

# Geophysical Research Letters®



## RESEARCH LETTER

10.1029/2022GL101414

### Key Points:

- Most of the nitrogen and organic matter were expelled to underlying unfrozen water in dissolved form, while a minor fraction was flocculated
- Oxidized and aromatic organic carbon was preferentially expelled to the underlying unfrozen water column after ice formation
- The apparent retention factor  $\text{DOC}_{\text{ice}}:\text{DOC}_{\text{before}}$  increased with increasing energy-rich aliphatic compounds in lake water

### Supporting Information:

Supporting Information may be found in the online version of this article.

### Correspondence to:

Y. Zhou and L. J. Tranvik,  
yqzhou@niglas.ac.cn;  
lars.tranvik@ebc.uu.se

### Citation:

Zhou, Y., Hiller, C., Andersson, S., Jakobsson, E., Zhou, L., Hawkes, J. A., et al. (2023). Selective exclusion of aromatic organic carbon during lake ice formation. *Geophysical Research Letters*, 50, e2022GL101414. <https://doi.org/10.1029/2022GL101414>

Received 23 SEP 2022

Accepted 31 JAN 2023

### Author Contributions:

**Conceptualization:** Yongqiang Zhou, Lars J. Tranvik

**Data curation:** Carolin Hiller, Sara Andersson, Elizabeth Jakobsson, Lei Zhou, Jeffery A. Hawkes, Dolly N. Kothawala





**Formal analysis:** Lei Zhou

**Funding acquisition:** Yongqiang Zhou, Lars J. Tranvik

© 2023. The Authors.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs License](#), which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

## Selective Exclusion of Aromatic Organic Carbon During Lake Ice Formation

Yongqiang Zhou<sup>1</sup> , Carolin Hiller<sup>2</sup>, Sara Andersson<sup>2</sup>, Elizabeth Jakobsson<sup>2</sup> , Lei Zhou<sup>3</sup>, Jeffery A. Hawkes<sup>4</sup> , Dolly N. Kothawala<sup>2</sup>, and Lars J. Tranvik<sup>2</sup> 

<sup>1</sup>Taihu Laboratory for Lake Ecosystem Research, State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing, China, <sup>2</sup>Department of Limnology, Uppsala University, Uppsala, Sweden, <sup>3</sup>State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China, <sup>4</sup>Analytical Chemistry, Department of Chemistry—BMC, Uppsala University, Uppsala, Sweden

**Abstract** Earth's lakes at northern latitudes are mostly ice-covered in winter. When lake water freezes, some organic matter dissolved in the water is excluded from the ice. We performed complementary field sampling and laboratory freeze-up experiments to explore how freeze-up may impact the partitioning and composition of dissolved organic matter (DOM) in boreal lakes. We found that  $16.2 \pm 4.7\%$  of dissolved organic carbon (DOC) was retained in the overlying ice,  $81.3 \pm 5.7\%$  of DOC was expelled to underlying unfrozen water, and  $1.3 \pm 0.7\%$  was expelled as flocs. During ice formation, nitrogen (TDN, total dissolved nitrogen), ions (specific conductance), and oxidized and aromatic DOM were preferentially expelled to the underlying water column. The apparent retention factor  $\text{DOC}_{\text{ice}}:\text{DOC}_{\text{before}}$  decreased from clearwater to brownwater lakes, that is, with increasing allochthonous DOC lost from lake ice, indicating that DOM exclusion from the ice cover will become more prevalent as lakes experience browning.

**Plain Language Summary** Lakes of the northern hemisphere are covered by ice in winter, yet how freeze-up may impact the partitioning and chemical composition of dissolved organic matter (DOM) remains largely unknown. Using field sampling and laboratory freeze-up experiments on a set of lakes where dissolved organic carbon ranged from 2.8 to 36.0 mg L<sup>-1</sup>, we provide some of the first insights into how freeze-up affects the allocation of dissolved and particulate organic matter in overlying ice cover and underlying unfrozen water. Variability in the molecular composition of DOM prior to and after ice formation showed that nitrogen (TDN, total dissolved nitrogen), ions (specific conductance), and oxidized and aromatic DOM were preferentially expelled to the underlying unfrozen water column, with a minor fraction of organic carbon being flocculated after ice formation. In summary, this study provides a better understanding of how ice formation influences the partitioning of DOM, and associated nutrients and which compounds are bioavailable.

## 1. Introduction

Lakes actively store, transport, and transform a large quantity of carbon received from the terrestrial environment. Despite covering <4% of the global non-glaciated land surface (Verpoorter et al., 2014), they are hotspots of carbon processing (Cole et al., 2007; Drake et al., 2018; Tranvik et al., 2018). In most lake waters, dissolved organic matter (DOM) is the largest pool of organic carbon and represents an important energy source for heterotrophs (Logue et al., 2016). At northern latitudes that harbor the majority of the Earth's inland waters, lakes are typically ice covered in the winter (Figures 1a and 1b) (Wang et al., 2021). When surface water freezes, gases, ions, and stored DOM is expelled from the advancing ice-water interface during crystal growth into the underlying water (Belzile et al., 2002; Jørgensen et al., 2015; Petrich & Eicken, 2010). The process of freezing can also promote DOM flocculation (Santibáñez et al., 2019). As ice crystals form, some DOM may be flocculated and excluded from the ice crystal junctions, and the flocculated material may settle to the sediment (Belzile et al., 2002; Santibáñez et al., 2019). DOM and nutrients expelled from the lake ice provide niches for active biological communities in the underlying unfrozen water column (Grosbois & Rautio, 2018; Hampton et al., 2017). The retention characteristics of solutes and DOM during lake ice formation may depend on ice growth conditions, ambient temperature, and the chemical composition of DOM (Belzile et al., 2002; Santibáñez et al., 2019). Unlike solutes, microbes are preferentially incorporated into the overlying ice during progressive freezing (Santibáñez et al., 2019). To understand how winter ice formation affects DOM dynamics and ecosystem

**Investigation:** Carolin Hiller, Sara Andersson, Elizabeth Jakobsson, Lei Zhou, Dolly N. Kothawala, Lars J. Tranvik

**Resources:** Jeffery A. Hawkes

**Supervision:** Lars J. Tranvik

**Writing – original draft:** Yongqiang Zhou

**Writing – review & editing:** Yongqiang Zhou, Lei Zhou, Jeffery A. Hawkes, Dolly N. Kothawala, Lars J. Tranvik

functioning, it is important to know the partitioning and compositional variability of DOM in the underlying water and overlying ice during freeze-up. Several studies have revealed that the concentration of dissolved organic carbon (DOC) in the unfrozen water column is higher than that in the surface ice (Belzile et al., 2002; Imbeau et al., 2021; Jørgensen et al., 2015). Other studies have unraveled the microbial segregation between the liquid and solid phases during lake ice formation (Santibáñez et al., 2019). However, the allocation of DOM and particulate organic matter (POM) including microbes and organic debris, and the molecular composition of DOM in the ice and unfrozen water fractions during ice formation remains unknown.

In this study, we aimed to characterize how freeze-up affects the allocation of DOM and POM (e.g., microbes and organic floculates) in ice and underlying unfrozen water, and the molecular composition of DOM prior to and after ice formation. We also aimed to assess how DOM retention varies across lakes with different concentrations of DOC, including a gradient of clear to brownwater lakes, and nutrient concentrations. DOM exclusion likely results from the tendency for ice to grow pure ice crystals and expel impurities (Belzile et al., 2002), particularly the most hydrophobic ions. Ice formation may favor heterotrophic processes and impact biogeochemical cycling of DOM and inorganic nutrients. We hypothesized that lakes with higher DOM aromaticity will result in a lower overall retention of DOM in lake ice during freeze-up as the aromatic DOM structures are hydrophobic (less water soluble) and accordingly will not be incorporated in the ice. Consequently, the aromatic DOM will concentrate in the underlying unfrozen water column and aliphatic DOM will be captured in the ice.

## 2. Materials and Methods

### 2.1. Lake Water and Ice Core Sampling

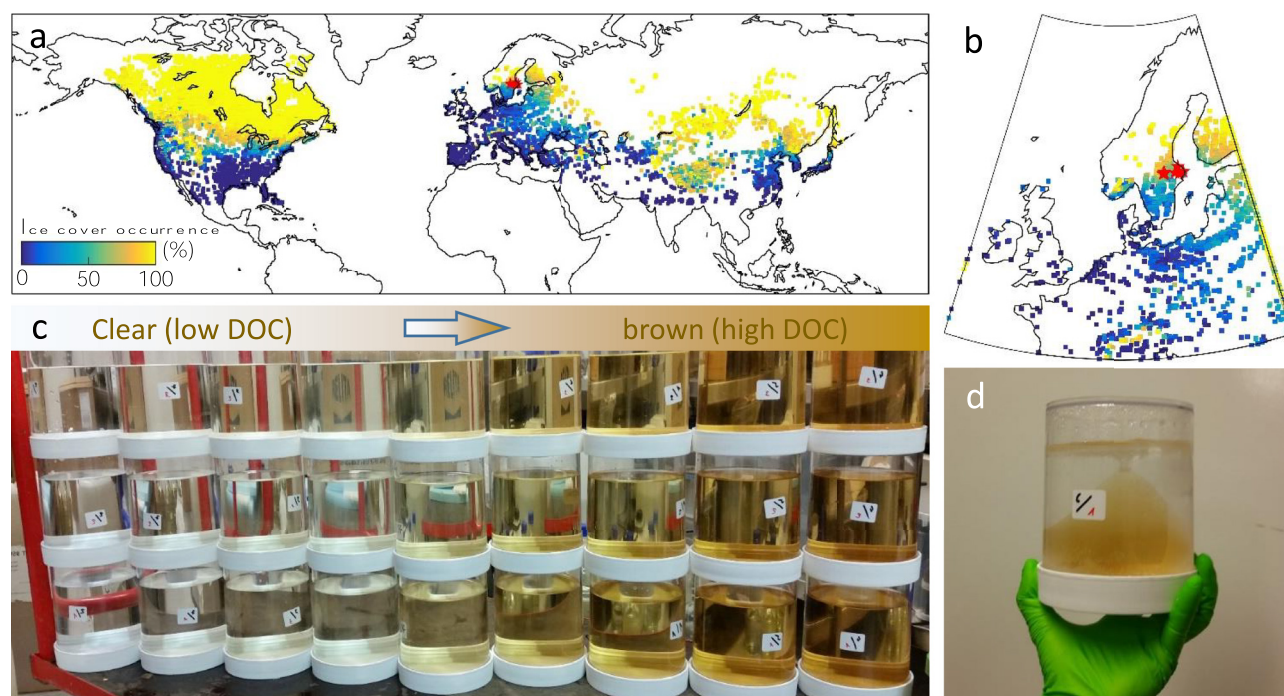
Lake water samples (in triplicates) were collected from nine Swedish freshwater lakes across a gradient of DOC concentrations (2.8–36.0 mg L<sup>-1</sup>) and specific ultraviolet absorbance SUVA<sub>254</sub> levels (1.22–5.23 L mgC<sup>-1</sup> m<sup>-1</sup>) during the ice free period in November 2017 after lake turnover and thus, all lakes were well-mixed. The same lakes were sampled again in March 2018 during the ice cover period to take samples of both the in situ ice layer and underlying unfrozen water (Figures 1a and 1b). However, two lakes (Lake Oppsveten and Lake Ljustjärn) were unreachable in March 2018 due to too high snow cover, resulting in seven lakes sampled (7 underlying lake water + 7 ice cores) × 3 replicates during the ice cover period (see Figure 1 and Table S1 in Supporting Information S1 for location). Thus, we directly compared paired samples from all nine lakes for the experimental ice formation experiment described in Section 2.2, and only seven lakes were compared for in situ ice formation (Supporting Information S1).

### 2.2. Laboratory Freeze-Up Experiment

A controlled laboratory freeze-up experiment was carried out to investigate the partitioning of ions, DOM, and POM between the overlying ice cover and underlying unfrozen water column during ice formation, and to determine how freeze-up changes the chemical composition of DOM. Triplicate aliquots of 1 L water taken from each of the nine lakes in November 2017 were poured into acid-washed PE (polyethylene) jars, with wide leak-proof screw caps. The PE jars were turned upside down and covered with styrofoam around the sides and bottom to allow freezing to occur mainly from above (Figure 1c). All jars were kept in darkness at –20°C for approximately 13 hr. At this timepoint, approximately 300 mL of lake water was still liquid, and roughly 700 mL of water was frozen within each jar (Figure 1d) (Supporting Information S1).

### 2.3. Water Chemistry and Optical Measurements of DOM

Specific conductance was measured as a surrogate of ion concentration (Belzile et al., 2002). Detailed information on the determination of specific conductance, pH, DOC, particulate organic carbon (POC), total dissolved nitrogen (TDN), total particulate nitrogen (TPN), DOM absorbance and fluorescence, along with data processing of DOM absorbance and fluorescence including parallel factor analysis (PARAFAC) can be found in the Supporting Information (Figures S1 and S2 in Supporting Information S1). Four humic-like (C1–C4) and two protein-like (C5–C6) components were identified and validated, and were statistically matched with components that share similar spectral shapes as identified earlier in other published studies using OpenFluor, an online spectral library (Murphy et al., 2014).



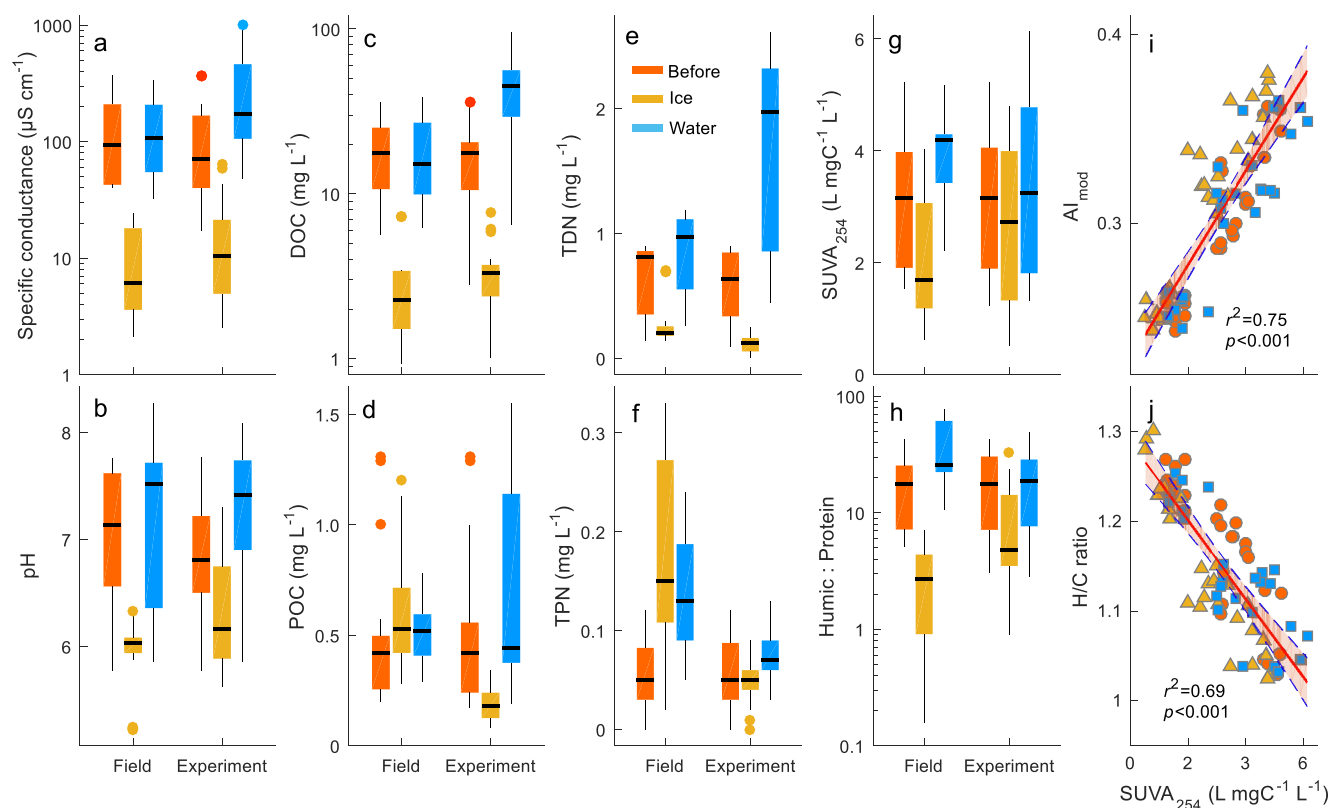
**Figure 1.** Sampling location and freeze-up experimental setup. (a) Lake ice cover occurrence (in %, defined as the frequency at which ice cover was detected in January to March using Landsat images) in the North Temperate Zone during 2015–2020 (data obtained from Wang et al. (2021)) (a), and enlarged map of Northern Europe showing the location of sampling sites in Sweden (b), red stars indicate the area where the nine Swedish lakes were sampled (a, b). (c) Photograph of freeze-up experimental setup. Jars (run in triplicates) with 1 L water samples collected from the nine Swedish lakes across a large DOC gradient (from clear to brown) in November 2017. (d) Samples were kept at  $-20^{\circ}\text{C}$  for approximately 13 hr and approximately 300 mL of water remained in a liquid state (the lower brown layer) while 700 mL of water was frozen (the upper transparent layer).

#### 2.4. Orbitrap Mass Spectrometry Measurements and Data Processing

Samples in triplicates collected from the nine lakes during the ice-free season in November 2017, and in the underlying water and overlying ice cover fractions during the freeze-up experiment (27 samples  $\times$  3 replicates) together with three blanks of Milli-Q water were passed through muffled Whatman GF/F filters ( $0.7\ \mu\text{m}$ ) and solid-phase extracted using 3 mL PPL cartridges (Agilent), and 80  $\mu\text{L}$  of sample were loaded to an autosampler syringe (Agilent 1100) and infused at 10  $\mu\text{L}/\text{min}$  by slowly pumping mobile phase (50% methanol) to the Orbitrap LTQ-Velos-Pro via electrospray ionization (ESI) (Hawkes et al., 2018) (Supporting Information S1). The modified aromaticity index ( $\text{AI}_{\text{mod}}$ ) was calculated for each formula and it increases with increasing DOM aromaticity (Koch & Dittmar, 2006, 2016). Compound categories were delineated according to the O/C and H/C cutoffs and  $\text{AI}_{\text{mod}}$ : (a) polycyclic condensed aromatics ( $\text{AI}_{\text{mod}} > 0.66$ ), (b) aromatics ( $0.66 \geq \text{AI}_{\text{mod}} > 0.5$ ), (c) highly unsaturated and phenolic formulae ( $\text{AI}_{\text{mod}} \leq 0.50$  and  $\text{H/C} < 1.5$ ), and (d) aliphatic compounds ( $2.0 > \text{H/C} \geq 1.5$  and  $N = 0$ ) (Fu et al., 2020; Kellerman et al., 2019).

#### 2.5. Statistical Analyses

The apparent retention factor of DOM-related parameters was calculated as the percentage ratio of the levels in the overlying ice fractions after ice formation to that in water before freezing, that is,  $C_{\text{Ice}}/C_{\text{Before}}$  and is in %. Statistical analyses, including mean values, standard deviations,  $t$ -test, analysis of variance (ANOVA) were performed with  $R \times 64$  4.0.5. Results of  $t$ -test, ANOVA, and linear or nonlinear fitting with  $p < 0.05$  were denoted as significant. Spearman rank correlations, together with boxplots and linear or nonlinear fittings were conducted using MATLAB R2019b.



**Figure 2.** Boxplots of specific conductance (a), pH (b), dissolved organic carbon (DOC) (c), particulate organic carbon (POC) (d), total dissolved nitrogen (TDN) (e), total particulate nitrogen (TPN) (f), specific UV absorbance at 254 nm, that is, ( $SUVA_{254}$ ) (g), and the ratio of summed fluorescence intensity of humic-like (C1 + C2 + C3 + C4) to protein-like (C5 + C6) components, (Humic: Protein) (h) for the samples collected during the ice-free season in November 2017 (“Before”), in the underlying unfrozen water (“Water”) and overlying ice (“Ice”) fractions during the ice covered season in March 2018 (shown as “Field results”), and during the freeze-up experiment (shown as “Experiment”). Note that in all panels, the “Before” category for the field results include only the seven reachable lakes in March 2018 shown in Table S1. Relationships between the specific ultraviolet absorbance of DOM ( $SUVA_{254}$ ) and relative-abundance (RA) weighted modified aromatic index, that is,  $AI_{mod}$  (i), RA-weighted hydrogen-to-carbon molar ratio, that is, H/C ratio (j).

### 3. Results

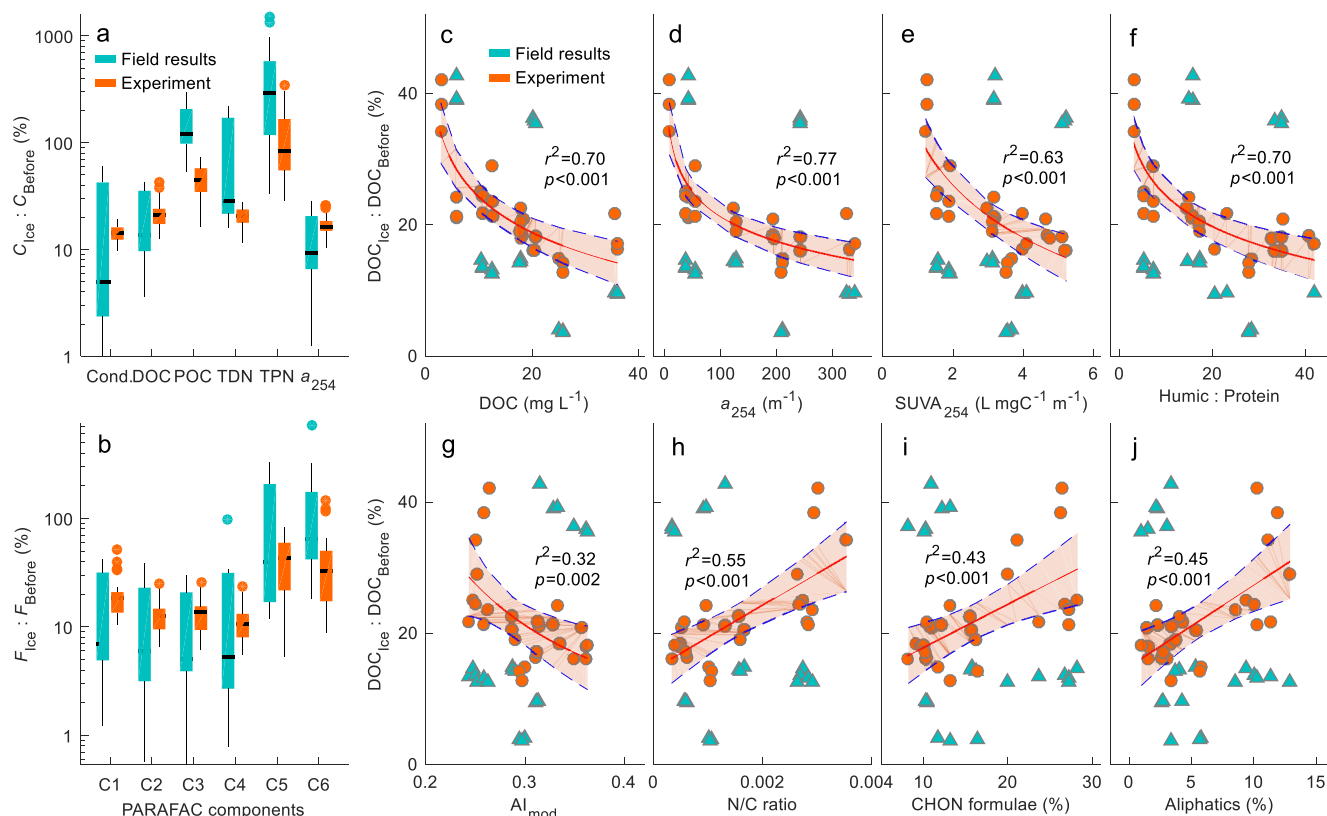
#### 3.1. Changes in Water Chemistry and Mass Balance During Ice Formation

Noticeable differences were apparent for water chemistry and DOM among the underlying unfrozen water column (“Water”), the overlying ice cover (“Ice”) after freeze-up, and before ice formation, that is, ice-free condition (“Before”), for both the field observations and laboratory experimental freeze-up.

In the field, we found notably higher specific conductance, pH, DOC,  $SUVA_{254}$ , and Humic: Protein for the “Before” and in the “Water” fraction than that in the “Ice” fraction (paired  $t$ -test,  $p < 0.001$ ) (Figure 2). In comparison, we found higher POC and TPN in the “Ice” fraction than that for the “Before” and in the “Water” fraction (Figure 2). During the ice-free season, DOC contributed  $97.1 \pm 1.5\%$  while POC contributed  $2.9 \pm 1.5\%$  of the total organic carbon pool (Figures 2c and 2d). During the ice cover season, based on the ice thickness and unfrozen water column depth, DOC in the “Water” contributed  $79.5 \pm 12.5\%$  and DOC in the “Ice” contributed  $14.4 \pm 10.1\%$ , while POC in the “Water” contributed  $2.8 \pm 1.3\%$  and POC in the “Ice” contributed  $3.3 \pm 2.0\%$  of the total water and ice column organic carbon pool (Figures 2c and 2d). In comparison, during the ice-free season, TDN contributed  $89.8 \pm 9.7\%$  while TPN contributed  $10.2 \pm 9.7\%$  of the total nitrogen pool (Figures 2e and 2f). During the ice cover season, TDN in the “Water” contributed  $81.1 \pm 12.2\%$  and TDN in the “Ice” contributed  $2.2 \pm 1.9\%$ , while TPN in the “Water” contributed  $15.7 \pm 11.2\%$  and TPN in the “Ice” contributed  $1.0 \pm 0.4\%$  of the total water and ice column nitrogen pool (Figures 2e and 2f).

For the experimental freeze-up, we found PE jars had an insignificant impact on the Milli-Q blank DOM optical composition (Figure S3 in Supporting Information S1). We found higher specific conductance, pH, DOC, POC,





**Figure 3.** Variability of dissolved organic matter (DOM) during freeze-up and linkages between the apparent retention factor of dissolved organic carbon (DOC) and DOM optical and mass indices. (a) The ratio of the concentrations of each parameter collected in the "Ice" fractions for the freeze-up experiment and field results to that before ice formation in November 2017 "Before,"  $C_{\text{Ice}} : C_{\text{Before}}$  of specific conductance (Cond.), DOC, particulate organic carbon (POC), total dissolved nitrogen (TDN), total particulate nitrogen (TPN), DOM absorption  $a_{254}$ , and (b) the apparent retention factor of fluorescence intensity  $F_{\text{Ice}} : F_{\text{Before}}$  of PARAFAC components C1–C6 for the field observations and the freeze-up experiment. Relationships between the apparent retention factor  $\text{DOC}_{\text{Ice}} : \text{DOC}_{\text{Before}}$  and (c) DOC, (d) DOM absorption  $a_{254}$ , (e) specific ultraviolet absorbance  $\text{SUVA}_{254}$ , (f) the ratio of humic-like (C1 + C2 + C3 + C4) to protein-like (C5 + C6) components (Humic : Protein), (g) modified aromaticity index  $\text{AI}_{\text{mod}}$ , (h) relative abundance normalized N/C molar ratio, (i) CHON-containing and (j) aliphatic formulae for the samples collected from the freeze-up experiment (red dots) and field observations (blue triangles). The fittings shown in panels (c–j) only apply to the data points for the freeze-up experiment.

TDN,  $\text{SUVA}_{254}$ , and Humic : Protein for the "Before" and in the "Water" fraction than that in the "Ice" fraction (paired  $t$ -test,  $p < 0.001$ ). We further found higher specific conductance, pH, DOC, TDN in the "Water" fraction than that in the "Before" ice formation (paired  $t$ -test,  $p < 0.001$ ). We found that freeze-up resulted in an elevated mean relative-abundance (RA)-weighted oxygen-to-carbon molar ratio (O/C ratio) and mean percentage of RA-weighted (%RA) polyphenolic compounds and a correspondingly lowered mean %RA of aliphatic compounds in the "Water" fraction than that in the "Before" ice formation (Figures 2i and 2j). We found DOC in the "Water" contributed  $81.3 \pm 5.7\%$  and DOC in the "Ice" contributed  $16.2 \pm 4.7\%$ , corresponding to a mean  $\text{DOC}_{\text{Ice}} : \text{DOC}_{\text{Before}}$  of  $22.0 \pm 7.0\%$ , while POC in the "Water" contributed  $1.3 \pm 0.7\%$  and POC in the "Ice" contributed  $1.2 \pm 0.8\%$  of the total water and ice column organic carbon pool (Figure 2). In comparison, TDN in the "Water" contributed  $76.3 \pm 5.8\%$  and TDN in the "Ice" contributed  $11.0 \pm 6.2\%$ , while TPN in the "Water"-contributed  $4.6 \pm 3.0\%$  and TPN in the "Ice" contributed  $8.0 \pm 6.1\%$  of the total water and ice column nitrogen pool (Figure 2).

### 3.2. Retention Factor of DOM and Potential Influencing Factors

For field observations in March 2018, the in situ retention factors, that is,  $C_{\text{Ice}} : C_{\text{Before}}$  for POC and TPN were higher than that for specific conductance, DOC, TDN, and  $a_{254}$  (Figure 3a). We found no significant relationship between  $\text{DOC}_{\text{Ice}} : \text{DOC}_{\text{Before}}$  and ice thickness (Figure S4). The apparent retention factors for the fluorescence intensity ( $F_{\text{Ice}} : F_{\text{Before}}$ ) of protein-like C5–C6 were notably higher than for the humic-like C1–C4 ( $t$ -test,  $p < 0.001$ ) (Figure 3b).

For the experimental freeze-up, the apparent retention factors followed the pattern seen for the field observations with higher  $C_{\text{Ice}}: C_{\text{Before}}$  recorded for POC and TPN than that for specific conductance, DOC, TDN, and  $a_{254}$  (Figure 3a). Furthermore, we found higher  $F_{\text{Ice}}: F_{\text{Before}}$  for protein-like components C5–C6 than that of the humic-like C1–C4 ( $t$ -test,  $p < 0.001$ ) (Figure 3b). We found that  $\text{DOC}_{\text{Ice}}: \text{DOC}_{\text{Before}}$  decreased significantly with increasing DOC ( $r^2 = 0.70$ ,  $p < 0.001$ ),  $a_{254}$  ( $r^2 = 0.77$ ,  $p < 0.001$ ),  $\text{SUVA}_{254}$  ( $r^2 = 0.63$ ,  $p < 0.001$ ), and Humic: Protein ( $r^2 = 0.70$ ,  $p < 0.001$ ) (Figures 3c–3f), while no significant relationship was found between  $\text{DOC}_{\text{Ice}}: \text{DOC}_{\text{Before}}$  and pH or between  $\text{DOC}_{\text{Ice}}: \text{DOC}_{\text{Before}}$  and specific conductance for the experiment. We found that  $\text{DOC}_{\text{Ice}}: \text{DOC}_{\text{Before}}$  increased with increasing Specific conductance  $\text{Ice}: \text{Specific conductance}_{\text{Before}}$ ,  $\text{TDN}_{\text{Ice}}: \text{TDN}_{\text{Before}}$ , and  $a_{254\text{Ice}}: a_{254\text{Before}}$  ( $p < 0.001$ ) (Figure S5 in Supporting Information S1).

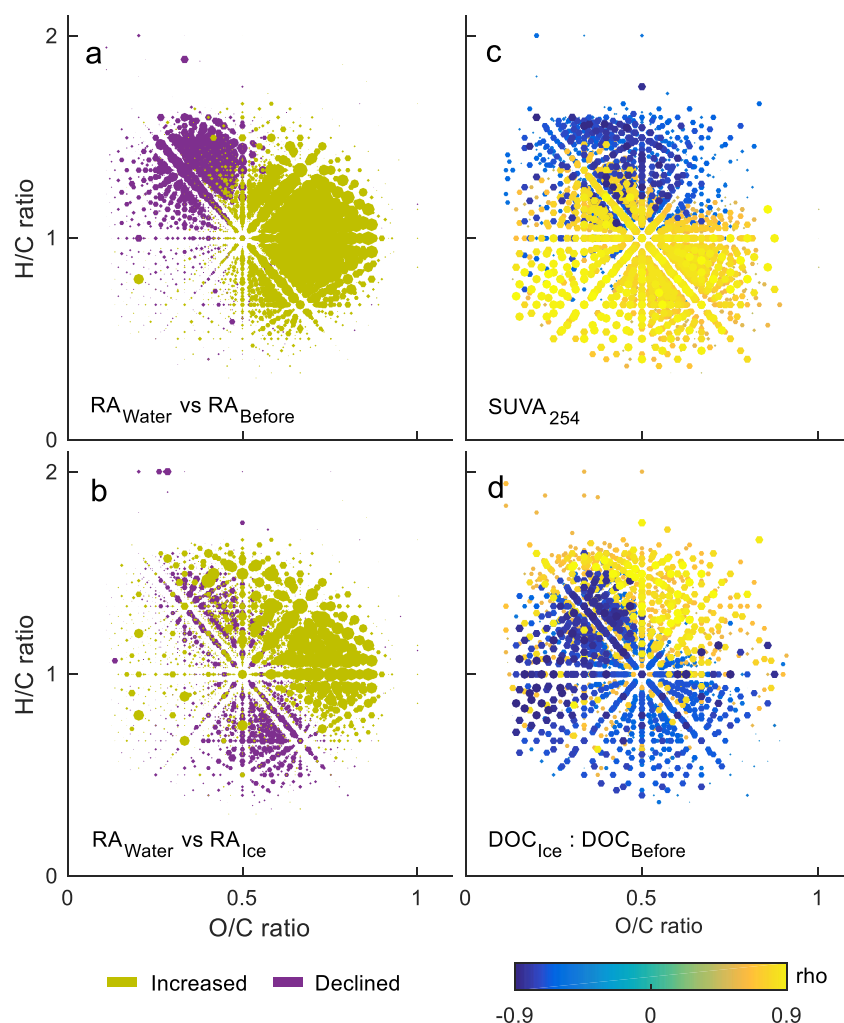
### 3.3. Molecular Variability Revealed Using High-Resolution Mass Spectrometry

We found that DOC was increasingly retained in the ice with decreasing overall aromaticity of the organic matter. Hence, retention factors ( $\text{DOC}_{\text{Ice}}: \text{DOC}_{\text{Before}}$ ) decreased significantly with an increase of modified aromaticity index ( $\text{AI}_{\text{mod}}$ ) for the freeze-up experiment ( $p = 0.002$ ) (Figure 3g). With increased relative contribution of N-containing or aliphatic compounds, DOC retention increased. Specifically, we found that  $\text{DOC}_{\text{Ice}}: \text{DOC}_{\text{Before}}$  increased with increasing RA-weighted N/C ratio ( $p < 0.001$ ), the percentage of RA-weighted (%RA) CHON-containing formulae ( $p < 0.001$ ), and %RA of aliphatic formulae for the freeze-up experiment ( $p < 0.001$ ) (Figures 3h–3j). We found that ice formation resulted in elevated levels of oxidized and aromatic DOM with higher  $m/z$  in the underlying unfrozen water of the experiment (Figure 4a; Tables S2 and S3 in Supporting Information S1). Compared with DOM in the “Before” samples, the relative abundance-weighted O/C and  $\text{AI}_{\text{mod}}$  increased, corresponding to a declined %RA of aliphatic formulae in the “Water” fraction than that before freezing (Figure 4a; Tables S2 and S3 in Supporting Information S1).

Results from ESI-Orbitrap and optical spectrometry were generally consistent. Hence,  $\text{SUVA}_{254}$  was positively linked to the mass-to-charge ( $m/z$ ) ratio,  $\text{AI}_{\text{mod}}$ , relative abundance of polyphenolic and polycyclic condensed aromatics, and negative H/C ratio, CHON-containing formulae, and aliphatic compounds (Figures 2i and 2j; Figure 4c; Figure S6, Table S3 in Supporting Information S1). Spearman rank correlation results further exhibited that  $\text{DOC}_{\text{Ice}}: \text{DOC}_{\text{Before}}$  was negatively correlated with the relative abundance of condensed aromatic and polyphenolic compounds, and positively correlated with the relative abundance of aliphatic formulae (Figure 4d; Table S3 in Supporting Information S1).

## 4. Discussion

Browning has been a widespread phenomenon in boreal lakes and is associated with a long-term gradual increase in the input of terrestrial DOC associated with high aromaticity (Brothers et al., 2014; Monteith et al., 2007). We found DOM prone to being expelled to the underlying water column in brown water lakes with high DOC and high aromaticity (Figure 3). The apparent retention factor  $\text{DOC}_{\text{Ice}}: \text{DOC}_{\text{Before}}$  increased with decreasing levels of DOC,  $a_{254}$ ,  $\text{SUVA}_{254}$ , Humic: Protein, and  $\text{AI}_{\text{mod}}$  (Figure 3) during the freezing-up experiment. This indicates that a greater fraction of DOC is retained in the overlying ice cover of lakes with low DOC concentrations, and thus the exclusion of organic matter will become more prevalent for lakes experiencing a greater extent of terrestrial inputs contributing to browning.  $\text{SUVA}_{254}$  and  $\text{AI}_{\text{mod}}$  increased with increasing DOM aromaticity (Koch & Dittmar, 2016; Weishaar et al., 2003). The increased exclusion of DOM across the DOC gradient in this study can be explained by the fact that allochthonous aromatic DOM structures with a darker color are generally more hydrophobic (Dilling & Kaiser, 2002; Kothawala et al., 2014; Patriarca et al., 2020), and hence may be preferentially expelled during ice formation. This means that underlying water during ice formation becomes increasingly concentrated in darker chromophoric DOM. In comparison, the more transparent and hydrophilic compounds typically have a smaller molecular size (Wu et al., 2007), and hence may be more prone to be encapsulated by ice crystals during ice formation. This is supported by higher mean apparent retention factors of protein-like than that of humic-like components in both the field observations and experimental results (Figure 3b). The protein-like components are typically associated with transparent DOM that is produced aquatically, and are often associated with higher bioavailability (Fellman et al., 2010; Hu et al., 2022; Kothawala et al., 2015). Thus, we can speculate that the bioavailable fraction of DOM is potentially selectively frozen, but further studies using water from freshwater systems with bioassays would be required to fully address this question. Compared with experimental results, the POC and TPN in the ice sampled in the field was notably higher in March 2018 (Figure 2). Possibly,



**Figure 4.** Molecular variability of DOM during lake ice formation. van Krevelen diagrams revealed by Orbitrap exhibiting the molecular formulae with increased or declined mean relative abundance (RA) in the underlying unfrozen water (“Water”) relative to before (“Before”), that is,  $RA_{\text{Water}}$  versus  $RA_{\text{Before}}$  (a), and in the “Water” fraction relative to the ice (“Ice”) fraction, that is,  $RA_{\text{Water}}$  versus  $RA_{\text{Ice}}$  (b), of the freeze-up experiment. Spearman correlation coefficients ( $\rho$ ) between the RA of molecules and specific UV absorbance at 254 nm, that is,  $SUVA_{254}$  (c), and between RA of molecules and the apparent retention factor  $DOC_{\text{Ice}} : DOC_{\text{Before}}$  (d). Note that only the statistically significant peaks are shown here.

this is due to a accumulation of organic particles in the upper layer of the lakes during onset of the ice cover in early winter, which typically takes place under calm conditions with limited mixing, compared to the well mixed samples from ca 1.5 m depth, used in the freeze-up experiment. This potentially also explains why the close relationships between apparent retention factor  $DOC_{\text{Ice}} : DOC_{\text{Before}}$  and optical and molecular compositional indices for the experiment do not apply for the field results (Figure 3). The close linkage between the apparent retention factors of DOC and TDN (Figure S5 in Supporting Information S1) can be explained by the fact that most TDN in those lakes is organic, rather than inorganic, and closely associated with DOC (Figure S7 in Supporting Information S1) (Kellerman et al., 2015; Kothawala et al., 2014).

Our controlled experiments showed that extensive exclusion of DOC occurred even when most of the water in experimental containers was frozen, with the experimental frozen water percentage being much higher than field observations (Table S1 in Supporting Information S1) (Belzile et al., 2002; Santibáñez et al., 2019). It is, therefore, not surprising that field lake ice thickness had no significant impact on the apparent retention of DOC (Figure S4 in Supporting Information S1) as the volume of water stored in ice typically makes up a minor fraction of the total lake volume in the studied lakes. In fact, for both the field and lab studies, we found the predominant fraction of DOC, TDN, ions (specific conductance), and  $a_{254}$  were excluded from the overlying ice

cover (Figure 2). In comparison, a limited fraction of POC and TPN were excluded during ice formation for both field and laboratory experimental freeze-up (Figure 3a), indicating that lake freeze-up and subsequent thaw does not to a large extent expel particles from the ice, and does not cause extensive flocculation of DOM into settling particulate form, a process that is significant in boreal lakes (von Wachenfeldt et al., 2008).

Ice formation resulted in an elevated proportion of oxygen-rich DOM (high O/C) with enhanced aromaticity in the underlying unfrozen water column as demonstrated by the elevated  $SUVA_{254}$ , O/C, and  $AI_{mod}$  values in the underlying water column compared with that of ice-free conditions in the freeze-up experiments, and indicated by similar patterns in field observations (Figure 2; Figure 4). The aromatic moieties of DOM contain almost entirely hydrophobic DOM (Dilling & Kaiser, 2002) and this fraction of DOM is preferentially expelled to the underlying unfrozen water (Table S3 in Supporting Information S1). We therefore conclude that the intrinsic composition of DOM, particularly water solubility, controls the overall retention of organic matter during ice formation. Aromatic DOM is associated with terrestrial soil organic-rich input and is highly photochemically labile (Drake et al., 2019; Stubbins et al., 2010; Wagner et al., 2019). Previous studies have revealed that photodegradation processes preferentially remove oxidized and aromatic DOM (Helms et al., 2008; Kellerman et al., 2015; Osburn et al., 2009).

Lake ice formation significantly expels ions and DOC into the underlying unfrozen water column (Imbeau et al., 2021), and thus can affect the transfer of energy and nutrients through food webs, as well as biogeochemical processes, which can ultimately have consequences for carbon cycling across northern aquatic landscapes (Belzile et al., 2002; Hazuková et al., 2021). Organic matter retained in the ice was aliphatic-rich material. The composition of organic carbon and nitrogen being enriched in the underlying unfrozen waters is highly relevant as it may provide important substrates for heterotrophic metabolism (Grosbois & Rautio, 2018; Pace et al., 2004; Vincent, 1981). In particular, recalcitrant and more aromatic DOM can be photochemically transformed into more bio-labile forms, however the extent of photo-degradation is variable depending on the extent of ultraviolet light transmission through the ice layer (Lindell et al., 1995; Wetzel et al., 1995). Nutrient supply together with underwater light climate and temperature control primary productivity of boreal lakes (Belzile et al., 2002). We anticipate that in future studies, it is relevant to consider the speed of freezing and periodicity of ice formation during the fall and spring shoulder seasons as related to the extent of ice retention.

The close associations between  $SUVA_{254}$  and RA-weighted  $m/z$ ,  $AI_{mod}$ , H/C, and other Orbitrap indices (Figure 2; Figure 4; Figure S6 in Supporting Information S1) demonstrate consistency between the two independent approaches to characterize the DOM. However, the similar mean RA-weighted  $m/z$  and  $AI_{mod}$ , while the significantly lower  $SUVA_{254}$  in the “Ice” compared with the “Before” fractions (Figure 2; Table S2 in Supporting Information S1), suggested that the results from optical and Orbitrap spectrometry are not fully consistent. This may be a result of different analytical windows of the two methods. The solid phase extraction prior to Orbitrap analysis does not retain carbohydrates, peptides, and sugars (Hawkes et al., 2019; Patriarca et al., 2020). Ionizable DOM detected via ESI-Orbitrap is often optically invisible, and most of the aromatic compounds in DOM are poorly ionized, that is, their relative contribution to ESI-Orbitrap results should be small, despite high abundance in terrestrial samples (Hawkes et al., 2019; Patriarca et al., 2020). The optical analysis, on the other hand, is limited to compounds that absorb light and emit fluorescence. Hence, it is not surprising that the two approaches are not fully consistent. In the case of the experiment the Orbitrap results appear to show an insignificant increase in %polyphenolics in the ice (Table S2 in Supporting Information S1), thus we keep our interpretation restricted to the optical metrics that were statistically significant (i.e., Figure 2g).

Our study demonstrates that during freezing of lake water, darker aromatic DOM is preferentially expelled to the unfrozen water column. In the ice-covered winter conditions, often representing half or more of the annual cycle, when northern lakes are mostly isolated from atmospheric and terrestrial inputs. Thus, internal cycling of carbon and nitrogen becomes the main source of nutrients nourishing microbial metabolisms (Santibáñez et al., 2019). The exclusion of organic carbon from ice to the underlying unfrozen water column is likely to enhance oxygen consumption during mineralization, and hence augment anoxic conditions and thereby the production of methane and subsequent emission upon ice breakup. In addition, the period of time surrounding ice melt may be relevant since a pool of labile DOM may be released back into the water column in a short period of time, and help support localized heterotrophic metabolism (Hazuková et al., 2021). Future studies with focus on freshwater systems examining changes in the bio-lability of DOM embedded in ice versus DOM excluded from ice in lake systems could help further our understanding of how these compositional shifts in DOM and nutrient stoichiometry (C:N



and C:P) influence the biological community. Global warming is likely to cause increase nutrient and DOM inputs to lakes, via increased runoff. Elevated primary productivity due to prolonged ice-free periods will likely favor the production of autochthonous DOM. Hence, global warming effects are expected to increase the abundance of (typically terrestrially-derived) aromatic as well as aliphatic (typically autochthonous) components, hence increasing DOM that is preferentially expelled as well as preferentially retained during ice formation. However, more importantly, the large-scale loss of lake ice (Huang et al., 2022), will also decrease the occurrence of internal ice-exclusion effects on organic matter.

## Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

## Data Availability Statement

Data used in this study are available at <https://dx.doi.org/10.6084/m9.figshare.21195799>.

## Acknowledgments

This study was jointly supported by funds to L. J. T. from the Knut and Alice Wallenberg Foundation (KAW 2013.0091 and 2018.0191) and from the Swedish Research Council (2014-04264). Y. Z. and L. Z. were supported by the Youth Innovation Promotion Association, CAS (2021312), the Third Xinjiang Scientific Expedition Program (2022xjkk0102), the Second Tibetan Plateau Scientific Expedition and Research Program (2019QZKK0202), NIGLAS Foundation (E1SL002 and 2022NIGLAS-CJH04), the Provincial Natural Science Foundation of Jiangsu (BK20220162 and BK20220041), and the Chinese Postdoctoral Science Foundation (BX2021325). We thank Jasmine Stavenow and Karelle Petrichor for their help with field sample collection, and Prof. Angelique White and the two anonymous reviewers for their helpful comments.

## References

- Belzile, C., Gibson, J. A. E., & Vincent, W. F. (2002). Colored dissolved organic matter and dissolved organic carbon exclusion from lake ice: Implications for irradiance transmission and carbon cycling. *Limnology & Oceanography*, 47(5), 1283–1293. <https://doi.org/10.4319/lo.2002.47.5.1283>
- Brothers, S., Köhler, J., Attermeyer, K., Grossart, H.-P., Mehner, T., Meyer, N., et al. (2014). A feedback loop links brownification and anoxia in a temperate, shallow lake. *Limnology & Oceanography*, 59(4), 1388–1398. <https://doi.org/10.4319/lo.2014.59.4.1388>
- Cole, J. J., Prairie, Y. T., Caraco, N. F., McDowell, W. H., Tranvik, L. J., Striegl, R. G., et al. (2007). Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon Budget. *Ecosystems*, 10(1), 172–185. <https://doi.org/10.1007/s10021-006-9013-8>
- Dilling, J., & Kaiser, K. (2002). Estimation of the hydrophobic fraction of dissolved organic matter in water samples using UV photometry. *Water Research*, 36(20), 5037–5044. [https://doi.org/10.1016/s0043-1354\(02\)00365-2](https://doi.org/10.1016/s0043-1354(02)00365-2)
- Drake, T. W., Raymond, P. A., & Spencer, R. G. M. (2018). Terrestrial carbon inputs to inland waters: A current synthesis of estimates and uncertainty. *Limnology and Oceanography Letters*, 3, 132–142. <https://doi.org/10.1002/lo2.10055>
- Drake, T. W., Van Oost, K., Barthel, M., Bauters, M., Hoyt, A. M., Podgorski, D. C., et al. (2019). Mobilization of aged and biolabile soil carbon by tropical deforestation. *Nature Geoscience*, 12(7), 541–546. <https://doi.org/10.1038/s41561-019-0384-9>
- Fellman, J. B., Hood, E., & Spencer, R. G. (2010). Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review. *Limnology & Oceanography*, 55(6), 2452–2462. <https://doi.org/10.4319/lo.2010.55.6.2452>
- Fu, Q. L., Fujii, M., & Kwon, E. (2020). Development and application of a high-precision algorithm for nontarget identification of organohalogenes based on ultrahigh-resolution mass spectrometry. *Analytical Chemistry*, 92(20), 13989–13996. <https://doi.org/10.1021/acs.analchem.0c02899>
- Grosbois, G., & Rautio, M. (2018). Active and colorful life under lake ice. *Ecology*, 99(3), 752–754. <https://doi.org/10.1002/ecy.2074>
- Hampton, S. E., Galloway, A. W., Powers, S. M., Ozersky, T., Woo, K. H., Batt, R. D., et al. (2017). Ecology under lake ice. *Ecology Letters*, 20(1), 98–111. <https://doi.org/10.1111/ele.12699>
- Hawkes, J. A., Radoman, N., Bergquist, J., Wallin, M. B., Tranvik, L. J., & Lofgren, S. (2018). Regional diversity of complex dissolved organic matter across forested hemiboreal headwater streams. *Scientific Reports*, 8(1), 16060. <https://doi.org/10.1038/s41598-018-34272-3>
- Hawkes, J. A., Sjöberg, P. J., Bergquist, J., & Tranvik, L. (2019). Complexity of dissolved organic matter in the molecular size dimension: Insights from coupled size exclusion chromatography electrospray ionization mass spectrometry. *Faraday Discussions*, 218, 52–71. <https://doi.org/10.1039/c8fd00222c>
- Hazuková, V., Burpee, B., McFarlane-Wilson, I., & Saros, J. (2021). Under ice and early summer phytoplankton dynamics in two Arctic lakes with differing DOC. *Journal of Geophysical Research: Biogeosciences*, 126(4), e2020JG005972. <https://doi.org/10.1029/2020jg005972>
- 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. *Limnology & Oceanography*, 53(3), 955–969. <https://doi.org/10.4319/lo.2008.53.3.0955>
- Hu, X., Zhou, Y., Zhou, L., Zhang, Y., Wu, L., Xu, H., et al. (2022). Urban and agricultural land use regulates the molecular composition and bio-lability of fluvial dissolved organic matter in human-impacted southeastern China. *Carbon Research*, 1(1), 19. <https://doi.org/10.1007/s44246-022-00020-6>
- Huang, L., Timmermann, A., Lee, S. S., Rodgers, K. B., Yamaguchi, R., & Chung, E. S. (2022). Emerging unprecedented lake ice loss in climate change projections. *Nature Communications*, 13(1), 5798. <https://doi.org/10.1038/s41467-022-33495-3>
- Imbeau, E., Vincent, W. F., Wauthy, M., Cusson, M., & Rautio, M. (2021). Hidden stores of organic matter in northern lake ice: Selective retention of terrestrial particles, phytoplankton and labile carbon. *Journal of Geophysical Research: Biogeosciences*, 126(8), e2020JG006233. <https://doi.org/10.1029/2020jg006233>
- Jørgensen, L., Stedmon, C. A., Kaartokallio, H., Middelboe, M., & Thomas, D. N. (2015). Changes in the composition and bioavailability of dissolved organic matter during sea ice formation. *Limnology & Oceanography*, 60(3), 817–830. <https://doi.org/10.1002/lno.10058>
- Kellerman, A. M., Arellano, A., Podgorski, D. C., Martin, E. E., Martin, J. B., Deuerling, K. M., et al. (2019). Fundamental drivers of dissolved organic matter composition across an Arctic effective precipitation gradient. *Limnology & Oceanography*, 65(6), 1217–1234. <https://doi.org/10.1002/lno.11385>
- Kellerman, A. M., Kothawala, D. N., Dittmar, T., & Tranvik, L. J. (2015). Persistence of dissolved organic matter in lakes related to its molecular characteristics. *Nature Geoscience*, 8(6), 454–457. <https://doi.org/10.1038/ngeo2440>
- Koch, B., & Dittmar, T. (2006). From mass to structure: An aromaticity index for high-resolution mass data of natural organic matter. *Rapid Communications in Mass Spectrometry*, 20(5), 926–932. <https://doi.org/10.1002/rcm.2386>
- Koch, B., & Dittmar, T. (2016). From mass to structure: An aromaticity index for high-resolution mass data of natural organic matter. *Rapid Communications in Mass Spectrometry*, 30(1), 250. <https://doi.org/10.1002/rcm.7433>

- 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(8), 1491–1505. <https://doi.org/10.1002/2015jg002946>
- Kothawala, D. N., Stedmon, C. A., Muller, R. A., Weyhenmeyer, G. A., Kohler, S. J., & Tranvik, L. J. (2014). Controls of dissolved organic matter quality: Evidence from a large-scale boreal lake survey. *Global Change Biology*, 20(4), 1101–1114. <https://doi.org/10.1111/gcb.12488>
- Lindell, M. J., Granéli, W., & Tranvik, L. J. (1995). Enhanced bacterial growth in response to photochemical transformation of dissolved organic matter. *Limnology & Oceanography*, 40(1), 195–199. <https://doi.org/10.4319/lo.1995.40.1.0195>
- Logue, J. B., Stedmon, C. A., Kellerman, A. M., Nielsen, N. J., Andersson, A. F., Laudon, H., et al. (2016). Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter. *The ISME Journal*, 10(3), 533–545. <https://doi.org/10.1038/ismej.2015.131>
- Monteith, D. T., Stoddard, J. L., Evans, C. D., de Wit, H. A., Forsius, M., Hogasen, T., et al. (2007). Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature*, 450(7169), 537–540. <https://doi.org/10.1038/nature06316>
- Murphy, K. R., Stedmon, C. A., Wenig, P., & Bro, R. (2014). OpenFluor—an online spectral library of auto-fluorescence by organic compounds in the environment. *Analytical Methods*, 6(3), 658–661. <https://doi.org/10.1039/c3ay41935e>
- Osburn, C. L., O'Sullivan, D. W., & Boyd, T. J. (2009). Increases in the longwave photobleaching of chromophoric dissolved organic matter in coastal waters. *Limnology & Oceanography*, 54(1), 145–159. <https://doi.org/10.4319/lo.2009.54.1.0145>
- Pace, M. L., Cole, J. J., Carpenter, S. R., Kitchell, J. F., Hodgson, J. R., Van de Bogert, M. C., et al. (2004). Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. *Nature*, 427(6971), 240–243. <https://doi.org/10.1038/nature02227>
- Patriarca, C., Balderrama, A., Moze, M., Sjöberg, P. J. R., Bergquist, J., Tranvik, L. J., & Hawkes, J. A. (2020). Investigating the ionization of dissolved organic matter by electrospray. *Analytical Chemistry*, 92(20), 14210–14218. <https://doi.org/10.1021/acs.analchem.0c03438>
- Petrich, C., & Eicken, H. (2010). Growth, structure and properties of sea ice. In D. N. Thomas & G. S. Diekmann (Eds.), *Sea ice* (2nd ed., pp. 23–77). Wiley-Blackwell Publishing.
- Santibañez, P. A., Michaud, A. B., Vick-Majors, T. J., D'Andrilli, J., Chiuchio, A., Hand, K. P., & Priscu, J. C. (2019). Differential incorporation of bacteria, organic matter, and inorganic ions into lake ice during ice formation. *Journal of Geophysical Research: Biogeosciences*, 124(3), 585–600. <https://doi.org/10.1029/2018jg004825>
- Stubbins, A., Spencer, R. G. M., Chen, H., Hatcher, P. G., Mopper, K., Hernes, P. J., et al. (2010). Illuminated darkness: Molecular signatures of Congo River dissolved organic matter and its photochemical alteration as revealed by ultrahigh precision mass spectrometry. *Limnology & Oceanography*, 55(4), 1467–1477. <https://doi.org/10.4319/lo.2010.55.4.1467>
- 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. *Limnology and Oceanography Letters*, 3(3), 41–48. <https://doi.org/10.1002/lol2.10068>
- Verpoorter, C., Kutser, T., Seekell, D. A., & Tranvik, L. J. (2014). A global inventory of lakes based on high-resolution satellite imagery. *Geophysical Research Letters*, 41(18), 6396–6402. <https://doi.org/10.1002/2014gl060641>
- Vincent, W. F. (1981). Production strategies in Antarctic inland waters: Phytoplankton eco-physiology in a permanently ice-covered lake. *Ecology*, 62(5), 1215–1224. <https://doi.org/10.2307/1937286>
- 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. *Limnology & Oceanography*, 53(6), 2416–2426. <https://doi.org/10.4319/lo.2008.53.6.2416>
- Wagner, S., Fair, J. H., Matt, S., Hosen, J., Raymond, P., Saiers, J., et al. (2019). Molecular hysteresis: Hydrologically-driven changes in riverine dissolved organic matter chemistry during a storm event. *Journal of Geophysical Research: Biogeosciences*, 124(4), 759–774. <https://doi.org/10.1029/2018JG004817>
- Wang, X., Feng, L., Gibson, L., Qi, W., Liu, J., Zheng, Y., et al. (2021). High-resolution mapping of ice cover changes in over 33,000 lakes across the North Temperate Zone. *Geophysical Research Letters*, 48(18), e2021GL095614. <https://doi.org/10.1029/2021gl095614>
- Weishaar, J. L., Aiken, G. R., Bergamaschi, B. A., Fram, M. S., Fujii, R., & Mopper, K. (2003). Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition of reactivity of dissolved organic matter. *Environmental Science & Technology*, 37(20), 4702–4708. <https://doi.org/10.1021/es030360x>
- Wetzel, R. G., Hatcher, P. G., & Bianchi, T. S. (1995). Natural photolysis by ultraviolet irradiance of recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism. *Limnology & Oceanography*, 40(8), 1369–1380. <https://doi.org/10.4319/lo.1995.40.8.1369>
- Wu, F., Kothawala, D., Evans, R., Dillon, P., & Cai, Y. (2007). Relationships between DOC concentration, molecular size and fluorescence properties of DOM in a stream. *Applied Geochemistry*, 22(8), 1659–1667. <https://doi.org/10.1016/j.apgeochem.2007.03.024>

## References From the Supporting Information

- Andersson, C. A., & Bro, R. (2000). The N-way toolbox for MATLAB. *Chemometrics and Intelligent Laboratory Systems*, 52, 1–4. [https://doi.org/10.1016/S0169-7439\(00\)00071-X](https://doi.org/10.1016/S0169-7439(00)00071-X)
- Attermeier, K., Catalán, N., Einarsdóttir, K., Freixa, A., Groeneveld, M., Hawkes, J. A., et al. (2018). Organic carbon processing during transport through boreal inland waters: Particles as important sites. *Journal of Geophysical Research: Biogeosciences*, 123(8), 2412–2428. <https://doi.org/10.1029/2018jg004500>
- Hawkes, J. A., Dittmar, T., Patriarca, C., Tranvik, L., & Bergquist, J. (2016). Evaluation of the orbitrap mass spectrometer for the molecular fingerprinting analysis of natural dissolved organic matter. *Analytical Chemistry*, 88(15), 7698–7704. <https://doi.org/10.1021/acs.analchem.6b01624>
- Hur, J., Jung, K. Y., & Schlautman, M. A. (2011). Altering the characteristics of a leaf litter-derived humic substance by adsorptive fractionation versus simulated solar irradiation. *Water Research*, 45(18), 6217–6226. <https://doi.org/10.1016/j.watres.2011.09.023>
- Johnston, S. E., Striegl, R. G., Bogard, M. J., Dornblaser, M. M., Butman, D. E., Kellerman, A. M., et al. (2020). Hydrologic connectivity determines dissolved organic matter biogeochemistry in northern high-latitude lakes. *Limnology & Oceanography*, 65(8), 1764–1780. <https://doi.org/10.1002/lno.11417>
- Kellerman, A. M., Dittmar, T., Kothawala, D. N., & Tranvik, L. J. (2014). Chemodiversity of dissolved organic matter in lakes driven by climate and hydrology. *Nature Communications*, 5(1), 3804. <https://doi.org/10.1038/ncomms4804>
- Kothawala, D. N., Murphy, K. R., Stedmon, C. A., Weyhenmeyer, G. A., & Tranvik, L. J. (2013). Inner filter correction of dissolved organic matter fluorescence. *Limnology and Oceanography: Methods*, 11(12), 616–630. <https://doi.org/10.4319/lom.2013.11.616>
- Lawaetz, A. J., & Stedmon, C. A. (2009). Fluorescence intensity calibration using the Raman scatter peak of water. *Applied Spectroscopy*, 63(8), 936–940. <https://doi.org/10.1366/000370209788964548>

- Maie, N., Scully, N. M., Pisani, O., & Jaffé, R. (2007). Composition of a protein-like fluorophore of dissolved organic matter in coastal wetland and estuarine ecosystems. *Water Research*, 41(3), 563–570. <https://doi.org/10.1016/j.watres.2006.11.006>
- Murphy, K. R., Stedmon, C. A., Graeber, D., & Bro, R. (2013). Fluorescence spectroscopy and multi-way techniques. PARAFAC. *Analytical Methods*, 5(23), 6557–6566. <https://doi.org/10.1039/c3ay41160e>
- Osburn, C. L., Wigdahl, C. R., Fritz, S. C., & Saros, J. E. (2011). Dissolved organic matter composition and photoreactivity in prairie lakes of the U.S. Great Plains. *Limnology & Oceanography*, 56(6), 2371–2390. <https://doi.org/10.4319/lo.2011.56.6.2371>
- Sobek, S., Nisell, J., & Fölster, J. (2011). Predicting the depth and volume of lakes from map-derived parameters. *Inland Waters*, 1(3), 177–184. <https://doi.org/10.5268/iw-1.3.426>
- Spencer, R. G. M., Butler, K. D., & Aiken, G. R. (2012). Dissolved organic carbon and chromophoric dissolved organic matter properties of rivers in the USA. *Journal of Geophysical Research*, 117(G3), G03001. <https://doi.org/10.1029/2011jg001928>
- Stedmon, C. A., & Markager, S. (2005). Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. *Limnology & Oceanography*, 50(2), 686–697. <https://doi.org/10.4319/lo.2005.50.2.0686>
- Zhou, Y., Davidson, T. A., Yao, X., Zhang, Y., Jeppesen, E., de Souza, J. G., et al. (2018). How autochthonous dissolved organic matter responds to eutrophication and climate warming: Evidence from a cross-continental data analysis and experiments. *Earth-Science Reviews*, 185, 928–937. <https://doi.org/10.1016/j.earscirev.2018.08.013>