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Dynamics of dissolved organic matter in boreal lakes and sediments

Juliana Valle das Neves

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"we can work

at our own pace

and still be

successful"

(Rupi Kaur)

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Abstract

Dissolved organic matter (DOM) is a highly complex mixture of organic molecules found in soils, sediments, fresh and marine waters. DOM is an important source of carbon and nutrients for many microbial degradation processes, in which it acts both as substrate and as electron acceptor, providing a range of ecosystem services. During anaerobic degradation, DOM is partially consumed and altered into new molecules, and greenhouse gases methane (CH_4) and carbon dioxide (CO_2) are produced.

In this study, our goals were threefolded. First, we wanted to prove that ultrahigh resolution mass spectrometry was a capable tool on tracing molecular changes in DOM during anaerobic degradation. Then, we further investigated water column and sediment pore water DOM to better understand its dynamics. And finally, we connected the freshwater DOM pool to the landscape context.

We used Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) to determine the molecular compositions of thousands of molecules and computed bulk parameters of solid-phase extractable DOM (SPE-DOM) isolated from lake water and surface sediments of ten Swedish boreal lakes. In parallel, laboratory sediment incubation was performed under anoxic conditions for 40 days. We determined CH_4 and CO_2 evolution, compared sediment pore water SPE-DOM pools before and after the incubation, and correlated the assigned molecular compositions with physico-chemical parameters, morphology, and landscape coverage.

Lake water SPE-DOM showed higher oxygenation but lesser proportions of nitrogen and sulfur than sediment pore water SPE-DOM. Incubation caused rather non-selective large-scale molecular transformations of SPE-DOM, with common trends and lake-specific alterations. Processing of SPE-DOM depended on key influencing variables, especially lake and catchment characteristics, and nutrients and DOC in lake waters. Processing of organic molecules exceeded mineralization by several orders of magnitude: CH_4 and CO_2 production rates accounted for small fractions of sedimentary organic carbon, whereas incubation-induced changes of bulk and molecular parameters were in the percent range. Incubation caused depletion of highly oxygenated and oxygen-deficient molecules, and formation of presumably non-labile fulvic acid type substances. Statistical analysis based on the common molecular compositions observed in all ten lakes were more influenced by the characteristics intrinsic to each lake and its catchment than by the anoxic incubation which the samples experienced.

Zusammenfassung

Gelöste organische Substanz (dissolved organic matter – DOM) ist eine hochkomplexe Mischung organischer Verbindungen, die in Böden, Sedimenten, Süß- und Meerwasser vorkommt. DOM ist eine wichtige Kohlenstoff- und Nährstoffquelle für viele mikrobielle Abbauprozesse, in denen es sowohl als Substrat als auch als Elektronenakzeptor fungiert und eine Reihe von Ökosystemleistungen bereitstellt. Beim anaeroben Abbau wird DOM teilweise verbraucht und in neue Moleküle umgewandelt, und es entstehen die Treibhausgase Methan (CH_4) und Kohlendioxid (CO_2).

In dieser Studie waren unsere Ziele dreifach. Zunächst wollten wir beweisen, dass die ultrahochauflösende Massenspektrometrie ein geeignetes Werkzeug zur Verfolgung molekularer Veränderungen in DOM während des anaeroben Abbaus ist. Dann untersuchten wir Wassersäulen- und Sedimentporenwasser-DOM weiter, um seine Dynamik besser zu verstehen. Und schließlich haben wir den Süßwasser-DOM-Pool mit dem Landschaftskontext verbunden.

Wir verwendeten Fourier-Transform-Ion-Zyklotron-Resonanz-Massenspektrometrie (FT-ICR-MS), um die molekulare Zusammensetzung von Tausenden von Molekülen zu bestimmen und berechneten Volumenparameter von festphasenextrahierbarem DOM (SPE-DOM), das aus Seewasser und Oberflächensedimenten von zehn schwedischen borealen Seen isoliert wurde. Parallel dazu wurde eine 40-tägige Laborsedimentinkubation unter anoxischen Bedingungen durchgeführt. Wir bestimmten die CH_4- und CO_2-Entwicklung, verglichen SPE-DOM-Pools im Sedimentporenwasser vor und nach der Inkubation und korrelierten die zugewiesenen molekularen Zusammensetzungen mit physikalisch-chemischen Parametern, Morphologie und Charakteristika der Einzugsgebiete.

Seewasser SPE-DOM zeigte eine höhere Sauerstoffanreicherung, aber geringere Anteile an Stickstoff und Schwefel als Sedimentporenwasser SPE-DOM. Die Inkubation verursachte ziemlich unselektive molekulare Transformationen von SPE-DOM im großen Maßstab mit gemeinsamen Trends und seespezifischen Veränderungen. Die Verarbeitung von SPE-DOM hing von wichtigen Einflussgrößen ab, insbesondere von See- und Einzugsgebietseigenschaften sowie Nährstoffen und DOC in Seewasser. Die Verarbeitung organischer Moleküle übertraf die Mineralisierung um mehrere Größenordnungen: Die CH_4- und CO_2-Produktionsraten machten kleine Anteile des sedimentären organischen Kohlenstoffs aus, wohingegen Änderungen molekularer Parameter im Prozentbereich beobachtet wurden. Die Inkubation verursachte eine Verarmung an stark sauerstoffangereicherten und sauerstoffarmen Molekülen und die Bildung von eher nicht-labilen Substanzen vom Fulvosäure-Typ. Die statistische Analyse der allen zehn Seen gemeinsamen Summenformeln wurde stärker von den charakteristischen Merkmalen eines jeden Sees und seinem Einzugsgebiet beeinflusst als von der anoxischen Inkubation der Proben.

List of publications

1. <u>Juliana Valle</u>, Michael Gonsior, Mourad Harir, Alex Enrich-Prast, Philippe Schmitt-Kopplin, David Bastviken, Ralf Conrad, and Norbert Hertkorn **(2018)**. Extensive processing of sediment pore water dissolved organic matter during anoxic incubation as observed by high-field mass spectrometry (FTICR-MS). *Water Research* (129), 252–263.

2. <u>Juliana Valle</u>, Michael Gonsior, Mourad Harir, Alex Enrich-Prast, Philippe Schmitt-Kopplin, David Bastviken, and Norbert Hertkorn **(2020)**. Molecular differences between water column and sediment pore water SPE-DOM in ten Swedish boreal lakes. *Water Research* (170), 115320.

3. <u>Juliana Valle</u>, Mourad Harir, Michael Gonsior, David Bastviken, Martin Karlson, Alex Enrich-Prast, Ralf Conrad, Philippe Schmitt-Kopplin, and Norbert Hertkorn **(2022)**. Influence of catchment in sediment pore water SPE-DOM molecular composition and degradation. (to be submitted)

Other, non-first author publications:

4. Michael Gonsior, <u>Juliana Valle</u>, Philippe Schmitt-Kopplin, Norbert Hertkorn, David Bastviken, Jenna Luek, Mourad Harir, Wanderley Bastos, and Alex Enrich-Prast **(2016)**. Chemodiversity of dissolved organic matter in the Amazon Basin. *Biogeosciences,* 13, 4279–4290.

5. Alex Enrich-Prast, Viviane Figueiredo, Fausto Machado-Silva, Roberta Bittencourt Peixoto, Leonardo Amora-Nogueira, Gabriela Cugler, Maria Carolina Barroso dos Santos, João Paulo de Sá Felizardo, <u>Juliana Valle</u>, Davi Pedroni Barreto, Luciene Valladares, Laís Rodrigues, Ana Lúcia Santoro, Luana Queiroz Pinho, Camila Negrão Signori, Ricardo Pollery, Eliane Silva, Humberto Marotta **(2022).** Inorganic nitrogen stimulates methane oxidation in coastal lagoon sediments. *Oecologia Australis,* 26, 326–338.

6. Siyu Li, Mourad Harir, Philippe Schmitt-Kopplin, Fausto Machado-Silva, Michael Gonsior, David Bastviken, Alex-Enrich-Prast, <u>Juliana Valle</u>, Norbert Hertkorn **(2022)**. Distinct non-conservative behavior of dissolved organic matter after mixing different types of Amazon River waters. (under review)

7. Siyu Li, Mourad Harir, David Bastviken, Philippe Schmitt-Kopplin, Michael Gonsior, Alex Enrich-Prast, <u>Juliana Valle</u>, Norbert Hertkorn **(2022)**. Dearomatization drives complexity generation in freshwater dissolved organic matter. (under review)

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List of Abbreviations

AI: aromaticity index

CDOM: chromophoric dissolved organic matter

CH_4: methane

CO_2: carbon dioxide

CRAM: carboxylic-rich alicyclic molecules

DBE: double bond equivalent

DIC: dissolved inorganic carbon

DOC: dissolved organic carbon

DOM: dissolved organic matter

EEM: excitation-emission matrix

ESI: electrospray ionization

FT-ICR: Fourier transformation ion cyclotron resonance

GC: gas chromatography

GDOM: groundwater dissolved organic matter

HCA: hierarchical cluster analysis

IRMS: isotope ratio mass spectrometry

KMD: Kendrick mass defect analysis

MS: mass spectrometry

MW: molecular weight

NOM: natural organic matter

OC: organic carbon

PARAFAC: parallel factor analysis

PCA: principal component analysis

PLS: partial least square

POC: particulate organic carbon

POM: particulate organic matter

sedDOM: sediment pore water dissolved organic matter

SOM: soil organic matter

SPE: solid phase extraction

TN: total nitrogen

TOC: total organic carbon

TP: total phosphorus

WTR: water retention time

Chapter 1

General Introduction

1. General Introduction

Natural organic matter (NOM) is found in all natural and engineered systems of soils, sediments, freshwaters, and marine waters. NOM is a highly complex mixture of compounds, that collectively exhibit a nearly continuous range of properties (size-reactivity continuum) (Amon and Benner, 1996a; Benner and Amon, 2015). Its chemical composition includes mainly carbon, hydrogen, and oxygen, with minor contributions from heteroatoms (e.g. nitrogen, sulfur, and phosphorus) (Hertkorn et al., 2008).

NOM is a relevant organic carbon (OC) stock on the planet. Its dissolved fraction is named dissolved organic matter (DOM), and acts as both substrate, source of nutrients, and electron acceptor for several biological and photochemical pathways (Carlson and Hansell, 2015), as well as providing a wide range of ecosystem services (Bolan et al., 2011; Ridgwell and Arndt, 2014; Schmidt et al., 2011; Senesi and Loffredo, 2005). In a modern context of global climate change, it is indispensable to understand the behavior of such organic molecules in the environment.

High-resolution mass spectra of solid phase extracted DOM (SPE-DOM) have been reported since the 1970s, but the introduction of Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometry, capable of measuring ions at both very high mass accuracy and resolution, has changed our ability to characterize SPE-DOM at the molecular level (Hertkorn et al., 2008; Schmitt-Kopplin et al., 2012; Schmitt-Kopplin and Hertkorn, 2007). Coupled to electrospray ionization (ESI), this instrument provides the necessary resolution to distinguish up to several thousand ions with unique masses within a DOM pool (usually with highest intensity around 350–700 Da) (Schmitt-Kopplin et al., 2012). Due to the high mass resolution and accuracy (typically <1 ppm for ions <400 Da), elemental formulas for most ions can be assigned with a high level of confidence. Since the number of isomers for any given mass increases rapidly with molecular weight, each unique mass probably represents a mixture of different isomers – ranging from hundreds to thousands and millions of isomers for any given molecular composition (Hertkorn et al., 2007; Schmitt-Kopplin et al., 2019).

Although high-resolution mass spectrometry (MS) does not allow for the full identification of new compounds, it has led to the discovery of important new compound classes, and it is proving to be a very powerful technique for detecting changes in DOM composition between

samples (Hertkorn et al., 2012, 2008; Repeta, 2015). However, there are still many degradation pathways which have not been elucidated at the molecular level.

1.1. DOM

The formation of NOM on earth preceded the evolution of life. NOM does not originate directly from a genetic code, such as proteins/peptides, carbohydrates, lipids, and other metabolites, neither acts in regulatory networks in life functions. Instead, NOM is generated through combined action of biotic and abiotic pathways, governed by common laws of thermodynamics and kinetics (Hertkorn et al., 2007).

Dissolved organic matter (DOM) is the dissolved or colloidal fraction of NOM, i.e., the portion that passes through a selected pore size filter (usually 0.7 or 0.45µm) (Brezonik and Arnold, 2011). The other fraction, i.e., larger than the pore size, is referred to as particulate organic matter (POM) (Kördel et al., 1997). NOM is the term used to refer to organic material in water, soil, or sediment derived from the activities of plants and animals (other than humans) in the natural environment (Brezonik and Arnold, 2011).

Among all environments, DOM is a complex mixture of aromatic and aliphatic hydrocarbon structures that have amide, carboxyl, hydroxyl, ketone, and various minor functional groups attached, that present a wide range of size (from 100 to 100,000 Da, when in colloidal form) and different polarity/solubility properties (Leenheer and Croué, 2003). Basic classification of DOM is shown in Figure 1.1.

DOM is generally expressed in terms of dissolved organic carbon (DOC) content, but the major elements accompanying carbon include oxygen, hydrogen, nitrogen, phosphorus, sulfur, and trace amounts of various cations including calcium, potassium, magnesium, and metal(loid)s including aluminum, iron, zinc, and copper (Bolan et al., 2011). CDOM (chromophoric dissolved organic matter) is the fraction of DOM which absorbs visible light and, therefore, appears colored (Raymond and Spencer, 2015).

Because the dissolved material is more available to microorganisms, DOM represents one of Earth's largest exchangeable reservoirs of organic material (Carlson and Hansell, 2015). DOM can act not only as a carbon source, but also as an electron acceptor (Carlson and Hansell,

2015), apart from controlling levels of dissolved oxygen, nitrogen, phosphorus, sulfur, numerous trace metals, and acidity (Leenheer and Croué, 2003).

For aquatic ecosystems, NOM is of central importance in understanding the acid-base chemistry, behavior of metal ions, and photochemistry of natural waters, and it has important ecological and water quality implications for the treatment and domestic use of water (Brezonik and Arnold, 2011). Actually, DOM is involved in range of different ecosystems services, such as soil aggregation and erosion control, mobilization and export of nutrients, and bioavailability and ecotoxicology of heavy metals (Bolan et al., 2011). Due to its importance to the ecosystem ecology, a details and comprehensive understanding of its molecular composition and behavior is needed.
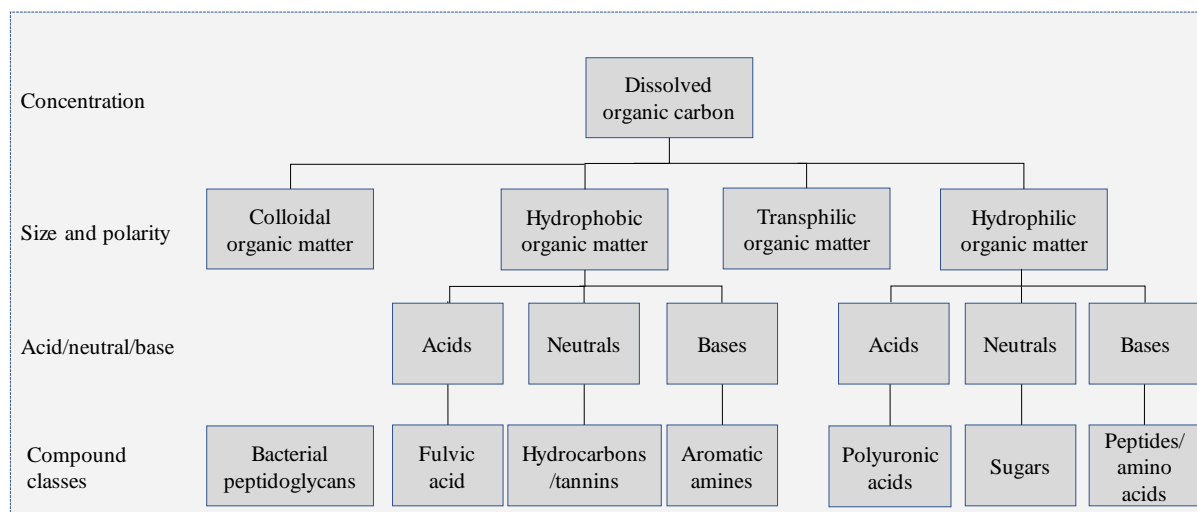
Figure 1.1. Classification of DOM adapted from *Leenheer & Croué (2003)*.

1.1.1. Soil DOM

In soils, DOM is the dissolved fraction of the soil organic matter (SOM), i.e., the soluble fraction. There are several sources of DOM input to soils (Table 1.1), but nearly all soil DOM derives from photosynthesis, including recently photosynthates – from leaf litter to root exudates – and decomposition and metabolic by-products of microbiologically processed SOM (Bolan et al., 2011).

Generally, DOM in the upper soil profile has a vegetation-type signature, with lignin-derived phenols and plant-derived carbohydrates (soluble organic compounds produced during litter and humus degradation in the organic rich compartment), while most subsoil DOM is of

microbial origin (Kaiser and Kalbitz, 2012; Schmidt et al., 2011). This reinforces the relevance of the landscape in the soil DOM composition.

Soil DOM consists mainly of low molecular weight carboxylic acids, amino acids, carbohydrates, and fulvic acids. From those, the first represents less than 10% of total DOM in most soil solutions and the last is typically the most abundant fractions of DOM (Bolan et al., 2011). Differently from other DOM components, soil fulvic acids are not defined by a unique chemical formula and do not belong to any of the known chemical classes of organic compounds. Fulvic acids consist of a heterogeneous mixture of relatively low molecular weight (500–2000 Da), colored organic molecules of mixed aliphatic and aromatic nature, and carries carboxylic and phenolic OH groups (Bolan et al., 2011).

Moreover, soil DOM is also rich in lipids, amino acids, carbohydrates, and black carbon. In general, soil DOM showed average O/C ratios of 0.28 – 0.47, average H/C ratios of 1.30 – 1.74, molecular weights of 380 – 417 Da and DBE values between 3.89 – 7.85 (Ohno et al., 2010). The later was lower than those verified for sediment, freshwater, and marine DOM. Besides, when compared to plant extracts, soil DOM showed a decrease in amino acids and a relative increase in lignin and carbohydrates. This is possibly a result of microbial uptake of N-containing molecules, release of water-soluble compounds during degradation, and intrinsic recalcitrance of lignin (Ohno et al., 2010).

Soil DOM undergoes different biological (biodegradation, biotransformation), chemical (sorption, complexation, photodegradation), and physical (leaching, eluviation) processes, that are regulated by biotic and abiotic factors, including pH, OC content, microbial activity, temperature, and moisture (Bolan et al., 2011). The effect of pH on soil DOM composition, for example, has been previously described (Roth et al., 2015). Here, higher DOC concentrations and lower pH values were related to increased molecular unsaturation and oxygenation, and larger molecular size. These molecular characteristics indicate that phenolic tannin compounds may be more abundant at a lower pH (Roth et al., 2015).

Roth et al. (2019) also investigated soil DOM as it moved through the soil profile. Their results indicated an increase of the weighted mean of m/z with soil depth, probably caused by both an absolute decline in abundance of low-molecular-mass compounds and by an absolute increase in abundance of mid- and high-molecular-mass compounds. Here, soil microbial community

was the most important predictor of the molecular DOM variation (25%), followed by the soil properties (19%) and root biomass (10%). However, more than 40% of the DOM variation remains unexplained, suggesting that other environmental variables contribute to DOM variability (Roth et al., 2019).

A high proportion of organic compounds in soils is attached to mineral particles, forming soil aggregates (Kögel-Knabner and Amelung, 2021). Solid-state NMR spectroscopy enables the characterization of insoluble compounds without extensive pretreatment and thus without chemical or physical changes (Abelmann et al., 2004). Solid-state ^{13}C NMR spectra have shown that physically protected carbon comprises functional groups also present in bulk soil OM. Interestingly, carbohydrates and peptide-like material, generally considered to be the most chemically and physically labile fractions of SOM, are present in residues after UV photo-oxidation. The presence of highly resistant peptide-like structures strongly suggests that preservation may originate from a physical protection mechanism (Knicker and Skjemstad, 2000).

Therefore, soil DOM persistence is not only a result of its molecular composition, but most relevant would be the environmental and biological mechanisms of preservation (Schmidt et al., 2011). A greater diversity of molecules, for example, could increase the cost of metabolism. Thus, some compounds might remain in soil despite their potentially easier metabolism, because investment in using rare molecules in soil solution are energetically less rewarding (Lehmann et al., 2020). Spatial heterogeneity and temporal variability may exacerbate the consequences of molecular diversity.

Based on that, recent studies have argued that SOM is a continuum of progressively decomposing organic compounds, instead of the common knowledge of large-molecular-size and persistent 'humic substances' formation in soils (Lehmann and Kleber, 2015). Nevertheless, Olk et al. (2019) have indicated, based on a literature review, that humic fractions are suitable for investigation of soil NOM. In this review, they have found evidence of the relative longevity of lignin residues, sometimes also in forms that currently popular chemical analyses do not recognize as lignin residues. Preferential degradation of carbohydrates and proteinaceous compounds and preferential accumulation of lipid-derived alkyl compounds with increasing age were found in several studies (Olk et al., 2019).

Table 1.1. Sources of dissolved organic matter input to soils, adapted from *Bolan et al. (2011)*.

Sources	Total Organic Matter (g C kg $^{-1}$)	Dissolved Organic Matter	
		g C kg $^{-1}$	% of total organic matter
Pasture Leys			
Brome grass	13.3	0.041	0.31
Clover	15.1	0.039	0.26
Crowtoe	10.4	0.036	0.35
Lucerne Cv. Longdong	11.4	0.038	0.32
Lucerne Cv. Saditi	10.9	0.036	0.33
Sainfoin	13.8	0.04	0.29
Sweet pea	10.2	0.034	0.33
Soil			
Forest soil - litter leachate	60	0.026	0.04
Arable soil	12	0.15	1.25
Soil under bermuda grass turf	8.1	0.3	3.7
Pasture soil	32 - 82.5	1.02 - 3.12	3.18 - 3.8
Organic amendments			
Sewage sludge	321 - 420	2.42 - 6	0.58 - 1.87
Paper sludge	281	7.19	2.56
Poultry litter	377	75.7	20.1
Mushroom compost	385	7.1	1.84
Fresh spent mushroom compost	288	133	46.2
Composted spent mushroom substrate	274	43.4	15.8
Separated cow manure	456	9.8	2.15
Poultry manure	425	8.18	1.92
Pig manure	296	6.13	2.07

1.1.2. Groundwater DOM

Groundwater comprises the major liquid freshwater reservoir on the planet and, therefore, is a major source of drinking water for consumption. Groundwater quality is a key environmental issue and is often assessed by DOC concentration. Besides, groundwater DOM (GDOM) serves as a carbon and energy source for heterotrophic metabolism and drives the bioremediation of many pollutants (Shen et al., 2015).

Anoxia in the stream-water-groundwater interface is regulated by hydrologic exchange with the stream surface, but consumption of dissolved oxygen at this interface is controlled by water residence time and respiration rate. Surface stream water enters the subsurface at locations of groundwater recharge (down welling zones), supplying oxygen to sediments, which is consumed during aerobic respiration (Jones and Mulholland, 2000). Depending on metabolic rates and contact time between water and sediments, OM mineralization proceeds via a set of microbial mediated alternative terminal electron-accepting processes, which strongly influence biogeochemical constituents and metabolic rates in the subsurface.

Surface plant litter and soil are important sources of GDOM (Baker et al., 2000); precipitation drives the transport of DOM from the surface through the soil column. Interactions between DOM and minerals during infiltration determine the selectivity of compounds retention within the soils (Hedges et al., 1994; Qualls and Haines, 1992). Lignin phenols, for example, known biomarkers of plant-derived DOM, were relatively depleted in GDOM, indicating substantial removal of plant-derived compounds during transportation (Shen et al., 2015). Moreover, small, and hydrophilic carbohydrates and amino acids eluted faster to the groundwater.

Therefore, DOM sorption processes were described as rather complex and related to various molecular properties, such as hydrophobicity, molecular size, and charge. Compared to surface DOM, GDOM also exhibited lower molecular weights (Shen et al., 2015). Besides, depletion of oxygen resulted in decrease of aromatic molecules and carbohydrates together with increase of aliphatic compounds (Einsiedl et al., 2007). GDOM was reported to contain small portions of both bioavailable and bacterial-derived DOM (Shen et al., 2015).

1.1.3. Freshwater DOM

Freshwater DOM contains many substances of both internal (autochthonous) and external (allochthonous) origin (Brezonik and Arnold, 2011). Autochthonous material includes organisms' self-maintenance and metabolism, and natural decay, whereas allochthonous matter is leached from plants and soils, as well from human discharge (Kördel et al., 1997). In this context, it is important to understand the influence of hydrologic flows and phenological events on the seasonal patterns of freshwater DOM in watersheds (Awad et al., 2017; Harun et al., 2016; Singh et al., 2014).

Significant seasonal and spatial variations in DOM have already demonstrated the effects of land use cover and precipitation amounts in open and closed catchments (Awad et al., 2017; Harun et al., 2016). Singh et al. (2014) have investigated a forested catchment for 4 years. They have found that seasonal differences in DOM were most pronounced for superficial flow paths (e.g. stormflow, litter leachate, throughfall and soil water) but muted or absent for groundwater and baseflow. This was attributed to the loss of DOM by sorption on mineral soil surfaces and/or microbial breakdown (Singh et al., 2014).

Current estimation of carbon discharge from inland waters to the ocean is about 0.9 Pg C year^{-1} (Cole et al., 2007; Tranvik et al., 2009; Ward et al., 2017). For 20 years now the importance of DOM in the transport of carbon has been recognized, and fluxes of terrestrial and freshwater DOM into the ocean were estimated to be approximately 0.25 Pg C year^{-1} (Hedges et al., 1997; Riedel et al., 2016). Inland waters provide a critical intersection between terrestrial and marine environments, but even if its importance has been finally highlighted, there are still knowledge gaps about carbon processing in such systems (Ward et al., 2017).

Due to its relevance in many biological and chemical processes, freshwater DOM structure and composition need to be investigated. In freshwater ecosystems, main DOM structural components consist of aliphatic, carboxylic-rich alicyclic molecules (CRAM), carbohydrates, lipids, peptides, and small amounts of aromatics (Sleighter and Hatcher, 2008). However, freshwater DOM from different environments (e.g. river, lakes, and estuaries) present considerable variance. Lignins and highly oxygenated tannins are mainly depleted in coastal DOM (Sleighter and Hatcher, 2008).

1.1.3.1. River and streams

The flux of riverine DOM from land to ocean is a major source of reduced carbon to marine environments, with biogeochemical cycling in coastal margins near riverine outflows dominated by the influx of terrestrial organic matter and nutrients (Raymond and Spencer, 2015). Riverine DOM was thought to be relatively stable, representing the degraded remains of vascular plant materials aged in soils (Hedges et al., 1997). Recently studies have shown, however, that DOC is younger than the particulate material, resulting from modern plant production (Mayorga et al., 2005).

Riverine DOM composition follows hydrological drivers. At higher discharge rates, for example at the beginning of spring in temperate and boreal regions (i.e., defrosting) or during flooded periods in tropical environments, DOM presents a more terrestrial character. On the other hand, at lower discharge rates, the microbial imprint is more relevant due to the increased residence time of DOM (Raymond and Spencer, 2015). Relative inputs from different land cover types are also readily apparent in stream and riverine DOM composition (Raymond and Spencer, 2015).

Although the terrestrial input is a major driver for stream and riverine DOM composition, it has been proposed that the longer the DOM is exposed to photo- and biodegradation, the more it resembles marine material (Dittmar et al., 2007; Stubbins et al., 2010). The combined effect of both degradation pathways seems to result in the accumulation of refractory molecules that share very similar molecular structures, independent of their original source (Jaffé et al., 2012).

Comparing the behavior of DOM in ten rivers around the globe, however, it became clear that despite the consistent removal of polyphenols, condensed polycyclic aromatics, and CDOM from riverine DOM pool, there were still dissimilarities between samples (Riedel et al., 2016). This suggests that even if there are general trends in DOM degradation in rivers and persistence of compounds with marine character, there are still individual features from each system.

When comparing compounds found only in the open ocean with unique compounds from the Amazon River, the first group had significantly higher H/C ratios (1.31 ± 0.14 compared to 0.88 ± 0.22), whereas riverine DOM was more aromatic (Medeiros et al., 2015). These findings are in agreement with previous studies that have shown that riverine sources add aromatic compounds to the coastal ocean (Ziolkowski and Druffel, 2010).

Several studies have shown the influence of terrestrial carbon to DOM composition in rivers and streams (Fasching et al., 2014; Kothawala et al., 2015). DOM composition in such systems is mainly based on aromatic compounds, carbohydrates, lignin-like compounds, amino acids, fatty acids, and, depending on the region, also elevated amounts of black carbon – due to recent increased fire in boreal forests (Fasching et al., 2014; Jaffé et al., 2012).

DOM in streams is characterized by presence of both unsaturated (H/C 1.0 – 1.5) and highly unsaturated, carboxyl-rich compounds (H/C < 1.0; O/C > 0.5). This indicates the concomitant presence of more persistent compounds in freshwater and marine environments, and DOM originated from terrestrial plants, which tend to release highly phenolic material to the watershed – that is subsequently oxidized and carboxylated in soils (Hawkes et al., 2018).

1.1.3.2. Lakes and wetlands

Besides its own autochthonous OC production, lakes and wetlands receive allochthonous input directly from terrestrial sources and via rivers and streams. However, due to the prolonged water residence time, DOM is more prone to flocculation and further sedimentation in such systems. Cycling of OC in lakes is now considered relevant for global carbon budgets because of the substantial processing of incoming DOM from the catchment, leading to burial of refractory organic matter in lake sediments simultaneously accompanied by large-scale formation and emissions of greenhouse gases (Tranvik et al., 2009). Therefore, selectivity and persistence of such DOM pools is highly important.

Although parameters such as climate and hydrology have relevant influence on freshwater DOM pool composition, molecular accumulation across 120 lake water samples showed a rapid saturation of unique compounds, such that 95% of the molecular richness was reached in only 45 lakes (Kellerman et al., 2014). This suggests that despite individual characteristics of each freshwater system, there is an impressive number of ubiquitous features, i.e., compounds that are overall present along the gradient of different lakes.

Commonly, lake DOM includes lipids, peptides, carbohydrates, N- and S-containing compounds, lignin- and tannin-like compounds (Goldberg et al., 2015; Gonsior et al., 2013; Kellerman et al., 2015, 2014; Zhang et al., 2014). These components are a result of precipitation, inflow and outflow of tributaries, water residence time and temperature. However, seasonal shifts in the compositions and structures of lake DOM are also observed.

For example, more proteins are produced in spring or summer time (Goldberg et al., 2015), a result of higher metabolic activity following higher temperatures.

A systematic study of DOM during and after cyanobacterial bloom (between summer and fall) has demonstrated the loss of HC-O units (commonly carbohydrates) and aliphatic oxygenated groups, whereas carbon bound methyl, methylene and methine carbon increased, leading to an overall molecular diversity (Zhang et al., 2014). In boreal lakes, seasonal differences in DOM pools between summer stratification and winter have been shown (Gonsior et al., 2013), which resulted in increased concentrations in the bottom due to DOC release from the sediments during winter and selective photodegradation in the water column during spring and summer.

In lake waters, selective degradation and preservation of DOM compounds depend on many factors, such as light, oxygen, and microbial community, among others. The oxidized aromatic compounds are preferentially removed during organic matter mineralization (photo- or biodegradation), while rather saturated aliphatic and N-containing compounds are more likely to persist in aquatic systems due to their recalcitrant characteristics (Kellerman et al., 2015, 2014).

The role of allochthonous DOM in flocculation is also relevant for the selectivity of DOM persistence in lake waters (von Wachenfeldt et al., 2009, 2008; von Wachenfeldt and Tranvik, 2008). Flocculation, and subsequent sedimentation, can be induced by microbial activity or light irradiation. Both biotic and abiotic processes remove certain DOM compounds groups from the water column to the sediments.

In wetlands with different sources of carbon, DOM pools were found to share many molecular features, such as aliphatics, CRAM, carbohydrates, peptides, aromatics, common groups of lignin- and tannin-like compounds, lipids and N-containing compounds, and also analogous fluorescence properties (Hertkorn et al., 2016). Regardless of the similarity, each sample differs in compositions due to specific environmental drivers or specific biogeochemical processes (Hertkorn et al., 2016).

Generally, the averaged elemental ratios (H/C 1.01 – 1.09; O/C 0.53 – 0.56) in wetland DOM shift to be more oxygenated and less saturated compared with lake DOM (H/C 1.08 – 1.16; O/C 0.42 – 0.43) (Hertkorn et al., 2016; Zhang et al., 2014). The lower degree of saturation

from compounds present in the wetland could be an indication of an increased number of aromatic compounds in that DOM pool.

1.1.4. Marine DOM

Most of the OC in the oceans is found in a dissolved state (Killops and Killops, 2005). In fact, the ocean inventory of DOC is comparable to the mass of inorganic C in the atmosphere (Fasham et al., 2001). The euphotic zone is the principal site of OM production in the open ocean, but despite DOM production's constraints regarding the magnitude of primary production, there are complementary mechanisms such as extracellular release by phytoplankton, grazer-mediated release and excretion, release via cell lysis, solubilization of detrital and sinking particles, and release from prokaryotes (Carlson and Hansell, 2015).

Recently, marine DOM has also been proposed to integrate the "biological pump" or "marine carbon pump". The biological pump is the mechanism of CO_2 fixation through photosynthesis in the upper ocean and its transportation to the deep ocean as sinking biogenic particles or DOM (Jiao et al., 2011, 2010). Most of this exported material is remineralized to CO_2, which accumulates in deep waters until it is eventually emitted to the atmosphere. However, a proportion of the fixed carbon is not oxidized but is instead stored for millennia as recalcitrant DOM (Jiao et al., 2011, 2010). The processes and mechanisms involved in the generation of this large carbon reservoir are not yet completely understood.

Investigation of marine DOM revealed its composition: amino acids and proteins, simple sugars, vitamins, fatty acids, polysaccharides, CRAM, lignin-like compounds, and very complex degradation products of unknown origin, such as humic substances and black carbon (Repeta, 2015). The proportion of each component, however, varies along the gradient from the costal zones to the open ocean.

Marine DOM samples collected along a transect from the littoral to the off-shore, however, have demonstrated the ubiquitous presence of a few N-containing compounds, which appeared to be refractory and produced solely in the marine environment (Gonsior et al., 2011). Moreover, studies in the open ocean were able to detect highly refractory compounds, such as CRAM, in marine DOM pool (Hertkorn et al., 2013, 2006), indicating the persistence of this group as well.

There is also a depth profile for DOM distribution in the oceans. For example, although carbohydrates, amino acids, lipids, and amino sugars constitute less than 25% of deep marine DOM, these compounds are present in higher percentage (25-50%) in surface marine DOM (Koch et al., 2005). However, marine DOM samples from different depths showed surprisingly stable H/C and O/C ratios, ranging between 1.378 – 1.373 and 0.731 – 0.741, from surface to profound replicates, respectively (Hertkorn et al., 2013).

Due to the leaching of OM from soils, rivers often have high concentrations of CDOM. However, most of CDOM research in the past decades have been fueled by marine studies. Combined with salinity, CDOM concentrations can be used to understand coastal mixing (Stedmon and Nelson, 2014). In a tropical estuary zone, for example, it has been shown that CDOM in the mangrove-fringe included both a microbial component, derived from phytoplankton debris, and also a refractory component, from allochthonous input (Tremblay et al., 2007), suggesting the persistence of some terrestrial compounds.

Nevertheless, DOM was found to become more aliphatic and less oxygenated along the gradient from rivers to the ocean, but a considerable proportion of common formulae remained (Sleighter and Hatcher, 2008). Approximately 1/3 of the formulae were common between marine and terrestrial DOM, which might be attributed to the presence of refractory DOM or arise from isomers with different chemical structures at the same formulae (Koch et al., 2005; Sleighter and Hatcher, 2008).

DOM in the marine environment can be transformed via biotic or abiotic pathways. Microbial production and degradation of marine DOM are complex processes, which occur simultaneously, but overall lead to the accumulation of recalcitrant DOM in the ocean (Flerus et al., 2012). On the other hand, photochemical changes in marine DOM have been shown to transform unsaturated aromatic compounds into more saturated molecules with higher H/C and O/C ratios (Gonsior et al., 2009).

In fact, photochemistry plays an important role in DOM dynamics in the open ocean. Both light availability for photosynthesis and the penetration of UV radiation within the marine environment are influenced by abiotic photochemical transformations. In addition to its control on UV light fields, DOM photochemistry strongly impacts the biogeochemical cycling of

biologically important elements in surface seawater, including mineral nutrients and trace metals (Mopper et al., 2014).

1.1.5. Sediment DOM

The definition of sediment refers to the loose particles of sand, clay, silt, and other substances that settle at the bottom of a water body (marine or freshwater ecosystems). In the sediments, an important fraction of OM is dissolved in the pore water or is water extractable (Chen and Hur, 2015). The sources of sediment DOM may be allochthonous (terrestrial higher plants-derived) and/or autochthonous (algae, macrophytes, and bacteria-derived).

Due to the complexity of the environment, with water mixing from different sources, sediment DOM in coastal zones is a mix between terrestrial-derived material and marine-derived DOM (Chen and Hur, 2015). DOM in marine sediment pore waters plays an important role in carbon and nitrogen remineralization and may also be involved in carbon burial. It also plays a role in pore water metal complexation, affecting dissolved metal and metal-complexing ligand fluxes from sediments (Burdige and Komada, 2015).

When compared to marine ecosystems, freshwater sediments tend to present higher concentrations of DOC. Besides, DOM in such sediments is dominated by terrestrial-derived sources (Chen and Hur, 2015). In boreal lakes, for example, allochthonous DOC input is the major source of OM in sediments (von Wachenfeldt et al., 2008), and it has been proposed that up to 50% of the carbon mineralization occurs in that compartment (Jonsson et al., 2001).

DOM in sediment pore waters is defined as a heterogeneous collection of organic compounds, ranging from relatively high molecular weight (HMW) macromolecules such as dissolved proteins or humic compounds, to smaller low molecular weight (LMW) molecules (e.g. individual amino acids or short-chain organic acids) (Burdige and Komada, 2015). A conceptual model for DOM cycling in sediments showing the different processes and the evolution of its molecular size is pictured in Figure 1.2.

In many sediments, pore water concentrations of DOM are up to an order of magnitude higher than bottom water values, possibly a result of the net production of DOM from degradation processes. Based on diffusive arguments alone, sediments are a potential source of DOM to the overlying waters (Burdige and Komada, 2015). DOM in sediment pore waters is not only more

abundant, but can also be more diverse than DOM regularly found in the water column (Schmidt et al., 2017).

In sediment pore water, DOM pools have shown an increased proportion of heteroatoms, i.e., N- and S-containing molecules, most likely a result of microbial activity (i.e., degradation of protein-like material) and sulfurization of DOM (Chen and Hur, 2015). Besides, marine sediment DOM has presented a wide range of H/C and O/C ratios with a large coverage in the van Krevelen diagram (Schmidt et al., 2017), while lake sediment DOM was rich in saturated compounds and/or depleted in oxygen-containing components, as compared with those in other ecosystems (i.e., freshwater, soil, and water treatment effluent) (Xu et al., 2016).

DOM samples from different systems have demonstrated to hold a large number of unique formulas, reinforcing the heterogeneity of the sediments along a given gradient (Schmidt et al., 2017). Sediment DOM includes a wide range of molecules, such as lipids, lignins, proteins, humic- and fulvic-like compounds, unsaturated hydrocarbons, condensed aromatics, and carbohydrates (Wang et al., 2013; Xu et al., 2016).
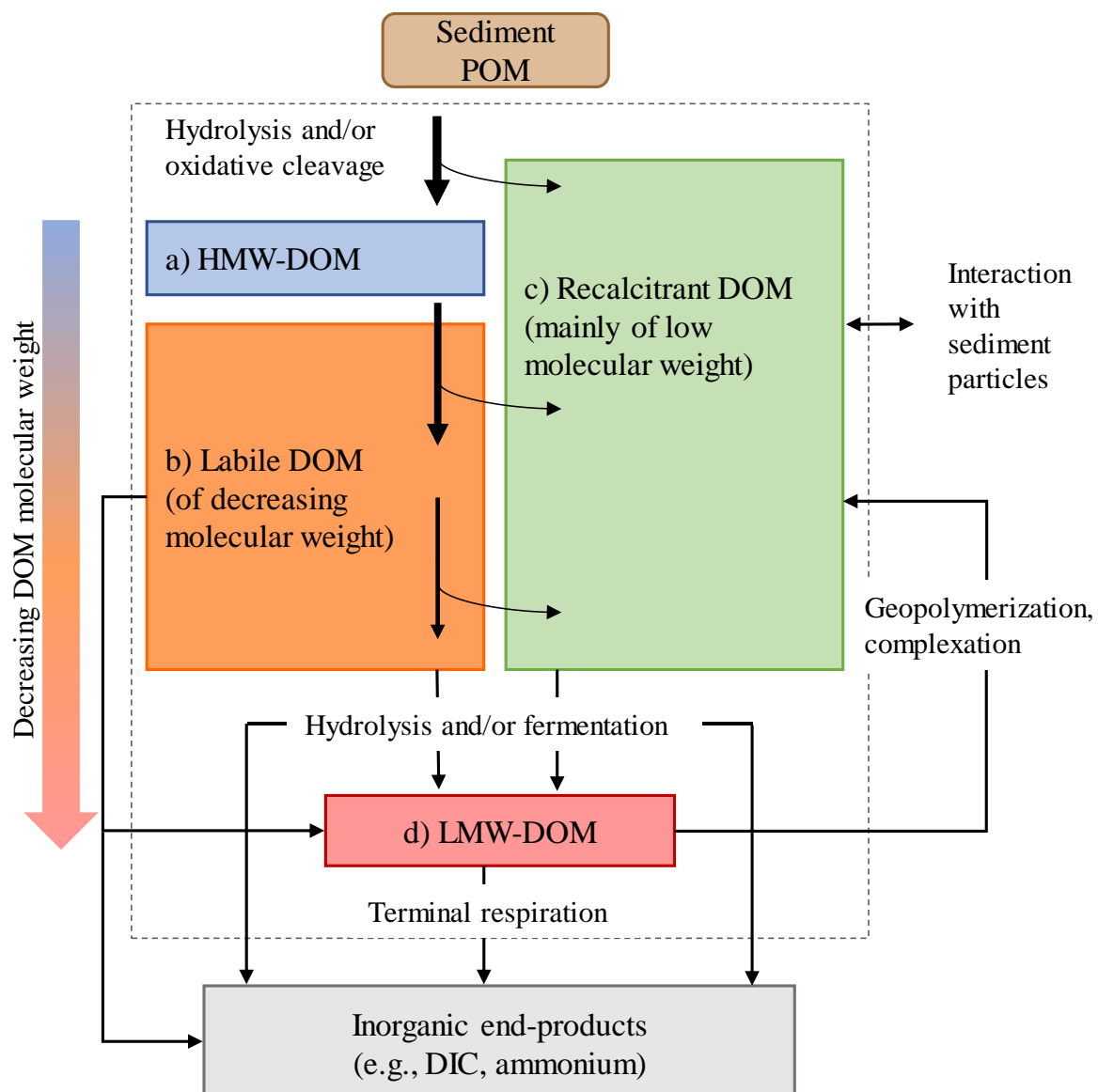
Figure 1.2. A conceptual model for DOM cycling in sediments, adapted from *Burdige & Komada (2015)*.

Although sediments receive input from autochthonous and allochthonous sources, terrestrial-derived DOM plays an important role in flocculation, being also relevant for the selectivity of DOM persistence in lake waters and sediments (Kellerman et al., 2015; von Wachenfeldt et al., 2009, 2008; von Wachenfeldt and Tranvik, 2008). Both via microbial and photochemical processes, DOM in the water column can form aggregates and particles, sinking into the sediments.

Most of DOM accumulated and produced in the sediments is believed to be overall recalcitrant (Burdige and Komada, 2015), and humic substances are the largest component of sedimentary OM, constituting 60-90% of the total OC pool in lacustrine sediments (Chen and Hur, 2015; Wang et al., 2013; Ziegelgruber et al., 2013). However, sediments are hotspots for microbial activity and increased degradation rates under (often) anoxic conditions may be explained by the selectivity of each DOM pool (Bastviken et al., 2004).

1.2. Organic carbon cycling: from terrestrial to aquatic systems – and the relevance of boreal regions

NOM in the forests and other terrestrial ecosystems is derived from the remains of organisms, e.g. plants and animals, and their waste products (Bolan et al., 2011; Carlson and Hansell, 2015; Hertkorn et al., 2008). In the water bodies, such as lakes and rivers, the same applies. However, the organic carbon present in the water bodies can be produced within the system (autochthonous material) or in the terrestrial environment and carried to the aquatic system (allochthonous material).

NOM production is fundamentally constrained by primary production, i.e., the amount of CO_2 fixed during photosynthesis (gross primary production). Part of this carbon is used to maintain the autotroph's metabolism, via respiration, and is returning to the atmosphere as CO_2. The remaining carbon (net primary production) is used to build tissues, which will provide structures for growth, reproduction and serve as food, directly or indirectly, for heterotrophs. This carbon fixed will ultimately originate NOM, via organism's excretion and debris (Carlson and Hansell, 2015).

In terrestrial environments, these organic remains can be directly degraded or stored in the soils, mostly by action of microorganisms. When degraded, the carbon returns to the atmosphere mainly as CO_2, but also as CH_4. When stored, this material will contribute to peat formation (Killops and Killops, 2005). Alternatively, this content can be leached into groundwater reservoirs, or carried and deposited in aquatic environments.

In lotic freshwater systems (i.e., rivers and streams), the OC will be transformed and consumed via different pathways. When complete mineralization occurs, the final gas products are released into the water column and possibly emitted to the atmosphere, while other inorganic nutrients are also made available. The OC is incorporated into the microorganism's biomass,

following to higher trophic levels, but the remaining OC can be transported with the current to other water bodies, such as lakes and the sea (Killops and Killops, 2005).

Within lakes, OC is also processed, mineralized, incorporated as biomass, and transferred into higher trophic levels. However, without the flowing current (lentic ecosystem), the remaining organic material is not transported somewhere else; instead, it persists in the water column and/or in the sediments. In the past, freshwater ecosystems were considered as a mere channel between terrestrial environments and the ocean (Schlesinger and Melack, 1981). Most recently inland waters have been acknowledged as important hotspots for carbon cycling (Battin et al., 2009; Cole et al., 2007).

The main factor contributing to freshwater systems to be such efficient bioreactors for degradation of terrestrial OM is the ideal set of conditions for microbial metabolism (Richey et al., 2002). In fact, OM is relatively short-lived in inland waters with a mean residence time of roughly 2.5 ± 4.7 years, compared to centennial to millennial-scale residence times in soils, oceans, and sediments (Catalán et al., 2016).

During DOM transport from inland waters to oceans, its composition is constantly changing due to microbial activity, flocculation and sedimentation, production by photosynthetic organisms, and photodegradation. All these processes operate simultaneously, driving the composition of the DOM pools in each habitat of aquatic ecosystems. Analyzing the aquatic continuum, a significant loss in DOM color and aromaticity was identified between inland and ocean waters (Massicotte et al., 2017).

Because OM, and more specifically DOM, is taking part in different processes and suffering compositional and structural transformations while serving as carbon and energy source, it is mostly relevant to have a comprehensive and detailed understanding of OM transport, mineralization and sinking along the continuum from terrestrial do aquatic ecosystems.

The boreal forest is the largest continuous land ecosystem on Earth, covering about 14% of the global vegetated surface. Approximately one third of global forests are boreal (almost 60% of the boreal region consists of forests), which are largely primary and dominated by conifers. In such forests, soils can be young and OM poor, but in some areas are well developed and layered. Formation of peat soils is also characteristic from that region (European Environment Agency, 2003; Olsson, 2009).

Most of the Boreal region lies less than 500 m above sea level and the north European ice sheet carved out numerous depressions, which combined with the effects of a cool and moist climate, has resulted in an exceptionally large number of lakes, rivers, and other wetlands. In fact, 21 out of Europe's 24 largest lakes occur in the region and at least three quarters of European natural lakes larger than 0.01 km^2 are boreal (European Environment Agency, 2003). Globally, the highest concentration, area, and perimeter of water bodies appear at boreal and arctic latitudes (Verpoorter et al., 2014).

In the current scenario of climate change, discussing the role of the boreal region is indispensable. Some models suggest that the growth of boreal forests will increase with higher temperatures, leading to more CO_2 sequestration from the atmosphere via photosynthesis. On the other hand, decomposition of OM in the soil is also expected to accelerate with warming. The net effect of boreal forests on the greenhouse gases concentration will depend on which of these competing feedbacks dominates (Hari and Kulmala, 2008).

It has been shown that average temperatures in the boreal region have increased almost twice the global rate in the past 100 years (Olsson, 2009). Due to its size and vulnerability, the boreal region plays an important role in the modern carbon cycle. The mechanisms by which OC is transformed and transported within and between terrestrial and aquatic environments are relevant to the possible scenarios of global warming. Moreover, understanding the dynamics of DOM in freshwater ecosystems in the boreal region could tell us a lot about the production and further emission of greenhouse gases to the atmosphere, since those are hotspots for mineralization (Battin et al., 2009; Cole et al., 2007).

One the of the aspects of rising temperatures is thermal stratification, which can prevent the mixing of different water masses and, therefore, increase residence time of organic carbon, resulting in a separation of water masses distinguished by DOM with contrasting UV exposure (Cory et al., 2015; Guo et al., 2018; Merck et al., 2012). It has been previously shown that DOM in the bottom waters closely resembled soil DOM, whereas both the concentration and character of DOM in surface water was reproduced by experimental photo-degradation of bottom water (Cory et al., 2015). Therefore, DOM degradation in CDOM-rich streams or ponds could be light-limited, and DOM preservation in bottom waters could be favored by stratification.

1.2.1. Transport

Water drives the biogeochemical dynamics of organic carbon previously described. Rainfall washes out atmospheric particles and OM deposited or secreted from vegetation into the soils. Together with plant material deposited in surface soils, this provides organic substrates for a considerable variety of soil organisms (Ward et al., 2017). In certain temperate and boreal environments, snowmelt is also a relevant source of DOM (Baker et al., 2000). The infiltration of water into soils and its movement over the landscape transports geochemical constituents vertically into groundwater and laterally into rivers and streams (Ward et al., 2017).

Once a raindrop precipitates, the water interacts with atmospheric particles and vapors, resulting in an enriched DOM content (Neu et al., 2016). As the rainfall passes through forest canopies and vegetation layers, it becomes increasingly enriched in NOM, while biological transformations also begin to rapidly convert organic substrates into inorganic carbon (Kaiser and Kalbitz, 2012; Ward et al., 2017). In consequence, waters draining the organic layers and the upper mineral soil horizons are rich in DOM, often causing intensive color (Kaiser and Kalbitz, 2012).

In that context, solubility of the molecules is a key factor in the distribution and evolution of NOM along the gradient of terrestrial to aquatic environments. The composition of OM along soil profiles is controlled by the interplay between stabilization/dissolution via mineral sorption/desorption, and microbial decomposition, which is dependent on microbial community composition/activity and redox/environmental conditions (Schmidt et al., 2011; Ward et al., 2017). Besides, differences in molecule's solubility results in a gradient of flow speed, from faster overland flow to relative slow base flow (Figure 1.3).
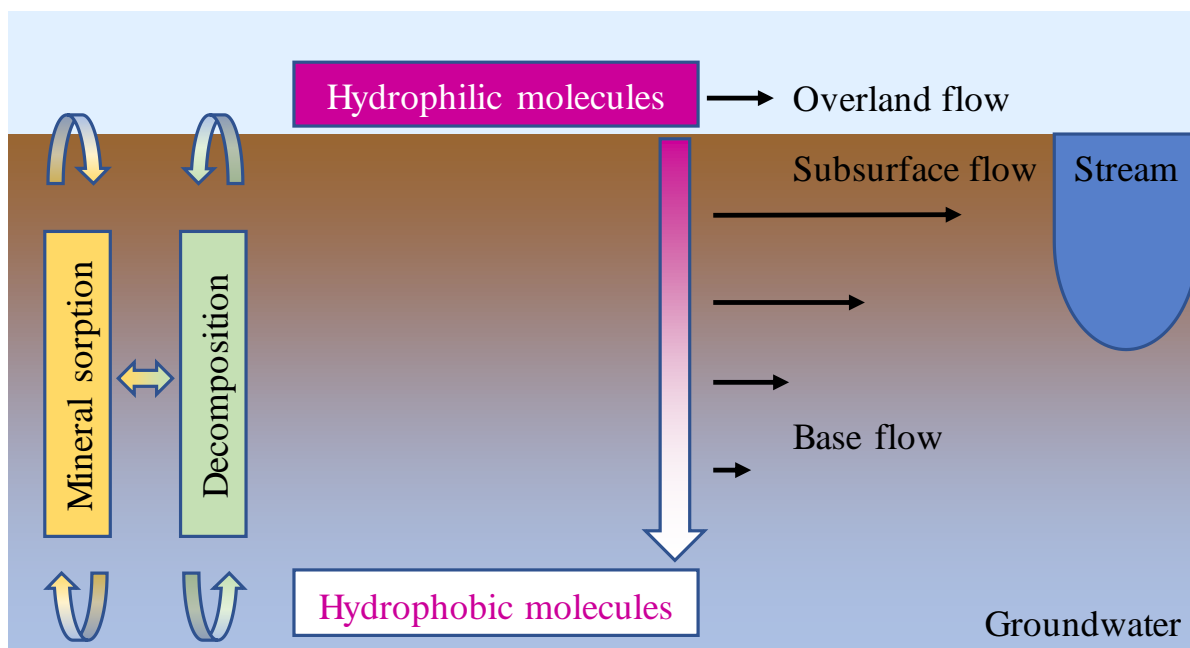
Figure 1.3. Conceptual model for mineral and microbial pathways that control OM composition along soil profiles, with preferential mobilization of more soluble molecules with correspondent flow to water bodies, adapted from *Ward et al. (2017).*

Like other solutes, DOM undergoes both sorption and complexation reactions in soils. Sorption results directly in the retention of DOM with soil components and subsequent decrease of its mobility and further degradation. However, complexation can result in the formation of both soluble and insoluble DOM–metal complexes (enhanced and retarded movement, respectively), thereby affecting movement and degradation in different ways (Bolan et al., 2011).

The molecular diversity of DOM in different samples, such as rainwater, throughfall, soil water, groundwater, and stream water has been already investigated in a boreal forest (Ide et al., 2017). The number of assigned molecular formulas identified by FT-ICR-MS ranged from 865 to 2194, revealing large DOM molecular diversity in the water samples. Additionally, the number of lignin-like compounds shared between different samples was larger than that of any other biomolecular class, indicating its persistence in the environment (Ide et al., 2017). They have suggested that labile molecules (e.g. simple carbohydrates, amino acids) are readily consumed by microbial activity and, therefore, non-labile compounds might regulate DOM molecular diversity in this gradient (Ide et al., 2017).

Because of its relevance to the global carbon budget, OC has been widely studied in boreal landscapes. For example, DOC concentrations from different fractions were determined at forest plots along an altitudinal gradient in eastern Norway (Clarke et al., 2005). It has been shown that DOC presented the following the order of concentration: precipitation < throughfall < soil pore water, reflecting the production of DOC both in the canopy and in the soil organic layer (Clarke et al., 2005).

Moreover, mean DOC concentrations in throughfall clearly decreased with altitude. In soil pore water, DOC presented the same trend, although less pronounced. This is probably a consequence of the decrease of biomass with altitude in that environment (Clarke et al., 2005). In the same study, significant positive correlations between DOC and [H^+] or electrical conductivity were also found, implying the contribution of DOC to solution acidity and the anionic charge, respectively (Clarke et al., 2005).

Similarly, concentrations and fluxes of DOC in precipitation, upland forest throughfall, stemflow and soil water, peatland pore water, beaver ponds, and streams from Canada have been also investigated (Moore, 2003). Here, similar patterns of DOC concentration in precipitation and throughfall have been observed. However, DOC sorption strength increased with depth in the soil profile, reducing the DOC concentrations in the soil (Moore, 2003).

Molecular composition and processing of DOM in boreal catchments has been fairly described. When compared to DOM pools from different regions (e.g. tundra, deciduous and tropical forest) in the USA, the boreal stream DOM pool presented higher content of black carbon, mostly a result of the increasing fire in the boreal region in recent years (Jaffé et al., 2012). However, overall molecular characteristics such as the amino acid distribution, the ^{13}C NMR patters, and formulas determined by FT-ICR mass spectrometry showed similar characteristics, suggesting comparable SOM influence and resembling degradation processes in the studied sites, despite the environmental differences (Jaffé et al., 2012).

Water is also responsible for carrying DOM from inland systems to the ocean. The flux of riverine DOM into to ocean is a major source of reduced carbon to marine environments, and biogeochemical cycling in coastal zones with riverine outflows is dominated by terrestrial OM and nutrients. DOM can undergo a variety of biogeochemical reactions in mixing regions (e.g.

river plumes, estuaries) that ultimately determines both the concentration and composition of DOM reaching the ocean (Raymond and Spencer, 2015).

Globally, the largest 25 rivers discharge more than 40% of the total flux of DOC from rivers to the ocean (McKee et al., 2004; Ward et al., 2017). Although those numbers are impressive, and large rivers do play an important role in such transport, smaller watersheds have to be considered, since they have less basin area for storing flood-driven sediments and are, thus, more likely to respond to event-driven floods (i.e., mobilizing allochthonous OM) (Milliman and Syvitski, 1992).

Several boreal streams have also been studied during 14 months in a Swedish catchment (Kothawala et al., 2015). Here, land cover explained 49% of the variability in DOM composition. In fact, all parallel factor analysis (PARAFAC) components were characteristic of terrestrial-derived material. In contrast, seasonal fluctuations in hydrology only contributed to 8% of the compositional variation of stream water DOM, while in-stream transformations to DOM were not detected (Kothawala et al., 2015).

Curiously, those findings suggest that low-order boreal streams act as a passive pipe, in contrast with the stablished literature of DOM processing in freshwater systems (Battin et al., 2009; Cole et al., 2007; Kothawala et al., 2015). They have proposed that in-stream processing of DOM is restricted by short water residence times, lower light penetration, lower proportions of labile OM, and reduced microbial activity – the latter ones being demonstrated by the undetectable protein-like fluorescence in DOM samples (Kothawala et al., 2015).

Nevertheless, investigations on a third order stream along a land-use gradient from pristine, agricultural to urban landscapes revealed a clear evolution on DOM composition along the river course (Kamjunke et al., 2019). Here, DOM pools showed an initial decrease of average oxidation and unsaturation followed by an increased relative abundance of CHNO and CHOS compounds introduced by agriculture and wastewater, respectively. In the headwaters, DOM composition was dominated by rather unsaturated CHO compounds (H/C $\leq$ 1), while at downstream sites aliphatic molecules were more abundant (Kamjunke et al., 2019).

Although terrestrial OC input has been already recognized, there are few studies that have taken the groundwater influence into account (Einarsdottir et al., 2017). Investigation on a small boreal lake has found that groundwater infiltration was responsible for 27% to the total DOC,

64% to the total DIC, and 96% to the total CH_4 loads from the catchment to the lake (Einarsdottir et al., 2017).

Therefore, it has been proposed that even in regions where lake hydrology is dominated by surface water inflow (e.g. streams), direct groundwater infiltration can represent an important carbon source to boreal lakes. Moreover, experimental data has suggested that DOC imported from the catchment via streams and groundwater was more photoreactive than the DOC of the lake water itself (Einarsdottir et al., 2017).

Another example of lower magnitude is the groundwater input in the oceans. The flow of coastal groundwater is considered low compared to rivers, but nutrient concentrations in such waters, however, can exceed that of surface water concentrations. Therefore, in a regional scale, the supply of OC and nutrients to the coastal ocean via submarine groundwater discharge can be more important than the supply by large rivers (Moore, 2010; Moore et al., 2008).

1.2.2. Transformation

Organic carbon mineralization is the process in which the carbon returns to its gaseous phase, mainly as CO_2, but a smaller fraction also as CH_4 (methanogenesis). There are different pathways for OC degradation, both biotic and abiotic, in aerobic and anaerobic conditions. Factors controlling OC degradation include environmental aspects, such as temperature, oxygen/other electron acceptors availability, and light exposure; biological ones (i.e., microbial community); and its own reactivity (Bastviken et al., 2004; Conrad, 1996; Findlay and Sinsabaugh, 2003; Killops and Killops, 2005).

DOM degradation can be a result of both photochemical and microbial processes; the former one prevailing in aquatic systems and the latter on soils (Bolan et al., 2011). Actually, photo-degradation was found responsible for higher proportions of molecular changes in riverine DOM pool than biodegradation (Seidel et al., 2016), although its proportion depends on the turbidity of the water body and light penetration (e.g. Bertilsson and Tranvik, 2000; Groeneveld et al., 2016; Medeiros et al., 2015; Remington et al., 2011; Spencer et al., 2009).

Another factor influencing photo-degradation is the input of terrestrial, highly colored, OM. It appears that allochthonous OM is more sensitive to light-mediated degradation (Koehler et al., 2012), most probably due to its capability of absorbing more radiation (Kothawala et al., 2014).

Therefore, photodegradation plays a bigger role in oligotrophic lakes compared to eutrophic ones (Bertilsson and Tranvik, 2000), but apparently it is limited in lakes with brown waters (Groeneveld et al., 2016).

In lakes, photochemical production of both dissolved inorganic carbon (DIC) and low molecular weight carboxylic acids has been previously described (Bertilsson and Tranvik, 2000). In light-mediated experiments, the loss of humic-like compounds and high molecular weight aromatics has also been shown (Mostovaya et al., 2016), together with the increased proportion of highly oxidized compounds (Gonsior et al., 2013).

Similarly, in riverine DOM, photochemical reactions have caused the loss of lignin (Spencer et al., 2009). Besides, decrease in O/C ratios and concomitant increase in saturation have also been described during photodegradation experiments (Gonsior et al., 2009; Seidel et al., 2015). These molecular changes can be explained by photochemical decarboxylation reactions, which are typically observed in photo-transformations of DOM (Amon and Benner, 1996b).

Likewise, decreased oxygenation and increased saturation have also been described for marine DOM (Gonsior et al., 2014). Marine photodegradation of DOM is, similarly to other environments, but perhaps more intensively, limited by photobleaching (i.e., decrease in light absorption capacity) (Brinkmann et al., 2003; Dainard et al., 2015; Gonsior et al., 2013; Mopper et al., 2014; Stedmon and Nelson, 2014). Nevertheless, photochemical reactions (alone or coupled with biotic processes) are responsible for the rapid removal of terrestrial DOM in coastal zones (Mopper et al., 2014), also because irradiation decreases the average molecular weight of terrestrial and estuarine DOM (Helms et al., 2008; Thomson et al., 2004).

According to the size-reactivity model (Amon and Benner, 1996a; Benner and Amon, 2015), biological degradation plays a dominant role in shaping the size spectrum of OM. The first step in DOM biodegradation is the breakdown of high molecular weight macromolecules, into either monomeric or polymeric low molecular weight DOM, the first being more labile and rapidly degrading into CO_2 or used in methanogenesis, while the latter is more refractory and often accumulates (Conrad, 1999).

Biological degradation processes are very diverse and comprise a set of diverse but established metabolic processing steps. The composition of microbial communities depends on a number of factors, including acidity and salinity, availability and type of organic substrates, and

terminal electron acceptors (Killops and Killops, 2005). DOM quality has proven to be an important determinant of the microbial communities developing in experimental and field studies (Carlson and Hansell, 2015).

Therefore, the microbial community is shaped according to the DOM pool in a given habitat (Blanchet et al., 2017). Previous studies have shown that adding DOM of different quantities and qualities to natural microbial assemblages yields significant differential responses among the major and minor groups of bacterioplankton (Alonso-Sáez et al., 2012; Landa et al., 2013; Nelson et al., 2013).

However, OM degradation in a specific setup depends on the available electron acceptors (Conrad, 1996). Oxygen (O_2) is the preferential electron acceptor and is used for OM oxidation until its depletion. Then, other oxidants are used following a thermodynamically-determined order, with nitrate (NO_3^-), manganese (Mn^{4+}), iron (Fe^{3+}), and sulfate (SO_4^{2-}) being consumed before CO_2 (i.e., methanogenesis) (Conrad, 1996).

Previous studies, however, have shown that degradable OM might be divided into distinct pools: one that is only degraded under oxic conditions, another that is degraded more rapidly under anoxic than oxic conditions, and a third that is degraded at similar rates under both oxic and anoxic conditions (Bastviken et al., 2004). Highly refractory, partially degraded OM, that can only be accessed using oxygenases or reactive oxygen species, probably belong to the first group (Bastviken et al., 2004).

Aerobic and anaerobic degradation reactions probably progress at about the same rate under identical conditions (i.e., fresh OM and unlimited supply of electron acceptors), but anaerobic degradation often appears slower because most of the labile organic components have already been removed, leaving mainly recalcitrant material. Moreover, depletion of electron acceptors is also a limitation (Killops and Killops, 2005).

In soils, limited oxygen (O_2) penetration it not only dependent on soil depth, but is also intimately related with natural flood events, such as floodplains, or flooded cultures (e.g. rice fields) (Achtnich et al., 1995; Fey and Conrad, 2000; Junk et al., 1989). Especially in systems with higher amounts of fresh OM and elevated temperatures, increased microbial activity leads to anoxia in the soils (Brown et al., 2004; Junk et al., 1989).

DOM decomposition rate in soils is influenced by several factors apart from O_2 availability, such as soil depth, land use, and soil fertility. Rates decrease with increasing soil depth and are lower in forest than in arable soils, for example. These factors are correlated, because microbial activity is limited by the bioavailability of OC as a substrate and/or by the supply of the essential nutrients N and P (Bolan et al., 2011).

In aquatic environments, the presence/absence of O_2 in the water column depends on seasonal stratification (Glissman et al., 2004; Huttunen et al., 2001). In case of thermal stratification in the water column, there is no mixing between the upper and the bottom layers and, therefore, O_2 does not reach deeper (Killops and Killops, 2005). Thus, the concentration of O_2 close to the sediments is highly influenced by water column stratification (Liikanen, Murtoniemi, & Tanskanen, 2002).

However, depletion of O_2 in the water column and in the sediments of aquatic environments is also enhanced by eutrophication (Antoniades et al., 2011; Fenchel et al., 1990; Zimmerman and Canuel, 2000). Increased proportions of nutrients in the water bodies lead to higher microbial activity (including algal bloom), promptly consuming all dissolved O_2 available and, thereafter, causing anoxia (Killops and Killops, 2005).

Anaerobic degradation processes are often generalized by the term fermentation, but strictly it only describes reactions in which an internal source of electron acceptors is used, not an external source like CO_2 (Killops and Killops, 2005). An example is fermentation of glucose to CO_2 and ethanol, which can be considered to involve an internal redox reaction whereby part of the substrate is oxidized (to CO_2) and part reduced (to C_2H_6O) (Killops and Killops, 2005).

Actually, fermentation can be performed by a series of microbial organisms and represents just one step of several anaerobic degradation pathways, such as methanogenesis (Conrad, 1996). Besides, organic substrates cannot be fermented further than acetate (Killops and Killops, 2005), which is one of the main precursors of CH_4 production (Nozhevnikova et al., 2007; Roden and Wetzel, 2003; Schwarz et al., 2007).

Microbial degradation in soils is performed by a wide range of bacteria, fungi, protists, and invertebrates. DOM is an important substrate for microorganisms, and it has been shown that up to ~40% of DOM in soil solution is microbiologically degradable (Bolan et al., 2011). Soil

respiration is the primary path by which CO_2 fixed by land plants returns to the atmosphere, with estimated flux around 75 Pg C year^{-1} (Schlesinger and Andrews, 2000). From the global budget of atmospheric methane, on the order of 0.5 Pg CH_4 year^{-1}, more than 30% comes from flooded soils (i.e., wetlands and rice fields) (Conrad, 2009).

Although microbial degradation of DOM in soils is important for the total carbon mineralization to the atmosphere (Hodgkins et al., 2014), detailed information about such degradation processes is still missing. DOM is known as a source of nutrients (Carlson and Hansell, 2015), and microbial degradation of DOM usually leaves an imprint. In fact, increased proportions of N- and S-containing compounds and accumulation of lignin- and lipid-like compounds have been observed in peat soils at an advanced stage of decomposition (Hodgkins et al., 2016, 2014).

Chemical characteristics and biodegradability of DOC collected from soil pore waters in boreal forest sites in Alaska have been investigated, and the specific UV absorbance values were high at all four sites, indicating high aromatic content in soil DOM. Hydrophobic acids were the dominant fraction at all sites, accounting for more than 50% of the total DOC (Wickland et al., 2007).

Here, vegetation leachates were also sampled and incubated. Wichland et al. (2007) have found that, during experimental microbial degradation, these leachates became more similar to each other and to the chemical characteristics of DOC in the soil pore waters. Fluorescence analysis have shown that protein-like material was preferentially transformed, while certain quinones increased in abundance (Wickland et al., 2007). Quinones (and hydroquinones) have been previously related to the fulvic acid metabolism (Amir et al., 2006).

Those results showed that microbial degradation can drive the preservation of SOM, and agreed with other studies which argued that molecular structure alone does not control SOM persistence, but physicochemical and biological influences from the surrounding environment reduce the probability of decomposition (Schmidt et al., 2011).

Black carbon, for example, comprises up to 40% of total SOM in grasslands and boreal forests (Preston and Schmidt, 2010). However, field experiments have shown higher degradation rates for fire-derived material than to the remaining bulk OM. Therefore, physical protection and

interactions with soil minerals might play a significant part in black carbon stability over long periods of time (Schmidt et al., 2011).

Although the importance of soil microbiota has already been recognized, there is still a knowledge gap in that area. Modern genetic and protein-based tools allow the quantification and description of soil microorganisms' abundance, taxonomic composition, and functioning. Nevertheless, the challenge remains of synthesizing this immense amount of detailed information and linking it to the rates and routes of SOM processing (Schmidt et al., 2011).

Molecular changes in DOM composition have also been studied in a permafrost thaw progression, with concomitant measurements of greenhouse gas production potential (Hodgkins et al., 2014). Here, thawing has led to succession in vegetation and, consequently, changes in OM chemistry; it has increased the proportion of low molecular weight compounds with lower aromaticity, lower organic oxygen content, and also enhanced amounts of microbial produced compounds in the DOM pool (Hodgkins et al., 2014).

Additionally, such changes have also influenced greenhouse gases production, increasing CO_2 and CH_4 production potential, the CH_4/CO_2 ratio, and a shift in the methanogenesis pathway from hydrogenotrophic ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$) to acetoclastic ($CH_3COOH \rightarrow CH_4 + CO_2$) (Hodgkins et al., 2014). Such biogeochemical changes are probably a result of both increased proportions of labile DOM and more frequently occurrence of anaerobic conditions due to the ice melting (i.e., flooded peats) (Hodgkins et al., 2014).

Similar trends have been found in another study of DOM composition in permafrost thaw peatland using FT-ICR-MS and optical measurements (Hodgkins et al., 2016). Fully-thawed spots have presented more N- and S-containing compounds, which were more saturated and more reduced, and indicated microbial-derived DOM, consistent with higher decomposition rates (Hodgkins et al., 2016).

Different, however, was the dominance of sphagnum in the intermediate thaw stage, driving the DOM composition towards a more aromatic, higher average molecular weight, and higher proportions of O/C compounds. Those characteristics are consistent with a higher abundance of phenolic compounds, which are released by Sphagnum and may accumulate due to inhibition of phenol oxidase activity by the acidic pH (Hodgkins et al., 2016).

Therefore, it has been proposed that Sphagnum presence might be one of the main drivers of DOM pool composition in boreal peatlands (Hodgkins et al., 2016). Such DOM pools are rich in more recalcitrant compounds (i.e., aromatic, higher-oxygen, and higher molecular weight), similarly to the non-labile compounds that have been proposed to regulate DOM molecular diversity in different water samples from a boreal forest (Ide et al., 2017).

Terrestrial DOM is not only relevant for carbon cycling in soils, but also in aquatic systems, both in the water and in the sediments (Battin et al., 2009; Ward et al., 2017). However, autochthonous DOM input is also relevant, especially when comparing sediment bacterial production and mineralization between lakes with a relatively high allochthonous influence. The sediment bacterial metabolism seems to increase with rising labile organic carbon produced within lakes (Gudasz et al., 2012).

In freshwater environments, anaerobic mineralization of OC has been estimated to account for $20 - 60\%$ of total carbon mineralization rates (Boon and Mitchell, 1995; Hamilton et al., 1995; Utsumi et al., 1998; Ward et al., 2017), from which up to 80% of the anaerobic activity being carried on by methanogenic bacteria. Lakes alone emit up to 48 Tg CH_4 $year^{-1}$, which exceeds methane emissions from the ocean (Ward et al., 2017).

Although microbial degradation of DOM in aquatic environments is highly important for the understanding of OC cycling, most studies investigate transformations occurring along a natural gradient and, therefore, coupled with photochemical reactions (e.g. Gonsior et al., 2016; Kellerman et al., 2015; Lambert et al., 2016; Murphy et al., 2018; Powers et al., 2018; Tremblay et al., 2007), instead of isolating the biological effects. However, some experiments with biodegradation of DOM were already capable of describe some patterns in DOM pools transformation.

Microbial degradation has been described as more selective than photochemical degradation. A general trend of both consumption and production of low molecular weight compounds (i.e., $250 - 800$ Da) has been shown (Reader et al., 2015). These select m/z-values have been proposed as potential biomarkers of microbial degradation due to their unique signature, and deserve further consideration in future studies (Reader et al., 2015).

In rivers and streams, biodegradation has been shown to selectively remove oxygenated unsaturated compounds (Kim et al., 2006; Medeiros et al., 2015), producing compounds with

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low O/C and high H/C ratios. Similar trends have been observed for DOM pools affected by photodegradation (Gonsior et al., 2009). However, contrasting results have been found in sediment flumes. Here, low molecular weight and oxygen-rich compounds were increased with bacterial activity, while high molecular weight and aliphatic, oxygen-poor molecules were negatively related to bacterial production (Kamjunke et al., 2017).

Dark incubation experiments with river water have also presented evidence for microbial degradation of the aromatic structures in lignin (Lu et al., 2016). According to this study, although light-induced degradation was higher in the first days of the experiment, microbial activity alone (i.e., dark treatment) accounted for double the potential of photo-degradation and microbial enhanced biodegradation of lignin combined (Lu et al., 2016). Sediment incubations have shown that lower molecular weight, labile, phytoplankton-derived compounds were degraded first, followed by petroleum-derived exogenous pollutants, and finally by higher MW polymeric plant material. This shift in utilization indicated a community succession and increased extracellular enzyme activities (Mahmoudi et al., 2017).

A study with 74 random streams in Sweden has analyzed DOM pool composition and transformation in such systems (Hawkes et al., 2018). Similarly, they have found that most of the compounds were ubiquitous to all samples and, therefore, unique mass assignments could be attributed to rare, low abundance peaks. Thus, although DOM concentration was different within the different streams, DOM pool composition was highly similar (Hawkes et al., 2018).

In this study, they have divided the DOM pool into three fractions in relation to its hydrophobic character. In both partially and highly hydrophobic fractions, DOM was composed of unsaturated compounds (H/C 1.0 – 1.5), whereas the hydrophilic fraction had highly unsaturated, carboxyl rich compounds (H/C < 1.0, O/C > 0.5). The first group (H/C > 1.0) tend to persist in freshwater and marine environments, while the latter reflects the terrestrial plant-derived character of DOM, which tend to release phenolic material (Hawkes et al., 2018).

In that context, Hawkes et al. (2018) have proposed that DOM of different boreal forested headwaters should react in similar ways during degradation, and that DOM composition should be rather similar across forested catchments already as it leaves the terrestrial environment into the streams, rather than becoming homogenous as they flow along the catchment and receive the influence of different headwaters and sub-catchments mix.

Large scale studies of DOM have also been done in boreal lakes (Kellerman et al., 2014; Kothawala et al., 2014). A total of 560 lakes distributed across Sweden have been investigated and it has been found that land cover (particularly the percentage of surface area covered with water in the catchment) was also a primary factor influencing the variability of PARAFAC components. Moreover, lake water retention time also influenced DOM quality (Kothawala et al., 2014).

These results suggest that, in addition to the individual characteristics of each lake, processes occurring in upstream water bodies have a significant influence on DOM quality. PARAFAC components with longer emission wavelengths (i.e., aromatic, humic-like compounds) were most reactive, while protein-like components were most persistent within lakes. This is probably a consequence of the fast internal production of such protein-like compounds, which are constantly renewed in the environment (Kothawala et al., 2014).

Therefore, Kothawala et al. (2014) have proposed that greater annual runoff might reduce lake water retention times, decreasing the opportunity for in-lake processing and primary production, shifting the quality of DOM to contain a greater proportion of humic-like, relative to protein-like OM. Since more aromatic and colored compounds are most effective at absorbing solar radiation, they expect it to be a relevant source of CO_2 due to photodegradation (Kothawala et al., 2014).

DOM molecular diversity in boreal lakes has also been described as driven by precipitation, temperature, and water residence time (Kellerman et al., 2014). Here, 120 Swedish lakes have been investigated and they proposed that terrestrially-derived DOM is preferentially lost as residence time increases, with warmer temperatures enhancing the production of N-containing compounds (i.e., microbial-derived) (Kellerman et al., 2014).

Similarly, they have suggested that increased mean annual precipitation would influence water retention time and, therefore, also runoff. These changes could enhance the abundance of polyphenols, reducing the influence of in-lake processing on DOM quality. They have found fire-derived compounds and polycyclic aromatics, vascular plant-derived polyphenols, and highly unsaturated and phenolic compounds to be positively correlated to mean annual precipitation (Kellerman et al., 2014).

Water retention time has also been shown to influence spectral characteristics of DOM, so that enhanced residence time represented a reduction in mean aromaticity (Köhler et al., 2013). Thus, it has been suggested that, with increased water retention time, concentration of allochthonous DOC decreases while autochthonous DOC increases; an effect that could prevent browning in some boreal lakes (Köhler et al., 2013).

Nevertheless, Panneer Selvam et al. (2019) have found that river mouths, which represent the extreme end-members in terms of land–water connectivity, had higher DOC photo-reactivity than most of the other aquatic ecosystems studied in the boreal landscape. These findings contrast with previous results which have shown that the DOC photo-reactivity tends to decrease as DOC is lost with increasing water residence time because of the increase in DOC exposure to sunlight and preferential loss of the aromatic, photo-reactive fractions of DOC (Panneer Selvam et al., 2019).

Since molecular composition of DOM can influence its degradation potential in the environment, the existence of priming effect (i.e., input of labile OC modifying OM decomposition rates, triggering the degradation of previously unreactive material [Bengtsson et al., 2018; Kuzyakov, 2010]) in boreal lakes has also been investigated (Catalán et al., 2015). However, experimental results have suggested that priming is unlikely to have a significant effect on bulk DOC degradation in these systems (Catalán et al., 2015).

With or without priming effect, the molecular changes derived from DOM transformation both via biological and photodegradation processes need to be further addressed. Biological transformations of DOM in boreal lake water have been studied in dark incubation experiments (Kothawala et al., 2012). Here, fluorescence components were identified with PARAFAC analysis before and after an incubation period of 3.5 years (Kothawala et al., 2012).

The results have shown that biodegradation and flocculation can also selectively alter the structural composition of lake water DOM. Two main humic-like components have been identified and presented contrasting behaviors; one was preferentially degraded, whereas the other accumulated. Although the chemical and molecular composition of these two peaks remain elusive, the differing responses of these two terrestrially-derived DOM peaks suggest differing reactivity, the first one being less recalcitrant than the latter (Kothawala et al., 2012).

The molecular composition and photo-reactivity of DOM as a function of lake depth has also been investigated in boreal systems (Gonsior et al., 2013). In this study, experiments with simulated light exposure for 24 hours have shown similar patterns of DOM degradation for lake water samples from the surface and right above the sediment in spring. However, the same experiment has been repeated in summer and surface samples showed less photobleaching, probably a result of the previous natural photobleaching that this DOM has already underwent in the natural environment (Gonsior et al., 2013).

Comparison between surface and deep SPE-DOM from a Swedish lake has also been done in April and June. In April, the relative abundance of highly oxidized organic compounds (O/C 0.50 – 0.87) was slightly decreased, whereas the lower oxidized (O/C < 0.45) organic compounds showed a small increase in the surface compared to deep sample. One explanation could be that more oxidized DOM is often also more water soluble (because the electronegativity of oxygen will introduce a polarization of covalent bonds and increase the overall solubility) and therefore is preferentially released from sediments (Gonsior et al., 2013).

In June, differences between surface and deep water were primarily associated with increased abundances of mass peaks assigned to relatively low H/C and high O/C ratio lower molecular weight compounds in the surface. Interestingly, those highly oxidized molecular formulas appeared in even higher relative abundances after the simulated light exposure experiments, indicating that these molecules were photochemical products that are labile and depleted by either microbial degradation or additional photochemical decarboxylation (Gonsior et al., 2013).

If the release of material from the sediments could be an important source of DOM to the bottom waters, it is also important to understand the mechanisms in which sedimentary OM is formed and settled. Flocculation of terrestrial DOC and subsequent sedimentation could lead to carbon sequestration by burial in lake sediments, since losses of OC from the water column via mineralization to CO_2 and via sedimentation were found to be equally important in boreal lakes (von Wachenfeldt and Tranvik, 2008).

1.2.3. Storage and internal turnover

Organic carbon preservation occurs when specific compounds are not completely mineralized and persist in the environments on a long-term scale (Killops and Killops, 2005). Lake

sediments, for example, represent regions of significant OM burial due to high sedimentation rates and burial efficiencies (Tranvik et al., 2009). However, DOM can also persist for long periods in the water column (Kellerman et al., 2015; Schmidt et al., 2011) and there are many factors contributing to OC storage.

Some of the abiotic known mechanisms that transform DOM into a recalcitrant material for enzymatic degradation are: polymerization of low molecular weight DOM (catalyzed by light and metal complexation); polymerization to macromolecular DOM; adsorption of labile DOM to colloids; assembling into gels that may undergo abrupt condensation (under high temperature and low pH conditions) (Carlson and Hansell, 2015).

In soils, sediments, and aquatic ecosystems, mineral bonding protects OM from microbial decomposition (Keil and Mayer, 2014). There are generally four types of interactions between OM and minerals: ligand exchange or sorption of small molecules to mineral surfaces; sorption of large molecules such as proteins to several mineral surface contact points; aggregation (i.e., chemical bonding or van der Waals attractions); and occlusion, which offers the most substantial protection of OM (Keil and Mayer, 2014). DOM becoming stable mineral-associated SOM, for example, has presented mean residence times of 100-200 years (Tipping et al., 2012).

Another factor determining selective preservation of OM in both terrestrial and aquatic environments is the sensitivity of organic molecules to O_2, or rather the dependence of microbial activity on O_2 content, and the exposure time (Arnarson and Keil, 2007; Burdige, 2007). Moreover, nutrient limitation, microbial community structure and specific metabolic repertoire, pressure, pH, and temperature also control where and when recalcitrant DOM becomes bioavailable (Carlson and Hansell, 2015), directly influencing OC preservation.

In soils, for instance, OM is efficiently recycled and, therefore, OC storage is mostly restricted to peat formation. The presence of large coal deposits in the sedimentary record suggests that peat formation was of greater importance in the past than it is today, when the annual accumulation rate is lower than that of organic-rich marine sediments (Killops and Killops, 2005).

The most recent paradigm on OM degradability in soils emphasizes the importance of the factors described above along with adaptations and structure of the microbial community

(Schmidt et al., 2011). These same principles apply to OM in the aqueous phase, where the majority of OM is mineralized soon after its synthesis. It has been shown that more than 99% of DOM is decomposed within a decade of being produced, with only a small fraction of molecules overturning on centennial to millennial time scales and accumulating in the ocean (Dittmar, 2014).

The photochemical and biological DOM degradation pathways (solely or coupled) contribute not only to the rapid removal of terrestrially derived OC in coastal waters, but also to the geochemical turnover of marine biologically refractory DOM (Mopper et al., 2014). Bio- and photodegradation can, additionally, induce flocculation and subsequent sedimentation of DOM, favoring its burial into the sediments (Guillemette et al., 2017; von Wachenfeldt et al., 2009, 2008; von Wachenfeldt and Tranvik, 2008).

Although marine and coastal sediments store large amounts of organic matter (Burdige, 2007, 2005) and marine OC burial rates may indeed be underestimated (Aufdenkampe et al., 2011), extensive research has shown that total OC burial rates in lakes and reservoirs are thought to be one to four times greater than the ocean and it has been suggested that total continental OM burial is an order of magnitude higher than marine burial (Einsele, 2001; Tranvik et al., 2009; Wilkinson and McElroy, 2007).

Recent calculations of CO_2 emission from anthropogenic and land-use change sources have been estimated between $9 - 10$ Pg C year^{-1} (Battin et al., 2009; Le Quéré et al., 2018). From that, 2.2 and 2.8 Pg C year^{-1} have been stored within marine and terrestrial biosphere, respectively (Battin et al., 2009). Therefore, it is important to have a detailed understanding of preserved organic matter.

Previous studies have shown that UV exposure alters the molecular composition of DOM (Gonsior et al., 2009; Kujawinski et al., 2009). The chemical composition of DOM dictates whether photochemical degradation will produce bio-available or bio-resistant compounds (Carlson and Hansell, 2015). The production of reactive oxygen species during photodegradation, for example, has been proposed as a mechanism that forms partially oxidized DOM, further reducing its bioavailability (Baltar et al., 2013).

Nevertheless, microbial degradation can also transform a fraction of labile DOM into compounds that resist further microbial degradation, being stored in the recalcitrant DOC pool

for months to millennia (Carlson and Hansell, 2015; Ogawa et al., 2001). Previous experimental mineralization studies have found that between 3 – 5% of the initial glucose was transformed to recalcitrant (i.e., humic-like) DOM within weeks of incubation (Gruber et al., 2006; Tranvik et al., 1993).

Recently, the molecular characteristics of persistent DOM have been studied using high resolution mass spectrometry. The term "island of stability" refers to a narrow range of molecular composition (H/C 1.17 ± 0.13; O/C 0.52 ± 0.10; molecular mass: 360 ± 28 and 497 ± 51 Da) that have been associated with the most persistent compounds in marine DOM (Lechtenfeld et al., 2014).

On natural gradient of connectivity with the catchment (i.e., river > lake > ocean), the contribution from polycyclic aromatic compounds decreased with the distance to the catchment (Kellerman et al., 2018). Moreover, the relative abundance of compounds in the range of "island of stability" increased at the same time, which was also coupled with sample age (Kellerman et al., 2018).

It has been proposed that both photochemical and biological degradation processes preferentially remove oxidized, aromatic compounds, whereas reduced, aliphatic and N-containing compounds persist in aquatic systems, either because they are resistant to degradation or tightly cycled (Kellerman et al., 2015). Therefore, DOM might be degraded along a gradient from aromatic to aliphatic compounds, and from a high to low nominal oxidation state of carbon.

Furthermore, molecular characteristics related to persistence of DOM have also been investigated in 109 Swedish lakes (Kellerman et al., 2015). In this study, ultrahigh-resolution mass spectrometry and optical spectroscopy have been used to investigate DOM properties, and they have found that degradation processes preferentially remove oxidized and aromatic compounds. Persistent compounds include reduced, aliphatic and N-containing molecules, which probably are either resistant to degradation or tightly cycled in aquatic systems (Kellerman et al., 2015).

The results suggested that elemental composition and basic structural features of individual molecules, such as aromaticity, nominal oxidation state of carbon, and elemental ratios (H/C, O/C, and N/C) influence their persistence in the environment. Thus, at the molecular level,

DOM is degraded along a gradient from aromatic to aliphatic compounds and from a high to low nominal oxidation state of carbon (Kellerman et al., 2015).

Flocculation of allochthonous DOM can be both microbially and light-induced (von Wachenfeldt et al., 2009, 2008). Laboratory experiments have shown that flocculation was enhanced by increased temperatures and addition of glucose, but it decreased by raising the pH. Besides, flocculation was similar under oxic and anoxic conditions and, for all treatments, flocculation was positively correlated to bacterial respiration (von Wachenfeldt et al., 2009).

However, bacterial biomass has represented a negligible fraction of the formed flocs. Their relevance to flocculation could be explained by the secretion of extracellular polymeric substances that possibly have a stabilizing effect on particles. Moreover, those polymeric components might have the capacity to adsorb other organic constituents, promoting the establishment of larger aggregates (von Wachenfeldt et al., 2009).

Light-mediated flocculation is also a relevant process in boreal lakes. In situ enclosure experiments with different light regimes have stimulated the formation of organic particles in lake waters at all incubation depths (von Wachenfeldt et al., 2008). Similarly to microbially induced flocculation, production of phytoplankton biomass was negligible, and allochthonous DOC was the most important precursor of the sinking particles (von Wachenfeldt et al., 2008).

Since flocculation can account for more than 20% of total DOC loss in lake waters (von Wachenfeldt et al., 2009, 2008), it is important to understand the dynamics of carbon preservation in lake sediments. Moreover, OC degradation in sediments has been estimated for a boreal catchment and it has been proposed that sediment OC mineralization was three times larger than OC burial, and contributed about 16% to the annual CO_2 emission (Chmiel et al., 2016).

Nevertheless, the preferential sequestration of terrestrially-derived OM in boreal lake sediments has been already shown (Guillemette et al., 2017). When comparing the fluorescence properties of sediments with settling material, they have found significant increase in humic-like components and decrease of protein-like component contribution. These results indicated that the first group was preferentially stored in the sediments, whereas the protein material must have been transformed (Guillemette et al., 2017).

On the other hand, it has also been suggested that degradation of OM transported through the inland water continuum might occur to a large extent via transition of DOC into more rapidly cycling POC in nature, for example, triggered by light (Attermeyer et al., 2018). In this way, particles would be a dominant pool of OC processing across the boreal aquatic continuum, partially sustained by replenishment via flocculation of DOC. In their study, Attermeyer et al. (2018) found microbial degradation rates of POC approximately 15 times higher compared to degradation of DOC. Rapid POC decay was accompanied by a shift in particulate C/N ratios, whereas DOM composition did not change at the time scale of incubations. Therefore, the mechanisms of transformation and/or storage of organic carbon are still not completely enlightened.

1.3. Ecological importance of DOM

DOM is commonly a major term in carbon, energy, and nutrient budgets for aquatic ecosystems and, as such, can have broad effects on food webs, heterotrophy, and nutrient retention/release (Findlay and Sinsabaugh, 2003). DOM acts as both substrate, source of nutrients, and electron acceptor for several biological and photochemical pathways (Carlson and Hansell, 2015), as well as providing a wide range of ecosystem services (Bolan et al., 2011; Ridgwell and Arndt, 2014; Schmidt et al., 2011; Senesi and Loffredo, 2005).

1.3.1. Energy

For the past decades, it has been well established that one of the fundamental roles that DOM plays in aquatic environments is to serve as a source of carbon and energy to microbial food webs (Bertilsson and Jones Jr, 2003; Pomeroy, 1974). However, for a long period, bacteria and other microorganisms were only recognized as decomposers in the traditional aquatic food web (Pomeroy et al., 2007).

It is believed that metabolism of particulate detritus (nonliving) and especially DOM from many pelagic and non-pelagic autochthonous and allochthonous sources dominates both material and energy fluxes in aquatic systems (Wetzel, 2003). For more than 30 years now, a model has been developed to describe such relations (Azam et al., 1983; Pomeroy, 1974).

The microbial loop is a model of pathways of carbon and nutrient cycling through microbial components of pelagic aquatic communities, in which DOM returns to higher trophic levels

via its incorporation into bacterial biomass and subsequent integration with the classic food chain (Azam et al., 1983). In other words, it channels energy and carbon via bacteria to protozoa, to larger zooplankton, and on to fishes and other carnivorous animals.

In fact, bacteria use nutrients and energy from diverse sources and populate the whole planet. In the ocean, for example, algae are responsible for most of the photosynthetic activity. Heterotrophic bacteria utilize DOM and POM, which they can digest with enzymes. Some bacteria/archaea oxidize inorganic chemicals for energy, and the carbon fixed into biomass serves as basis for food webs in diverse ecosystems. Earth's ocean is most certainly a sea of microbes; without them it would be a very different place, less hospitable to all life (Pomeroy et al., 2007).

Depth integrated primary production for the world ocean is between $30 - 8543$ mg C m^{-2} d^{-1}, but DOM concentrations, however, are maintained in a remarkably narrow range of $34 - 80$ µmol kg^{-1} C (Hansell et al., 2009). This striking offset between the range in primary production rates and DOC concentrations indicates that several biotic and abiotic DOM removal processes are at work (Carlson and Hansell, 2015).

Although the research that generated the concept of the microbial loop was almost exclusively carried out in marine environments, we can safely apply it to inland waters (Tranvik, 1992). In both environments, a substantial fraction of primary production is transformed into DOM through extracellular release from algal and cyanobacterial cells, and this DOM is subsequently utilized by heterotrophic bacteria, which make up a large biomass with large turnover rate (Tranvik, 1992).

Processes that produce, consume, and transform DOM are important in the overall cycling of carbon, energy, and nutrients in aquatic ecosystems. However, not all DOM is biologically labile or even biodegradable. It has been proposed that a small pool of biologically labile molecules is cycled rapidly and, despite the lower concentrations, the rapid turnover of these labile molecules could satisfy the energy and nutrient requirements of aerobic heterotrophic bacteria (e.g. Bunte and Simon, 1999; Fuhrman and Ferguson, 1986; Keil and Kirchman, 1991; Skoog et al., 1999).

An alternative scenario is that a large pool of more refractory DOM, primarily humic substances, provides a significant supplement to the heterotrophic metabolism supported by

monomers (Kaplan and Newbold, 2003; Tranvik, 1992). Although biologically less labile and perhaps less energy yielding than monomeric or complex polysaccharides, the increased concentration of the humic compounds might compensate for slower rates of catabolism (Kaplan and Newbold, 2003). Biodegradation of terrestrially derived macromolecules in freshwater ecosystems has been already described (Ward et al., 2013).

1.3.2. Nutrients

Although DOM is a smaller fraction of SOM, it is a major vehicle for the transport and loss of N and P from forest ecosystems (Bolan et al., 2011; Qualls and Haines, 1991). In aquatic ecosystems, DOM comprises the major form of organic matter, playing a significant role in aquatic food webs, mediating the availability of dissolved nutrients and metals, and modifying the optical properties of water bodies (Findlay and Sinsabaugh, 2003).

The labile DOM pool is of particular biological significance because it is rapidly consumed, supporting the metabolic energy and nutrient demands of heterotrophic prokaryotes, and turning over on time scales of hours to days (Carlson and Hansell, 2015; Killops and Killops, 2005). However, DOM is not only relevant as a source of nutrients from biological degradation processes. It has been shown that DOM photolysis releases nutrients to algae and bacteria (Moran and Covert, 2003). While N cycling is controlled mainly by biological reactions, both abiotic and biotic processes control P and S dynamics (Bolan et al., 2011).

DOM is linked directly and indirectly to several ecosystem processes and its metabolism can affect nutrient balances of aquatic ecosystems (Findlay and Sinsabaugh, 2003). Input of bioavailable DOM into nutrient-rich waters, for example, can lead to significant conversion of inorganic nutrients to organic forms. Conversely, the organic nutrients can be released by microbial metabolism of DOM with obvious effects on the likelihood of eutrophication of waters (Findlay and Sinsabaugh, 2003).

Among other inorganic and organic forms, nutrients can associate with OM by complexation (e.g. complexed Cu) or incorporation, including microbial biomass (e.g. N, P, and S) (Bolan et al., 2011). The mineralization–immobilization dynamic regulates the release of nutrients that are associated with DOM, affecting mobility and bioavailability. The mineralization process involves the conversion of plant unavailable organic forms of nutrients into plant available inorganic forms by soil microorganisms, while immobilization is the reverse process in which

the plant available nutrients are converted to plant unavailable organic compounds (Bolan et al., 2011).

Since nutrients are often incorporated in organic biomass, DOM dynamics are an integral factor in the mobilization and loss of nutrients in the environment. For example, it has been found that about half of the dissolved N and P in throughfall of a forested catchment was in organic form (Qualls et al., 1991). The role of DOM utilization by microorganisms in the nutrients cycling has also been shown in freshwater ecosystems (Wetzel, 1992). In lakes, for example, positive relationships between bacteria number and nutrient concentrations have been found (Currie, 1990; Nürnberg and Shaw, 1998).

The availability and mineralization of nutrients in aquatic systems are in close relationship with the eutrophication state. In fact, increasing the availability of nutrients enhances primary production, which then provides more substrates for heterotrophic microorganisms. Simultaneously, microbial degradation of OM mineralizes OC into gases (e.g. CO_2, CH_4, N_2O) and mobilizes nutrients (Liikanen et al., 2002).

Moreover, sediments from freshwater and marine environments are also hotspots for microbial remineralization of nutrients to the water column (Burdige and Komada, 2015; Droppo, 2001). Previous studies have shown great fluxes of nutrients from both oxic and anoxic sediment interfaces to the water column in lakes, which can be enhanced by increasing temperatures and primary production rates (Liikanen et al., 2003, 2002). Nutrient release from the sediments can occur due to the natural diffusion from one compartment to the other, but can also be enhanced by gas ebullition (Bastviken, 2009).

1.3.3. Complexation with metals

DOM plays an important role at the formation of carcinogenic and mutagenic disinfection by-products in drinking water treatment, and the transport and reactivity of toxic substances such as mercury (Raymond and Spencer, 2015). Many studies have shown evidence for DOM-induced mobilization of metals (e.g. Ashworth and Alloway, 2008; Khokhotva and Waara, 2010; Wong et al., 2007; Yang et al., 2008).

Specifically, humic substances have a great potential to form water-soluble complexes with toxic metals and organics, affecting the bioavailability of these toxicants and potentially

increasing their transportation range (Killops and Killops, 2005). Actually, apart from their contribution to the fluorescence signal of terrestrial CDOM, humic compounds have been recognized as an efficient transport for metals and nutrients to the coast, where they are released (Sipler and Bronk, 2014).

Humic material may contain different metals, which are incorporated into its macromolecular structure. Although mostly through coordination to COOH groups, phenols and sulfides are also credible coordination sites for metal ions (Haitzer et al., 2003; Hertkorn et al., 2004). The stability and further transport of metals ions is ensured because they are surrounded by and bonded to suitable chelating groups, mostly carboxylic acids (Bolan et al., 2011; Killops and Killops, 2005).

Natural environmental changes can regulate the release of complexed toxicants as they promote the opening of humic substance's structure. The interaction of metal ions with OM is mainly affected by redox potential and pH. The oxidation state of a metal ion and the availability of anions that may compete with organic moieties for the metal are controlled by these factors. For example, lowering the acidity to pH ~2 can release most of the humic-bound iron (Fe), because carboxylic group become protonated to COOH making it difficult for metal ions to coordinate (Killops and Killops, 2005).

Nevertheless, the interaction of humic compounds with metals is complex and for the series of metals mercury(II) [Hg(II)], Fe(III), lead (Pb), copper (Cu), aluminum (Al), nickel (Ni), chromium(III) [Cr(III)], cadmium (Cd), zinc (Zn), cobalt (Co) and manganese (Mn), it has been found that Hg and Fe are the most efficiently adsorbed, and Co and Mn the least (Kerndorff and Schnitzer, 1980). Besides, the binding power of Fe(II) ions with humic substances appears to be greater than that of Fe(III) (Killops and Killops, 2005).

Reactive Fe and OC are intimately associated in soils and sediments. In acid forest soils, Al and Fe can form relatively stable complexes with DOM and may thus be mobilized and transported in the soil profile (Marschner and Kalbitz, 2003). DOM structure alteration during complexation has been previously demonstrated by changes in fluorescence (Blaser et al., 1999) and molecular size (Jandl and Sletten, 1999).

Previous studies have assumed that complexed DOM is stabilized against biodegradation due to toxic effects from the metals, especially Al, but experimental data indicated that

complexation of potentially toxic metals by DOM would not necessarily reduce its biodegradability but instead could even enhance microbial activity by reducing the free metal ion concentrations and thus their toxicity (Marschner and Kalbitz, 2003). Both inhibiting, enhancing and, null effects of Al additions on the mineralization of different DOM fractions from forest floor solutions have been reported (Jandl and Sletten, 1999)

In fen soils, for example, it has been demonstrated that precipitation of iron hydroxides at the oxic surface layer removed a large fraction of DOM via coagulation, and that further oxygenation of anoxic fen pore water rapidly removed more than 90% of dissolved iron and almost 30% of DOC (Riedel et al., 2013). Moreover, while vascular plant-derived aromatic and pyrogenic compounds were preferentially retained, most of the carboxyl-rich aliphatic acids remained in solution. It has been proposed that redox interfaces, which are ubiquitous in marine and terrestrial settings, act as selective barriers, limiting the flux of land-derived DOM to oceanic waters (Riedel et al., 2013).

Mass spectrometry has been proven as a very useful tool to significantly facilitate and speed up structural characterization of such complexes, confirming complexation of certain humic fractions with specific metals, tracking correlations between metal complexation and molecular formula composition, and isolating metal-humic complexes in real-time (Stenson, 2009). In aquatic ecosystems, spectral parameters have been found to correlate with the total amounts of DOM-bound Pb(II) and to the extent of Pb(II) bound by carboxylic groups (Xu et al., 2018).

Experiments relating the effect of light-induced DOM degradation and metal toxicity in aquatic environments have been performed. It was expected that DOC concentrations would decrease with UVB irradiation and that metal toxicity would be enhanced following the decreased of DOC concentration. However, significant effects have only been demonstrated in brown-waters, while changes were less evident in the clear-water sample (Winch et al., 2002).

The role of salinity in metals bioavailability is still unresolved. By mixing riverine with marine water, it has been long proposed that flocculation of OC, humic substances, and metals – such as Fe, Al, and Mn – increased with increasing in salinity (Sholkovitz, 1976). However, recent experiments in an Arctic estuary have found that salinity does not play a relevant role for DOC and metals removal (Chupakova et al., 2018).

1.3.4. Anthropogenic influence, climate change, and expected consequences

Since the industrial revolution, in the 18th century, atmospheric concentrations of CO_2 and CH_4 are rapidly increasing due to massive burning of fossil fuels and related manufacturing commodities (Ciais et al., 2013). Growing population and the consequent increase in consumption also led to rising of agriculture and livestock, with practices such as deforestation, irrigation, and burning, which also contribute to elevated levels of greenhouse gases in the atmosphere (Ciais et al., 2013). Current atmospheric concentrations of CO_2 and CH_4 are, respectively, 1.5 and 2.5 times higher than the ones in 1750 (Le Quéré et al., 2018; Saunois et al., 2016).

With continuous increase of global population, fossil fuel emissions kept growing and in recent years have increased by 65% (i.e., from 1990 to 2014) (Le Quéré et al., 2015). Actually, it has been responsible for more than 80% of total CO_2 emissions since 1959, while land-use change represented 18% (Le Quéré et al., 2018). Atmospheric concentrations of CO_2 and CH_4 reached 405 ppm and 1810 ppb, respectively (Le Quéré et al., 2018; Saunois et al., 2016).

The global carbon budget for the last decade estimates fossil fuel CO_2 emissions to be around 9 Pg C year^{-1} (Battin et al., 2009; Le Quéré et al., 2018), whereas emissions from land use and land use change contributed with approximately 1.5 Pg C year^{-1} (Le Quéré et al., 2018). Based on available data, fossil CO_2 emissions for 2018 were projected to increase by 2.7% (Le Quéré et al., 2018).

CH_4 emissions, although smaller in number, are highly relevant due to its long atmospheric residence time and radiative properties, which results in a warming potential of approx. 23 times greater than CO_2 (Bastviken, 2009). The global budget for methane is estimated in 0.5 Pg CH_4 year^{-1}, from which 36% are a direct result of anthropogenic activity, such as fossil fuel and biomass burning, sewage treatment, and landfills, while ruminants and rice fields are responsible for another 27% – emissions enhanced by extensive livestock and agriculture (Conrad, 2009). Lakes emit roughly 8 – 48 Tg CH_4 year^{-1} (6 – 16% of natural emission), exceeding methane emissions from the ocean (Bastviken, 2009; Bastviken et al., 2011, 2004; Kirschke et al., 2013).

Greenhouse gases have that name because they naturally warm the planet and make life possible, but increased proportion of such gases in the atmosphere promotes climate change

(IPCC, 2013). Apart from intensification of extreme natural events (e.g. drought, intense rain, heat waves, etc.), current concentrations of greenhouse gases have promoted an increase in 0.85 ℃ in the planet surface (land and ocean) since the industrial revolution (IPCC, 2013).

Higher temperatures in the oceans, among other consequences, decrease CO_2 solubility in the water, reducing the primary production/fixation potential (Ciais et al., 2013). In general, warmer temperatures also promote increased microbial activity (Brown et al., 2004), which result in higher OM degradation rates and further emissions of CO_2 and CH_4 (Davidson and Janssens, 2006; Duc et al., 2010; Gudasz et al., 2015). Recently, photodegradation of DOM has also been shown as a temperature sensitive process (Porcal et al., 2015).

In lakes, warmer temperatures can change even the sediment dynamics (Liikanen et al., 2002). It has been shown that CO_2 production from the sediments increases with temperature for both oxic and anoxic conditions, and that higher temperatures also enhance the transport of nutrients from the sediment to the water column, accelerating the eutrophication process and promoting CH_4 production in anoxic sediments (Liikanen et al., 2002).

Theoretically, higher CO_2 concentration in the atmosphere positively feeds back the photosynthetic activity of autotrophic organisms, increasing their consumption of CO_2 (Killops and Killops, 2005). However, higher photosynthetic rates, ultimately, promote DOM production (Carlson and Hansell, 2015). Increased availability of DOC could also lead to increased proportions of carbon mineralization and further greenhouse gases production (Conrad et al., 2011).

Nevertheless, anthropogenic direct and indirect greenhouse gases emissions (i.e., burning of fossil fuels or input of DOC in the environments, leading to enhanced mineralization and further gas emissions) cannot be compensated by such natural feedback (IPCC, 2013). The impacts of human action in the carbon cycle have been already demonstrated (Ciais et al., 2013; Le Quéré et al., 2018, 2015, 2014; Saunois et al., 2016).

Besides, in the modern world, increased primary production does not necessarily mean carbon sequestration for a long-term period. In fact, extensive agriculture and cattle farming comprehend a series of practices that increases the emissions of greenhouse gases to the atmosphere, such as fire and deforestation (Le Quéré et al., 2018). Moreover, land use changes also lead to transformation of DOM pool composition.

For example, previous studies with stable isotopic analysis of different fractions of OM have demonstrated that its signature has changed due to substitution of forests by pasture (Bernardes et al., 2004). While C4 grasses present in pastures have δ^{13}C values varying between –11 and –14‰, forest C3 leaves have characteristically lower δ^{13}C values, between –28 and –34‰. They have found a change in the δ^{13}C of the fine OM fraction from –27.2‰ to –20.5‰ when a small stream leaves the forest and enters a pasture (Bernardes et al., 2004).

A similar study, investigating different streams which had either forest or pasture as a primary source for allochthonous OM, has found the same trends (Deegan et al., 2011). Here, all OM fractions in the forest stream had isotopic values consistent with being completely derived from inputs of terrestrial C3 vegetation, whereas the δ^{13}C of OM in the pasture streams was enriched in comparison, suggesting inputs of C4 grasses or algae (Deegan et al., 2011).

Land use change can also damage the soil structure, because OM is essential in stabilizing the entire soil matrix (Bolan et al., 2011). Agricultural practices have proven to be responsible for reduction in soil aggregation due to changes in DOM composition and release of redox-sensitive elements (e.g. Fe and Mn) that act as a binding material and, therefore, contribute to increased rates of soil erosion (Bolan et al., 2011).

Groeneveld et al. (2020) have performed laboratory experiments with addition of clay particles to 30 water samples collected throughout Sweden. Their results suggest that freshly produced aquatic DOM is less susceptible to adsorption than more terrestrial material. Moreover, the percentage DOM adsorbed in the experiments greatly exceeds the actual adsorption taking place in boreal inland waters across all studied systems. These results illustrate the potential impact of mineral erosion, for example, as a result of agriculture, mining, or forestry practices, on the availability, transport, and composition of organic carbon in inland waters (Groeneveld et al., 2020).

Enhanced input of terrestrial DOM in aquatic water bodies, either due to land use change or higher erosion rates, could also change the biogeochemical dynamics of DOM biodegradation (Fasching et al., 2014). It has been shown that terrigenous DOM is respired by microorganisms rather than incorporated into their biomass, and this would channel terrigenous carbon to the pool of CO_2 potentially outgassing from streams into the atmosphere (Fasching et al., 2014).

Increased concentrations of DOC and other nutrients (mainly N and P) carried to water bodies leads to eutrophication of freshwater systems and further transport of great amounts of OC to the oceans (Antoniades et al., 2011; Ward et al., 2017). Cultural eutrophication (i.e., anthropogenic) is mainly related to land use change, such as agriculture/pasture and urbanization (Gücker et al., 2016). For example, Evans et al. (2017) have found that trophic status strongly regulates whether water bodies act as net dissolved organic carbon sources or sinks, and that rates of both DOC production and consumption can be predicted from water residence time.

Fluvial DOM and POM composition have been investigated through C/N ratios, $\delta^{13}C$, and optical measurements including excitation emission matrix fluorescence with parallel factor analysis (EEM–PARAFAC), and results have demonstrated a shift toward a more microbial/algal and less plant/soil-derived character as human disturbance increased (Lambert et al., 2017). Additionally, changes in pH and autochthonous DOC input along the continuum have been suggested to sustain high DOC photo-mineralization throughout continental aquatic networks (Panneer Selvam et al., 2019).

Previous comparison of DOM composition between surface and groundwaters has shown lower amounts of CDOM, lower contributions of humic-like compounds, and higher proportions of microbially-derived and protein-like DOM in the groundwater pool. Although it has been shown that human activity does influence autochthonous OM production, stable isotope data has suggested that the influence of human activity on terrestrial OM transfer is subjected to different controls (Lambert et al., 2017).

Johnston et al. (2020) have observed a relative decrease in CDOM in more hydrologically isolated lakes, without a corresponding decrease in DOC concentration. Indicators of allochthonous DOM suggested that little allochthonous DOM was supplied to many of these hydrologically isolated lakes. They have proposed that decreased lake hydrologic connectivity, driven by ongoing climate change (i.e., decreased precipitation, warming temperatures), will reduce allochthonous DOM contributions and shift lakes toward lower CDOM systems with ecosystem-scale ramifications for heat transfer, photochemical reactions, productivity, and ultimately their biogeochemical function (Johnston et al., 2020).

A study analyzing DOM in catchments dominated by natural vegetation, pasture, intensive agriculture, and urban land cover, has found that the latter is the most damaging to biogeochemical dynamics (Gücker et al., 2016). Whereas pasture catchments had lower stream DOM concentration, with lower C/N and C/P ratios, agricultural catchments had higher DOM export, with lower C/P, and urban catchments had higher DOM concentration and export, with lower C/N and higher C/P ratios (Gücker et al., 2016).

Moreover, sewage discharge from urbanized landscaped into catchments can supply – together with higher concentrations of nutrients – chemical pollutants, pesticides, and heavy metals that accumulate in the water and the sediments (Antoniades et al., 2011; Bolan et al., 2011; Das, 2005). Previous data has shown that the solubility of DOM in a sewage-sludge-amended soil is positively associated with the solubility of Cu, Ni, and Pb (Ashworth and Alloway, 2008).

OM solubility fluctuates both temporally and spatially, and is controlled by several soil-specific (e.g. native organic-matter content, mineralogy, abundance of cations and anions) and environmental factors (e.g. temperature, soil moisture content, rainfall, land use, and timing of inputs of organic material) (Ashworth and Alloway, 2008). Therefore, the current scenario of anthropogenic driven climate change could have an even greater impact in the way DOM remedies the bioavailability of heavy metals in the environment.

Mass spectrometry analysis of effluent DOM has shown that its molecular composition is different from DOM in a natural environment (Schmitt-Kopplin et al., 2011). Data has demonstrated that effluent DOM was enriched with S-containing compounds, probably a consequence of the addition of surfactants such as linear alkyl benzene sulfonates, their co-products dialkyl tetralin sulfonates, and their biodegraded metabolites (e.g. sulfophenyl carboxylic acids) (Schmitt-Kopplin et al., 2011).

Human activity also leads to air pollution. A current problem in our atmosphere is the ozone depletion, caused by the emissions of different pollutants, which leads to increased UV-radiation on the planet (IPCC, 2013; Killops and Killops, 2005). While ozone levels are increasing in the troposphere, they are decreasing in the stratosphere by the action of anthropogenic gases, particularly the xenobiotic chlorofluorocarbons, which have been widely used as refrigerants, blowing/propulsion agents, and cleaning agents for electronic components (Killops and Killops, 2005).

Photobleaching of DOM, a recognized process in aquatic environments, could be enhanced by the higher levels of UV-radiation. That is concerning because DOM acts as a screening filter for aqueous microorganisms against excessive and potentially harmful UV-radiation (Brinkmann et al., 2003). Thus, changing DOM molecular properties in aquatic environments could threaten its protection to several microbial communities.

Finally, besides land use change or pollution, another anthropogenic impact in the biogeochemical cycles is the landscape change. Human intervention in river hydraulics is known to change the dynamics of both CO_2 and CH_4 production and emissions (Crawford et al., 2016; Crawford and Stanley, 2016). Reservoirs impede the water flow, trapping the suspended sediment load in rivers, changing the flux of sediments in the watershed (Latrubesse et al., 2017).

At the same time that OM burial associated with these sediments can represent an important carbon sink in these environments, they also become hotspots for CH_4 production and subsequent emissions to the atmosphere (DelSontro et al., 2010; Maeck et al., 2013; Sobek et al., 2012). Another effect of increased sedimentation is the enhancing of light penetration and consequent autochthonous primary production. The growth of the phytoplankton community is an important source of labile OM that fuels microbial degradation (Ward et al., 2017).

Reservoir greenhouse gases emissions can be further amplified by the flooding of dry land (Ward et al., 2017). A study tracking the changes in a reservoir after 10 years of flooding has shown that, during that period, more than 20% of the flooded OC was mineralized and lost to the atmosphere (Abril et al., 2005).

1.4. Final considerations

It is extremely important to understand how the ongoing fossil fuel greenhouse gases emissions, and the consequent changes in climate, could affect DOC cycling in natural environments (Ridgwell and Arndt, 2014). DOM dynamics (both synthesis and degradation) are influenced by the following factors: rising atmospheric CO_2 concentrations; altered photosynthesis and biomass formation; increased temperature; elevated metabolic rates from microorganisms; increased stratification of water bodies; among others.

The dissolved fraction of carbon pool in the environments is responsible for sustaining an important part of food webs, both via direct energy transfer and nutrients availability (Bolan et al., 2011; Carlson and Hansell, 2015; Pomeroy et al., 2007; Pomeroy, 1974). Besides, DOM also protects living organisms and landscape structure both physically and chemically (e.g. erosion control, UV-radiation protection, bioavailability of heavy metals) (Ashworth and Alloway, 2008; Bolan et al., 2011; Brinkmann et al., 2003; Raymond and Spencer, 2015).

DOM complexity and importance is already recognized (Hertkorn et al., 2008), and its molecular composition and dynamics have been studies in a wide range of environments (e.g. D'Andrilli et al., 2002; Gonsior et al., 2016; Kellerman et al., 2018; Roth et al., 2014; Seidel et al., 2014; Tremblay et al., 2007; Zhao et al., 2017). Nevertheless, there is still important information missing about the mechanisms through which DOM is microbially mineralized or preserved.

Because carbon cycling in terrestrial and aquatic environments is largely driven by DOM (Bolan et al., 2011; Burdige and Komada, 2015; Carlson and Hansell, 2015; Raymond and Spencer, 2015), more studies investigating the mechanisms of its complete mineralization to greenhouse gases but also the molecular selectivity in DOM preservation are needed, especially in freshwater environments considered hotspots for microbial degradation (Battin et al., 2009; Cole et al., 2007).

While some processes can positively reinforce DOM mineralization and greenhouse gases emissions, some may stimulate higher carbon burial efficiency in land and ocean sediments. Therefore, educated knowledge of DOM structure, composition and processes will aid political decisions in a changing world.

1.5. References

Abelmann, K., Totsche, K.U., Knicker, H., Kögel-Knabner, I., 2004. CP dynamics of heterogeneous organic material: Characterization of molecular domains in coals. Solid State Nucl. Magn. Reson. 25, 252–266.

Abril, G., Guérin, F., Richard, S., Delmas, R., Galy-Lacaux, C., Gosse, P., Tremblay, A., Varfalvy, L., Dos Santos, M.A., Matvienko, B., 2005. Carbon dioxide and methane emissions and the carbon budget of a 10-year old tropical reservoir (Petit Saut, French Guiana). Global Biogeochem. Cycles 19, 1–16.

Achtnich, C., Bak, F., Conrad, R., 1995. Competition for electron donors among nitrate reducers, ferric iron reducers, sulfate reducers, and methanogens in anoxic paddy soil. Biol Fertil Soils 65–72.

Alonso-Sáez, L., Sánchez, O., Gasol, J.M., 2012. Bacterial uptake of low molecular weight organics in the subtropical Atlantic: Are major phylogenetic groups functionally different? Limnol. Oceanogr. 57, 798–808.

Amir, S., Hafidi, M., Lemee, L., Bailly, J.R., Merlina, G., Kaemmerer, M., Revel, J.C., Ambles, A., 2006. Structural characterization of fulvic acids, extracted from sewage sludge during composting, by thermochemolysis-gas chromatography-mass spectrometry. J. Anal. Appl. Pyrolysis 77, 149–158.

Amon, R.M.W., Benner, R., 1996a. Bacterial utilization of different size classes of dissolved organic matter. Limnol. Oceanogr. 41, 41–51.

Amon, R.M.W., Benner, R., 1996b. Photochemical and microbial consumption of dissolved organic carbon and dissolved oxygen in the Amazon River system. Geochim. Cosmochim. Acta 60, 1783–1792.

Antoniades, D., Michelutti, N., Quinlan, R., Blais, J.M., Bonilla, S., Douglas, M.S.V., Pienitz, R., Smol, J.P., Vincenta, W.F., 2011. Cultural eutrophication, anoxia, and ecosystem recovery in Meretta Lake, High Arctic Canada. Limnol. Oceanogr. 56, 639–650.

Arnarson, T.S., Keil, R.G., 2007. Changes in organic matter-mineral interactions for marine sediments with varying oxygen exposure times. Geochim. Cosmochim. Acta 71, 3545–3556.

Ashworth, D.J., Alloway, B.J., 2008. Influence of dissolved organic matter on the solubility of heavy metals in sewage-sludge-amended soils. Commun. Soil Sci. Plant Anal. 39, 538–550.

Attermeyer, K., Catalán, N., Einarsdottir, K., Freixa, A., Groeneveld, M., Hawkes, J.A., Bergquist, J., Tranvik, L.J., 2018. Organic Carbon Processing During Transport Through Boreal Inland Waters: Particles as Important Sites. J. Geophys. Res. Biogeosciences 123, 2412–2428.

Aufdenkampe, A.K., Mayorga, E., Raymond, P.A., Melack, J.M., Doney, S.C., Alin, S.R., Aalto, R.E., Yoo, K., 2011. Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. Front. Ecol. Environ. 9, 53–60.

Awad, J., van Leeuwen, J., Chow, C.W.K., Smernik, R.J., Anderson, S.J., Cox, J.W., 2017. Seasonal variation in the nature of DOM in a river and drinking water reservoir of a closed catchment. Environ. Pollut. 220, 788–796.

Azam, F., Fenchel, T., Field, J., Gray, J., Meyer-Reil, L., Thingstad, F., 1983. The Ecological Role of Water-Column Microbes in the Sea. Mar. Ecol. Prog. Ser. 10, 257–263.

Baker, M.A., Valett, H.M., Dahm, C.N., 2000. Organic carbon supply and metabolism in a shallow groundwater ecosystem. Ecology 81, 3133–3148.

Baltar, F., Reinthaler, T., Herndl, G.J., Pinhassi, J., 2013. Major Effect of Hydrogen Peroxide on Bacterioplankton Metabolism in the Northeast Atlantic. PLoS One 8, 2–9.

Bastviken, D., 2009. Methane, in: Likens, G.E. (Ed.), Encyclopedia of Inland Waters. Elsevier, Oxford, pp. 783–805.

Bastviken, D., Persson, L., Odham, G., Tranvik, L.J., 2004. Degradation of dissolved organic matter in oxic and anoxic lake water. Limnol. Oceanogr. 49, 109–116.

Battin, T.J., Luyssaert, S., Kaplan, L. A., Aufdenkampe, A.K., Richter, A., Tranvik, L.J., 2009. The boundless carbon cycle. Nat. Geosci. 2, 598–600.

Bengtsson, M.M., Attermeyer, K., Catalán, N., 2018. Interactive effects on organic matter processing from soils to the ocean: are priming effects relevant in aquatic ecosystems? Hydrobiologia 822, 1–17.

Benner, R., Amon, R.M.W., 2015. The Size-Reactivity Continuum of Major Bioelements in the Ocean. Ann. Rev. Mar. Sci. 7, 185–205.

Bernardes, M.C., Martinelli, L.A., Krusche, A. V., Gudeman, J., Moreira, M., Victoria, R.L., Ometto, J.P.H.B., Ballester, M.V.R., Aufdenkampe, A.K., Richey, J.E., Hedges, J.I., 2004. Riverine organic matter composition as a function of land use changes, Southwest Amazon. Ecol. Appl. 14, 263–279.

Bertilsson, S., Jones Jr, J.B., 2003. Supply of Dissolved Organic Matter to Aquatic Ecosystems: Autochthonous Sources, in: Findlay, S.E.G., Sinsabaugh, R.L. (Eds.), Aquatic Ecosystems - Interactivity of Dissolved Organic Matter. Academic Press, San Diego, pp. 3–25.

Bertilsson, S., Tranvik, L.J., 2000. Photochemical transformation of dissolved organic matter in lakes. Limnol. Oceanogr. 45, 753–762.

Blanchet, M., Pringault, O., Panagiotopoulos, C., Lefèvre, D., Charrière, B., Ghiglione, J.F., Fernandez, C., Aparicio, F.L., Marrasé, C., Catala, P., Oriol, L., Caparros, J., Joux, F., 2017. When riverine dissolved organic matter (DOM) meets labile DOM in coastal

waters: changes in bacterial community activity and composition. Aquat. Sci. 79, 27–43.

Blaser, P., Heim, A., & Luster, J., 1999. Total Luminescence Spectroscopy of NOM-Typing Samples and their Aluminium Complexes. Environment International 25(2), 285–293.

Bolan, N.S., Adriano, D.C., Kunhikrishnan, A., James, T., McDowell, R., Senesi, N., 2011. Chapter 1 – Dissolved Organic Matter: Biogeochemistry, Dynamics, and Environmental Significance in Soils (First Edition). Advances in Agronomy. Elsevier Inc., Academic Press, Volume 110, 1–75.

Boon, P.I., Mitchell, A., 1995. Methanogenesis in the sediments of an Australian freshwater wetland: Comparison with aerobic decay, and factors controlling methanogenesis. FEMS Microbiol. Ecol. 18, 175–190.

Brezonik, P.L., Arnold, W.A., 2011. Water Chemistry - An Introduction to the Chemistry of Natural and Engineered Aquatic Systems. Oxford University Press, New York.

Brinkmann, T., Sartorius, D., Frimmel, F.H., 2003. Photobleaching of humic rich dissolved organic matter. Aquat. Sci. 65, 415–424.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V., West, G.B., 2004. Toward a metabolic theory of ecology. Ecology 85, 1771–1789.

Bunte, C., Simon, M., 1999. Bacterioplankton turnover of dissolved free monosaccharides in a mesotrophic lake. Limnol. Oceanogr. 44, 1862–1870.

Burdige, D.J., 2007. Preservation of Organic Matter in Marine Sediments: Controls, Mechanisms, and an Imbalance in Sediment Organic Carbon Budgets? Chem. Rev. 107, 467–485.

Burdige, D.J., 2005. Burial of terrestrial organic matter in marine sediments: A re-assessment. Global Biogeochem. Cycles 19, GB4011.

Burdige, D.J., Komada, T., 2015. Sediment Pore Waters. In Biogeochemistry of Marine Dissolved Organic Matter (Second Edition). Elsevier Inc., Academic Press, 535–577.

Carlson, C. A., Hansell, D. A., 2015. Chapter 3 – DOM Sources, Sinks, Reactivity, and Budgets. In Biogeochemistry of Marine Dissolved Organic Matter (Second Edition). Elsevier Inc, Academic Press, 65–126.

Catalán, N., Kellerman, A.M., Peter, H., Carmona, F., Tranvik, L.J., 2015. Absence of a priming effect on dissolved organic carbon degradation in lake water. Limnol. Oceanogr. 60, 159–168.

Catalán, N., Marcé, R., Kothawala, D.N., Tranvik, L.J., 2016. Organic carbon decomposition rates controlled by water retention time across inland waters. Nat. Geosci. 9, 501–504.

Chen, M., Hur, J., 2015. Pre-treatments, characteristics, and biogeochemical dynamics of dissolved organic matter in sediments: a review. Water Res. 79, 10–25.

Chmiel, H.E., Kokic, J., Denfeld, B.A., Einarsdóttir, K., Wallin, M.B., Koehler, B., Isidorova, A., Bastviken, D., Ferland, M.-È., Sobek, S., 2016. The role of sediments in the carbon budget of a small boreal lake. Limnol. Oceanogr. 61, 1814–1825.

Chupakova, A.A., Chupakov, A. V., Neverova, N. V., Shirokova, L.S., Pokrovsky, O.S., 2018. Photodegradation of river dissolved organic matter and trace metals in the largest European Arctic estuary. Sci. Total Environ. 622–623, 1343–1352.

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., Jones, C., Quéré, C. Le, Myneni, R.B., Piao, S., Thornton, P., 2013. Carbon and Other Biogeochemical Cycles, in: Stocker, T.F., Qin, D., Plattner, Gi.-K., Tignor, M.M.B., Allen, S.K., Boschung, J. (Eds.), Climate Change 2013: The Physical Science Basis Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY, USA, pp. 465–570.

Clarke, N., Røsberg, I., Aamlid, D., 2005. Concentrations of dissolved organic carbon along an altitudinal gradient from Norway spruce forest to the mountain birch/alpine ecotone in Norway. Boreal Environ. Res. 10, 181–189.

Cole, J.J., Prairie, Y.T., Caraco, N.F., McDowell, W.H., Tranvik, L.J., Striegl, R.G., Duarte, C.M., Kortelainen, P., Downing, J. a., Middelburg, J.J., Melack, J., 2007. Plumbing the Global Carbon Cycle: Integrating Inland Waters into the Terrestrial Carbon Budget. Ecosystems 10, 172–185.

Conrad, R., 2009. The global methane cycle: recent advances in understanding the microbial processes involved. Environ. Microbiol. Rep. 1, 285–292.

Conrad, R., 1999. Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. FEMS Microbiol. Ecol. 28, 193–202.

Conrad, R., 1996. Soil Microorganisms as Controllers of Atmospheric Trace Gases (H_2, CO, CH_4, OCS, N_2O, and NO). Microbiol. Rev. 60, 609–640.

Conrad, R., Noll, M., Claus, P., Klose, M., Bastos, W.R., Enrich-Prast, A., 2011. Stable carbon isotope discrimination and microbiology of methane formation in tropical anoxic lake sediments. Biogeosciences 8, 795–814.

Cory, R.M., Harrold, K.H., Neilson, B.T., Kling, G.W., 2015. Controls on dissolved organic matter (DOM) degradation in a headwater stream: the influence of photochemical and hydrological conditions in determining light-limitation or substrate-limitation of photo-degradation. Biogeosciences 12, 6669–6685.

Crawford, J.T., Loken, L.C., Stanley, E.H., Stets, E.G., Dornblaser, M.M., Striegl, R.G., 2016. Basin scale controls on CO_2 and CH_4 emissions from the Upper Mississippi River. Geophys. Res. Lett. 43, 1973–1979.

Crawford, J.T., Stanley, E.H., 2016. Controls on methane concentrations and fluxes in streams draining human-dominated landscapes. Ecol. Appl. 26, 1581–1591.

Currie, D.J., 1990. Large-scale variability and interactions among phytoplankton, bacterioplankton, and phosphorus. Limnol. Oceanogr. 35, 1437–1455.

D'Andrilli, J., Dittmar, T., Koch, B.P., Purcell, J.M., Marshall, A.G., Cooper, W.T., 2002. Comprehensive characterization of marine dissolved organic matter by Fourier transform ion cyclotron resonance mass spectrometry with electrospray and atmospheric pressure photoionization. Rapid Commun. Mass Spectrom. 24, 1779–1784.

Dainard, P.G., Guéguen, C., McDonald, N., Williams, W.J., 2015. Photobleaching of fluorescent dissolved organic matter in Beaufort Sea and North Atlantic Subtropical Gyre. Mar. Chem. 177, 630–637.

Das, B.K., 2005. Environmental pollution impact on water and sediments of Kumaun lakes, Lesser Himalaya, India: a comparative study. Environ. Geol. 49, 230–239.

Davidson, E. A., Janssens, I. A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173.

Deegan, L.A., Neill, C., Haupert, C.L., Ballester, M.V.R., Krusche, A. V., Victoria, R.L., Thomas, S.M., de Moor, E. , 2011. Amazon deforestation alters small stream structure, nitrogen biogeochemistry and connectivity to larger rivers. Biogeochemistry 105, 53–74.

DelSontro, T., McGinnis, D.F., Sobek, S., Ostrovsky, I., Wehrli, B., 2010. Extreme Methane Emissions from a Swiss Hydropower Reservoir: Contribution from Bubbling Sediments. Environ. Sci. Technol. 44, 2419–2425.

Dittmar, T., 2014. Chapter 7 – Reasons Behind the Long-Term Stability of Dissolved Organic Matter. In Biogeochemistry of Marine Dissolved Organic Matter (Second Edition). Elsevier Inc., Academic Press, 369–388.

Dittmar, T., Whitehead, K., Minor, E.C., Koch, B.P., 2007. Tracing terrigenous dissolved organic matter and its photochemical decay in the ocean by using liquid chromatography/mass spectrometry. Mar. Chem. 107, 378–387.

Droppo, I.G., 2001. Rethinking what constitutes suspended sediment. Hydrol. Process. 15, 1551–1564.

Duc, N.T., Crill, P., Bastviken, D., 2010. Implications of temperature and sediment characteristics on methane formation and oxidation in lake sediments. Biogeochemistry 100, 185–196.

Einarsdottir, K., Wallin, M.B., Sobek, S., 2017. High terrestrial carbon load via groundwater to a boreal lake dominated by surface water inflow. J. Geophys. Res. Biogeosciences 122, 15–29.

Einsele, G., 2001. Atmospheric carbon burial in modern lake basins and its significance for the global carbon budget. Glob. Planet. Change 30, 167–195.

Einsiedl, F., Hertkorn, N., Wolf, M., Frommberger, M., Schmitt-Kopplin, P., Koch, B.P., 2007. Rapid biotic molecular transformation of fulvic acids in a karst aquifer. Geochim. Cosmochim. Acta 71, 5474–5482.

Evans, C.D., Futter, M.N., Moldan, F., Valinia, S., Frogbrook, Z., Kothawala, D.N., 2017. Variability in organic carbon reactivity across lake residence time and trophic gradients. Nat. Geosci. 10, 832–835.

Fasching, C., Behounek, B., Singer, G.A., Battin, T.J., 2014. Microbial degradation of terrigenous dissolved organic matter and potential consequences for carbon cycling in brown-water streams. Sci. Rep. 4, 1–7.

Fasham, M.J.R., Balino, B.M., Bowles, M.C., Anderson, R., Archer, D., Bathmann, U., Boyd, P., Buesseler, K., Burkill, P., Bychkov, A., Carlson, C., Chen, C.-T.A., Doney, S., Ducklow, H., Emerson, S., Feely, R., Feldman, G., Garcon, V., Hansell, D., Hanson, R., Harrison, P., Honjo, S., Jeandel, C., Karl, D., Le Borgne, R., Liu, K.-K., Lochte, K., Louanchi, F., Lowry, R., Michaels, A., Monfray, P., Murray, J., Oschlies, A., Platt, T., Priddle, J., Quinones, R., Ruiz-Pino, D., Saino, T., Sakshaug, E., Shimmield, G., Smith, S., Smith, W., Takahashi, T., Tréguer, P., Wallace, D., Wanninkhof, R., Watsen, A., Willebrand, J., Wong, C.S., 2001. A New Vision of Ocean Biogeochemistry After a Decade of the Joint Global Ocean Flux Study (JGOFS). AMBIO – A Journal of the Human Environment – Special Report N. 10. Stockholm.

Fenchel, T., Kristensen, L., Rasmussen, L., 1990. Water column anoxia: vertical zonation of planktonic protozoa. Mar. Ecol. Prog. Ser. 62, 1–10.

Fey, A., Conrad, R., 2000. Effect of Temperature on Carbon and Electron Flow and on the Archaeal Community in Methanogenic Rice Field Soil. Appl. Environ. Microbiol. 66, 4790–4797.

Findlay, S.E.G., Sinsabaugh, R.L. (Eds.), 2003. Aquatic Ecosystems - Interactivity of Dissolved Organic Matter. Academic Press, San Diego.

Flerus, R., Lechtenfeld, O.J., Koch, B.P., McCallister, S.L., Schmitt-Kopplin, P., Benner, R., Kaiser, K., Kattner, G., 2012. A molecular perspective on the ageing of marine dissolved organic matter. Biogeosciences 9, 1935–1955.

Fuhrman, J., Ferguson, R., 1986. Nanomolar concentrations and rapid turnover of dissolved free amino acids in seawater: agreement between chemical and microbiological measurements. Mar. Ecol. Prog. Ser. 33, 237–242.

Glissman, K., Chin, K.-J., Casper, P., Conrad, R., 2004. Methanogenic Pathway and Archaeal Community Structure in the Sediment of Eutrophic Lake Dagow: Effect of Temperature. Microb. Ecol. 48, 389–399.

Goldberg, S.J., Ball, G.I., Allen, B.C., Schladow, S.G., Simpson, a. J., Masoom, H., Soong, R., Graven, H.D., Aluwihare, L.I., 2015. Refractory dissolved organic nitrogen accumulation in high-elevation lakes. Nat. Commun. 6, 6347.

Gonsior, M., Hertkorn, N., Conte, M.H., Cooper, W.J., Bastviken, D., Druffel, E., Schmitt-Kopplin, P., 2014. Photochemical production of polyols arising from significant photo-transformation of dissolved organic matter in the oligotrophic surface ocean. Mar. Chem. 163, 10–18.

Gonsior, M., Peake, B.M., Cooper, W.T., Podgorski, D., D'Andrilli, J., Cooper, W.J., 2009. Photochemically Induced Changes in Dissolved Organic Matter Identified by Ultrahigh Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. Environ. Sci. Technol. 43, 698–703.

Gonsior, M., Peake, B.M., Cooper, W.T., Podgorski, D.C., D'Andrilli, J., Dittmar, T., Cooper, W.J., 2011. Characterization of dissolved organic matter across the Subtropical Convergence off the South Island, New Zealand. Mar. Chem. 123, 99–110.

Gonsior, M., Schmitt-kopplin, P., Bastviken, D., 2013. Depth-dependent molecular composition and photo-reactivity of dissolved organic matter in a boreal lake under winter and summer conditions. Biogeosciences 10, 6945–6956.

Gonsior, M., Valle, J., Schmitt-Kopplin, P., Hertkorn, N., Bastviken, D., Luek, J., Harir, M., Bastos, W., Enrich-Prast, A., 2016. Chemodiversity of Dissolved Organic Matter in the Amazon Basin. Biogeosciences Discuss. 1–21.

Groeneveld, M., Catalán, N., Attermeyer, K., Hawkes, J., Einarsdóttir, K., Kothawala, D., Bergquist, J., Tranvik, L., 2020. Selective Adsorption of Terrestrial Dissolved Organic Matter to Inorganic Surfaces Along a Boreal Inland Water Continuum. J. Geophys. Res. Biogeosciences 125.

Groeneveld, M., Tranvik, L.J., Natchimuthu, S., Koehler, B., 2016. Photochemical mineralisation in a boreal brown water lake: Considerable temporal variability and minor contribution to carbon dioxide production. Biogeosciences 13, 3931–3943.

Gruber, D.F., Simjouw, J.P., Seitzinger, S.P., Taghon, G.L., 2006. Dynamics and characterization of refractory dissolved organic matter produced by a pure bacterial culture in an experimental predator-prey system. Appl. Environ. Microbiol. 72, 4184–4191.

Gücker, B., Silva, R.C.S., Graeber, D., Monteiro, J.A.F., Boëchat, I.G., 2016. Urbanization and agriculture increase exports and differentially alter elemental stoichiometry of dissolved organic matter (DOM) from tropical catchments. Sci. Total Environ. 550, 785–792.

Gudasz, C., Bastviken, D., Premke, K., Steger, K., Tranvik, L.J., 2012. Constrained microbial processing of allochthonous organic carbon in boreal lake sediments. Limnol. Oceanogr. 57, 163–175.

Gudasz, C., Sobek, S., Bastviken, D., Koehler, B., Tranvik, L.J., 2015. Temperature sensitivity of organic carbon mineralization in contrasting lake sediments. J. Geophys. Res. 120, 1215–1225.

Guillemette, F., von Wachenfeldt, E., Kothawala, D.N., Bastviken, D., Tranvik, L.J., 2017. Preferential sequestration of terrestrial organic matter in boreal lake sediments. J. Geophys. Res. Biogeosciences 122, 863–874.

Guo, J. qiang, Liang, S. kang, Li, X. jiao, Li, W., Wang, Y. fei, Su, R. guo, 2018. Composition and bioavailability of dissolved organic matter in different water masses of the East China sea. Estuar. Coast. Shelf Sci. 212, 189–202.

Haitzer, M., Aiken, G.R., Ryan, J.N., 2003. Binding of mercury(II) to aquatic humic substances: Influence of pH and source of humic substances. Environ. Sci. Technol. 37, 2436–2441.

Hamilton, S.K., Sippel, S.J., Melack, J.M., 1995. Oxygen depletion and carbon dioxide and methane production in waters of the Pantanal wetland of Brazil. Biogeochemistry 30, 115–141.

Hansell, D., Carlson, C., Repeta, D., Schlitzer, R., 2009. Dissolved Organic Matter in the Ocean: A Controversy Stimulates New Insights. Oceanography 22, 202–211.

Harun, S., Baker, A., Bradley, C., Pinay, G., 2016. Spatial and seasonal variations in the composition of dissolved organic matter in a tropical catchment: the Lower Kinabatangan River, Sabah, Malaysia. Environ. Sci. Process. Impacts 18, 137–150.

Hawkes, J.A., Radoman, N., Bergquist, J., Wallin, M.B., Tranvik, L.J., Löfgren, S., 2018. Regional diversity of complex dissolved organic matter across forested hemiboreal headwater streams. Sci. Rep. 8, 16060.

Hedges, J. I., Cowie, G. L., Richey, J. E., Quay, P. D., Benner, R., Strom, M., & Forsberg, B. R., 1994. Origins and processing of organic matter in the Amazon River as indicated by carbohydrates and amino acids. Limnology and Oceanography 39(4), 743–761.

Hedges, J.I., Keil, R.G., Benner, R., 1997. What happens to terrestrial organic matter in the ocean? Org. Geochem. 27, 195–212.

Helms, J.R., Stubbins, A., Ritchie, J.D., Minor, E.C., Kieber, D.J., Mopper, K., 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnol. Oceanogr. 53, 955–969.

Hertkorn, N., Benner, R., Frommberger, M., Schmitt-Kopplin, P., Witt, M., Kaiser, K., Kettrup, A., Hedges, J., 2006. Characterization of a major refractory component of marine dissolved organic matter. Geochim. Cosmochim. Acta 70, 2990–3010.

Hertkorn, N., Frommberger, M., Witt, M., Koch, B.P., Schmitt-Kopplin, P., Perdue, E.M., 2008. Natural Organic Matter and the Event Horizon of Mass Spectrometry. Anal. Chem. 80, 8908–8919.

Hertkorn, N., Harir, M., Cawley, K.M., Schmitt-Kopplin, P., Jaffé, R., 2016. Molecular characterization of dissolved organic matter from subtropical wetlands: A comparative study through the analysis of optical properties, NMR and FTICR/MS. Biogeosciences 13, 2257–2277.

Hertkorn, N., Harir, M., Koch, B.P., Michalke, B., Grill, P., Schmitt-Kopplin, P., 2012. High field NMR spectroscopy and FTICR mass spectrometry: powerful discovery tools for the molecular level characterization of marine dissolved organic matter from the South Atlantic Ocean. Biogeosciences Discuss. 9, 745–833.

Hertkorn, N., Harir, M., Koch, B.P., Michalke, B., Schmitt-Kopplin, P., 2013. High-field NMR spectroscopy and FTICR mass spectrometry: Powerful discovery tools for the molecular level characterization of marine dissolved organic matter. Biogeosciences 10, 1583–1624.

Hertkorn, N., Perdue, E.M., Kettrup, A., 2004. A potentiometric and ^{113}Cd NMR study of cadmium complexation by natural organic matter at two different magnetic field strengths. Anal. Chem. 76, 6327–6341.

Hertkorn, N., Ruecker, C., Meringer, M., Gugisch, R., Frommberger, M., Perdue, E.M., Witt, M., Schmitt-Kopplin, P., 2007. High-precision frequency measurements: indispensable tools at the core of the molecular-level analysis of complex systems. Anal. Bioanal. Chem. 389, 1311–1327.

Hodgkins, S.B., Tfaily, M.M., McCalley, C.K., Logan, T. a, Crill, P.M., Saleska, S.R., Rich, V.I., Chanton, J.P., 2014. Changes in peat chemistry associated with permafrost thaw increase greenhouse gas production. PNAS 111, 5819–5824.

Hodgkins, S.B., Tfaily, M.M., Podgorski, D.C., McCalley, C.K., Saleska, S.R., Crill, P.M., Rich, V.I., Chanton, J.P., Cooper, W.T., 2016. Elemental composition and optical properties reveal changes in dissolved organic matter along a permafrost thaw chronosequence in a subarctic peatland. Geochim. Cosmochim. Acta 187, 123–140.

Huttunen, J.T., Hammar, T., Alm, J., Silvola, J., Martikainen, P.J., 2001. Greenhouse gases in non-oxygenated and artificially oxygenated eutrophied lakes during winter stratification. J. Environ. Qual. 30, 387–394.

Ide, J., Ohashi, M., Takahashi, K., Sugiyama, Y., Piirainen, S., Kortelainen, P., Fujitake, N., Yamase, K., Ohte, N., Moritani, M., Hara, M., Finér, L., 2017. Spatial variations in the molecular diversity of dissolved organic matter in water moving through a boreal forest in eastern Finland. Sci. Rep. 7, 42102.

IPCC, 2013. Climate Change 2013: The Physical Science Basis. Cambridge University Press, New York.

Jaffé, R., Yamashita, Y., Maie, N., Cooper, W.T., Dittmar, T., Dodds, W.K., Jones, J.B., Myoshi, T., Ortiz-Zayas, J.R., Podgorski, D.C., Watanabe, A., 2012. Dissolved Organic Matter in Headwater Streams: Compositional Variability across Climatic Regions of North America. Geochim. Cosmochim. Acta 94, 95–108.

Jandl, R., Sletten, R.S., 1999. Mineralization of Forest Soil Carbon: Interactions with Metals. J. Plant Nutr. Soil Sci. 162, 623–629.

Jiao, N., Herndl, G.J., Hansell, D.A., Benner, R., Kattner, G., Wilhelm, S.W., Kirchman, D.L., Weinbauer, M.G., Luo, T., Chen, F., Azam, F., 2011. The microbial carbon pump and the oceanic recalcitrant dissolved organic matter pool. Nat. Rev. Microbiol. 9, 555.

Jiao, N., Herndl, G.J., Hansell, D.A., Benner, R., Kattner, G., Wilhelm, S.W., Kirchman, D.L., Weinbauer, M.G., Luo, T., Chen, F., Azam, F., 2010. Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. Nat. Rev. Microbiol. 8, 593–599.

Johnston, S.E., Striegl, R.G., Bogard, M.J., Dornblaser, M.M., Butman, D.E., Kellerman, A.M., Wickland, K.P., Podgorski, D.C., Spencer, R.G.M., 2020. Hydrologic connectivity determines dissolved organic matter biogeochemistry in northern high-latitude lakes. Limnol. Oceanogr. 65, 1764–1780.

Jones, J.B., Mulholland, P.J. (Eds.), 2000. Streams and Ground Waters. Elsevier Inc., Academic Press.

Jonsson, A., Meili, M., Bergström, A.-K., Jansson, M., 2001. Whole-lake mineralization of allochthonous and autochthonous organic carbon in a large humic lake (Örträsket, N. Sweden). Limnol. Oceanogr. 46, 1691–1700.

Junk, W., Bayley, P.B., Sparks, R.E., 1989. The flood pulse concept in river-floodplain-systems. Can. J. Fish. Aquat. Sci. 106, 110–127.

Kaiser, K., Kalbitz, K., 2012. Cycling downwards - dissolved organic matter in soils. Soil Biol. Biochem. 52, 29–32.

Kamjunke, N., Hertkorn, N., Harir, M., Schmitt-Kopplin, P., Griebler, C., Brauns, M., von Tümpling, W., Weitere, M., Herzsprung, P., 2019. Molecular change of dissolved organic matter and patterns of bacterial activity in a stream along a land-use gradient. Water Res. 164, 114919.

Kamjunke, N., von Tümpling, W., Hertkorn, N., Harir, M., Schmitt-Kopplin, P., Norf, H., Weitere, M., Herzsprung, P., 2017. A new approach for evaluating transformations of dissolved organic matter (DOM) via high-resolution mass spectrometry and relating it to bacterial activity. Water Res. 123, 513–523.

Kaplan, L.A., Newbold, J.D., 2003. The Role of Monomers in Stream Ecosystem Metabolism, in: Findlay, S.E.G., Sinsabaugh, R.L. (Eds.), Aquatic Ecosystems - Interactivity of Dissolved Organic Matter. Academic Press, San Diego, pp. 97–120.

Keil, R., Kirchman, D., 1991. Contribution of dissolved free amino acids and ammonium to the nitrogen requirements of heterotrophic bacterioplankton. Mar. Ecol. Prog. Ser. 73, 1–10.

Keil, R.G., Mayer, L.M., 2014. Mineral Matrices and Organic Matter, in: Treatise on Geochemistry. Elsevier, pp. 337–359.

Kellerman, A.M., Dittmar, T., Kothawala, D.N., Tranvik, L.J., 2014. Chemodiversity of dissolved organic matter in lakes driven by climate and hydrology. Nat. Commun. 5, 3804.

Kellerman, A.M., Guillemette, F., Podgorski, D.C., Aiken, G.R., Butler, K.D., Spencer, R.G.M., 2018. Unifying Concepts Linking Dissolved Organic Matter Composition to Persistence in Aquatic Ecosystems. Environ. Sci. Technol. 52, 2538–2548.

Kellerman, A.M., Kothawala, D.N., Dittmar, T., Tranvik, L.J., 2015. Persistence of dissolved organic matter in lakes related to its molecular characteristics. Nat. Geosci. 8, 454-457.

Kerndorff, H., Schnitzer, M., 1980. Sorption of metals on humic acid. Geochim. Cosmochim. Acta 44, 1701–1708.

Khokhotva, O., Waara, S., 2010. The influence of dissolved organic carbon on sorption of heavy metals on urea-treated pine bark. J. Hazard. Mater. 173, 689–696.

Killops, S.D., Killops, V.J., 2005. Introduction to Organic Geochemistry, Second. ed. Blackwell Publishing, Oxford, UK.

Kim, S., Kaplan, L.A., Hatcher, P.G., 2006. Biodegradable dissolved organic matter in a temperate and a tropical stream determined from ultra-high resolution mass spectrometry. Limnol. Oceanogr. 51, 1054–1063.

Knicker, H., Skjemstad, J.O., 2000. Nature of organic carbon and nitrogen in physically protected organic matter of some Australian soils as revealed by solid-state ^{13}C and ^{15}N NMR spectroscopy. Soil Res. 38, 113.

Koch, B.P., Witt, M., Engbrodt, R., Dittmar, T., Kattner, G., 2005. Molecular formulae of marine and terrigenous dissolved organic matter detected by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Geochim. Cosmochim. Acta 69, 3299–3308.

Koehler, B., Von Wachenfeldt, E., Kothawala, D., Tranvik, L.J., 2012. Reactivity continuum of dissolved organic carbon decomposition in lake water. J. Geophys. Res. Biogeosciences 117, 1–14.

Kögel-Knabner, I., Amelung, W., 2021. Soil organic matter in major pedogenic soil groups. Geoderma 384, 114785.

Köhler, S.J., Kothawala, D., Futter, M.N., Liungman, O., Tranvik, L.J., 2013. In-Lake Processes Offset Increased Terrestrial Inputs of Dissolved Organic Carbon and Color to Lakes. PLoS One 8, 1–12.

Kördel, W., Dassenakis, M., Lintelmann, J., Padberg, S., 1997. The Importance of Natural Organic Material for Environmental Processes in Waters and Soils. Pure Appl. Chem. 69, 1571–1600.

Kothawala, D.N., Ji, X., Laudon, H., Ågren, A.M., Futter, M.N., Köhler, S.J., Tranvik, L.J., 2015. The relative influence of land cover, hydrology, and in-stream processing on the composition of dissolved organic matter in boreal streams. Journal of Geophysical Research: Biogeosciences 120, 1491–1505.

Kothawala, D.N., Stedmon, C.A., Müller, R.A., Weyhenmeyer, G.A., Köhler, S.J., Tranvik, L.J., 2014. Controls of dissolved organic matter quality: Evidence from a large-scale boreal lake survey. Glob. Chang. Biol. 20, 1101–1114.

Kothawala, D.N., von Wachenfeldt, E., Koehler, B., Tranvik, L.J., 2012. Selective loss and preservation of lake water dissolved organic matter fluorescence during long-term dark incubations. Sci. Total Environ. 433, 238–246.

Kujawinski, E.B., Longnecker, K., Blough, N.V., Del Vecchio, R., Finlay, L., Kitner, J.B., Giovannoni, S.J., 2009. Identification of possible source markers in marine dissolved organic matter using ultrahigh resolution mass spectrometry. Geochim. Cosmochim. Acta 73, 4384–4399.

Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter. Soil Biol. Biochem. 42, 1363–1371.

Lambert, T., Bouillon, S., Darchambeau, F., Massicotte, P., Borges, A.V., 2016. Shift in the chemical composition of dissolved organic matter in the Congo River network. Biogeosciences 13, 5405–5420.

Lambert, T., Bouillon, S., Darchambeau, F., Morana, C., Roland, F.A.E., Descy, J.-P., Borges, A. V., 2017. Effects of human land use on the terrestrial and aquatic sources of fluvial organic matter in a temperate river basin (The Meuse River, Belgium). Biogeochemistry 136, 191–211.

Landa, M., Cottrell, M.T., Kirchman, D.L., Blain, S., Obernosterer, I., 2013. Changes in bacterial diversity in response to dissolved organic matter supply in a continuous culture experiment. Aquat. Microb. Ecol. 69, 157–168.

Latrubesse, E.M., Arima, E.Y., Dunne, T., Park, E., Baker, V.R., d'Horta, F.M., Wight, C., Wittmann, F., Zuanon, J., Baker, P.A., Ribas, C.C., Norgaard, R.B., Filizola, N., Ansar, A., Flyvbjerg, B., Stevaux, J.C., 2017. Damming the rivers of the Amazon basin. Nature 546, 363–369.

Le Quéré, C., Andrew, R.M., Friedlingstein, P., Sitch, S., Hauck, J., Pongratz, J., Pickers, P.A., Korsbakken, J.I., Peters, G.P., Canadell, J.G., Arneth, A., Arora, V.K., Barbero, L., Bastos, A., Bopp, L., Chevallier, F., Chini, L.P., Ciais, P., Doney, S.C., Gkritzalis, T., Goll, D.S., Harris, I., Haverd, V., Hoffman, F.M., Hoppema, M., Houghton, R.A., Hurtt, G., Ilyina, T., Jain, A.K., Johannessen, T., Jones, C.D., Kato, E., Keeling, R.F.,

Goldewijk, K.K., Landschützer, P., Lefèvre, N., Lienert, S., Liu, Z., Lombardozzi, D., Metzl, N., Munro, D.R., Nabel, J.E.M.S., Nakaoka, S., Neill, C., Olsen, A., Ono, T., Patra, P., Peregon, A., Peters, W., Peylin, P., Pfeil, B., Pierrot, D., Poulter, B., Rehder, G., Resplandy, L., Robertson, E., Rocher, M., Rödenbeck, C., Schuster, U., Schwinger, J., Séférian, R., Skjelvan, I., Steinhoff, T., Sutton, A., Tans, P.P., Tian, H., Tilbrook, B., Tubiello, F.N., van der Laan-Luijkx, I.T., van der Werf, G.R., Viovy, N., Walker, A.P., Wiltshire, A.J., Wright, R., Zaehle, S., Zheng, B., 2018. Global Carbon Budget 2018. Earth Syst. Sci. Data 10, 2141–2194.

Le Quéré, C., Moriarty, R., Andrew, R.M., Peters, G.P., Ciais, P., Friedlingstein, P., Jones, S.D., Sitch, S., Tans, P., Arneth, A., Boden, T.A., Bopp, L., Bozec, Y., Canadell, J.G., Chini, L.P., Chevallier, F., Cosca, C.E., Harris, I., Hoppema, M., Houghton, R.A., House, J.I., Jain, A.K., Johannessen, T., Kato, E., Keeling, R.F., Kitidis, V., Klein Goldewijk, K., Koven, C., Landa, C.S., Landschützer, P., Lenton, A., Lima, I.D., Marland, G., Mathis, J.T., Metzl, N., Nojiri, Y., Olsen, A., Ono, T., Peng, S., Peters, W., Pfeil, B., Poulter, B., Raupach, M.R., Regnier, P., Rödenbeck, C., Saito, S., Salisbury, J.E., Schuster, U., Schwinger, J., Séférian, R., Segschneider, J., Steinhoff, T., Stocker, B.D., Sutton, A.J., Takahashi, T., Tilbrook, B., van der Werf, G.R., Viovy, N., Wang, Y.-P., Wanninkhof, R., Wiltshire, A., Zeng, N., 2015. Global carbon budget 2014. Earth Syst. Sci. Data 7, 47–85.

Le Quéré, C., Peters, G.P., Andres, R.J., Andrew, R.M., Boden, T.A., Ciais, P., Friedlingstein, P., Houghton, R.A., Marland, G., Moriarty, R., Sitch, S., Tans, P., Arneth, A., Arvanitis, A., Bakker, D.C.E., Bopp, L., Canadell, J.G., Chini, L.P., Doney, S.C., Harper, A., Harris, I., House, J.I., Jain, A.K., Jones, S.D., Kato, E., Keeling, R.F., Klein Goldewijk, K., Körtzinger, A., Koven, C., Lefèvre, N., Maignan, F., Omar, A., Ono, T., Park, G.H., Pfeil, B., Poulter, B., Raupach, M.R., Regnier, P., Rödenbeck, C., Saito, S., Schwinger, J., Segschneider, J., Stocker, B.D., Takahashi, T., Tilbrook, B., Van Heuven, S., Viovy, N., Wanninkhof, R., Wiltshire, A., Zaehle, S., 2014. Global carbon budget 2013. Earth Syst. Sci. Data 6, 235–263.

Lechtenfeld, O.J., Kattner, G., Flerus, R., McCallister, S.L., Schmitt-Kopplin, P., Koch, B.P., 2014. Molecular transformation and degradation of refractory dissolved organic matter in the Atlantic and Southern Ocean. Geochim. Cosmochim. Acta 126, 321–337.

Leenheer, J.A., Croué, J.-P., 2003. Peer Reviewed: Characterizing Aquatic Dissolved Organic Matter. Environ. Sci. Technol. 37, 18A-26A.

Lehmann, J., Hansel, C.M., Kaiser, C., Kleber, M., Maher, K., Manzoni, S., Nunan, N., Reichstein, M., Schimel, J.P., Torn, M.S., Wieder, W.R., Kögel-Knabner, I., 2020. Persistence of soil organic carbon caused by functional complexity. Nat. Geosci. 13, 529–534.

Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60–68.

Liikanen, A.N.U., Huttunen, J.T., Murtoniemi, T., 2003. Spatial and seasonal variation in greenhouse gas and nutrient dynamics and their interactions in the sediments of a boreal eutrophic lake. Biogeochemistry 65, 83–103.

Liikanen, A.N.U., Murtoniemi, T., Tanskanen, H., 2002. Effects of temperature and oxygen availability on greenhouse gas and nutrient dynamics in sediment of a eutrophic mid-boreal lake. Biogeochemistry 59, 269–286.

Lu, C.-J., Benner, R., Fichot, C.G., Fukuda, H., Yamashita, Y., Ogawa, H., 2016. Sources and Transformations of Dissolved Lignin Phenols and Chromophoric Dissolved Organic Matter in Otsuchi Bay, Japan. Front. Mar. Sci. 3, 1–12.

Maeck, A., Delsontro, T., McGinnis, D.F., Fischer, H., Flury, S., Schmidt, M., Fietzek, P., Lorke, A., 2013. Sediment trapping by dams creates methane emission hot spots. Environ. Sci. Technol. 47, 8130–8137.

Mahmoudi, N., Beaupré, S.R., Steen, A.D., Pearson, A., 2017. Sequential bioavailability of sedimentary organic matter to heterotrophic bacteria. Environ. Microbiol. 19, 2629–2644.

Marschner, B., Kalbitz, K., 2003. Controls of bioavailability and biodegradability of dissolved organic matter in soils. Geoderma 113(3–4), 211–235.

Massicotte, P., Asmala, E., Stedmon, C., Markager, S., 2017. Global distribution of dissolved organic matter along the aquatic continuum: across rivers, lakes and oceans. Sci. Total Environ. 609, 180–191.

Mayorga, E., Aufdenkampe, A.K., Masiello, C.A., Krusche, A. V, Hedges, J.I., Quay, P.D., Richey, J.E., Brown, T.A., 2005. Young organic matter as a source of carbon dioxide outgassing from Amazonian rivers. Nature 436, 538–541.

McKee, B.A., Aller, R.C., Allison, M.A., Bianchi, T.S., Kineke, G.C., 2004. Transport and transformation of dissolved and particulate materials on continental margins influenced by major rivers: Benthic boundary layer and seabed processes. Cont. Shelf Res. 24, 899–926.

Medeiros, P.M., Seidel, M., Ward, N.D., Carpenter, E.J., Gomes, H.R., Niggemann, J., Krusche, A. V., Richey, J.E., Yager, P.L., Dittmar, T., 2015. Fate of the Amazon River dissolved organic matter in the tropical Atlantic Ocean. Global Biogeochem. Cycles, 667–690.

Merck, M.F., Neilson, B.T., Cory, R.M., Kling, G.W., 2012. Variability of in-stream and riparian storage in a beaded arctic stream. Hydrol. Process. 26, 2938–2950.

Milliman, J.D., Syvitski, J.P.M., 1992. Geomorphic/Tectonic Control of Sediment Discharge to the Ocean: The Importance of Small Mountainous Rivers. J. Geol. 100, 525–544.

Moore, T.R., 2003. Dissolved organic carbon in a northern boreal landscape. Global Biogeochem. Cycles 17(4), 1109.

Moore, W.S., 2010. The Effect of Submarine Groundwater Discharge on the Ocean. Ann. Rev. Mar. Sci. 2, 59–88.

Moore, W.S., Sarmiento, J.L., Key, R.M., 2008. Submarine groundwater discharge revealed by ^{228}Ra distribution in the upper Atlantic Ocean. Nat. Geosci. 1, 309–311.

Mopper, K., Kieber, D.J., Stubbins, A., 2014. Chapter 8 – Marine Photochemistry of Organic Matter: Processes and Impacts. In Biogeochemistry of Marine Dissolved Organic Matter (Second Edition). Elsevier Inc., Academic Press, 389–450.

Moran, M.A., Covert, J.S., 2003. Photochemically Mediated Linkages between Dissolved Organic Matter and Bacterioplankton, in: Findlay, S.E.G., Sinsabaugh, R.L. (Eds.), Aquatic Ecosystems - Interactivity of Dissolved Organic Matter. Academic Press, San Diego, pp. 244–262.

Mostovaya, A., Koehler, B., Guillemette, F., Brunberg, A.K., Tranvik, L.J., 2016. Effects of compositional changes on reactivity continuum and decomposition kinetics of lake dissolved organic matter. J. Geophys. Res. Biogeosciences 121, 1733–1746.

Murphy, K.R., Timko, S.A., Gonsior, M., Powers, L.C., Wünsch, U.J., Stedmon, C.A., 2018. Photochemistry Illuminates Ubiquitous Organic Matter Fluorescence Spectra. Environ. Sci. Technol. 52, 11243–11250.

Nelson, C.E., Goldberg, S.J., Wegley K., L., Haas, A.F., Smith, J.E., Rohwer, F., Carlson, C.A., 2013. Coral and macroalgal exudates vary in neutral sugar composition and differentially enrich reef bacterioplankton lineages. ISME J. 7, 962–979.

Neu, V., Ward, N.D., Krusche, A. V., Neill, C., 2016. Dissolved Organic and Inorganic Carbon Flow Paths in an Amazonian Transitional Forest. Front. Mar. Sci. 3, 1–15.

Nozhevnikova, A.N., Nekrasova, V., Ammann, A., Zehnder, A.J.B., Wehrli, B., Holliger, C., 2007. Influence of temperature and high acetate concentrations on methanogenesis in lake sediment slurries. FEMS Microbiol. Ecol. 62, 336–44.

Nürnberg, G.K., Shaw, M., 1998. Productivity of clear and humic lakes: Nutrients, phytoplankton, bacteria. Hydrobiologia 382, 97–112.

Ogawa, H., Amagai, Y., Koike, I., Kaiser, K., Benner, R., 2001. Production of Refractory Dissolved Organic Matter by Bacteria. Science 292, 917–920.

Ohno, T., He, Z., Sleighter, R.L., Honeycutt, C.W., Hatcher, P.G., 2010. Ultrahigh resolution mass spectrometry and indicator species analysis to identify marker components of soil- and plant biomass-derived organic matter fractions. Environ. Sci. Technol. 44, 8594–8600.

Olk, D.C., Bloom, P.R., Perdue, E.M., McKnight, D.M., Chen, Y., Farenhorst, A., Senesi, N., Chin, Y.-P., Schmitt-Kopplin, P., Hertkorn, N., Harir, M., 2019. Environmental and Agricultural Relevance of Humic Fractions Extracted by Alkali from Soils and Natural Waters. J. Environ. Qual. 48, 217–232.

Panneer Selvam, B., Lapierre, J.-F., Soares, A.R.A., Bastviken, D., Karlsson, J., Berggren, M., 2019. Photo-reactivity of dissolved organic carbon in the freshwater continuum. Aquat. Sci. 81, 57.

Pomeroy, L., LeB. Williams, P.J., Azam, F., Hobbie, J.E., 2007. The Microbial Loop. Oceanography 20(2), 28–33.

Pomeroy, L.R., 1974. Food Changing Paradigm. Bioscience 24, 499–504.

Porcal, P., Dillon, P.J., Molot, L.A., 2015. Temperature dependence of photodegradation of dissolved organic matter to dissolved inorganic carbon and particulate organic carbon. PLoS One 10, 1–15.

Powers, L.C., Luek, J.L., Schmitt-kopplin, P., Barbara, J., Magen, C., Cooper, L.W., Gonsior, M., 2018. Seasonal changes in dissolved organic matter composition in Delaware Bay, USA in March and August 2014. Org. Geochem.

Preston, C.M., Schmidt, M.W.I., 2010. Black (pyrogenic) carbon in boreal forests: a synthesis of current knowledge and uncertainties. Biogeosciences Discuss. 3, 211–271.

Qualls, R.G., Haines, B.L., 1992. Biodegradability of dissolved organic matter in forest throughfall, soil solution and stream water. Soil Sci. Soc. Am. J. 56, 578–586.

Qualls, R.G., Haines, B.L., 1991. Geochemistry of Dissolved Organic Nutrients in Water Percolating through a Forest Ecosystem. Soil Sci. Soc. Am. J. 55, 1112–1123.

Qualls, R.G., Haines, B.L., Swank, W.T., 1991. Fluxes of Dissolved Organic Nutrients and Humic Substances in a Deciduous Forest. Ecology 72, 254–266.

Raymond, P.A., Spencer, R.G.M., 2015. Chapter 11 – Riverine DOM. In Biogeochemistry of Marine Dissolved Organic Matter (Second Edition). Elsevier Inc., Academic Press, 509–533.

Reader, H.E., Stedmon, C.A., Nielsen, N.J., Kritzberg, E.S., 2015. Mass and UV-visible spectral fingerprints of dissolved organic matter: sources and reactivity. Front. Mar. Sci. 2, 1–10.

Remington, S., Krusche, A., Richey, J., 2011. Effects of DOM photochemistry on bacterial metabolism and CO_2 evasion during falling water in a humic and a whitewater river in the Brazilian Amazon. Biogeochemistry 105, 185–200.

Repeta, D.J., 2015. Chapter 2 – Chemical Characterization and Cycling of Dissolved Organic Matter. In Biogeochemistry of Marine Dissolved Organic Matter (Second Edition). Elsevier Inc., Academic Press, 21–63.

Richey, J.E., Melack, J.M., Aufdenkampe, A.K., Ballester, V.M., Hess, L.L., 2002. Outgassing from Amazonian rivers and wetlands as a large tropical source of atmospheric CO_2. Nature 416, 617–620.

Ridgwell, A., Arndt, S., 2014. Why Dissolved Organics Matter: DOC in Ancient Oceans and Past Climate Change, Second Edi. ed, Biogeochemistry of Marine Dissolved Organic Matter: Second Edition. Elsevier Inc.

Riedel, T., Zak, D., Biester, H., Dittmar, T., 2013. Iron traps terrestrially derived dissolved organic matter at redox interfaces. PNAS 110, 10101–10105.

Riedel, T., Zark, M., Vähätalo, A. V., Niggemann, J., Spencer, R.G.M., Hernes, P.J., Dittmar, T., 2016. Molecular Signatures of Biogeochemical Transformations in Dissolved Organic Matter from Ten World Rivers. Front. Earth Sci. 4.

Roden, E.E., Wetzel, R.G., 2003. Competition between Fe(III)-reducing and methanogenic bacteria for acetate in iron-rich freshwater sediments. Microb. Ecol. 45, 252–258.

Roth, V.-N., Dittmar, T., Gaupp, R., Gleixner, G., 2015. The molecular composition of dissolved organic matter in forest soils as a function of pH and temperature. PLoS One 10, e0119188.

Roth, V.-N., Dittmar, T., Gaupp, R., Gleixner, G., 2014. Ecosystem-Specific Composition of Dissolved Organic Matter. Vadose Zo. J. 13, vzj2013.09.0162.

Roth, V.-N., Lange, M., Simon, C., Hertkorn, N., Bucher, S., Goodall, T., Griffiths, R.I., Mellado-Vázquez, P.G., Mommer, L., Oram, N.J., Weigelt, A., Dittmar, T., Gleixner, G., 2019. Persistence of dissolved organic matter explained by molecular changes during its passage through soil. Nat. Geosci. 12, 755–761.

Saunois, M., Bousquet, P., Poulter, B., Peregon, A., Ciais, P., Canadell, J.G., Dlugokencky, E.J., Etiope, G., Bastviken, D., Houweling, S., Janssens-Maenhout, G., Tubiello, F.N., Castaldi, S., Jackson, R.B., Alexe, M., Arora, V.K., Beerling, D.J., Bergamaschi, P., Blake, D.R., Brailsford, G., Brovkin, V., Bruhwiler, L., Crevoisier, C., Crill, P., Covey, K., Curry, C., Frankenberg, C., Gedney, N., Höglund-Isaksson, L., Ishizawa, M., Ito, A., Joos, F., Kim, H.-S., Kleinen, T., Krummel, P., Lamarque, J.-F., Langenfelds, R., Locatelli, R., Machida, T., Maksyutov, S., McDonald, K.C., Marshall, J., Melton, J.R., Morino, I., Naik, V., O&apos;Doherty, S., Parmentier, F.-J.W., Patra, P.K., Peng, C., Peng, S., Peters, G.P., Pison, I., Prigent, C., Prinn, R., Ramonet, M., Riley, W.J., Saito, M., Santini, M., Schroeder, R., Simpson, I.J., Spahni, R., Steele, P., Takizawa, A., Thornton, B.F., Tian, H., Tohjima, Y., Viovy, N., Voulgarakis, A., van Weele, M., van der Werf, G.R., Weiss, R., Wiedinmyer, C., Wilton, D.J., Wiltshire, A., Worthy, D., Wunch, D., Xu, X., Yoshida, Y., Zhang, B., Zhang, Z., Zhu, Q., 2016. The global methane budget 2000–2012. Earth Syst. Sci. Data 8, 697–751.

Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle. Biogeochemistry 48, 7–20.

Schlesinger, W.H., Melack, J.M., 1981. Transport of organic carbon in the world's rivers. Tellus 33, 172–187.

Schmidt, F., Koch, B.P., Goldhammer, T., Elvert, M., Witt, M., Lin, Y.S., Wendt, J., Zabel, M., Heuer, V.B., Hinrichs, K.U., 2017. Unraveling signatures of biogeochemical processes and the depositional setting in the molecular composition of pore water DOM across different marine environments. Geochim. Cosmochim. Acta 207, 57–80.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56.

Schmitt-Kopplin, P., Gonsior, M., Hertkorn, N., Ishida, K.P., Cooper, W.J., Jeung, M.K., Rosso, D., Song, W., Zwartjes, M., Tseng, L.Y., 2011. Molecular characterization of effluent organic matter identified by ultrahigh resolution mass spectrometry. Water Res. 45, 2943–2953.

Schmitt-Kopplin, P., Harir, M., Tziotis, D., Gabelica, Z., Hertkorn, N., 2012. Ultrahigh resolution Fourier Transform ion cyclotron resonance mass spectrometry for the analysis of natural organic matter from various environmental systems. Comprehensive Environmental Mass Spectrometry, 443–459.

Schmitt-Kopplin, P., Hemmler, D., Moritz, F., Gougeon, R.D., Lucio, M., Meringer, M., Müller, C., Harir, M., Hertkorn, N., 2019. Systems chemical analytics: Introduction to the challenges of chemical complexity analysis. Faraday Discuss. 218, 9–28.

Schmitt-Kopplin, P., Hertkorn, N., 2007. Ultrahigh resolution mass spectrometry. Anal. Bioanal. Chem. 389, 1309–1310.

Schwarz, J.I.K., Lueders, T., Eckert, W., Conrad, R., 2007. Identification of acetate-utilizing Bacteria and Archaea in methanogenic profundal sediments of Lake Kinneret (Israel) by stable isotope probing of rRNA. Environ. Microbiol. 9, 223–37.

Seidel, M., Beck, M., Riedel, T., Waska, H., Suryaputra, I.G.N. a, Schnetger, B., Niggemann, J., Simon, M., Dittmar, T., 2014. Biogeochemistry of dissolved organic matter in an anoxic intertidal creek bank. Geochim. Cosmochim. Acta 140, 418–434.

Seidel, M., Dittmar, T., Ward, N.D., Krusche, A. V., Richey, J.E., Yager, P.L., Medeiros, P.M., 2016. Seasonal and spatial variability of dissolved organic matter composition in the lower Amazon River. Biogeochemistry 131, 281–302.

Seidel, M., Yager, P.L., Ward, N.D., Carpenter, E.J., Gomes, H.R., Krusche, A. V., Richey, J.E., Dittmar, T., Medeiros, P.M., 2015. Molecular-level changes of dissolved organic matter along the Amazon River-to-ocean continuum. Mar. Chem. 177, 218–231.

Senesi, N., Loffredo, E., 2005. Soil Humic Substances, in: Steinbüchel, A., Hofrichter, M. (Eds.), Biopolymers Online. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp. 247–299.

Shen, Y., Chapelle, F.H., Strom, E.W., Benner, R., 2015. Origins and bioavailability of dissolved organic matter in groundwater. Biogeochemistry 122, 61–78.

Sholkovitz, E.R., 1976. Flocculation of dissolved organic and inorganic matter during the mixing of river water and seawater. Geochim. Cosmochim. Acta 40, 831–845.

Singh, S., Inamdar, S., Mitchell, M., McHale, P., 2014. Seasonal pattern of dissolved organic matter (DOM) in watershed sources: Influence of hydrologic flow paths and autumn leaf fall. Biogeochemistry 118, 321–337.

Sipler, R.E., Bronk, D.A., 2014. Dynamics of Dissolved Organic Nitrogen, Second Edi. ed, Biogeochemistry of Marine Dissolved Organic Matter: Second Edition. Elsevier Inc.

Skoog, A., Biddanda, B., Benner, R., 1999. Bacterial utilization of dissolved glucose in the upper water column of the Gulf of Mexico. Limnol. Oceanogr. 44, 1625–1633.

Sleighter, R.L., Hatcher, P.G., 2008. Molecular characterization of dissolved organic matter (DOM) along a river to ocean transect of the lower Chesapeake Bay by ultrahigh resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Mar. Chem. 110, 140–152.

Sobek, S., Delsontro, T., Wongfun, N., Wehrli, B., 2012. Extreme organic carbon burial fuels intense methane bubbling in a temperate reservoir. Geophys. Res. Lett. 39, 2–5.

Spencer, R.G.M., Stubbins, A., Hernes, P.J., Baker, A., Mopper, K., Aufdenkampe, A.K., Dyda, R.Y., Mwamba, V.L., Mangangu, A.M., Wabakanghanzi, J.N., Six, J., 2009. Photochemical degradation of dissolved organic matter and dissolved lignin phenols from the Congo River. J. Geophys. Res. Biogeosciences 114, 1–12.

Stedmon, C.A., Nelson, N.B., 2014. Chapter 10 – The Optical Properties of DOM in the Ocean. In Biogeochemistry of Marine Dissolved Organic Matter (Second Edition). Elsevier Inc., Academic Press, 481-508.

Stenson, A.C., 2009. Fourier transform ion cyclotron resonance mass spectral characterization of metal-humic binding. Rapid Commun. mass Spectrom. RCM 23, 465–476.

Stubbins, A., Spencer, R.G.M., Chen, H., Hatcher, P.G., Mopper, K., Hernes, P.J., Mwamba, V.L., Mangangu, A.M., Wabakanghanzi, J.N., Six, J., 2010. Illuminated darkness: Molecular signatures of Congo River dissolved organic matter and its photochemical alteration as revealed by ultrahigh precision mass spectrometry. Limnol. Oceanogr. 55, 1467–1477.

Thomson, J., Parkinson, A., Roddick, F.A., 2004. Depolymerization of Chromophoric Natural Organic Matter. Environ. Sci. Technol. 38, 3360–3369.

Tipping, E., Chamberlain, P.M., Fröberg, M., Hanson, P.J., Jardine, P.M., 2012. Simulation of carbon cycling, including dissolved organic carbon transport, in forest soil locally enriched with [14]C. Biogeochemistry 108, 91–107.

Tranvik, L.J., 1992. Allochthonous dissolved organic matter as an energy source for pelagic bacteria and the concept of the microbial loop. Hydrobiologia 229, 107–114.

Tranvik, L.J., Downing, J.A., Cotner, J.B., Loiselle, S.A., Striegl, R.G., Ballatore, T.J., Dillon, P., Finlay, K., Fortino, K., Knoll, L.B., Kortelainen, P.L., Kutser, T., Larsen, S., Laurion, I., Leech, D.M., McCallister, S.L., McKnight, D.M., Melack, J.M., Overholt, E., Porter, J.A., Prairie, Y., Renwick, W.H., Roland, F., Sherman, B.S., Schindler, D.W., Sobek, S., Tremblay, A., Vanni, M.J., Verschoor, A.M., von Wachenfeldt, E., Weyhenmeyer, G.A., 2009. Lakes and reservoirs as regulators of carbon cycling and climate. Limnol. Oceanogr. 54, 2298–2314.

Tranvik, L.J., Sherr, E.B., Sherr, B.F., 1993. Uptake and utilization of "colloidal DOM'' by heterotrophic flagellates in seawater." Mar. Ecol. Prog. Ser. 92, 301–309.

Tremblay, L.B., Dittmar, T., Marshall, A.G., Cooper, W.J., Cooper, W.T., 2007. Molecular characterization of dissolved organic matter in a North Brazilian mangrove porewater and mangrove-fringed estuaries by ultrahigh resolution Fourier Transform-Ion Cyclotron Resonance mass spectrometry and excitation/emission spectroscopy. Mar. Chem. 105, 15–29.

Utsumi, M., Nojiri, Y., Nakamura, T., Nozawa, T., Otsuki, A., Takamura, N., Watanabe, M., Seki, H., 1998. Dynamics of dissolved methane and methane oxidation in dimictic Lake Nojiri during winter. Limnol. Oceanogr. 43, 10–17.

Verpoorter, C., Kutser, T., Seekell, D.A., Tranvik, L.J., 2014. A global inventory of lakes based on high-resolution satellite imagery. Geophys. Res. Lett. 41, 6396–6402.

von Wachenfeldt, E., Bastviken, D., Tranvik, L.J., 2009. Microbially induced flocculation of allochthonous dissolved organic carbon in lakes. Limnol. Oceanogr. 54, 1811–1818.

von Wachenfeldt, E., Sobek, S., Bastviken, D., Tranvik, L.J., 2008. Linking allochthonous dissolved organic matter and boreal lake sediment carbon sequestration: The role of light-mediated flocculation. Limnol. Oceanogr. 53, 2416–2426.

von Wachenfeldt, E., Tranvik, L.J., 2008. Sedimentation in Boreal Lakes—The Role of Flocculation of Allochthonous Dissolved Organic Matter in the Water Column. Ecosystems 11, 803–814.

Wang, Y., Zhang, D., Shen, Z., Feng, C., Chen, J., 2013. Revealing Sources and Distribution Changes of Dissolved Organic Matter (DOM) in Pore Water of Sediment from the Yangtze Estuary. PLoS One 8, e76633.

Ward, N.D., Bianchi, T.S., Medeiros, P.M., Seidel, M., Richey, J.E., Keil, R.G., Sawakuchi, H.O., 2017. Where Carbon Goes When Water Flows: Carbon Cycling across the Aquatic Continuum. Front. Mar. Sci. 4, 1–27.

Ward, N.D., Keil, R.G., Medeiros, P.M., Brito, D.C., Cunha, A.C., Dittmar, T., Yager, P.L., Krusche, A.V., Richey, J.E., 2013. Degradation of terrestrially derived macromolecules in the Amazon River. Nat. Geosci. 6, 530–533.

Wetzel, R.G., 2003. Dissolved Organic Carbon: Detrital Energetics, Metabolic Regulators, and Drivers of Ecosystem Stability of Aquatic Ecosystems, in: Findlay, S.E.G., Sinsabaugh, R.L. (Eds.), Aquatic Ecosystems - Interactivity of Dissolved Organic Matter. Academic Press, San Diego, pp. 455–478.

Wetzel, R.G., 1992. Gradient-dominated ecosystems: sources and regulatory functions of dissolved organic matter in freshwater ecosystems. Hydrobiologia 229, 181–198.

Wickland, K.P., Neff, J.C., Aiken, G.R., 2007. Dissolved Organic Carbon in Alaskan Boreal Forest: Sources, Chemical Characteristics, and Biodegradability. Ecosystems 10, 1323–1340.

Wilkinson, B.H., McElroy, B.J., 2007. The impact of humans on continental erosion and sedimentation. Geol. Soc. Am. Bull. 119, 140–156.

Winch, S., Ridal, J., Lean, D., 2002. Increased metal bioavailability following alteration of freshwater dissolved organic carbon by ultraviolet B radiation exposure. Environ. Toxicol. 17, 267–274.

Wong, J.W.C., Li, K.L., Zhou, L.X., Selvam, A., 2007. The sorption of Cd and Zn by different soils in the presence of dissolved organic matter from sludge. Geoderma 137, 310–317.

Xu, H., Guo, L., Jiang, H., 2016. Depth-dependent variations of sedimentary dissolved organic matter composition in a eutrophic lake: Implications for lake restoration. Chemosphere 145, 551–559.

Xu, H., Yan, M., Li, W., Jiang, H., Guo, L., 2018. Dissolved organic matter binding with Pb(II) as characterized by differential spectra and 2D UV–FTIR heterospectral correlation analysis. Water Res. 144, 435–443.

Yang, Y., Liang, L., Wang, D., 2008. Effect of dissolved organic matter on adsorption and desorption of mercury by soils. J. Environ. Sci. 20, 1097–1102.

Zhang, F., Harir, M., Moritz, F., Zhang, J., Witting, M., Wu, Y., Schmitt-Kopplin, P., Fekete, A., Gaspar, A., Hertkorn, N., 2014. Molecular and structural characterization of dissolved organic matter during and post cyanobacterial bloom in Taihu by combination of NMR spectroscopy and FTICR mass spectrometry. Water Res. 57, 280–294.

Zhao, Z., Gonsior, M., Luek, J., Timko, S., Ianiri, H., Hertkorn, N., Schmitt-Kopplin, P., Fang, X., Zeng, Q., Jiao, N., Chen, F., 2017. Picocyanobacteria and deep-ocean fluorescent dissolved organic matter share similar optical properties. Nat. Commun. 8, 1–10.

Ziegelgruber, K.L., Zeng, T., Arnold, W.A., Chin, Y.P., 2013. Sources and composition of sediment pore-water dissolved organic matter in prairie pothole lakes. Limnol. Oceanogr. 58, 1136–1146.

Zimmerman, A.R., Canuel, E.A., 2000. A geochemical record of eutrophication and anoxia in Chesapeake Bay sediments: Anthropogenic influence on organic matter composition. Mar. Chem. 69, 117–137.

Ziolkowski, L.A., Druffel, E.R.M., 2010. Aged black carbon identified in marine dissolved organic carbon. Geophys. Res. Lett. 37, L16601.

Chapter 2

Material and Methods

2. Material and Methods

2.1. Sampling location

Sediment and water samples were collected from ten boreal lakes in the Malingsbo area, Sweden. Although the lakes investigated are geographically close, they vary in both size, depth and organic carbon content. Övre Skärsjön is the largest and deepest one, with 165 ha of area and 32 m of maximum depth, whereas Svarttjärn is the smallest with just 0.7 ha of areas and maximum depth of 7 m. The difference in area correlate with water residence time (Hanson et al., 2011); lake Svarttjärn, for example shows exceptionally high drainage ratio at short water residence time (von Wachenfeldt and Tranvik, 2008). In turn this leads to different average age of the surface sediments and of the DOC found in the lakes (Evans et al., 2017). Ljustjärn was an exception among the lakes used in this study, because it was influenced by groundwater inflow and received low amounts of organic matter from the rather tiny drainage area, resulting in a low DOC concentration and light color of the water (von Wachenfeldt and Tranvik, 2008).

2.2. Sediment sample preparation

Five replicates of surface sediment samples (upper 10 cm) were collected from a boat approximately at the deepest water depth of the lake using a small gravity corer. The samples were homogenized and transferred into 125 ml polyethylene bottles, which were completely filled with sediment sample, avoiding air bubbles inside. The bottles were kept cold (4ºC), stored in the dark and shipped by air to the Max Planck Institute for Terrestrial Microbiology, Marburg, Germany, for incubation experiments.

2.3. Land coverage determination

The watershed area, as well as the percentage of cover by wetland, forest, open water, and open land, were computed using ArcGIS software, version 10.3.1, with the Swedish National DEM (2 m raster). First, we ran the Flow Direction tool to get flow direction rasters (https://desktop.arcgis.com/en/arcmap/10.3/tools/spatial-analyst-toolbox/how-flow-direction-works.htm).

Then we used the flow direction raster in combination with a basemap (aerial photography) to identify outlets (called pour-points in ArcMap) for each lake. Lastly, we ran the Watershed tool

using the flow direction raster and the pour-points as input (https://desktop.arcgis.com/en/arcmap/10.3/tools/spatial-analyst-toolbox/watershed.htm).

2.4. Chemical analysis

Water samples from the lakes were analyzed for Dissolved Organic Carbon (DOC), Total Nitrogen (TN) and Total Phosphorus (TP). DOC was measured with a TOC analyzer (Shimadzu 5000, Japan). TN and TP were analyzed colourimetrically after persulfate oxidation using an AA3 auto-analyzer (Bran Luebbe, Germany). Organic carbon (OC) content in the sediments was measured in one of the replicates of each sample. Samples were acidified (to remove possible carbonates) and dried. The carbon content was analyzed at the Centre for Stable Isotope Research and Analysis (KOSI) at Göttingen University (Germany) using an elemental analyzer (NA 2500, CE Instruments, Rodano, Italy).

2.5. DOM extraction

The increasing interest on molecular composition of DOM lead to the application and development of several different methodologies of DOM extraction. Solid phase extraction (SPE) has become an established and widely used DOM isolation method combining ease of use and high extraction efficiency (Dittmar et al., 2008; Green et al., 2014; Kim et al., 2003b). Here, we decided to use PPL cartridges because of its demonstrated efficiency and suitability to freshwater samples (Li et al., 2016b; Raeke et al., 2016). Similar to other methods of SPE isolation, the styrene−divinylbenzene copolymer (PPL)-based SPE is also prone to chemical selectivity, depending on the extraction conditions, because it relies on interactions between polydisperse organic molecules of considerable structural variance and a modified styrene divinylbenzene-type stationary phase (Li et al., 2016b). Nevertheless, on a systematic evaluation of 24 commercial SPE cartridges, Li et al. (2017) have found that DOC recoveries were relatively high with polymer-based sorbents (e.g. PPL) but more variable with silica-based sorbents. PPL cartridges achieving up to 90% DOC recovery have been previously shown (Li et al., 2016a). Moreover, sorbents with non-polar functionalities, such as PPL readily retain large proportions of non-polar compounds from freshwater DOM which is the biogeochemically most evolved and diverse assembly of molecules (Dittmar et al., 2008; Li et al., 2017, 2016b). Compounds not isolated by PPL based SPE likely comprise large (bio)molecules which become irreversibly adsorbed and metabolite-like very hydrophilic

compounds which are not retained at all. Selective enrichment of freshwater DOM by SPE is also less critical for subsequent FT-ICR MS analysis, because those fractions that are not sufficiently recovered have comparatively small effects on the mass spectra (Raeke et al., 2016).

The lake water DOM was extracted by a previously described solid phase extraction (SPE) method (Dittmar et al., 2008). Sediment pore water DOM was extracted by an adapted solid phase extraction, based on the same method described by Dittmar et al. (2008). The pore water of about 2 ml sediment was isolated by centrifugation and the leftover sediment was then washed three times with 1 ml ultra-pure water (LC-MS Chromasolv®, Sigma-Aldrich). The supernatants of the centrifugation and washing steps were combined. Then, approximately 4 ml liquid sample was acidified to pH 2 with formic acid and passed through a SPE cartridge (Bond Elut PPL, 100 mg, 1 ml, Agilent). The cartridge had been activated with 1 ml methanol (LC-MS Chromasolv®, Sigma-Aldrich) and rinsed with 1 ml acidified pure water (pH 2) prior to extraction. In the end, DOM was eluted with 500 µl methanol. This procedure was repeated for all five replicates, before and after the incubation experiment (total of ten samples per lake). Flerus et al. (2011) have shown hydrogen–deuterium (H/D) exchange and esterification reactions in samples extracted with methanol and stored at 20°C, but such reactions had no substantial influence on the most important parameters of the FT-ICR MS data evaluation – i.e. weighted average H/C and O/C ratios, DBE, and Kendrick diagrams. Their results suggest that SPE with methanol is possible, but cold storage of the extracts is strongly recommended, because potential reactions like methanolysis (Flerus et al., 2011; Hertkorn et al., 2016) can be decreased to a minimum at cold temperatures. Our eluates were stored in the freezer (-20 ºC) until mass spectrometry analysis.

2.6. Laboratory experiments

A similar incubation experiment has been described elsewhere (Conrad et al., 2011). Approximately 9 ml of sediment were placed into 27 ml sterile glass vials, closed with rubber stoppers and flushed with nitrogen gas (N_2). Then, the sediments were incubated at 15°C, and methane (CH_4) and carbon dioxide (CO_2) concentrations were measured regularly in the headspace by gas chromatography during the incubation period of 40 days. Because metabolic rates of microorganisms are strongly influenced by temperature (Brown et al., 2004), here we

measured a potential CH_4 and CO_2 production rate at 15°C. Water temperature at the bottom of the Swedish lakes do not reach such temperatures all year round.

Organic carbon (OC) content in the sediments was measured in one of the replicates of each sample at the end of the incubation experiment. Samples were acidified (to remove possible carbonates) and dried. The C content was analyzed at the Centre for Stable Isotope Research and Analysis (KOSI) at Göttingen University (Germany) using an elemental analyzer (NA 2500, CE Instruments, Rodano, Italy) connected with an isotope ratio mass spectrometer (IRMS; Finnigan MAT Deltaplus, Bremen, Germany; data not shown) coupled via an interface (ConFlo III; Thermo Electron Cooperation, Bremen, Germany).

2.7. FT-ICR MS analysis

In the past decades, Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry coupled with electrospray ionization (ESI) in positive [ESI(+)] and negative [ESI(-)] mode has been used extensively to analyze the molecular composition of NOM and to elucidate chemical and biological transformations in a wide range of environmental compartments (Schmitt-Kopplin et al., 2012). The main reason for that choice relies on the ultra-high mass resolution and mass accuracy of the method, capable of revealing an unprecedented representation of thousands of molecular formulae directly out of mixtures (Hertkorn et al., 2007; Schmitt-Kopplin et al., 2012; Schmitt-Kopplin and Hertkorn, 2007). The key information which enabled the positive identification of molecular formulas composed solely of C, H, and O, based on accurate mass determination, resulted from the finding that any mass spacing Δm (the mass difference between neighboring C,H,O-molecules if sorted according to mass) of any C,H,O- molecules in the mass range from 0-1000 Da was large compared with standard high-field FT-ICR mass resolution (Hertkorn et al., 2008). For molecular determination of heteroatoms, functional network reconstruction is based on a restricted list of selected small molecular units with defined Δm, corresponding to common chemical functional group equivalents (which can be commonly inserted between any C–C and C–H bond, such as CH_2, H_2, O, CO_2, S, SO_3, NH, PO_3, ΔC and ΔS) and transformations (Tziotis et al., 2011). Functional network reconstruction corresponds to a multiple Kendrick analogue mass defect analysis and generates all homologous series according to chosen transformations simultaneously (Tziotis et al., 2011).

High resolution FT-ICR MS is already an stablish method to investigate changes in sedimentary DOM (D'Andrilli et al., 2010; Herzsprung et al., 2017; Hodgkins et al., 2016; Schmidt et al., 2017, 2011, 2009; Seidel et al., 2014; Tremblay et al., 2007) and bacterial activity (D'Andrilli et al., 2019; Hodgkins et al., 2014; Kamjunke et al., 2019, 2017; Medeiros et al., 2015; Seidel et al., 2016).

FT-ICR MS was also applied to investigate and characterize DOC from different sources. DOM from various rivers, bogs, and soil sites were investigated for their molecular patterns, and unique molecular formulae for each ecosystem were identified and their main characteristics compared (Roth et al., 2014). It has been suggested that the lack of nitrogen-containing compounds in the surface water and the lower molecular size in the soil water could be related to higher microbial activity and reworking in soils, whereas the lack of aromatic compounds in surface waters could be the result of photo degradation. Their results demonstrated that tannin molecular formulae seemed to be particularly suited markers for forest systems (Roth et al., 2014).

In another study, samples covering a wide spectrum of DOM molecular composition, such as groundwaters, rivers, lakes, and marine waters, were investigated (Kellerman et al., 2018). Their results have shown that modern Δ^{14}C-DOC and optical properties reflecting increased aromaticity, such as carbon specific UV absorbance at 254 nm (SUVA254), were directly related to polyphenolic and polycyclic aromatic compounds, whereas enriched δ^{13}C-DOC and optical properties reflecting autochthonous end-members were positively correlated to more aliphatic compounds (Kellerman et al., 2018).

Unit-resolution mass spectra of NOM samples are commonly characterized by a rather continuous envelope, accounting for many hundreds of signals (with a maximum intensity around 350-700 Da), but showing only a single intensity which integrates across all mass peaks, and by the occurrence of several regular mass spacing patterns (Hertkorn et al., 2008). The mass peaks which represent pairs of molecules of similar structure and function, like CH_2 homologues ($\Delta m = 14.01565$ Da), are widely separated on the mass axis. In general, any pair of mass peaks separated by less than 2.1057 Da, irrespective of its position on the mass axis and independent of its composition ($C_nH_mO_qZ_\Sigma$; where Z is any combination of elements) necessarily represents considerably dissimilar chemical structures (Hertkorn et al., 2008; Schmitt-Kopplin et al., 2012). Mass spacings based on methylene ($\Delta m = \pm14$ Da) and double-

bond equivalents (DBE, $\Delta m = \pm 2$ Da) dominate, simply because they refer to these most fundamental building blocks of organic chemistry. Unfortunately, linear combinations of methylene and DBE cannot be distinguished from oxygen-containing fragments such as H_2O ($\Delta m = \pm 18$ Da), CO ($\Delta m = \pm 28$ Da), and CO_2 ($\Delta m = \pm 44$ Da) at this level of resolution, leaving descriptive mass listing and mass comparison as one of the few remaining options for mass analysis (Hertkorn et al., 2008; Schmitt-Kopplin et al., 2012).

Nevertheless, the analysis of DOM through high resolution MS presents challenges in data comparison and interpretation among laboratories operating instruments with differing performance and user operating conditions. Zherebker et al. (2020) have performed an interlaboratory comparison in which 5 different FT-ICR MS with electrospray ionization were used to resolve the molecular composition of six samples of humic substances from varied sources. The results from Suwannee River have shown that, despite the differences in the number and abundance of the assigned formulae, the calculated number-averaged parameters revealed similarities between the spectra examined. The van Krevelen diagrams obtained for the same sample were characterized by similar general features of molecular distribution, but with substantial differences in the exact location of molecular species. Here, the ratio of oxidized to reduced species varied among the laboratories, which could be explained by different equipment adjustments for analysis. The obtained results highlighted the importance of the development of statistical tools for data treatment, which could explore the unique and common features of HS samples analyzed by different FT-ICR MS instruments. The reliable similarity among the data was achieved only by transforming a singular point (stoichiometry) in van Krevelen diagram into a sizeable pixel (a number of closely located stoichiometries), which can be calculated from the population density distribution (Zherebker et al., 2020).

Hawkes et al. (2020), on the other hand, have analyzed four identically prepared DOM samples in 16 different laboratories, using 17 commercially purchased instruments. The instruments identified ~1000 common ions in both negative and positive ESI modes over a wide range of m/z values and chemical space, as determined by van Krevelen diagrams. Calculated metrics of abundance-weighted average indices (H/C, O/C, aromaticity, and m/z) of the commonly detected ions showed that hydrogen saturation and aromaticity were consistent for each reference sample across the instruments, while average mass and oxygenation were more affected by differences in instrument type and settings (Hawkes et al., 2020).

However, the incredible resolution of FT-ICR MS alone is not enough, because structural information will remain elusive when mass spectrometry is used without additional techniques capable of resolving isomers from peaks of identical mass (Hertkorn et al., 2008). Mass spectra acquired without fragmentation or ion-mobility discrimination consolidate all isomeric structures of molecular ions in a single mass peak. Accordingly, the compositional space of molecules represents the isomer-filtered complement of the entire space of molecular structures. The transformation of the total structural space of molecules into its compositional space is equivalent to its projection on a highly resolved molecular-weight axis and amounts to an extreme data reduction, while crucial molecular-level information is retained. The compositional space is quantized as defined by the laws of chemical binding and it will increase with molecular size and number of contributing elements.

In our investigations, negative electrospray ionization Fourier transform ion cyclotron resonance [ESI(-)] FT-ICR mass spectra were acquired using a 12T Bruker Solarix mass spectrometer (Bruker Daltonics, Bremen, Germany) and an Apollo II ESI source in negative mode (Hertkorn et al., 2016). It has been shown before that both ESI, atmospheric pressure chemical ionization (APCI), and atmospheric pressure photo ionization (APPI), generated a higher number of resolved signals and unique molecular formulas in the negative ion mode than in the positive ion mode (Hertkorn et al., 2008). Generally, molecular formulas that arise from negative ion mode also provide more extensive coverage of theoretically possible H/C and O/C atomic ratios in van Krevelen plots (and thus greater compositional diversity) than molecular formulas obtained from positive ion mode (Hertkorn et al., 2008). Negative ESI is also widely used in NOM research because it preferentially ionizes oxygen-rich molecules, such as carboxylic acids (COOH), which are abundant in such samples (Hertkorn et al., 2008).

SPE samples were injected using a 223 Sample Changer - Gilson, Inc (Middleton, USA) with an adapted in-house made software to FT-ICR-MS. Nebulizer gas pressure, drying gas pressure, and the source heater temperature were 138 kPa, 103 kPa, and 200°C, respectively. The spectra were acquired with a time domain of 4 MW and five hundred scans were accumulated for each mass spectrum. All spectra were first externally calibrated on clusters of arginine in MeOH (0.57 µmol/L) and internally calibrated using appropriate reference mass lists of common natural organic matter molecules, reaching accuracy values lower than 500 ppb. Data processing was done using Compass Data Analysis 4.1 (Bruker, Bremen, Germany)

and formula assignment by an in-house made software (NetCalc) (Tziotis et al., 2011). The molecular formula assignments were based on the following elements: $^{1}H_{0-200}$, $^{12}C_{0-100}$, $^{16}O_{0-80}$, $^{32}S_{0-3}$, $^{14}N_{0-3}$ as well as the $^{13}C_{0-1}$ and $^{34}S_{0-1}$ isotopomers. The generated formulae were validated by setting sensible chemical constraints [N rule, O/C ratio ≤ 1, H/C ratio ≤ 2n + 2 (C_nH_{2n+2})]. Restriction on nitrogen and sulfur atoms to counts up to three was based on previous studies of natural organic matter (NOM) where no mass assignments with more than three atoms from both were regularly observed (Gonsior et al., 2011; Hertkorn et al., 2013).

Ions common to all five replicates were included in our calculations; to account for variance in-between replicates, molecular formulas derived from peaks with intensity lower than 3 • 106 (~10^{-4} of maximum amplitude) were not considered here and then, respective bulk parameters were computed from mass peak intensity weighted values. The final assigned molecular formulas were categorized into groups containing CHO, CHNO, CHOS and CHNOS molecular compositions, which were used to reconstruct the group-selective mass spectra. For molecular compositions common to all five replicates, number and percentage of molecular formulas containing CHO, CHNO, CHOS or CHNOS molecular series and the computed average of H, C, N, O, S atoms and H/C, O/C, C/N, C/S, as well as m/z (mass-to-charge) ratios, and other relevant bulk parameters.

By direct analysis of mass spectra and by the use of mathematical data analysis and data sorting schemes, such as Kendrick mass analysis (Kendrick, 1963) and van Krevelen diagrams (van Krevelen, 1950) both the intrinsic order of the entire, quantized compositional space and the substance-specific informative order that is obtained from FTICR MS of complex materials can be readily compared and contrasted (Rivas-Ubach et al., 2018).

Plots of the assigned molecular formulas were visualized based on their elemental ratios using van Krevelen plots (van Krevelen, 1950). The standard display is a projection of two elemental ratios, commonly H/C versus O/C derived from assigned molecular formulas, along two axes; here, each data point reflects a given elemental ratio regardless of the molecular mass (Schmitt-Kopplin et al., 2012). Since major chemical classes typically found in DOM show characteristic H/C and O/C ratios, these usually cluster within a specific region of the diagram (Kim et al., 2003a). Thus, patterns in van Krevelen diagrams of DOM reflect not only the source material, but may also indicate changes in bulk DOM composition due to degradation and hint at possible

chemical reactions during DOM evolution (methylation, oxidation and hydrogenation, for example) (Kim et al., 2003a).

Another important visualization tool of FT-ICR mass spectra is the Kendrick mass defect analysis (KMD) which is not restricted to assigned molecular compositions but shows the entire complement of molecular compositions (Hughey et al., 2001). A useful extension is the KMD/z* diagram (Shakeri Yekta et al., 2012) in which the Kendrick mass defect is divided by an empirical, integer sorting parameter z*, introduced by (Hsu et al., 1992). z* was later found useful because the majority of molecular compositions in common organic matter could be described by two simple equations: DBE – O = -0.5z* + 1, and DBE – O = -0.5z* - 6 (DBE: double bond equivalent) (Stenson et al., 2003). Further rearrangement and introduction of a "family score" (Stenson, 2009, 2008) provided very powerful sorting schemes of the NOM (natural organic matter) compositional space (Green and Perdue, 2015; Perdue and Green, 2015). In KMD/z* versus mass diagrams (Shakeri Yekta et al., 2012), molecular compositions with identical KMD/z* represent unique homologous series, which then appear on horizontal lines. Naturally, the most relevant homologous series present in DOM are methylene- and DBE-based moieties, reflecting mass difference of homologous molecules $\Delta m(CH_2)$ by 14.0156 Da (providing horizontal lines in KMD/z* diagrams), $\Delta m(H_2)$ by 2.0157 Da (providing upward sloped lines in KMD/z* diagrams) and $\Delta m(CH_4-O)$ by 36.4 mDa (providing vertical lines in KMD/z* diagrams) (Gonsior et al., 2014; Hsu et al., 1992; Perdue and Green, 2015; Shakeri Yekta et al., 2012; Stenson et al., 2003).

An alternative projection of the CHO-compositional space is the depiction of the average oxidation state (OS_av) according to OS_av = $2 \times$ O/C - H/C (Kroll et al., 2011). This has been done for individual CHO compounds as well as for the consolidated MS-derived intensity weighted average of all detected CHO compounds, before and after incubation. Here, these OS_av values have been sorted in ranks of 0.1 width. Alternative indices like NOSC [nominal oxidation state of carbon; (LaRowe and Van Cappellen, 2011)] were not considered, because these would have required severe estimates about (average) oxidation states of the heteroatoms sulfur and nitrogen, respectively, which are not warranted at present.

2.8. Statistical analysis

Specific compounds which showed increased or decreased relative abundance prior and after the incubation experiment were computed using profile search in Hierarchical Cluster Explorer, with a threshold of 0.9 (search method: model-based; distance measure: Pearson's R. Hierarchical Cluster Analysis (HCA) was carried by Hierarchical Cluster Explorer 3.0 software (Human-Computer Interaction Lab, University of Maryland, College Park, http://www.cs.umd.edu/hcil/multi-cluster/). We used average linkage (UPGMA) method and the similarity measure was based on the Pearson correlation coefficient. Principal Component Principal Component Analysis (PCA) and Partial Least Square (PLS) analysis were performed with the software SIMCA-P 9.0 (Umetrics AB, Sweden). All molecular compositions which were attributed to have increased and decreased in relative abundance had significantly changed between the different incubation states ($p < 0.05$, Mann Whitney test, GraphPad Prism 5.0). Paired T-test and Correlation were performed on Excel (Microsoft Office). Determination of unique molecular formulas in each compartment was done based on presence and absence.

2.9. References

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V., West, G.B., 2004. Toward a metabolic theory of ecology. Ecology 85, 1771–1789.

Conrad, R., Noll, M., Claus, P., Klose, M., Bastos, W.R., Enrich-Prast, A., 2011. Stable carbon isotope discrimination and microbiology of methane formation in tropical anoxic lake sediments. Biogeosciences 8, 795–814.

D'Andrilli, J., Chanton, J.P., Glaser, P.H., Cooper, W.T., 2010. Characterization of dissolved organic matter in northern peatland soil porewaters by ultra high resolution mass spectrometry. Org. Geochem. 41, 791–799.

D'Andrilli, J., Junker, J.R., Smith, H.J., Scholl, E.A., Foreman, C.M., 2019. DOM composition alters ecosystem function during microbial processing of isolated sources. Biogeochemistry 142, 281–298.

Dittmar, T., Koch, B., Hertkorn, N., Kattner, G., 2008. A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. Limnol. Oceanogr. Methods 6, 230–235.

Evans, C.D., Futter, M.N., Moldan, F., Valinia, S., Frogbrook, Z., Kothawala, D.N., 2017. Variability in organic carbon reactivity across lake residence time and trophic gradients. Nat. Geosci. 10, 832–835.

Flerus, R., Koch, B.P., Schmitt-Kopplin, P., Witt, M., Kattner, G., 2011. Molecular level investigation of reactions between dissolved organic matter and extraction solvents using FT-ICR MS. Mar. Chem. 124, 100–107.

Gonsior, M., Hertkorn, N., Conte, M.H., Cooper, W.J., Bastviken, D., Druffel, E., Schmitt-Kopplin, P., 2014. Photochemical production of polyols arising from significant photo-transformation of dissolved organic matter in the oligotrophic surface ocean. Mar. Chem. 163, 10–18.

Gonsior, M., Peake, B.M., Cooper, W.T., Podgorski, D.C., D'Andrilli, J., Dittmar, T., Cooper, W.J., 2011. Characterization of dissolved organic matter across the Subtropical Convergence off the South Island, New Zealand. Mar. Chem. 123, 99–110.

Green, N.W., Perdue, E.M., 2015. Fast graphically inspired algorithm for assignment of molecular formulae in ultrahigh resolution mass spectrometry. Anal. Chem. 87, 5086–5094.

Green, N.W., Perdue, E.M., Aiken, G.R., Butler, K.D., Chen, H., Dittmar, T., Niggemann, J., Stubbins, A., 2014. An intercomparison of three methods for the large-scale isolation of oceanic dissolved organic matter. Mar. Chem. 161, 14–19.

Hanson, P.C., Hamilton, D.P., Stanley, E.H., Preston, N., Langman, O.C., Kara, E.L., 2011. Fate of allochthonous dissolved organic carbon in lakes: A quantitative approach. PLoS One 6 (7), e21884.

Hawkes, J.A., D'Andrilli, J., Agar, J.N., Barrow, M.P., Berg, S.M., Catalán, N., Chen, H., Chu, R.K., Cole, R.B., Dittmar, T., Gavard, R., Gleixner, G., Hatcher, P.G., He, C., Hess, N.J., Hutchins, R.H.S., Ijaz, A., Jones, H.E., Kew, W., Khaksari, M., Palacio Lozano, D.C., Lv, J., Mazzoleni, L.R., Noriega-Ortega, B.E., Osterholz, H., Radoman, N., Remucal, C.K., Schmitt, N.D., Schum, S.K., Shi, Q., Simon, C., Singer, G., Sleighter, R.L., Stubbins, A., Thomas, M.J., Tolic, N., Zhang, S., Zito, P., Podgorski, D.C., 2020. An international laboratory comparison of dissolved organic matter composition by high resolution mass spectrometry: Are we getting the same answer? Limnol. Oceanogr. Methods 18, 235–258.

Hertkorn, N., Frommberger, M., Witt, M., Koch, B.P., Schmitt-Kopplin, P., Perdue, E.M., 2008. Natural Organic Matter and the Event Horizon of Mass Spectrometry. Anal. Chem. 80, 8908–8919.

Hertkorn, N., Harir, M., Cawley, K.M., Schmitt-Kopplin, P., Jaffé, R., 2016. Molecular characterization of dissolved organic matter from subtropical wetlands: A comparative study through the analysis of optical properties, NMR and FTICR/MS. Biogeosciences 13, 2257–2277.

Hertkorn, N., Harir, M., Koch, B.P., Michalke, B., Schmitt-Kopplin, P., 2013. High-field NMR spectroscopy and FTICR mass spectrometry: Powerful discovery tools for the molecular level characterization of marine dissolved organic matter. Biogeosciences 10, 1583–1624.

Hertkorn, N., Ruecker, C., Meringer, M., Gugisch, R., Frommberger, M., Perdue, E.M., Witt, M., Schmitt-Kopplin, P., 2007. High-precision frequency measurements: indispensable tools at the core of the molecular-level analysis of complex systems. Anal. Bioanal. Chem. 389, 1311–1327.

Herzsprung, P., von Tümpling, W., Wendt-Potthoff, K., Hertkorn, N., Harir, M., Schmitt-Kopplin, P., Friese, K., 2017. High field FT-ICR mass spectrometry data sets enlighten qualitative DOM alteration in lake sediment porewater profiles. Org. Geochem. 108, 51–60.

Hodgkins, S.B., Tfaily, M.M., McCalley, C.K., Logan, T. a, Crill, P.M., Saleska, S.R., Rich, V.I., Chanton, J.P., 2014. Changes in peat chemistry associated with permafrost thaw increase greenhouse gas production. PNAS 111, 5819–24.

Hodgkins, S.B., Tfaily, M.M., Podgorski, D.C., McCalley, C.K., Saleska, S.R., Crill, P.M., Rich, V.I., Chanton, J.P., Cooper, W.T., 2016. Elemental composition and optical properties reveal changes in dissolved organic matter along a permafrost thaw chronosequence in a subarctic peatland. Geochim. Cosmochim. Acta 187, 123–140.

Hsu, C.S., Qian, K., Chen, Y.C., 1992. An innovative approach to data analysis in hydrocarbon characterization by on-line liquid chromatography-mass spectrometry. Anal. Chim. Acta 264, 79–89.

Hughey, C.A., Hendrickson, C.L., Rodgers, R.P., Marshall, A.G., Qian, K., 2001. Kendrick Mass Defect Spectrum: A Compact Visual Analysis for Ultrahigh-Resolution Broadband Mass Spectra. Anal. Chem. 73, 4676–4681.

Kamjunke, N., Hertkorn, N., Harir, M., Schmitt-Kopplin, P., Griebler, C., Brauns, M., von Tümpling, W., Weitere, M., Herzsprung, P., 2019. Molecular change of dissolved organic matter and patterns of bacterial activity in a stream along a land-use gradient. Water Res. 164, 114919.

Kamjunke, N., von Tümpling, W., Hertkorn, N., Harir, M., Schmitt-Kopplin, P., Norf, H., Weitere, M., Herzsprung, P., 2017. A new approach for evaluating transformations of dissolved organic matter (DOM) via high-resolution mass spectrometry and relating it to bacterial activity. Water Res. 123, 513–523.

Kellerman, A.M., Guillemette, F., Podgorski, D.C., Aiken, G.R., Butler, K.D., Spencer, R.G.M., 2018. Unifying Concepts Linking Dissolved Organic Matter Composition to Persistence in Aquatic Ecosystems. Environ. Sci. Technol. 52, 2538–2548.

Kendrick, E., 1963. A Mass Scale Based on CH = 14.0000 for High Resolution Mass Spectrometry of Organic Compounds. Anal. Chem. 35, 2146–2154.

Kim, S., Kramer, R.W., Hatcher, P.G., 2003a. Graphical Method for Analysis of Ultrahigh-Resolution Broadband Mass Spectra of Natural Organic Matter, the Van Krevelen Diagram. Anal. Chem. 75, 5336–5344.

Kim, S., Simpson, A.J., Kujawinski, E.B., Freitas, M.A., Hatcher, P.G., 2003b. High resolution electrospray ionization mass spectrometry and 2D solution NMR for the analysis of DOM extracted by C18 solid phase disk. Org. Geochem. 34, 1325–1335.

Kroll, J.H., Donahue, N.M., Jimenez, J.L., Kessler, S.H., Canagaratna, M.R., Wilson, K.R., Altieri, K.E., Mazzoleni, L.R., Wozniak, A.S., Bluhm, H., Mysak, E.R., Smith, J.D., Kolb, C.E., Worsnop, D.R., 2011. Carbon oxidation state as a metric for describing the chemistry of atmospheric organic aerosol. Nat. Chem. 3, 133–139.

LaRowe, D.E., Van Cappellen, P., 2011. Degradation of natural organic matter: A thermodynamic analysis. Geochim. Cosmochim. Acta 75, 2030–2042.

Li, Y., Harir, M., Lucio, M., Gonsior, M., Koch, B.P., Schmitt-Kopplin, P., Hertkorn, N., 2016a. Comprehensive structure-selective characterization of dissolved organic matter by reducing molecular complexity and increasing analytical dimensions. Water Res. 106, 477–487.

Li, Y., Harir, M., Lucio, M., Kanawati, B., Smirnov, K., Flerus, R., Koch, B.P., Schmitt-Kopplin, P., Hertkorn, N., 2016b. Proposed guidelines for solid phase extraction of Suwannee River dissolved organic matter. Anal. Chem. acs.analchem.5b04501.

Li, Y., Harir, M., Uhl, J., Kanawati, B., Lucio, M., Smirnov, K.S., Koch, B.P., Schmitt-Kopplin, P., Hertkorn, N., 2017. How representative are dissolved organic matter

(DOM) extracts? A comprehensive study of sorbent selectivity for DOM isolation. Water Res. 116, 316–323.

Medeiros, P.M., Seidel, M., Ward, N.D., Carpenter, E.J., Gomes, H.R., Niggemann, J., Krusche, A. V., Richey, J.E., Yager, P.L., Dittmar, T., 2015. Fate of the Amazon River dissolved organic matter in the tropical Atlantic Ocean. Global Biogeochem. Cycles 667–690.

Perdue, E.M., Green, N.W., 2015. Isobaric Molecular Formulae of C, H, and O – A View from the Negative Quadrants of van Krevelen Space. Anal. Chem. 150204032255009.

Raeke, J., Lechtenfeld, O.J., Wagner, M., Herzsprung, P., Reemtsma, T., 2016. Selectivity of solid phase extraction of freshwater dissolved organic matter and its effect on ultrahigh resolution mass spectra. Environ. Sci. Process. Impacts 18, 918–927.

Rivas-Ubach, A., Liu, Y., Bianchi, T.S., Tolić, N., Jansson, C., Paša-Tolić, L., 2018. Moving beyond the van Krevelen Diagram: A New Stoichiometric Approach for Compound Classification in Organisms. Anal. Chem. 90, 6152–6160.

Roth, V.-N., Dittmar, T., Gaupp, R., Gleixner, G., 2014. Ecosystem-Specific Composition of Dissolved Organic Matter. Vadose Zo. J. 13, vzj2013.09.0162.

Schmidt, F., Elvert, M., Koch, B.P., Witt, M., Hinrichs, K.-U., 2009. Molecular characterization of dissolved organic matter in pore water of continental shelf sediments. Geochim. Cosmochim. Acta 73, 3337–3358.

Schmidt, F., Koch, B.P., Elvert, M., Schmidt, G., Witt, M., Hinrichs, K.-U., 2011. Diagenetic transformation of dissolved organic nitrogen compounds under contrasting sedimentary redox conditions in the Black Sea. Environ. Sci. Technol. 45, 5223–9.

Schmidt, F., Koch, B.P., Goldhammer, T., Elvert, M., Witt, M., Lin, Y.S., Wendt, J., Zabel, M., Heuer, V.B., Hinrichs, K.U., 2017. Unraveling signatures of biogeochemical processes and the depositional setting in the molecular composition of pore water DOM across different marine environments. Geochim. Cosmochim. Acta 207, 57–80.

Schmitt-Kopplin, P., Harir, M., Tziotis, D., Gabelica, Z., Hertkorn, N., 2012. Ultrahigh resolution Fourier Transform ion cyclotron resonance mass spectrometry for the analysis of natural organic matter from various environmental systems. Compr. Environ. Mass Spectrom. 443–459.

Schmitt-Kopplin, P., Hertkorn, N., 2007. Ultrahigh resolution mass spectrometry. Anal. Bioanal. Chem. 389, 1309–1310.

Seidel, M., Beck, M., Riedel, T., Waska, H., Suryaputra, I.G.N.A., Schnetger, B., Niggemann, J., Simon, M., Dittmar, T., 2014. Biogeochemistry of dissolved organic matter in an anoxic intertidal creek bank. Geochim. Cosmochim. Acta 140, 418–434.

Seidel, M., Dittmar, T., Ward, N.D., Krusche, A. V., Richey, J.E., Yager, P.L., Medeiros, P.M., 2016. Seasonal and spatial variability of dissolved organic matter composition in the lower Amazon River. Biogeochemistry 131, 281–302.

Shakeri Yekta, S., Gonsior, M., Schmitt-Kopplin, P., Svensson, B.H., 2012. Characterization of Dissolved Organic Matter in Full Scale Continuous Stirred Tank Biogas Reactors Using Ultrahigh Resolution Mass Spectrometry: A Qualitative Overview. Environ. Sci. Technol. 46, 12711–12719.

Stenson, A.C., 2009. Fourier transform ion cyclotron resonance mass spectral characterization of metal-humic binding. Rapid Commun. Mass Spectrom., 23, 465–476.

Stenson, A.C., 2008. Reversed-Phase Chromatography Fractionation Tailored to Mass Spectral Characterization of Humic Substances. Environ. Sci. Technol. 42, 2060–2065.

Stenson, A.C., Marshall, A.G., Cooper, W.T., 2003. Exact Masses and Chemical Formulas of Individual Suwannee River Fulvic Acids from Ultrahigh Resolution Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectra. Anal. Chem. 75, 1275–1284.

Tremblay, L.B., Dittmar, T., Marshall, A.G., Cooper, W.J., Cooper, W.T., 2007. Molecular characterization of dissolved organic matter in a North Brazilian mangrove porewater and mangrove-fringed estuaries by ultrahigh resolution Fourier Transform-Ion Cyclotron Resonance mass spectrometry and excitation/emission spectroscopy. Mar. Chem. 105, 15–29.

Tziotis, D., Hertkorn, N., Schmitt-Kopplin, P., 2011. Kendrick-analogous network visualisation of ion cyclotron resonance Fourier transform mass spectra: improved options for the assignment of elemental compositions and the classification of organic molecular complexity. Eur. J. Mass Spectrom. 17, 415–421.

van Krevelen, D.W., 1950. Graphical-statistical method for the study of structure and reaction processes of coal. Fuel 29, 269–284.

von Wachenfeldt, E., Tranvik, L.J., 2008. Sedimentation in Boreal Lakes—The Role of Flocculation of Allochthonous Dissolved Organic Matter in the Water Column. Ecosystems 11, 803–814.

Zherebker, A., Kim, S., Schmitt-Kopplin, P., Spencer, R.G.M., Lechtenfeld, O., Podgorski, D.C., Hertkorn, N., Harir, M., Nurfajin, N., Koch, B., Nikolaev, E.N., Shirshin, E.A., Berezin, S.A., Kats, D.S., Rukhovich, G.D., Perminova, I. V., 2020. Interlaboratory comparison of humic substances compositional space as measured by Fourier transform ion cyclotron resonance mass spectrometry (IUPAC Technical Report). Pure Appl. Chem. 92, 1447–1467.

Chapter 3

Extensive processing of sediment pore water dissolved organic matter during anoxic incubation as observed by high-field mass spectrometry (FTICR-MS)

3. Extensive processing of sediment pore water dissolved organic matter during anoxic incubation as observed by high-field mass spectrometry (FTICR-MS)

3.1. Abstract

Dissolved organic matter (DOM) contained in lake sediments is a carbon source for many microbial degradation processes, including aerobic and anaerobic mineralization. During anaerobic degradation, DOM is partially consumed and transformed into new molecules while the greenhouse gases methane (CH_4) and carbon dioxide (CO_2) are produced. In this study, we used ultrahigh resolution mass spectrometry to trace differences in the composition of solid-phase extractable (PPL resin) pore water DOM (SPE-DOM) isolated from surface sediments of three boreal lakes before and after 40 days of anoxic incubation, with concomitant determination of CH_4 and CO_2 evolution. CH_4 and CO_2 production detected

By gas chromatography varied considerably among replicates and accounted for fractions of ~2–4 x 10^{-4} of sedimentary organic carbon for CO_2 and ~0.8–2.4 x 10^{-5} for CH_4. In contrast, the relative changes of by gas chromatography varied considerably among replicates and accounted for fractions of ~2–4 x 10^{-4} key bulk parameters during incubation, such as relative proportions of molecular series, elemental ratios, average mass and unsaturation, were regularly in the percent range (1–3% for compounds decreasing and 4–10% for compounds increasing), i.e. several orders of magnitude higher than mineralization alone. Computation of the average carbon oxidation state in CHO molecules of lake pore water DOM revealed rather non-selective large-scale transformations of organic matter during incubation, with depletion of highly oxidized and highly reduced CHO molecules, and formation of rather non-labile fulvic acid type molecules. In general, proportions of CHO compounds slightly decreased. Nearly saturated CHO and CHOS lipid-like substances declined during incubation: these rather commonplace molecules were less specific indicators of lake sediment alteration than the particular compounds, such as certain oxygenated aromatics and carboxyl-rich alicyclic acids (CRAM) found more abundant after incubation. There was a remarkable general increase in many CHNO compounds during incubation across all lakes. Differences in DOM transformation between lakes corresponded with lake size and water residence time. While in the small lake Svarttjärn, CRAM increased during incubation, lignin-and tannin-like

compounds were enriched in the large lake Bisen, suggesting selective preservation of these rather non-labile aromatic compounds rather than recent synthesis. SPE-DOM after incubation may represent freshly synthesized compounds, leftover bulk DOM which is primarily composed of intrinsically refractory molecules and/or microbial metabolites which were not consumed in our experiments. In spite of a low fraction of the total DOM being mineralized to CO_2 and CH_4, the more pronounced change in molecular DOM composition during the incubation indicates that diagenetic modification of organic matter can be substantial compared to complete mineralization.

3.2. Author contribution

Juliana Valle collected samples from Swedish lakes in August 2012; David Bastviken generated funding in support of this research. Juliana Valle performed gas evolution measurements at the facilities of Ralf Conrad. Alex Enrich-Prast, David Bastviken and Ralf Conrad contributed to the study design. Juliana Valle, Michael Gonsior and Mourad Harir performed the FTICR/MS acquisition, data analyses and participated in data interpretation. Philippe Schmitt-Kopplin provided support for the FTICR/MS analyses and general data interpretations. Data interpretation was performed by all authors. Juliana Valle, Mourad Harir, Michael Gonsior, David Bastviken and Norbert Hertkorn actively participated in the writing of the manuscript; all authors provided significant input on the final manuscript.

Chapter 4

Molecular differences between water column and sediment pore water SPE-DOM in ten Swedish boreal lakes

4. Molecular differences between water column and sediment pore water SPE-DOM in ten Swedish boreal lakes

4.1. Abstract

Boreal lakes are considered hot spots of dissolved organic matter (DOM) processing within the global carbon cycle. This study has used FT-ICR mass spectrometry and comprehensive data evaluation to assess the molecular differences of SPE-DOM between lake column water SPE-DOM and sedimentary pore water SPE-DOM in 10 Swedish boreal lakes of the Malingsbo area, which were selected for their large diversity of physicochemical and morphological characteristics. While lake column water is well mixed and fairly oxygenated, sedimentary pore water is subject to depletion of oxygen and to confinement of molecules. Robust trends were deduced from molecular compositions present in all compartments and in all ten lakes ("common compositions") with recognition of relative abundance. Sedimentary pore water SPE-DOM featured higher proportions of heteroatoms N and S, higher average H/C ratios in presence of higher DBE/C ratios, and higher average oxygenation than lake column water SPE-DOM. These trends were observed in all lakes except Ljustjärn, which is a ground water fed kettle lake with an unique lake biogeochemistry. Analogous trends were also observed in case of single or a few lakes and operated also for compounds present solely in either lake column water or sedimentary pore water. Unique com- pounds detected in either compartments and/or in a few lakes showed higher molecular diversity than the "common compositions". Processing of DOM molecules in sediments included selective preservation for polyphenolic compounds and microbial resynthesis of selected molecules of considerable diversity.

4.2. Author contribution

Juliana Valle, Michael Gonsior, Philippe Schmitt-Kopplin, and Norbert Hertkorn collected samples from Swedish lakes in August 2012. David Bastviken generated funding in support of this research. Ales Enrich-Prast and David Bastviken contributed to the study design. Juliana Valle, Michael Gonsior and Mourad Harir performed the FT-ICR/MS acquisition, data analyses and participated in data interpretation. Philippe Schmitt-Kopplin provided support for the FT-ICR/MS analyses and general data interpretations. Data interpretation was performed by all authors. Juliana Valle, Mourad Harir and Norbert Hertkorn actively participated in the writing of the manuscript; all authors provided significant input on the final manuscript.

Chapter 5

Influence of catchment in sediment pore water SPE-DOM molecular composition and degradation

5. Influence of catchment in sediment pore water SPE-DOM molecular composition and degradation

5.1. Abstract

Dissolved organic matter (DOM) is one of the largest exchangeable reservoirs of organic material in the planet, and connects hydrological and element cycles. Most of the carbon degradation occurs in freshwater ecosystems, considered hotspots of carbon cycling, and the boreal region has the highest density of lakes on Earth. In this study, we investigated ten boreal lakes with distinct characteristics. We determined the catchment area and land coverage using ArcGIS and applied ultrahigh resolution mass spectrometry to trace differences in the composition of solid-phase extractable sediment pore water DOM (SPE-sedDOM) isolated from surface sediments before and after 40 days of anoxic incubation, with concomitant determination of methane (CH_4) and carbon dioxide (CO_2) evolution. Lakes were mainly located in pristine areas, presenting catchment basins with at least 67% of forest. CH_4 and CO_2 production rates varied considerably among lakes, with maximum of 26.14 ($\pm$ 2.25) and 252.92 ($\pm$ 44.46) nmol g_{dw}^{-1} d^{-1}, respectively. We found that among all lakes, intrinsic characteristics from each system and its surroundings seemed to be more relevant to the SPE-sedDOM pools than the anoxic incubation to which the samples were exposed to. Molecular changes in SPE-sedDOM pools during anoxic incubation comprised a gradual transformation of both reduced and oxidized DOM (fatty acid like and carboxylic rich DOM, respectively) to fulvic acid and tannin-like DOM with intermediate oxidation state of carbon.

5.2. Introduction

Boreal forests contain large portions of the total carbon stored in forest vegetation and soils (Dixon et al., 1994). The boreal region also accounts for more than half of European natural lakes (European Environment Agency, 2003) and presents the highest concentration of lakes and highest proportionate surface area on the planet (Verpoorter et al., 2014). This is relevant for carbon cycling, because freshwater ecosystems carry appreciable abundance of dissolved organic matter (DOM) and have been considered hotspots for carbon cycling (Battin et al., 2009; Cole et al., 2007). Organic carbon carried from terrestrial environments to water bodies, primarily in a dissolved state (Kortelainen et al., 2006), can build up boreal lake sediments via flocculation and subsequent sedimentation of DOM (Gudasz et al., 2012; von Wachenfeldt et al., 2008; von Wachenfeldt and Tranvik, 2008).

Catchment parameters, such as area and land coverage, are expected to influence DOM composition (Kellerman et al., 2014), microbial cycling and production of greenhouse gases resulting from aerobic and anaerobic processing of DOM in boreal lakes (Duc et al., 2010; Sobek et al., 2009). Parameters intrinsic to lakes like surface area and water residence time (WTR) also influence DOM concentration and composition (Evans et al., 2017). While autochthonous organic matter deposition is an important source of labile carbon in the sediments (Casper et al., 2003; Schulz and Conrad, 1995), terrestrial input was considered to be less labile to microbial activity (Bianchi, 2011).

The specific molecular fingerprint of DOM depends on the ecosystem characteristics and, therefore, forest DOM would supply a different signature than that of rivers, for example (Roth et al., 2014). Previous investigation of DOM persistence in boreal lakes have shown that reduced, aliphatic, and N-containing compounds could be more resistant to degradation, accumulating in freshwater systems, whereas oxidized, aromatic compounds would be removed (Kellerman et al., 2015). Recent research, however, has proposed that, despite its assumed recalcitrance, allochthonous DOM promoted increased microbial growth when compared to autochthonous material (Stadler et al., 2020). In this context, it would be valuable to understand the sources and characteristics of persistent DOM in lake sediments, since most of the degradation processes occurs in that compartment (Jonsson et al., 2001).

DOM processing depends on hydrological and element cycles under respective ecosystem conditions (Battin et al., 2009; Tranvik et al., 2018). Because the dissolved material is more available than particulates to microorganisms, DOM represents one of the largest exchangeable reservoirs of organic material in the planet, acting as both substrate, source of nutrients, and electron acceptor for several biological and photochemical pathways (Carlson and Hansell, 2015). Under anaerobic conditions, such as commonly found in lake sediments, DOM can be a substrate for production of greenhouse gases CO_2 and CH_4 (e.g. by methanogenesis) (Bastviken, 2009; Bastviken et al., 2004). It has been estimated that anaerobic carbon degradation contributes to 20 – 60% of total carbon mineralization rates in freshwater environments, with methanogenesis making up to 80% of microbial activity (Ward et al., 2017).

DOM can be resolved into individual molecular compositions by ultrahigh resolution Fourier Transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) (Gonsior et al., 2013; Schmitt-Kopplin et al., 2012). FT-ICR-MS has been previously used to study the spatial variations in DOM composition within a boreal catchment (Ide et al., 2017), to describe the relationship between DOM chemodiversity in Swedish lakes and hydrology and climate parameters (Kellerman et al., 2014), and also to demonstrate the influence of temperature and pH in soil DOM (Roth et al., 2015). Molecular level differences between SPE-DOM in the water column and the sediment pore water from ten boreal lakes have also been described (Valle et al., 2020), as well as changes in SPE-DOM during anaerobic degradation for three of the ten boreal lakes (Valle et al., 2018). In this study, highly oxygenated and reduced compounds (lipids) were depleted during incubation, while fulvic acid-like compounds were produced. However, the molecular patterns of DOM processing during anoxic incubation apparently depended strongly on lake characteristics. Thus, further investigation is needed to understand the mechanisms acting on DOM transformation and persistence on a larger scale.

Here, we used electrospray ionization (ESI) FT-ICR-MS to characterize DOM molecular composition from sediment pore water from ten boreal lakes before and after anoxic incubation, and measured production of greenhouse gases CO_2 and CH_4 during the experiment. We also determined the extension and land coverage of the catchments. By applying these methods and acquiring other relevant physicochemical parameters, we aimed at answering the following research questions: (a) how does the overall chemodiversity of DOM varies between

different boreal lake sediments; (b) what are the significant environmental parameters influencing DOM composition in the studied system; c) how do these parameters influence DOM mineralization into CO_2 and CH_4. Comprehensive understanding of these relationships would substantially improve our current knowledge of carbon cycling in sediments.

5.3. Material and Methods

5.3.1. Site description

We collected water column and sediment samples from ten boreal lakes in the Malingsbo area, Sweden, in August 2012 (Figure 5.1). Description of the investigated lakes can be found in Table 5.1.
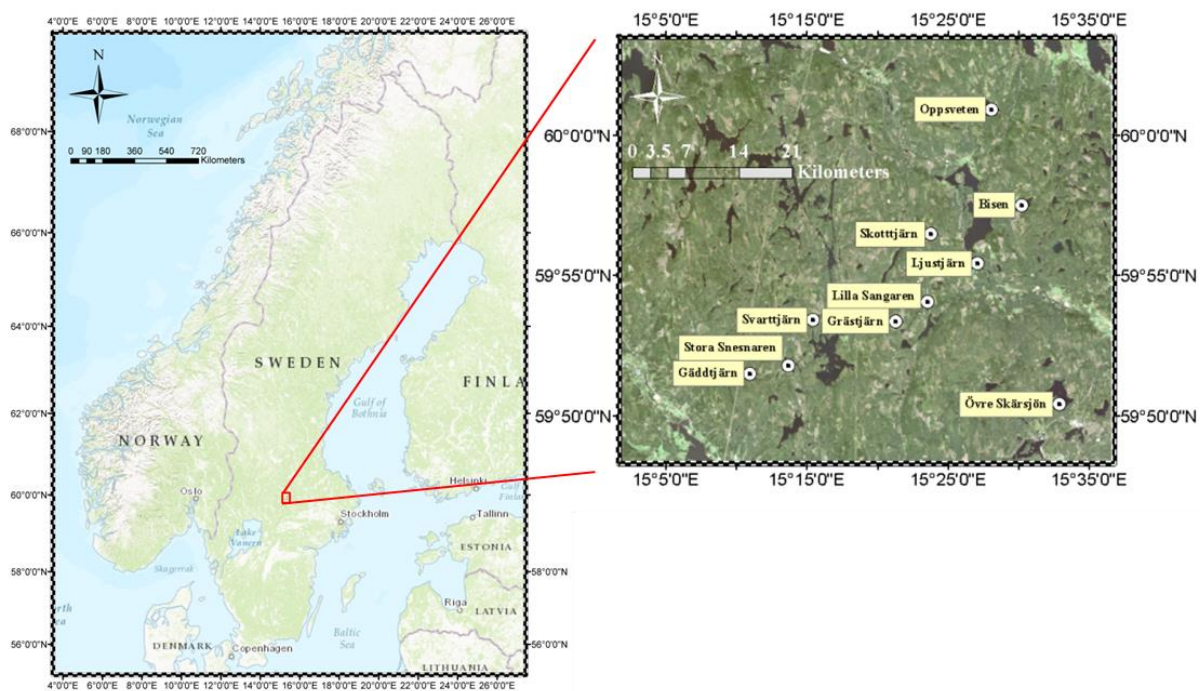
Figure 5.1. Map of sampling area (Malingsbo, Sweden), with detailed location of the ten boreal lakes studied.

5.3.2. Sampling

We sampled from a boat positioned approximately at the deepest part of the lake. A gravity corer was used to take five superficial (first 10 cm) sediment samples from each lake, approximately at the same location. Sediment samples were homogenized and transferred into 125 ml polyethylene bottles, which were carefully filled, avoiding air bubbles inside. The sediment bottles were kept cold (4 ℃) and stored in the dark, before shipped by air to the Max Planck Institute for Terrestrial Microbiology (Marburg, Germany) for further experiments.

5.3.3. Land coverage determination

The watershed area, as well as the percentage of cover by wetland, forest, open water, and open land (Table 5.1), were calculated using ArcGIS software, version 10.3.1, with the Swedish National DEM (2m raster). First, we ran the Flow Direction tool to get flow direction rasters (https://desktop.arcgis.com/en/arcmap/10.3/tools/spatial-analyst-toolbox/how-flow-direction-works.htm). Then we used the flow direction raster in combination with a basemap (aerial photography) to identify outlets (called pour-points in ArcMap) for each lake. Lastly, we ran the Watershed tool using the flow direction raster and the pour-points as input (https://desktop.arcgis.com/en/arcmap/10.3/tools/spatial-analyst-toolbox/watershed.htm).

5.3.4. Chemical analysis

In laboratory, samples were submitted to solid phase extraction (SPE). Detailed SPE-sedDOM extraction was described elsewhere (Valle et al., 2018). The eluates were stored in the freezer (-20 ℃) until mass spectrometry analysis. Moreover, we analyzed water samples from the lakes for Dissolved Organic Carbon (DOC), Total Nitrogen (TN) and Total Phosphorus (TP) (Valle et al., 2020). Organic carbon (sed OrgC) and nitrogen (sed N) content in the sediments was measured in one of the replicates of each sample, which was acidified (to remove possible carbonates) and dried. Samples were analyzed at the Centre for Stable Isotope Research and Analysis (KOSI) at Göttingen University (Germany) using an elemental analyzer (NA 2500, CE Instruments, Rodano, Italy).

5.3.5. Incubation experiment

An analogous incubation experiment has been described elsewhere (Conrad et al., 2011, 2007; Valle et al., 2018). Approximately 9 ml of sediment were placed into 27 ml sterile glass vials

and closed with rubber stoppers, with anoxic headspace (N_2). Then, the sediments were incubated in the dark at 15°C. The observed CH_4 and CO_2 production rates represent the potential degradation of the microbial community and allow for timescales practical in laboratory experiments (Hodgkins et al., 2014). During the incubation period of 40 days, CH_4 and CO_2 concentrations were measured regularly (Figure 5.2 and Table S1) by gas chromatography as described before (Conrad et al., 2011; Valle et al., 2018).

Table 5.1. Physico-chemical and environmental parameters of the ten boreal lakes investigated.

	Gäddtjärn	Stora Snesnaren	Svarttjärn	Grästjärn	Lilla Sången	Skotttjärn	Ljustjärn	Bisen	Oppsveten	Övre Skärsjön
water TN (μg l^{-1})	392	214	502	335	275	632	169	281	444	402
water TP (μg l^{-1})	9	6.1	15.1	8.7	11.4	15	6.5	6.5	9.2	8.2
water DOC (mg l^{-1})	15.5	10.1	25.7	17.4	6.8	24.9	3.9	7.3	19	11.2
water pH	4.5	5.6	4.8	4.9	6.3	4.6	6.3	6.1	NA	5.5
max depth (m)	10	26	7	8	20	5	9	17	11	32
lake area (ha)	7	48	0.7	9	24	3	12	43	65	165
sed OrgC (%)	24.6	16.4	8.5	17.4	18.2	26.7	34.8	13	21.2	10.6
sed N (%)	1.5	1	0.4	1.1	1.2	1.8	2.1	0.9	1.3	0.7
sed. water content (%)	91.4	92.1	79	90.1	90.8	92.3	93.5	91.5	91.4	88.9
δ^{13}Corg (‰)	-28.5	-28.3	-28.1	-29.3	-28.2	-30.1	-31.5	-27.7	-28.4	-27.4
watershed area (ha)	226	1338.2	146.7	101.6	184.6	80	40.8	440	1723.2	893.9
wetland (%)	4.3	4.3	5.3	8.4	1.7	6.8	0.7	3.5	7.4	9.8
forest (%)	92.2	78.1	93.3	82.5	83.6	83.3	67.9	82.5	87.9	69.0
water (%)	3.5	16.5	1.4	9.1	13.2	3.4	31.4	9.8	3.9	20.2
open land (%)	0	1.2	0	0	1.4	6.6	0	4.3	0.8	1.1

5.3.6. FT-ICR mass spectrometry

Detailed description of the mass spectrometry methods can be found elsewhere (Valle et al., 2020, 2018). We used a 12T Bruker Solarix mass spectrometer (Bruker Daltonics, Bremen, Germany) and an Apollo II electrospray ionization (ESI) source in negative mode (Hertkorn et al., 2016) to acquire Fourier transform ion cyclotron resonance [ESI(-)] FT-ICR mass spectra. SPE samples were injected using a 223 Sample Changer - Gilson, Inc (Middleton, USA) with an adapted in-house made software to FT-ICR-MS. Although our results might be biased by the choice of negative ionization alone (Hertkorn et al., 2008), we believe this source would provide suitable results for comparison of our samples, since it has been shown that molecular formulas of natural organic matter (NOM) obtained with ESI (-) presented larger coverage of molecular diversity within the van Krevelen diagrams than molecular formulas derived from positive ion mode.

Data processing was done using Compass Data Analysis 4.1 (Bruker, Bremen, Germany) and formula assignment by an in-house made software (NetCalc) (Tziotis et al., 2011). Molecular formulas were assigned to peaks with a minimum signal-to-noise ratio of 3. The molecular formula assignments were based on the following elements: $^{1}H_{0-200}$, $^{12}C_{0-100}$, $^{16}O_{0-80}$, $^{32}S_{0-3}$, $^{14}N_{0-3}$ as well as the $^{13}C_{0-1}$ and $^{34}S_{0-1}$ isotopomers. The generated formulae were validated by setting sensible chemical constraints [N rule, O/C ratio ≤ 1, H/C ratio $\leq 2n + 2$ (C_nH_{2n+2})]. We restricted nitrogen and sulfur atoms to three based on previous studies of NOM where no mass assignments with more than three atoms from both were regularly observed (Hertkorn et al., 2013; Schmitt-Kopplin et al., 2011).

Based on the similarity level of the replicates as computed by Hierarchical Cluster Analysis (HCA), we defined our data set with the three replicates that were more similar and filtered the assigned mass peaks that were common to these replicates (Table S2). Respective bulk parameters were computed from mass peak intensity weighted value. The final assigned molecular formulas were categorized into groups containing CHO, CHNO, CHOS and CHNOS molecular compositions, which were used to reconstruct the group-selective mass spectra (Schmitt-Kopplin et al., 2012).

Van Krevelen diagrams (van Krevelen, 1950) were used for visualization of the data. Based on their elemental ratios, commonly H/C versus O/C derived from assigned molecular formulas,

each data point is a projection of a given elemental ratio regardless of the molecular mass. Main chemical classes found in DOM often show characteristic H/C and O/C ratios and cluster within specific regions of van Krevelen diagrams (Kim et al., 2003; Rivas-Ubach et al., 2018). KMD/z* diagram is also a useful visualization tool (Shakeri Yekta et al., 2012; Valle et al., 2020). Here, the Kendrick mass defect (KMD) (Hughey et al., 2001) is divided by an empirical, integer sorting parameter z*, introduced by Hsu et al. (1992) which is defined by the molecular moiety under investigation. Molecular compositions with identical KMD/z* represent unique homologous series.

5.3.7. Statistical analysis

We used the Hierarchical Cluster Explorer 3.0 (http://www.cs.umd.edu/hcil/multi-cluster/) to perform HCA. The clustering of the dataset was done using Average Linkage (UPGMA) method and the Pearson correlation coefficient (Pearson's r) as the similarity/distance measure. Based on the HCA, we used the "profile search" tool from HCE 3.0, choosing a search method (model-based), a distance measure (Pearson's r) and a threshold (0.9). With these settings, we selected mass peaks that were increased in relative abundance in a chosen subset but decreased in another one (Valle et al., 2018). Extraction of individual features from each lake was done, because no meaningful information was recovered when trying to select formulas that were increased in each of the sub-groups defined by statistical analysis. Principal Component Analysis (PCA) and Partial Least Square (PLS) analysis were performed with the software SIMCA-P 9.0 (Umetrics AB, Sweden). Paired T-test and Correlation were performed on Excel (Microsoft Office).

5.4. Results

5.4.1. Environmental parameters

The ten boreal lakes investigated in this study demonstrate a broad range of physico-chemical parameters of lake waters and sediments, as well as the differences in size and land coverage within their watersheds (e.g. Attermeyer et al., 2018; Grahn et al., 2006; Groeneveld et al., 2020; Gudasz et al., 2015; Koehler et al., 2012; Peter et al., 2017; Steger et al., 2011; von Wachenfeldt and Tranvik, 2008) (Table 5.1). DOC content in the water column ranged between 3.9 and 25.7 mg l^{-1}. TN and TP varied between 169-632 µg l^{-1} and 6.1-15.1 µg l^{-1}, respectively.

Whereas most of the lakes presented majority of the watershed covered with forests (69-93.3%), the area covered with open waters ranged between 1.4 and 31.4%.

Regarding sediment parameters, the boreal lakes presented more similar values. With a few exceptions, most of the sediments had pronounced water content (>90%) and a relatively similar sed N; sed orgC was more variable, ranging between 8.5 and 34.8%, while the δ^{13}Corg values were on average 28.75‰ with more limited variation. Isotopically light organic carbon (~32‰) is often measured in eutrophic lakes with anoxic hypolimnion, where it is probably caused by sedimentation of anaerobic microbial biomass, whereas higher values (~26‰) can be found in oligotrophic lakes (Conrad et al., 2009, 2007).

5.4.2. Greenhouse gas production experiment

The evolution of greenhouse gases production during the 40 days incubation are depicted in Figure 5.2. The sediment incubation experiments showed almost instantaneously CH_4 production, starting without any lag phase in all replicates. This is a sign of the absence of other electron acceptors (e.g. nitrate, sulfate) in the sediments, since their presence inhibits methanogenesis (Conrad, 2002). Compared to tropical and temperate lakes (Conrad et al., 2010, 2009), our methanogenesis rates were extremely low. Nevertheless, measurements done by Duc et al. (2010) in lake Svarttjärn have shown similar CH_4 production rates as ours (9 nmol d-1 gdw-1 at 10 ℃).

Our experiments also revealed an unequal production of CO_2 and CH_4 (Table S1). CH_4 production rates accounted for maximal 15% of CO_2 values for all lake sediments. Additionally, the ratios of CO_2 and CH_4 production did not behave uniformly among the boreal lakes. Skotttjärn, for example, presented the highest CH_4 production rate (26.14 ± 2.25 nmol gdw-1 d-1), more than double the average methane production of the other lakes. Its CO_2 production rate (171.09 ± 11.96 nmol gdw-1 d-1), however, was lower than two other lakes in this study.

Lake Skotttjärn is the shallowest lake in the study (Table 5.1). In contrast, most of the deepest lakes presented lower CH_4 production rates: Stora S., Övre S., and Bisen. In fact, maximal depth was negatively correlated with CH_4 production (r = –0.67). CO_2 production rates, on the contrary, were higher in lakes Stora S. and Ljustjärn, but there was no significant correlation between CO_2 production rates and lake depth.

Both CH4 and CO2 production rates were positively correlated with sed OrgC and sed N. For CH4 production, a slightly positive correlation was detected, with $r = 0.58$ and $r = 0.61$, for sed OrgC and sed N, respectively. As for CO2 production, the positive correlation was stronger, with $r = 0.80$ for sed OrgC and $r = 0.78$ for sed N. Although CH4 and CO2 production rates were not strongly correlated with the concentrations of nutrients in the water, the ratio CH4/CO2 was positively related with all parameters (TN: $r = 0.80$, TP: $r = 0.90$, and DOC: $r = 0.82$).
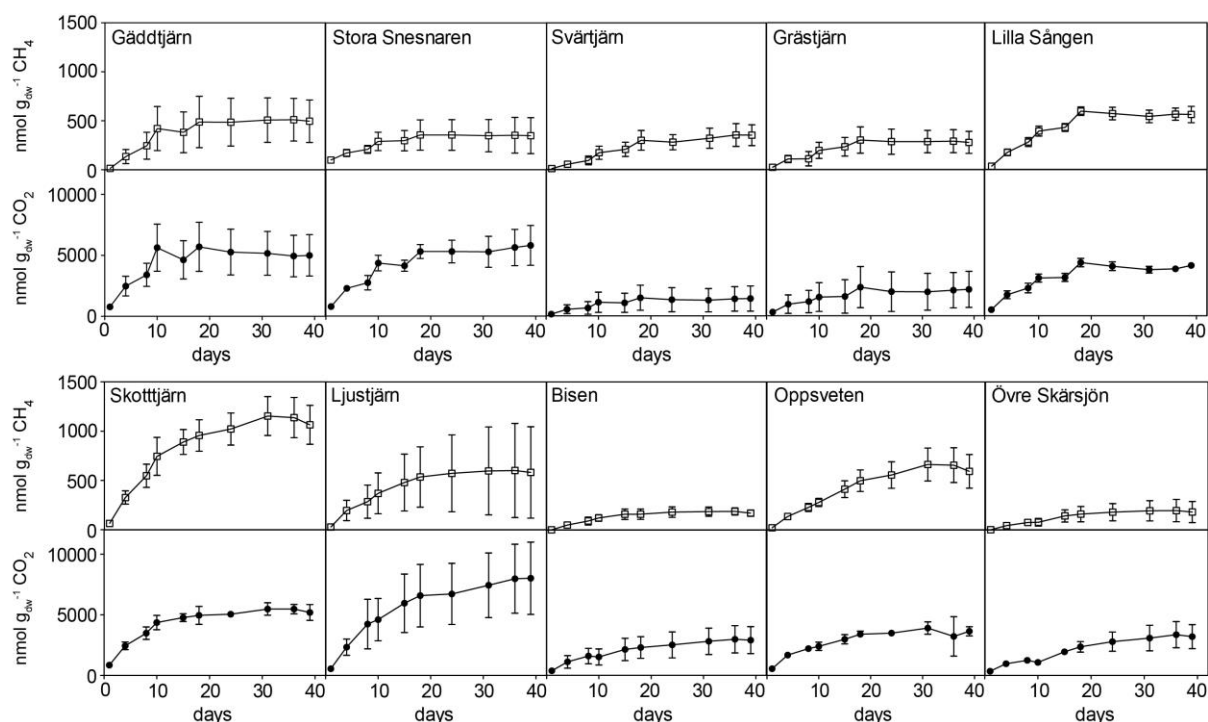
Figure 5.2. CH_4 and CO_2 concentrations during the anaerobic incubation experiment for ten boreal lakes. Values shown are average from five replicates and error bars represent standard error.

5.4.3. Compositional characteristics of SPE-DOM

Molecular compositions of lake sediment pore water SPE-DOM before and after anoxic incubation experiment were identified by means of high mass accuracy and mass resolution typical of FT-ICR mass spectrometry (Hertkorn et al., 2012, 2008, 2007; Schmitt-Kopplin et al., 2012). We will refer to SPE-sedDOM before incubation as "original", whereas SPE-sedDOM pools after incubation are named "incubated".

Overall, spectra showed increased molecular diversity among all samples following incubation. More than 4000 molecular identified formulae were detected for each lake, both before and

after the incubation experiment (Table S2). Given the inevitable heterogeneity within sediments concerning pore water DOM, microbial abundance and diversity, and the course of incubation, reproducibility of mass peaks was also high, with an average of 74% of shared molecular formulas among three replicates for each lake sediment sample. The presence of unique compounds in each replicate, however, corroborates an appreciable chemical diversity and heterogeneity of carbon cycling in these sediments (Krause et al., 2009; Siljanen et al., 2011).

On average, CHO compounds accounted for 63% of the assigned formulas for each lake sediment. Lake Ljüstjärn incubated presented the lowest proportion, with only 49% of CHO compounds within its SPE-DOM pool. Among all lake sediments, CHOS and CHNO compounds represented, on average, 12% and 25% of the SPE-DOM pool, respectively.

5.4.4. Clustering of lakes based on molecular compositions of SPE-DOM

FTMS-based PCA and HCA of molecular compositions of original and incubated SPE-sedDOM clustered the ten investigated lakes based on the relative abundance of molecular assignments of SPE-DOM pools (Figure 5.3, Figure S1). First, all common molecular assignments between original and incubated samples were analyzed. Here, van Krevelen and mass-edited H/C diagrams, as well as the difference FTMS spectra, showed compounds with higher relative abundance in incubated and original SPE-sedDOM (Figure 5.3). Especially for CHO and CHNO compounds, the van Krevelen diagrams showed a somehow complementary distribution. While original samples presented CHO compounds with rather average O/C and H/C ratios (central distribution in the diagram), the incubated samples presented higher abundance of CRAM and also highly oxygenated and unsaturated compounds (lignin- and tannin-like). For CHNO compounds, there is a clear shift on the oxygenation: original samples presented higher abundance of molecules with increased O/C ration, whereas incubated samples showed increased proportions of molecules with lower O/C ratios (Figure 5.3).

Second, PCA and HCA clustering were computed separately within original and incubated samples (Figure S1; panels A-D). Van Krevelen and mass-edited H/C ratio diagrams of molecular formulas depicted the increased relative abundance in each lake in relationship to the others (Figure S1; panels E-H). Here, lakes were clustered in five different groups by both PCA and HCA, with two of them comprising only a single lake each (group 1: Ljustjärn; group

2: Bisen). The other groups distributed as follows: group 3 (Gäddtjärn, Grästjärn, and Lilla S.), group 4 (Stora S. and Övre S.), and group 5 (Svarttjärn, Skottjärn, and Oppsveten) (Figure S1). Most interestingly, SPE-sedDOM from original and incubated sediments clustered similarly when statistical analyses were done for both samples simultaneously and in the same five groups for individual statistical analysis, indicating that intrinsic characteristics of each lake were more relevant for classification than the degradation status of the SPE-DOM pools (Valle et al., 2018).

Figure 5.3. FTMS-based PCA (panel A) and HCA (panel B) of molecular compositions common to original and incubated SPE-sedDOM, among all ten lakes using normalized spectra (total mass peak amplitude = 100%). Panel C: difference FTMS spectra showing compounds with higher relative abundance in incubated SPE-sedDOM (purple; positive amplitude), and original SPE-sedDOM (blue; negative amplitude). Van Krevelen and mass-edited H/C diagrams showing CHO, CHNO, CHOS, CHNOS compounds which were relatively more abundant in original SPE-sedDOM (Panel D) and which were relatively more abundant in incubated SPE-sedDOM (Panel E).

Moreover, replicates from each sample were close together, indicating a good reproducibility of our method. Another interesting aspect is that some groups ended up being closer to each other after incubation (*groups 3, 4 and 5* – Figure 5.3, Figure S1). This could be an indication that microbial degradation of DOM happens similarly across lakes, resulting in SPE-DOM pools which are more resembling to each other than the original pools. *Groups 1* and *2*, which comprise Ljustjärn and Bisen, respectively, can be considered some sort of exception among the boreal lakes investigated in this study.

Ljustjärn is known for being influenced by groundwater (von Wachenfeldt and Tranvik, 2008), which is so far unknown for the other lakes. Besides, the rather low input of OM from a tiny drainage area results in decreased concentration of DOC and light color of the water. Moreover, based on past coring, we know Ljustjärn sediments have a peaty layer below the sand layer. There must have been extensive benthic macrophyte growth or floating sphagnum mats that sank to the bottom at some point of its history. On a molecular level, Ljustjärn presented an increased amount of CHOS compounds of lower saturation and oxygenation, which were not highly abundant in the other lakes (Figure S1).

Bisen, on the other hand, is one the largest lakes in our study; even larger were Övre S. (165 ha) > Oppsveten > Stora S. > Bisen (43ha) (Table 5.1). Since WTR is, among other factors, a function of area and depth, it followed the same gross order. Perhaps because of unique characteristics of its watershed and the almost 83% of pine forest land coverage, Bisen presented an increased amount of highly oxygenated lignin- and tannin-like compounds, higher than the other lakes (Figure S1).

Group 3 represented rather average lakes (Gäddtjärn, Grästjärn, and Lilla S.). About all the physico-chemical and hydrological parameters of these lakes showed near average distribution (Table 5.1). Similarly, the molecular signature of SPE-DOM pools from these lakes also did not show peculiarities that would differentiate them from the other lakes investigated (Figure S1), except an increased relative abundance of rather unsaturated CHO compounds.

Group 4 contained the rather large lakes Stora S. and Övre S., which also have relatively large watershed areas and higher proportions of water coverage in their catchments (Table 5.1). Van Krevelen diagrams (Figure S1) demonstrated increased amounts of compounds with higher levels of saturation and oxygenation, especially among the incubated samples for both lakes.

Group 5 contained the lakes with highest values for water DOC, TN, and TP – Svarttjärn, Skottjärn, and Oppsveten (Table 5.1), and presented a rather restricted distribution of heteroatoms in the center area of the van Krevelen diagram at the near average H/C and O/C ratios (Figure S1). Svarttjärn showed a particularly increased proportion of highly saturated and oxygen-deficient CHOS compounds in the original sample. Skotttjärn, on the other hand, presented increased amounts of lignin- and tannin-like CHO compounds on the incubated sample.

We have used HCA to identify mass peaks that were more abundant in each individual lake in relation the others (Figure S1). Gäddtjärn showed a similar distribution of specific compounds before and after incubation, with slightly broader coverage of the van Krevelen diagram after incubation, indicative of increased molecular diversity mainly in two groups: one of highly saturated and another of unsaturated compounds – in a range of O/C ratios (0.1 – 0.6). Stora S. presented a shift from more oxygenated compounds to a broader distribution from original to incubated samples. Svarttjärn had an increased proportion of lipid-like CHOS compounds before incubation, which were progressively lost during degradation.

On the contrary, Grästjärn, Lilla S., and Oppsvetten did not present significant compounds with increased relative abundance in relation to the other lakes, in both original and incubated samples. Skotttjärn, however, showed higher proportions of lignin-like compounds after incubation. Ljustjärn presented the same CHNO compounds before and after incubation, but there was an increase of CHOS compounds with low O/C ratios. Bisen showed a higher number of lignin-like compounds both before and after incubation (CHO and CHNO). Övre S. had a broader distribution of specific compounds with increased relative abundance before incubation, with compounds of incubated sample being more restricted to high saturation and mostly high oxygenation.

5.4.5. Influence of environmental parameters on the SPE-DOM composition

According to the PCA and HCA-based clustering, environmental characteristics obviously affected the molecular composition of SPE-sedDOM. Nevertheless, the goal was to deeply understand the influence of a given set of environmental parameters on specific mass assignments. Therefore, Partial Least Square (PLS) analysis was performed with both original and incubated SPE-sedDOM data (Figures 5.4 and 5.5). Original and incubated samples

presented similar results in PLS too. Based on that, we decided to extract the molecular formulas mostly related to each group of parameters and compare the original and incubated SPE-sedDOM pools for each case, based on their relative abundance.

Figure 5.4. FTMS-based PLS model from original SPE-sed DOM (panel A) and detailed (CHO; CHOS; CHNO; CHNOS) van Krevelen and mass-edited H/C diagrams of the corresponding molecular assignments which were extracted from the model and are influenced by the following parameters: watershed area, type of land cover, depth, area, and δ^{13}orgC (panel B); water DOC, TN and TP (panel C); sediment orgC and sediment N (panel D); sediment water content and pH (panel E).

First, we had a closer look into the parameters: watershed area, type of land coverage, lake area and depth, and sedimentary δ13orgC. Original SPE-sedDOM showed high abundance of highly oxygenated CHO and CHNO molecules, such as lignin- and tannin-like compounds (Figure 5.4), and rather oxygenated CHOS compounds. Incubated SPE-sedDOM also showed elevated proportions of highly oxygenated CHO and CHNO compounds, whereas the distribution of CHOS compounds on the van Krevelen diagram covered a broader range of O/C ratios (Figure 5.5). These results agreed with the high proportion of forests among all catchment areas, since vascular plants are the main source of lignin.

Second, we extracted molecular assignments which were more related with the water concentration parameters (DOC, TN, and TP). Here, for original SPE-sedDOM, we can observe that among CHO compounds, there is a somehow restricted distribution in some areas with unsaturated and/or low oxygen content, such as lignin-like and CRAM (Figure 5.4). Among CHOS compounds, we can identify lipid-like and regular NOM signatures, whereas CHNO compounds are unspecific. As for incubated SPE-sedDOM, a similar pattern was observed, with however loss of CHOS lipid-like compounds (Figure 5.5).

The third group of parameters considered here was sediment orgC and sediment N. Among original SPE-sedDOM pools, we could identify lipid-like CHO compounds, unspecific CHOS compounds, and CHNO compounds presenting common NOM signatures with rather low oxygen content and molecular weight (Figure 5.4). As for incubated samples, the presence of saturated CHO compounds, such as lipid-like, was more pronounced (Figure 5.5). Unspecific CHOS compounds with regular NOM signature and rather unsaturated CHNO compounds were also observed.

Last, we investigated the compounds which were more related to sediment water content and pH. Here, especially for original samples, very few assigned mass peaks were correlated with this group of variables (Figure 5.4). Only a small number of CHO and CHNO compounds were depicted in the van Krevelen diagrams, with no clear significant distribution. Compounds distribution was more diverse for incubated SPE-sedDOM (Figure 5.5). Here, highly saturated CHO and CHOS compounds were present, such as lipid-like, as well as rather unsaturated CHNO compounds.
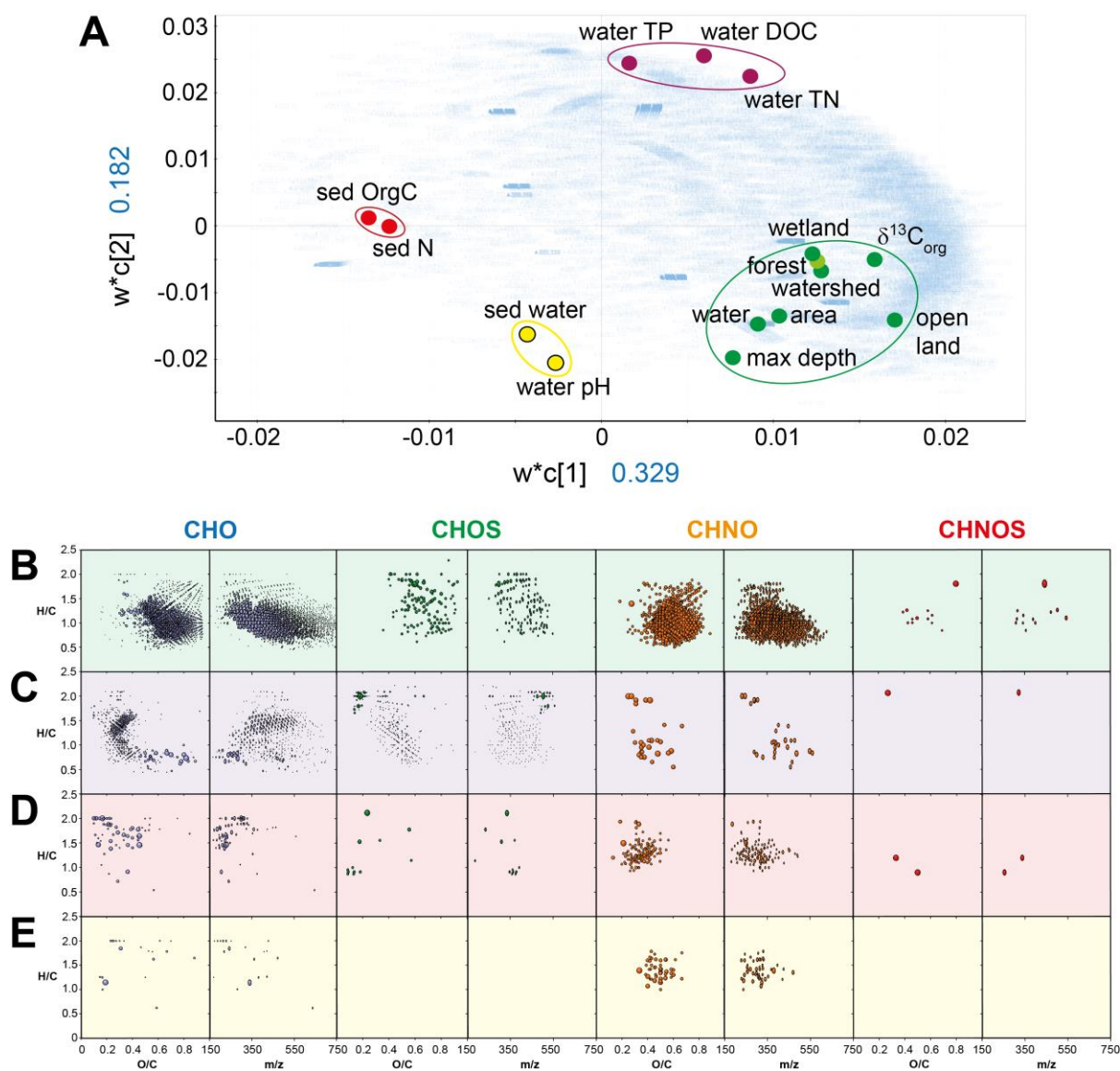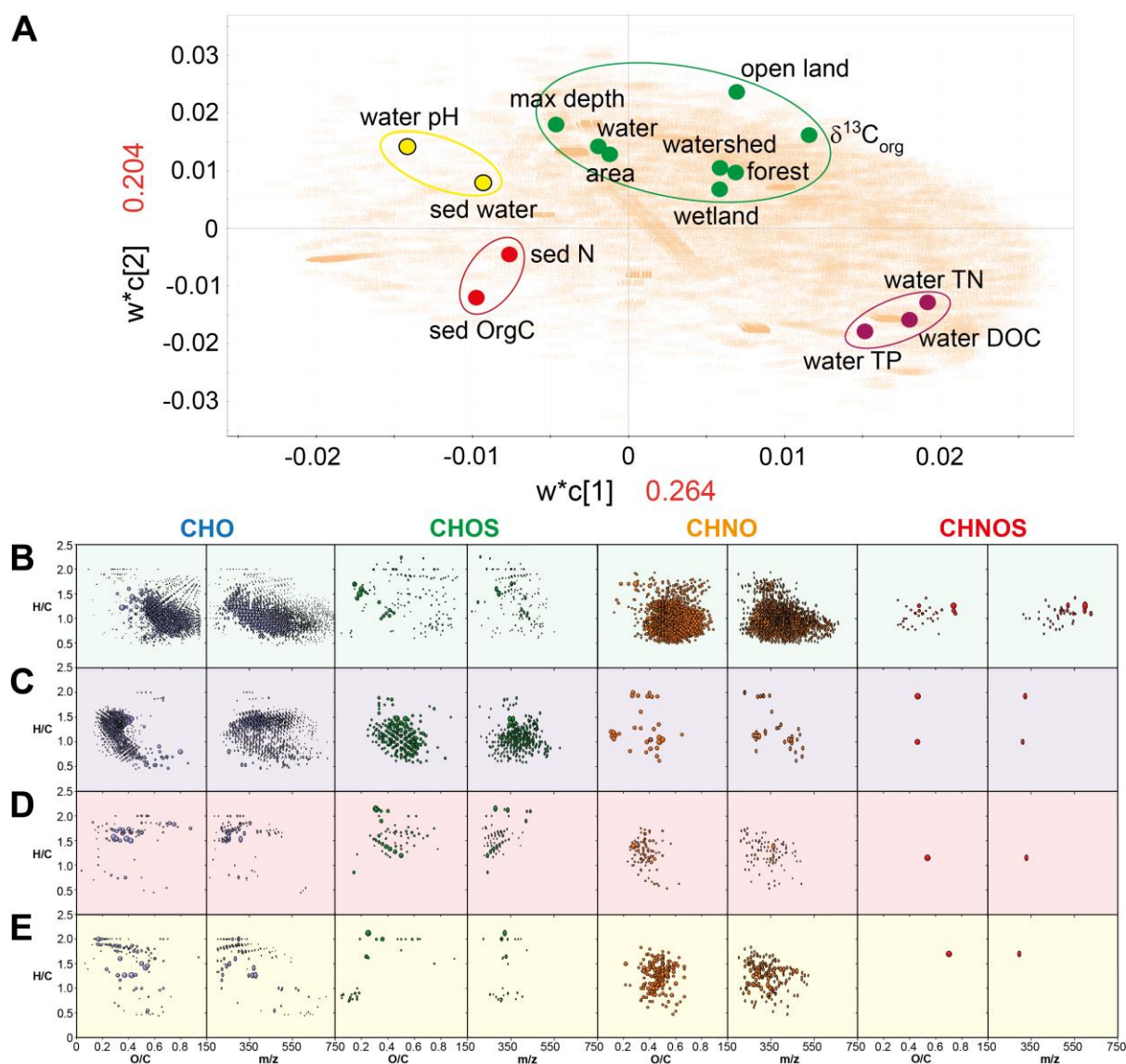
Figure 5.5. FTMS-based PLS model from incubated SPE-sedDOM (panel A) and detailed (CHO; CHOS; CHNO; CHNOS) van Krevelen and mass-edited H/C diagrams of the corresponding molecular assignments which were extracted from the model and are influenced by the following parameters: watershed area, type of land cover, depth, area, and δ13orgC (panel B); water DOC, TN and TP (panel C); sediment orgC and sediment N (panel D); sediment water content and pH (panel E).

5.4.6. Molecular changes in SPE-sedDOM pool during incubation

Summarized FTMS-derived van Krevelen (Figure S2), mass edited H/C (Figure S3), and KMD/z* versus m/z diagrams (Figure S4) from original, consumed, produced, and incubated SPE-sedDOM can be seen on the Appendix. Consumed and produced compounds were determined by means of HCA (Valle et al., 2018). Except for Svarttjärn, Övre S., and Oppsvetten (which number remained virtually the same), all SPE-sedDOM pools showed increased numbers of assigned mass peaks after incubation, accompanied by decrease of the mass weighted average. Increased chemodiversity of SPE-sedDOM pools after incubation was observed, especially regarding CHOS and CHNO compound, which were also more abundant in incubated samples.

FTMS-based van Krevelen and mass-edited H/C diagrams of the sum of unique molecular compositions present only in original and only in incubated samples – depicted according to CHO, CHOS, CHNO, and CHNOS molecular series – can be seen on Figure S5. Here, despite extensive averaging, mass assignments distribution in the van Krevelen diagrams showed again an increased molecular diversity in samples after incubation.

Despite the dominance of CHO compounds in all SPE-DOM pools, computed average H/C and O/C ratios showed that incubation caused an overall trend of increasing saturation and decreasing oxygenation (Table S2). The molecular changes in SPE-sedDOM from ten boreal lakes during incubation agreed with results from boreal lakes Svärttjärn, Grästjärn, and Bisen (Valle et al., 2018). Eight out of ten lakes (exceptions were Oppsvetten and Övre S.) showed significant differences between assigned mass peaks of original and incubated samples (T-test, $p < 0.05$). The alternate display of common molecular compositions between original and incubated SPE-sedDOM bulk characteristics (Table S2) produced sawtooth-like patterns indicative of rather regular variations between those materials for nine lakes (Valle et al., 2020), again with greater exception of lake Ljustjärn (Figure S6).

The average H/C and O/C ratios presented an opposite trend, with the H/C ratio being usually higher on the incubated samples, whereas the O/C ratio was higher on the original samples. The relative proportions of CHO compounds were larger in the original SPE-sedDOM, whereas the relative proportions of heteroatoms were increased in incubated samples. DBE generally increased after incubation, whereas the relative unsaturation as expressed by DBE/C ratio

decreased in most samples after incubation (Figure S6, Table S2). Differences in DBE/C ratios between original and incubated samples, however, was very subtle and would not have been possible with rounding the values.

In general, mass weighted average decreased from original to incubated pore water SPE-DOM, with exception of Skotttjärn, where average mass increased during incubation (Figure S6; Table S2). This indicated that in general, microbial degradation broke down larger molecules into smaller compounds. C/N and C/S ratios were generally decreasing after incubation, demonstrating the increase on molecular diversity with elevated proportions of heteroatoms after anaerobic microbial degradation. The higher abundance of CHNO and CHOS compounds in incubated sediment SPE-DOM likely resulted from the bioactivity during anaerobic degradation, which produced a succession of microbial metabolites (Herzsprung et al., 2010; Hodgkins et al., 2016, 2014; Roth et al., 2019, 2014; Seidel et al., 2014).

Lakes Skotttjärn and Bisen showed enhanced production/persistence of lignin- and tannin-like compounds. Lakes Stora S., Oppsveten and Övre S. presented some degradation of highly oxygenated CHO compounds, which also resemble lignin- and tannin-like compounds. Lake sediments from Gäddtjärn, Stora S., Lilla S., and to a lesser degree also Svarttjärn and Oppsveten, showed the production of more saturated compounds with lower O/C ratios.

5.4.7. Average oxidation state of carbon in CHO compounds during incubation

Another relevant projection of the CHO-compositional space is the depiction of the average oxidation state (OS_av) according to $OS\_av = 2 \times O/C - H/C$ (Kroll et al., 2011), which has been computed for individual CHO compounds before and after incubation (Figure 5.6). In agreement with previously published results (Valle et al., 2018), alternative indices like NOSC (nominal oxidation state of carbon; LaRowe and Van Cappellen, 2011) were not computed, because these would have required an estimate of heteroatoms (S and N) oxidation states, which are unknown at present.

Here, we sorted the OS_av values of original and incubated samples, and the difference "original minus incubated" in ranks of 0.1 width and distributed them in histograms to detect relative losses and gains of compounds according to redox status. All organic matter molecules detected in our study presented average oxidation states between -1.8 and +1.3 (Figure 5.6), in between the endmembers CH_4 (OS_av = -4) and CO_2 (OS_av = +4). In addition, the difference

"original minus incubated" was calculated to detect relative losses and gains of compounds according to redox status.

Interestingly, a common trend among all lake sediments was the preferential depletion of most oxidized CHO molecules, which contained several oxygen-containing functional groups. Except for Skottjärn, all CHO compounds in our SPE-sedDOM pools presented the same behavior. Two main groups were preferentially depleted during the incubation experiment: one contained high-energy substrates of considerable saturation (e.g. palmitic acid $C_{16}H_{32}O_2$, OS_av = -1.75 [lipid]; linoleic acid, $C_{18}H_{32}O_2$, OS_av = -1.55 [lipid]). The other contained molecules with elevated OS_av probably representing e.g. syringic acid, $C_9H_{10}O_2$, OS_av = 0 [aromatic acid]; acetic acid, $C_2H_4O_2$, OS_av = 0 [aliphatic acid]; glucose, $C_6H_{12}O_6$, OS_av = 0 [carbohydrate]; citric acid, $C_6H_8O_7$, OS_av = 1.167 [oxygenated aliphatic acid].
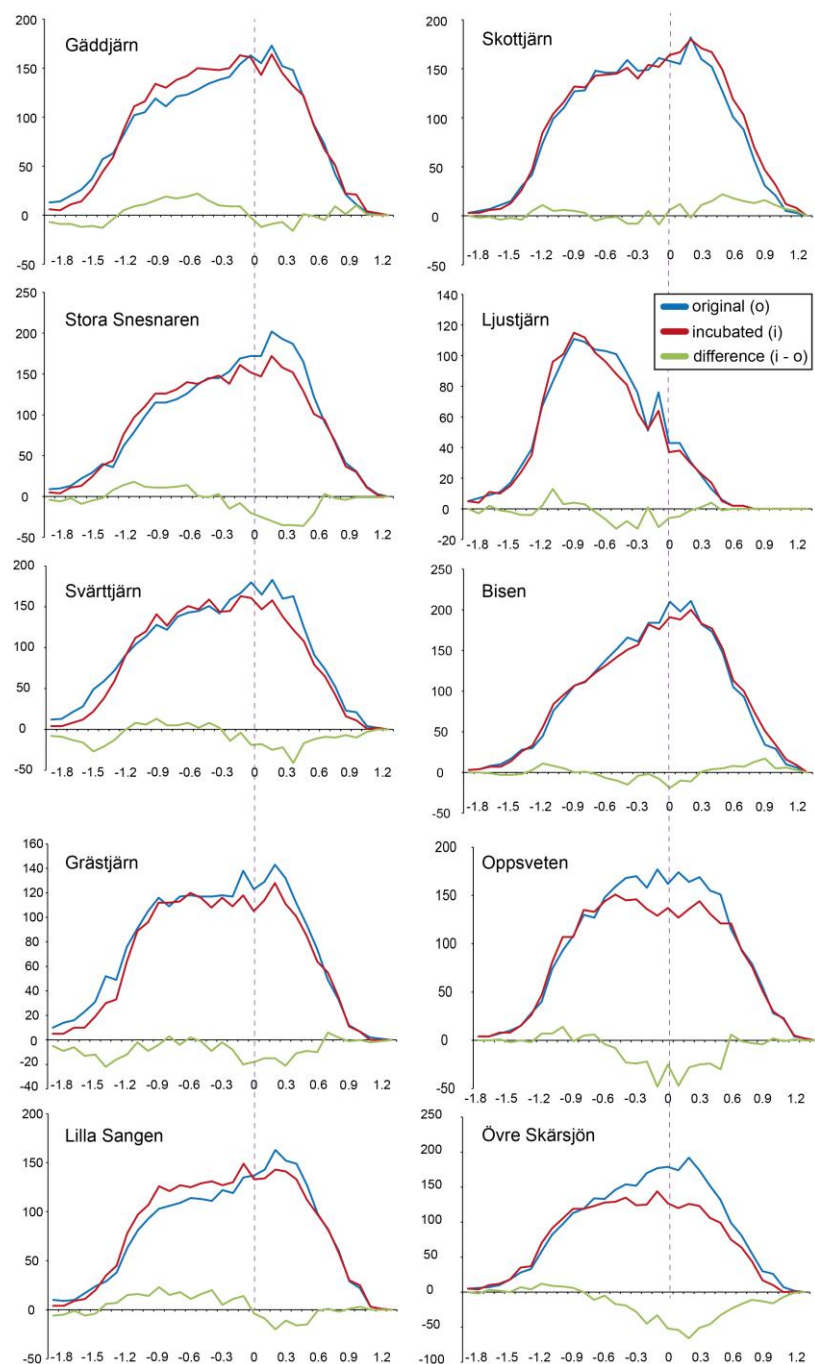
Figure 5.6. Distribution of average carbon oxidation state OS_av in CHO compounds only, sorted in increments of ±0.1 of nominal carbon oxidation state (Kroll et al., 2011) in 10 lake sediments before and after incubation; color code: blue, original SPE-sedDOM; red: incubated SPE-sedDOM; green: difference in counts: original minus incubated. It appears that the extent of relative changes progressively decreases with ever increasing lake size and water residence time. At the bottom, several reference compounds (common metabolites) are indicated: PalA, palmitic acid $C_{16}H_{32}O_2$, OS_av = -1.75 (lipid); linoleic acid, $C_{18}H_{32}O_2$, OS_av = -1.55 (lipid); FA, fulvic acid, OS_av = -0.52 (LaRowe and Van Cappellen, 2011); SyrA, syringic acid, $C_9H_{10}O_2$, OS_av = 0 (aromatic acid); HOAc, acetic acid, $C_2H_4O_2$, OS_av = 0 (aliphatic acid); glucose, $C_6H_{12}O_6$, OS_av = 0 (carbohydrate); CitA, citric acid, $C_6H_8O_7$, OS_av = 1.167 (oxygenated aliphatic acid).

5.5. Discussion

5.5.1. Microbial degradation as seen by greenhouse gases production

The immediate production of CH_4 is a sign of the absence of other electron acceptors (e.g. nitrate, sulfate) in the sediments, since their presence inhibits methanogenesis (Conrad, 2002). Compared to tropical and temperate lakes (Conrad et al., 2010, 2009), our methanogenesis rates were extremely low, but agreed with measurements done by Duc et al. (2010) in lake Svarttjärn. In fact, a study among almost 300 lakes has found that emissions of CH_4 from boreal lakes were lower than those from tropical lakes (Sanches et al., 2019).

The variability of CH_4 dynamics in boreal lakes has been shown before (Juutinen et al., 2008). The mechanisms behind the unequal production of CO_2 and CH_4, however, are still not completely clear. The most likely explanation for low CH_4/CO_2 ratios is that organic compounds such as humic acids serve as electron acceptors, allowing the oxidation of other organic compounds to CO_2 (Gao et al., 2019; Keller et al., 2009; Klüpfel et al., 2014). The oxidation of organic matter by electron acceptors such as O_2, NO^{3-}, Fe^{3+}, Mn^{4+}, SO_4^{2-}, and humic substances, as well as the partial fermentation of high molecular-weight into lower molecular-weight organic matter, are also potential sources of CO_2 in the sedimentary environment (Corbett et al., 2015).

Anaerobic microbial communities can use DOM as a surrogate for oxygen, and can transfer electrons generated from DOM mineralization onto reactive DOM molecules. These compounds feature reduction potentials midway to other electron acceptor systems (i.e. NO^{3-}, Fe^{3+}, Mn^{4+}, and SO_4^{2-}) such that DOM may mediate a range of anaerobic respiration pathways (Lau and del Giorgio, 2020). Formally, this is a disproportionation of bulk OM to reduced OM compounds plus CO_2 and thus, may be called fermentation of OM (Keller et al., 2009).

Other relevant findings from our results include the computation of average oxidation state of carbon in CHO compounds during incubation (OS_av). The loss of (the highly oxidized molecules) H_2O and CO_2 during anoxic incubation (in case of exclusive methanogenesis, H_2O would be taken up instead of being released) is a plausible reason for the apparent reduction of the residual organic matter. Precursor molecules will likely contain several oxygen-containing functional groups, making those more polar and reactive than common aliphatic compounds

(Burdige, 2007), and incubation produced metabolites that were on average more saturated and rather depleted in oxygen. Both features reduce OS_av.

Here, groups containing compounds with negative OS_av values and molecules with elevated OS_av were preferentially depleted during the incubation experiment (Figure 5.6). Fermenting organic matter with a markedly negative carbon oxidation state has been previously described during methanogenesis in boreal sediments (Clayer et al., 2020, 2018). The negative carbon oxidation state value corresponds more closely to a mixture of fatty acids and fatty alcohols than to carbohydrates (e.g. CH_2O), which would have yielded equivalent CH_4 and CO_2 production rates (Clayer et al., 2018). It has been proposed that at even more negative carbon oxidation state of the fermenting molecules, the CH_4/CO_2 production ratio is progressively increased (Arning et al., 2016; Clayer et al., 2018). Disproportional production of CO_2 and CH_4 was also observed in our study, but with an inversed pattern.

In accordance with our results, previous records demonstrated that anaerobically derived DOM was depleted in oxygen, showing a lower energy yield per unit C oxidized (LaRowe and Van Cappellen, 2011; Lau and del Giorgio, 2020). This means its selective preservation may offset a lower thermodynamic drive when using electron acceptors other than oxygen. The thermodynamic drive depends also on products, and not only on metabolic substrates; when products accumulate, reaction rates may decrease to maintain reaction equilibrium. Thus, microbes diversify the range of produced compounds trying to overcome this limitation, which in turn favors a higher diversity in both the aquatic microbial community and the resulting DOM chemical composition in anoxic habitats (Dadi et al., 2017; Lau and del Giorgio, 2020).

5.5.2. Zoom in: molecular insights into microbial degradation

Although a greater portion of molecular assignments were common between our replicates, the presence of unique compounds in each replicate corroborates an appreciable chemical diversity and heterogeneity of carbon cycling in these sediments (Krause et al., 2009; Siljanen et al., 2011), which possibly contributes to the high observed variability of CH_4 and CO_2 production rates. Previous experiments in the Amazonian floodplain have shown that bacterial community composition shifts are tightly coupled with DOM quality (Melo et al., 2020).

Like other studies in freshwater ecosystems, bulk sedDOM was dominated by unsaturated and phenolic compounds that appeared to persist in the environment over much longer timescales

than the labile pool (Figure S2) (Johnston et al., 2019; Kellerman et al., 2015). These findings suggest either a degradation pathway from more labile to more stable organic matter, or an overall persistence of highly unsaturated and phenolic compounds with rapid cycling of labile material (Johnston et al., 2019).

Our results presented SPE-DOM pools dominated by CHO compounds, similar to what has been found with lake sediment DOM obtained with different extraction methods (Kurek et al., 2020). Despite high proportions of CHO compounds when compared to original samples, increased proportions of CHOS and CHNO molecules were found in all incubated samples. Computed average H/C and O/C ratios showed an overall trend of increasing saturation and decreasing oxygenation during the incubation (Table S2). Analogous trends of DOM evolution with decrease of average oxidation and increase of saturation, accompanied by an increased relative abundance of CHNO and CHOS compounds has been described along a river course with external inputs from agriculture and wastewater (Kamjunke et al., 2019). In this study, bacterial abundance and activity were also positively related to more aliphatic components (H/C > 1). D'Andrilli et al. (2019) have performed incubations with different leachate sources, in which the biological processing of DOM presented different short- and long-term patterns. After two days of incubation, there was a decrease in heterogeneity accompanied by the increase in saturation. By the end of the 44 days experiment, however, hydrogen saturation was at its lowest point and abundance of heteroatoms was even larger than at the beginning of the experiment. Since we did not evaluate DOM composition changes in short time intervals, we cannot confirm that analogous behavior occurred in our incubations under anaerobic conditions.

Our results for the molecular changes in sedDOM from ten boreal lakes during incubation agreed with the results previously published by Valle et al. (2018). Here, all sedDOM samples showed decrease of the mass weighted average after the anoxic experiment, and preferential consumption of aliphatic compounds. Previous experiments have suggested an accumulation of aliphatic compounds (possibly leached from fresh plant material) during autumn, which were rapidly depleted in spring (Johnston et al., 2019). They have proposed that most of endmember source leachates contain more energy-rich aliphatic compounds compared to lake bulk DOM, suggesting rapid turnover of the labile pool sourced from leachate DOM (Johnston et al., 2019).

5.5.3. Zoom out: from catchment to lake sediment DOM

The increased proportion of highly oxygenated CHO (i.e. lignin- and tannin-like compounds) and CHNO molecules presented in our results agrees with the land coverage data, which revealed elevated proportion of forests among all catchment areas. Moreover, the apparent persistence of such compounds in lakes with larger areas and commonly larger WRT, agrees with the concept of slower degradation rates in such systems. It has been proposed that rates of both DOC production and consumption can be predicted from WRT (Evans et al., 2017). Their results suggested a dominant role of rapid light-driven removal in water bodies with a short WRT, whereas in water bodies with longer WRT, slower biotic production and consumption processes are dominant and counterbalance one another.

Selective degradation of DOM in a gradient of WRT has already been shown (Kellerman et al., 2014). There, relative abundance of compounds with increased saturation was inversely related with longer WRT, and such lakes were believed to host a greater proportion of autochthonous DOM. Our investigations on individual SPE-sedDOM pools for each lake showed that larger boreal lakes (Stora S, Lilla S., Bisen, Oppsveten, and Övre S.) presented mainly unsaturated compounds with relatively higher degree of oxygenation (data not shown). Johnston et al. (2020) have proposed that as lakes become more hydrologically isolated with longer WRT, photodegradation and relative dilution from autochthonous production decrease the allochthonous DOM to values typically seen in other hydrologically isolated environments such as saline prairie lakes.

Nevertheless, even if some lakes present longer WRT with slower or selective degradation of DOM, freshwater ecosystems can still be considered hotspots for organic carbon mineralization. The half-life of organic carbon is shorter in inland waters (2.5 ± 4.7 years) than in terrestrial and marine ecosystems (Catalán et al., 2016). The long-term stability of marine DOC is thought to emerge from basic physiological properties of the degrading microbial community, and the resulting interactions between the individual microbial members through substrate units (Mentges et al., 2019). Persistence of DOC could be explained by the following mechanisms: (a) if there is no supply of DOC, the microbes go almost extinct after the initial bloom and thus "leave behind" a residual concentration of DOC, and (b) if there is supply of DOC, microbes maintain a stable population on the long-term and therefore the DOC

concentration reaches a steady-state where uptake, release, and supply are balanced (Mentges et al., 2019).

But persistence of DOM in aquatic environments has also been thought to rely on biological transformation. Increased humification driven by bacterial activity has been reported in both marine (Lønborg et al., 2015; Ogawa et al., 2001) and freshwaters (Fasching et al., 2014; Stadler et al., 2020). Therefore, we know that degradation and persistence of DOM in freshwater ecosystem is not only dependent on the original DOM pool composition. Nevertheless, although general patterns of DOM consumption and transformation might be independent of source material, DOM composition plays a large role in ecosystem function on short- and long-term scales affecting the mechanisms of processing (D'Andrilli et al., 2019).

Our results also confirmed that, despite general trends on the molecular signature of DOM throughout the degradation process occur, in-lake specific characteristics, reflecting external (catchment basin) and internal input of organic matter, as well as the microbiology of each lake, are very relevant. Experiments in which model microbial communities were exposed to a terrestrially derived DOM substrate highlighted that different bacterial communities (i.e. different compositions) were able to degrade the same amount of DOM (Logue et al., 2016). Nevertheless, both the temporal pattern of degradation and, most importantly, the compounds that were degraded significantly different.

Stadler et al. (2020) have compared DOM reactivity between the inlet and outlet of a lake and found no clear lake effect on DOM biodegradability. These results contradicted emerged despite clear differences in DOM composition with high terrestrial influence in the inlet and pronounced autochthony at the outlet. It points toward the potential of high microbial plasticity regarding DOM consumption of various quality, supported also by the highest bacterial growth rates observed at the lake inlet (Stadler et al., 2020).

Because lake sediment is both influenced by autochthonous and allochthonous input of organic matter, different microbiology dynamics in both oxic and anoxic conditions, and complex mineral sorption mechanisms, it could be considered an intermediate compartment between water column and soil. In this context, our results are not only relevant for the understanding of freshwater ecosystems, but they also leave open questions for deeper investigation on universal concept of DOM cycling (Kothawala et al., 2021). Although our findings also suggest

that DOM initial composition is one of the major drivers in organic matter degradation and persistence (Catalán et al., 2021; Kellerman et al., 2015), we still could not define a unanimous role of microbial communities.

5.6. Conclusions

Catchment characteristics and biotic and abiotic lake-specific processing mechanisms produced an appreciable chemodiversity of sedimentary pore water SPE-DOM in ten Swedish boreal lakes, comprising a substantial core of ubiquitous DOM molecules at near average degree of oxygenation and unsaturation supplemented by a considerable range of individual molecules with an overall higher compositional diversity. Four key groups of influencing variables effected both the composition of sedimentary pore water SPE-DOM and its molecular alterations during anoxic incubation: nutrients and DOC in lake waters, sedimentary organic carbon and nitrogen, water content and pH, as well as lake and catchment characteristics.

Incubation caused rather non-selective large-scale molecular transformations of SPE-DOM that exceeded mineralization by several orders of magnitude. CH_4 and CO_2 production rates accounted for rather small fractions of sedimentary organic carbon (max. 4×10^{-4}) whereas incubation-induced changes of bulk and molecular parameters were in the percent range. Incubation caused depletion of highly oxygenated and oxygen-deficient molecules, and formation of heteroatom-rich CHNO and CHOS compounds, and presumably non-labile fulvic acid type substances. Overall, lake specific parameters determined not only open lake water SPE-DOM as previously recognized but also the individuality of its sedimentary pore water DOM composition and its susceptibility to anoxic processing. This implies that land management will have considerable effects on lake molecular and microbial diversity and that protective measures against release of harmful substances in all these ecosystem compartments will affect the entire food chain from bottom-up, i.e. from molecules to food webs.

5.7. References

Arning, E.T., van Berk, W., Schulz, H.-M., 2016. Fate and behaviour of marine organic matter during burial of anoxic sediments: Testing CH_2O as generalized input parameter in reaction transport models. Mar. Chem. 178, 8–21.

Attermeyer, K., Catalán, N., Einarsdottir, K., Freixa, A., Groeneveld, M., Hawkes, J.A., Bergquist, J., Tranvik, L.J., 2018. Organic Carbon Processing During Transport Through Boreal Inland Waters: Particles as Important Sites. J. Geophys. Res. Biogeosciences 123, 2412–2428.

Bastviken, D., 2009. Methane, in: Likens, G.E. (Ed.), Encyclopedia of Inland Waters. Elsevier, Oxford, pp. 783–805.

Bastviken, D., Cole, J., Pace, M., Tranvik, L.J., 2004. Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. Global Biogeochem. Cycles 18, 1–12.

Battin, T.J., Luyssaert, S., Kaplan, L. a., Aufdenkampe, A.K., Richter, A., Tranvik, L.J., 2009. The boundless carbon cycle. Nat. Geosci. 2, 598–600.

Bianchi, T.S., 2011. The role of terrestrially derived organic carbon in the coastal ocean: a changing paradigm and the priming effect. PNAS 108, 19473–81.

Burdige, D.J., 2007. Preservation of Organic Matter in Marine Sediments: Controls, Mechanisms, and an Imbalance in Sediment Organic Carbon Budgets? Chem. Rev. 107, 467–485.

Carlson, C. A., Hansell, D. A., 2015. Chapter 3 – DOM Sources, Sinks, Reactivity, and Budgets. In Biogeochemistry of Marine Dissolved Organic Matter (Second Edition). Elsevier Inc, Academic Press, 65–126.

Casper, P., Chim Chan, O., Furtado, A.L.S., Adams, D.D., 2003. Methane in an acidic bog lake: The influence of peat in the catchment on the biogeochemistry of methane. Aquat. Sci. - Res. Across Boundaries 65, 36–46.

Catalán, N., Marcé, R., Kothawala, D.N., Tranvik, L.J., 2016. Organic carbon decomposition rates controlled by water retention time across inland waters. Nat. Geosci. 9, 501–504.

Catalán, N., Pastor, A., Borrego, C.M., Casas-Ruiz, J.P., Hawkes, J.A., Gutiérrez, C., Schiller, D., Marcé, R., 2021. The relevance of environment vs. composition on dissolved organic matter degradation in freshwaters. Limnol. Oceanogr. 66(2), 306–320.

Clayer, F., Gélinas, Y., Tessier, A., Gobeil, C., 2020. Mineralization of organic matter in boreal lake sediments: rates, pathways, and nature of the fermenting substrates. Biogeosciences 17, 4571–4589.

Clayer, F., Moritz, A., Gélinas, Y., Tessier, A., Gobeil, C., 2018. Modeling the carbon isotope signatures of methane and dissolved inorganic carbon to unravel mineralization pathways in boreal lake sediments. Geochim. Cosmochim. Acta 229, 36–52.

Cole, J.J., Prairie, Y.T., Caraco, N.F., McDowell, W.H., Tranvik, L.J., Striegl, R.G., Duarte, C.M., Kortelainen, P., Downing, J. a., Middelburg, J.J., Melack, J., 2007. Plumbing the Global Carbon Cycle: Integrating Inland Waters into the Terrestrial Carbon Budget. Ecosystems 10, 172–185.

Conrad, R., 2002. Control of microbial methane production in wetland rice fields. Nutr. Cycl. Agroecosystems 64, 59–69.

Conrad, R., Chan, O.-C., Claus, P., Casper, P., 2007. Characterization of methanogenic Archaea and stable isotope fractionation during methane production in the profundal sediment of an oligotrophic lake (Lake Stechlin, Germany). Limnol. Oceanogr. 52, 1393–1406.

Conrad, R., Claus, P., Casper, P., 2009. Characterization of stable isotope fractionation during methane production in the sediment of a eutrophic lake, Lake Dagow, Germany. Limnol. Oceanogr. 54, 457–471.

Conrad, R., Klose, M., Claus, P., Enrich-Prast, A., 2010. Methanogenic pathway, 13 C isotope fractionation, and archaeal community composition in the sediment of two clear-water lakes of Amazonia. Limnol. Oceanogr. 55, 689–702.

Conrad, R., Noll, M., Claus, P., Klose, M., Bastos, W.R., Enrich-Prast, A., 2011. Stable carbon isotope discrimination and microbiology of methane formation in tropical anoxic lake sediments. Biogeosciences 8, 795–814.

Corbett, J.E., Tfaily, M.M., Burdige, D.J., Glaser, P.H., Chanton, J.P., 2015. The relative importance of methanogenesis in the decomposition of organic matter in northern peatlands. J. Geophys. Res. Biogeosciences 120, 280–293.

D'Andrilli, J., Junker, J.R., Smith, H.J., Scholl, E.A., Foreman, C.M., 2019. DOM composition alters ecosystem function during microbial processing of isolated sources. Biogeochemistry 142, 281–298.

Dadi, T., Harir, M., Hertkorn, N., Koschorreck, M., Schmitt-Kopplin, P., Herzsprung, P., 2017. Redox Conditions Affect Dissolved Organic Carbon Quality in Stratified Freshwaters. Environ. Sci. Technol. 51, 13705–13713.

Dixon, R.K., Browne, S., Houghton, R.A., Soloman, A.M., Trexler, M.C., Wosniewski, J., 1994. Carbon pools and flux of global forest ecosystems. Science 263, 185–190.

Duc, N.T., Crill, P., Bastviken, D., 2010. Implications of temperature and sediment characteristics on methane formation and oxidation in lake sediments. Biogeochemistry 100, 185–196.

European Environment Agency, 2003. The Boreal biogeographical region - numerous lakes, vast coniferous forests dominate. Copenhagen.

Evans, C.D., Futter, M.N., Moldan, F., Valinia, S., Frogbrook, Z., Kothawala, D.N., 2017. Variability in organic carbon reactivity across lake residence time and trophic gradients. Nat. Geosci. 10, 832–835.

Fasching, C., Behounek, B., Singer, G.A., Battin, T.J., 2014. Microbial degradation of terrigenous dissolved organic matter and potential consequences for carbon cycling in brown-water streams. Sci. Rep. 4, 1–7.

Gao, C., Sander, M., Agethen, S., Knorr, K.H., 2019. Electron accepting capacity of dissolved and particulate organic matter control CO_2 and CH_4 formation in peat soils. Geochim. Cosmochim. Acta 245, 266–277.

Gonsior, M., Schmitt-kopplin, P., Bastviken, D., 2013. Depth-dependent molecular composition and photo-reactivity of dissolved organic matter in a boreal lake under winter and summer conditions. Biogeosciences 10, 6945–6956.

Grahn, E., Karlsson, S., Düker, a, 2006. Sediment reference concentrations of seldom monitored trace elements (Ag, Be, In, Ga, Sb, Tl) in four Swedish boreal lakes--comparison with commonly monitored elements. Sci. Total Environ. 367, 778–90.

Groeneveld, M., Catalán, N., Attermeyer, K., Hawkes, J., Einarsdóttir, K., Kothawala, D., Bergquist, J., Tranvik, L., 2020. Selective Adsorption of Terrestrial Dissolved Organic Matter to Inorganic Surfaces Along a Boreal Inland Water Continuum. J. Geophys. Res. Biogeosciences 125.

Gudasz, C., Bastviken, D., Premke, K., Steger, K., Tranvik, L.J., 2012. Constrained microbial processing of allochthonous organic carbon in boreal lake sediments. Limnol. Oceanogr. 57, 163–175.

Gudasz, C., Sobek, S., Bastviken, D., Koehler, B., Tranvik, L.J., 2015. Temperature sensitivity of organic carbon mineralization in contrasting lake sediments. J. Geophys. Res. 120, 1215–1225.

Hertkorn, N., Benner, R., Frommberger, M., Schmitt-Kopplin, P., Witt, M., Kaiser, K., Kettrup, A., Hedges, J., 2006. Characterization of a major refractory component of marine dissolved organic matter. Geochim. Cosmochim. Acta 70, 2990–3010.

Hertkorn, N., Frommberger, M., Witt, M., Koch, B.P., Schmitt-Kopplin, P., Perdue, E.M., 2008. Natural Organic Matter and the Event Horizon of Mass Spectrometry. Anal. Chem. 80, 8908–8919.

Hertkorn, N., Harir, M., Cawley, K.M., Schmitt-Kopplin, P., Jaffé, R., 2016. Molecular characterization of dissolved organic matter from subtropical wetlands: A comparative study through the analysis of optical properties, NMR and FTICR/MS. Biogeosciences 13, 2257–2277.

Hertkorn, N., Harir, M., Koch, B.P., Michalke, B., Grill, P., Schmitt-Kopplin, P., 2012. High field NMR spectroscopy and FTICR mass spectrometry: powerful discovery tools for the molecular level characterization of marine dissolved organic matter from the South Atlantic Ocean. Biogeosciences Discuss. 9, 745–833.

Hertkorn, N., Harir, M., Koch, B.P., Michalke, B., Schmitt-Kopplin, P., 2013. High-field NMR spectroscopy and FTICR mass spectrometry: Powerful discovery tools for the molecular level characterization of marine dissolved organic matter. Biogeosciences 10, 1583–1624.

Hertkorn, N., Ruecker, C., Meringer, M., Gugisch, R., Frommberger, M., Perdue, E.M., Witt, M., Schmitt-Kopplin, P., 2007. High-precision frequency measurements: indispensable tools at the core of the molecular-level analysis of complex systems. Anal. Bioanal. Chem. 389, 1311–1327.

Hodgkins, S.B., Tfaily, M.M., McCalley, C.K., Logan, T. a, Crill, P.M., Saleska, S.R., Rich, V.I., Chanton, J.P., 2014. Changes in peat chemistry associated with permafrost thaw increase greenhouse gas production. PNAS. 111, 5819–5824.

Hsu, C.S., Qian, K., Chen, Y.C., 1992. An innovative approach to data analysis in hydrocarbon characterization by on-line liquid chromatography-mass spectrometry. Anal. Chim. Acta 264, 79–89.

Hughey, C.A., Hendrickson, C.L., Rodgers, R.P., Marshall, A.G., Qian, K., 2001. Kendrick Mass Defect Spectrum: A Compact Visual Analysis for Ultrahigh-Resolution Broadband Mass Spectra. Anal. Chem. 73, 4676–4681.

Ide, J., Ohashi, M., Takahashi, K., Sugiyama, Y., Piirainen, S., Kortelainen, P., Fujitake, N., Yamase, K., Ohte, N., Moritani, M., Hara, M., Finér, L., 2017. Spatial variations in the molecular diversity of dissolved organic matter in water moving through a boreal forest in eastern Finland. Sci. Rep. 7, 42102.

Johnston, S.E., Bogard, M.J., Rogers, J.A., Butman, D., Striegl, R.G., Dornblaser, M., Spencer, R.G.M., 2019. Constraining dissolved organic matter sources and temporal variability in a model sub-Arctic lake. Biogeochemistry 146, 271–292.

Johnston, S.E., Striegl, R.G., Bogard, M.J., Dornblaser, M.M., Butman, D.E., Kellerman, A.M., Wickland, K.P., Podgorski, D.C., Spencer, R.G.M., 2020. Hydrologic connectivity determines dissolved organic matter biogeochemistry in northern high-latitude lakes. Limnol. Oceanogr. 65, 1764–1780.

Jonsson, A., Meili, M., Bergström, A.-K., Jansson, M., 2001. Whole-lake mineralization of allochthonous and autochthonous organic carbon in a large humic lake (Örträsket, N. Sweden). Limnol. Oceanogr. 46, 1691–1700.

Juutinen, S., Rantakari, M., Kortelainen, P., Huttunen, J.T., Larmola, T., Alm, J., Silvola, J., Martikainen, P.J., 2008. Methane dynamics in different boreal lake types. Biogeosciences Discuss. 5, 3457–3496.

Kamjunke, N., Hertkorn, N., Harir, M., Schmitt-Kopplin, P., Griebler, C., Brauns, M., von Tümpling, W., Weitere, M., Herzsprung, P., 2019. Molecular change of dissolved organic matter and patterns of bacterial activity in a stream along a land-use gradient. Water Res. 164, 114919.

Keller, J.K., Weisenhorn, P.B., Megonigal, J.P., 2009. Humic acids as electron acceptors in wetland decomposition. Soil Biol. Biochem. 41, 1518–1522.

Kellerman, A.M., Dittmar, T., Kothawala, D.N., Tranvik, L.J., 2014. Chemodiversity of dissolved organic matter in lakes driven by climate and hydrology. Nat. Commun. 5, 3804.

Kellerman, A.M., Kothawala, D.N., Dittmar, T., Tranvik, L.J., 2015. Persistence of dissolved organic matter in lakes related to its molecular characteristics. Nat. Geosci. 8, 454–457.

Kim, S., Kramer, R.W., Hatcher, P.G., 2003. Graphical Method for Analysis of Ultrahigh-Resolution Broadband Mass Spectra of Natural Organic Matter, the Van Krevelen Diagram. Anal. Chem. 75, 5336–5344.

Klüpfel, L., Piepenbrock, A., Kappler, A., Sander, M., 2014. Humic substances as fully regenerable electron acceptors in recurrently anoxic environments. Nat. Geosci. 7, 195–200.

Koehler, B., Von Wachenfeldt, E., Kothawala, D., Tranvik, L.J., 2012. Reactivity continuum of dissolved organic carbon decomposition in lake water. J. Geophys. Res. Biogeosciences 117, 1–14.

Kortelainen, P., Mattsson, T., Finér, L., Ahtiainen, M., Saukkonen, S., Sallantaus, T., 2006. Controls on the export of C, N, P and Fe from undisturbed boreal catchments, Finland. Aquat. Sci. 68, 453–468.

Kothawala, D.N., Kellerman, A.M., Catalán, N., Tranvik, L.J., 2021. Organic Matter Degradation across Ecosystem Boundaries: The Need for a Unified Conceptualization. Trends Ecol. Evol. 36, 113–122.

Krause, S., Lüke, C., Frenzel, P., 2009. Spatial heterogeneity of methanotrophs: A geostatistical analysis of pmoA-based T-RFLP patterns in a paddy soil. Environ. Microbiol. Rep. 1, 393–397.

Kroll, J.H., Donahue, N.M., Jimenez, J.L., Kessler, S.H., Canagaratna, M.R., Wilson, K.R., Altieri, K.E., Mazzoleni, L.R., Wozniak, A.S., Bluhm, H., Mysak, E.R., Smith, J.D., Kolb, C.E., Worsnop, D.R., 2011. Carbon oxidation state as a metric for describing the chemistry of atmospheric organic aerosol. Nat. Chem. 3, 133–139.

Kurek, M.R., Harir, M., Shukle, J.T., Schroth, A.W., Schmitt-Kopplin, P., Druschel, G.K., 2020. Chemical fractionation of organic matter and organic phosphorus extractions from freshwater lake sediment. Anal. Chim. Acta 1130, 29–38.

LaRowe, D.E., Van Cappellen, P., 2011. Degradation of natural organic matter: A thermodynamic analysis. Geochim. Cosmochim. Acta 75, 2030–2042.

Lau, M.P., del Giorgio, P., 2020. Reactivity, fate and functional roles of dissolved organic matter in anoxic inland waters. Biol. Lett. 16, 20190694.

Logue, J.B., Stedmon, C.A., Kellerman, A.M., Nielsen, N.J., Andersson, A.F., Laudon, H., Lindström, E.S., Kritzberg, E.S., 2016. Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter. ISME J. 10, 533–545.

Lønborg, C., Yokokawa, T., Herndl, G.J., Àlvarez-Salgado, X.A., 2015. Production and degradation of fluorescent dissolved organic matter in surface waters of the eastern north Atlantic ocean. Deep Sea Res. Part I Oceanogr. Res. Pap. 96, 28–37.

Melo, M.L., Kothawala, D.N., Bertilsson, S., Amaral, J.H., Forsberg, B., Sarmento, H., 2020. Linking dissolved organic matter composition and bacterioplankton communities in an Amazon floodplain system. Limnol. Oceanogr. 65, 63–76.

Mentges, A., Feenders, C., Deutsch, C., Blasius, B., Dittmar, T., 2019. Long-term stability of marine dissolved organic carbon emerges from a neutral network of compounds and microbes. Sci. Rep. 9, 1–13.

Ogawa, H., Amagai, Y., Koike, I., Kaiser, K., Benner, R., 2001. Production of refractory dissolved organic matter by bacteria. Science 292, 917–920.

Peter, S., Agstam, O., Sobek, S., 2017. Widespread release of dissolved organic carbon from anoxic boreal lake sediments. Inl. Waters 7, 151–163.

Rivas-Ubach, A., Liu, Y., Bianchi, T.S., Tolić, N., Jansson, C., Paša-Tolić, L., 2018. Moving beyond the van Krevelen Diagram: A New Stoichiometric Approach for Compound Classification in Organisms. Anal. Chem. 90, 6152–6160.

Roth, V.-N., Dittmar, T., Gaupp, R., Gleixner, G., 2015. The molecular composition of dissolved organic matter in forest soils as a function of pH and temperature. PLoS One 10, e0119188.

Roth, V.-N., Dittmar, T., Gaupp, R., Gleixner, G., 2014. Ecosystem-Specific Composition of Dissolved Organic Matter. Vadose Zo. J. 13, vzj2013.09.0162.

Sanches, L.F., Guenet, B., Marinho, C.C., Barros, N., de Assis Esteves, F., 2019. Global regulation of methane emission from natural lakes. Sci. Rep. 9, 255.

Schmitt-Kopplin, P., Gonsior, M., Hertkorn, N., Ishida, K.P., Cooper, W.J., Jeung, M.K., Rosso, D., Song, W., Zwartjes, M., Tseng, L.Y., 2011. Molecular characterization of effluent organic matter identified by ultrahigh resolution mass spectrometry. Water Res. 45, 2943–2953.

Schmitt-Kopplin, P., Harir, M., Tziotis, D., Gabelica, Z., Hertkorn, N., 2012. Ultrahigh resolution Fourier Transform ion cyclotron resonance mass spectrometry for the

analysis of natural organic matter from various environmental systems. Compr. Environ. Mass Spectrom. 443–459.

Schulz, S., Conrad, R., 1995. Effect of algal deposition on acetate and methane concentrations in the profundal sediment of a deep lake (Lake Constance). FEMS Microbiol. Ecol. 16, 251–260.

Shakeri Yekta, S., Gonsior, M., Schmitt-Kopplin, P., Svensson, B.H., 2012. Characterization of Dissolved Organic Matter in Full Scale Continuous Stirred Tank Biogas Reactors Using Ultrahigh Resolution Mass Spectrometry: A Qualitative Overview. Environ. Sci. Technol. 46, 12711–12719.

Siljanen, H.M.P., Saari, A., Krause, S., Lensu, A., Abell, G.C.J., Bodrossy, L., Bodelier, P.L.E., Martikainen, P.J., 2011. Hydrology is reflected in the functioning and community composition of methanotrophs in the littoral wetland of a boreal lake. FEMS Microbiol. Ecol. 75, 430–445.

Sobek, S., Durisch-Kaiser, E., Zurbrügg, R., Wongfun, N., Wessels, M., Pasche, N., Wehrli, B., 2009. Organic carbon burial efficiency in lake sediments controlled by oxygen exposure time and sediment source. Limnol. Oceanogr. 54, 2243–2254.

Stadler, M., Ejarque, E., Kainz, M.J., 2020. In-lake transformations of dissolved organic matter composition in a subalpine lake do not change its biodegradability. Limnol. Oceanogr. 65, 1554–1572.

Steger, K., Premke, K., Gudasz, C., Sundh, I., Tranvik, L.J., 2011. Microbial biomass and community composition in boreal lake sediments. Limnol. Oceanogr. 56, 725–733.

Tranvik, L.J., Cole, J.J., Prairie, Y.T., 2018. The study of carbon in inland waters-from isolated ecosystems to players in the global carbon cycle. Limnol. Oceanogr. Lett. 3, 41–48.

Tziotis, D., Hertkorn, N., Schmitt-Kopplin, P., 2011. Kendrick-analogous network visualisation of ion cyclotron resonance Fourier transform mass spectra: improved options for the assignment of elemental compositions and the classification of organic molecular complexity. Eur. J. Mass Spectrom. 17, 415–421.

Valle, J., Gonsior, M., Harir, M., Enrich-Prast, A., Schmitt-Kopplin, P., Bastviken, D., Conrad, R., Hertkorn, N., 2018. Extensive processing of sediment pore water dissolved organic matter during anoxic incubation as observed by high-field mass spectrometry (FTICR-MS). Water Res. 129, 252–263.

Valle, J., Harir, M., Gonsior, M., Enrich-Prast, A., Schmitt-Kopplin, P., Bastviken, D., Hertkorn, N., 2020. Molecular differences between water column and sediment pore water SPE-DOM in ten Swedish boreal lakes. Water Res. 170, 115320.

van Krevelen, D.W., 1950. Graphical-statistical method for the study of structure and reaction processes of coal. Fuel 29, 269–284.

Verpoorter, C., Kutser, T., Seekell, D.A., Tranvik, L.J., 2014. A global inventory of lakes based on high-resolution satellite imagery. Geophys. Res. Lett. 41, 6396–6402.

von Wachenfeldt, E., Sobek, S., Bastviken, D., Tranvik, L.J., 2008. Linking allochthonous dissolved organic matter and boreal lake sediment carbon sequestration: The role of light-mediated flocculation. Limnol. Oceanogr. 53, 2416–2426.

von Wachenfeldt, E., Tranvik, L.J., 2008. Sedimentation in Boreal Lakes—The Role of Flocculation of Allochthonous Dissolved Organic Matter in the Water Column. Ecosystems 11, 803–814.

Ward, N.D., Bianchi, T.S., Medeiros, P.M., Seidel, M., Richey, J.E., Keil, R.G., Sawakuchi, H.O., 2017. Where Carbon Goes When Water Flows: Carbon Cycling across the Aquatic Continuum. Front. Mar. Sci. 4, 1–27.

Chapter 6

General Discussion, Conclusions & Perspectives

6. General Discussion, Conclusions & Perspectives

6.1. DOM processing (under anaerobic conditions)

Anoxic OM-rich sediments from lakes and floodplains are responsible for large amounts of methane production. In fact, lakes emit more methane than the ocean: roughly 6 – 16% of natural global CH_4 emissions (Bastviken, 2009; Bastviken et al., 2011, 2004; Kirschke et al., 2013). In freshwater environments it has been estimated that anaerobic carbon degradation contributes to 20 – 60% of total carbon mineralization rates, with methanogenesis making up 30 – 80% of this anaerobic activity. As such, it has been estimated that OM delivered to sediments via suspended particles (DOM from the water column can be flocculated by different processes, sedimented, and form the sedimentary OM pool) might contribute with more than half of methane production (Ward et al., 2017; and references therein).

In that context, it is important to keep in mind that results found in this thesis can only account as potential greenhouse gas production rates. Sediment samples from boreal lakes were incubated at 15°C – a temperature that they could probably reach in a few years, but that so far has been mostly unlikely. Additionally, laboratory incubation experiments for greenhouse gas production do not take into consideration all sorts of processes happening both in the sediment and in the water column that can prevent such gases from reaching the atmosphere.

The high variability of CH_4 production in our results are common for boreal lakes (Juutinen et al., 2008), but the mechanisms behind the disproportional production of CO_2 and CH_4, however, are still not completely clear (Conrad, 2020a). The most likely explanation for low CH_4/CO_2 ratios is that some organic compounds (e.g. humic acids) serve as electron acceptors, allowing the oxidation of other substances to CO_2 (Gao et al., 2019; Keller et al., 2009; Klüpfel et al., 2014). Anaerobic microbial communities can use DOM as a surrogate for oxygen – fermentation of OM (Keller et al., 2009) – such that they can transfer electrons generated from DOM mineralization onto a reactive fraction within DOM (Lau and del Giorgio, 2020).

Sediment pore water, predominantly an anoxic habitat, presented bulk DOM dominated by unsaturated and phenolic compounds that appeared to persist in the environment over much longer timeframes than the labile pool. This suggests either a degradation pathway from more labile to more stable organic matter, or an overall persistence of highly unsaturated and phenolic compounds with rapid cycling of labile material (Johnston et al., 2019; Kellerman et

al., 2015). Additionally, our sedimentary SPE-DOM presented an elevated proportion of CHO compounds (Kurek et al., 2020).

Nevertheless, among all lakes, there is a general trend of increasing proportions of CHOS and CHNO compounds in samples after the anaerobic incubation experiment (Valle et al., 2018). Computed average H/C and O/C ratios showed an overall trend of increasing saturation and decreasing oxygenation during the incubation. The same trends of DOM evolution with decrease of average oxidation and increase of saturation, accompanied by an increased relative abundance of CHNO and CHOS compounds have been described before in different setups and time frames (D'Andrilli et al., 2019; Kamjunke et al., 2019).

Eight out of ten lakes (exceptions were Oppsvetten and Övre S.) showed significant differences between original and incubated assigned mass peaks (T-test, $p < 0.05$). Here, all sediment pore water SPE-DOM samples showed reduction of the mass weighted average after the anoxic experiment (Valle et al., to be submitted). Moreover, aliphatic compounds were primarily consumed, which agrees with previous data that demonstrated the rapid turnover of aliphatic DOM in lake bulk DOM pool (Johnston et al., 2019). However, we cannot distinguish whether these molecules were consumed in the sediment, or even adsorbed to minerals on the solid phase by complexation, and thereby decreased in the pore water (Herzsprung et al., 2017).

Other relevant findings from our results reside on the computed average oxidation state of carbon in CHO compounds during incubation. The apparent reduction of the residual organic matter was most probably an indirect consequence of the loss of H_2O and CO_2 during anoxic incubation (in case of exclusive methanogenesis, H_2O would be taken up instead of being released). Such molecules will necessarily contain several oxygen-containing functional groups, making those more polar and reactive than common aliphatic compounds (Burdige, 2007).

Interestingly, in addition to high-energy substrates of considerable saturation (OS_av ~ -1.7 – -1.1; possibly bioavailable lipids), molecules with elevated OS_av (OS_av ~ 0 – 1.2; organic acids) were also preferentially depleted. Fermenting organic matter with a markedly negative carbon oxidation state has been previously described during methanogenesis in boreal sediments (Clayer et al., 2020, 2018). The negative carbon oxidation state value corresponds

more closely to a mixture of fatty acids and fatty alcohols than to carbohydrates (e.g. CH_2O), which would have yielded equivalent CH_4 and CO_2 production rates (Clayer et al., 2018).

Even though our results showed a greater proportion of common compounds between the samples, the presence of unique compounds corroborates an appreciable chemical diversity and heterogeneity of carbon cycling in these sediments (Krause et al., 2009; Siljanen et al., 2011). It has been previously shown that bacterial community composition shifts are tightly coupled with DOM quality (Melo et al., 2020). Therefore, the molecular diversity of DOM could be one of the reasons behind the observed variability of CH_4 and CO_2 production rates among all lakes. The original composition of OM exerts a great influence on pathways of microbial degradation. Cellulose, for example, when degraded via anaerobic microbial methane production, results in $> 67\%$ of CH_4 produced by acetoclastic and $< 33\%$ hydrogenotrophic methanogenesis. However, the relative contribution of hydrogenotrophic methanogenesis increases if the organic material is more reduced than saccharides, e.g. lipids (Conrad, 2020a).

The increase in molecular diversity on our DOM samples after the incubation can also be explained by the basic fact that those molecules were processed under anaerobic conditions. It has been shown that when products accumulate, reaction rates may decrease to maintain reaction equilibrium; therefore, thermodynamic drive depends not only on metabolic substrates, but also on products. Anaerobically derived DOM is depleted in oxygen, showing a lower energy yield per unit of carbon oxidized (LaRowe and Van Cappellen, 2011; Lau and del Giorgio, 2020). This means its selective preservation may offset a lower thermodynamic drive when using electron acceptors other than oxygen. The microbial community then diversifies the range of produced compounds trying to overcome this limitation, which in turn favors higher diversity in the resulting DOM composition (Dadi et al., 2017; Lau and del Giorgio, 2020).

Nevertheless, a dependable assessment of the general and the lake-specific complex chemical transformations happening during anaerobic DOM processing required different mathematical approaches: mass spectrometry-based assignment of molecular compositions, computation of the average carbon oxidation state in CHO molecules, averaging across replicates, consideration of gain and loss of relative mass peak amplitude before and after incubation, and depiction of individual CHO, CHOS and CHNO molecular series.

In summary, such assessments revealed that anaerobic processing and transformation of pore water DOM in sediments was much more extensive than revealed just from formation of the degradation end-products CO_2 and CH_4. We obtained molecular evidence for the gradual transformation from clearly reduced and oxidized DOM (fatty acid like and carboxylic rich DOM, respectively) to fulvic acid and tannin-like DOM with intermediate oxidation state – that may arise from selective preservation. There were also clear signs of increasing the relative abundance of nitrogen-containing DOM of likely microbial origin

6.2. DOM dynamics in the sediment-water interface

Like the bulk characteristics of individual lakes, the molecular diversity of lake water and sediment pore water SPE-DOM as observed by negative ESI-FT-ICR mass spectra presented general and individual trends that helped us to comprehend the dynamics of DOM processing in boreal lakes. Distinct clustering between water column and sediment pore water SPE-DOM was observed in HCA and PCA for nine lakes except Lustjärn. In general, SPE-DOM pools from a given compartment (i.e., lake water column or sedimentary SPE-DOM) were more similar among themselves than pools from different compartments within identical lakes (Valle et al., 2020).

For most of the lakes, with exception of Grästjärn, Lilla Sången, and Ljustjärn, sediment pore water SPE-DOM showed larger counts of assigned mass peaks than the water column SPE-DOM pool. Sediment pore water SPE-DOM molecules covered a larger area in van Krevelen diagrams for all boreal lakes, reflecting a higher molecular diversity of sedimentary SPE-DOM, related to synthesis and selective preservation of organic molecules in sediments (Raven et al., 2016; Roth et al., 2019). This is expected because sediment pore water composition depends on distinct and heterogeneous consortia of microorganisms separated by open ground (Singer et al., 2010) as well as mineral composition and texture with much steeper gradients of concentration, composition and redox conditions than observed in the well mixed open water column (Burdige, 2001; Komada et al., 2013; Krause et al., 2009; Siljanen et al., 2011).

Another common feature was the higher proportion of heteroatom-containing CHOS and CHNO compounds in sediment pore water SPE-DOM compared with lake water column SPE-DOM. The higher abundance of heteroatoms in sediment SPE-DOM likely results from an intense bioactivity in the sediments, which will produce a succession of microbial metabolites

in complex food webs (Herzsprung et al., 2010; Hodgkins et al., 2016, 2014; Roth et al., 2019, 2014; Seidel et al., 2014). The fact that not any single CHOS compound was relatively more abundant in the lake water indicates either chemo-selective removal of CHOS compounds, e.g. by photooxidation (Ossola et al., 2019) or/and rather effective production of CHOS compounds in the sediment, e.g. by reductive sulfurization under conditions of oxygen limitation (Hebting et al., 2006).

Molecular formulas present in the water column SPE-DOM pool were usually more saturated than the ones in the sediment pore water. Additionally, sediment pore water SPE-DOM was overall more oxygenated than water column SPE-DOM (Valle et al., 2020). The molecular compositions common to both lake water column and sediment pore water SPE-DOM showed a narrower and more even distribution, indicative of substantial averaging between these two compartments. Nevertheless, water samples were taken just below the surface, and not right above the sediment. An investigation of the bottom waters in comparison with sediment and surface water could have shown molecular assignments with a broader distribution.

Our data indicated higher proportions of aliphatic compounds and smaller proportions of oxygenated compounds in lake water SPE-DOM. One plausible reason is photodegradation of DOM in the well mixed and oxygenated open water column which initiates partial photo-mineralization to produce the oxygen-rich byproducts CO_2 and H_2O (Amaral et al., 2013; Chupakova et al., 2018; Roth et al., 2014; Wolf et al., 2018). Hence, carbon in the form of CO_2 and various oxygen-rich functional groups would no longer be available for resynthesis of DOM molecules; this leads to an apparent deoxygenation of residual DOM even in case of its oxidative degradation (Einsiedl et al., 2007). Aromatic and unsaturated compounds are in general susceptible to photo-degradation owing to their chromophoric groups (Gonsior et al., 2009; Roth et al., 2014; von Wachenfeldt et al., 2009).

In general, trends observed for unique compounds were analogous to those observed for abundance-weighted common molecular compositions. While a sizable proportion of lake water CHOS compounds occupied the same area as CRAM, the CHOS compounds in sediment pore water reflected rather saturated lipid-like compounds and S-derivatives of lignin- and tannin-like compounds, possibly modified products of these rather stable constituents (Gonsior et al., 2016; Herzsprung et al., 2010). Furthermore, both common and unique compositions showed an apparent trend towards an increased mass weighted average in the sediment pore

water when compared to the water column samples. It might indicate the preferential microbial processing of low mass molecules and/or selective preservation of larger size molecules (Hodgkins et al., 2016; Roth et al., 2019; Seidel et al., 2014), and selective preservation of more refractory compounds such as polyphenols (Guillemette et al., 2017; Kellerman et al., 2015; Valle et al., 2018).

The transition from lake water column to sedimentary pore water itself is initiated by flocculation (biotic flocculation POM, abiotic flocculation e photochemistry and metal complexation); sinking particles accumulate at the bottom of the lakes and impede the rather unrestricted mixing of water, which is characteristic of the (commonly oxic) lake water column. Diffusion from organic rich particles and microbial processing of DOM quickly build up higher DOC concentrations and lead to depletion of oxygen, depending on individual conditions (Liikanen et al., 2003). Production of N-rich microbial metabolites and large-scale sulfurization reactions under conditions of oxygen limitation produced a substantial range of CHNO and CHOS compounds (Hodgkins et al., 2016; Shakeri Yekta et al., 2012).

6.3. Molecular signature of DOM

Our results revealed that selectivity of DOM degradation in the mixed and oxic water column and subsequent processing/preservation of DOM in the sediments left distinct imprints on DOM composition among boreal lakes, notwithstanding individual characteristics of each system. The increased proportion of highly oxygenated CHO and CHNO molecules (i.e., lignin- and tannin-like compounds) presented in our results agrees with the land coverage data, which presented elevated proportion of forests among all catchment areas (Valle et al., to be submitted). Whereas surface waters display lower abundance of aromatic compounds and heteroatoms, these tend to accumulate in soils and sediments, where photo-degradation does not happen and higher microbial activity rates are observed (Roth et al., 2014).

Moreover, the somehow persistence of lignin- and tannin-like compounds in lakes with larger areas and, therefore, larger water residence time (WRT), agrees with the concept of slower degradation rates in such systems (Catalán et al., 2016; Evans et al., 2017; Kellerman et al., 2014). Lakes with short WRT are allegedly dominated by rapid light-driven DOM removal, whereas in water bodies with longer WRT, slower biotic production and consumption processes rule and counterbalance one another. Our investigations on unique compounds for

each lake showed that larger boreal lakes (Stora S., Lilla S., Bisen, Oppsveten, and Övre S.) presented mainly unsaturated compounds with relatively higher degree of oxygenation, which would indicate an increased proportion of allochthonous organic matter preservation.

It has been proposed that initial differences in bioavailability might not exert a strong control on the mineralization of DOC at the landscape scale, considering that WRT are typically in the order of years (Koehler et al., 2012). Nevertheless, different studies have shown that even if the role of environment on shaping the bacterial community composition is relevant, the intrinsic nature of the DOM source exerts a stronger control on DOM degradation and preservation (Catalán et al., 2021; Kellerman et al., 2015).

Preservation of DOM can be explained both by molecular composition and biological activity. Increased humification driven by bacterial activity has been reported in both marine (Lønborg et al., 2015; Ogawa et al., 2001) and freshwaters (Fasching et al., 2014; Stadler et al., 2020). DOC persistence has been explained by the following mechanisms: (a) if there is no supply of DOC, the microbes go almost extinct after the initial bloom and thus "leave behind" a residual concentration of DOC, and (b) if there is supply of DOC, microbes maintain a stable population on the long-term and therefore the DOC concentration reaches a steady-state where uptake, release, and supply are balanced (Mentges et al., 2019). Nevertheless, DOM composition has been suggested to play a large role in ecosystem function on short- and long-term scales affecting the mechanisms of processing (D'Andrilli et al., 2019).

Studies with soil OM suggest that DOM is not intrinsically recalcitrant, but instead persists in soil because of simultaneous consumption, transformation, and synthesis. It has been observed that plant-derived molecules were first broken down into molecules containing a large proportion of low molecular weight compounds, which in turn became less abundant during soil passage, whereas larger molecules, depleted in plant-related lignin-like substances, became more abundant (Roth et al., 2019). Since we only analyzed the upper 10 cm of the sediment, it could be that we found molecules with microbial signature (CHNO and CHOS compounds), but also several of those low molecular weight lignin-like assignments which have not yet been totally transformed.

Regardless of general trends on the molecular signature of SPE-DOM assessed throughout the degradation process, our results confirmed the idea that in-lake specific characteristics,

determined both by the external (catchment basin) and internal input of organic matter, and the microbiology of each lake, are highly relevant (Logue et al., 2016; Stadler et al., 2020). Here, we could separate the ten boreal lakes into five different groups and detect subtle differences among DOM pools and processing dynamics, reinforcing the idea that individual characteristics could be more relevant than general processes in which these lakes are inserted.

Lake sediments are both influenced by autochthonous and allochthonous input of organic matter, different microbiology dynamics in both oxic and anoxic conditions, and complex mineral sorption mechanisms. Therefore, knowledge from both aquatic and soil biogeochemistry has been applied to this compartment. In this context, our results are not only relevant for the understanding of freshwater ecosystems, but they also leave open questions for deeper investigation on unified concepts of DOM cycling (Kellerman et al., 2018; Kothawala et al., 2021). Although our findings also suggest DOM initial composition as one of the major drivers in organic matter degradation and persistence (Catalán et al., 2021; Kellerman et al., 2015), we still could not unambiguously determine a clearly defined role of the microbial communities (Logue et al., 2016).

6.4. Conclusions & Perspectives

We live in a changing world. For the past decades the prognostics of the scientific reports regarding climate change have been warning us about the severity of the problem and each forecast aggravates the future scenarios. It is just natural that the non-scientific community stays alarmed by the big catastrophic events, but forgets that many answers lay on the small details. Although microscopic, DOM is a very relevant organic carbon stock on Earth.

DOM is found in all natural and engineered systems of soils, sediments, freshwaters, and marine waters. This dissolved fraction of carbon pool is responsible for a series of environmental services. It sustains an important part of food webs, both via direct energy transfer and nutrients availability (Bolan et al., 2011; Carlson and Hansell, 2015; Pomeroy et al., 2007; Pomeroy, 1974), and protects living organisms and landscape structure both physically and chemically (e.g. erosion control, UV-radiation protection, bioavailability of heavy metals) (Ashworth and Alloway, 2008; Bolan et al., 2011; Brinkmann et al., 2003; Raymond and Spencer, 2015). DOM complexity and importance is already recognized (Hertkorn et al., 2008), and its molecular composition and dynamics have been studies in a

wide range of environments (e.g. D'Andrilli et al., 2002; Gonsior et al., 2016; Kellerman et al., 2018; Roth et al., 2014; Seidel et al., 2014; Tremblay et al., 2007; Zhao et al., 2017).

DOM dynamics (both synthesis and degradation) are influenced by many factors. It is extremely important to understand how the ongoing fossil fuel greenhouse gases, the landscape changes, and the consequent changes in climate, could affect OM cycling in natural environments (Ridgwell and Arndt, 2014). Carbon cycling in terrestrial and aquatic environments is largely driven by DOM (Bolan et al., 2011; Burdige and Komada, 2015; Carlson and Hansell, 2015; Raymond and Spencer, 2015), and freshwater environments are currently considered hotspots for microbial degradation (Battin et al., 2009; Cole et al., 2007; Ward et al., 2017). Landscape changes, for example, leading to increased mineral erosion, can change the patterns of DOM adsorption (Groeneveld et al., 2020).

Here, we revealed important information about the mechanisms through which DOM is processed from the water column to the sediment, microbially mineralized into greenhouse gases, or preserved in boreal lakes:

1. Anaerobic processing of DOM can be traced through ultra-high resolution mass spectrometry. Here, negative electrospray ionization Fourier transform ion cyclotron [ESI(-)] FT-ICR mass spectra was used to analyze sediment pore water samples recovered via solid phase extraction (SPE-DOM) before and after incubation experiments. Furthermore, the relative change during incubation of key bulk parameters (e.g. relative proportions of molecular series, elemental ratios, average mass, and unsaturation) was regularly several orders of magnitude higher than mineralization alone. Therefore, anaerobic processing and transformation of pore water DOM in sediments was much more extensive than revealed just from detection of CO_2 and CH_4.

2. Our results demonstrate that high resolution mass spectra of freshwater DOM in lakes followed by mathematical data analysis revealed significant molecular changes between lake water, sedimentary pore water, and during anoxic incubation. These in turn contributed to a better understanding of mechanisms governing the temporal evolution of carbon chemical environments in freshwater ecosystems.

3. Microbial degradation of DOM in surface sediments of boreal lakes is a process capable of increasing the molecular diversity while decreasing the average size of molecular compounds.

4. Higher molecular diversity was closely related to the presence of heteroatoms after microbial processing. Increased relative abundance of nitrogen-containing DOM is likely a result of microbial activity, enhanced especially in the sediment compartment.

5. We found molecular evidence for the gradual transformation from clearly reduced and oxidized DOM (fatty acid like and carboxylic rich DOM, respectively) to fulvic acid and tannin-like DOM with intermediate oxidation state.

6. Despite lake-specific features in each of the boreal lakes studied, both common and unique compounds showed similar trends when lake water column and sediment pore water SPE-DOM pools were compared. In general, SPE-DOM from each compartment (i.e., lake water column or sediment pore water) were more similar among themselves than pools from different compartments within identical lakes. These findings highlight the importance of understanding photochemical and biological transformations of DOM prior sedimentation and storage.

7. Although some compounds were unique in each lake, most of the DOM pool was ubiquitous, presenting a high proportion of lignin- and tannin-like persistent compounds, closely related with land cover characteristics.

8. Among ten boreal lakes, intrinsic characteristics from each system and its catchments seemed to be more relevant to the SPE-DOM pools than the anoxic incubation to which the samples were exposed to.

9. Four key groups of influencing variables effected both the composition of sedimentary pore water SPE-DOM and its molecular alterations during anoxic incubation: nutrients and DOC in lake waters, sedimentary organic carbon and nitrogen, water content and pH, as well as lake and catchment characteristics.

10. Lake specific parameters determined not only open lake water SPE-DOM as previously recognized but also the individuality of its sedimentary pore water DOM composition and its susceptibility to anoxic processing.

While some processes can positively reinforce DOM mineralization and greenhouse gases emissions, some may stimulate higher carbon burial efficiency in land and ocean sediments. Terrestrial allochthonous DOM in freshwater systems, for example, seem to be more susceptible to adsorption to mineral particles from increasing erosion (Groeneveld et al., 2020). Therefore, educated knowledge of DOM structure, composition, and degradation/preservation processes have the power to support better informed political decisions.

Additionally, more research is needed in tropical areas. Although boreal regions present an extensive coverage of freshwater ecosystems and one of the highest concentrations of lakes, tropical environments are of great interest. This is not only a result of higher microbial activity rates, favored by warmer temperatures all year round, but also because most of the tropical areas are in under-development countries in the global south – the ones which will be deeply affected by climate change in the near future.

In this context, more experiments and investigations on DOM dynamics are indispensable. It would be interesting to better understand DOM degradation and storage in different layers of lake sediment (Herzsprung et al., 2017). It is known that, only through diffusion, oxygen cannot penetrate in deeper layers of the sediment. Over the past few years, however, the description of cable bacteria in different types of sediment and flooded soils has shown that oxygen consumption is often coupled to the oxidation of sulfide generated by degradation of organic matter in deeper, oxygen-free layers (Burdorf et al., 2017; Müller et al., 2016; Pfeffer et al., 2012; Sandfeld et al., 2020; Scholz et al., 2020).

Not only the mineralization of DOM over various sediment layers should be investigated, but also the temporal behavior and distribution. Incubations with multiple replicates that were to be analyzed in different time frames would also be relevant to unveil short- and long-term DOM modifications. Understanding of DOM dynamics in different intervals would be especially helpful to better comprehend and manage flooded areas (Conrad, 2020b).

Another important aspect is that the ratios of CH_4/CO_2 production found here were much lower than the theoretical ratio of 1.0 that is expected. Although the oxidation of other organic compounds to CO_2 could be one the reasons for that, other explanations cannot be ruled out – i.e., methanogenic conversion of OM operating at a ratio different from $CH_4/CO_2 = 1.0$, or partial anaerobic oxidation of the produced CH_4 to CO_2. Deeper investigation is necessary to fully comprehend the mechanisms behind OM degradation and further greenhouse gas productions under anaerobic conditions.

6.5. References

Amaral, J.H.F., Suhett, A.L., Melo, S., Farjalla, V.F., 2013. Seasonal variation and interaction of photodegradation and microbial metabolism Seasonal variation and interaction of photodegradation and microbial metabolism of DOC in black water Amazonian ecosystems. Aquat. Microb. Ecol. 70, 157–168.

Ashworth, D.J., Alloway, B.J., 2008. Influence of dissolved organic matter on the solubility of heavy metals in sewage-sludge-amended soils. Commun. Soil Sci. Plant Anal. 39, 538–550.

Bastviken, D., 2009. Methane, in: Likens, G.E. (Ed.), Encyclopedia of Inland Waters. Elsevier, Oxford, pp. 783–805.

Bastviken, D., Cole, J., Pace, M., Tranvik, L.J., 2004. Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. Global Biogeochem. Cycles 18, 1–12.

Bastviken, D., Tranvik, L.J., Downing, J.A., Crill, P.M., Enrich-Prast, A., 2011. Freshwater Methane Emissions Offset the Continental Carbon Sink. Science 331, 50.

Battin, T.J., Luyssaert, S., Kaplan, L. a., Aufdenkampe, A.K., Richter, A., Tranvik, L.J., 2009. The boundless carbon cycle. Nat. Geosci. 2, 598–600.

Bolan, N.S., Adriano, D.C., Kunhikrishnan, A., James, T., McDowell, R., Senesi, N., 2011. Dissolved Organic Matter. Biogeochemistry, Dynamics, and Environmental Significance in Soils., 1st ed, Advances in Agronomy. Elsevier Inc.

Brinkmann, T., Sartorius, D., Frimmel, F.H., 2003. Photobleaching of humic rich dissolved organic matter. Aquat. Sci. 65, 415–424.

Burdige, D., 2001. Dissolved organic matter in Chesapeake Bay sediment pore waters. Org. Geochem. 32, 487–505.

Burdige, D.J., 2007. Preservation of Organic Matter in Marine Sediments: Controls, Mechanisms, and an Imbalance in Sediment Organic Carbon Budgets? Chem. Rev. 107, 467–485.

Burdige, D.J., Komada, T., 2015. Sediment Pore Waters. In Biogeochemistry of Marine Dissolved Organic Matter (Second Edition). Elsevier Inc., Academic Press, 535–577.

Burdorf, L.D.W., Tramper, A., Seitaj, D., Meire, L., Hidalgo-Martinez, S., Zetsche, E.-M., Boschker, H.T.S., Meysman, F.J.R., 2017. Long-distance electron transport occurs globally in marine sediments. Biogeosciences 14, 683–701.

Carlson, C. A., Hansell, D. A., 2015. Chapter 3 – DOM Sources, Sinks, Reactivity, and Budgets. In Biogeochemistry of Marine Dissolved Organic Matter (Second Edition). Elsevier Inc, Academic Press, 65–126.

Catalán, N., Marcé, R., Kothawala, D.N., Tranvik, L.J., 2016. Organic carbon decomposition rates controlled by water retention time across inland waters. Nat. Geosci. 9, 501–504.

Catalán, N., Pastor, A., Borrego, C.M., Casas-Ruiz, J.P., Hawkes, J.A., Gutiérrez, C., Schiller, D., Marcé, R., 2021. The relevance of environment vs. composition on dissolved organic matter degradation in freshwaters. Limnol. Oceanogr. 66(2), 306–320.

Chupakova, A.A., Chupakov, A. V., Neverova, N. V., Shirokova, L.S., Pokrovsky, O.S., 2018. Photodegradation of river dissolved organic matter and trace metals in the largest European Arctic estuary. Sci. Total Environ. 622–623, 1343–1352.

Clayer, F., Gélinas, Y., Tessier, A., Gobeil, C., 2020. Mineralization of organic matter in boreal lake sediments: rates, pathways, and nature of the fermenting substrates. Biogeosciences 17, 4571–4589.

Clayer, F., Moritz, A., Gélinas, Y., Tessier, A., Gobeil, C., 2018. Modeling the carbon isotope signatures of methane and dissolved inorganic carbon to unravel mineralization pathways in boreal lake sediments. Geochim. Cosmochim. Acta 229, 36–52.

Cole, J.J., Prairie, Y.T., Caraco, N.F., McDowell, W.H., Tranvik, L.J., Striegl, R.G., Duarte, C.M., Kortelainen, P., Downing, J. a., Middelburg, J.J., Melack, J., 2007. Plumbing the Global Carbon Cycle: Integrating Inland Waters into the Terrestrial Carbon Budget. Ecosystems 10, 172–185.

Conrad, R., 2020a. Importance of hydrogenotrophic, aceticlastic and methylotrophic methanogenesis for methane production in terrestrial, aquatic and other anoxic environments: A mini review. Pedosphere 30, 25–39.

Conrad, R., 2020b. Methane Production in Soil Environments—Anaerobic Biogeochemistry and Microbial Life between Flooding and Desiccation. Microorganisms 8(6), 881.

D'Andrilli, J., Dittmar, T., Koch, B.P., Purcell, J.M., Marshall, A.G., Cooper, W.T., 2002. Comprehensive characterization of marine dissolved organic matter by Fourier transform ion cyclotron resonance mass spectrometry with electrospray and atmospheric pressure photoionization. Rapid Commun. Mass Spectrom. RCM 24, 1779–1784.

D'Andrilli, J., Junker, J.R., Smith, H.J., Scholl, E.A., Foreman, C.M., 2019. DOM composition alters ecosystem function during microbial processing of isolated sources. Biogeochemistry 142, 281–298.

Dadi, T., Harir, M., Hertkorn, N., Koschorreck, M., Schmitt-Kopplin, P., Herzsprung, P., 2017. Redox Conditions Affect Dissolved Organic Carbon Quality in Stratified Freshwaters. Environ. Sci. Technol. 51, 13705–13713.

Einsiedl, F., Hertkorn, N., Wolf, M., Frommberger, M., Schmitt-Kopplin, P., Koch, B.P., 2007. Rapid biotic molecular transformation of fulvic acids in a karst aquifer. Geochim. Cosmochim. Acta 71, 5474–5482.

Evans, C.D., Futter, M.N., Moldan, F., Valinia, S., Frogbrook, Z., Kothawala, D.N., 2017. Variability in organic carbon reactivity across lake residence time and trophic gradients. Nat. Geosci. 10, 832–835.

Fasching, C., Behounek, B., Singer, G.A., Battin, T.J., 2014. Microbial degradation of terrigenous dissolved organic matter and potential consequences for carbon cycling in brown-water streams. Sci. Rep. 4, 1–7.

Gao, C., Sander, M., Agethen, S., Knorr, K.H., 2019. Electron accepting capacity of dissolved and particulate organic matter control CO_2 and CH_4 formation in peat soils. Geochim. Cosmochim. Acta 245, 266–277.

Gonsior, M., Peake, B.M., Cooper, W.T., Podgorski, D., D'Andrilli, J., Cooper, W.J., 2009. Photochemically Induced Changes in Dissolved Organic Matter Identified by Ultrahigh Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. Environ. Sci. Technol. 43, 698–703.

Gonsior, M., Valle, J., Schmitt-Kopplin, P., Hertkorn, N., Bastviken, D., Luek, J., Harir, M., Bastos, W., Enrich-Prast, A., 2016. Chemodiversity of dissolved organic matter in the Amazon Basin. Biogeosciences 13, 4279–4290.

Groeneveld, M., Catalán, N., Attermeyer, K., Hawkes, J., Einarsdóttir, K., Kothawala, D., Bergquist, J., Tranvik, L.J., 2020. Selective Adsorption of Terrestrial Dissolved Organic Matter to Inorganic Surfaces Along a Boreal Inland Water Continuum. J. Geophys. Res. Biogeosciences 125, 1-22.

Guillemette, F., von Wachenfeldt, E., Kothawala, D.N., Bastviken, D., Tranvik, L.J., 2017. Preferential sequestration of terrestrial organic matter in boreal lake sediments. J. Geophys. Res. Biogeosciences 122, 863–874.

Hebting, Y., Schaeffer, P., Behrens, A., Adam, P., Schmitt, G., Schneckenburger, P., Bernasconi, S.M., Albrecht, P., 2006. Biomarker evidence for a major preservation pathway of sedimentary organic carbon. Science 312, 1627–1631.

Hertkorn, N., Frommberger, M., Witt, M., Koch, B.P., Schmitt-Kopplin, P., Perdue, E.M., 2008. Natural Organic Matter and the Event Horizon of Mass Spectrometry. Anal. Chem. 80, 8908–8919.

Herzsprung, P., Hertkorn, N., Friese, K., Schmitt-Kopplin, P., 2010. Photochemical degradation of natural organic sulfur compounds (CHOS) from iron-rich mine pit lake pore waters--an initial understanding from evaluation of single-elemental formulae using ultra-high-resolution mass spectrometry. Rapid Commun. Mass Spectrom. 24, 2909–2924.

Herzsprung, P., von Tümpling, W., Wendt-Potthoff, K., Hertkorn, N., Harir, M., Schmitt-Kopplin, P., Friese, K., 2017. High field FT-ICR mass spectrometry data sets enlighten qualitative DOM alteration in lake sediment porewater profiles. Org. Geochem. 108, 51–60.

Hodgkins, S.B., Tfaily, M.M., McCalley, C.K., Logan, T.A., Crill, P.M., Saleska, S.R., Rich, V.I., Chanton, J.P., 2014. Changes in peat chemistry associated with permafrost thaw increase greenhouse gas production. PNAS 111, 5819–5824.

Hodgkins, S.B., Tfaily, M.M., Podgorski, D.C., McCalley, C.K., Saleska, S.R., Crill, P.M., Rich, V.I., Chanton, J.P., Cooper, W.T., 2016. Elemental composition and optical properties reveal changes in dissolved organic matter along a permafrost thaw chronosequence in a subarctic peatland. Geochim. Cosmochim. Acta 187, 123–140.

Johnston, S.E., Bogard, M.J., Rogers, J.A., Butman, D., Striegl, R.G., Dornblaser, M., Spencer, R.G.M., 2019. Constraining dissolved organic matter sources and temporal variability in a model sub-Arctic lake. Biogeochemistry 146, 271–292.

Juutinen, S., Rantakari, M., Kortelainen, P., Huttunen, J.T., Larmola, T., Alm, J., Silvola, J., Martikainen, P.J., 2008. Methane dynamics in different boreal lake types. Biogeosciences Discuss. 5, 3457–3496.

Kamjunke, N., Hertkorn, N., Harir, M., Schmitt-Kopplin, P., Griebler, C., Brauns, M., von Tümpling, W., Weitere, M., Herzsprung, P., 2019. Molecular change of dissolved organic matter and patterns of bacterial activity in a stream along a land-use gradient. Water Res. 164, 114919.

Keller, J.K., Weisenhorn, P.B., Megonigal, J.P., 2009. Humic acids as electron acceptors in wetland decomposition. Soil Biol. Biochem. 41, 1518–1522.

Kellerman, A.M., Dittmar, T., Kothawala, D.N., Tranvik, L.J., 2014. Chemodiversity of dissolved organic matter in lakes driven by climate and hydrology. Nat. Commun. 5, 3804.

Kellerman, A.M., Guillemette, F., Podgorski, D.C., Aiken, G.R., Butler, K.D., Spencer, R.G.M., 2018. Unifying Concepts Linking Dissolved Organic Matter Composition to Persistence in Aquatic Ecosystems. Environ. Sci. Technol. 52, 2538–2548.

Kellerman, A.M., Kothawala, D.N., Dittmar, T., Tranvik, L.J., 2015. Persistence of dissolved organic matter in lakes related to its molecular characteristics. Nat. Geosci. 8, 454-457.

Kirschke, S., Bousquet, P., Ciais, P., Saunois, M., Canadell, J.G., Dlugokencky, E.J., Bergamaschi, P., Bergmann, D., Blake, D.R., Bruhwiler, L., Cameron-Smith, P., Castaldi, S., Chevallier, F., Feng, L., Fraser, A., Heimann, M., Hodson, E.L., Houweling, S., Josse, B., Fraser, P.J., Krummel, P.B., Lamarque, J.-F., Langenfelds, R.L., Le Quéré, C., Naik, V., O'Doherty, S., Palmer, P.I., Pison, I., Plummer, D., Poulter, B., Prinn, R.G., Rigby, M., Ringeval, B., Santini, M., Schmidt, M., Shindell, D.T., Simpson, I.J., Spahni, R., Steele, L.P., Strode, S.A., Sudo, K., Szopa, S., van der Werf, G.R., Voulgarakis, A., van Weele, M., Weiss, R.F., Williams, J.E., Zeng, G., 2013. Three decades of global methane sources and sinks. Nat. Geosci. 6, 813–823.

Klüpfel, L., Piepenbrock, A., Kappler, A., Sander, M., 2014. Humic substances as fully regenerable electron acceptors in recurrently anoxic environments. Nat. Geosci. 7, 195–200.

Koehler, B., Von Wachenfeldt, E., Kothawala, D., Tranvik, L.J., 2012. Reactivity continuum of dissolved organic carbon decomposition in lake water. J. Geophys. Res. Biogeosciences 117, 1–14.

Komada, T., Burdige, D.J., Crispo, S.M., Druffel, E.R.M., Griffin, S., Johnson, L., Le, D., 2013. Dissolved organic carbon dynamics in anaerobic sediments of the Santa Monica Basin. Geochim. Cosmochim. Acta 110, 253–273.

Kothawala, D.N., Kellerman, A.M., Catalán, N., Tranvik, L.J., 2021. Organic Matter Degradation across Ecosystem Boundaries: The Need for a Unified Conceptualization. Trends Ecol. Evol. 36, 113–122.

Krause, S., Lüke, C., Frenzel, P., 2009. Spatial heterogeneity of methanotrophs: A geostatistical analysis of pmoA-based T-RFLP patterns in a paddy soil. Environ. Microbiol. Rep. 1, 393–397.

Kurek, M.R., Harir, M., Shukle, J.T., Schroth, A.W., Schmitt-Kopplin, P., Druschel, G.K., 2020. Chemical fractionation of organic matter and organic phosphorus extractions from freshwater lake sediment. Anal. Chim. Acta 1130, 29–38.

LaRowe, D.E., Van Cappellen, P., 2011. Degradation of natural organic matter: A thermodynamic analysis. Geochim. Cosmochim. Acta 75, 2030–2042.

Lau, M.P., del Giorgio, P., 2020. Reactivity, fate and functional roles of dissolved organic matter in anoxic inland waters. Biol. Lett. 16, 20190694.

Liikanen, A.N.U., Huttunen, J.T., Murtoniemi, T., 2003. Spatial and seasonal variation in greenhouse gas and nutrient dynamics and their interactions in the sediments of a boreal eutrophic lake. Biogeochemistry 65, 83–103.

Logue, J.B., Stedmon, C.A., Kellerman, A.M., Nielsen, N.J., Andersson, A.F., Laudon, H., Lindström, E.S., Kritzberg, E.S., 2016. Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter. ISME J. 10, 533–545.

Lønborg, C., Yokokawa, T., Herndl, G.J., Àlvarez-Salgado, X.A., 2015. Production and degradation of fluorescent dissolved organic matter in surface waters of the eastern north Atlantic ocean. Deep Sea Res. Part I Oceanogr. Res. Pap. 96, 28–37.

Melo, M.L., Kothawala, D.N., Bertilsson, S., Amaral, J.H., Forsberg, B., Sarmento, H., 2020. Linking dissolved organic matter composition and bacterioplankton communities in an Amazon floodplain system. Limnol. Oceanogr. 65, 63–76.

Mentges, A., Feenders, C., Deutsch, C., Blasius, B., Dittmar, T., 2019. Long-term stability of marine dissolved organic carbon emerges from a neutral network of compounds and microbes. Sci. Rep. 9, 1–13.

Müller, H., Bosch, J., Griebler, C., Damgaard, L.R., Nielsen, L.P., Lueders, T., Meckenstock, R.U., 2016. Long-distance electron transfer by cable bacteria in aquifer sediments. ISME J. 10, 2010–2019.

Ogawa, H., Amagai, Y., Koike, I., Kaiser, K., Benner, R., 2001. Production of refractory dissolved organic matter by bacteria. Science 292, 917–920.

Ossola, R., Tolu, J., Clerc, B., Erickson, P.R., Winkel, L.H.E., McNeill, K., 2019. Photochemical Production of Sulfate and Methanesulfonic Acid from Dissolved Organic Sulfur. Environ. Sci. Technol. 53, 13191–13200.

Pfeffer, C., Larsen, S., Song, J., Dong, M., Besenbacher, F., Meyer, R.L., Kjeldsen, K.U., Schreiber, L., Gorby, Y.A., El-Naggar, M.Y., Leung, K.M., Schramm, A., Risgaard-Petersen, N., Nielsen, L.P., 2012. Filamentous bacteria transport electrons over centimetre distances. Nature 491, 218–221.

Pomeroy, L., leB. Williams, P., Azam, F., Hobbie, J., 2007. The Microbial Loop. Oceanography 20, 28–33.

Pomeroy, L.R., 1974. Food Changing Paradigm. Bioscience 24, 499–504.

Raven, M.R., Sessions, A.L., Fischer, W.W., Adkins, J.F., 2016. Sedimentary pyrite $\delta^{34}S$ differs from porewater sulfide in Santa Barbara Basin: Proposed role of organic sulfur. Geochim. Cosmochim. Acta 186, 120–134.

Raymond, P.A., Spencer, R.G.M., 2015. Chapter 11 – Riverine DOM. In Biogeochemistry of Marine Dissolved Organic Matter (Second Edition). Elsevier Inc., Academic Press, 509–533.

Ridgwell, A., Arndt, S., 2014. Chapter 1 – Why Dissolved Organics Matter: DOC in Ancient Oceans and Past Climate Change. In Biogeochemistry of Marine Dissolved Organic Matter (Second Edition). Elsevier Inc., Academic Press, 1-20.

Roth, V.-N., Dittmar, T., Gaupp, R., Gleixner, G., 2014. Ecosystem-Specific Composition of Dissolved Organic Matter. Vadose Zo. J. 13, vzj2013.09.0162.

Roth, V.-N., Lange, M., Simon, C., Hertkorn, N., Bucher, S., Goodall, T., Griffiths, R.I., Mellado-Vázquez, P.G., Mommer, L., Oram, N.J., Weigelt, A., Dittmar, T., Gleixner, G., 2019. Persistence of dissolved organic matter explained by molecular changes during its passage through soil. Nat. Geosci. 12, 755–761.

Sandfeld, T., Marzocchi, U., Petro, C., Schramm, A., Risgaard-Petersen, N., 2020. Electrogenic sulfide oxidation mediated by cable bacteria stimulates sulfate reduction in freshwater sediments. ISME J. 14, 1233–1246.

Scholz, V. V., Meckenstock, R.U., Nielsen, L.P., Risgaard-Petersen, N., 2020. Cable bacteria reduce methane emissions from rice-vegetated soils. Nat. Commun. 11, 1–5.

Seidel, M., Beck, M., Riedel, T., Waska, H., Suryaputra, I.G.N. a, Schnetger, B., Niggemann, J., Simon, M., Dittmar, T., 2014. Biogeochemistry of dissolved organic matter in an anoxic intertidal creek bank. Geochim. Cosmochim. Acta 140, 418–434.

Shakeri Yekta, S., Gonsior, M., Schmitt-Kopplin, P., Svensson, B.H., 2012. Characterization of Dissolved Organic Matter in Full Scale Continuous Stirred Tank Biogas Reactors Using Ultrahigh Resolution Mass Spectrometry: A Qualitative Overview. Environ. Sci. Technol. 46, 12711–12719.

Siljanen, H.M.P., Saari, A., Krause, S., Lensu, A., Abell, G.C.J., Bodrossy, L., Bodelier, P.L.E., Martikainen, P.J., 2011. Hydrology is reflected in the functioning and community composition of methanotrophs in the littoral wetland of a boreal lake. FEMS Microbiol. Ecol. 75, 430–445.

Singer, G., Besemer, K., Schmitt-Kopplin, P., Hödl, I., Battin, T.J., 2010. Physical Heterogeneity Increases Biofilm Resource Use and Its Molecular Diversity in Stream Mesocosms. PLoS One 5, e9988.

Stadler, M., Ejarque, E., Kainz, M.J., 2020. In-lake transformations of dissolved organic matter composition in a subalpine lake do not change its biodegradability. Limnol. Oceanogr. 65, 1554–1572.

Tremblay, L.B., Dittmar, T., Marshall, A.G., Cooper, W.J., Cooper, W.T., 2007. Molecular characterization of dissolved organic matter in a North Brazilian mangrove porewater and mangrove-fringed estuaries by ultrahigh resolution Fourier Transform-Ion Cyclotron Resonance mass spectrometry and excitation/emission spectroscopy. Mar. Chem. 105, 15–29.

Valle, J., Gonsior, M., Harir, M., Enrich-Prast, A., Schmitt-Kopplin, P., Bastviken, D., Conrad, R., Hertkorn, N., 2018. Extensive processing of sediment pore water dissolved organic matter during anoxic incubation as observed by high-field mass spectrometry (FTICR-MS). Water Res. 129, 252–263.

Valle, J., Harir, M., Gonsior, M., Enrich-Prast, A., Schmitt-Kopplin, P., Bastviken, D., Hertkorn, N., 2020. Molecular differences between water column and sediment pore water SPE-DOM in ten Swedish boreal lakes. Water Res. 170, 115320.

von Wachenfeldt, E., Bastviken, D., Tranvik, L.J., 2009. Microbially induced flocculation of allochthonous dissolved organic carbon in lakes. Limnol. Oceanogr. 54, 1811–1818.

Ward, N.D., Bianchi, T.S., Medeiros, P.M., Seidel, M., Richey, J.E., Keil, R.G., Sawakuchi, H.O., 2017. Where Carbon Goes When Water Flows: Carbon Cycling across the Aquatic Continuum. Front. Mar. Sci. 4, 1–27.

Wolf, R., Thrane, J.-E., Hessen, D.O., Andersen, T., 2018. Modelling ROS formation in boreal lakes from interactions between dissolved organic matter and absorbed solar photon flux. Water Res. 132, 331–339.

Zhao, Z., Gonsior, M., Luek, J., Timko, S., Ianiri, H., Hertkorn, N., Schmitt-Kopplin, P., Fang, X., Zeng, Q., Jiao, N., Chen, F., 2017. Picocyanobacteria and deep-ocean fluorescent dissolved organic matter share similar optical properties. Nat. Commun. 8, 1–10.

Appendix

Supplementary Information for Chapter 5

Table S1. CH_4 and CO_2 production rates (mean ± SE) measured during experimental incubation of ten boreal lake sediments.

Lake	CH_4		CO_2		Ratio CH_4/CO_2
	nmol g_{dw}^{-1} d^{-1}	SE	nmol g_{dw}^{-1} d^{-1}	SE	%
1. Gäddtjärn	11.84	2.05	140.86	23.47	8.41
2. Stora S.	6.44	2.36	194.56	35.45	3.31
3. Svarttjärn	11.82	2.57	77.55	5.91	15.24
4. Grästjärn	8.88	1.54	113.45	11.59	7.83
5. Lilla S.	12.66	0.97	131.34	2.07	9.64
6. Skotttjärn	26.14	2.25	171.09	11.96	15.28
7. Ljustjärn	13.46	5.29	252.92	44.46	5.32
8. Bisen	5.9	1.38	107.21	19.50	5.50
9. Oppsveten	16.02	2.35	127.44	6.19	12.57
10. Övre S.	4.77	1.39	123.72	25.04	3.86

Table S2. Counts of common mass peaks between three replicates of SPE-sedDOM in the ten boreal lakes as computed from negative electrospray (ESI) 12T FTICR mass spectra for singly charged ions, before and after anoxic incubation experiments.

	Gäddtjärn original	Gäddtjärn incubated	Stora S. original	Stora S. incubated	Svarttjärn original	Svarttjärn incubated	Grästjärn original	Grästjärn incubated	Lilla S. original	Lilla S. incubated
total number assigned mass peaks	5709	6443	5924	6210	6583	6340	4642	4735	4972	5949
formulas shared in replicates [%]	76%	72%	77%	76%	76%	71%	76%	68%	78%	74%
CHO compounds (%)	2843 (65%)	2908 (62%)	3081 (68%)	2925 (62%)	3280 (66%)	2838 (63%)	2445 (69%)	2201 (68%)	2571 (67%)	2667 (61%)
CHOS compounds (%)	504 (12%)	524 (11%)	479 (11%)	539 (11%)	777 (16%)	671 (15%)	375 (11%)	253 (8%)	401 (10%)	465 (11%)
CHNO compounds (%)	1003 (23%)	1234 (26%)	995 (22%)	1266 (27%)	926 (19%)	1011 (22%)	725 (20%)	764 (24%)	889 (23%)	1256 (29%)
CHNOS compounds (%)	3 (0%)	3 (0%)	3 (0%)	2 (0%)	2 (0%)	3 (0%)	3 (0%)	1 (0%)	0 (0%)	3 (0%)
total number common assigned mass peaks (%)	4353 (100%)	4669 (100%)	4558 (100%)	4732 (100%)	4985 (100%)	4523 (100%)	3548 (100%)	3219 (100%)	3861 (100%)	4391 (100%)
average H [%]	43.0	44.3	41.1	45.1	44.1	44.0	45.7	46.9	42.9	45.0
average C [%]	38.1	37.7	38.1	37.1	37.5	37.8	37.1	37.3	37.8	37.5
average O [%]	18.5	17.4	20.5	17.1	18.0	17.8	16.7	15.1	18.9	16.9
average N [%]	0.24	0.30	0.19	0.24	0.14	0.25	0.21	0.29	0.24	0.32
average S [%]	0.22	0.28	0.14	0.46	0.25	0.22	0.23	0.45	0.21	0.38
computed average H/C ratio	1.13	1.18	1.08	1.21	1.17	1.16	1.23	1.26	1.14	1.20
computed average O/C ratio	0.48	0.46	0.54	0.46	0.48	0.47	0.45	0.40	0.50	0.45
computed average C/N ratio	156.2	124.9	201.3	154.3	259.7	151.5	173.1	129.2	158.6	117.2
computed average C/S ratio	175.7	134.7	272.8	81.1	152.1	172.4	157.9	82.8	178.4	97.5
mass weighted average	385.2	378.8	404.7	375.4	398.6	386.4	367.3	359.6	387.4	371.9
DBE	9.5	9.2	9.8	9.1	9.3	9.1	8.5	8.9	9.4	9.0
DBE/C	0.515	0.500	0.528	0.503	0.492	0.490	0.476	0.494	0.514	0.501

|	Skotttjärn original	Skotttjärn incubated	Ljustjärn original	Ljustjärn incubated	Bisen original	Bisen incubated	Oppsveten original	Oppsveten incubated	Övre S. original	Övre S. incubated
total number assigned mass peaks	6248	6794	3132	4194	6383	7171	6316	6322	6039	5745
formulas shared in replicates [%]	77%	75%	71%	63%	78%	74%	77%	72%	77%	67%
CHO compounds (%)	2948 (62%)	3092 (61%)	1346 (61%)	1297 (49%)	3151 (64%)	3205 (61%)	2995 (61%)	2722 (60%)	2945 (63%)	2445 (64%)
CHOS compounds (%)	700 (15%)	714 (14%)	284 (13%)	515 (20%)	466 (9%)	501 (9%)	689 (14%)	615 (14%)	534 (11%)	399 (10%)
CHNO compounds (%)	1129 (24%)	1257 (25%)	580 (26%)	764 (29%)	1320 (27%)	1534 (29%)	1195 (24%)	1194 (26%)	1164 (25%)	1003 (26%)
CHNOS compounds (%)	11 (0%)	44 (1%)	2 (0%)	62 (2%)	11 (0%)	37 (1%)	13 (0%)	17 (0%)	5 (0%)	1 (0%)
total number common assigned mass peaks (%)	4788 (100%)	5107 (100%)	2212 (100%)	2638 (100%)	4948 (100%)	5277 (100%)	4892 (100%)	4548 (100%)	4648 (100%)	3848 (100%)
average H [%]	42.0	41.6	51.1	51.6	40.4	42.1	42.6	44.5	41.8	46.0
average C [%]	38.3	38.4	35.2	35.4	37.9	37.7	37.9	37.6	37.7	36.9
average O [%]	19.4	19.5	13.0	11.9	21.3	19.6	19.2	17.4	20.2	16.4
average N [%]	0.21	0.22	0.41	0.45	0.24	0.24	0.23	0.23	0.24	0.23
average S [%]	0.15	0.23	0.28	0.67	0.09	0.36	0.14	0.30	0.13	0.43
computed average H/C ratio	1.10	1.08	1.45	1.46	1.07	1.12	1.12	1.19	1.11	1.25
computed average O/C ratio	0.51	0.51	0.37	0.34	0.56	0.52	0.51	0.46	0.53	0.45
computed average C/N ratio	178.6	176.8	84.9	78.6	156.6	157.9	162.6	163.5	158.4	159.9
computed average C/S ratio	261.7	170.1	124.2	52.6	423.6	105.2	264.5	126.3	301.4	85.5
mass weighted average	394.7	403.5	328.7	326.0	411.0	403.1	393.6	384.8	394.6	371.2
DBE	9.5	10.1	6.2	6.3	9.8	10.2	9.2	9.2	9.2	8.7
DBE/C	0.513	0.532	0.370	0.378	0.527	0.544	0.498	0.496	0.509	0.484
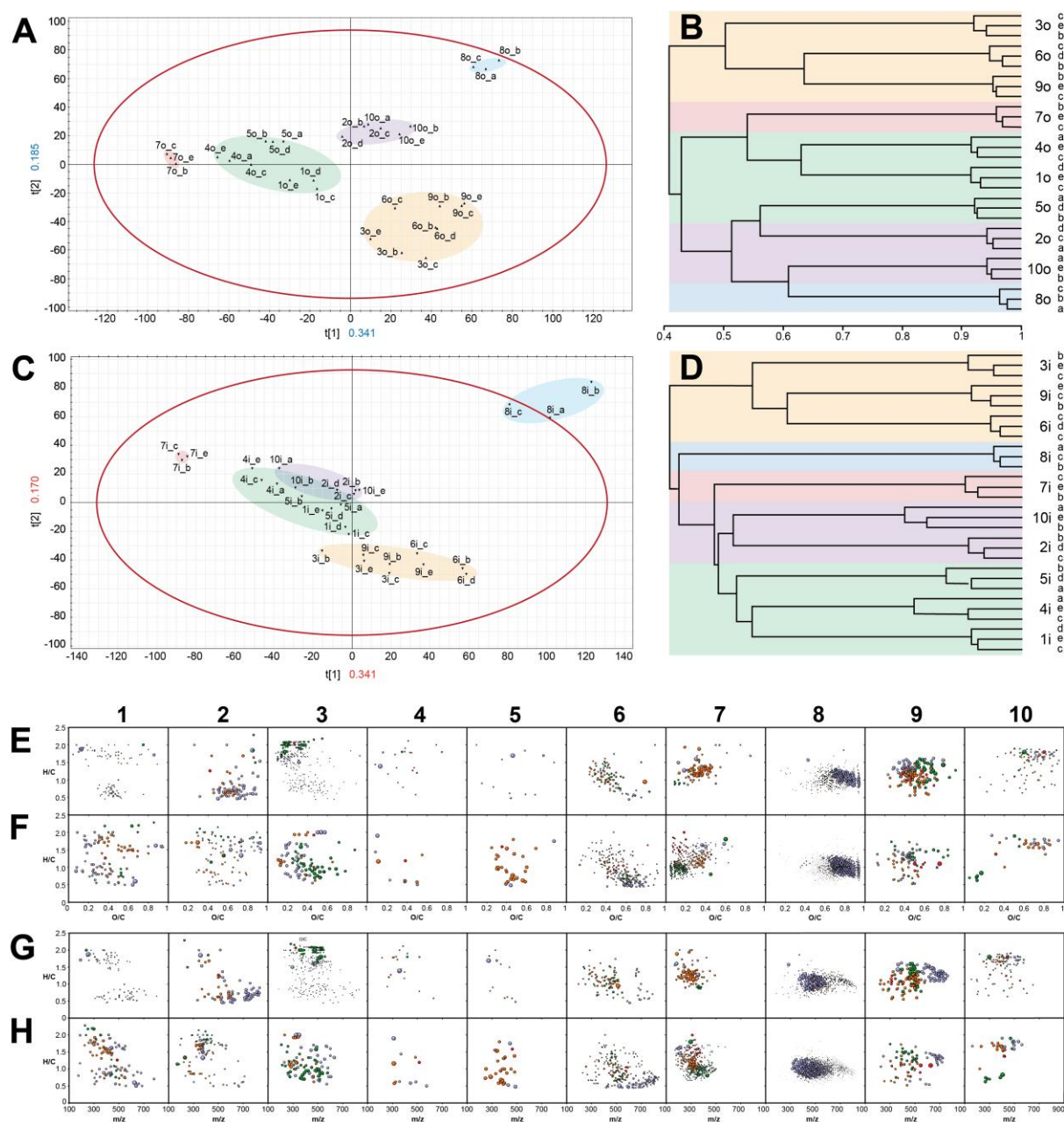
Figure S1. FTMS-based PCA (panels A and C) and HCA (panels B and D) of molecular compositions common to original (panels A and B) and incubated (panels C and D) SPE-sedDOM pools, among ten boreal lakes using normalized spectra (total mass peak amplitude = 100%) and recognition of the respective relative abundances of assigned molecular compositions in individual incubation status. FTMS-derived van Krevelen (panels E-F) and mass-edited H/C diagrams (panels G-H) showing individual CHO, CHNO and CHOS compounds for each lake which were relatively more abundant in this lake compared to the others. Panels E and G: original samples. Panels F and H: incubated samples.

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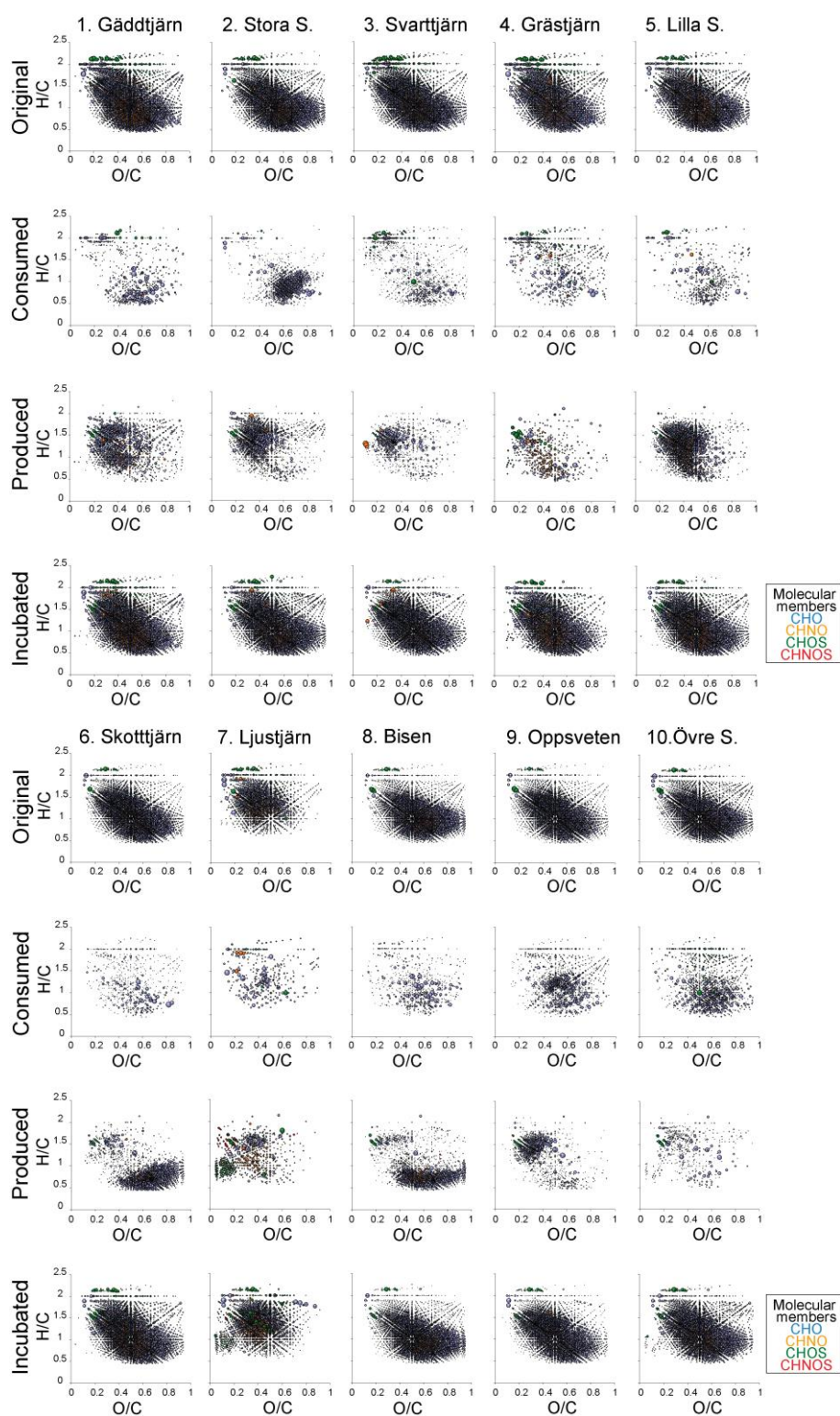
Figure S2. FTMS-derived van Krevelen diagrams of common molecular compositions observed in all three replicates of original and incubated SPE-sedDOM from ten boreal lakes, color-coded according to molecular series. Consumed and produced compounds were computed based on increasing or decreasing abundance of molecules during incubation; the bubble area corresponds to relative mass peak amplitude.
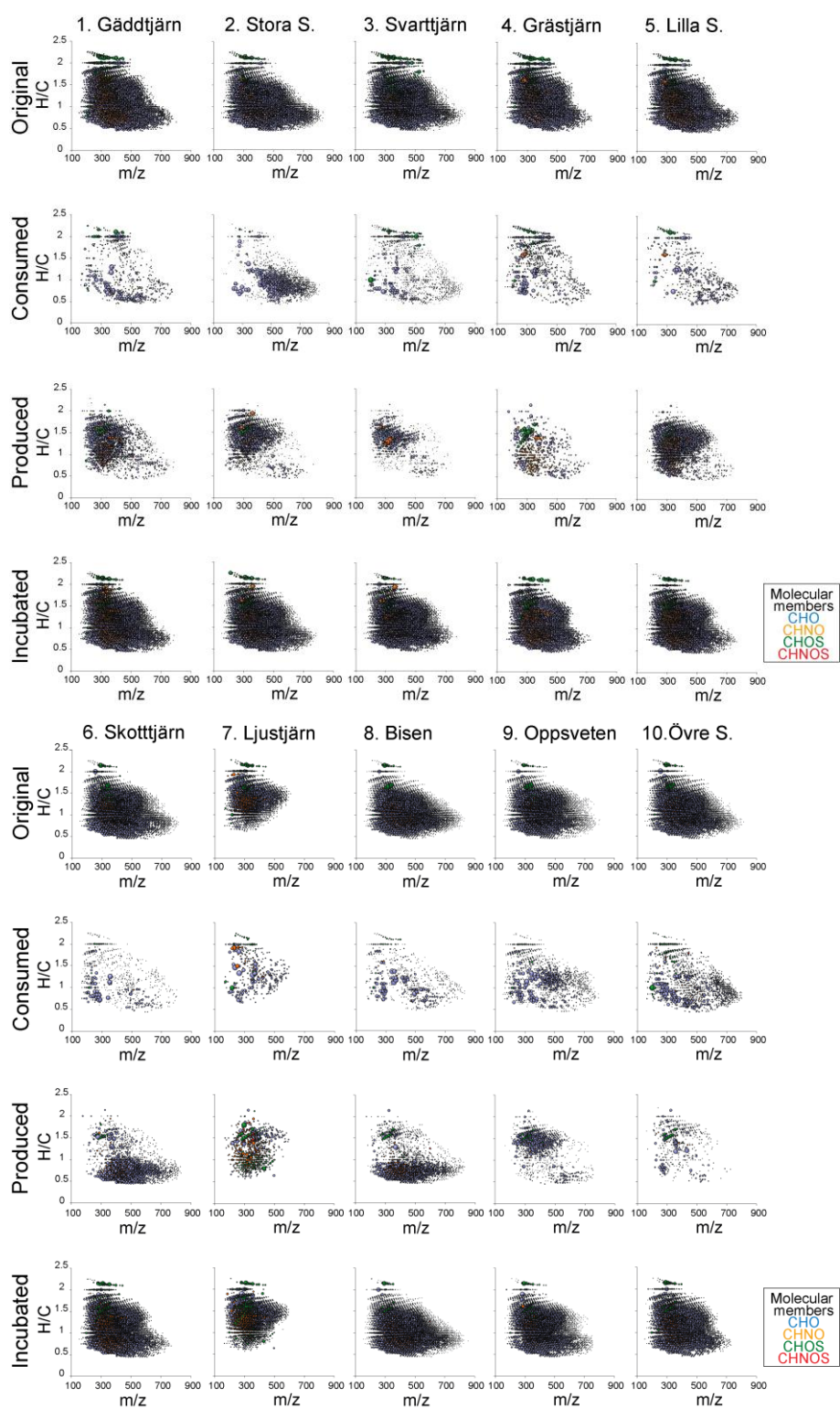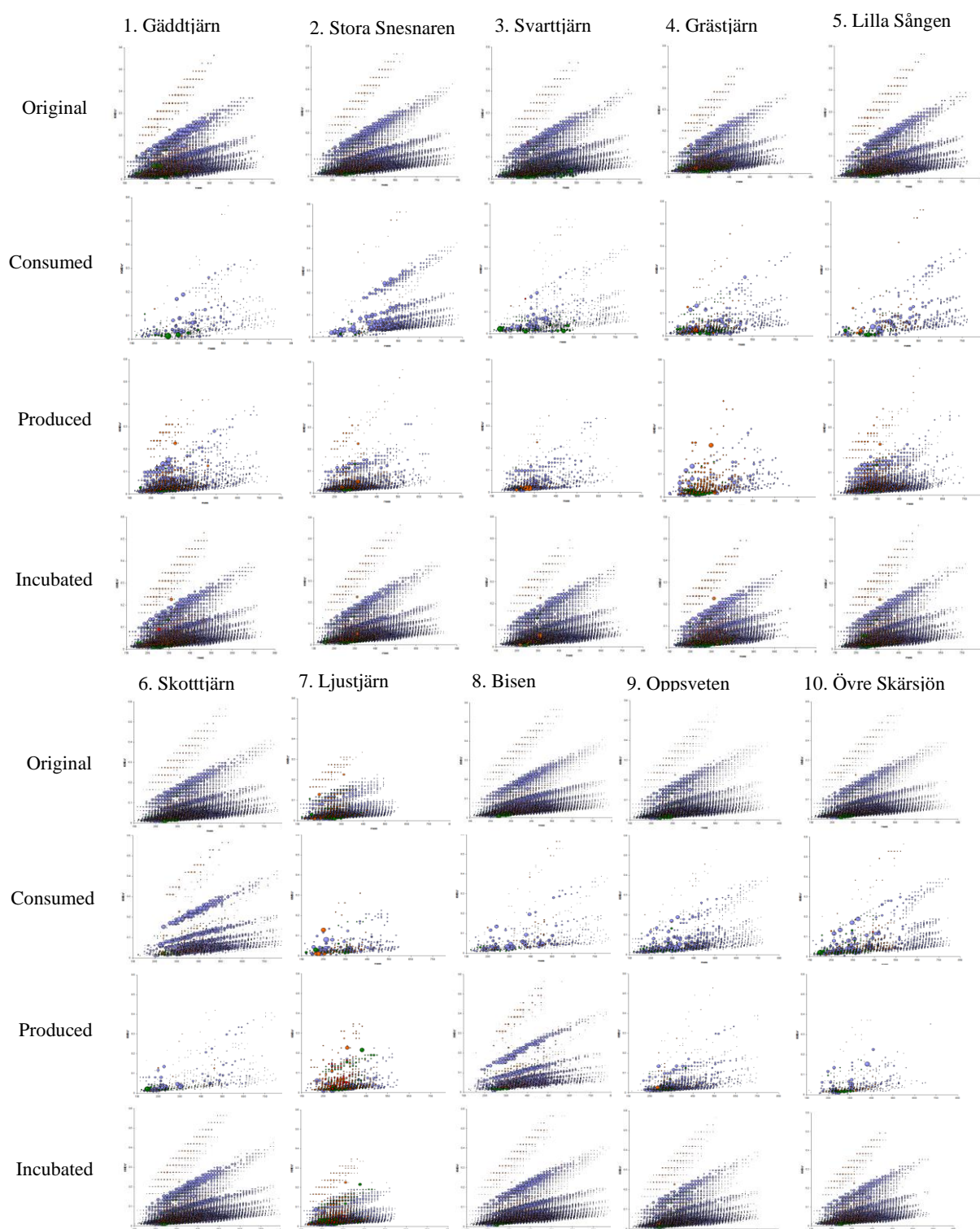
Figure S3. FTMS-derived mass edited H/C diagrams of common molecular compositions observed in all three replicates of original and incubated SPE-sedDOM from ten boreal lakes, color-coded according to molecular series. Consumed and produced compounds were computed based on increasing or decreasing abundance of molecules during incubation; the bubble area corresponds to relative mass peak amplitude.

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Figure S4. FTMS-derived KMD/z* versus m/z diagrams of common molecular compositions observed in all three replicates of original and incubated SPE-sedDOM from ten boreal lakes, color-coded according to molecular series. Consumed and produced compounds were computed based on increasing or decreasing abundance of molecules during incubation; the bubble area corresponds to relative mass peak amplitude.
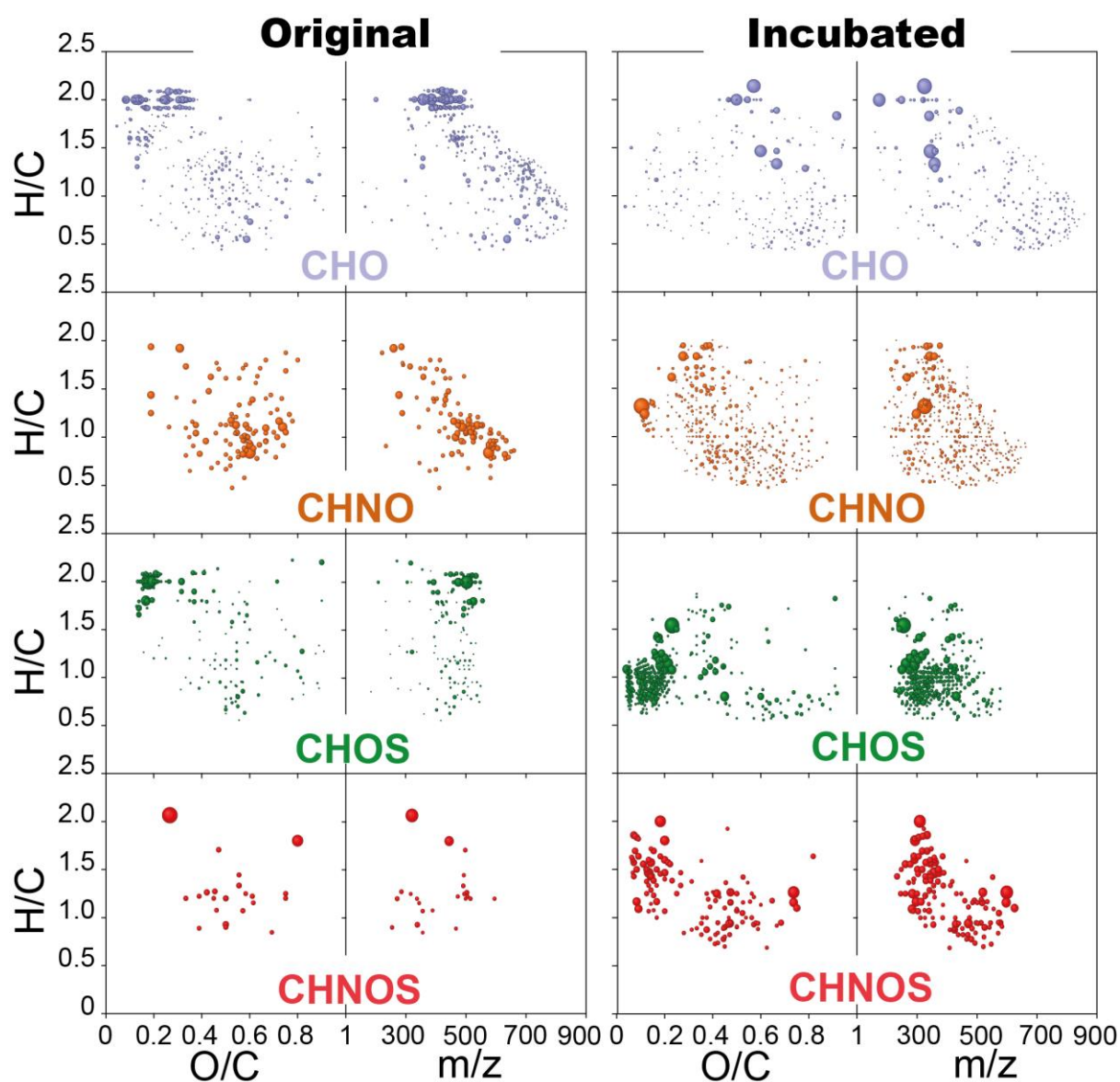
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Figure S5. FTMS-derived van Krevelen and mass-edited H/C diagrams of the sum of unique molecular compositions present only in original and only incubated samples, depicted here according to CHO (blue), CHOS (green), CHNO (orange), and CHNOS (red) molecular series; the bubble area corresponds to relative mass peak amplitude.
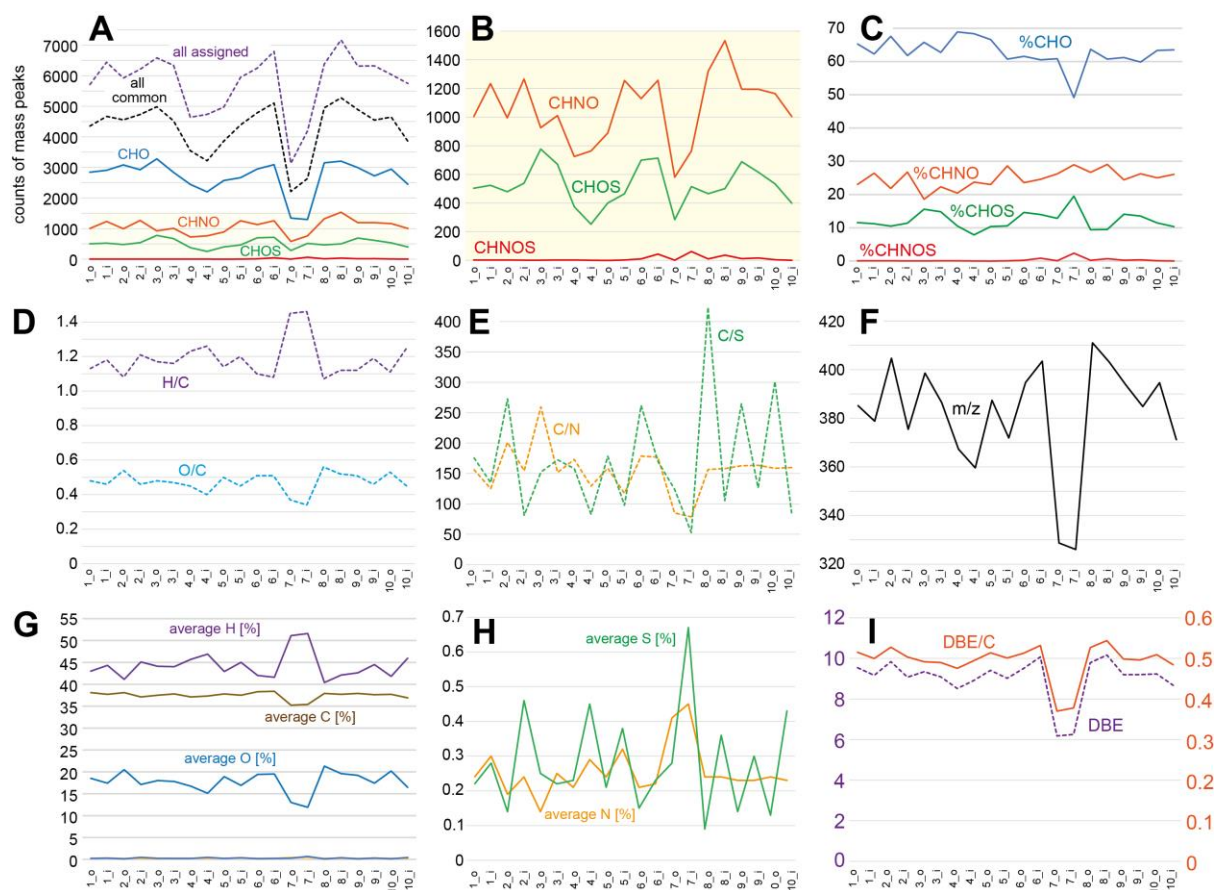
Figure S6. Distribution of FTMS-derived average computed bulk parameters (Table S2) for assigned masses from the original and incubated sediment pore water SPE-DOM of all ten boreal lakes, based on recognition of relative abundance in normalized mass spectra.