L10_083	4952	400	9.04	18.5	9.7	0.13	0.53	1.14	137.67	786.35

Supplementary Information to Manuscript 4

“Mobilization, mineralization and turnover of permafrost-derived organic matter”

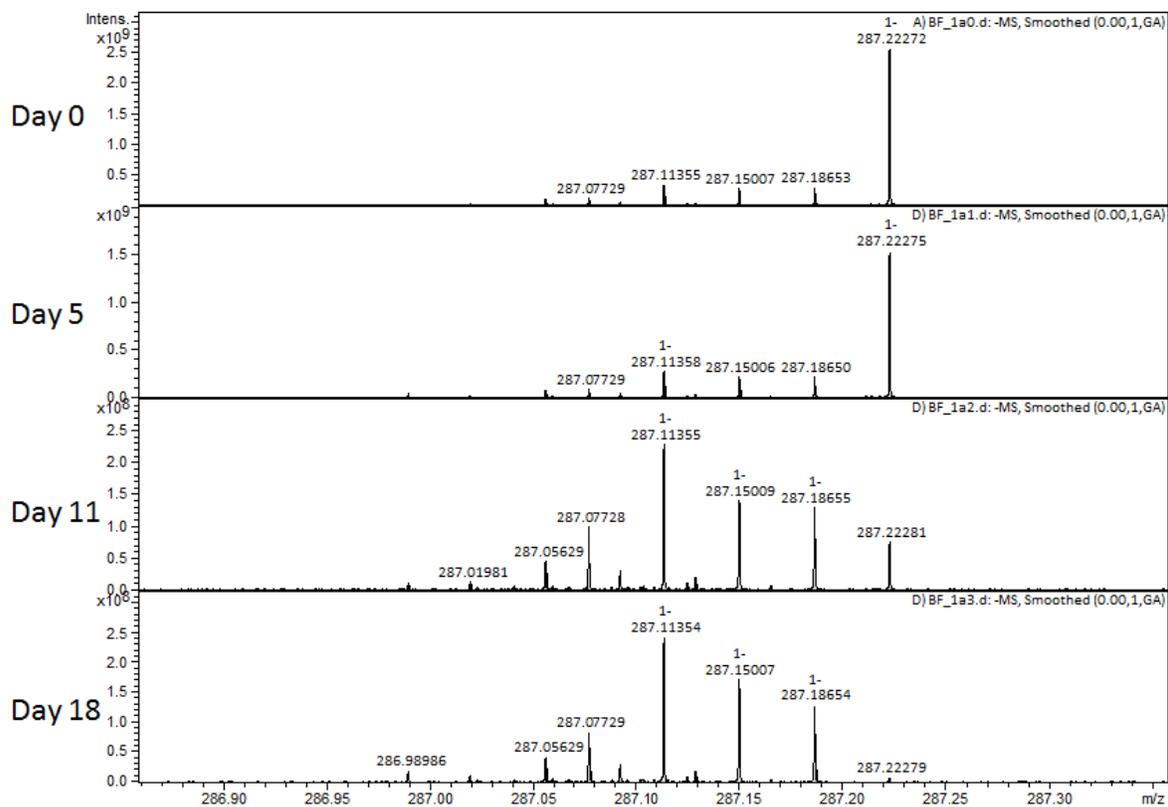


Fig. M4_S1. Molecular bio signatures on the nominal mass of 287 and their vanishing over the incubation experiments.

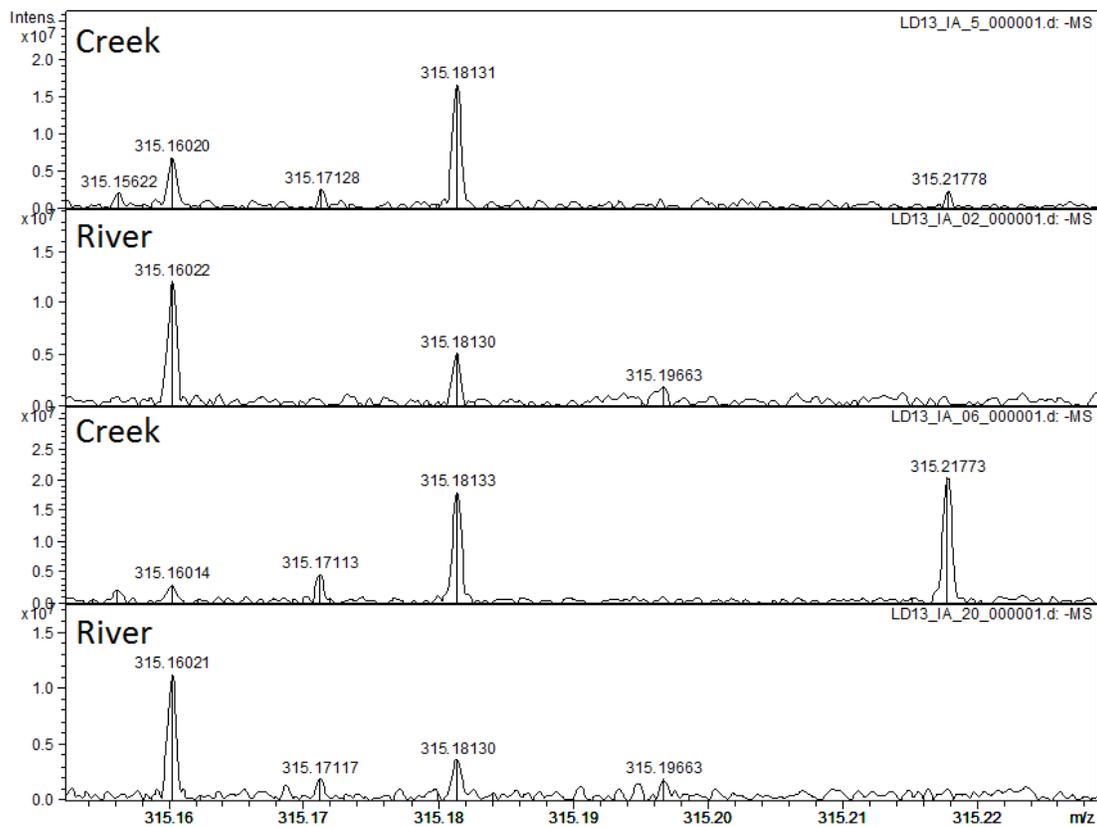


Fig. M4-S2. Differences in molecular signatures of DOM from Kurungnakh permafrost outflows and adjacent waters of Olenekskaia branch of the Lena River. 315 m/z.

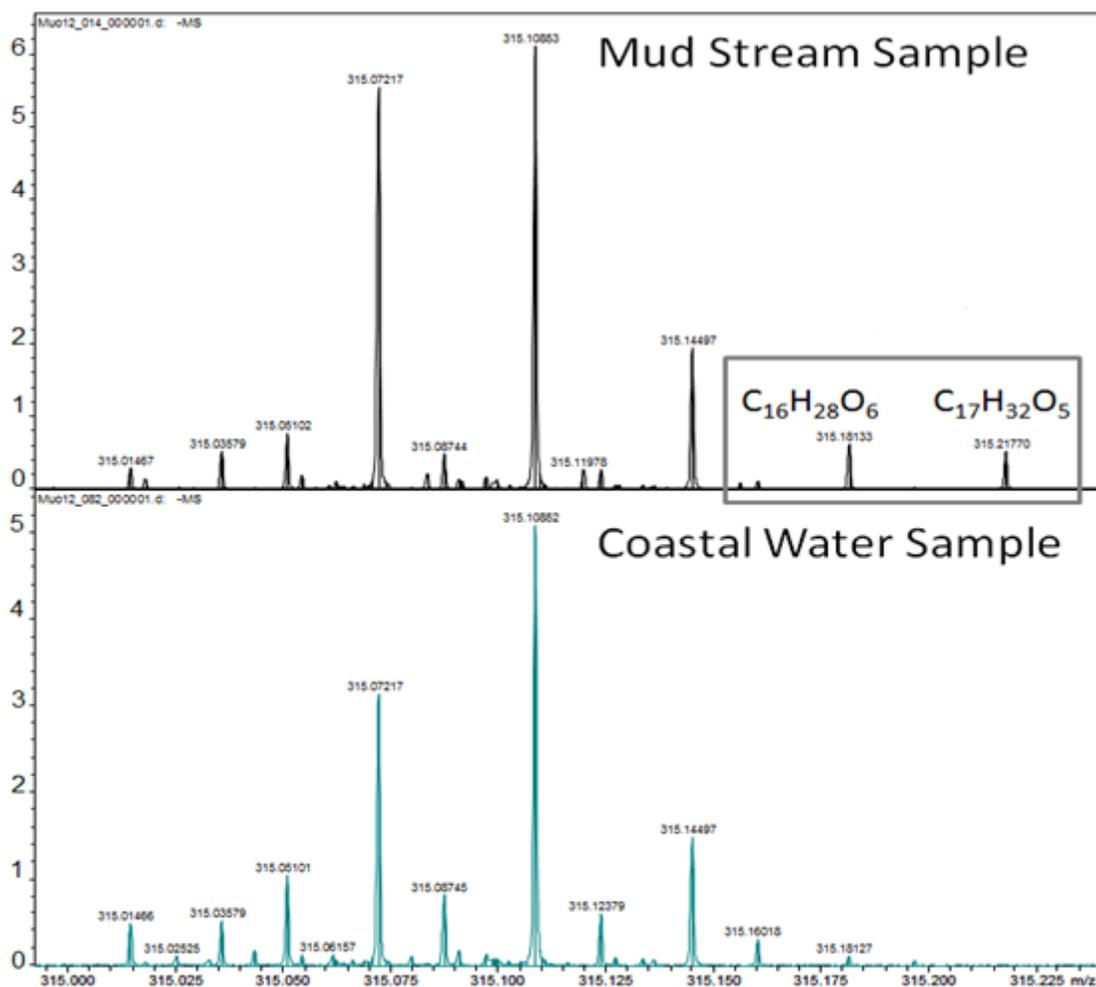


Fig. M4-S3. Differences in molecular signatures of DOM from the Muostakh Island permafrost outflows and adjacent waters of the coastal Laptev Sea. 315 m/z.

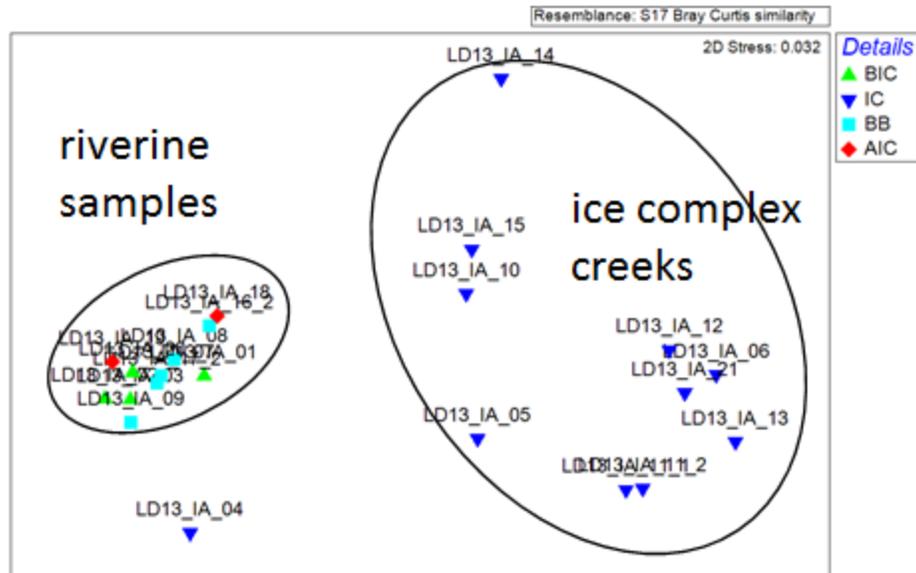
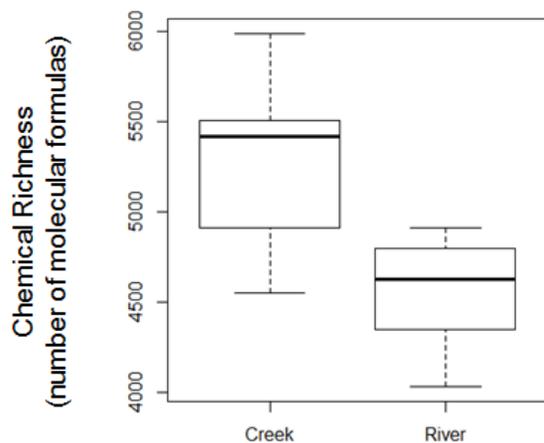


Fig. M4-S4. Results of multidimensional scaling based on molecular data for the Kurungnakh Island permafrost outflows and Olenekskaya channel DOM samples.



Results of t-test:

Welch Two Sample t-test data:
 Richness_data\$Richness by Richness_data\$Type
 $t = 4.6356$, $df = 18.409$, $p\text{-value} = 0.0001946$
 alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval:
 381.9959 1013.4167
 sample estimates: mean in group Creek: 5267.091
 mean in group River: 4569.385

Fig. M4-S5. Differences in chemical richness of the permafrost and riverine DOM.

Addendum

The addendum to this thesis contains the official reprints of published manuscripts 1 and 2.

Molecular Mapping of Sorbent Selectivities with Respect to Isolation of Arctic Dissolved Organic Matter as Measured by Fourier Transform Mass Spectrometry

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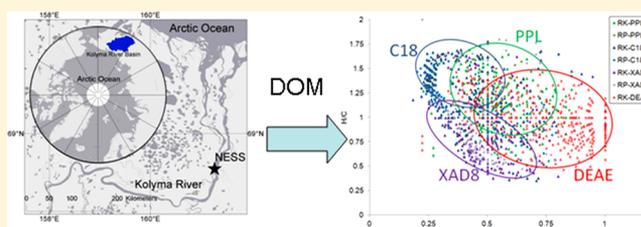
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S Supporting Information

ABSTRACT: The objectives of this study were to identify molecular features characteristic to arctic DOM from the Kolyma River basin and to elucidate structural imprints induced by a choice of the sorption technique. To achieve this goal, DOM was isolated from the Kolyma River basin with a use of three nonionic sorbents: Amberlite XAD-8 resin, PPL and C18 - SPE cartridges, and one anion exchanging resin—diethylaminoethyl (DEAE) -cellulose. The structural studies were conducted with a use of electrospray ionization Fourier Transform Ion Cyclotron Resonance (ESI FT-ICR) mass spectrometry and liquid state ¹H NMR spectroscopy. The DOM isolates obtained with a use of PPL and C18 cartridges were characterized with higher content of aliphatic compounds as compared to XAD-8 and DEAE-isolates. In total, for all arctic DOM isolates we observed predominance of hydrogen saturated compounds with high H/C values of identified formulas from FT-ICR MS data. ¹H NMR spectroscopy studies have confirmed this trend and revealed high contribution of alkyl-chain protons into the spectral density of the arctic DOM reaching 43% for PPL isolates.



INTRODUCTION

Identification of molecular features of dissolved organic matter (DOM) of permafrost origin discharged by the arctic rivers into the ocean is a subject of intense research due to growing contribution of dissolved organic carbon (DOC) released by permafrost thaw into the global carbon cycle.¹ The major focus of those studies is a search of molecular markers which would be characteristic of the DOM originating from the arctic systems.^{2,3} The main challenge here is molecular complexity of freshwater DOM represented by extremely heterogeneous ensemble of natural organic constituents which include lipids, terpenoids, lignin-like-structures, polyphenols, carbohydrates, and proteinaceous structures.⁴ As a result, Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) and ¹H NMR spectroscopy with their unprecedented resolution capacity became the methods of choice in investigating molecular features of DOM.^{5–14} However, application of these high resolution magnetic resonance techniques is very demanding with respect to the sample preparation procedures,

in particular, to a choice of appropriate extraction protocol.^{15–17}

Recently, solid phase extraction (SPE) on prepacked cartridges (e.g., C18 or PPL) has been proposed for DOM isolation from marine waters as an alternative to the long-standing practice of column extraction using Amberlite XAD resins.¹⁸ The major advantages of this new technique as compared to XAD8-technique are much higher purity of the DOM isolates and higher extraction efficiency.^{18–20} As a result, the SPE-isolates are much better suited to the purposes of ESI FT-ICR MS studies which attracted substantial interest of the DOM researchers to this extraction technique.¹⁵ However, comparative studies on molecular compositions and structures of the DOM isolates obtained with a use of XAD-resins and SPE-cartridges are very scarce. At the same time, they are of

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ultimate importance for reaching unbiased conclusions on structures and properties of DOM from the largely unknown sources such as runoffs of thawing permafrost discharged into the arctic rivers.

This motivated us to conduct a study on molecular variations within the same source of arctic DOM induced by a choice of the isolation technique. As a source of arctic DOM we used the Kolyma River and its tributary—the Panteleikha River, which are located in northeastern Siberia. The Kolyma River has earth's largest watershed that is completely underlain by continuous permafrost.²¹ The permafrost here contains very high amount of organic carbon (14 ± 8) kg m⁻³ which gives rise to mud springs in the summer with concentration of organic carbon achieving 60 mg L⁻¹.²² For DOM isolation we have used three nonionic polymeric sorbents: Amberlite XAD-8 resin, PPL- and C18- SPE cartridges which extract protonated (hydrophobicized) species of DOM after acidification, and one alternative sorbent—a weak anion exchanging resin (diethylaminoethyl (DEAE)-cellulose) which extracts negatively charged DOM compartments. To enable comparative study of molecular features of the DOM isolates, we applied ESI FT-ICR mass spectrometry and ¹H NMR spectroscopy.

MATERIALS AND METHODS

Site Description and Sampling. The DOM sampling took place during the July 2011 Polaris Project expedition to the Kolyma River basin (www.thepolarisproject.org). The study site was located near the North-East Science Station of the Russian Academy of Science (NESS), near Cherskiy, Sakha Republic, Russia (Figure 1).

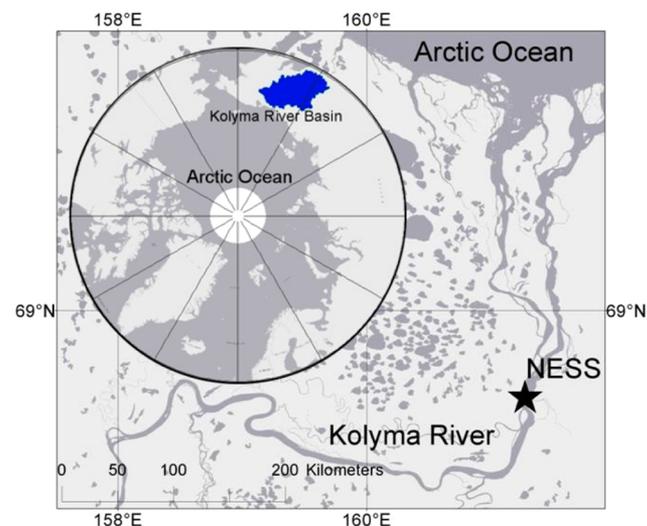


Figure 1. Location of the North-East Science Station of Russian Academy of Sciences (NESS) near the Kolyma and Panteleikha Rivers, Sakha Republic, Russia (designated with a black star).

Water was sampled two times from the main stem of the Kolyma River at 68.74858 N, 161.27721 E, and once from its tributary - the Panteleikha River - at 68.731769 N, 161.27721 E. The water sample size used for DOM isolation was 40 L. The water samples were placed into 20 L LDPE containers and transported to the NESS laboratory.

Materials and Chemicals. In-line filters (0.45 μ m) (AquaPrep 600, Gelman Sciences) were used for water filtration. DEAE cellulose (Whatman, fiber), Amberlite XAD-

8 resin (Rohm and Haas Ltd.), Varian Bond Elut PPL cartridges (Mega Pack, 6 g, 60 mL), and Varian Bond Elut C18 cartridges (Mega Pack, 6 g, 60 mL) were used for isolation of DOM. Amberlite 120R in H-form (Rohm and Haas Ltd.) was used for desalting alkaline extracts. Concentrated HCl (reagent grade) was used for samples acidification, 0.1 M NaOH (reagent grade) was used for DOM elution from resins. HPLC-grade methanol was used for DOM elution from cartridges. XAD-8 resin and DEAE cellulose sorbents were precleaned according to manufacturers recommendations to eliminate sorbent bleeding which could otherwise contaminate the isolated DOM samples.

Water Samples Preparation. The sampled water was filtered through 0.45 μ m in-line filters using peristaltic pump for removing particulates. It was further acidified with HCl until pH 2 in case of PPL, C18, and XAD8, or used as it is, in case of DEAE cellulose.

DOM Extraction Using PPL and C18 Cartridges. DOM isolation with a use of BondElut cartridges was performed as described by Dittmar et al.¹⁸ Prior to use, the cartridges were activated by rinsing with one volume of methanol and washed with two volumes of acidified Milli-Q water. The acidified water sample (40 L) was discharged through the cartridge with a flow rate of 40 mL·min⁻¹. Then, the DOM-loaded cartridge was switched off the pump, dried, and stored in the dark at 2 °C. All loaded cartridges were further transported to the lab facilities at the Lomonosov Moscow State University, Russia. The cartridge was dried with nitrogen and then eluted with 30 mL of methanol at a flow rate of 2 mL·min⁻¹. The obtained extracts were rotor-evaporated to dryness and stored at -20 °C in the dark. The corresponding samples were assigned as PPL-RK for the Kolyma River sample and PPL-RP for the Panteleikha River sample (PPL-cartridge isolate) and C18-RK, C18-RP (C18-cartridge isolate).

XAD Isolation Technique. The standard protocol for DOM isolation from natural waters was used.²⁰ In brief, the filtered water sample (40 L) was acidified to pH 2 and discharged through the column (2 × 20 cm) packed with 60 g of XAD-8 resin. The loaded XAD-8 resin was stored in the dark at 2 °C and transported to the lab facilities of the Lomonosov MSU. The resin was eluted with 0.1 M NaOH. The obtained alkaline extract was immediately passed through the cation-exchanging resin in H-form. The desalted samples were dried in the vacuum oven at 40 °C, and stored in the darkness at -20 °C. The samples were designated as XAD-RK (Kolyma River) and XAD-RP (Panteleikha River).

DEAE Isolation Technique. DOM extraction with DEAE-cellulose was performed according to the method outlined by Ivanova et al.¹⁹ The pretreated DEAE-cellulose (60 g on dry weight basis) was packed into 2.5 × 40 cm column. The sample of filtered water (40 L) was discharged through the column at a flow rate of 10 mL·min⁻¹. Elution and desalting procedures were as described for XAD8-resin. The isolation was conducted only for the Kolyma River water. The corresponding sample was designated as DEAE-RK.

Sorbent-Blanks Preparation for FT-ICR MS Measurements. The sorbent blanks for FT-ICR MS measurements were prepared by reproducing DOM desorption protocols on the unloaded sorbents. Methanol was used for rinsing PPL and C18 cartridges, while 0.1 M NaOH was used in case of XAD8 and DEAE-cellulose. The methanol-eluates from PPL and C18 cartridges were further evaporated to dryness. In case of the alkali eluates, they were first desalted using cation-exchanging

Table 1. Extraction Efficiency of the Sorbents Studied and Elemental Compositions of the Isolated DOM Samples from the Kolyma River (RK) and Panteleikha River (RP)

DOM sample	extraction efficiency ^a	content of element, % mass on ash-free basis				atomic ratio	
	%	C	H	N	O ^b	H/C	C/N
C18-RK	67 ± 5	52.2	5.50	1.03	41.27	1.3	51
PPL-RK	68 ± 5	50.9	5.22	1.07	42.81	1.2	48
XAD-8-RK	50 ± 3	49.0	4.49	0.95	45.56	1.1	56
DEAE-RK	82 ± 7	41.1	4.21	1.09	53.60	1.2	37
C18-RP	65 ± 5	52.1	5.54	1.40	40.96	1.3	43
PPL-RP	67 ± 6	52.8	5.93	1.28	40.01	1.3	48
XAD8-RP	47 ± 3	43.0	4.08	0.81	61.9	1.1	62

^aExtraction efficiency was determined as a difference between concentrations of DOC at the inlet and outlet of the column and corrected to the sample volume treated. ^bOxygen was calculated by mass balance as a difference (O% = 100%-C%-H%-N%).

resin, and then evaporated to dryness. The obtained residues were added with 1 mL methanol prior to FT-ICR MS analysis.

Elemental Analysis. All solid DOM samples were analyzed for CHN compositions using elemental analyzer (Vario EL, Germany). Ash content was determined manually. The content of oxygen was calculated as a difference after correction for the content of ash: O% = 100%-C%-H%-N%.

Dissolved Organic Carbon Measurement. DOC concentrations for filtered acidified (pH 2) water samples were determined as nonpurgeable total organic carbon by high temperature combustion (680 °C) on a Shimadzu TOC-VCPN analyzer equipped with a Pt catalyst (Shimadzu Scientific Instruments) which was located at the NESS station. Samples were sparged for 5 min immediately prior to analysis with the same ultrahigh purity oxygen that was used as a carrier gas in the TOC analyzer. Standards (potassium hydrogen phthalate) were analyzed immediately prior to the analysis of samples. Water for the standards was prepared by deionization and distillation. All samples were analyzed in triplicate. Standard deviations (SD) for DOC concentrations within the range from 0 to 20 mg·L⁻¹ did not exceed 2%. Initial DOC concentrations in water samples from the Kolyma and Panteleikha Rivers were 6.5 ± 0.1 and 9.7 ± 0.2 mg·L⁻¹, respectively (±stands for SD (*n* = 3)).

Extraction Efficiency Determination. Extraction efficiencies were determined on the basis of a decrease in the DOC content in the initial and processed water samples of 40 L. For this calculation, we determined DOC concentration in filtered water sample immediately before and after it was passed through the sorbent. An amount of intercepted DOC was calculated as a difference in DOC concentrations of initial and processed water samples multiplied with a volume of the sample (40 L). Ratio of the intercepted amount of DOC to its initial content in the bulk water sample was used as an estimate of the extraction efficiency of the sorbent.

Electrospray Ionization FT-ICR MS Analysis. A commercial LTQ FT Ultra mass spectrometer with a 7 T superconducting magnet equipped with ion max electrospray ion source (Thermo Electron Corp., Bremen, Germany) located at the Institute of Biochemical Physics of RAS was used for all mass spectral measurements. An electrospray ionization (ESI) source was used to generate negative charged molecular ions. Experimental conditions were as follows: needle voltage -2.9 kV, no sheath and auxiliary gas flow, tube lens voltage -160 V, heated capillary temperature 200 °C.

The solid DOM samples were dissolved in methanol immediately before FT-ICR MS analysis, to minimize formation of methyl esters. The sample concentration was 0.5

g DOM·L⁻¹ (or 0.2 g DOC·L⁻¹). Full scan MS spectra (*m/z* 200–900) were acquired in the FT-ICR with resolution *R* = 400k at *m/z* 400. The automatic gain control (AGC) target was set to 1 × 10⁶ corresponding to the number of ions accumulated in the linear ion trap and transferred to the ICR cell. The average FT-ICR mass spectrum was a sum of 500 consecutive scans. FT-ICR data were obtained as processed mass spectra with an associated peak list using Qual Browser version 2.0 (Thermo Electron Corporation, Bremen, Germany).

The formula assignments on the basis of the FT-ICR MS data were made using the lab-made “Transhumus” software designed by A. Grigoriev, which is based on previously described total mass difference statistics algorithm.²³ The isotopes ¹²C (maximum number of atoms for molecular formula computation: 50), ¹H (100), ¹⁶O (30), ¹⁴N(2), and ¹³C(1) were included in the calculations. Error threshold in formula assignments was set to ±0.5 ppm, intensity threshold was 1%. In this work we therefore focused on the most abundant ions by “intensity”-excluding peaks belonging to potential contaminations. For all ions the mass accuracy (measured as the root mean squared (rms) errors for the given mass) was below 2 ppm in the mass range from 250 to 650 *m/z*. The corresponding histogram of rms versus *m/z* covering 250–650 Da with data every 100 Da is given in Figure S1 in the Supporting Information (SI). We also provide an estimate for percentage of assigned molecular peaks within selected mass ranges (SI Figure S1). It did not vary substantially from low to high molecular weight peaks. That is why full range of ion masses was used for further data treatment. ¹³C peaks were used for quality assurance and removed from the final data set. The blanks were measured for each sorbent yielding the sorbent-specific peaks of individual compounds, which did not demonstrate broad distributions characteristic of DOM patterns. We removed these sorbent-specific peaks from the peak lists of the corresponding DOM isolates before the formula assignment.

¹H NMR Spectroscopy. ¹H NMR spectra were acquired using a Bruker Avance 400 NMR spectrometer operating at 400 MHz proton frequency. Sample preparation was conducted as described elsewhere.²⁴ In brief, a weight of 15 mg of solid DOM sample was placed into NMR tube, attached to vacuum line and dried for 6 h at 45 °C for complete water removal. The dry sample was then dissolved in 0.7 mL of anhydrous DMSO-*d*₆ (Merck, Germany 99,95 isotopic purity) under vacuum conditions. The spectra were acquired in a 5 mm tube using 90° excitation pulses (90(¹H) = 9 μs relaxation delay, 150 scans). Fourier transformation, phase correction and integration were

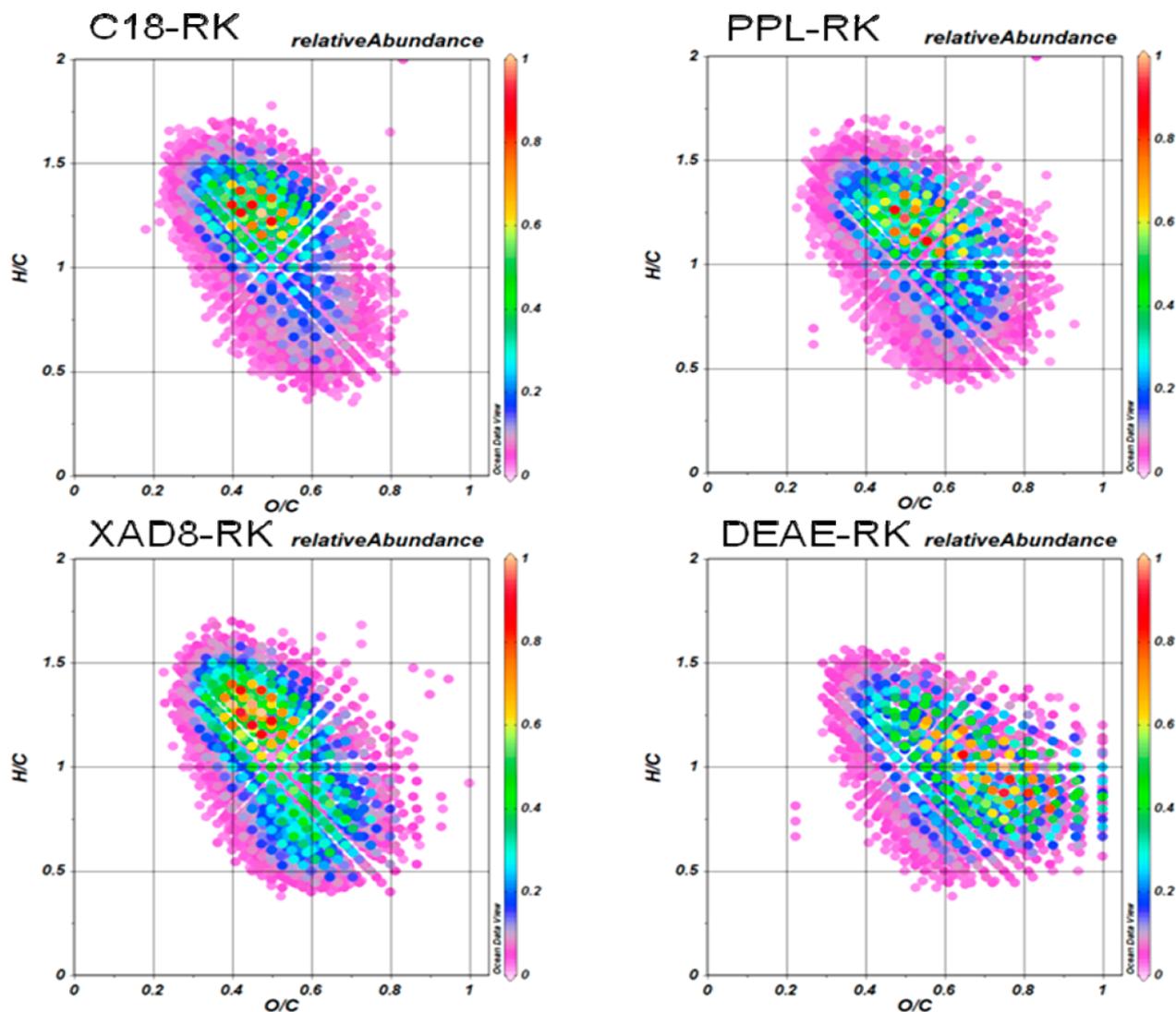


Figure 2. Van Krevelen diagrams for the Kolyma River DOM obtained from the assigned formulas (CHON) in each sample: (a) C18-RK, (b) PPL-RK, (c) XAD8-RK, and (d) DEAE-RK. Color codes correspond to relative peak abundance in FT-ICR mass spectra. Diagrams were plotted using Ocean Data View software.

performed using ACD-laboratories software Version 10 (Advanced Chemistry Development, Canada). As a reference for proton assignments, a signal of residual protons of DMSO- d_6 located at 2.5 ppm was used. The integration of spectral density was conducted using the following assignments (ppm): 0.5–2.05—protons of alkyl chains (CH_n —protons), 3.2–2.05—protons of alkyl chains located in α -position to electro-negative functional group or aromatic ring (α - CH_n), 3.2–6, “hetero-aliphatic” protons attached to O or N substituted C atom or to the corresponding heteroatom (CH_n -O(N)H); 6–11.5, aromatic protons (C_{Ar} -OH + C_{Ar} -H); 11.5–15.0, protons of carboxyl groups COO-H.

RESULTS AND DISCUSSION

Isolation of the Arctic DOM Using Different Sorbents.

To achieve the goals of this study, we isolated DOM from the Kolyma River and its tributary—the Panteleikha River using three nonionic adsorbents. In addition, an anion exchanging resin was used for isolation of DOM from the Kolyma river. The selected sorbents could be put into the following ascending order with regard to their polarity: C18 < PPL \approx XAD-8 \ll

DEAE. The extraction efficiency of the sorbents studied and the elemental compositions of the corresponding DOM isolates are summarized in Table 1.

It can be seen that DEAE-cellulose with its extraction efficiency of 82% had the highest affinity for various DOM compartments which corroborates well the previous findings.¹⁷ The PPL and C18 cartridges had comparable extraction efficiency on the level of 67–68% which is in line with the reported data.¹⁸ The lowest extraction efficiency had XAD-8 resin (50%) which is on the high end of extraction efficiencies observed for most unpolluted uncolored waters (30–50%).²⁵ We have to stress here that the above values of extraction efficiencies were calculated as a difference in DOC concentrations of the acidified water sample before and after it was passed through the cartridge. Hence, they do not account for possible irreversible sorption of DOM compartments on the cartridge, and the real DOM recoveries might be smaller. The C18 samples were characterized with the highest H/C values indicative of the maximum content of aliphatic saturated structures in this sample. This was to expect from the highly hydrophobic nature of the corresponding sorbent. The lowest

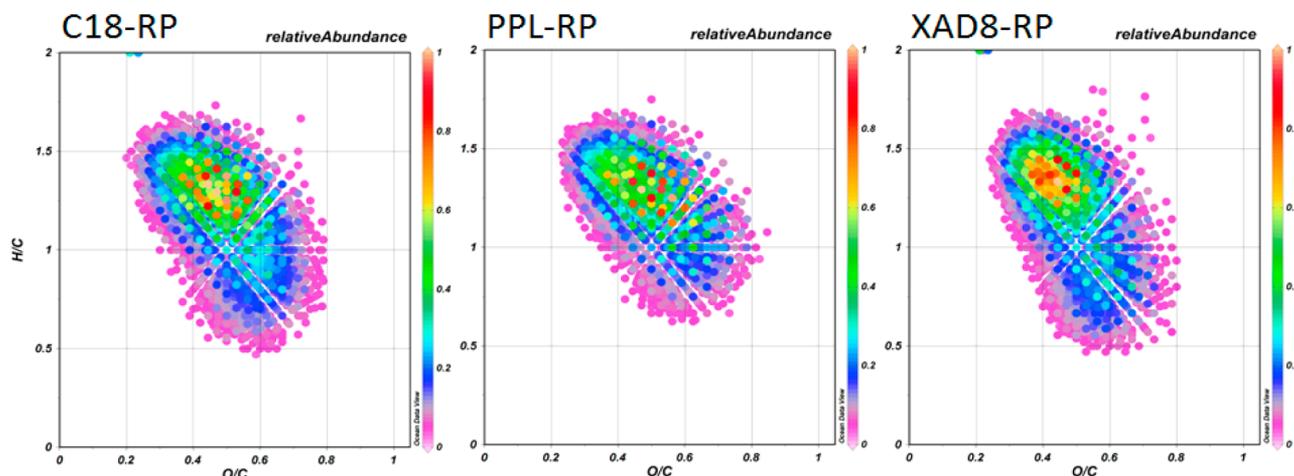


Figure 3. Van Krevelen diagrams for the Panteleikha River DOM obtained from the assigned formulas (CHON) in each sample: (a) C18-RP, (b) PPL-RP, (c) XAD8-RP. Color codes correspond to relative peak abundance in FT-ICR Mass Spectra. Diagrams were plotted using Ocean Data View software.

H/C values were observed for XAD8 samples, which might reflect preferred sorption of aromatic DOM constituents. Among the nonionic sorbents, PPL and C18 isolates were characterized with the highest content of nitrogen. The DEAE-isolate was characterized with the highest oxygen content indicating enrichment with oxygenated compounds. It can be concluded that a use of three nonionic sorbents yielded the DOM samples with much more similar elemental compositions as compared to the DEAE-cellulose isolate.

FT-ICR Mass Spectrometry of the Arctic DOM Isolated with Different Sorbents. All DOM isolates studied were analyzed using FT-ICR mass spectrometry under identical conditions to enable appropriate comparison of the data obtained. The corresponding mass spectra are shown in Figure S2 in SI. They were characterized with maximum peak density located between 400 and 600 m/z similar to those reported for other aquatic DOM.^{6,26,27} The obtained mass spectra were processed by assigning molecular formulas ($C_xH_yO_zN_n$) to individual peaks, which were plotted in the corresponding Van Krevelen diagrams.

Figure 2. shows Van Krevelen diagrams with relative peak magnitude intensities for DOM samples isolated from the Kolyma River using the four sorbents studied. The most remarkable differences in peak magnitude distribution were characteristic of the sample isolated with DEAE-cellulose. It was characterized with the highest abundance of formulas intrinsic to most oxidized, tannin-like components. C18-RK, PPL-RK, XAD8-RK samples showed similar intensity distributions with a predominance of aliphatic components in the observed molecular formulas. XAD8-RK sample was characterized with the highest abundance of unsaturated molecular components located in the bottom-left corner of VK diagram. At the same time, C18 sample was dominated with hydrogen-saturated components as compared to other samples.

It was of importance to compare the above trends observed for the samples isolated from the Kolyma River with those for the Panteleikha River. Their similarity for two independent sample sets would be indicative of reproducibility of the data obtained. The corresponding comparison was conducted for three nonionic sorbents which were applied for isolation of DOM from the Panteleikha River. Figure 3 shows the corresponding VK diagrams.

Visual inspection of the VK diagrams for DOM from the Panteleikha River allows us to conclude about substantial similarity of these molecular trends to those observed for the Kolyma River. Again, we could see the largest contribution of saturated components for C18-RP sample, the highest abundance of unsaturated components was seen within XAD8-RP sample, and the most dense population within the nitrogenous components was observed for PPL-RP.

Upon examining Van Krevelen diagrams for the seven DOM samples from the Kolyma River basin studied, a conclusion can be made on prevalently aliphatic character of this DOM reflected in higher density of hydrogen-saturated compounds with $H/C > 1.0$ as compared with the corresponding data on DOM from temperate or tropical regions.^{15,28} To illustrate an impact of sorption selectivities on molecular compositions of the DOM isolates, we show in Figure 4 locations of unique identifications which were present only in the DOM isolates obtained with a use of the given sorbent, and absent in all other isolates. Unique identifications together with common identifications are also shown in Figures S3 and S4 in the SI.

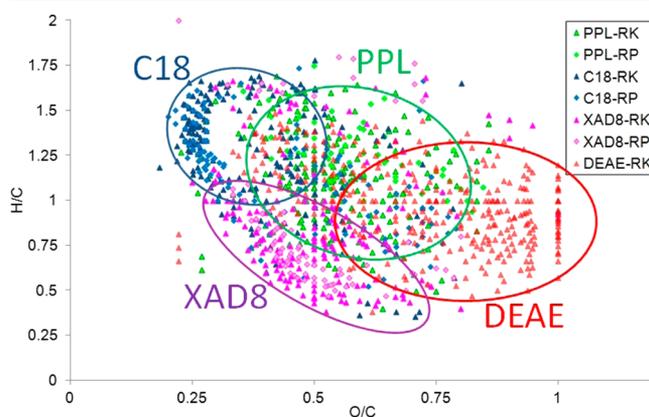


Figure 4. Molecular mapping of sorbent selectivities with respect to DOM compartments isolated from the rivers Kolyma and Panteleikha for C18- and PPL-SPE cartridges, XAD-8 resin and DEAE-cellulose. Blue dots designate unique CHON-compositions in the C18-RK and C18-RP samples, green dots—in the PPL-RK and PPL-RP samples, purple dots—in the XAD8-RK and XAD8-RP samples, and red dots—in the DEAE-RK sample.

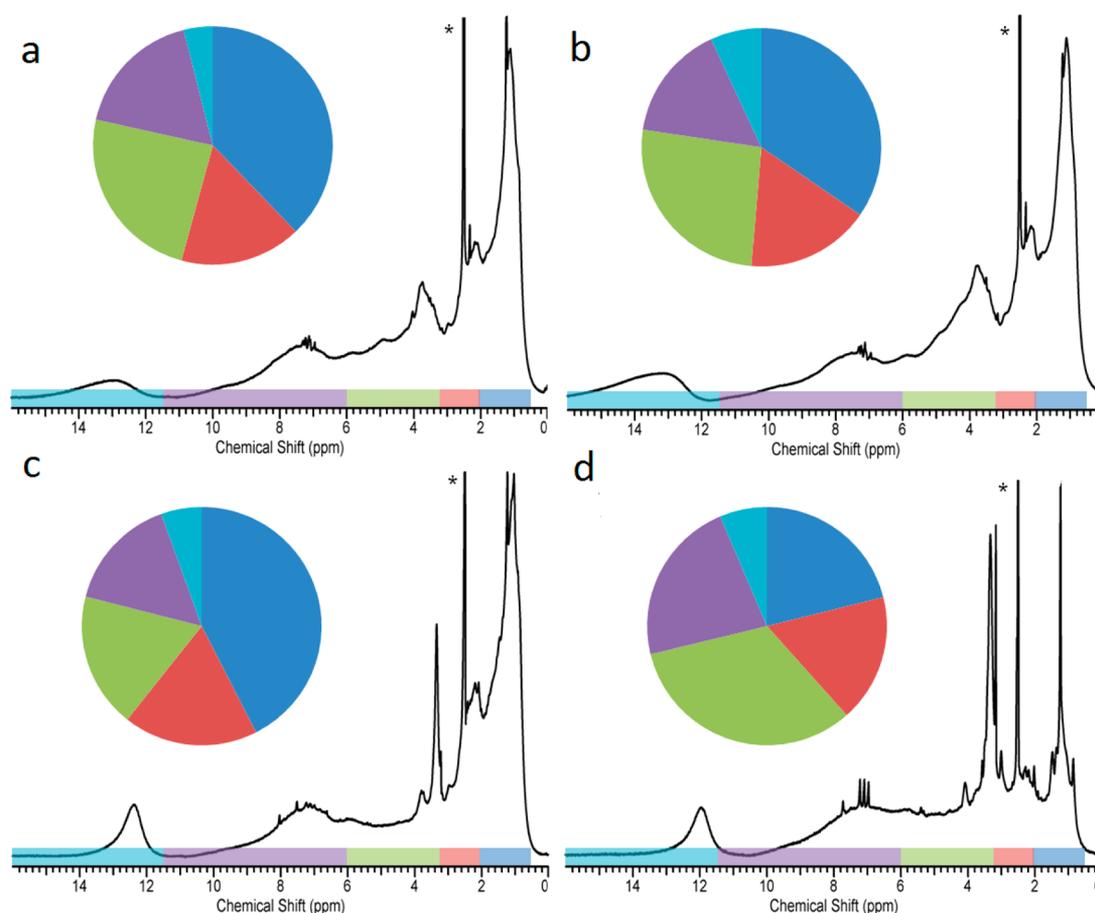


Figure 5. ^1H NMR spectra of the Kolyma River DOM isolated by four different sorbents: (a) C18, (b) PPL, (c) XAD-8, (d) DEAE-cellulose. Starred peak designates residual protons of DMSO. The pie diagrams represent proton distributions in each sample where dark blue represents CH_n protons; red, $\alpha\text{-CH}_n$ protons; green, $\text{CH}_n\text{O(N)H}$ -protons; violet, $\text{C}_A\text{H(OH)}$ protons; light blue – COOH protons.

The patterns in Van Krevelen diagrams followed rather consistently the trends expected from the sorption mechanisms specific to the sorbent used (Figure 4). A use of the most nonpolar C18 SPE cartridge lead to much more abundant pools of the saturated aliphatic compartments located in the range of lipids and terpenoids (here and below we refer to molecular mapping of Van Krevelen diagram based on the model sets of major NOM precursors as described elsewhere).^{29,30} At the same time, a use of more polar PPL-cartridge induced enhancement of the DOM isolates with protein-derived moieties (upper central part of the diagram)²⁹ and carboxyl-rich alicyclic moieties (CRAM)¹⁴ with H/C values ranging from 1.0 to 1.6 and O/C values from 0.25 to 0.6. XAD-8 isolates were characterized with much richer pool of more condensed aromatics, which is characteristic of flavonoids or higher molecular weight lignins with prevailing H/C values from 0.5 to 1.0 and O/C values from 0.3 to 0.6. DEAE-RK sample had the most specific character designated with rich pool of highly oxidized structures present in polyhydroxyphenols as well as in oxyhydroxyaromatic acids, and carbohydrates, characteristic of hydrolyzable tannins. This is in line with anion exchanging properties of this sorbent.

The obtained results allow us to conclude that care should be exercised to avoid misinterpretation upon relating molecular features of DOM isolated with a use of different extraction technique to the particular source of its origin. For example, in case of SPE and XAD8 it can be expected that a use of the

former will lead to the more aliphatic-rich DOM isolate as compared to the latter regardless of the DOM source. To support this statement, the more structure-specific studies on the DOM isolates were conducted using ^1H NMR spectroscopy.

^1H NMR Spectroscopy of the Arctic DOM Isolated with Different Sorbents. ^1H NMR spectra acquired for the samples from the Kolyma River are shown in Figure 5, and from the Panteleikha River, in Figure S5 in the SI. All spectra were characterized with well resolved and intense resonances in the range of aliphatic protons dominated by methylene protons of alkyl chains. Quantitative assessment of proton distributions is given in Table 2. It is also visualized for the samples from the Kolyma River as pie diagrams shown in Figure 5.

For both arctic rivers studied, the DOM samples isolated with a use of nonionic sorbents (XAD-8, PPL and C18) were rather similar in the proton distributions, while the DEAE-RK sample was very different from all others (Figure 5). The major difference was a lesser amount of alkyl-chain protons and higher contribution of the carbohydrate- and aromatic protons. These features corroborate well the findings of FT-ICR MS on enrichment of this sample with highly oxidized polar compounds which could be assigned to hydrolyzable tannins containing both carbohydrates and gallic acids in their structures.

At the same time, all other samples were characterized with very high content of aliphatic alkane protons varying from 35 to

Table 2. Distribution of Integral Spectral Density in ^1H NMR Spectra Acquired on DOM from the Rivers Kolyma and Panteleikha Isolated with a Use of the Four Different Sorbents

DOM sample	CH_n	a-CH_n	$\text{CH}_n\text{O(N)H}$	$\text{C}_A\text{H(O)H}$	COOH
	0.5–2.05 ppm	2.05–3.2 ppm	3.2–6.0 ppm	6–11.5 ppm	11.5–16 ppm
C18-RK	38	16	24	18	4
PPL-RK	35	17	26	16	7
XAD8-RK	43	18	18	16	5
DEAE-RK	21	17	33	22	6
C18-RP	42	18	26	8	6
PPL-RP	41	20	24	8	6
XAD8-RP	37	24	28	7	5

43%. These alkyl-chain fragments could belong to side chains of proteins as well as to linear terpenoid compounds.^{13,14,31} A lack of higher content of aromatic protons which could be expected from multiple unsaturated species which are uniquely present in XAD8-sample (Figure 4) can be explained by “invisibility” of condensed or highly substituted aromatic structures for ^1H NMR spectroscopy due to a lack of protons on those aromatic carbons.

In general, comparison of all ^1H NMR spectra acquired for DOM from the Kolyma River and the Panteleikha River allows us to conclude that the most profound structural feature intrinsic to all samples studied is extremely high content of alkyl-chain protons located in the region from 0.5 to 2.05 ppm (up to 43%). This might be related to the dominance of poorly degraded, hydrogen-rich structures in the pool of the arctic DOM in the Kolyma River basin DOM. This was to expect from the uniqueness of the main source of its origin—least degraded ancient organic carbon on Earth preserved in the Yedoma permafrost deposited in the Kolyma River lowland.²¹ This corroborates well the recent findings on high bioavailability and molecular characterization of permafrost DOM.^{1,32,33}

Summarizing results of our investigations we would recommend a use of SPE-technique (in particular, PPL sorbent) for DOM extraction from natural waters. It yields rather representative pool of DOM components, and it is much easier to handle experimentally as compared to XAD8-technique. Particular advantage of this technique for the Arctic region is higher affinity of PPL cartridge to dominating aliphatic compartments as compared to XAD8 resin. On the other side, if conclusions on specific structural features of the DOM formed under arctic conditions are sought, the SPE-isolates should be compared only to the corresponding SPE isolates: they cannot be directly compared to the pool of data obtained previously for the XAD8-DOM isolates.

■ ASSOCIATED CONTENT

📄 Supporting Information

The results of quality assurance of formula assignments are obtained on the example of XAD-8-RK sample and given in Figure S1. ESI FT-ICR mass spectra are shown in Figure S2. 2D Van Krevelen diagrams for the Kolyma and Panteleikha Rivers with common and unique identifications are shown in Figures S3 and S4. ^1H NMR spectra of the Panteleikha River DOM are shown in Figure S5. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

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Origin-specific molecular signatures of dissolved organic matter in the Lena Delta

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Abstract Large Arctic rivers discharge significant amounts of dissolved organic matter (DOM) into the Arctic Ocean. We sampled natural waters of the Lena River, the Buor-Khaya Bay (Laptev Sea), permafrost melt water creeks, ice complex melt water creeks and a lake. The goal of this study was to characterize the molecular DOM composition with respect to different water bodies within the Lena Delta. We aimed at an identification of source-specific DOM molecular markers and their relative contribution to DOM of different origin. The molecular characterization was

performed for solid-phase extracted DOM by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). Average dissolved organic carbon concentrations in the original samples were $490 \pm 75 \mu\text{mol C L}^{-1}$ for riverine and bay samples and $399 \pm 115 \mu\text{mol C L}^{-1}$ for permafrost melt water creeks. Average TDN concentrations were elevated in the permafrost melt waters ($19.7 \pm 7.1 \mu\text{mol N L}^{-1}$) in comparison to the river and the bay (both $13.2 \pm 2.6 \mu\text{mol N L}^{-1}$). FT-ICR MS and statistical tools demonstrated that the origin of DOM in the Lena Delta was systematically reflected in its molecular composition. Magnitude weighted parameters calculated from MS data (O/C_{wa} , H/C_{wa} , C/N_{wa}) highlighted preliminary sample discrimination. The highest H/C_{wa} of 1.315 was found for DOM in melt

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water creeks in comparison to 1.281 for river and 1.230 for the bay samples. In the bay samples we observed a higher fraction of oxygen-rich components which was reflected in an O/C_{wa} ratio of 0.445 in comparison to 0.425 and 0.427 in the river and creeks, respectively. From the southernmost location to the bay a relative depletion of nitrogenous molecular markers and an enrichment of oxidized DOM components occurred. The highest contribution of nitrogenous components was indicative for creeks reflected in a C/N_{wa} of 104 in comparison to 143 and 176 in the river and bay, respectively. These observations were studied on a molecular formula level using principal component and indicator value analyses. The results showed systematic differences with respect to water origin and constitute an important basis for a better mechanistic understanding of DOM transformations in the changing Arctic rivers.

Keywords Arctic · Lena Delta · Permafrost · DOM · FT-ICR MS · Molecular markers

Introduction

The large rivers discharge huge amounts of freshwater into the Arctic Ocean. Arctic rivers drain vast areas characterized by variable vegetation and soil conditions (Bhatt et al. 2010; Lantuit et al. 2009) which are important sources of dissolved organic carbon (DOC) in the Arctic Ocean. Dissolved organic matter (DOM) is involved in a wide range of biogeochemical processes in the Arctic river watersheds (Yenisei, Ob, Lena, Indigirka, Kolyma, Mackenzie and Yukon) (Roehm et al. 2009; Wickland et al. 2012) and in the Arctic Ocean (Amon and Meon 2004; Dittmar and Kattner 2003). It supports microbial growth, affects primary production and therefore plays an important role in carbon and nutrient cycling (Le Fouest et al. 2013).

The water (McClelland et al. 2004; Peterson et al. 2002) and DOC discharge (Frey and McClelland 2009; Semiletov et al. 2011) into the Arctic Ocean are expected to change in future due to increasing temperatures and subsequent permafrost thawing and degradation (Zhao-ping et al. 2010). The largest and most vulnerable source of organic matter is represented by the organic-rich permafrost (Engelhaupt

2008). The estimated amount of organic carbon accumulated in permafrost is $\sim 1,700$ Pg of carbon (Tarnocai et al. 2009). The degradation and mobilization of this carbon pool would have critical implications for microbial processes, primary production and carbon cycling in the Arctic and the Arctic Ocean Basin interior (Frey and McClelland 2009; Schuur et al. 2008). DOM represents the most mobile part in these soils and is therefore of particular importance when studying organic carbon fluxes in changing permafrost environments. DOM is a highly complex mixture of organic compounds with different chemical properties (Thurman 1985). In the context of global element cycles it remains an unresolved issue which fractions of DOM are bioavailable or recalcitrant (Abbt-Braun and Frimmel 2002; Jiao et al. 2011) and it is one of the most important challenges in the field of DOM research to develop a better mechanistic understanding of the processes, which determine the wide range of residence times (Flerus et al. 2012; Lechtenfeld et al. 2014).

The Lena River is one of the largest rivers in the Arctic, and permafrost underlies 78–93 % of the watershed with continuous permafrost extending south to 50°N (Zhang et al. 1999). Estimates for the annual discharge of DOC range between 4.1–4.9 Tg C year⁻¹ (Dittmar and Kattner 2003; Opsahl et al. 1999) and 5.6–5.8 Tg C year⁻¹ (Holmes et al. 2012; Raymond et al. 2007). The annual discharge of the Lena River particulate organic carbon (POC) was estimated to be 0.38 Tg C year⁻¹ (Semiletov et al. 2011) which is less than one tenth of the contribution of DOC emphasizing the importance of DOM to the total organic carbon discharge. Late summer runoff of the Lena River is dominated by DOM input from organic-rich soils, ice complexes, and the mires of northern Asia. Hydrologic processes mobilize organic carbon stored in the permafrost soils and carry it downstream (Guo et al. 2007).

The majority of existing studies, which addressed carbon fluxes, sources and transformation of organic matter in the Arctic used bulk DOC, isotopic signatures and targeted biomarker approaches. Several studies used isotopic and biomarker approaches to investigate DOM and particulate organic matter fluxes in the Buor-Khaya Bay of the Laptev Sea (Karlsson et al. 2011) and other Arctic regions (Cooke et al. 2009; Vonk et al. 2008; Yunker et al. 1995). Carbon isotope and lipid biomarker studies in the Buor-Khaya

Bay demonstrated that a large terrestrial input from the Lena River leaves a significant imprint on the surface water POC composition (Karlsson et al. 2011). The young fluvial/alluvial POC pool is relatively buoyant, organic carbon rich and bioavailable, which is consistent with clear spatial trends of rapidly degrading POC throughout the Buor-Khaya Bay surface water (Karlsson et al. 2011). Most of the annual discharge of lignin (an unambiguous terrestrial tracer) occurs during the 2 months of spring freshet with extremely high lignin concentrations and a lignin phenol composition indicative of fresh vegetation from boreal forests. At low flow conditions DOM was dominated by older, soil, peat and wetland-derived DOC (Doxaran et al. 2012). Despite several studies on DOM molecular markers in the Lena River (Doxaran et al. 2012; Kraberg et al. 2013; Lara et al. 1998), there is still a gap in our understanding of regional biogeochemical processes involving DOM from different sources (i.e., riverine, soil and permafrost).

In the permafrost influenced Kolyma River the composition and age of DOM was analyzed during different seasons using radiocarbon analysis (Neff et al. 2006). The bulk DOM in winter and spring is modern with a high concentration of lignin. At the end of summer and in autumn, the age of DOM becomes significantly older indicating that the DOM generation changed from surface to deep soils or other terrestrial sources of old and more refractory DOM. The Lena River and Kolyma River may have similar mechanisms and trends of organic matter release. However, integral DOM signals such as isotopic composition or radiocarbon age and more specific signals from other molecular markers have to be combined with new analytical tools and markers on a molecular level for a better understanding of the Lena River Delta ecosystem processes. Identification of new groups of biomarkers will also broaden our current knowledge of changing processes in the Arctic rivers caused by climate change.

There is still a gap of knowledge of the quality of DOM in Arctic rivers which is an important factor to assess its bioavailability and degradability. Untargeted analytics such as Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) allows to cover the complexity of the molecular pattern of DOM and to identify molecular markers and their transformation pathways in the pool of DOM molecules (Stubbins et al. 2010). Molecular markers, which

demonstrate not only the most dramatic but also individual changes, may also represent major biogeochemical processes. FT-ICR MS already demonstrated its applicability in a number of studies on DOM molecular compositions (Hertkorn et al. 2008; Nebbioso and Piccolo 2013; Schmidt et al. 2011). These studies take into account thousands of molecular components of DOM. Further data analysis allows understanding what presence/absence (or changes in relative biomarker contribution) of different markers reflect on the biogeochemical level. Previous studies demonstrated that the application of statistical tools simplifies data interpretation (Koch et al. 2008; Kujawinski et al. 2009; Sleighter et al. 2010). Recent FT-ICR MS studies on Arctic DOM observed latitude and pH driven trends in the molecular composition of DOM in the Yenisey River (Roth et al. 2013). The authors showed that the climatic gradients along the latitude (from south to north) were related to a higher abundance of low molecular weight components, nitrogen-containing components and an increase in the degree of unsaturation of riverine DOM. For boreal lakes of Sweden it was demonstrated that parameters such as precipitation, water residence time and mean annual temperature are reflected in the DOM composition and its chemodiversity (Kellerman et al. 2014). In Arctic fjords, FT-ICR MS indicated the rapid turnover of DOM (Osterholz et al. 2014).

The aims of this research were to quantify DOC and TDN concentrations in different waters of the Lena Delta and to identify source-specific molecular markers of DOM and their relative contribution to the Lena River, melt water creeks and Buor-Khaya Bay DOM samples for an advanced understanding of DOM compositional changes in the permafrost dominated riverine region. The broad molecular view derived by FT-ICR MS is expected to expand our current knowledge about the processes involving DOM in the Lena Delta.

Materials and methods

The field sampling campaign took place in August 2009 in the Lena Delta. The highest mean air temperatures in this region occur in July (10.1 °C) and August (8.5 °C) (Boike et al. 2013). Several DOM sources were sampled including Lena River channels,

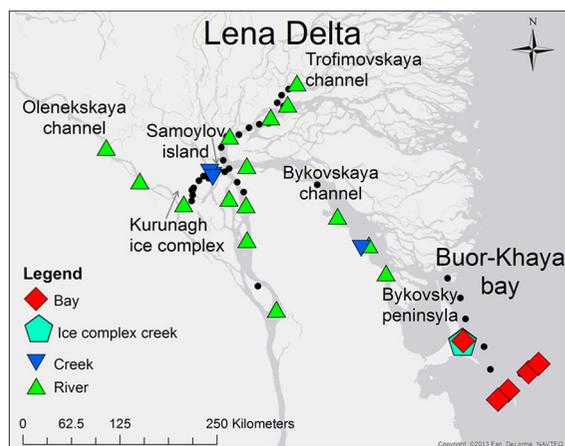


Fig. 1 Lena Delta ($73^{\circ}0'N$, $127^{\circ}0'E$) and sampling stations of the 2009 campaign. Riverine samples (*triangles*), bay samples (*diamonds*), permafrost creek samples (*upside down triangles*), ice complex melt water creeks (*pentagons*) and water samples used only for DOC and TDN analyses (*dots*)

permafrost melt water creeks (including ice complex creeks), a permafrost lake and the Buor-Khaya Bay, Laptev Sea (Fig. 1). The majority of melt water creeks and lakes were sampled on Samoylov Island. Creeks and lakes were connected and represented different parts of the Samoylov Island system. Ice complex melt water creeks derived from the ice complex on the Bykovsky Peninsula. All samples except the bay were fresh waters. In the bay the range of salinity varied from 0 to 2.

Water was sampled from different depths using a Niskin water sampler or pre-cleaned glass bottles for creek sampling. Samples were filtered through $0.7\ \mu\text{m}$ GF/F filters (Whatman, pre-combusted, 4 h, $450\ ^{\circ}\text{C}$). DOC and total dissolved nitrogen (TDN) concentrations were measured using high temperature catalytic oxidation (TOC-VCPN, Shimadzu). Water samples were acidified in the auto sampler to remove inorganic carbon and analyzed directly.

DOM samples were concentrated directly in the field using pre-packed solid phase extraction (SPE) cartridges (PPL, Varian). SPE was applied for enrichment and purification (i.e., removal of inorganic constituents). After extraction, the cartridges were dried with nitrogen and stored at $-20\ ^{\circ}\text{C}$. Back in the lab, the cartridges were eluted with methanol (Lichrosolv, Merck). The DOC content of SPE-DOM was determined by evaporation of $50\ \mu\text{l}$ methanol extract and re-dissolved in ultra-pure water. Details of the

method are described in (Dittmar et al. 2008). The extraction efficiency varied from 30 to 60 %.

Fourier transform ion cyclotron resonance mass spectrometry

Ultra-high resolution mass spectra for 31 Lena Delta DOM samples were acquired in one batch on a Bruker Apex 12 Qe FT-ICR MS equipped with a 12T superconducting magnet and an APOLLO II electrospray source. SPE-DOM methanol extracts were adjusted to $\sim 200\ \mu\text{mol C L}^{-1}$ DOC concentrations by dilution with methanol/water (1:1). Samples were analyzed with electrospray ionization (ESI) in negative ionization mode. The average FT-ICR mass spectrum was composed of 512 consecutive scans. The spectra were recalibrated internally by characteristic DOM ions ($[\text{C}_{16}\text{H}_{19}\text{O}_8]^{-}$ —339.10854 m/z; $[\text{C}_{17}\text{H}_{21}\text{O}_9]^{-}$ —369.11911 m/z; $[\text{C}_{19}\text{H}_{23}\text{O}_{10}]^{-}$ —411.12967 m/z; $[\text{C}_{21}\text{H}_{25}\text{O}_{12}]^{-}$ —469.13515 m/z; $[\text{C}_{24}\text{H}_{29}\text{O}_{14}]^{-}$ —541.15628 m/z; $[\text{C}_{28}\text{H}_{35}\text{O}_{14}]^{-}$ —595.20323 m/z; $[\text{C}_{28}\text{H}_{35}\text{O}_{15}]^{-}$ —611.19814 m/z; $[\text{C}_{31}\text{H}_{39}\text{O}_{15}]^{-}$ —651.22944 m/z; $[\text{C}_{33}\text{H}_{41}\text{O}_{16}]^{-}$ —693.24001 m/z). All ions were singly charged.

FT-ICR MS data was evaluated in the mass range of 200–500 m/z. Molecular formulas were assigned for peaks with a signal to noise ratio of more than 4 and a mass accuracy window of ± 0.5 ppm (Koch et al. 2007, 2005) and included following isotopes: ^{12}C (0– ∞), ^{13}C (0–1), ^1H (0– ∞), ^{16}O (0– ∞), ^{14}N (0–2), ^{32}S (0–1). The nitrogen rule was applied and thresholds for elemental ratios were: $\text{O/C} \leq 1.2$, $\text{H/C} \leq 2\text{C} + 2 + \text{N}$ (Koch et al. 2005). ^{13}C isotopes were used for quality control (verification of the parent ion) but removed from the final dataset. FT-ICR MS provided exact masses of singly charged ions which allowed calculations of corresponding molecular formulas. It is important to note that each molecular formula can represent a number of isomers (Hertkorn et al. 2008). In this article, for the ease of readability, we generally refer to molecular formulas as “compounds”, “components”, “markers” or “constituents”. We assigned $\sim 55\%$ of peaks within the chosen thresholds. The number of assigned formulas (excluding ^{13}C isotopes) varied from 2,737 to 3,601.

FT-ICR MS data exploration and analysis

The peak magnitudes were normalized to the sum of intensities of all identified molecular formulas of the

molecular mass region. Peak magnitude weighted-average elemental ratios O/C_{wa} , H/C_{wa} , C/N_{wa} , double bond equivalents (DBE) and mass defects were calculated from the assigned formulas. Magnitude weighted parameters X_{wa} were calculated according to $X_{wa} = \sum_{i=1}^n X_i * M_i / \sum_{i=1}^n M_i$ where X is e.g., O/C , H/C or C/N , i is the individual molecular formula and M is the relative mass peak intensity (Sleighter et al. 2010). We prepared a data matrix for samples with molecular formulas as parameters where the peak magnitude corresponded to a parameter value. This data matrix was used for hierarchical clustering, principal component analysis (PCA) and indicator value analysis. For the statistical data processing, we only considered formulas, which were observed at least in five samples. By this, we avoided differences between samples, which resulted from discrete signal to noise threshold and FT-ICR MS signal fluctuation and tried to avoid false formula assignments. Molecular components revealed by FT-ICR MS were visualized using van Krevelen diagrams as ratios of elements: O/C and H/C (van Krevelen 1950).

Cluster analysis was based on Bray–Curtis similarity (Bray and Curtis 1957) and performed using commercial software (PRIMER v.6; PRIMER-E Ltd, UK). For principal component analysis (PCA, “Chemometrics” package, R) we used a similar approach as described in Sleighter et al. 2010.

Indicator value analysis was performed according to a previous approach (Dufrene and Legendre 1997). We used molecular peaks (assigned formulas) as species and the relative intensity of peaks as species abundance. We renormalized peak intensities in each sample to a lowest intensity and rounded obtained values to integer values. By this, we mimic species counts and minimum “specie” value in each sample was 1 compare (Kellerman et al. 2014). For the indicator value analysis we used R (“labdsv” package).

Results

Dissolved organic carbon and total dissolved nitrogen

The average DOC concentrations were $492 \pm 79 \mu\text{mol C L}^{-1}$ for river water, $484 \pm 55 \mu\text{mol C L}^{-1}$

for bay samples and $399 \pm 115 \mu\text{mol C L}^{-1}$ for permafrost melt water creeks. Ice complex influenced samples from the Olenekskaya channel west of the Kurunagh showed higher DOC concentrations ($657 \pm 8 \mu\text{mol C L}^{-1}$) compared to the riverine samples. It is, however, difficult to test the statistical significance because of the small number of Olenekskaya channel samples ($n = 4$) compared to riverine samples ($n = 51$). The average TDN concentrations were $13.1 \pm 2.8 \mu\text{mol N L}^{-1}$ for river, $13.7 \pm 1.7 \mu\text{mol N L}^{-1}$ for bay samples, and $19.7 \pm 7.1 \mu\text{mol N L}^{-1}$ for permafrost melt waters. DOC and TDN in ice complex melt water creeks were highly variable but also showed the highest concentrations of the whole Lena Delta system reaching average values of $3,910 \pm 1,780 \mu\text{mol C L}^{-1}$ and $220 \pm 138 \mu\text{mol N L}^{-1}$, respectively. DOC/DON molar elemental ratios of the SPE-extracts, which do not contain inorganic nitrogen, were 63 ± 17 for riverine samples, 56 ± 14 for bay samples, 35 ± 11 for permafrost melt waters and 33 ± 4 for ice complex samples (complete DOC and TDN data available online: <http://dx.doi.org/10.1594/PANGAEA.831765>; average DOC and TDN values available in supplementary information (SI) in Table SI-1 and Fig. SI-1).

Molecular characterization of Lena Delta DOM

FT-ICR MS characterization resulted in typical monomodal peak magnitude distributions for all DOM spectra (Fig. 2).

The typical peak distribution spanned a mass range from 200 (lower limit of the mass detector) to 600 m/z (Fig. 2). We identified, on average, 3,100 formulas (excluding ^{13}C isotopologues) in each sample (detailed information in Table SI-2). The molecular patterns on single nominal masses revealed pronounced molecular differences between samples from the Buor-Khaya Bay, melt water creeks and Lena River (Fig. 2b–d). Particular differences were found in the relative peak magnitude of nitrogen containing mass peaks (e.g., $[\text{C}_{17}\text{H}_{20}\text{N}_1\text{O}_{10}]^-$, $[\text{C}_{18}\text{H}_{24}\text{N}_1\text{O}_9]^-$, $[\text{C}_{19}\text{H}_{28}\text{N}_1\text{O}_8]^-$). The highest relative magnitudes of nitrogenous peaks were characteristic for the permafrost melt water creek samples, the lowest for the Buor-Khaya Bay samples.

Molecular differences between samples were reflected in their peak magnitude weighted average

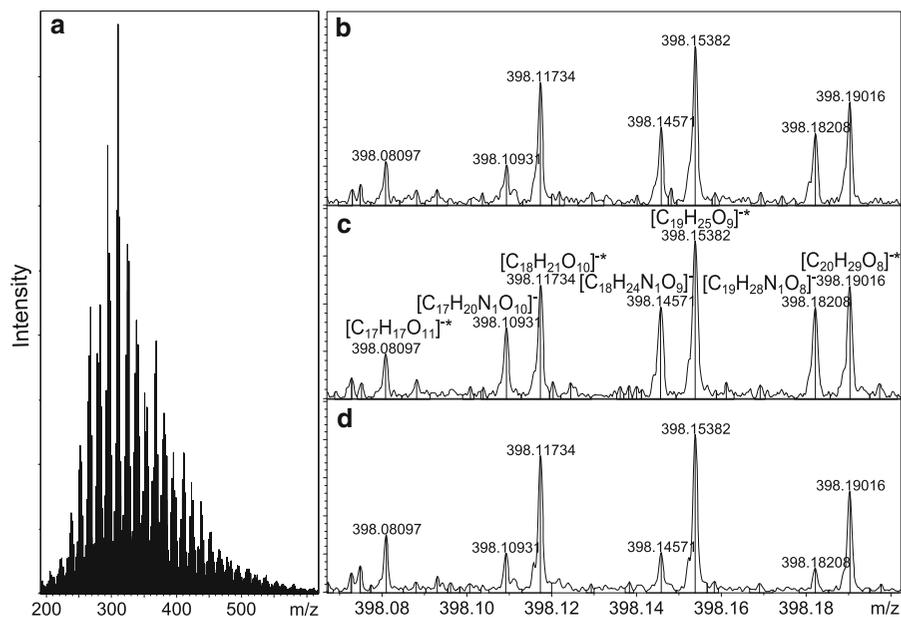


Fig. 2 Characteristic ESI negative FT-ICR mass spectrum for Lena Delta DOM with **a** mass range of 200–600 m/z, **b** mass spectrum at nominal mass 398 for a typical Lena River sample,

c melt water creek sample, and **d** Buor-Khaya Bay sample. Asterisks indicate the presence of one ^{13}C isotope in the assigned formula

elemental ratios. Compared to the weighted average H/C and O/C ratios for riverine samples (1.281 ± 0.013 and 0.425 ± 0.006 , respectively), Buor-Khaya Bay samples showed lower weighted average H/C ratios ranging from 1.217 to 1.241 (mean 1.230 ± 0.009), and higher O/C ratios of 0.445 ± 0.002 . For the permafrost melt water creek/lake DOM, the O/C and H/C ratios were 0.427 ± 0.007 and 1.315 ± 0.013 , respectively. DOM in ice complex melt water creeks was more oxidized than in the other creek samples. Weighted average O/C and H/C ratios in the ice complex DOM samples were 0.445 ± 0.007 and 1.268 ± 0.024 , respectively. Highest magnitude weighted average C/N ratios were observed for the bay samples (C/N: 176 ± 23) followed by the riverine (C/N: 143 ± 17) and creek/lake samples (C/N: 104 ± 15). Detailed information on weighted average elemental ratios are available in Table SI-3 and Fig. SI-2.

Molecular fingerprints

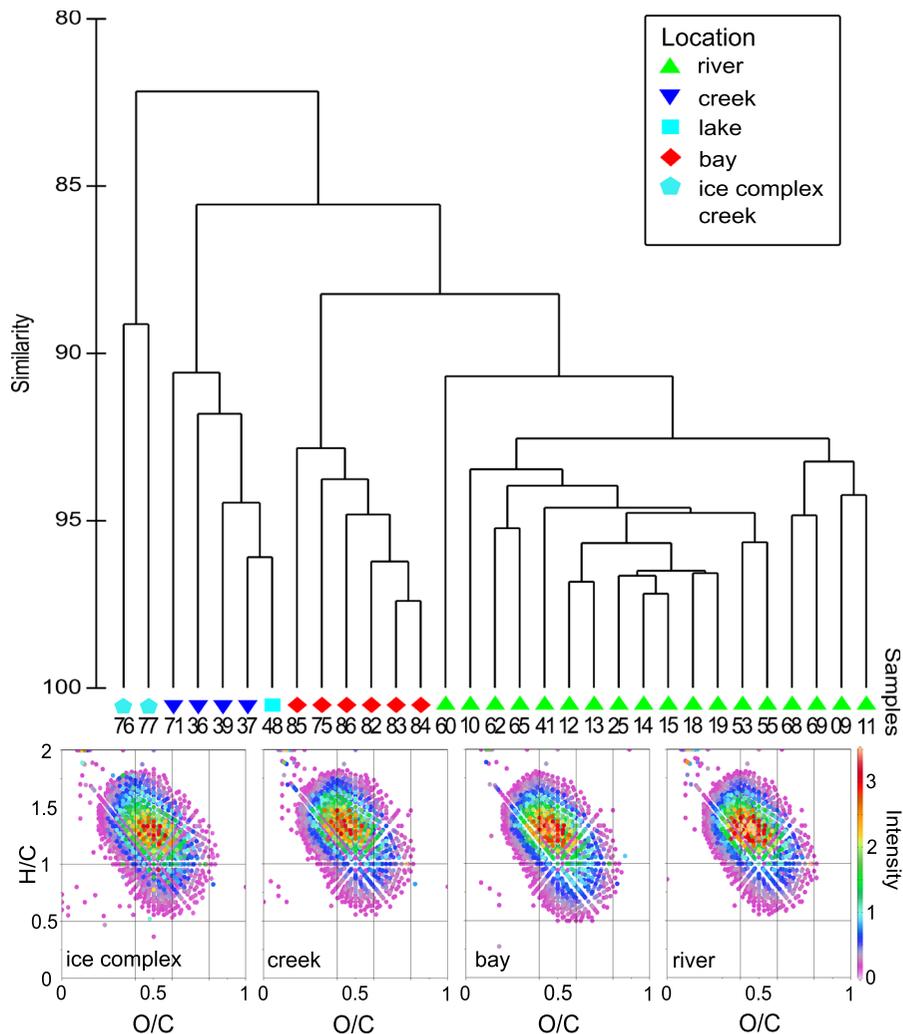
Hierarchical cluster analysis yielded four major clusters which clearly corresponded to the sample origin (Fig. 3): Lena River, Buor-Khaya Bay, permafrost creeks (including the lake sample) and ice complex

creeks. Samples derived from the Bykovsky Peninsula melt water creeks (ice complex samples 76 and 77) were most dissimilar from all other samples and formed a separate cluster. Samples from the same station in the river but from different depths (samples 14/15 and 12/13) showed a highly similar molecular composition (Bray–Curtis similarity > 95). Although samples showed a high degree of similarity (Van Krevelen diagrams, Fig. 3), it was still possible to differentiate sources based on the molecular formula information.

To elucidate the molecular differences we calculated the average relative peak magnitude for each sample type identified by the cluster analysis. The average results for each formula containing C, H, O and N were presented as their elemental H/C and O/C ratios in the van Krevelen diagrams (Fig. 3). For the determination of those formulas which were responsible for the sample variability in the cluster analysis, we applied principal component analysis (PCA) for the same data matrix (Fig. 4).

The PCA highlighted the molecular differences of the various sample types. In total, 56.7 % of variance could be explained by the first two principal components (PC, Fig. 4). PC1 and PC2 explained 31.5 and 25.2 % of the variance, respectively. Since PC3

Fig. 3 Hierarchical cluster analysis (Bray–Curtis similarity) and van Krevelen diagrams for “average samples”. Molecular differences were observed between a ice complex melt water creeks (*pentagons*, $n = 2$), creek (*triangles*, $n = 4$) plus lake samples (*square*, $n = 1$), Buor-Khaya Bay samples (*diamonds*, $n = 6$) and Lena River samples (*green triangles*, $n = 18$). For each sample type, all identified molecular formulas containing C, H, O and N are represented by their molecular H/C and O/C ratio (van Krevelen diagram). The *color code* represents the average relative peak magnitude



explained only 9 %, only the first two principal components were used for the data interpretation. Bour-Khaya Bay and permafrost melt water creeks were primarily separated on the PC1 axis whereas riverine samples showed exclusively negative PC2 values. The only exception which also showed negative PC2 values was the creek sample (sample 36) which was collected near the research station on Samoylov Island.

To identify specific molecular DOM markers for each source of DOM, the molecular formulas with the highest absolute PCA loadings for PC1 and PC2 were analyzed (Fig. 4). The combined loadings were irregularly distributed; some regions had a higher and others a lower density of data points. The majority of $C_xH_yO_z$ identifications showed negative loadings of PC1 whereas nitrogenous compounds ($C_xH_yO_zN_1$ and

$C_xH_yO_zN_2$) had positive PC1 loadings. Therefore, $C_xH_yO_zN_{1-2}$ had a higher contribution to sample variance as compared to $C_xH_yO_z$. Sulfur containing compounds did not contribute considerably to the sample variability. Additionally, negative PC1 loadings were characteristic for identifications with the highest molecular masses. For the identification and generalization of molecular drivers responsible for the sample groupings and to understand the nature of molecular differences and transformations of DOM the 500 highest positive/negative eigenvalues for each principal component were used and plotted in van Krevelen diagrams (Fig. 5).

The Buor-Khaya Bay samples (negative PC1 values, Fig. 4) showed a predominance of oxygen-rich components and nitrogenous compounds were almost

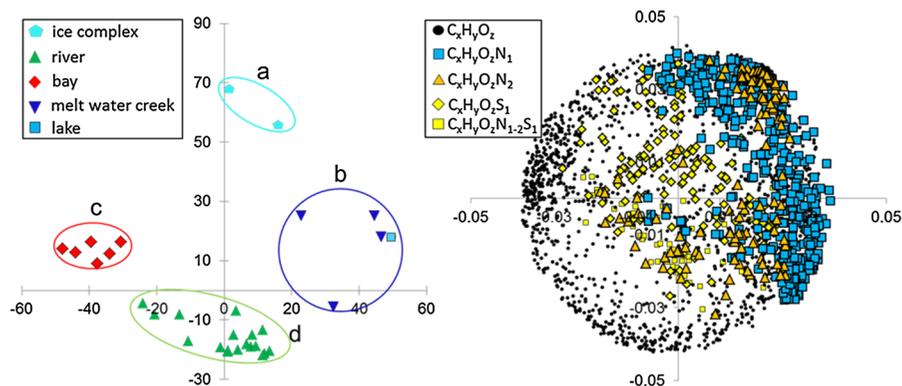
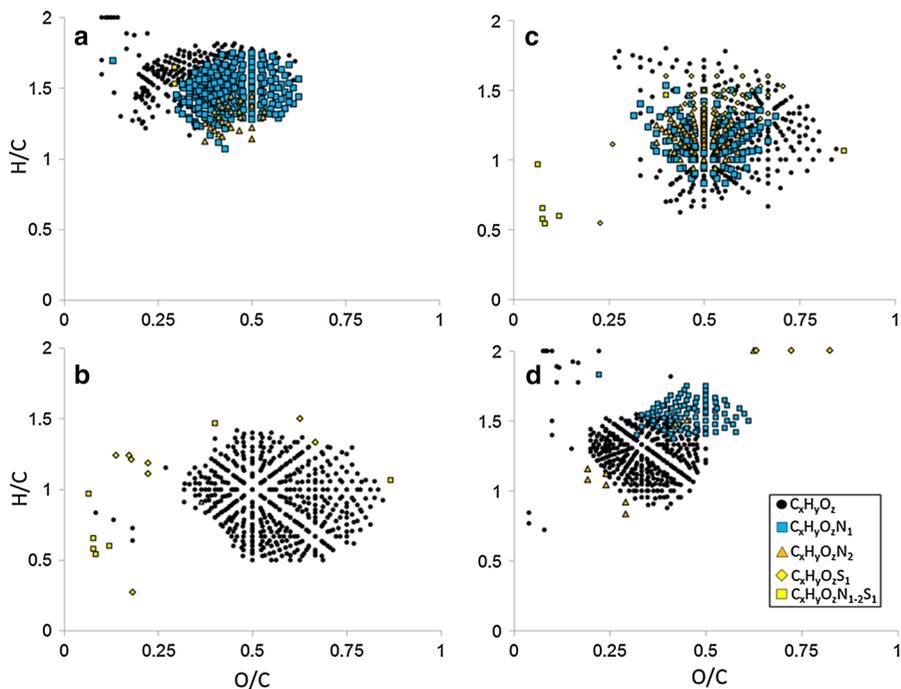


Fig. 4 Principal component analysis. PC1 and PC2 explained 56.7 % of the variance in the biplot (left panel): **a** ice complex creek DOM samples (pentagons), **b** permafrost creeks and lake DOM samples (upside down triangles and square), **c** Buor-Khaya Bay samples (diamonds), **d** Lena River DOM samples

(triangles). The PCA loadings highlighted the molecular formulas with respect to nitrogen and sulfur content (right panel): no nitrogen and sulfur (circles), one nitrogen atom (squares), two nitrogen atoms (triangles), one sulfur (light diamonds), sulfur and nitrogen (light squares)

Fig. 5 Van Krevelen plots for the 500 highest positive/negative PC loadings: **a** 500 positive eigenvalues for PC1, **b** 500 negative for PC1, **c** 500 positive PC2, **d** 500 negative for PC2. $C_xH_yO_z$ molecular formulas (circles) were distinguished from $C_xH_yO_zN_1$ (squares), $C_xH_yO_zN_2$ (triangles), $C_xH_yO_zS_1$ (light diamonds), and $C_xH_yO_zN_{1-2}S_1$ (light squares). (Color figure online)



absent in formulas which showed negative PC1 loadings (Fig. 5b). Formulas with high positive PC1 loadings contained many N-containing formulas (Figs. 4, 5a). The clear separation of compounds with positive and negative PC1 loadings in the van Krevelen diagrams particularly reflected the differences in chemical compositions and properties and the relation to sample origin.

High PC2 values were characteristic for bay and creek water samples with the highest values for ice complex creek samples. The $C_xH_yO_z$ identifications with the highest PC2 loadings had high O/C ratios, but were more hydrogen saturated compared to the lowest PC1 loadings. There was also a core of nitrogenous compounds with the “center of mass” at O/C \sim 0.55 and H/C \sim 1.2 (Fig. 5c). The contribution of

molecular formulas with low PC2 scores to the bay sample variability was minor and formulas with high positive PC2 loadings did not explain variance in the bay samples. Molecular components with negative PC2 loadings (Fig. 5d) mostly represent riverine-dominant DOM molecular formulas. We have visualized molecular transformation trends on the upstream–downstream transect. This information with applied procedures is available in the supplementary materials.

Indicator peaks supported the sample groups (permafrost melt water creeks, ice complex creeks, river and bay samples) assigned in the cluster analysis (Fig. 6). Using only significant indicator values ($p < 0.05$; Fig. 6), bay samples had high O/C ratios and a relatively high degree of unsaturation in comparison to ice complex, creek and river DOM samples. Creek DOM samples had highly aliphatic indicator molecular markers. Riverine indicator markers took an intermediate position between the bay and creek DOM samples (Fig. 6). It is difficult to judge representativeness of the indicators of ice complex creek DOM due to the low number of samples ($n = 2$). Using the available data, riverine indicators (Fig. 6) match well with the PCA loadings (negative PC2 values; Fig. 5d). Therefore, river-dominant DOM compounds exposed a relative depletion of oxygen (O/C ranged from 0.25 to 0.5) and high H/C ratios (ranging from 1 to 1.55; (Fig. 6, Figs. SI-4/5 and Table SI-4)).

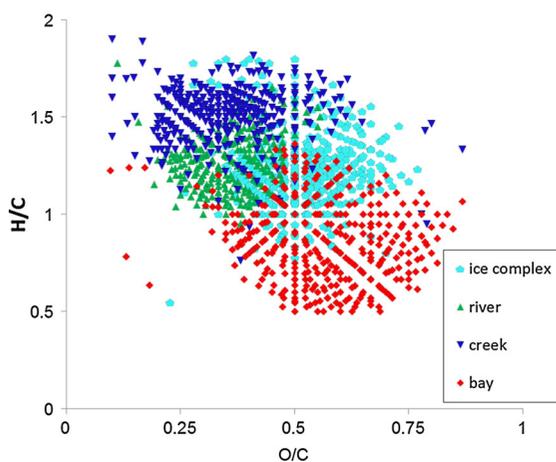


Fig. 6 Van Krevelen diagrams with indicator peaks determined by indicator value analysis (indicator values of Dufrène and Legendre)

DOM classification

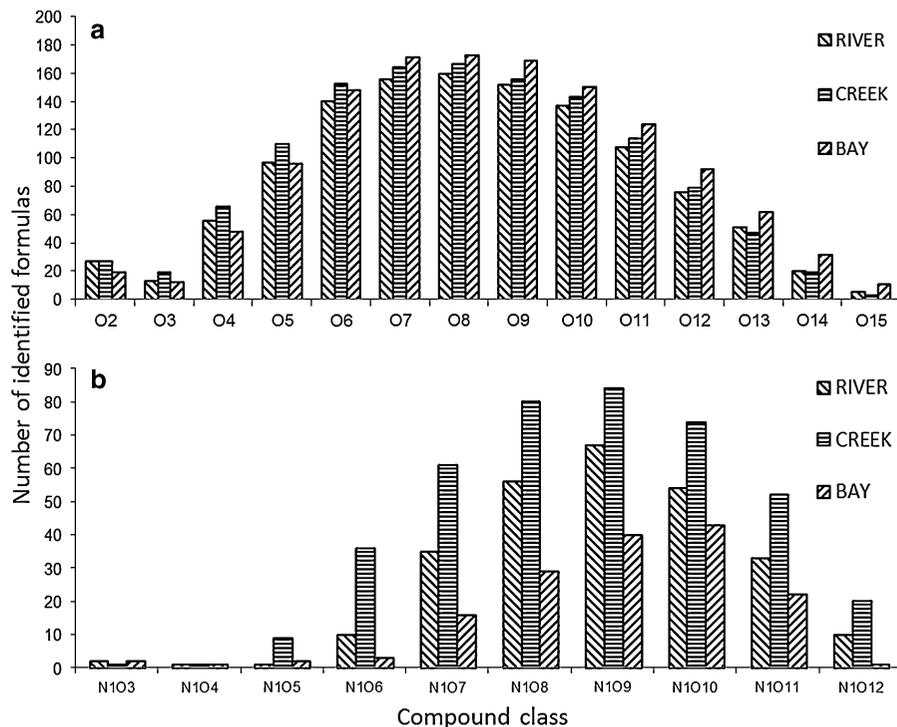
Based on the information derived from the PCA, the individual formulas were examined in more detail. The series of formulas which solely contained C, H, and O (Fig. 5a) revealed that compounds with higher numbers of oxygen clearly prevailed in the bay samples. This observation fit well with the prevalent oxygenated formulas in the bay samples (Fig. 5b). The classes O₁–O₆ of the bay samples had the lowest number of identifications compared with the riverine and creek samples. However, in the compound classes with >5 oxygen atoms, the bay samples showed the highest number of oxygen-rich identifications (Fig. 7).

Compounds containing one nitrogen atom were predominantly detected in river and creek samples (Fig. 6b and Fig. 7). The lowest abundance of this compound class was observed for the bay samples. A similar trend was found for compounds containing two nitrogen atoms. These results agreed well with the PCA loadings.

Discussion

Little is known about the role, dynamics and transformation of DOM in the Arctic ecosystem in particular with regard to permafrost, its thawing and predicted changes due to global warming. The average DOC concentration of about 500 $\mu\text{mol C L}^{-1}$ and TDN concentration of 13 $\mu\text{mol N L}^{-1}$ in the Lena River in the summer 2009 were similar to values presented in previous studies (Lara et al. 1998; Lobbes et al. 2000). DON concentrations in the summer months are the highest within the seasons (Holmes et al. 2012), potentially because of the high number of very different DOM sources with less degraded material. Highest DOC concentrations are characteristic for the freshet period. Elevated DON values in late summer represent a source shift of organic matter in the river, likely because of increasing contribution of permafrost organic matter. Freshet periods are characterized by a large contribution of organic matter from surface layers of soils (Finlay et al. 2006). In late summer, the contribution of surface soil layer DOM decreases and the contribution of deeper soil horizons increases. An increase of soil derived organic matter in the permafrost influenced Kolyma River was inferred from the changes in the radiocarbon age of DOC in late summer (Neff et al. 2006). Since it

Fig. 7 **a** Classification of $C_xH_yO_z$ identified molecular formulas based on the number of oxygen atoms per identification. **b** Classification of $C_xH_yO_zN_1$ identified molecular formulas based on the number of oxygen atoms per identification



was demonstrated that organic matter released by permafrost degradation could be more labile, this organic matter will affect regional microbial activity and primary production in the Lena River and the inner Laptev Sea. Decomposition of about 34 % of DOC was characteristic for Yedoma (ice complex) melt water streams and of 17–33 % for river–creek water mixtures (Vonk et al. 2013). We did not observe that DOC concentrations increased continuously on the way from the most upstream to the downstream sample. Thus, deltaic permafrost emitted DOM obviously did not affect riverine DOC concentration probably due to high mineralization rates and dilution by the huge river water volume.

DOC and TDN values for ice complex melt waters were extremely high. Several melt water creeks with very high DOC concentrations drain the massive ice complex on Kurunagh Island and discharge into the Olenekskaya channel. These DOM pulses probably caused the elevated DOC concentration in the channel ($660 \mu\text{mol C L}^{-1}$). Since the Olenekskaya channel discharges only ~ 6.5 % as compared to the total Lena River water (Fedorova et al. 2013) ice complex derived DOC contributes only very little to river DOC in the entire Lena Delta. The few ice complexes

are sporadically distributed (Are and Reimnitz 2000). They are syngenetically frozen, fine-grained deposits and are built of large amounts of ground ice and massive ice wedges. The ice complexes also contain considerable amounts of fossil organic carbon that might become accessible due to permafrost thawing (Khvorostyanov et al. 2008; Schirmermeister et al. 2011) but it is unknown if this material is labile or refractory.

DOC and TDN concentrations in permafrost melt waters were highly variable and ~ 10 times lower than in the ice complex derived water. In contrast to the river and bay, the DOC concentrations of the melt water creeks were more variable and were slightly lower in DOC and higher in TDN. Permafrost melt waters are exposed to more environmental factors in comparison with the river. The influence of vegetation type, intense exposure to the light, different contributions of active organic matter layers and nutrients are more pronounced (Rodionov et al. 2007). Also, mixing processes, which are intense in the river, might be less important in the creeks resulting in a higher DOC and TDN variability.

Molar C/N ratios of DOM and SPE-DOM were different but the trends were similar. Inorganic nitrogenous nutrients are almost negligible in summer

(Holmes et al. 2012; Lobbes et al. 2000) and thus DON is only slightly lower than TDN determined in our study. SPE-DOM contains only traces of inorganic material, and therefore TDN concentrations were equivalent to DON. The C/N ratios did not allow discrimination of riverine and bay samples, although ratios were slightly lower in the bay samples which however were still mostly dominated by Lena River water with almost no salinity. The high C/N ratios are typical for the Arctic rivers (e.g., Lobbes et al. 2000) decreasing by a factor of two in the open Laptev Sea (Kattner et al. 1999). DOM of riverine and bay samples were reduced in DON compared to the creeks. The C/N ratios of this ice complex runoff (~ 33) reflect a high contribution of relatively fresh material released by the thawing process. This is further evidence for the lability and degradability of organic matter deposited in permafrost. The DOM may also originate from thawing of long-term frozen particulate material. In contrast, the particulate material in the Lena River is mostly allochthonous, soil-derived organic matter accumulated along the enormous length of the river. Autochthonous production is low because of the nutrient limitation and the high turbidity.

The obvious and interesting changes in the relative nitrogen content of the samples were reflected in the FT-ICR MS analyses. This supports the observations derived from bulk DOC and TDN measurements that during the transport of DOM from the thawing permafrost into the melt water creeks and the river and finally the Buor-Khaya Bay a continuous decline of nitrogen containing molecules occurred (Figs. 4, 7). The N-containing formulas in Fig. 5a (those enriched in the permafrost creeks and lake DOM) are mostly aliphatic (based on their H/C ratios) and could likely be related to peptide-like moieties (based on their O/C ratio). Although FT-ICR MS is a semi-quantitative method, it clearly demonstrated the relative depletion of nitrogenous compounds in the bay samples. The O/C_{wa} , H/C_{wa} and C/N_{wa} ratios exposed the general enrichment of creek DOM samples by nitrogenous components and vice versa in the bay samples, which were more oxidized. The clearly higher C/N ratios calculated from the weighted average FT-ICR MS data than from the bulk data are due to the strict intensity threshold used in FT-ICR MS data processing, which excludes the low intensity nitrogenous mass peaks. Nevertheless, the trend was similar to the

bulk measurements. Hierarchical clustering of the FT-ICR MS data showed that the origin of DOM was systematically reflected in its molecular composition also beyond the contribution of nitrogen containing compounds. Principal component analysis also highlighted the importance of nitrogenous and oxidized components for the molecular variance of DOM in the creek, river and Buor-Khaya Bay samples. Even the different types of creeks could be distinguished: permafrost melt water creeks differed from organic-rich ice complex melt water creeks. Obviously, the type of permafrost also affected the composition of released DOM.

On a smaller spatial scale, as studied on Samoylov Island (samples 48, 36, 37, 39), the composition of all DOM samples was very similar. Even the molecular composition of the lake sample matched with the creek samples. Creeks on Samoylov Island drain the lakes and therefore, lake and creek DOM samples have the same origin that is the active layer of permafrost and the local vegetation. The similarity of the one single lake sample and the creeks suggests that the prevailing molecular pattern from the lake sample remained largely unaltered in the creek run-off on its short way ($<1,000$ m) to the Lena River.

The molecular changes from river to bay might be partially explained by precipitation/flocculation of less-hydrophilic molecules (Lantuit et al. 2012) although there was no pronounced salinity gradient in the bay (maximum salinity of 2). This observation opposed previous findings. For example, compositional differences of DOM presented for marine and mangrove systems (Koch et al. 2005) and for a river–ocean transect in the Chesapeake Bay (Sleighter and Hatcher 2008) showed that DOM tends to become more aliphatic and depleted in oxygen-rich molecules from inshore to offshore. However, the bay samples in our study were still largely influenced by the riverine DOM and therefore did not represent marine waters. Also, it was shown that the phytoplankton communities are different in the Lena Delta and Buor-Khaya Bay due to the increasing amounts of seawater mixed to the riverine freshwater (Kraberg et al. 2013). These observations might be more important for molecular differences between river and bay samples than physico-chemical processes due to the slight salinity gradient. Open ocean studies have shown that the molecular composition of DOM differs between biological provinces (Koch and Kattner 2012). Also,

previously it was demonstrated that DOM photo-degradation resulted in production of highly oxidized organic molecules, similar to molecular components dominated in the bay samples of our study (Gonsior et al. 2013). Since summers in the Arctic are characterized with intense 24 h sun activity, the observable molecular characteristics of the bay samples could also be a result of riverine DOM photo-degradation.

Source-specific and source-dominant molecular markers will allow in future estimates of the various contributions to DOM which integrates signals from diverse sources (e.g., riverine and estuarine DOM). Since Lena River is affected by the climate change driven factors (discharge increase, permafrost degradation, changes in vegetation) and permafrost-derived DOM believed is to be labile, quantification of its contribution to riverine DOM and analysis of its further fate is important.

Conclusions

Bulk DOM parameters matched with the DOM molecular fingerprints, especially with focus on the nitrogenous fraction. FT-ICR MS was successfully employed as a tool for non-targeted biogeochemical studies of DOM in the Lena Delta. DOM samples were clearly differentiated according to source. DOM systematic molecular characteristics with respect to water origin potentially allow understanding of processes and transformations of DOM. Origin-systematic molecular composition is originating from different mechanisms of DOM formation. Therefore, our study supports the identification of processes and environmental parameters responsible for molecular DOM fingerprints. Special attention needs to be addressed to the quantification of DON fluxes in the region.

The compositional changes could present a major direction of DOM mineralization and transformation processes in the deltaic system of the Lena River. Since DOM is an important player in the carbon cycle the advanced understanding of DOM mineralization which results in CO₂ emission could provide additional knowledge on dynamics of changes in DOM. Understanding of these dynamics will allow predictions and record changes in carbon cycling. Climate change can potentially cause an increase of the active permafrost layer in the Arctic which will increasingly contribute to the amount of soil-derived organic matter

in the rivers (Gustafsson et al. 2011). Since we demonstrated significant differences of the DOM molecular composition of melt water creeks and the Lena River, the growing contribution by permafrost DOM might also change the riverine biogeochemistry in the Lena Delta and the Laptev Sea with consequence on the ecosystem.

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