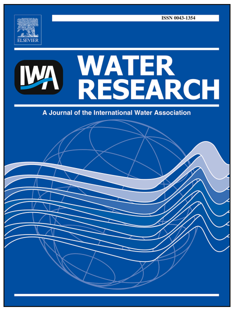

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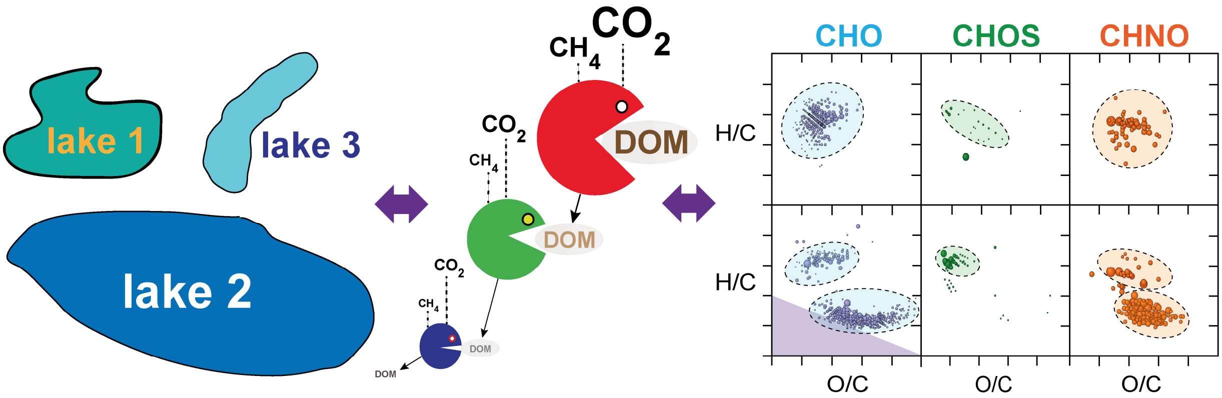
lake 1
lake 3
lake 2
CO2
CH4
DOM
CHO
CHOS
CHNO
H/C
H/C
O/C
O/C
O/C

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

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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 ~2-4×10^{-4} of sedimentary organic carbon for CO_2 and ~0.8-2.4×10^{-5} for CH_4. In contrast, the relative changes of 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 magnitudes 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 of 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, carboxyl-rich alicyclic molecules (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.

Keywords: solid phase extraction, DOM, FTICR-MS, sediment, methane, anaerobic respiration

1. Introduction

Dissolved organic matter (DOM) represents one of the largest active carbon pools and also one of the most complex mixtures of organic molecules on Earth (Hertkorn et al., 2007). Organic matter cycling in lakes is now considered relevant for global carbon budgets because of 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 carbon dioxide (CO_2) and methane (CH_4) from lakes (Tranvik et al., 2009). It has been proposed that allochthonous (terrigenous) dissolved organic carbon (DOC) input is the major source of organic matter in boreal lake sediments (von Wachenfeldt et al., 2008), and that up to 50% of the carbon mineralization occurs in the sediments (Jonsson et al., 2001). However, many studies have only used bulk parameters such as organic carbon and nitrogen contents as indicators of organic matter quality and lability (Bernardes et al., 2004; Conrad et al., 2011).

Methanogenesis is considered the last step in microbial degradation of sediment organic matter, and occurs after the depletion of other inorganic electron acceptors, such as oxygen (O_2), nitrate (NO_3^-), ferric iron (Fe^{3+}), and sulfate (SO_4^{2-}) (Conrad, 1996). During anaerobic organic matter degradation first the major (bio)polymers are decomposed to oligomers and monomers, followed by fermentation reactions which produce fatty acids, alcohols, acetate, CO_2 and hydrogen (H_2) – the last three being the main substrates of methanogenesis (Conrad et al., 2011). Nevertheless, degradation of organic matter under methanogenic conditions can account for a major share of total organic matter metabolism (20-56 %; Bastviken, 2009). Previous studies have found a positive relationship between CH_4 production rates and the content of organic carbon as well as a preferential degradation of algal material (Conrad et al., 2011; Schulz and Conrad, 1995; West et al., 2012). However, the chemical nature of the organic substances that are converted to CO_2 and CH_4 remain largely unknown, although sequential processing of various carbon pools has been reported (Mahmoudi et al., 2017).

DOM plays an important role in the transfer of energy and matter in aquatic ecosystems, and the anaerobic decomposition of sediment DOM can be extensive. On the example of lake Gäddtjärn, Chmiel et al. (2016) found that, even when sedimentary organic carbon mineralization contributed less than one fifth to the total lake CO_2 emissions, sediment mineralization still overcame carbon burial about three-fold. In addition, regarding the potential for DOM under different conditions, the total degradation potential of lake water DOM was found highest under oxic conditions, but about one tenth of DOM was promptly and exclusively mineralized under anoxic conditions (Bastviken et al., 2004). Extensive anoxic processing of DOM has also been demonstrated in a variety of soil and marine conditions (Hodgkins et al., 2014, 2016; Schmidt et al., 2017; Seidel et al., 2014). A typical feature of previous studies of anaerobic DOM decomposition is that they are based on formation of degradation end-products, such as CO_2 or CH_4. However, transformations of DOM that does not lead to formation of end-products could potentially be very important in generating significant metabolic energy for food webs, as well as resulting in new DOM molecules that progressively become more refractory and are later preserved in the sediments as a carbon sink. There is also a possibility that microbial processing leads to a gradual shift from terrestrial plant derived DOM to more microbial derived DOM (Einsiedl et al., 2007). Here we assess the relative magnitudes of the anaerobic DOM-to-DOM transformations versus the complete DOM degradation to CO_2 and CH_4, hypothesizing, that both are equally important. We also ask how the DOM pool is altered in terms of elemental composition, oxidation state, and change in distribution of molecular formulas during decomposition, under the hypothesis that the relative abundance of reprocessed or microbial refractory DOM compounds should get progressively stronger over time, and hence differ between lakes having different sizes and residence times.

Ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), which can resolve thousands of individual molecular formulae within natural DOM samples (Hertkorn et al., 2008), has been already been successfully used to characterize DOM from freshwater environments (Einsiedl et al., 2007; Gonsior et al., 2016; Jaffé et al., 2012; Kellerman et al. 2015), to trace changes in DOM composition after exposure to photo- and biodegradation along natural gradients (Gonsior et al., 2014, 2009; Medeiros et al., 2015; Ide et al., 2017) and incubation experiments (Hodgkins et al., 2014; Medeiros et al., 2015; Seidel et al., 2016). FTICR mass spectrometry has also been applied to investigate pore water in freshwater, estuarine and marine sediments (Seidel et al., 2014; Schmidt et al., 2009, 2011, 2017; Tremblay et al., 2007) and soils (D'Andrilli et al., 2010; Hodgkins et al., 2014, 2016). Mass spectrometry has also revealed changes of DOM in bog and fen pore water profiles of peatlands (D'Andrilli et al., 2010; Hodgkins et al., 2016), and possible relations with increased production of the greenhouse gases CH_4 and CO_2 in permafrost environments (Hodgkins et al., 2014). In this study we have used negative mode electrospray ionization (ESI) FTICR mass spectrometry to determine the extent and molecular selectivity of DOM alterations in boreal lake sediment pore water during experimental anoxic incubation, alongside with determination of CH_4 and CO_2 production.

2. Material and Methods

2.1. Site description

Sediment was collected from three lakes in the Malingsbo area, Sweden (Svarttjärn: 59°53’25.12”N 15°15’26.68”E; Grästjärn: 59°53'18.43"N 15°21'22.64"E; Bisen: 59°57′17.36″N 15°29′37.44″E), in August 2012 (Fig. 1). The lakes investigated are geographically close, but vary in both size and depth (Table 1). Bisen is the largest and deepest one, with 43 ha of area and 17 m of maximum depth. Grästjärn and Svarttjärn have an area of 9.5 ha and 0.7 ha, and a maximum depth of 8 m and 7 m, respectively (see Table 1 for lake characteristics). The difference in lake area and volume causes variable water residence time (Hanson et al., 2011), which in turn influences the average age of the surface sediments and of the DOC found in the lakes. While lake Bisen is expected to have the longest residence time, lake Svarttjärn shows exceptionally high drainage ratio and short water residence time (von Wachenfeldt and Tranvik, 2008).

2.2. Sampling

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 with minimal exposure to air. 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 (4 ºC), stored in the dark and shipped by air to the Max Planck Institute for Terrestrial Microbiology, Marburg, Germany, for incubation experiments. In the field, O_2 profiles in the water column were acquired using an oximeter (Hach HQ40 optical DO sensor) with a depth resolution of 0.5 and 1.0 m.

2.3. Incubation experiment

An analogous incubation experiment has been described elsewhere (Conrad et al., 2007, 2011). Approximately 9 ml of sediment were placed into 27 ml sterile glass vials and closed with rubber stoppers. Vials were then flushed with nitrogen gas (N_2) and vortexed, sequentially and repeatedly, ensuring that all initial gas phase was composed solely of nitrogen. Initial gas measurements proved CH_4 concentration in the headspace to be zero. Then, the sediments were incubated in the dark at 15°C, higher than the actual sediment temperature (Table 1). The observed CH_4 and CO_2 production rates represent the potential of the microbial community and allow for timescales practical in laboratory experiments (Hodgkins et al., 2014). CH_4 and CO_2 concentrations were measured regularly during the incubation period of 40 days (Fig. 2). Gas samples (250 µl) were taken from the headspace and injected in the Gas Chromatograph (Shimadzu GC-8A, Japan). 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 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).

2.4. Chemical analyses

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 autoanalyzer (Bran Luebbe, Germany). The pore water DOM was extracted by a previously described, adapted solid phase extraction (SPE) method (Dittmar et al., 2008). For each replicate, 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 per replicate 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). The eluates were stored in the freezer (-20 ºC) until mass spectrometry analysis.

2.5. FTICR Mass Spectrometry

Negative electrospray ionization Fourier transform ion cyclotron resonance [ESI(-)] FTICR mass spectra were acquired using 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). 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. Further information can be found in Support Online Information.

2.6. Statistical analysis

Hierarchical Cluster Analysis (HCA) was performed using the Hierarchical Clustering Explorer 3.0 (HCE; http://www.cs.umd.edu/hcil/multi-cluster/), with clustering of the dataset using the 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. 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). Principal Component Analysis (PCA) was done with the software SIMCA-P 9.0 (Umetrics AB, Sweden).

3. Results and Discussion

3.1. CH_4 and CO_2 production rates

Anoxic incubation of lake sediment samples resulted in continually increasing concentrations of CH_4 and CO_2 in the gas phase (Fig. 2). Release of CH_4 started without lag phase, indicating that reduction of nitrate, ferric iron, sulfate, etc., was minimal, and that methanogenesis was the dominant degradation process of organic matter (Conrad, 2007). Considerable variability in gas production rates was observed among replicate sediment cores. The production rates of CH_4 varied also among the different lakes. The highest CH_4 production rate (11.8 ± 5.8 nmol d^{-1} g_{dw}^{-1}) was found in sediments of Svarttjärn, followed by Grästjärn (8.9 ± 3. 5 nmol d^{-1} g_{dw}^{-1}) and Bisen (5.9 ± 3.1 nmol d^{-1} g_{dw}^{-1}). Rates of CO_2 production, however, were more similar in Svarttjärn, Grästjärn and Bisen (77.6 ± 5.9, 113.5 ± 11.6 and 107.2 ± 19.5 nmol d^{-1} g_{dw}^{-1}, respectively). The CH_4 production rates were low when compared with tropical and temperate lake sediments (Conrad et al., 2009, 2010, 2011). Duc et al. (2010) have measured CH_4 production in lake Svarttjärn and found similar values (9 nmol d^{-1} g_{dw}^{-1}) at 10°C. Lake sediments in Alaska (Lofton et al., 2015) also presented variable CH_4 production, ranging from 1.7 up to 118 nmol d^{-1} g_{dw}^{-1}.

Cumulative release of gaseous CO_2 during incubation accounted for small fractions of ~2-4×10^{-4} of total sedimentary carbon. Evolution of CH_4 accounted for even lower fractions of only ~0.8-2×10^{-5} (Fig. 2). While complete degradation of organic matter under exclusive methanogenic conditions should result in about equal amounts of CO_2 and CH_4, organic matter degradation here occurred to a larger extent by oxidation to CO_2. Such excess oxidation to CO_2 is only possible if an oxidant is available. If O_2, nitrate, sulfate, ferric iron and other inorganic electron acceptors were not available (as indicated by the instantaneous production of CH_4), organic compounds (e.g. humic acids) must have served as oxidants (Corbett et al., 2015; Klüpfel et al., 2014; Yavitt and Seidmann-Zager, 2006).

3.2. Compositional characteristics of SPE-DOM

Molecular compositions of lake sediment porewater SPE-DOM before and after incubation were identified by virtue of high mass accuracy and mass resolution typical of FTICR mass spectrometry (see Supporting Online Information for comments on the depiction of molecular diversity of SPE-DOM by mass spectrometry). Common mass peaks that were present in all five replicate samples before and after incubation exceeded 3000 counts in sediment from Grästjärn and 4000 counts for

Svarttjärn and Bisen (Table 2). With the exception of CHNOS compounds, which were comparatively less abundant, the counts of CHO, CHNO and CHOS molecular series between individual replicates showed larger relative variance of incubated compared with original SPE-DOM samples (Table S1, S2). The relative variance commonly changed in the order CHNOS > CHNO ≈ CHOS > CHO molecular series (Table S2).

On average, ~60-70% of all assigned molecular compositions were common to all five replicate sediment cores in each lake (Table 2). 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.

Hierarchical Cluster Analysis (HCA) of FTICR mass spectra showed a clear separation according to the different lakes (minimum similarity: 0.55), consistent with studies on the molecular diversity of DOM in aquatic systems (Ide et al., 2017) (Fig. S1). DOM compositions before and after incubation differed considerably for all three lakes (minimum similarity: 0.83), suggesting turnover of DOM by combined effects of microbial production and degradation or chemical transformations during the incubation experiment. However, lake-specific characteristics (Table 1) were more influential for DOM composition than changes during the incubation, and differences among the replicates were always larger for incubated versus original samples (Fig. S2).

Analogous results were obtained from Principal Component Analysis (PCA). Again, clustering of SPE-DOM compositions according to lakes was more pronounced than clustering according to incubation or replication (Fig. S2). The relative variance of incubated samples always exceeded that of original samples, while the fraction of molecular compositions common to all five replicates was always smaller for original than for incubated samples (Table 2). Both trends were related and suggested increasing chemical diversity caused by incubation (Fig. S1, S2).

When comparing the FTICR mass spectra of sediment pore water SPE-DOM from the three boreal lakes before and after incubation, the compounds declining in relative abundance during incubation were less abundant (~1-3%) than those increasing in relative abundance (~4-10%)(Fig. S3). Here, Svarttjärn and in particular Bisen showed substantial mass range (Fig. S3) and bandwidth of molecular compositions (Fig. 3) increasing in relative abundance after incubation. CO_2 and CH_4 are unambiguously defined molecules and easily quantified; these are likely to result from mineralization of carbon present in original organic matter, mediated by activity of microorganisms (Bastviken, 2009; Tranvik et al., 2009). In our experiments, the relative turnover of carbon ranged from $2\text{-}4\times10^{-4}$ (CO_2), which was likely an underestimate of total produced CO_2 because bicarbonate was not considered, and $<1\text{-}2\times10^{-5}$ (CH_4) of total sedimentary organic carbon (Fig. 2). In contrast, the relative change during incubation of key bulk parameters, such as relative proportions of molecular series, elemental ratios, average mass and unsaturation, was regularly in the percent range (Table 2; Fig. S3); i.e. several orders of magnitudes higher than mineralization alone. This implies that during incubation, the SPE-DOM transformation was far more extensive than the readily observed mineralization, which is commonly regarded as a measure of microbial activity.

Although the consistent increase of apparent chemical diversity during incubation in all three lakes contrasts with a simple notion of degradation of organic matter from larger to smaller molecules as expressed in the familiar size-reactivity continuum model (Amon and Benner, 1996; Benner and Amon, 2014), analogous trends were reported for anoxic marine sediments (Seidel et al., 2014). While the original SPE-DOM samples represent highly processed biogeochemical materials with smooth mass peak distributions resulting from the massive projection of many isomers on one given molecular composition (Hertkorn et al., 2007, 2008), the incubated samples probably contained higher proportions of remnant biomolecules originating from microbial transformations. Although common polydisperse biogeochemical materials such as organic matter are arguably much more complex mixtures than typical biological samples (Hertkorn et al., 2007), the latter may include large proportions of microbial metabolites that might produce recognizable patterns in the mass spectra. In our study, patterning might apply to (the rather few) compounds decreasing during incubation (Fig. S3), suggesting preferential removal of specific CHO- and CHOS-based lipid-like compounds (Fig. 4; Table S3).

In general, more than 60% of the detected mass peaks were assigned to CHO compounds, followed by CHNO molecules (17-28%), CHOS molecules (8-15%) and a few CHNOS compounds (0.4-1.3%), respectively. Despite the substantial molecular diversity of DOM, the chemical diversity of lipid-like

(H/C ratio >1.7; O/C ratio <0.3) and carbohydrate-like DOM (H/C ratio ~1.5; O/C ratio >0.7) was limited. This likely resulted from activity of hydrolytic microorganisms (Shakeri Yekta et al., 2012; Walker and Wilson, 1991), which is expected for lake sediments anaerobic degradation, since hydrolysis is the first step in methanogenesis and other organic matter degradation processes (Bastviken, 2009; Conrad, 1999).

While the computed average O/C ratios of SPE-DOM uniformly declined during incubation, the respective H/C values stayed near constant in Svarttjärn, whereas those in Grästjärn and Bisen increased considerably (Table 2). Microbial activity has been suggested as responsible for analogous trends in the sediments of the Black Sea (Schmidt et al., 2017), in peat soils in Sweden (Hodgkins et al., 2016), and in biodegradation experiments in the Amazon River (Medeiros et al., 2015; Seidel et al., 2016). Reactive functional groups of organic matter, which often contain oxygen, are expected to react more frequently than unfunctionalized aliphatics, and the oxygen-rich molecules H_2O and CO_2 are commonly released during organic matter processing (Einsiedl et al., 2007), likely contributing to the observed relative depletion of oxygen in organic matter during incubation (Evans and Fuchs, 1988; Ghattas et al., 2017).

Van Krevelen and KMD/z* diagrams for original and incubated sediment pore water SPE-DOM from the three boreal lakes were determined for initial and final samples and for respective molecular compositions growing or declining in abundance during incubation (Fig. 3, 4, S4). These diagrams demonstrated coverage of a rather large area of the CHO, CHNO and CHOS compositional space for initial and final SPE-DOM, indicative of a remarkable molecular diversity of these organic molecules which exceeds that of common DOM present in the water column of boreal lakes (Gonsior et al., 2013). Furthermore, production of CHNO compounds with average H/C and O/C ratios of ~0.5-2.0 and ~0.2-0.8, respectively, was found in all three boreal lake sediments (Fig. 4). Production of N-containing compounds might be related to protein degradation and formation during anaerobic degradation of biomass-derived substrates (Schmidt et al., 2011; Shakeri Yekta et al., 2012), or from microbial biomass (Seidel et al., 2016).

While CHNO and CHNOS compounds covered a smaller area in the van Krevelen diagrams than CHO and CHOS compounds, CHNO compounds, specifically, were mostly limited to average H/C and O/C ratios of ~ 0.5-2 and ~0.2-0.8, respectively (Fig. 4). In addition, CHO compounds covered a relatively large mass range (~200-850 Da). In comparison, CHNO and CHNOS compounds were smaller than ~700 Da, and CHOS compounds were almost exclusively restricted to <600 Da. The relative gain of heteroatom-containing molecules during incubation seemed to be a common feature among the three lakes. Increased proportions of CHNO and CHOS compounds were also observed in Swedish peat soils at an advanced stage of decomposition (Hodgkins et al., 2014, 2016). In our study, lower mass compounds ($m/z < 500$) showed higher H/C ratios (i.e. were more aliphatic) than higher mass compounds (Fig. 3) among consumed and produced compounds. The average mass of DOM molecules in general declined during incubation (Table 2). Nevertheless, a number of DOM molecules formed during incubation were of high mass (Fig. 3, S3, S4, S5).

Summary van Krevelen, mass-edited H/C ratio and KMD/z* diagrams of original and incubated SPE-DOM showed extensive coverage and overlap of the CHO, CHNO and CHOS compositional space, precluding clear assessment of molecular changes caused by incubation (Fig. 3, S4). KMD/z* diagrams revealed extended (n ~ 12-20) CH_2-based homologous series for original and incubated samples for CHO compounds and shorter ones (n ~ 6-10) for CHNO and CHOS compounds (Fig. S4). Anoxic incubation of sediment caused some uniform progression of key bulk parameters (Table 2): the average mass of SPE-DOM decreased, in line with greenhouse gas release from organic matter. The proportions of CHO compounds consistently decreased after incubation whereas the proportions of CHNO compounds uniformly increased. Higher abundances of CHNO compounds might be an indication of intensive microbial activity (Hodgkins et al., 2016; Roth et al., 2014; Seidel et al., 2014). Rather non labile CHNO compounds were present after incubation; hence, incubation affected the coupled carbon and nitrogen cycles in lake sediments (Hedges and Oades, 1997). Oxygen content as well as O/C ratios of the residual SPE-DOM decreased following incubation, partially because oxidized substrates H_2O and CO_2 had been liberated. In contrast, average carbon, average double bond equivalent (DBE) and DBE/C values were less affected, indicating that C-based molecular skeletons were rather modified by corresponding functional groups than fundamentally rearranged during incubation.

3.3. Changes according to CHO-, CHNO-, CHOS- and CHNOS molecular series in SPE-DOM

Two stages of sorting were required to enable a meaningful assessment of lake sediment processing by anoxic incubation. First, the exclusive recognition of molecular compositions common to all five respective replicates alleviated the considerable intrinsic variance of the individual incubation experiments (which likely reflected the variance of organic matter molecules and that of the temporal evolution of microorganisms; Fig. S5, S6; Table S1, S2) and enabled the detection of specific organic matter transformation within lake sediments. Second, the separate display of CHO, CHNO, CHOS and CHNOS molecular series common to all five replicates removed projection and clearly revealed distinct trends for each of the three lake sediments investigated in this study. Summary van Krevelen, mass-edited H/C ratio and KMD/z* diagrams for presumably consumed and produced compounds during the anoxic incubation experiments were computed for three selected individual samples each (Fig. S5, S6), and for common molecular compositions present in all five replicates of the six incubation experiments (Fig. 3, 4, S4 , Table 2, S1, S2).

In addition, a display for individual CHO, CHNO, CHOS and CHNOS molecular series in van Krevelen and KMD/z* diagrams was provided for common molecular compositions found in all five replicates (Fig. 4, S4). While the figures displaying common molecular compositions appeared more distinct and “less noisy” than those resulting from individual SPE-DOM samples, a visual comparison of SPE-DOM samples before and after incubation still could not retrieve relevant information in any of these diagrams. The high abundance of CHO molecules in particular, and the broad coverage of the CHO, CHNO and CHOS chemical space at average H/C and O/C ratios (Hertkorn et al., 2007) by SPE-DOM both prior to and after incubation precluded easy determination of changes in composition by visual inspection. Finally, relative changes of normalized mass peak amplitudes were computed by means of “profile search” within HCA, providing depiction of compounds decreasing and increasing in relative abundance during incubation. When CHO, CHNO and CHOS molecular series were shown separately, distinct DOM processing during incubation according to lake was clearly revealed in van Krevelen and KMD/z* diagrams (Fig. 4, S6). In general, the following changes were observed (all Tables):

- Loss of certain compounds during incubation was mainly restricted to near saturated CHO and CHOS lipid-like compounds (H/C ratio > 1.8) and seemed less significant than gain of specific compounds (Fig. 3, 4, S3).
- Svarttjärn showed formation of CHO and CHNO-based CRAM molecules (carboxylic-rich alicyclic molecules) (Fig. 4) (Hertkorn et al., 2006) which are considered exemplary microbial products (Bianchi, 2011). A minor suite of common CHOS organic matter molecules of near average H/C and O/C ratio was also produced.
- Grästjärn showed related but less specific trends: two groups of oxygen-deficient lipid-like CHOS compounds with variable extent of unsaturation (H/C ratio ~1.5; O/C ratio < 1.1) and an extended group of unsaturated CHNO compounds had appeared following incubation. A small proportion of supposedly aromatic CHOS compounds (H/C ratio < 1.1; O/C ratio < 0.2) was observed in Grästjärn after incubation (Fig. 4). Analogous CHOS compounds have also been found in SPE-DOM samples from Amazonian rivers and German mine pit lake pore water (Gonsior et al., 2016; Herzsprung et al., 2010). These compounds could result from sulfurization of aromatic CHO compounds by biological sulfide production under anoxic conditions (Shakeri Yekta et al., 2012).
- Bisen exhibited the most unequivocal chemical evolution of sediment organic matter. Here, an extended set of CHO- and CHNO-based CRAM had been generated (although less distinct than in Svarttjärn), and a particular set of unsaturated sulfolipid-like compounds. Most important, however, was a very specific array of rather unsaturated (H/C ratio < 1) lignin- and tannin-like CHO compounds which was found more abundant in Bisen after incubation. Another, peculiar set of unsaturated CHNO compounds (H/C ratio < 1.1; mass range m/z ~ 300-500; Fig. 4) with unclear attribution at present deserves further observance.
- It is noteworthy that both Grästjärn and Bisen showed substantial depletion of oxygen after incubation, which was almost exclusively restricted to their CHOS compounds. This most likely indicates reduction of sulfur in CHOS compounds, probably originating from sulfate reduction (Table S4).

Compounds found more abundant after incubation may represent molecules synthesized during incubation or, alternatively, compounds being less efficiently degraded during incubation. In case of

Bisen, general considerations about relative persistence of lake organic matter compound classes (Kellerman et al., 2015; Mahmoudi et al., 2017) suggest that the lignin- and tannin-like CHO compounds found at elevated relative abundance after incubation represent less efficiently degraded compounds under anoxic conditions (Seidel et al., 2014) which were already present in original lake Bisen sediments (leftovers of incubation) rather than newly synthesized ones. Tannin-like compounds have been found characteristic of forest soil leachates (Roth et al., 2014), and the evolution of SPE-DOM conforms to the land coverage present in Bisen catchment, which shows ~84% of coniferous and mixed forest.

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

An alternative projection of the CHO-compositional space is the depiction of the average oxidation state (OS_av) according to OS_av = 2 × O/C - H/C (Kroll et al., 2011) which has been computed for individual CHO compounds as well as for the consolidated MS-derived intensity weighted average of all detected CHO compounds, before and after incubation (Fig. 5). 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. Here, these OS_av values have been sorted in ranks of 0.1 width. All organic matter molecules displayed average oxidation states between -1.8 and +1.3, i. e. a range smaller than that between the endmembers CH_4 (OS_av = -4) and CO_2 (OS_av = +4). In addition, the difference "original minus incubated" was shown to demonstrate relative losses and gains of compounds according to redox status. Remarkably, OS_av of the consolidated complement of all CHO compounds became more reduced after incubation (change towards more negative values in the order Svarttjärn > Grästjärn > Bisen; Fig. 5). The apparent reduction of the residual organic matter was most probably an indirect consequence of the loss of (the highly oxidized molecules) H_2O (in case of exclusive methanogenesis, H_2O would be taken up instead of being released) and CO_2 during incubation. Interestingly, oxidized CHO molecules, which contained several oxygen-containing functional groups, became preferentially depleted after anoxic incubation. This behavior resembles that of aerobic processing of organic matter in a karst environment in which some reduced organic carbon compounds with relatively small O/C and high H/C ratio were formed, although the bulk of the organic matter became oxidized (Einsiedl et al., 2007). Remarkably, 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. Such molecules will necessarily contain several oxygen-containing functional groups, making those more polar and reactive than common aliphatic compounds (Burdige, 2007).

Microbial redox reactions rely on transfer of electrons from electron donor substrates to terminal electron acceptors and must be thermodynamically favorable (LaRowe and Van Cappellen, 2011;

Thullner et al., 2007). Reduced carbon in the form of organic matter is often the ultimate source of electrons in sediments, and environmental redox conditions progressively change during degradation or incubation. The bioenergetic potential of organic matter and of its individual molecular constituents is related to its oxidation state as expressed by OS_av. The Gibbs ΔG_{ox} energy for oxidation half reactions of organic molecules depends on their molecular composition and structures, and is commonly inversely related with the oxidation state, even when ΔG_{ox} is normalized to the number of electrons released (LaRowe and Van Cappellen, 2011). Hence, the removal of electrons from an organic compound becomes thermodynamically more favorable with increasing OS_av (Kroll et al., 2011; LaRowe and Van Cappellen, 2011). In Svarttjärn and Grästjärn, potentially easily bioavailable lipid-like compounds as well as oxygenated compounds were preferentially depleted during incubation (cf. above; Fig. 5) whereas Bisen showed similar trends with however lesser distinction. These underlying fundamental trends were not disclosed by the differential mass analysis shown above (sections 3.2 and 3.3), which had revealed lake-specific organic matter transformations based on relative mass peak amplitude differences exceeding a certain threshold (e.g. for CHO compounds; Fig. 4). It is therefore highly probable that a directional redox evolution of organic matter during incubation as expressed by OS_av will apply to fundamentally different chemical structures which then may occupy diverging sections within van Krevelen and mass-edited H/C ratio diagrams. Notably, compounds with similar OS_av as found in fulvic acids (OS_av ~ -0.52; Kroll et al., 2011; LaRowe and Van Cappellen, 2011) had grown in abundance after incubation (Fig. 5). Fulvic acids represent the highly processed, water soluble complement of humic substances (Hertkorn et al., 2002), It appears that the extent of relative changes progressively decreased with ever increasing lake size and water residence time. Here, the increase of molecular diversity in pore water DOM (Seidel et al., 2014) could be explained due to the accumulation of non-labile DOM in the sediments, when compared to the water column (Kellerman et al., 2014).

4. Conclusion

This study revealed rather non-selective large scale transformations of organic matter during incubation and lake-specific, extensive molecular level alterations during anaerobic degradation of sediment pore water DOM obtained from boreal lakes. A dependable assessment of the general and the lake-specific, complex chemical transformations happening during anaerobic DOM processing required 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. Here, 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. 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 was obtained. There were also clear signs of increasing the relative abundance of nitrogen-containing DOM of likely microbial origin. This study highlights the importance to link biogeochemical process studies with studies of molecular composition using high resolution non-target approaches. Further studies could clarify effects of study design on DOM processing in natural and artificial systems.

Author contributions

J.V. collected samples from Swedish lakes in August 2012; D.B. generated funding in support of this research. J.V. performed gas evolution measurements at the facilities of R.C. A.P., D.B. and R.C. contributed to the study design. J.V., M.G. and M.H. performed the FTICR/MS acquisition, data analyses and participated in data interpretation. P.S.-K. provided support for the FTICR/MS analyses and general data interpretations. Data interpretation was performed by all authors. J.V., M.H., M.G., D.B., and N.H. actively participated in the writing of the manuscript; all authors provided significant input on the final manuscript.

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

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Tables and Captions

Table 1. GPS coordinates, physico-chemical parameters from water column and sediments, and greenhouse gases production rates (± SE) from three Swedish lakes studied.

item	**Svarttjärn**	**Grästjärn**	**Bisen**
position	59°53'26.50" N 15°15'27.00" E	59°53'23.30" N 15°21'17.50" E	59°57'20.60" N 15°21'38.00" E
area [km^2]	0.007	0.09	0.43
maximum depth [m]	7	8	17
pH	4.8	4.9	6.1
DOC lake water [mg l^{-1}]	25.7	17.4	7.3
lake water total phosphorus TP [µg l^{-1}]	15.1	8.7	6.5
lake water total nitrogen TN [µg l^{-1}]	502	335	281
sedimentary organic carbon [%]	8.5	17.4	13.0
sedimentary organic nitrogen [%]	0.4	1.1	0.9
CH_4 evolution [nmol g dw^{-1} d^{-1}]	11.8 ± 2.6	8.9 ± 1.5	5.9 ± 1.4
CO_2 evolution [nmol g dw^{-1} d^{-1}]	77.6 ± 75.9	113.5 ± 11.6	107.2 ± 19.5
depth / temperature	6.5 m / 5.3^{o}C	4.5 m / 10.9^{o}C	13 m / 6.8^{o}C
O_2 saturation %	1.2	7.6	3.6
mg/L oxygen	0.15	0.82	0.42

Table 2. Counts of common mass peaks between five replicates of sediment pore water SPE-DOM in the three studied lakes (Svarttjärn, Grästjärn, and Bisen) before and after incubation as computed from negative electrospray (ESI) 12 T FTICR mass spectra for singly charged ions.

members of molecular series	**Svärtjärn original**	**Svärtjärn incubated**	**Grästjärn original**	**Grästjärn incubated**	**Bisen original**	**Bisen incubated**
consolidated count of assigned mass peaks detected in any of the five replicates	6555	6695	4821	4898	6401	7090
total count of assigned mass peaks common to all five replicates	4571	4203	3316	3018	4473	4592
CHO compounds	3100 (68%)	2693 (64%)	2299 (69%)	2083 (69%)	2919 (65%)	2900 (63%)
CHOS compounds	686 (15%)	614 (15%)	352 (11%)	240 (8%)	420 (9%)	418 (9%)
CHNO compounds	784 (17%)	895 (21%)	664 (20%)	695 (23%)	1132 (25%)	1263 (28%)
CHNOS compounds	1 (0.02%)	1 (0.02%)	1 (0.03%)	0 (0%)	2 (0.04%)	11 (0.24%)
proportion of formulas shared in all five replicates	70%	63%	69%	62%	70%	65%
average H [%]	43.7	43.9	45.1	47.6	40.2	42.2
average C [%]	37.7	37.8	37.3	37.1	37.9	37.7
average O [%]	18.3	17.9	17.2	14.6	21.5	19.5
average N [%]	0.1	0.2	0.2	0.2	0.2	0.2
average S [%]	0.2	0.2	0.2	0.5	0.1	0.3
computed average H/C ratio	1.16	1.16	1.21	1.28	1.06	1.12
computed average O/C ratio	0.49	0.47	0.46	0.39	0.57	0.52
computed average C/N ratio	292	188	187	182	174	169
computed average C/S ratio	165	166	169	68	448	113
average carbon oxidation state of CHO compounds	-0.183	-0.214	-0.215	-0.219	0.059	-0.056
average DBE	9.0	8.8	8.1	7.4	9.8	9.2
average DBE/C	0.5	0.5	0.5	0.4	0.5	0.5
mass weighted average [Da]	400.3	388.6	369.7	358.3	412.9	399.7

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

Juliana Valle [a], *Michael Gonsior* [b], *Mourad Harir* [a,c], *Alex Enrich-Prast* [d,e*],
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Figures and Captions

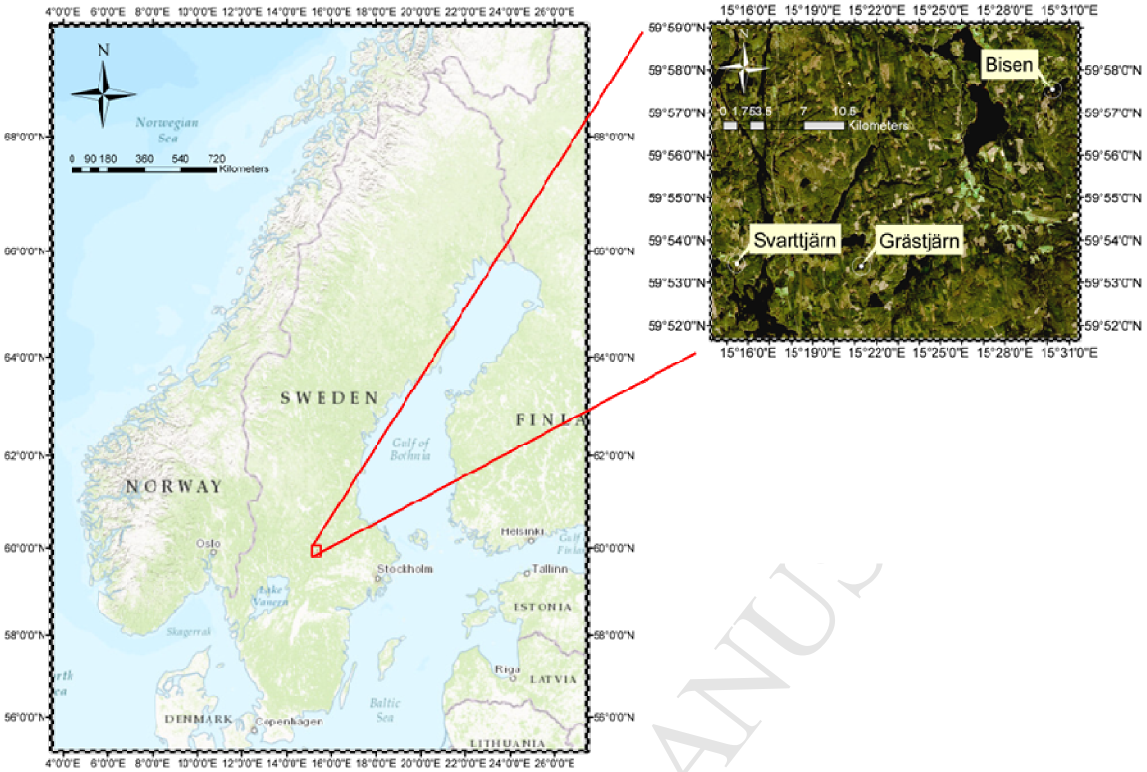

Figure 1. Maps of sampling areas.

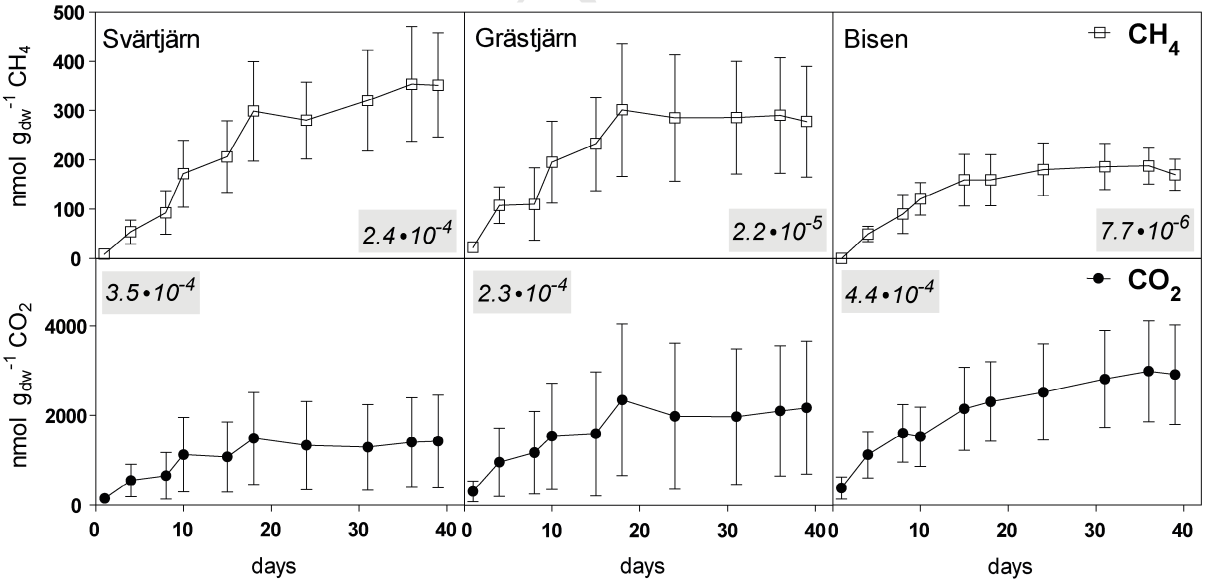

Figure 2. CH_4 and CO_2 concentrations during the anaerobic incubation experiment. Values shown are average from five replicates and error bars represent standard error. Gray shaded values denote fraction of carbon evolved after 40 days of incubation in the form of CO_2 and CH_4 with respect to organic carbon present in dry sediment after incubation.

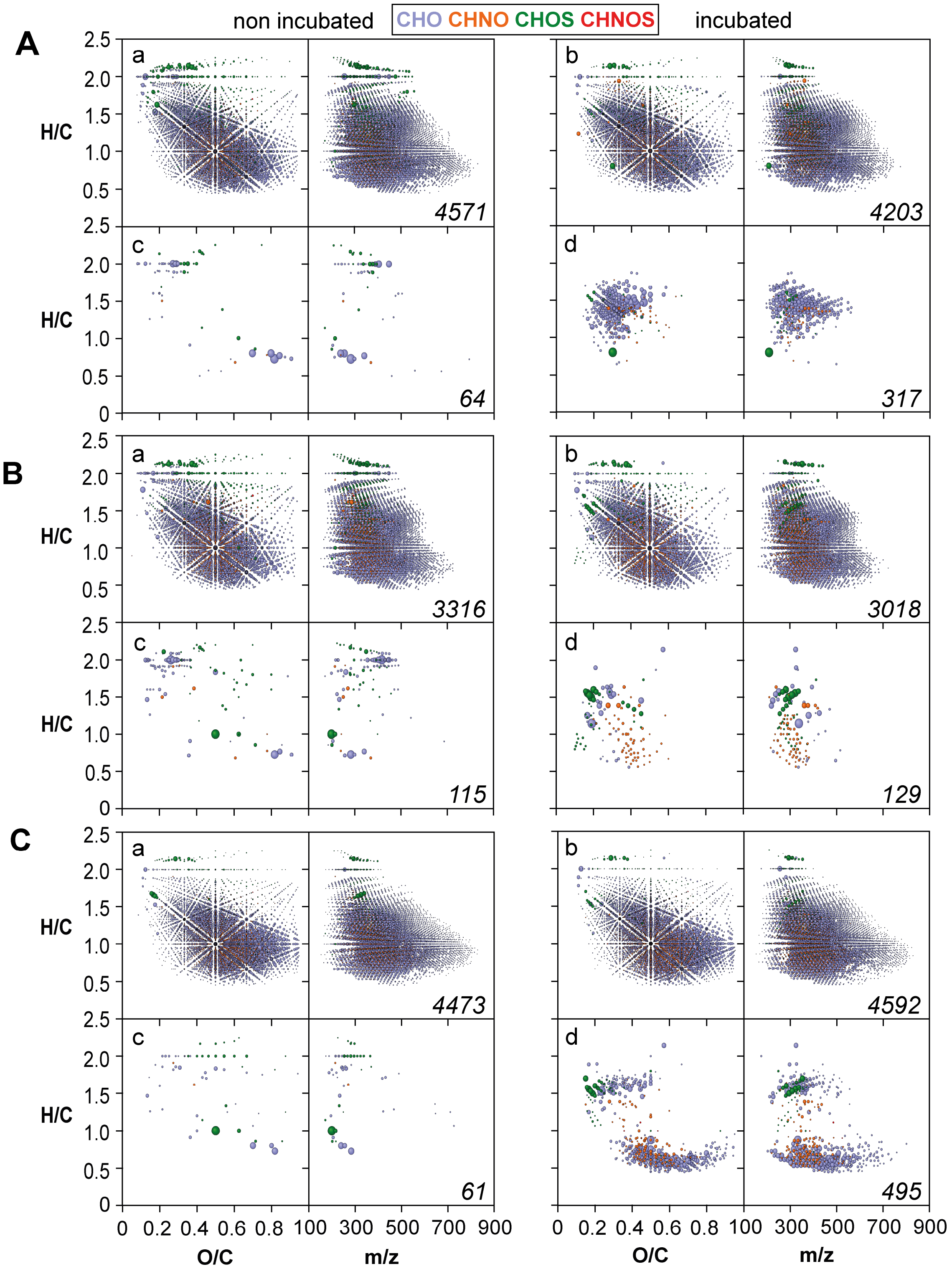

Figure 3. FTMS-derived van Krevelen diagrams of common molecular compositions observed in all five replicates of original and incubated sediment pore water SPE-DOM from boreal lakes (A) Svattjärn, (B) Grästjärn and (C) Bisen, provided with total counts of molecular compositions (overlay) and color-coded according to CHO (blue), CHOS (green), CHNO (orange), and CHNOS (red) molecular series. Panel a: all compounds observed in the original sample; panel b: all compounds observed in the incubated sample; panel c: compounds declining in abundance during the incubation (presumably consumed compounds); panel d: compounds increasing in abundance during the incubation (presumably produced compounds). Van Krevelen diagrams derived from individual SPE-DOM samples (Figures S5 and S6) show individual characteristics and variance, resulting in larger counts and nominal chemical diversity of observed molecular compositions (Table 2), thereby obscuring common trends of SPE-DOM evolution associated with incubation.

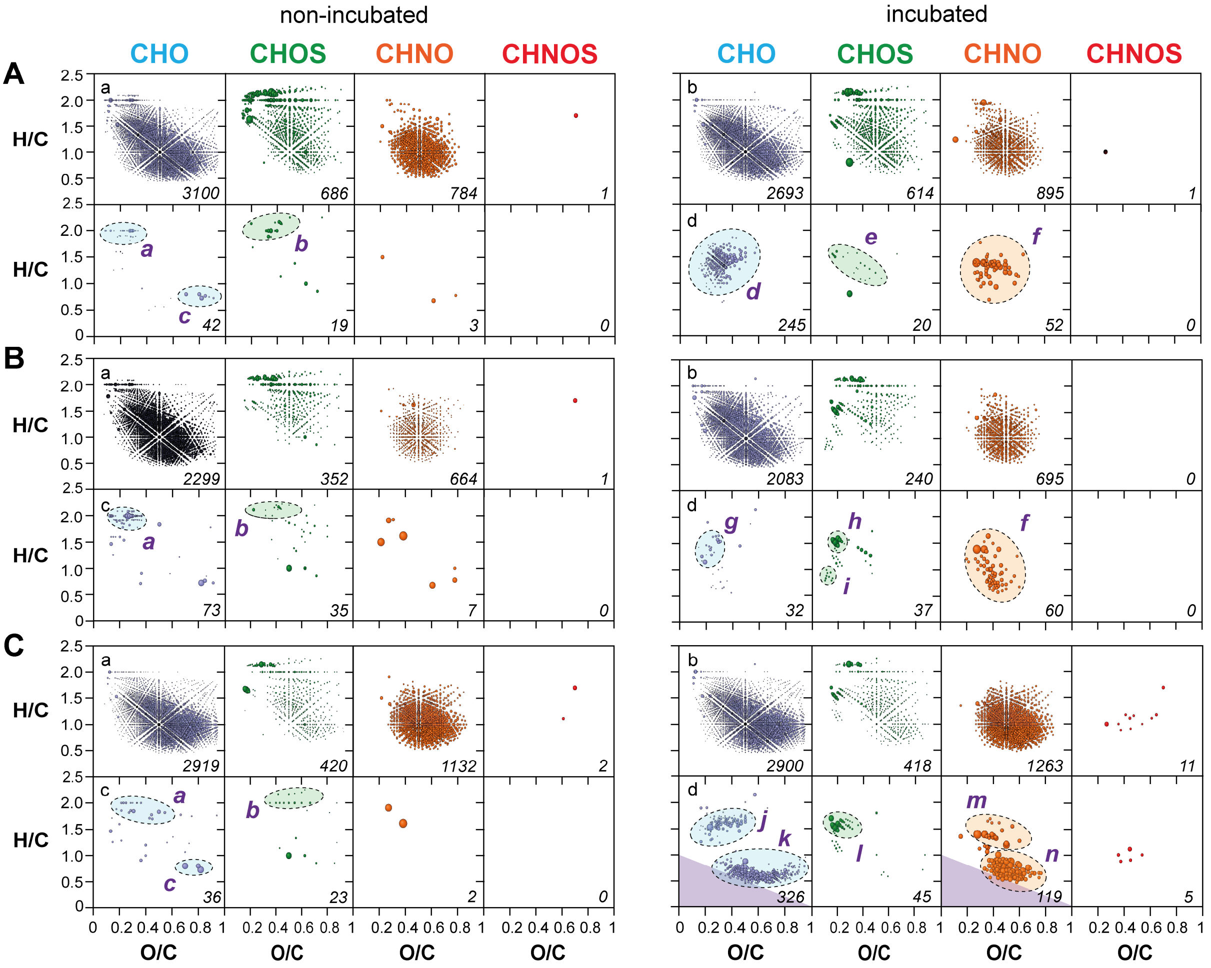

Figure 4. FTMS derived van Krevelen diagrams of common molecular compositions observed in all five replicates of original and incubated sediment pore water SPE-DOM from boreal lakes (A) Svattjärn, (B) Grästjärn and (3) Bisen, provided with counts of molecular compositions and color-coded according to CHO (blue), CHOS (green), CHNO (orange), and CHNOS (red) individual molecular series. Panel a: all compounds observed in the original sample; panel b: all compounds observed in the incubated sample; panel c: compounds declining in abundance during the incubation (presumably consumed compounds); panel d: compounds increasing in abundance during the incubation (presumably produced compounds). Van Krevelen diagrams derived from individual SPE-DOM samples (Figure S6) show individual characteristics and variance (Tables S1 and S2), resulting in larger counts and nominal chemical diversity of observed molecular compositions (Table 2), thereby obscuring common trends of SPE-DOM evolution associated with incubation. Attribution of key structures (purple, italics): *a*: aliphatic CHO-lipid like compounds; *b*: aliphatic CHOS lipid-like compounds; *c*: highly oxygenated, tannin-like compounds; *d*: carboxylic-rich alicyclic molecules (CRAM; Hertkorn et al., 2006); *e*: common CHOS organic matter; *f*: rather unsaturated and oxygenated CHNO compounds of yet unclear provenance; *g*: unsaturated CHO-lipids; *h*: unsaturated CHOS lipids; *i*: aromatic CHOS compounds; *j*: carboxylic-rich alicyclic molecules (CRAM; Hertkorn et al., 2006); *k*: lignin- and tannin-like compounds; *l*: unsaturated CHOS lipid-like compounds; *m*: common CHNO organic matter; *n*: nitrogen-containing lignin- and tannin-like compounds. The aromaticity index AI (Koch and Dittmar, 2006) provided (purple triangles) denotes presence of aromatic compounds.

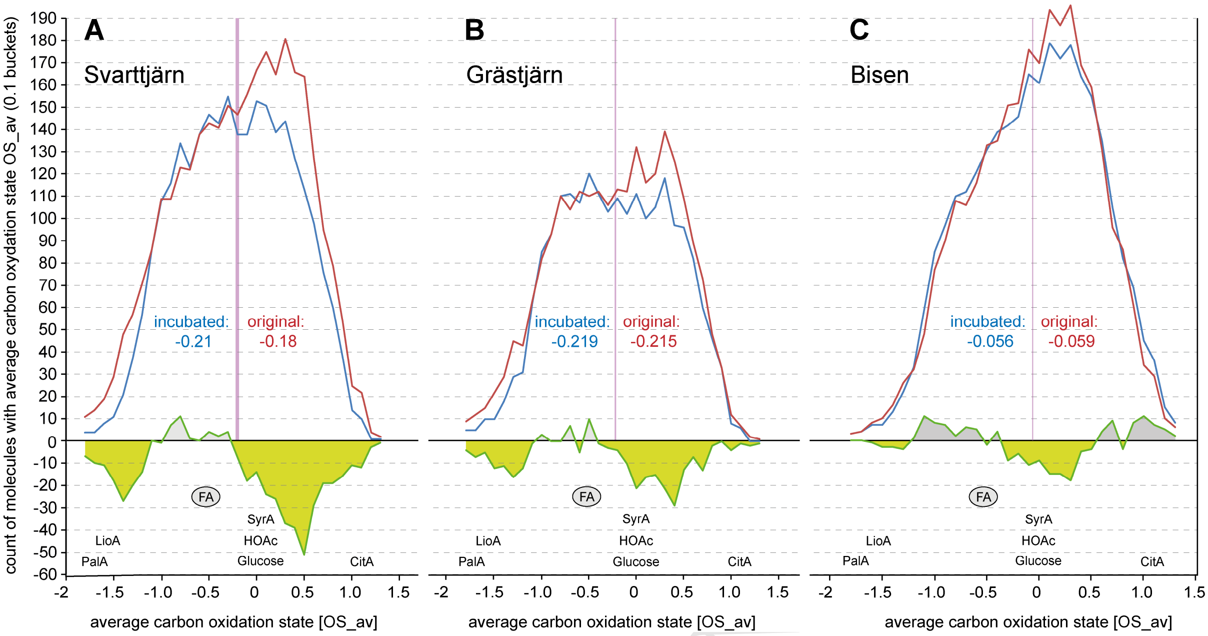

Figure 5. Distribution of average carbon oxidation state OS_av in CHO compounds only, sorted in increments of ±0.1 of nominal carbon oxidation state (Kroll et al., 2011) in three lake sediments before and after incubation; color code: red, original SPE-DOM; blue: SPE-DOM after incubation; green: difference in counts: original minus incubated; yellow-green: compounds decreased during incubation; gray: compounds increased after incubation. 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 (Le Rowe et al., 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).

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Highlights

DOM processing by anaerobic incubation greatly exceeds production of greenhouse gases CO_2 and CH_4

DOM after incubation may represent microbial metabolites and recalcitrant original DOM

Lake-specific DOM processing was observed