Spatiotemporal patterns of dissolved organic matter in Boreal Shield lakes and ice of the Laurentian Great Lakes

A Thesis submitted to the Committee on Graduate Studies in Partial Fulfillment of the Requirement for the Degree of Master of Science in the Faculty of Arts and Science

TRENT UNIVERSITY

Peterborough, Ontario,

Canada

Environmental and Life Sciences M.Sc. Graduate Program

May 2024

Abstract

Spatiotemporal patterns of dissolved organic matter in Boreal Shield lakes and ice of the Laurentian Great Lakes

Anthony J. Arsenault

Dissolved organic matter (DOM) plays a vital role in lake ecosystems, yet its temporal and spatial variations in lakes remain poorly understood. This study investigates DOM dynamics in boreal lakes during the warm season and the Laurentian Great Lakes ice during winter. Sampling 10 boreal lakes in early and late June 2022, revealed subtle changes in DOM in the epilimnion and hypolimnion related to factors such as water residence time and stratification strength. In ice from the Great Lakes, lower dissolved organic carbon concentrations and a higher proportion of protein-like DOM were found compared to water, which mostly contained terrestrial-like DOM. Ice DOM composition varied with factors such as ice thickness, water nutrients, and DOM concentration in ice and water. In addition, we found that the potential release of protein-like DOM from ice to the water during spring melt is considerable and may fuel heterotrophic microbial metabolism.

Keywords: dissolved organic carbon, dissolved organic matter composition, lake and watershed characteristics, seasonal transformations, lake ice, microbial metabolism

Preface

This thesis is structured in manuscript format. For Chapters 2 and 3, Chapter 1 serves as a general introduction, providing background information, and Chapter 4 offers a conclusion. Chapters 2 and 3 are intended for publication, and Chapter 3 has undergone a thorough review by each coauthor. These chapters use the pronoun “we” to acknowledge coauthors' contributions. Additionally, due to conceptual similarities between Chapters 2 and 3, efforts were made to minimize repetition.

Chapter 2:

A. J. Arsenault, M. J. Paterson, S. Klemet-N’Guessan, M. I. Denga, M. A. Xenopoulos. 2023. Subtle seasonal transformations of dissolved organic matter in Boreal Shield lakes and their relationship to lake and watershed characteristics.

Chapter 3:

*A. J. Arsenault, *N.J.T. Pearce, T. Ozersky, K. Shchapov, H. J. Carrick, J. D. Chaffin, M. L. Coleman, W. J. S. Currie, J. P. Doubek, R. Eveleth, C. M. Godwin, R. M. McKay, M. D. Rennie, M. R. Twiss, D. Uzarski, H. Vanderploeg, T. Vick-Majors, A. Zastepa, M. A. Xenopoulos. 2023. Fingerprints of dissolved organic matter composition in ice of the Laurentian Great Lakes. (To be submitted to *Geophysical Research Letters*).

*These authors contributed equally to this work: AJA and NJTP

Acknowledgments

I would like to start by thanking my supervisor, Dr. Marguerite Xenopoulos, for her guidance and constructive feedback. I would also like to thank my committee members, Dr. Michael Paterson and Dr. Jean-Francois Koprivnjak, for their feedback and support. Thanks also to lab research associate Dr. Nolan Pearce for his assistance regarding technical and statistical matters, as well as lab manager Dr. Oghenemise Abirhire for being quick to order lab supplies and field equipment. Additionally, thanks to my lab mates/friends in the Xenopoulos/Frost Lab for their support, reassurance, and for the fun times.

Furthermore, thanks to my lab mates Sandra Klemet-N'Guessan and Miracle Denga for their long hours of help with field and lab work while we were at the IISD-ELA. Thanks to all the IISD-ELA staff who helped with field and lab work, especially members of Hydrolim, Fish Crew, and Chemistry Lab. Additionally, thanks to the numerous researchers and students involved in the data collection (braved the cold) and lab analysis for the binational “Winter Grab” project, in which I used dissolved organic matter data for my second thesis chapter. Thanks especially to Dr. Tedy Ozersky at the University of Minnesota Duluth, who was the lead organizer and coordinator for the Winter Grab project.

Additionally, I would like to thank my immediate and extended family members for their unwavering support, encouragement, and belief in me throughout the process.

Table of contents

List of Figures

Chapter 2

Chapter 3

Appendices

List of Tables

Chapter 3

Appendices

Chapter 1: General Introduction

Dissolved organic matter: an overview

Dissolved organic matter (DOM) is a heterogeneous mixture (e.g., humic acids, fulvic acids, tannins, proteins, lipids, and carbohydrates) derived primarily from the decomposition of terrestrial plant matter but also from aquatic plants and phytoplankton. DOM in lakes that is terrestrially derived, e.g., from terrestrial plant detritus or organic soils, is referred to as "allochthonous DOM" and is often aromatic (flat, ring-shaped organic molecules with alternating single and double bonds). DOM that is aquatically derived, e.g., from phytoplankton, is referred to as "autochthonous DOM" and is often aliphatic (organic molecules with straight or branched chain structures). Moreover, DOM represents a large portion of the global carbon pool; for instance, the total amount of carbon contained in DOM within freshwaters and the oceans combined is roughly the same as the amount of carbon in the atmosphere (Hedges, 1992). Hence, it is important to consider DOM in climate change models, especially in freshwaters as they are usually net emitters of carbon to the atmosphere (Pollard, 2022).

Benefits to aquatic ecosystems

DOM provides many benefits to aquatic ecosystems. For instance, chromophoric DOM (CDOM), which is the fraction of DOM that absorbs light, attenuates ultraviolet (UV) radiation near the surface of lakes that could otherwise damage the DNA of aquatic organisms (Tedetti & Sempéré, 2006). Also, light attenuation by CDOM can reduce the amount of heating in the profundal zone of lakes which can shift the thermocline to shallower depths providing a larger habitat for cold-water fish species, such as lake trout (Tanentzap et al. 2008; Gunn et al. 2003). Moreover, DOM plays an important role in binding heavy metals, such as aluminum, which reduces metal availability to aquatic organisms (Graham et al., 2013). In addition, DOM provides a source of food for aquatic food webs and in some lakes, the base of the food web is primarily derived from DOM via heterotrophic bacterial production instead of autotrophic production (D'Andrilli et al., 2019).

Spectroscopic properties

DOM absorbs visible and UV light at different wavelengths depending on its molecular structure. A portion of DOM also fluoresces at different wavelengths. Absorbance and fluorescence spectrophotometry are often employed to assess the molecular composition of DOM to infer its source, degree of processing, or reactivity. Various DOM composition indices can be produced from spectrophotometry. Some commonly used indices include specific ultraviolet absorbance at 254 nm, spectral slope ratio, fluorescence index, freshness index, and humification index.

The specific ultraviolet absorption at 254 nm ($SUVA_{254}$), which is the absorbance at 254 nm divided by DOC concentration (to correct for variations in the total amount of organic carbon), is commonly used to assess the aromatic content of DOM (Weishaar et al., 2003). Weishaar et al. (2003) explored the relationship between $SUVA_{254}$ and aromaticity using ^{13}C-nuclear magnetic resonance (^{13}C-NMR) across a large number of samples of humic DOM and found a strong association ($r^2 > 0.97$). High $SUVA_{254}$ levels are commonly observed in lakes in spring due to terrestrial inputs from spring rains and snow melt, with subsequent declines, although occasional spikes may occur after heavy summer rain events (Raudina et al., 2022;

Burd et al., 2018). Higher $SUVA_{254}$ values have been shown to be positively correlated with the ability of DOM to bind iron (Fujii et al., 2014). Additionally, iron (III) can interfere with $SUVA_{254}$ by increasing absorbance values (Poulin et al., 2014; Weishaar et al., 2003), yet this can be corrected for by reducing iron (III) to iron (II) as it has negligible absorbance (Doane & Horwáth, 2010).

Helms et al. (2008) established the spectral slope ratio (S_R), which they found exhibits an inverse correlation with molecular weight. This ratio is calculated by dividing the absorbance slope within the 275–295 nm range ($S_{275–295}$) by the slope within the 350–400 nm range ($S_{350-400}$). Helms et al., 2008 completed irradiation experiments of Dismal Swamp water samples of both high and low molecular weight DOM and found that $S_{275–295}$ tended to increase while $S_{350-400}$ decreased (an overall increase in S_R). Hence, S_R is often used to assess changes in molecular weight due to photodegradation.

The fluorescence index (FI) is often used to determine the source of DOM. It is computed as the ratio of the emission intensity at a wavelength of 450 nm to that at 500 nm, with an excitation wavelength of 370 nm. McKnight et al. (2001) showed that microbially sourced DOM tends to have an FI of ~1.9, while terrestrially sourced DOM often has an FI of ~1.4. FI has been shown to increase with a higher percentage of cropland, possibly due to higher production of microbial-like DOM resulting from increased nutrient levels (Wilson & Xenopoulos, 2009).

Freshness index (β: α) is the ratio between relatively fresh DOM (β) to degraded DOM (α). The measurement involves calculating the ratio between the maximum emission intensity in the range of 380 nm to 400nm (β) and the maximum emission intensity observed in the range of 420 to 435 nm (α), using an excitation wavelength of 310 nm (Wilson & Xenopoulos, 2009; Parlanti et al., 2000). Higher β:α values have been associated with larger autochthonous microbial production (Wilson & Xenopoulos, 2009), yet β:α may also be high with fresh allochthonous sources.

The humification index (HIX) serves as a metric for assessing the degree of humification of DOM (Zsolnay et al., 1999). Higher values indicate increased decomposition and the conversion of organic materials into humic substances, or an increased presence of terrestrial, humic material. HIX is often calculated as the ratio of the sum of the fluorescence intensity between 435 and 480 nm and between 300 and 345 nm at a fixed excitation wavelength of 254 nm (Zsolnay et al., 1999).

In addition, a technique called "parallel factor analysis" (PARAFAC) has emerged as an effective means for analyzing the composition of DOM from a diverse range of compounds. PARAFAC enables the decomposition of complex fluorescence data (excitation emission matrices; EEMS) into specific DOM components, thereby aiding in the identification of unique fluorophores within a mixture (Stedmon et al., 2003). Various PARAFAC models have been developed with different numbers of DOM components. For instance, Williams et al. (2013) created a PARAFAC model with seven DOM components: C1 (ubiquitous humic-like), C2-C3 (terrestrial humic-like), C4 (soil fulvic-like), C5 (microbial humic-like), C6 (anthropogenic humic-like), and C7 (protein-like). Applying PARAFAC to fluorescence data allows researchers to discern distinct sources of DOM fluorescence, providing a more detailed understanding of the molecular composition.

Brownification

The concentration or quantity of DOM (measured as dissolved organic carbon; DOC) is increasing in many parts of the northern hemisphere, especially the boreal forest, through a phenomenon known as brownification (Stackpoole et al. 2017). This is due to recovery from acid deposition as well as climatological factors, such as increases in precipitation (e.g., increased runoff) and temperature (e.g., increased decomposition of soil organic matter) (de Wit et al. 2021; Chow et al. 2017; Couture et al. 2012; Zhang et al. 2010). Brownification is concerning as it can limit benthic primary production, which, in turn, leads to reduced hypolimnetic oxygen levels, which can have negative effects on aquatic organisms (van Dorst et al., 2019; Seekell et al., 2015). Furthermore, brownification can increase the occurrence of harmful disinfection byproducts created from water treatment plants (Ritson et al., 2014). In addition, brownification may promote toxic cyanobacterial blooms due to nutrients associated with DOM, and certain cyanobacteria taxa are tolerable of low light conditions (Senar et al., 2019).

Spatial and temporal patterns

Dissolved organic matter composition and concentration has been shown to vary from lake to lake based on various lake and watershed characteristics. For instance, lakes in natural and forested watersheds tend to display more terrestrial or allochthonous-like signatures, whereas lakes in agricultural or urbanized catchments tend to display more microbial or autochthonous-like signatures (Williams et al., 2016). Also, the number of wetlands in a catchment is often positively correlated with DOC and terrestrial-like DOM (Warner & Saros, 2019; Xenopoulos et al., 2003), while the mean slope of a catchment (Frost et al., 2006; Xenopoulos et al., 2003; Rasmussen et al., 1989) and water residence time (Hall et al., 2019; Kellerman et al., 2014) are often negatively correlated with DOC and terrestrial-like DOM.

DOM composition has been shown to vary temporally in lakes. In the spring, DOM throughout the water column tends to be relatively homogenous and terrestrial-like in composition due to the import of terrestrial DOM following snowmelt and spring rains, and spring turnover which mixes DOM throughout the water column (Berg et al., 2022; Oswald & Branfireun, 2014). However, as conditions get drier during the summer and allochthonous inputs are reduced, DOM in the epilimnion tends to be phototransformed from high molecular weight, terrestrial-like to low molecular weight, microbial-like compounds (Berg et al., 2022; Dadi et al., 2017; Gonsior et al., 2013). This is especially the case for lakes with high water residence times as there is more time for processing of DOM (Hall et al., 2019; Curtis & Schindler, 1997). Also, a density gradient from stratification causes the top layer of water to not mix with the bottom layer, so DOM in the hypolimnion remains allochthonous-like as it is not exposed to much solar radiation (Berg et al., 2022). Furthermore, in the presence of sufficient nutrients promoting algal growth or blooms, there is a potential rise in protein-like DOM during the summer in the epilimnion, from algal exudation (Berg et al., 2022; Mangal et al., 2016; Meon & Kirchman, 2001). In addition, it is possible that the proportion of lake perimeter with wetlands, the proportion of wetlands in the catchment, and the mean slope of a catchment may affect the rate of transformation of epilimnetic DOM by altering surface DOM inputs, yet no studies have linked these variables to seasonal transformations of DOM composition.

Winter DOM dynamics in lakes

Winter is a long period for lakes in the northern hemisphere. With climate change, there have been observed decreases in both ice cover and duration (Higgins et al., 2021; Imrit & Sharma, 2021). However, significant research gaps persist in the study of winter lake dynamics (Ozersky et al., 2021). These research gaps are mainly due to the logistical and safety challenges associated with sampling lakes in winter. Nonetheless, recent research has emphasized the important role lake ice has in the ecology of lakes (Cavaliere et al. 2021; Hampton et al. 2017;

Jansen et al., 2021). For example, ice provides a habitat for filamentous diatoms and various microbes (Santibáñez et al., 2019; D'souza et al., 2013). Lake ice has also been found to play a role in the lake-atmosphere heat balance due to the albedo effect which reduces solar penetration into the water, thereby reducing heating (Austin & Colman 2007).

Although there has been some progress in winter lake studies, dissolved organic matter dynamics in lakes during winter remain largely unstudied. The existing literature, primarily for small boreal and temperate lakes, has shown that ice plays a role in DOM dynamics. For instance, the presence of ice has been shown to affect the composition and concentration of DOM under the ice (Kurek et al., 2022). Moreover, lake ice has been shown to have a lower quantity of DOM than the underlying water, and preferentially incorporates protein-like compounds and excludes terrestrial-like compounds as it forms (Zhou et al. 2023; Belzile et al. 2002). However, it is not known if the proportions of different DOM compounds in ice vary in water bodies that span large spatial scales with different limnological conditions (e.g., ice conditions and nutrients) such as the Laurentian Great Lakes. Additionally, the protein-like DOM stored in ice may be a source of bioavailable carbon to heterotrophic microbes when the ice melts in the spring (Imbeau et al., 2021). No studies have quantified this release.

Objectives

This thesis has two primary objectives. The first is to better understand seasonal changes of DOM within the water column of Boreal Shield lakes and relate these changes to a suite of lake and watershed variables. Understanding the seasonal distribution of DOM in the water column of lakes is crucial as factors such as brownification may alter the vertical distribution of DOM, potentially impacting the ecology of lakes. The second objective of this thesis is to examine the patterns of DOM composition and quantity found in the ice of the Laurentian Great Lakes. This involves identifying the key variables influencing these patterns and evaluating whether the ice could serve as a significant contributor of protein-like DOM to the water during the spring ice melt. Understanding the role of lake ice in DOM dynamics is important, especially its function in storing protein-like DOM, which may be beneficial to aquatic microbes following ice melt. This feature may be lost due to declining ice cover under climate change.

Chapter 2: Subtle seasonal transformations of dissolved organic matter in Boreal Shield lakes and their relationship to lake and watershed characteristics

Abstract

Dissolved organic matter (DOM) is a crucial component of lake ecosystems, influencing the carbon cycle. However, much is unknown about the temporal and spatial variations of DOM within lakes. This study investigates the short-term dynamics of DOM composition and dissolved organic carbon (DOC) in the epilimnion and hypolimnion of 10 lakes at the IISD Experimental Lakes Area. Sampling occurred in early and late June 2022, following an unusually cold and wet spring. We assessed whether DOC and various DOM indicators increased or decreased between samplings in the epilimnion and hypolimnion, and if these changes were related to multiple lake and watershed parameters. In the epilimnion, DOC increased across all lakes, and in most lakes, there were subtle increases in microbial-like DOM and subtle decreases in terrestrial-like DOM. The hypolimnion exhibited inconsistent changes in DOM composition and DOC, except for DOM molecular weight which increased for most lakes. Some relationships were found between DOM indicators and lake and watershed variables in the epilimnion and hypolimnion, respectively. For instance, decreases in terrestrial humic-like DOM in the epilimnion were inversely related to lake area, and increases in DOM molecular weight in

the hypolimnion were positively related to water residence time. However, there were a lack of relationships with several lake and watershed variables, e.g., nutrient levels, which may suggest that these variables do not contribute to DOM seasonal transformations or could be due to the short sampling period, small sample size, or the potential influence of a wetter than normal spring.

Introduction

Dissolved organic matter (DOM) is a crucial part of lake ecosystems and the carbon cycle. The carbon component of DOM, known as dissolved organic carbon (DOC), is the largest pool of organic carbon in lake water, constituting up to 97% in some boreal lakes (Kortelainen et al., 2006). Much is unknown about how DOM is distributed in lakes over seasons and locations, and within the water column. These patterns are important to understand because shifts such as more algal blooms or increased DOC (browning) could alter normal distributions of DOM in the water column of lakes, which could have implications for lake ecosystems and the carbon cycle.

DOM composition in the water column of lakes changes seasonally. After spring turnover and during the summer, the molecular weight of DOM tends to decrease in epilimnetic waters, with a rise in aliphatic or microbial-like compounds (Berg et al., 2022; Dadi et al., 2017; Gonsior et al., 2013). Simultaneously, in hypolimnetic waters, DOM composition either maintains its high molecular weight, often characterized by aromatic, terrestrial-like or humic-like compounds, or experiences a relative increase in such high molecular weight substances (Berg et al., 2022; Dadi et al., 2017; Gonsior et al., 2013). This is related to the phototransformation of high molecular weight compounds to low molecular weight compounds in the epilimnion that is aided by a density gradient (from stratification) that prevents vertical mixing, essentially locking high molecular weight compounds in the hypolimnion (Zhang et al., 2023; Berg et al., 2022). This pattern can be further influenced by aromatic compounds being released from the sediment or iron during periods of anoxia (Dadi et al., 2017), and algae near the surface of the lake exuding low molecular weight compounds (Zhang et al., 2013; Meon & Kirchman, 2001).

Water residence time can play a large role in seasonal DOM transformations in lakes. For example, longer water residence times can allow for more phototransformations to occur in epilimnetic waters or other in-lake processes, such as flocculation or microbial respiration, compared to shorter water residence times which are characterized by more constant inputs and outputs of DOM (Hall et al., 2019; Curtis & Schindler, 1997; Rasmussen et al., 1989). Moreover, lake and watershed parameters such as lake perimeter wetlands, the proportion of wetlands in catchment, and mean slope of catchment may affect the rate or extent of DOM transformation during the summer in lakes by altering DOM surface inputs. Wetlands are a large source of humic-like DOM to lakes, especially treed and perimeter wetlands (Warner & Saros, 2019; Xenopoulos et al., 2003); and watershed slope can influence the quantity of terrestrial-like DOM entering a lake (Frost et al., 2006; Rasmussen et al., 1989). Additionally, nutrients may alter DOM composition throughout the water column of lakes by increasing microbial decomposition of DOM or increasing algal production, which may exude autochthonous-like DOM (Meon & Kirchman, 2001).

In this study, 10 lakes of different DOC concentrations and nutrient levels at the International Institute for Sustainable Development - Experimental Lakes Area (IISD-ELA), which is located in the Boreal Shield in Northwestern Ontario, Canada, were sampled once in early June and again in late June 2022. The objectives of this study were to 1) assess if DOM composition was different in the epilimnion compared to the hypolimnion during both samplings, 2) determine if there were consistent changes among lakes in DOM composition and DOC in the epilimnion and hypolimnion, respectively, between both samplings, and 3) evaluate if changes in DOM composition were related to lake specific DOC and nutrient levels, water residence time, mean slope of catchment, proportion of lake perimeter with wetlands, proportion of catchment area with wetlands, and stratification strength change. The sampling period followed an unusually wet and cold spring with a late ice out, which may have affected our results. For example, a late ice out would likely have delayed stratification, and wetter conditions may have meant higher hydrological connectivity with increased export of DOM from the catchment (Johnston et al., 2020; Pace & Cole, 2002).

Methods

Site description

The IISD - ELA is located in Northwestern Ontario about 52 km east southeast of Kenora, Ontario (49°40’N and 93°44’W) (Brunskill and Schindler 1971). It is in the boreal forest and on the southern edge of the Precambrian Shield (Schindler *et al*., 1996). The climate is characteristic of the humid continental Köppen Dfb classification with warm, moist summers and dry, cold winters (Desloges, 2000). Due to the IISD - ELA’s remote location, the lakes are generally free of human influence (Zhang et al., 2010).

Field sampling

Water samples for DOM/DOC, chlorophyll *a* (Chl-*a*), and nutrients were collected from the epilimnion (1 m below surface) and hypolimnion (1 m above sediment) of 10 lakes (L114, L223, L224, L227, L239, L304, L373, L378, L442, and L626) of varying DOC and lake and watershed characteristics (Table A1), between June 6th to June 10th, 2022 (1st sampling) and again between June 26th and June 30th, 2022 (2nd sampling). L114 top samples broke during transport and L239 bottom samples were contaminated, so these depths were excluded from our DOM spectral analyses. The lakes had recently undergone spring turnover and only begun to undergo stratification prior to the first sampling. In 2022, ice-out occurred on May 13th, which was later than the long-term average. Furthermore, the total rainfall for April-June 2022 was 391 mm, which was 73% higher than the average 30-year total rainfall for April-June (225 mm). Moreover, two of our study lakes, L227 and L304, were receiving phosphorus additions as part of a eutrophication experiment, while the rest of our study lakes are oligotrophic. Water samples were collected at the center buoy of each lake (deepest part of the lake) 1 m below the surface and 1 m above the sediment using a combination of peristaltic pumps, Van Dorn samplers, and integrated samplers, then poured into 1L HDPE bottles (kept out of sunlight) and stored in a refrigerator until filtering. Temperature profiles were measured using an RBR XRX-620 multi-function probe, and Secchi depth was measured using a Secchi disk. Additionally, we obtained DOM data from epilimnion water samples collected by the IISD-ELA field crew (Hydrolim) from L223, L239, L373, L378, and L626, June to September 2022, to compare DOM/DOC results from our shorter sampling period (June) for 10 lakes.

Analytical Methods

For DOM/DOC, 200 mL of water from each sample was filtered through 0.7 µm GF/F filters, then through 0.22 µm pore-size polycarbonate Whatman PCTE filters and stored in 125mL ashed amber bottles and refrigerated until DOM/DOC analysis. The GF/F filters were frozen in aluminum foil for later Chl-*a* analysis. UV-persulfate acidification (Non-Purgeable Organic Carbon method) via a Shimadzu Total Organic Carbon Analyzer (TOC-VWP) was used to measure DOC concentration (mg L^{-1}) in the 0.22 μm filtered water. Water for total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) was filtered using Whatman GF/C filters and analyzed with methods described in Stainton et al. (1977), by the IISD-ELA's chemistry laboratory. For Chl-*a*, filters were put in 20 mL of a 95% ethanol solution and placed in a fridge (4 °C) for 24 hours (cold ethanol extraction). This extracted solution was measured for fluorescence on a Varian Cary Eclipse Fluorometer (Agilent Technologies, Mississauga, ON, Canada) at wavelengths of 440 to 660 nanometers (nm). Fluorescence was converted to Chl-*a* concentration using concentrations of known Chl-*a* standards.

DOM spectral analyses

DOM composition was determined using absorbance and fluorescence spectroscopic techniques. A Varian Cary 50 Bio UV-Visible spectrophotometer (Agilent Technologies) was used to measure absorbance over a wavelength range of 230 to 800 nm, at 5 nm increments. The absorbance at 254 nm (a254) was divided by DOC concentration (mg L^{-1}) to obtain the specific UV absorbance ($SUVA_{254}$), which is indicative of aromaticity (Weishaar et al., 2003). Excitation emission matrices (EEMs) were created using a Varian Cary Eclipse Fluorometer via methods described in Williams et al. (2013). Four DOM indices were measured using the EEM and absorbance data: spectral slope ratio (S_R), a proxy for molecular size (Helms et al. 2008); freshness index (β/α), which reflects the degree of freshness (Wilson & Xenopoulos, 2009; Parlanti et al., 2000); fluorescence index (FI), a proxy of source (McKnight et al., 2001); and humification index (HIX), reflecting the degree of humification (Zsolnay et al., 1999). In addition, we extracted seven DOM components using parallel factor analysis (PARAFAC) from models derived earlier (Williams et al. 2013, 2016; Williams and Xenopoulos, 2023). The seven components of DOM composition include C1, which is ubiquitous humic-like; C2 to C3, which are terrestrial humic-like; C4, which is soil fulvic-like; C5, which is microbial humic-like; C6, which is anthropogenic microbial humic-like; and C7, which is protein-like, e.g., tryptophan-like fluorescence (Williams and Xenopoulos, 2023; Williams et al., 2013, 2016;).

Lake and watershed parameters

The total wetland area in the catchment, catchment area, lake surface area, and mean catchment slope were extracted from the Ontario Watershed Information Tool (Government of Ontario, 2023). Lake volume calculations were conducted through ArcGIS Pro 3.0 (Esri Inc, 2022) and Python 3.0 (Van Rossum and Drake, 2009) utilizing lake surface area and depth digital elevation models (DEMs) as input data. The depth DEMs were generated by extrapolating survey data from bathymetry transects. To calculate the proportion of lake perimeter with wetlands, firstly, shapefiles of wetland area were retrieved from Ontario GeoHub (Ontario Ministry of Natural Resources and Forestry, 2022), uploaded into ArcGIS Pro 3.0 (Esri Inc, 2022), and 50-meter (m) buffers were created around the lakes to calculate the proportion of lake perimeter with wetlands. A proxy for water residence time was calculated by dividing lake volume by lake catchment area. Stratification strength was calculated as the Schmidt Stability Index (unitless and is used to assess the stability of a water body with respect to the exchange of gases) (Idso, 1973) using the "schmidt.stability" function with the "rLakeAnalyser" package in R version 4.2.1 (Winslow et al., 2019). The Schmidt Stability Index incorporates water temperature measurements at various depths and cross-sectional areas of the lake at various depths.

Statistical analyses

All statistical analyses and plots were carried out using R version 4.2.1 (R Core Team, 2021). To assess if DOM composition was different in the epilimnion compared to the hypolimnion for both samplings, principal component analysis (PCA) plots were created using the R packages "ggplot2" and "ggfortify" with the functions "prcomp" and "autoplot" (Horikoshi et al., 2023; Wickham et al., 2023) for first and second sampling, respectively. Epilimnion and hypolimnion observations (scores) were color-coded to visualize if epilimnion and hypolimnion samples differed in DOM composition (e.g., form separate clusters). Furthermore, four additional PCAs were performed to examine changes in overall DOM composition (PC1 scores) between samplings: two for the epilimnion (one for the first sampling and one for the second sampling) and two for the hypolimnion (one for the first sampling and one for the second sampling). PC1 scores were extracted from these PCAs. Prior to displaying changes between samplings (via ladder plots), PC1 scores from the 2nd sampling were aligned to the scores from the 1st sampling (for epilimnion and hypolimnion, separately) via Procrustes analysis, using the R "vegan" package and "procrustes" function (Oksanen et al., 2022) to minimize differences in translation, rotation, and scaling to enable more accurate comparisons.

To test if changes in DOM indices (delta variables) from the first to second sampling among lakes were significantly correlated to our lake and watershed parameters, correlation matrices were performed using the R "metan" package (Olivoto, 2023). Significant relationships were plotted individually as linear regression plots. The delta DOM indices were ΔDOC, ΔS_R, $\Delta SUVA_{254}$, ΔC1, ΔC7, and ΔPC1 scores. The lake and watershed variables were the proportion of lake perimeter with wetlands, the proportion of catchment area with wetlands, change in stratification strength, water residence time, mean slope of catchment, and DOC, TDN, and TDP concentrations. DOC, TDN, and TDP were averages of the first and second sampling to represent the typical levels in our study lakes. In addition, the delta variables and lake and watershed variables were scaled for normality as part of the correlation matrix procedure in R.

Results

Physicochemical conditions

The temperature in the epilimnion (1 m) increased from the first to the second sampling for all lakes (Figure 2.1a) but was rather constant in the hypolimnion except for L114 (Figure 2.1g). Stratification strength increased for all lakes from the first to second sampling, except for L114 which is very shallow (max ~5 m depth), so it would not be expected to properly stratify (Figure 2.1b), hence why the temperature also increased considerably in that lake in the hypolimnion (Figure 2.1g). There was a small decrease in TDP and TDN (epilimnetic) for most lakes from first to second sampling (Figure 2.1c and d), except for L304 and L227 which saw large increases (were receiving P additions). Changes in Chl-*a* (epilimnetic) were inconsistent, for both the epilimnion and hypolimnion, except for L227 and L304 which saw substantial increases due to P additions (Figure 2.1e and h). For the most part, Secchi depth appears to have increased for the lower DOC lakes (e.g., L224, L626, and L373) (became clearer) but decreased for the higher DOC lakes (became browner) (Figure 2.1f, Table A1).

Trends in DOM/DOC

For the first sampling, DOM composition separated into microbial, protein-like (as indicated by higher C7, S_R, and $\beta:\alpha$) and terrestrial, humic-like (as indicated by higher $SUVA_{254}$, C1, C2, etc.)

along the PC1 axis, which explained most of the variation in the data (Figure 2.2a). However, DOM composition did not separate based on water column location (i.e., epilimnion and hypolimnion samples did not form two separate clusters on the PCA plot) (Figure 2.2a), which indicates that DOM composition was similar in the epilimnion compared to the hypolimnion. This was also the case for the second sampling (Figure 2.2b).

Moreover, for the epilimnion, all lakes exhibited an increase in DOC, and most lakes had an increase in S_R (inversely related to molecular weight) (Figure 2.3a and b). $SUVA_{254}$ (aromaticity) did not show any consistent trends (Figure 2.3c), however C1 (ubiquitous humic-like DOM) decreased for most lakes (Figure 2.3d). C7 (protein-like DOM) and PC1 (overall microbial-like DOM) (Figure 2.3e and f) increased for most lakes. Epilimnetic samples from a subset of our lakes (n = 5), from June to September, showed more pronounced changes than June for our 10 study lakes for DOC, $SUVA_{254}$, and S_R (Figure 2.4). Aside from S_R, which decreased for most lakes, there were no consistent changes in DOM indices and DOC for the hypolimnion during our sampling period for our 10 study lakes (Figure 2.3g-l).

Relationships with delta DOM variables and lake and watershed characteristics

Some relationships were found between changes in DOM variables (ΔDOC, ΔS_R, $\Delta SUVA_{254}$, ΔC1, ΔC7, and ΔPC1) and lake and watershed parameters (proportion of lake perimeter with wetlands, proportion of catchment area with wetlands, change in stratification strength, water residence time, mean slope, DOC, TDP, and TDN) (Figure 2.5, Table A2).

In the epilimnion, lake area was inversely related to ΔC1 and positively with ΔPC1 (Figure 2.5a and b), e.g., larger decreases in humic-like DOM were correlated with smaller lake area, and larger decreases in microbial-like DOM were correlated with larger lake area. Change in stratification strength was positively related to $\Delta SUVA_{254}$ and $\Delta C1$, and negatively with $\Delta PC1$ (Figure 2.5c, d, and e), e.g., larger decreases in aromaticity and humic-like DOM were correlated with smaller increases in stratification strength, while larger decreases in microbial-like DOM were correlated with larger increases in stratification strength. In addition, the proportion of lake perimeter with wetlands, the proportion of catchment area with wetlands, mean slope of catchment, water residence time, and DOC, TDN, and TDP levels did not show significant relationships with delta DOM variables (Table A2).

In the hypolimnion, perimeter wetlands were positively related to ΔDOC (Figure 2.5f), e.g., larger decreases in DOC were correlated with more lake perimeter wetland area. Changes in stratification strength were inversely related to ΔS_R, e.g., lakes that saw larger increases in stratification strength saw larger decreases in low molecular weight DOM (Figure 2.5g). Water residence time was positively correlated to ΔS_R (Figure 2.5h), e.g., lakes with longer water residence times had larger relative decreases in low molecular weight DOM. Mean slope of catchment was negatively related to $\Delta C1$ (Figure 2.5i), e.g., lakes that saw larger decreases in humic-like DOM tended to have smaller mean slopes. In addition, the proportion of catchment area with wetlands, lake area, DOC, TDN, and TDP did not show significant relationships with delta DOM variables (Table A2).

Discussion

We observed subtle changes in DOM composition and concentration between the first and second sampling in June 2022 at the IISD-ELA, with shifts from terrestrial-like to microbial-like in the epilimnion for most lakes and overall, inconsistent changes in the hypolimnion. Such patterns are consistent with past research (e.g., Berg et al., 2022). Moreover, we observed some relationships between DOM changes and lake and watershed variables in the epilimnion and hypolimnion. Our results indicate that DOM transformations in lakes can occur, albeit subtly, over a relatively short period and can be related to various lake and watershed characteristics.

Overall DOM composition: Epilimnion vs hypolimnion

For the first sampling, overall DOM composition in the epilimnion did not differentiate from that in the hypolimnion. The lakes had recently undergone spring turnover and had only begun to stratify so DOM composition would be expected to be relatively homogenous throughout the water column (Berg et al., 2022). However, for the second sampling, there was also no clear separation in DOM composition between the epilimnion and hypolimnion, possibly because not enough time had passed for pronounced DOM transformations to occur (less than one month elapsed between samplings).

Additionally, an interesting observation is that DOM appears to be terrestrial or humic-like in L227 and L304 for both samplings and depths even though they were receiving nutrients and experienced high algal production, which often produces protein or microbial-like DOM (Zhang et al., 2013; Meon & Kirchman, 2001). These two lakes lack stream inflows but do contain wetlands in their catchments which may contribute to terrestrial-like DOM (Warner & Saros, 2019; Xenopoulos et al., 2003). In addition, aquatic macrophytes may have been contributing to terrestrial-like DOM (Zhang et al., 2013), or even algal production or decomposition as it has been found to produce DOM with humic-like fluorescence (Lobus et al., 2022).

DOM changes in epilimnion and hypolimnion

In the epilimnion, most lakes saw subtle decreases in humic-like DOM and molecular weight of DOM (increase in S_R) and subtle increases in microbial and protein-like DOM (C7). This suggests that terrestrial-like compounds were being phototransformed to low molecular weight, microbial, and protein-like compounds (Berg et al., 2022; Dadi et al., 2017; Gonsior et al., 2013). Also, we saw a general small decrease in dissolved nutrients from the first to second sampling (except for the nutrient addition lakes, L227 and L304) suggesting that phytoplankton had been using up nutrients and potentially increasing in biomass as well as exuding low molecular weight compounds (Zhang et al., 2013; Kritzberg et al., 2004; Meon & Kirchman, 2001). Yet, there were no consistent increases in Chl-*a* concentration (except for L227 and L304), which is often used as a proxy for phytoplankton abundance. However, Chl-*a* is not always an accurate measure of phytoplankton biomass (Sherbo et al., 2023; Girdner et al. 2020). All lakes did, however, see an increase in DOC, which may have been due to autochthonous production or imports from the watershed due to a wetter than normal spring (Johnston et al., 2020; Oswald & Branfireun, 2014). Additionally, for a subset of our study lakes sampled from June to September, there were pronounced changes in DOC, S_R, and $SUVA_{254}$, suggesting that a longer sampling period for our 10 study lakes may have yielded more marked changes.

For the hypolimnion, most lakes saw an increase in molecular weight of DOM (decrease in S_R). This decrease in S_R is consistent with previous studies showing the hypolimnion tends to become more concentrated with high molecular weight compounds following stratification (Zhang et al., 2023, Berg et al., 2022; Dadi et al., 2017). No consistent changes were observed for DOC and the other DOM indices, which may be expected since conditions should be relatively stable due to minimal phototransformations from lack of (or little) sunlight in hypolimnion waters. However, other processes in the hypolimnion that may affect DOM composition and concentration can still occur such as microbial transformations, redox reactions, flocculation, and sedimentary release of DOM (Berg et al., 2022).

Relationships with lake and watershed parameters

In the epilimnion, we observed that increases in stratification strength were correlated with increases in aromatic/humic-like DOM (e.g., $SUVA_{254}$ and C1) and decreases in microbial-like DOM. These observations are interesting, as it would be expected that as stratification strength increases, there would be a reduction in aromatic/humic-like compounds and an increase in microbial-like compounds due to less mixing of aromatic compounds from the hypolimnion with epilimnetic water combined with photobleaching (Zhang et al., 2023; Berg et al., 2022). It is possible, however, that terrestrial inputs from the catchment continued throughout our sampling period due to high hydrological connectivity from above average precipitation in the preceding several months (Johnston et al., 2020; Oswald & Branfireun, 2014). Moreover, lakes that experienced larger decreases in humic-like DOM (C1) and larger increases in microbial-like DOM tended to have smaller surface areas, which is not what would be anticipated as larger lakes often have longer water residence times providing more opportunities for photobleaching and in-lake processing of humic-like DOM (Rasmussen et al., 1989). It is possible, however, that photodegradation of humic-like compounds may have been more noticeable in lakes with small surface areas since most of our small lakes were headwater lakes without inflows, so they would not be receiving constant inputs of terrestrial DOM, although they would still receive runoff from the catchment (Van Stan & Stubbins, 2018).

In the hypolimnion, we observed that lakes with more perimeter wetland areas experienced higher decreases in DOC. This observation is interesting, considering that perimeter wetlands have been found to be large sources of DOC to lakes (Xenopoulos et al., 2003). However, it could be that inputs of DOC from perimeter wetlands while a lake is stratified does not necessarily lead to more DOC in the hypolimnion since the hypolimnion is essentially sealed off, and another factor in these lakes is likely driving a faster decrease, e.g., heterotrophic microbial respiration. Furthermore, lakes with larger mean slopes tended to experience smaller decreases in terrestrial/humic-like DOM (C1), thus less respiration or degradation of this DOM. Past research has shown that the import of terrestrially derived DOM into lakes is lower with a larger mean catchment slope (Frost et al., 2006; Xenopoulos et al., 2003; Rasmussen et al., 1989), so it would be expected that there would be larger decreases in this DOM under higher mean slopes (less replenishment). However, as mentioned previously, what occurs in the hypolimnion may not be a direct response to surface inputs due to isolation from the epilimnion from stratification, and another variable in these lakes with steeper slopes must be resulting in less decreases in terrestrial-like DOM. Moreover, lakes with longer water residence times had larger decreases in low molecular weight DOM. This may be because over the summer, the relative amount of low molecular weight compounds often increases in the epilimnion and high molecular weight compounds increase in the hypolimnion (Berg et al., 2022), which may be aided by longer water residence times as there is more time for transformations to occur (Hall et al., 2019; Curtis & Schindler, 1997). Similarly, lakes with greater increases in stratification strength had larger decreases in low molecular weight DOM. This is likely due to reduced mixing of low molecular weight DOM from the epilimnion with the hypolimnion during stronger stratification regimes (Zhang et al., 2023).

In addition, in the epilimnion, there were no relationships between DOM delta variables and area of lake perimeter with wetlands, proportion of catchment area with wetlands, mean slope of catchment, water residence time, and average DOC, TDN, and TDP levels. While in the hypolimnion, there were no relationships between delta DOM variables and the proportion of catchment area with wetlands, lake area, and DOC, TDN, and TDP levels. The lack of relationships with such lake and watershed variables in the epilimnion and hypolimnion may be because of the relatively small sample size of lakes, the short duration of our sampling period, terrestrial input associated with a wetter-than-normal spring (may have masked some relationships), or simply due to no effect of these variables.

Conclusions

This was the first study that examined relationships between a suite of lake and watershed variables and DOM composition transformations in the water column of lakes, particularly the hypolimnion, during the summer. Although we saw only subtle changes in DOM composition, and a few correlations with lake and watershed characteristics, this study has shown that DOM transformations can occur in lakes during a short amount of time at the onset of summer. Continued research on this topic should enhance our understanding of the seasonal dynamics of DOM within lakes and its complex relationship with lake and watershed characteristics, which is particularly relevant in the context of catchment and lake changes due to anthropogenic activity and elevated DOC concentrations associated with brownification.

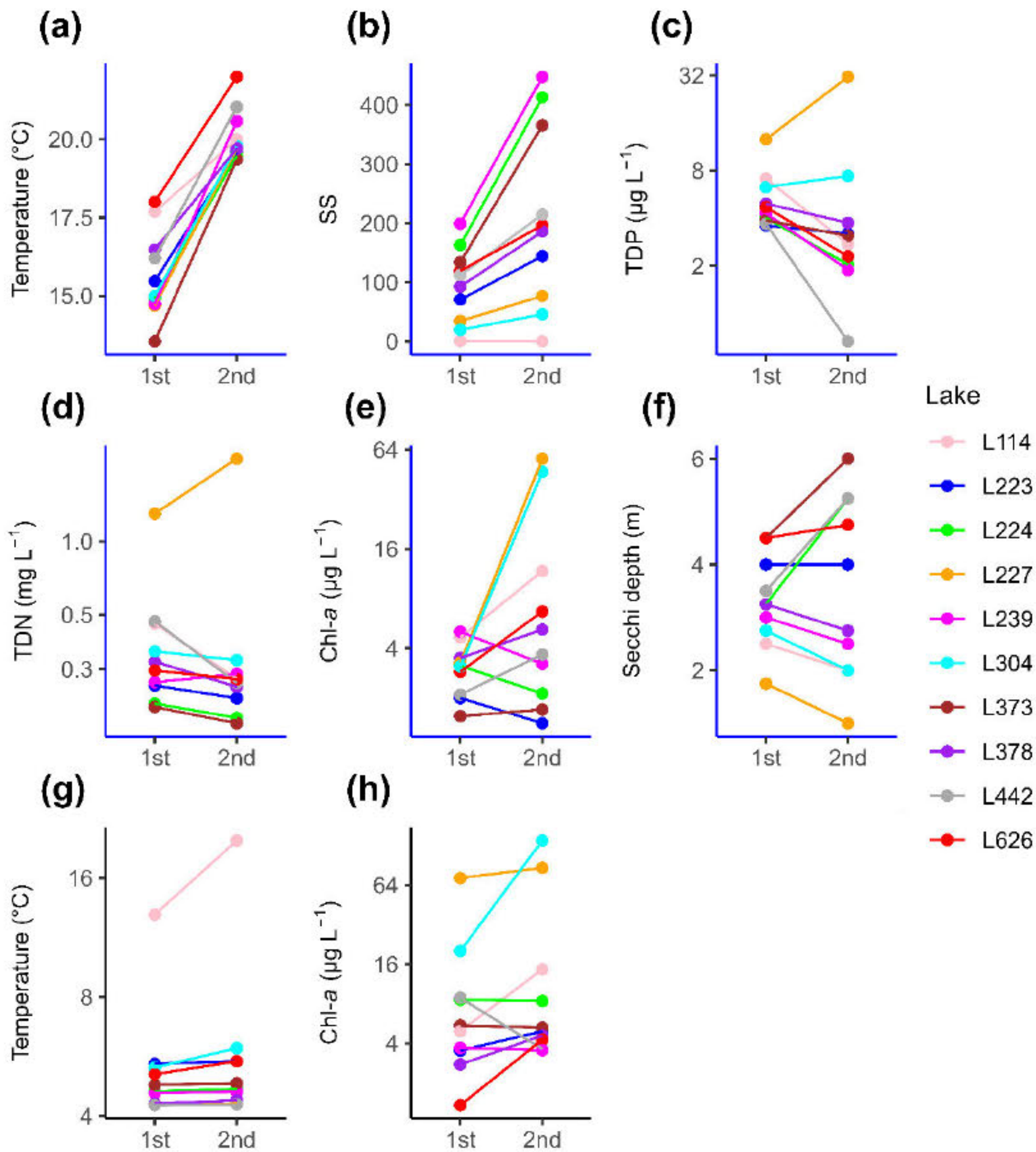

Figure 2.1. Ladder plots illustrating changes in physicochemical conditions from the first sampling (early June) to the second sampling (late June). SS is stratification strength. Plots a to f are for the epilimnion (blue axis lines) while g and h are for the hypolimnion (black axis lines).

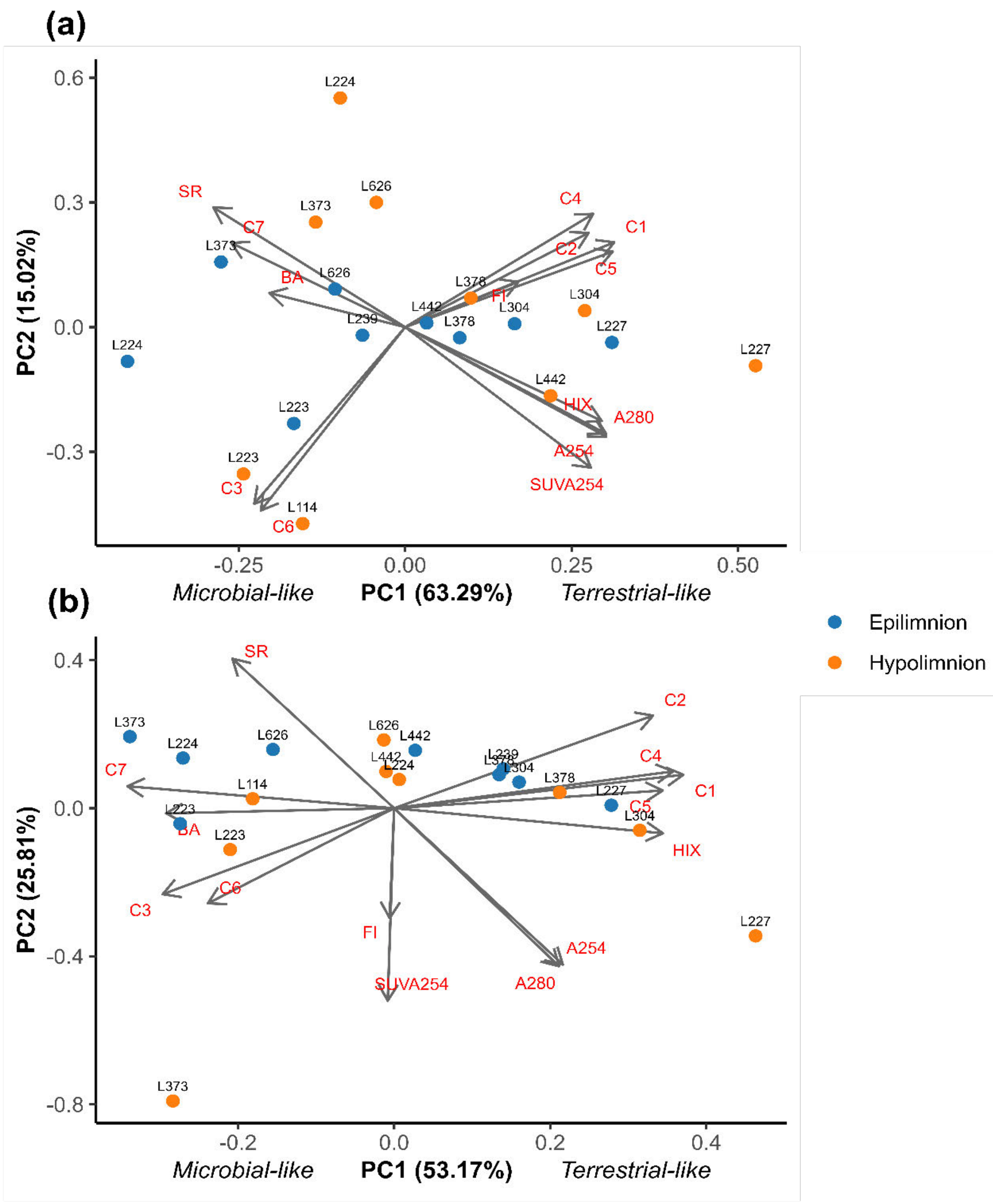

Figure 2.2. PCA plots for the first sampling (a) and second sampling (b) with blue dots representing the epilimnion and orange dots representing the hypolimnion. For both plots, to the left of 0 on the PC1 axis represents microbial-like DOM, and on the right, it represents terrestrial-like DOM.

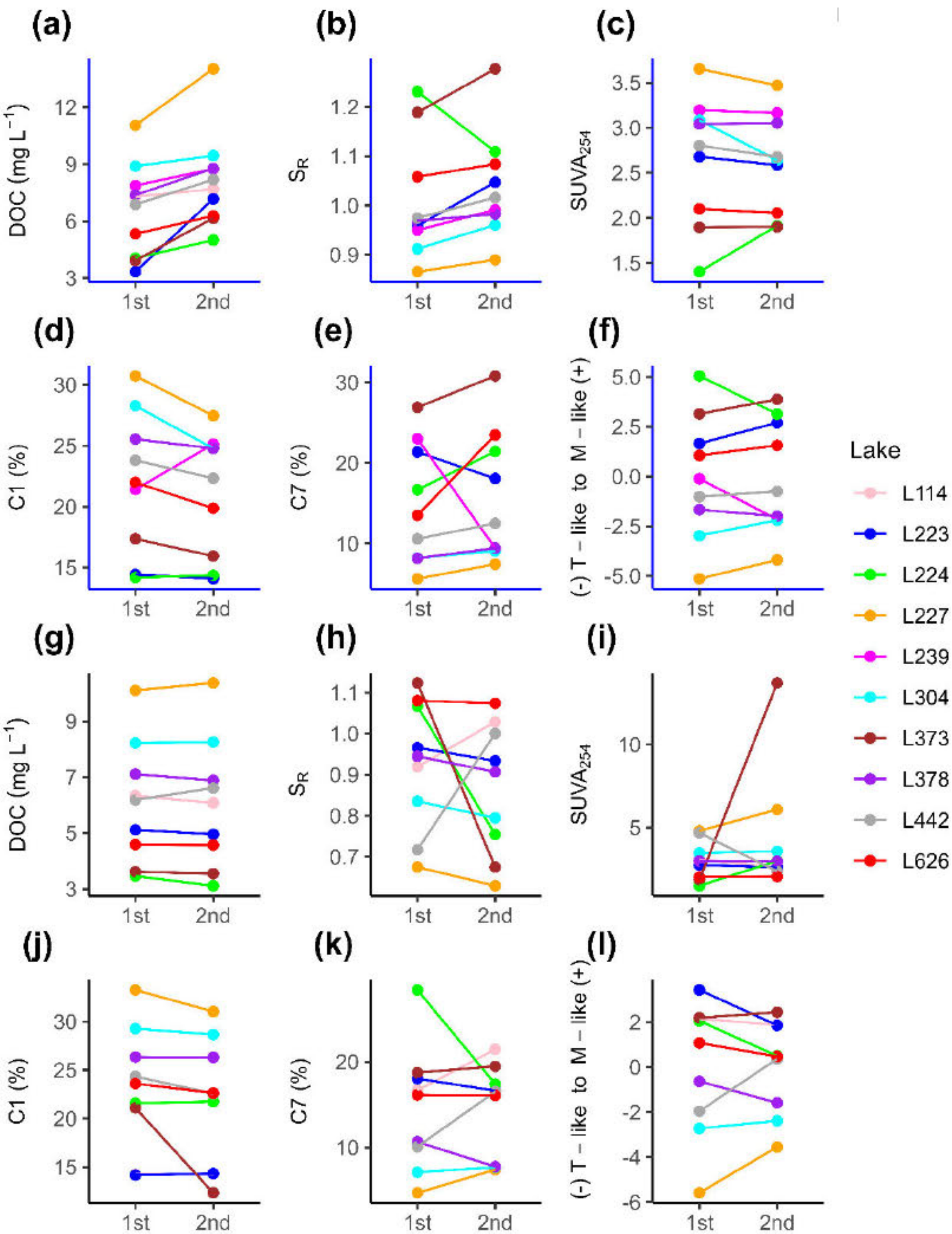

Figure 2.3. Ladder plots displaying changes in DOM indices between first and second sampling, represented by lines. T-like is terrestrial-like and M-like is microbial-like. Plots a-f (blue axis lines) are for the epilimnion, and g to l (black axis lines) are for the hypolimnion.

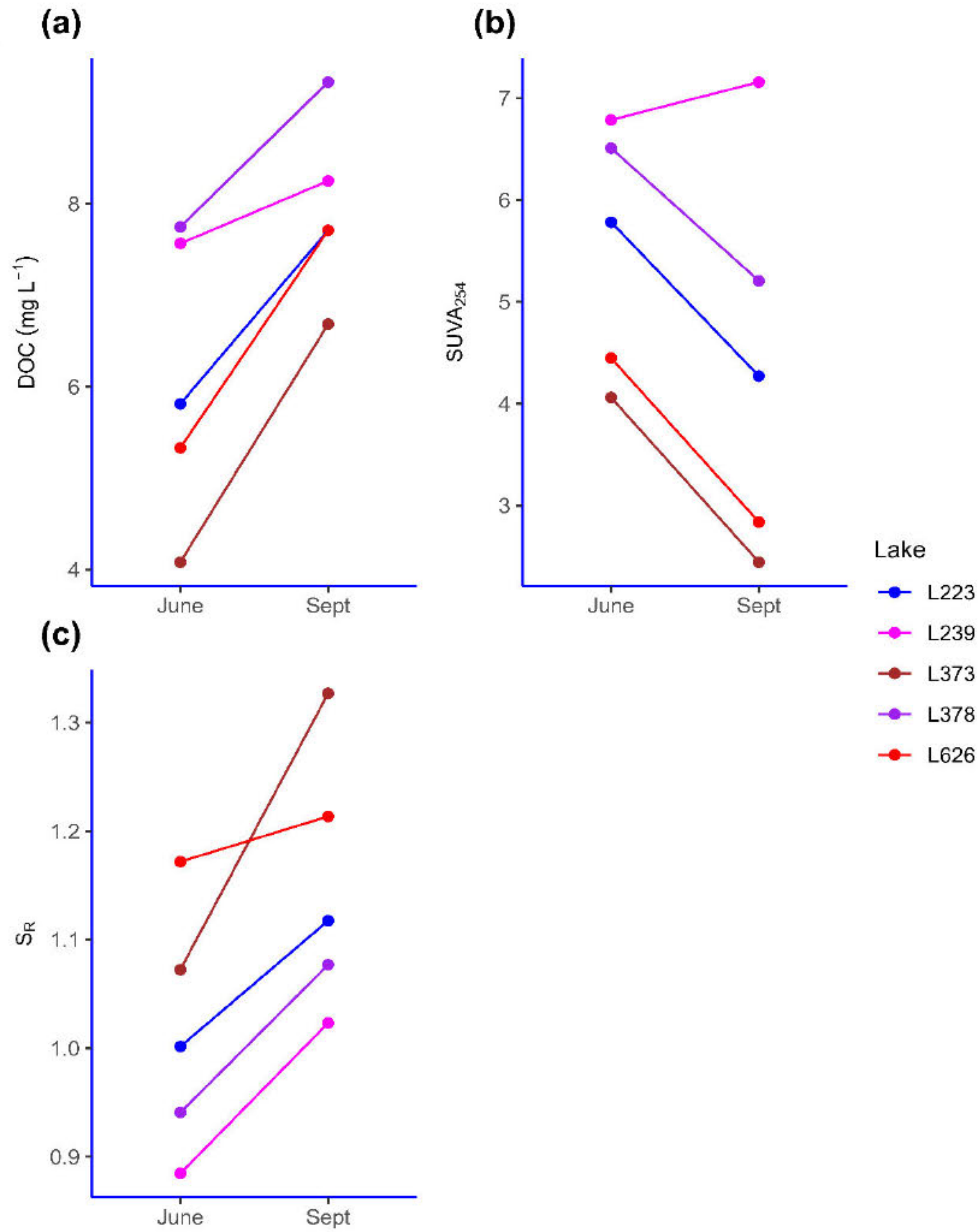

Figure 2.4. Ladder plots displaying changes in (a) DOC, (b) $SUVA_{254}$, and (c) S_R from June to September 2022 for 5 lakes for the epilimnion.

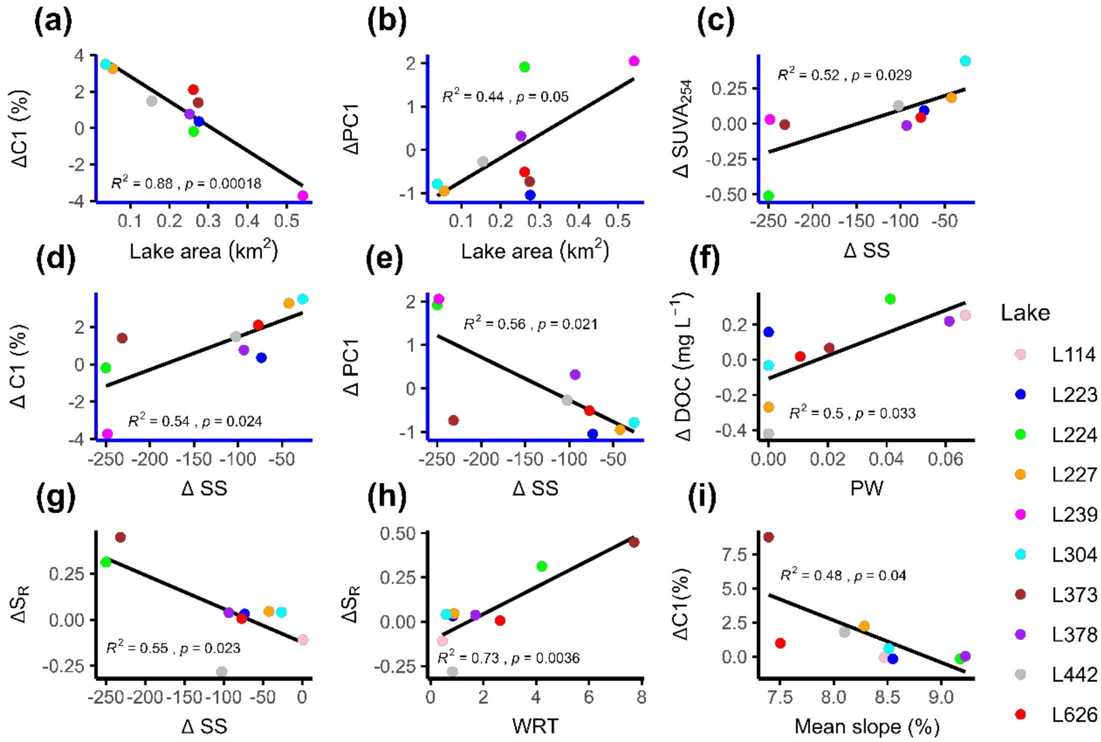

Figure 2.5. Significant relationships between various DOM composition indices and DOC and some lake and watershed variables for the epilimnion (a-e; blue axis lines) and hypolimnion (f-i; black axis lines). SS stands for stratification strength, and WRT is for water residence time.

Chapter 3: Fingerprints of dissolved organic matter composition in ice of the Laurentian Great Lakes

Abstract

Dissolved organic matter (DOM) plays a vital role in lakes, but its behavior in winter is poorly understood. We assessed differences in DOM between lake ice and water across the Laurentian Great Lakes. The quantity of DOM (dissolved organic carbon; DOC) was lower in ice compared to water. Protein-like DOM was dominant in ice, while terrestrial-like DOM was dominant in water. The amount of protein-like DOM in ice varied by site based on factors such as ice thickness and water DOM composition, nutrients, and DOC. Calculations of protein-like, microbial-like, and terrestrial-like DOM storage in ice under different ice cover scenarios revealed considerable contributions to the upper water column during ice melt (up to 25.82%). However, these contributions are expected to diminish with projected climate change-induced reductions in ice cover and duration. Understanding these dynamics is crucial for assessing the impact on microbial metabolism in the upper water column.

Introduction

Over the last several decades, lakes in the northern hemisphere have experienced a significant decrease in the extent and duration of ice cover (Sadro and Xenopoulos 2022; Imrit & Sharma 2021; Higgins et al., 2021; Wang et al., 2012). Although winter remains understudied for lake ecosystems, ice cover is often considered a “master variable” (Ozersky et al., 2021) that controls many physical and biogeochemical aspects of lakes (Cavaliere et al., 2021; Hampton et al., 2017; Jansen et al., 2021).

Winter and lake ice research on the dynamics of dissolved organic matter (DOM) is limited compared to other limnological variables, despite its significance in lake ecosystems and the global carbon cycle (Tranvik et al., 2009). Most studies of ice DOM have focused on glacier and sea ice rather than freshwater lake ice. Glacier ice contributes bioavailable carbon to downstream riverine and marine microbial communities, while sea ice has high dissolved organic carbon (DOC) concentrations due to brine channels that concentrate DOM, which freshwater ice lacks (Lawson et al., 2014; Hood et al., 2009; Müller et al., 2013). Yet, the few studies on lake ice DOM suggest that certain carbon components are selectively incorporated during formation with ice serving as a mechanism of transient storage for this DOM (Kurek et al., 2022; Zhou et al. 2023; Santibáñez et al., 2019; Belzile et al., 2002).

Previous research on lake ice DOM has revealed lower DOC levels in ice compared to water, with DOM being primarily protein-like in ice and terrestrial-like in water (Zhou et al., 2023; Santibáñez et al., 2019; Song et al., 2019; Belzile et al., 2002). Preferential retention of protein-like DOM and exclusion of terrestrial/humic-like DOM during ice formation are suggested to explain these findings (Zhou et al., 2023). Additionally, humic compounds that remain in ice may be phototransformed into microbial-like compounds during periods with limited snow cover and clear ice (Kurek et al., 2022; Belzile et al., 2002). It is further possible that protein-like DOM originates from algal or bacterial cells within or on the ice (Imbeau et al., 2021; Santibáñez et al., 2019; Guo et al., 2017; McKay et al., 2015), including filamentous diatoms on the underside of the ice (D'souza et al., 2013; Bondarenko et al., 2012). The protein and microbial-like DOM stored in lake ice may serve as an important source of carbon for planktonic microbes after ice melt and may not be available during spring melt with future loss of lake ice associated with climate change (Imbeau et al., 2021).

DOM composition in lake ice may vary across large spatial scales with different ice conditions and lake trophic status. For example, the amount of snow on the ice and the clarity of the ice may affect the phototransformation of DOM in or below the ice (Kurek et al., 2022; Belzile et al., 2002). Also, differences in ice thickness and type of ice may affect the capacity of ice to store protein-like DOM (Imbeau et al., 2021; Belzile et al., 2002). Moreover, nutrient-rich lakes may support more algal production that can exude protein-like DOM into the water, which can be incorporated into ice during formation (Imbeau et al., 2021).

To better understand patterns of DOM in lake ice across a large spatial scale, we utilized a binational (coordinated across 19 research institutions in Canada and the USA) sampling campaign ('Winter Grab'; Sola, 2022) to obtain samples of lake ice and water collected across all five Laurentian Great Lakes (hereafter Great Lakes) during approximately maximum winter ice cover (mid-February 2022). We sought to: 1) examine DOM composition and DOC in ice and water across the Great Lakes; 2) relate observed variation in ice DOM to environmental and ice conditions; and 3) estimate the relative contributions of the main DOM components to the upper water column after ice melt under different ice cover scenarios. To date, no studies have created a budget for the quantity of different DOM components that can be stored in as well as released from lake ice to the water during ice melt. Hence, our results should illustrate if ice acts as an important source of DOM to the water column following ice melt, which could be an important temporary source of carbon for heterotrophic microbes.

Methods

Sample collection

At 21 of the total 49 Winter Grab sampling sites, ice cores were obtained by drilling three holes into the ice (3 cm apart), using an ice auger, and the center triangular piece of ice was removed with an ice saw. The ice core was put in a Whirl-Pak polyethylene bag and placed in a cooler. Water samples for DOM, nutrients, and chlorophyll *a* (Chl-*a*) were taken from immediately below the surface using a Van Dorn sampler, stored in acid-washed containers, and kept in the dark at 4°C prior to laboratory processing. Once in the lab, the ice was kept in the dark and left to melt in the fridge (4°C).

Analytical Methods

Samples for DOM/DOC were filtered through 47 mm diameter Whatman GF/F glass fiber filters and subsequently through 47 mm diameter 0.2 µm pore-size Whatman PCTE filters, then stored in pre-combusted amber glass bottles in a dark refrigerator (4°C). DOC (mg L^{-1}) was measured via UV-persulfate acidification (Non-Purgeable Organic Carbon method) with a Shimadzu Total Organic Carbon Analyzer (TOC-VWP). Water for TDP and NO_3 was filtered, via syringe filtration, through 0.2 µm cellulose nitrate filters and frozen (-20°C) in HDPE Nalgene bottles. The cellulose filters were folded and placed into foil-wrapped 15 mL centrifuge tubes and frozen (-20°C) for later Chl-*a* analysis. TDP (µg L^{-1}) was determined using a potassium persulfate digestion method (Murphy & Riley, 1962; Wetzel & Likens, 1991) and a SEAL Analytical AQ400 autoanalyzer with US EPA-119-A method (United States Environmental Protection Agency, 1993). NO_3 was measured using the AQ2 EPA-127-A method with a SEAL Analytical AQ400 Discrete Analyzer (United States Environmental Protection Agency, 1993). For Chl-*a*, the cellulose filters were extracted in 90% acetone for 18 hours in the dark (in a refrigerator), and extracts were analyzed with a Turner Designs 10-AU fluorometer (Welschmeyer, 1994).

DOM spectral analyses

DOM composition was analyzed through absorbance and fluorescence spectrophotometry. Absorbance was measured using a Varian Cary 50 Bio UV-Visible spectrophotometer (Agilent Technologies, Mississauga, ON, Canada) from 800 to 230 nm at intervals of 5 nm. The specific UV absorbance at 254 nm ($SUVA_{254}$), indicating aromaticity, was determined by dividing the absorbance at 254 nm by DOC concentration (mg L^{-1}) (Weishaar et al., 2003). Excitation emission matrices (EEMs) were generated with a Varian Cary Eclipse Fluorometer (Agilent Technologies) using the methods described in Williams et al. (2013). The absorbance and EEM data were used to calculate four DOM indices: spectral slope ratio (S_R) for molecular size (Helms et al., 2008), fluorescence index (FI) for source (McKnight et al., 2001), freshness index (β/α) for proportion of recently microbially derived carbon (β) to highly decomposed carbon (α) (Wilson & Xenopoulos, 2009; Parlanti et al., 2000), and humification index (HIX) for degree of humification (Zsolnay et al., 1999). Additionally, seven DOM components were extracted using parallel factor analysis (PARAFAC) from previously derived models (Williams et al. 2013, 2016; Williams and Xenopoulos 2023). The seven components are C1 to C3, terrestrial humic-like; C4, soil fulvic-like; C5, microbial humic-like; C6, anthropogenic microbial humic-like; and C7, protein-like.

Calculations for storage of DOM components in ice

To approximate the transient storage and release of DOM to the water column upon ice melt, we used a budget approach to estimate the mass of DOM components in both ice and water. For simplicity, PARAFAC components C1 to C3 were added together to represent terrestrial humic-like DOM (DOM_T), C5 and C6 were added together to represent microbial humic-like DOM

(DOM_{MH}), and C7 represented protein-like DOM (DOM_{P}). Approximate concentrations of each DOM component (DOM_{T}, DOM_{MH}, and DOM_{P}) were estimated by multiplying the site-specific DOC concentration by the site-specific DOM proportion of each DOM component for both ice and water. This was then averaged for each lake. Indeed, this conversion of PARAFAC components to units of carbon is not 1:1 as each component is comprised of different fluorophores that vary in relative amounts of molecular carbon (Williams et al., 2013; Stedmon et al., 2003). Here, we convert DOM to DOC to construct a mass budget of individual PARAFAC components irrespective of spatiotemporal variability. Following our budget approach, the mass of each DOM component in lake ice was determined by multiplying the PARAFAC DOC concentration in ice by the ice volume (product of lake surface area, proportion of ice coverage, and ice thickness) for each of the Great Lakes. For this exercise, three different ice condition scenarios were used to illustrate the potential range in transient DOM storage, the ice conditions at the time of sampling (mid-February 2022, which was an average ice cover year), the historical maximum ice conditions, and the historical minimum ice conditions. Ice data was obtained from the Great Lakes Environmental Research Laboratory (US Department of Commerce, 2023). The mass of each DOM component in lake water was determined by multiplying the DOC concentrations in water (February 2022) by the volume of water in the upper 3 m, to represent the upper water column, for each of the Great Lakes. The mass of each DOM component in ice (for each ice cover scenario) was divided by the sum of the mass in the ice and the mass in water to obtain an estimate of the relative contribution from ice to the upper water column following ice melt. The volume of water in ice (using correction factor = 0.92 to account for the fact that ice shrinks ~8% when it melts) and the upper water column was further used with this mass balance to approximate the spring concentration of each DOM component to be compared relative to winter concentrations.

Statistical Analyses

All statistical analyses and figures for this study were completed in R version 4.2.1 (R Core Team, 2021). We used a paired t-test to isolate site-specific differences in DOC and DOM between ice and water to determine if any variables significantly ($\alpha = 0.05$) differed between ice and water. To explore differences in DOC and DOM in water between sites with ($n = 21$) and without ice ($n = 6$), a principal component analysis (PCA), using the package "ggbiplot" and function "prcomp" in R (R Core Team, 2021; Vu, 2023), was used to reduce the dimensionality of the multivariate DOM data and visually assess patterns among groups of sites. For additional support, a non-parametric Wilcoxon Rank Sum test was used to statistically compare ($\alpha = 0.05$) and evaluate differences in individual DOM composition indices in the water at sites with and without ice.

A Partial Least Squares (PLS) regression was used to relate observed variation in ice DOM to environmental and ice conditions. Individual PLS models were generated for each dependent variable, including 6 PARAFAC components (C1, C2, C3, C5, C6, and C7). Independent variables included DOC_{Water}, DOC_{Ice}, Chl-*a*, NO_3, TDP, ice thickness, snow depth, and corresponding PARAFAC components in water (e.g., $C1_{Water}$ for $C1_{Ice}$, $C2_{Water}$ for $C2_{Ice}$, etc.). All variables (independent and dependent) were cube root transformed and Z-standardized to improve normality. The model with the lowest root mean squared error of prediction and a Q_2 value > 0.0975 indicated predictiveness (Abdi & Williams, 2010). VIP scores were used to rank the influence of each independent variable on the dependent variable where independent variables with VIP < 0.8 were considered weak, 0.8 < VIP < 1 moderate, and VIP > 1 strong. The sign (positive or negative) of regression coefficients was used to signify the direction of association. Biplots were used to visualize predictive PLS models, with independent variables located near the origin (0,0) considered less predictive than variables situated a greater distance from the origin. PLS analysis was performed in R with the "pls" and "plsVarSel" packages (Liland et al., 2023a, 2023b).

Results

DOC/DOM in ice and water

DOC was significantly lower in the ice than the water (Figure 3.1a, Table 3.1). Indicators of low molecular weight (S_R), freshness (β: α), and protein-like DOM (C7) were significantly higher in the ice compared to the water (Figure 3.1b, c, and e; Table 3.1). DOM indicators of humification (HIX) and terrestrial, humic material (C1-C3) were significantly lower in the ice than the water (Figure 3.1d and e, Table 3.1). In contrast, there was no significant difference in $SUVA_{254}$ (aromaticity), C4 (soil fulvic-like), or C5 (microbial humic-like) between ice and water (Table 3.1). For water DOM composition, PCA revealed considerable overlap between sites with and without ice (Figure A1). PCA results were supported by Wilcoxon Rank Sum analyses that showed no significant differences (p-values > 0.05) for any DOM composition indicator between sites with and without ice.

Variability in ice DOM composition across sites

The PARAFAC components $C1_{Ice}$, $C2_{Ice}$, $C3_{Ice}$, $C5_{Ice}$, $C6_{Ice}$, and $C7_{Ice}$ varied considerably among sites, with coefficient of variations of 0.58, 0.51, 0.78, 0.6, 2.12, and 0.4, respectively. PLS regression models were significant for $C2_{Ice}$ ($Q^2 = 0.0986$, $R^2X = 75.46$, $R^2Y = 64.24$, components = 3) and $C7_{Ice}$ ($Q^2 = 0.1695$, $R^2X = 75.32$, $R^2Y = 66.22$, components = 3) but not for $C1_{Ice}$, $C3_{Ice}$, $C5_{Ice}$, and $C6_{Ice}$ ($Q^2 < 0.0975$). $C2_{Ice}$ was strongly associated with NO_3 (+), DOC_{Ice} (+) and ice thickness (-), and moderately with $C2_{Water}$ (+), TDP (+) and snow depth (-). $C2_{Ice}$ was only weakly associated with DOC_{Water} and Chl-*a*. $C7_{Ice}$ was strongly associated with $C7_{Water}$ (+), ice thickness (+), NO_3 (-) and DOC_{Ice} (-), and moderately with Chl-*a* (+), TDP (-), and DOC_{Water} (+). $C7_{Ice}$ was only weakly associated with snow depth. The PLS biplots for $C2_{Ice}$ and $C7_{Ice}$ are shown in Figure 3.2.

Ice as a repository of DOM

The relative contribution of DOM to the upper water column in kilograms (kg) after ice melt, as approximated through our mass budget approach, varied with DOM component. Upon ice melt, DOM_P (protein-like DOM) from the ice could comprise between 1.94% to 18.37% of DOM_P in the upper water column of the Great Lakes for a year of average ice conditions and up to 25.82% for a year of maximum ice conditions (Table 3.2). DOM_{MH} (microbial humic-like), which had lower proportions in ice, would comprise between 0.39% to 5.07% of the DOM in the water after ice melt during a year of average ice conditions and up to 11.59% for a year of maximum ice conditions (Table 3.2). Additionally, DOM_T (terrestrial-like), which had the lowest proportions in ice could comprise between 0.09% to 1.18% in the water after ice melt during a year of average ice conditions and up to 5.32% during a year of maximal ice conditions (Table 3.2). Hence, based on our calculations, ice stores substantially more DOM under higher ice cover scenarios, especially protein-like DOM, that can be released to the upper water column upon ice melt. However, despite ice largely being a source of DOM to the upper water column, ice melt was found to dilute DOM concentrations in some cases (spring concentrations, Table 3.2).

Discussion

We found that DOC was generally lower in ice compared to water, and that DOM associated with lake ice was mainly comprised of protein-like compounds whereas DOM in underlying water was mostly humic-like compounds. These results are consistent with studies on small humic lakes (e.g., Zhou et al. 2023; Song et al., 2019; Belzile et al. 2002). In the Great Lakes, we observed considerable ice and water DOM composition variability. Variation in ice DOM composition was linked to some explanatory variables such as ice thickness, nutrient levels, and DOC, but much variation remained unexplained. DOM in lake ice has been suggested to be an important reservoir of bioavailable carbon to planktonic microbes after ice melt (Zhou et al., 2023; Imbeau et al., 2021). Here, we approximated, for the first time, DOM storage in ice and showed that it can contribute to lake DOC upon spring melt, especially protein-like DOM components (up to 25.82% in kg), which would be readily available to microbes. Release of lake ice DOC upon ice melt may only represent a minor increase in water DOC concentrations because of dilution, however the absolute contributions (kg) of protein-like DOM are pronounced. It remains unclear what specific mixture of protein-like DOM (Murphy et al., 2014) is released from the ice and how cryo-transformation (Chen et al., 2016) alters these molecules' reactive moieties. Yet, it is likely that the contribution of this DOM pool to microbial communities will be lost with the disappearance of lake ice due to climate change.

Variability in ice DOM composition across sites

The proportion of $C2_{Ice}$, indicative of terrestrial humic-like DOM, varied across sites and this was primarily associated with DOC_{Ice}, ice thickness, and nutrients. A positive association of $C2_{Ice}$ with DOC_{Ice} indicates that terrestrial-like DOM is higher when more DOC is present in the ice (e.g., Inamdar et al., 2012; Yamashita et al., 2010). A positive association with water nutrients could be simply because nutrients are often transported with terrestrial DOM in river plumes (Marcarelli et al., 2019) and many of our sampling sites were located near river mouths due to accessibility constraints in the winter. Ice thickness was inversely related to $C2_{Ice}$, which indicates that less humic-like DOM is retained the thicker ice becomes (Zhou et al. 2023).

The positive association of $C7_{Ice}$ with $C7_{Water}$ can be explained by the selective incorporation of protein-like compounds into ice as it forms, as mentioned previously (Zhou et al. 2023). A negative association between $C7_{Ice}$ and nutrients may be because, as mentioned previously, nutrients are often associated with terrestrial-like DOM or DOC in river plumes (Marcarelli et al., 2019) and DOC is often inversely related to protein-like DOM in ice (Imbeau et al. 2021), which we also found to be the case in the ice. The positive association of $C7_{Ice}$ with ice thickness reflects how protein-like DOM in ice can become more concentrated the thicker and older ice becomes (Zhou et al. 2023; Imbeau et al. 2021). There was a moderate association between Chl-*a* in the water and $C7_{Ice}$. It is possible that Chl-*a* in the ice, which we did not measure, may have been higher due to potential incorporation of algal cells within ice or filamentous diatoms on the underside, which can exude proteinaceous compounds (Imbeau et al. 2021; D'souza et al., 2013).

Of the variables selected for PLS regression (i.e., nutrients, ice thickness, snow depth, Chl-*a*, DOC_{Ice}, and DOC_{water}), none explained variation in the terrestrial humic-like C1 and C3, microbial humic-like C5, and urban microbially humic-like C6 in the ice. Other variables not accounted for may have been driving their variation across sites, such as quality or type of ice (i.e., black vs white ice). These properties may influence the amount of light that is able to pass through the ice that may subsequently phototransform humic-like DOM in the ice as well as in the water beneath the ice (Kurek et al., 2022; Belzile et al., 2002). Future studies should thoroughly note the type of ice and formation timeline at each site, as well as light penetration.

Contribution of DOM from ice to water

We found that, during extensive ice cover, substantial protein-like and moderate microbial-like DOM can be stored and released from the ice to the upper water column during ice melt, but a rather small amount of terrestrial-like DOM can be stored and released. This result highlights the role lake ice has as a repository of and potential source of carbon to microbes in the upper water column following ice melt (Imbeau et al. 2021). However, ice DOC concentrations in the Great Lakes varied substantially and were as low as 0.33 mgL^{-1}. Relative to the DOC in the upper water column and dilution from the water content of melting ice and snow, lake ice DOC would not be a substantial source in these low DOC areas. However, despite low concentrations, it is possible that the protein-like DOM released from ice may be preferentially respired by microbes compared to protein-like DOM already in the water from prior to ice melt. Additionally, higher DOC lakes or lakes with higher trophic status where ice entrains greater quantities and/or proportions of protein-like DOM may have a greater influence on spring conditions.

Conclusions

We found that the DOM fingerprint of lake ice was dominated by protein-like compounds and varied with limnological conditions. The protein-like DOM present in lake ice has the potential to be an important source of bioavailable carbon to microbes in the upper water column following ice melt but is highly context dependent when accounting for the dilution factor of water from melted ice and snow and the relative amount of protein-like DOM already in the water. Future studies should examine microbial production before and after ice melt and see if it is related to pulses of DOM from melting ice. This knowledge gap also raises questions about the potential climate feedback implications associated with the interaction between lake ice, DOM, and microbial activity, which should be explored.

Table 3.1. Mean difference in ice minus water for dissolved organic carbon and dissolved organic matter composition indices and their associated p-values and t-values with 17 degrees of freedom.

	Description	Mean difference	*p*-value	t-value
DOC	Dissolved organic carbon (mg L^{-1})	(-) 2.98±2.75	<0.001	4.61
$SUVA_{254}$	Specific UV absorbance at 254 nm (L $mg^{-1}m^{-1}$); higher values indicate greater aromaticity	(-) 0.43±3.01	0.548	0.61
HIX	Humification index; higher values indicate greater degree of humification (unitless)	(-) 4.98±2.64	<0.001	-8.00
β:α	Freshness index; proportion of recently microbially derived carbon (β) to highly decomposed carbon (α) (unitless)	(+) 0.12±0.18	0.013	2.75
S_R	Spectral slope; inversely correlated with molecular weight; higher values suggest photobleaching (unitless)	(+) 0.64±0.68	<0.001	4.04
C1	Ubiquitous humic-like (%)	(-) 7.87±5.11	<0.001	-6.53
C2	Terrestrial humic-like (%)	(-) 11.56±11.21	<0.001	-4.37
C3	Terrestrial humic-like (%)	(-) 10.09±7.58	<0.001	-5.65
C4	Soil fulvic-like (%)	(-) 1.7±3.60	0.061	-2.01
C5	Microbial humic-like (%)	(-) 1.30±4.24	0.209	-5.07
C6	Urban microbial-like (%)	(-) 6.2±5.18	<0.001	-1.31
C7	Protein-like (tryptophan-like) (%)	(+) 38.72±19.22	<0.001	-8.54

Note: (-) indicates a negative mean difference (lower in ice compared to water samples) and (+) a positive mean difference (higher in ice compared to water samples).

Table 3.2. Percentage of ice cover and corresponding relative contribution (%Cont) of protein-like, terrestrial-like, and microbial humic-like DOM to the upper water column after ice melt corresponding to the year of highest maximum (max) ice cover, February 14th, 2022, and year of lowest max for all 5 of the Great Lakes. Also included are the winter concentration (Winter mgL^{-1}) and spring concentration (Spring mgL^{-1}) of each component in the water.

		DOM_P			DOM_{MH}			DOM_T		
	%Ice	%Cont	Winter mgL^{-1}	Spring mgL^{-1}	%Cont	Winter mgL^{-1}	Spring mgL^{-1}	%Cont	Winter mgL^{-1}	Spring mgL-1
Superior										
Highest max	100.00	24.43	0.58	0.64	11.59	0.40	0.38	5.32	1.39	1.23
Feb 2022	45.68	6.40	0.58	0.59	2.70	0.40	0.40	1.18	1.39	1.35
Lowest max	8.50	0.99	0.58	0.58	0.40	0.40	0.40	0.17	1.39	1.38
Michigan										
Highest max	93.10	7.86	0.58	0.57	3.63	0.68	0.63	0.23	3.63	3.26
Feb 2022	37.50	3.20	0.58	0.57	1.44	0.68	0.66	0.09	3.63	3.48
Lowest max	12.40	0.96	0.58	0.58	0.43	0.68	0.67	0.03	3.63	3.58
Huron										
Highest max	98.20	25.82	0.54	0.60	9.17	0.60	0.54	1.99	3.05	2.55
Feb 2022	60.60	8.87	0.54	0.56	2.75	0.60	0.58	0.57	3.05	2.89
Lowest max	22.80	3.10	0.54	0.55	0.92	0.60	0.59	0.19	3.05	2.99
Erie										
Highest max	100.00	24.10	0.33	0.35	7.02	0.61	0.54	2.33	2.44	2.04
Feb 2022	82.30	18.37	0.33	0.34	5.07	0.61	0.56	1.66	2.44	2.14
Lowest max	5.40	0.40	0.33	0.33	0.09	0.61	0.61	0.03	2.44	2.44
Ontario										
Highest max	86.20	6.46	0.61	0.61	1.33	0.66	0.63	0.77	4.25	3.99
Feb 2022	22.90	1.94	0.61	0.61	0.39	0.66	0.65	0.22	4.25	4.17
Lowest max	1.90	0.04	0.61	0.61	0.01	0.66	0.66	0.01	4.25	4.24

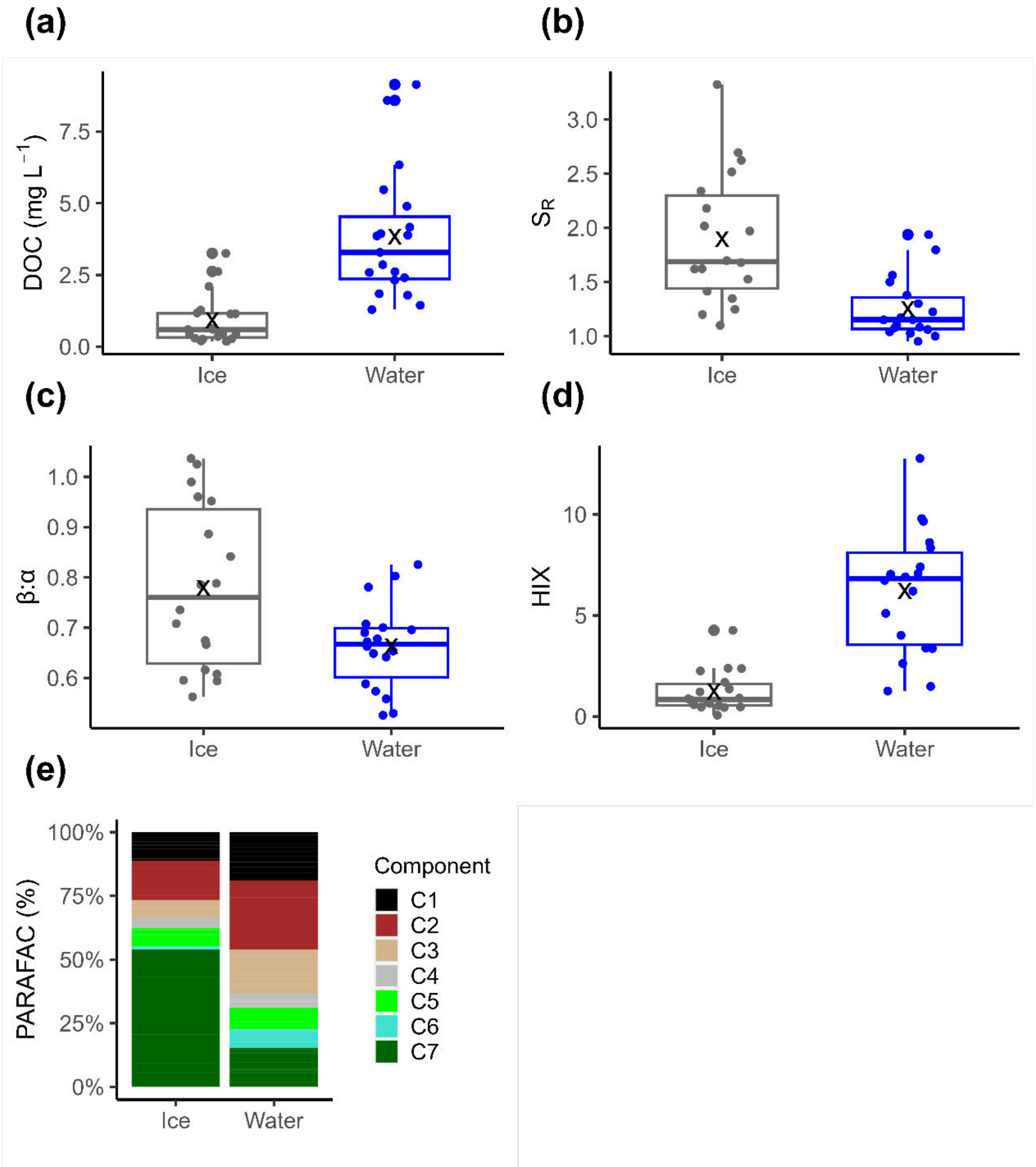

Figure 3.1. Box plots for (a) DOC (mg L^{-1}), (b) spectral slope ratio (S_R), (c) freshness index (β:α), (d) humification index (HIX), and (e) PARAFAC percentages for ice and water. n=18

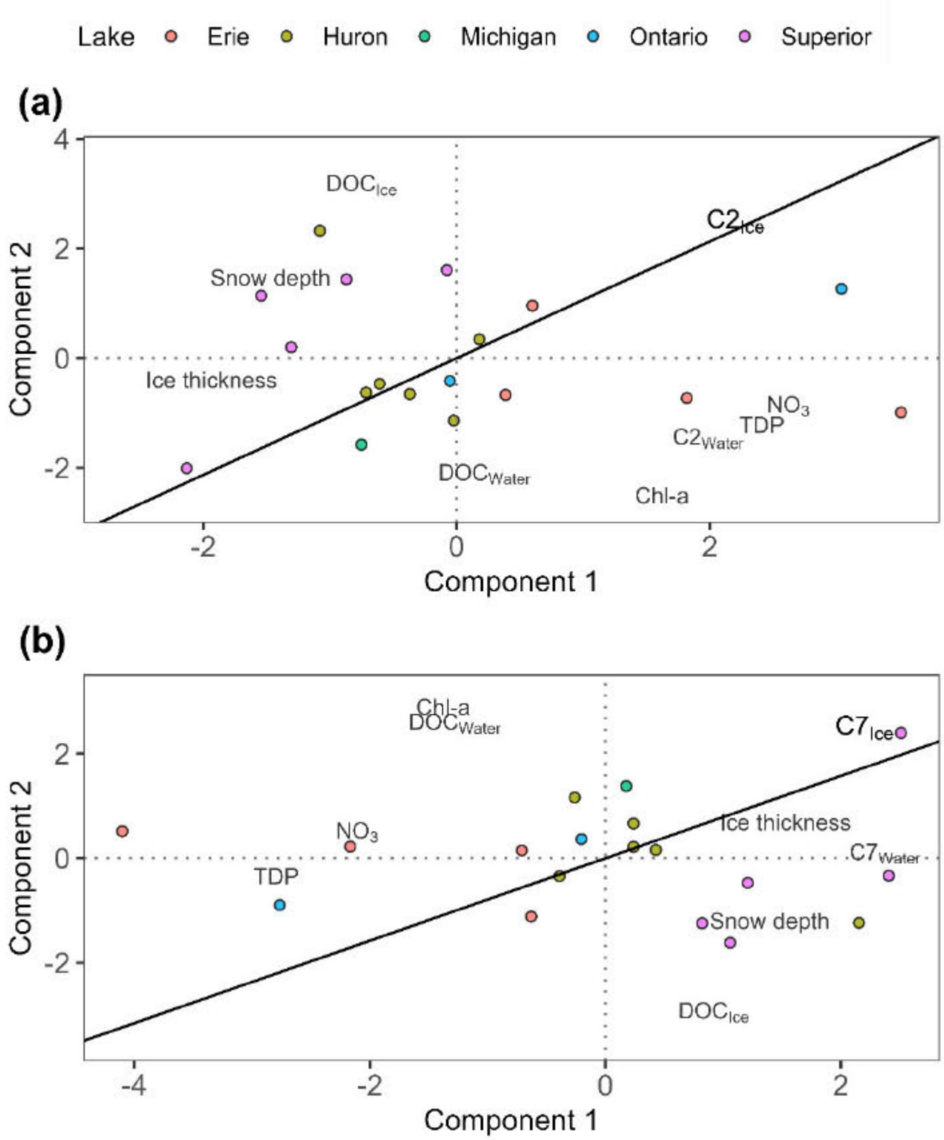

Figure 3.2. PLS biplots for (a) $C2_{Ice}$ (terrestrial humic-like) and (b) $C7_{Ice}$ (protein-like) display the predictor variables in relation to the response variable as well as scores (observations) color-coded for each lake.

Chapter 4: General Conclusion

The first part of this thesis evaluated changes in dissolved organic matter in 10 boreal lakes of varied lake and catchment characteristics from early to late June 2022, which followed an unusually wet and cold spring. We observed subtle changes in DOM composition in the epilimnion from the first to the second sampling, with DOM generally decreasing in molecular weight, likely due to photobleaching or autochthonous production. Changes were less consistent for the hypolimnion. In the epilimnion, some of the variability in DOM change was related to lake area and change in stratification strength. While in the hypolimnion, some variability was related to the proportion of lake perimeter with wetlands, change in stratification strength, water residence time, and mean slope of catchment. The correlations observed with lake and watershed characteristics and changes in DOM composition illustrate that lake and watershed factors can contribute to seasonal DOM dynamics in lakes. However, the rather nuanced changes of DOM over our study period and lack of extensive relationships with lake and watershed variables may be due to the short duration of our sampling (only 1 month), small sample size, or increased terrestrial import associated with a wetter-than-normal spring.

The second part of this thesis assessed DOM in lake ice in the Great Lakes in February 2022. It was found that ice tended to have lower DOC than the underlying water and that ice contained more protein-like DOM than the water, which contained mostly terrestrial-like DOM. We also observed considerable variability in the type of DOM in ice across sites. This variability was primarily related to ice thickness, water nutrients, and DOC. In addition, we underscored the role of lake ice as a temporary storage unit of protein-like DOM that may be an important source of carbon for planktonic microbes following ice melt. However, we found that this contribution is relatively minimal in the Great Lakes due to dilution of melted ice water and concentrations of DOM already in the water. Nonetheless, it is possible that microbes may preferentially respire this DOM released from ice, and loss of ice with climate change may have implications for spring microbial production in the upper water column.

This thesis underscores the significance of incorporating spatial and limnological factors into the analysis of DOM variability in lakes as well as lake ice, applicable not only to small lakes but also to expansive systems such as the Great Lakes. This type of research is important as threats to lakes, such as loss of ice and brownification, may vary depending on spatial and limnological conditions.

References

Abdi, H., & Williams, L. J. (2010). Principal component analysis. *WIREs Computational Statistics*, *2*(4), 433–459. https://doi.org/10.1002/wics.101

Austin, J. A., & Colman, S. M. (2007). Lake Superior summer water temperatures are increasing more rapidly than regional air temperatures: A positive ice-albedo feedback. *Geophysical Research Letters*, *34*(6). https://doi.org/10.1029/2006GL029021

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 and Oceanography*, *47*(5), 1283–1293. https://doi.org/10.4319/lo.2002.47.5.1283

Berg, S., Peterson, B., Mcmahon, K., & Remucal, C. (2022). Spatial and Temporal Variability of Dissolved Organic Matter Molecular Composition in a Stratified Eutrophic Lake. *Journal of Geophysical Research: Biogeosciences*, *127*. https://doi.org/10.1029/2021JG006550

Bondarenko, N. A., Belykh, O. I., Golobokova, L. P., Artemyeva, O. V., Logacheva, N. F., Tikhonova, I. V., Lipko, I. A., Kostornova, T. Ya., Parfenova, V. V., Khodzher, T. V., Ahn, T.-S., & Zo, Y.-G. (2012). Stratified distribution of nutrients and extremophile biota within freshwater ice covering the surface of Lake Baikal. *The Journal of Microbiology*, *50*(1), 8–16. https://doi.org/10.1007/s12275-012-1251-1

Brunskill, G. J., & Schindler, D. W. (1971). Geography and Bathymetry of Selected Lake Basins, Experimental Lakes Area, Northwestern Ontario. *Journal of the Fisheries Research Board of Canada*, *28*(2), 139–155. https://doi.org/10.1139/f71-028

Burd, K., Tank, S. E., Dion, N., Quinton, W. L., Spence, C., Tanentzap, A. J., & Olefeldt, D. (2018). Seasonal shifts in export of DOC and nutrients from burned and unburned peatland-rich catchments, Northwest Territories, Canada. *Hydrology and Earth System Sciences*, *22*(8), 4455–4472. https://doi.org/10.5194/hess-22-4455-2018

Cavaliere, E., Fournier, I. B., Hazuková, V., Rue, G. P., Sadro, S., Berger, S. A., Cotner, J. B., Dugan, H. A., Hampton, S. E., Lottig, N. R., McMeans, B. C., Ozersky, T., Powers, S. M., Rautio, M., & O'Reilly, C. M. (2021). The Lake Ice Continuum Concept: Influence of Winter Conditions on Energy and Ecosystem Dynamics. *Journal of Geophysical Research: Biogeosciences*, *126*(11), e2020JG006165. https://doi.org/10.1029/2020JG006165

Chen, J., Xue, S., Lin, Y., Wang, C., Wang, Q., & Han, Q. (2016). Effect of freezing–thawing on dissolved organic matter in water. *Desalination and Water Treatment*, *57*(37), 17230–17240. https://doi.org/10.1080/19443994.2015.1085913

Chow, M. F., Lai, C.-C., Kuo, H.-Y., Lin, C.-H., Chen, T.-Y., & Shiah, F.-K. (2017). Long Term Trends and Dynamics of Dissolved Organic Carbon (DOC) in a Subtropical Reservoir Basin. *Water*, *9*(7), Article 7. https://doi.org/10.3390/w9070545

Couture, S., Houle, D., & Gagnon, C. (2012). Increases of dissolved organic carbon in temperate and boreal lakes in Quebec, Canada. *Environmental Science and Pollution Research*, *19*(2), 361–371. https://doi.org/10.1007/s11356-011-0565-6

Curtis, P. J., & Schindler, D. W. (1997). Hydrologic control of dissolved organic matter in low-order Precambrian Shield lakes. *Biogeochemistry*, *36*(1), 125–138. https://doi.org/10.1023/A:1005787913638

D'Andrilli, J., Junker, J. R., Smith, H. J., Scholl, E. A., & Foreman, C. M. (2019). DOM composition alters ecosystem function during microbial processing of isolated sources. *Biogeochemistry*, *142*(2), 281–298. https://doi.org/10.1007/s10533-018-00534-5

Doane, T. A., & Horwáth, W. R. (2010). Eliminating interference from iron(III) for ultraviolet absorbance measurements of dissolved organic matter. *Chemosphere*, *78*(11), 1409–1415. https://doi.org/10.1016/j.chemosphere.2009.12.062

D'souza, N. A., Kawarasaki, Y., Gantz, J. D., Lee, R. E., Beall, B. F. N., Shtarkman, Y. M., Koçer, Z. A., Rogers, S. O., Wildschutte, H., Bullerjahn, G. S., & McKay, R. M. L. (2013). Diatom assemblages promote ice formation in large lakes. *The ISME Journal*, *7*(8), Article 8. https://doi.org/10.1038/ismej.2013.49

Dadi, T., Harir, M., Hertkorn, N., Koschorreck, M., Schmitt-Kopplin, P., & Herzsprung, P. (2017). Redox Conditions Affect Dissolved Organic Carbon Quality in Stratified Freshwaters. *Environmental Science & Technology*, *51*(23), 13705–13713. https://doi.org/10.1021/acs.est.7b04194

de Wit, H. A. de, Stoddard, J. L., Monteith, D. T., Sample, J. E., Austnes, K., Couture, S., Fölster, J., Higgins, S. N., Houle, D., Hruška, J., Krám, P., Kopáček, J., Paterson, A. M., Valinia, S., Dam, H. V., Vuorenmaa, J., & Evans, C. D. (2021). Cleaner air reveals growing influence of climate on dissolved organic carbon trends in northern headwaters. *Environmental Research Letters*, *16*(10), 104009. https://doi.org/10.1088/1748-9326/ac2526

Desloges, J. R. (2000). *Physical Geography of Ontario*. https://www.academia.edu/21397461/Physical_Geography_of_Ontario

Esri Inc. (2022). *ArcGIS Pro* (Version 3.0). Esri Inc. https://www.esri.com/en-us/arcgis/products/arcgis-pro/overview

Frost, P. C., Larson, J. H., Johnston, C. A., Young, K. C., Maurice, P. A., Lamberti, G. A., & Bridgham, S. D. (2006). Landscape predictors of stream dissolved organic matter concentration and physicochemistry in a Lake Superior river watershed. *Aquatic Sciences*, *68*(1), 40–51. https://doi.org/10.1007/s00027-005-0802-5

Fujii, M., Imaoka, A., Yoshimura, C., & Waite, T. D. (2014). Effects of Molecular Composition of Natural Organic Matter on Ferric Iron Complexation at Circumneutral pH. *Environmental Science & Technology*, *48*(8), 4414–4424. https://doi.org/10.1021/es405496b

Girdner, S., Mack, J., & Buktenica, M. (2020). Impact of nutrients on photoacclimation of phytoplankton in an oligotrophic lake measured with long-term and high-frequency data: Implications for chlorophyll as an estimate of phytoplankton biomass. *Hydrobiologia*, *847*(8), 1817–1830. https://doi.org/10.1007/s10750-020-04213-1

Gonsior, M., Schmitt-Kopplin, P., & Bastviken, D. (2013). Depth-dependent molecular composition and photo-reactivity of dissolved organic matter in a boreal lake under winter and summer conditions. *Biogeosciences*, *10*(11), 6945–6956. https://doi.org/10.5194/bg-10-6945-2013

Government of Ontario. (2023). Ontario Watershed Information Tool (OWIT). https://www.ontario.ca/page/ontario-watershed-information-tool-owit

Graham, A. M., Aiken, G. R., & Gilmour, C. C. (2013). Effect of Dissolved Organic Matter Source and Character on Microbial Hg Methylation in Hg–S–DOM Solutions. *Environmental Science & Technology*, *47*(11), 5746–5754. https://doi.org/10.1021/es400414a

Gunn, J., Steedman, R. J., & Ryder, R. (2003). *Boreal Shield Watersheds: Lake Trout Ecosystems in a Changing Environment*. CRC Press. https://doi.org/10.1201/9780203495087

Guo, S., Stevens, C. A., Vance, T. D. R., Olijve, L. L. C., Graham, L. A., Campbell, R. L., Yazdi, S. R., Escobedo, C., Bar-Dolev, M., Yashunsky, V., Braslavsky, I., Langelaan, D. N., Smith, S. P., Allingham, J. S., Voets, I. K., & Davies, P. L. (2017). Structure of a 1.5-MDa adhesin that binds its Antarctic bacterium to diatoms and ice. *Science Advances*, *3*(8), e1701440. https://doi.org/10.1126/sciadv.1701440

Hall, B. D., Hesslein, R. H., Emmerton, C. A., Higgins, S. N., Ramlal, P., & Paterson, M. J. (2019). Multidecadal carbon sequestration in a headwater boreal lake. *Limnology and Oceanography*, *64*(S1), S150–S165. https://doi.org/10.1002/lno.11060

Hampton, S. E., Galloway, A. W. E., Powers, S. M., Ozersky, T., Woo, K. H., Batt, R. D., Labou, S. G., O'Reilly, C. M., Sharma, S., Lottig, N. R., Stanley, E. H., North, R. L., Stockwell, J. D., Adrian, R., Weyhenmeyer, G. A., Arvola, L., Baulch, H. M., Bertani, I., Bowman Jr., L. L., … Xenopoulos, M. A. (2017). Ecology under lake ice. *Ecology Letters*, *20*(1), 98–111. https://doi.org/10.1111/ele.12699

Hedges, J. I. (1992). Global biogeochemical cycles: Progress and problems. *Marine Chemistry*, *39*(1), 67–93. https://doi.org/10.1016/0304-4203(92)90096-S

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 and Oceanography*, *53*(3), 955–969. https://doi.org/10.4319/lo.2008.53.3.0955

Higgins, S. N., Desjardins, C. M., Drouin, H., Hrenchuk, L. E., & van der Sanden, J. J. (2021). The Role of Climate and Lake Size in Regulating the Ice Phenology of Boreal Lakes. *Journal of Geophysical Research: Biogeosciences*, *126*(3), e2020JG005898. https://doi.org/10.1029/2020JG005898

Hood, E., Fellman, J., Spencer, R. G. M., Hernes, P. J., Edwards, R., D'Amore, D., & Scott, D. (2009). Glaciers as a source of ancient and labile organic matter to the marine environment. *Nature*, *462*(7276), Article 7276. https://doi.org/10.1038/nature08580

Horikoshi, M., Tang, Y., Dickey, A., Grenié, M., Thompson, R., Selzer, L., Strbenac, D., Voronin, K., & Pulatov, D. (2023). *ggfortify: Data Visualization Tools for Statistical Analysis Results* (0.4.16) [R package]. https://cran.r-project.org/web/packages/ggfortify/index.html

Idso, S. B. (1973). On the concept of lake stability1. *Limnology and Oceanography*, *18*(4), 681–683. https://doi.org/10.4319/lo.1973.18.4.0681

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

Imrit, M. A., & Sharma, S. (2021). Climate Change is Contributing to Faster Rates of Lake Ice Loss in Lakes Around the Northern Hemisphere. *Journal of Geophysical Research: Biogeosciences*, *126*(7), e2020JG006134. https://doi.org/10.1029/2020JG006134

Inamdar, S., Finger, N., Singh, S., Mitchell, M., Levia, D., Bais, H., Scott, D., & McHale, P. (2012). Dissolved organic matter (DOM) concentration and quality in a forested mid-Atlantic watershed, USA. *Biogeochemistry*, *108*(1/3), 55–76.

Jansen, J., MacIntyre, S., Barrett, D. C., Chin, Y.-P., Cortés, A., Forrest, A. L., Hrycik, A. R., Martin, R., McMeans, B. C., Rautio, M., & Schwefel, R. (2021). Winter Limnology: How do Hydrodynamics and Biogeochemistry Shape Ecosystems Under Ice? *Journal of Geophysical Research: Biogeosciences*, *126*(6), e2020JG006237. https://doi.org/10.1029/2020JG006237

Johnston, S. E., Striegl, R. G., Bogard, M. J., Dornblaser, M. M., Butman, D. E., Kellerman, A. M., Wickland, K. P., Podgorski, D. C., & Spencer, R. G. M. (2020). Hydrologic connectivity determines dissolved organic matter biogeochemistry in northern high-latitude lakes. *Limnology and 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), Article 1. https://doi.org/10.1038/ncomms4804

Kortelainen, P., Mattsson, T., Finér, L., Ahtiainen, M., Saukkonen, S., & Sallantaus, T. (2006). Controls on the export of C, N, P and Fe from undisturbed boreal catchments, Finland. *Aquatic Sciences*, *68*(4), 453–468. https://doi.org/10.1007/s00027-006-0833-6

Kritzberg, E. S., Cole, J. J., Pace, M. L., Granéli, W., & Bade, D. L. (2004). Autochthonous versus allochthonous carbon sources of bacteria: Results from whole-lake 13C addition experiments. *Limnology and Oceanography*, *49*(2), 588–596. https://doi.org/10.4319/lo.2004.49.2.0588

Kurek, M. R., Frey, K. E., Guillemette, F., Podgorski, D. C., Townsend-Small, A., Arp, C. D., Kellerman, A. M., & Spencer, R. G. M. (2022). Trapped Under Ice: Spatial and Seasonal Dynamics of Dissolved Organic Matter Composition in Tundra Lakes. *Journal of Geophysical Research: Biogeosciences*, *127*(4), e2021JG006578. https://doi.org/10.1029/2021JG006578

Lawson, E. C., Wadham, J. L., Tranter, M., Stibal, M., Lis, G. P., Butler, C. E. H., Laybourn-Parry, J., Nienow, P., Chandler, D., & Dewsbury, P. (2014). Greenland Ice Sheet exports labile organic carbon to the Arctic oceans. *Biogeosciences*, *11*(14), 4015–4028. https://doi.org/10.5194/bg-11-4015-2014

Liland, K. H., Mehmood, T., & Sæbø, S. (2023a). *plsVarSel: Variable Selection in Partial Least Squares* (0.9.10) [Computer software]. https://cran.r-project.org/web/packages/plsVarSel/index.html

Liland, K. H., Mevik, B.-H., Wehrens, R., & Hiemstra, P. (2023b). *pls: Partial Least Squares and Principal Component Regression* (2.8-3) [R package]. https://cran.r-project.org/web/packages/pls/index.html

Lobus, N. V., Glushchenko, A. M., Osadchiev, A. A., Maltsev, Y. I., Kapustin, D. A., Konovalova, O. P., Kulikovskiy, M. S., Krylov, I. N., & Drozdova, A. N. (2022). Production of Fluorescent Dissolved Organic Matter by Microalgae Strains from the Ob and Yenisei Gulfs (Siberia). *Plants*, *11*(23), 3361. https://doi.org/10.3390/plants11233361

Mangal, V., Stock, N. L., & Guéguen, C. (2016). Molecular characterization of phytoplankton dissolved organic matter (DOM) and sulfur components using high resolution Orbitrap mass spectrometry. *Analytical and Bioanalytical Chemistry*, *408*(7), 1891–1900. https://doi.org/10.1007/s00216-015-9295-9

Marcarelli, A. M., Coble, A. A., Meingast, K. M., Kane, E. S., Brooks, C. N., Buffam, I., Green, S. A., Huckins, C. J., Toczydlowski, D., & Stottlemyer, R. (2019). Of Small Streams and Great Lakes: Integrating Tributaries to Understand the Ecology and Biogeochemistry of Lake Superior. *JAWRA Journal of the American Water Resources Association*, *55*(2), 442–458. https://doi.org/10.1111/1752-1688.12695

McKay, R. M. L., Prášil, O., Pechar, L., Lawrenz, E., Rozmarynowycz, M. J., & Bullerjahn, G. S. (2015). Freshwater ice as habitat: Partitioning of phytoplankton and bacteria between ice and water in central European reservoirs. *Environmental Microbiology Reports*, *7*(6), 887–898. https://doi.org/10.1111/1758-2229.12322

McKnight, D. M., Boyer, E. W., Westerhoff, P. K., Doran, P. T., Kulbe, T., & Andersen, D. T. (2001). Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography*, *46*(1), 38–48. https://doi.org/10.4319/lo.2001.46.1.0038

Meon, B., & Kirchman, D. L. (2001). Dynamics and molecular composition of dissolved organic material during experimental phytoplankton blooms. *Marine Chemistry*, *75*(3), 185–199. https://doi.org/10.1016/S0304-4203(01)00036-6

Müller, S., Vähätalo, A. V., Stedmon, C. A., Granskog, M. A., Norman, L., Aslam, S. N., Underwood, G. J. C., Dieckmann, G. S., & Thomas, D. N. (2013). Selective incorporation of dissolved organic matter (DOM) during sea ice formation. *Marine Chemistry*, *155*, 148–157. https://doi.org/10.1016/j.marchem.2013.06.008

Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, *27*, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

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

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M. D., Durand, S., … Weedon, J. (2022). *vegan: Community Ecology Package* (2.6-4) [R package]. https://cran.r-project.org/web/packages/vegan/index.html

Olivoto, T. (2023). *metan: Multi Environment Trials Analysis* (1.18.0) [R package]. https://cran.r-project.org/web/packages/metan/index.html

Ontario Ministry of Natural Resources and Forestry. (2022). Ontario Geohub. https://geohub.lio.gov.on.ca/

Oswald, C. J., & Branfireun, B. A. (2014). Antecedent moisture conditions control mercury and dissolved organic carbon concentration dynamics in a boreal headwater catchment. *Water Resources Research*, *50*(8), 6610–6627. https://doi.org/10.1002/2013WR014736

Ozersky, T., Bramburger, A. J., Elgin, A. K., Vanderploeg, H. A., Wang, J., Austin, J. A., Carrick, H. J., Chavarie, L., Depew, D. C., Fisk, A. T., Hampton, S. E., Hinchey, E. K., North, R. L., Wells, M. G., Xenopoulos, M. A., Coleman, M. L., Duhaime, M. B., Fujisaki-

Manome, A., McKay, R. M., … Zastepa, A. (2021). The Changing Face of Winter: Lessons and Questions From the Laurentian Great Lakes. *Journal of Geophysical Research: Biogeosciences*, *126*(6), e2021JG006247. https://doi.org/10.1029/2021JG006247

Pace, M. L., & Cole, J. J. (2002). Synchronous variation of dissolved organic carbon and color in lakes. *Limnology and Oceanography*, *47*(2), 333–342. https://doi.org/10.4319/lo.2002.47.2.0333

Parlanti, E., Wörz, K., Geoffroy, L., & Lamotte, M. (2000). Dissolved organic matter fluorescence spectroscopy as a tool to estimate biological activity in a coastal zone submitted to anthropogenic inputs. *Organic Geochemistry*, *31*(12), 1765–1781. https://doi.org/10.1016/S0146-6380(00)00124-8

Pollard, P. C. (2022). Globally, Freshwater Ecosystems Emit More CO2 Than the Burning of Fossil Fuels. *Frontiers in Environmental Science*, *10*. https://www.frontiersin.org/articles/10.3389/fenvs.2022.904955

Poulin, B. A., Ryan, J. N., & Aiken, G. R. (2014). Effects of Iron on Optical Properties of Dissolved Organic Matter. *Environmental Science & Technology*, *48*(17), 10098–10106. https://doi.org/10.1021/es502670r

Raudina, T. V., Smirnov, S. V., Lushchaeva, I. V., Istigechev, G. I., Kulizhskiy, S. P., Golovatskaya, E. A., Shirokova, L. S., & Pokrovsky, O. S. (2022). Seasonal and Spatial Variations of Dissolved Organic Matter Biodegradation along the Aquatic Continuum in the Southern Taiga Bog Complex, Western Siberia. *Water*, *14*(23), Article 23. https://doi.org/10.3390/w14233969

R Core Team. (2021). *R: A language and environment for statistical computing* (Version 4.1.2) [Software]. R Foundation for Statistical Computing. https://www.R-project.org/

Rasmussen, J. B., Godbout, L., & Schallenberg, M. (1989). The humic content of lake water and its relationship to watershed and lake morphometry. *Limnology and Oceanography*, *34*(7), 1336–1343. https://doi.org/10.4319/lo.1989.34.7.1336

Ritson, J. P., Graham, N. J. D., Templeton, M. R., Clark, J. M., Gough, R., & Freeman, C. (2014). The impact of climate change on the treatability of dissolved organic matter (DOM) in upland water supplies: A UK perspective. *Science of The Total Environment*, *473–474*, 714–730. https://doi.org/10.1016/j.scitotenv.2013.12.095

Sadro, S., Xenopoulos, M. A. (2022, July 11). *A Future Without Ice Cover*. Eos. http://eos.org/editors-vox/a-future-without-ice-cover

Santibáñez, P. A., Michaud, A. B., Vick-Majors, T. J., D'Andrilli, J., Chiuchiolo, 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

Schindler, D. W., Bayley, S. E., Parker, B. R., Beaty, K. G., Cruikshank, D. R., Fee, E. J., Schindler, E. U., & Stainton, M. P. (1996). The effects of climatic warming on the properties of boreal lakes and streams at the Experimental Lakes Area, northwestern Ontario. *Limnology and Oceanography*, *41*(5), 1004–1017. https://doi.org/10.4319/lo.1996.41.5.1004

Seekell, D. A., Lapierre, J.-F., & Karlsson, J. (2015). Trade-offs between light and nutrient availability across gradients of dissolved organic carbon concentration in Swedish lakes: Implications for patterns in primary production. *Canadian Journal of Fisheries and Aquatic Sciences*, *72*(11), 1663–1671. https://doi.org/10.1139/cjfas-2015-0187

Senar, O. E., Creed, I. F., Strandberg, U., & Arts, M. T. (2019). Browning reduces the availability—But not the transfer—Of essential fatty acids in temperate lakes. *Freshwater Biology*, *64*(12), 2107–2119. https://doi.org/10.1111/fwb.13399

Sherbo, B. A. H., Tonin, J., Paterson, M. J., Hann, B. J., Kozak, J., & Higgins, S. N. (2023). The effects of terrestrial dissolved organic matter on phytoplankton biomass and productivity in boreal lakes. *Freshwater Biology*, *68*(12), 2109–2119. https://doi.org/10.1111/fwb.14178

Sola, C. (2022, February 23). *The Winter Grab: First-of-its-kind Great Lakes winter sampling takes place*. Canadian Geographic. https://canadiangeographic.ca/articles/the-winter-grab-first-of-its-kind-great-lakes-winter-sampling-takes-place/

Song, K., Wen, Z., Jacinthe, P.-A., Zhao, Y., & Du, J. (2019). Dissolved carbon and CDOM in lake ice and underlying waters along a salinity gradient in shallow lakes of Northeast China. *Journal of Hydrology*, *571*, 545–558. https://doi.org/10.1016/j.jhydrol.2019.02.012

Stackpoole, S. M., Stets, E. G., Clow, D. W., Burns, D. A., Aiken, G. R., Aulenbach, B. T., Creed, I. F., Hirsch, R. M., Laudon, H., Pellerin, B. A., & Striegl, R. G. (2017). Spatial and temporal patterns of dissolved organic matter quantity and quality in the Mississippi River Basin, 1997–2013. *Hydrological Processes*, *31*(4), 902–915. https://doi.org/10.1002/hyp.11072

Stainton, M. P., Capel, M. J., & Armstrong, F. A. J. (1977). *The Chemical Analysis of Fresh Water* (2nd ed.). Fisheries and Environment Canada, Fisheries and Marine Service. Miscellaneous Special Publication No. 25, 180 p. http://hdl.handle.net/1993/33786

Stedmon, C. A., Markager, S., & Bro, R. (2003). Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. *Marine Chemistry*, *82*(3), 239–254. https://doi.org/10.1016/S0304-4203(03)00072-0

Tanentzap, A., Yan, N., Keller, B., Girard, R., Heneberry, J., Gunn, J., Hamilton, D., & Taylor, P. (2008). Cooling Lakes While the World Warms: Effects of Forest Regrowth and Increased Dissolved Organic Matter on the Thermal Regime of a Temperate, Urban Lake. *Limnology and Oceanography*, *53*, 404–410. https://doi.org/10.2307/40006181

Tedetti, M., & Sempéré, R. (2006). Penetration of Ultraviolet Radiation in the Marine Environment. A Review. *Photochemistry and Photobiology*, *82*(2), 389–397. https://doi.org/10.1562/2005-11-09-IR-733

Tranvik, L. J., Downing, J. A., Cotner, J. B., Loiselle, S. A., Striegl, R. G., Ballatore, T. J., Dillon, P., Finlay, K., Fortino, K., Knoll, L. B., Kortelainen, P. L., Kutser, T., Larsen, Soren., Laurion, I., Leech, D. M., McCallister, S. L., McKnight, D. M., Melack, J. M., Overholt, E., … Weyhenmeyer, G. A. (2009). Lakes and reservoirs as regulators of carbon cycling and climate. *Limnology and Oceanography*, *54*(6part2), 2298–2314. https://doi.org/10.4319/lo.2009.54.6_part_2.2298

United States Environmental Protection Agency, Office of Research and Development. (1993). Environmental Monitoring Systems Laboratory (Cincinnati, Ohio). Methods for the determination of inorganic substances in environmental samples (Vol. 600).

US Department of Commerce. (2023). *NOAA Great Lakes Environmental Research Lab*. Retrieved November 24, 2023, from https://www.glerl.noaa.gov/

van Dorst, R. M., Gårdmark, A., Svanbäck, R., Beier, U., Weyhenmeyer, G. A., & Huss, M. (2019). Warmer and browner waters decrease fish biomass production. *Global Change Biology*, *25*(4), 1395–1408. https://doi.org/10.1111/gcb.14551

Van Rossum, G., & Drake, F. L. (2009). *Python 3 Reference Manual*. Scotts Valley, CA: CreateSpace.

Van Stan, J. T., & Stubbins, A. (2018). Tree-DOM: Dissolved organic matter in throughfall and stemflow. *Limnology and Oceanography Letters*, *3*(3), 199–214. https://doi.org/10.1002/lol2.10059

Vu, V. (2023). *Ggbiplot* [R code]. https://github.com/vqv/ggbiplot (Original work published 2011)

Wang, J., Bai, X., Hu, H., Clites, A., Colton, M., & Lofgren, B. (2012). Temporal and Spatial Variability of Great Lakes Ice Cover, 1973–2010. *Journal of Climate*, *25*(4), 1318–1329. https://doi.org/10.1175/2011JCLI4066.1

Warner, K. A., & Saros, J. E. (2019). Variable responses of dissolved organic carbon to precipitation events in boreal drinking water lakes. *Water Research*, *156*, 315–326. https://doi.org/10.1016/j.watres.2019.03.036

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 and Reactivity of Dissolved Organic Carbon. *Environmental Science & Technology*, *37*(20), 4702–4708. https://doi.org/10.1021/es030360x

Welschmeyer, N. A. (1994). Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. *Limnology and Oceanography*, *39*(8), 1985–1992. https://doi.org/10.4319/lo.1994.39.8.1985

Wetzel, R. G., & Likens, G. E. (1991). Inorganic Nutrients: Nitrogen, Phosphorus, and Other Nutrients. In R. G. Wetzel & G. E. Likens (Eds.), *Limnological Analyses* (pp. 81–105). Springer. https://doi.org/10.1007/978-1-4757-4098-1_7

Wickham, H., Chang, W., Henry, L., Pedersen, T. L., Takahashi, K., Wilke, C., Woo, K., Yutani, H., Dunnington, D., Posit, & PBC. (2023). *ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics* (3.4.4) [R package]. https://cran.r-project.org/web/packages/ggplot2/index.html

Williams, C. J., & Xenopoulos, M. A. (2023). 7-component PARAFAC model, dataset, and matlab code of aquatic dissolved organic matter composition in North American Great Lakes Region (Version 2) [Code]. Figshare. https://doi.org/https://doi.org/10.6084/m9.figshare.24018450.v2

Williams, C. J., Frost, P. C., & Xenopoulos, M. A. (2013). Beyond best management practices: Pelagic biogeochemical dynamics in urban stormwater ponds. *Ecological Applications*, *23*(6), 1384–1395. https://doi.org/10.1890/12-0825.1

Williams, C. J., Frost, P. C., Morales-Williams, A. M., Larson, J. H., Richardson, W. B., Chiandet, A. S., & Xenopoulos, M. A. (2016). Human activities cause distinct dissolved organic matter composition across freshwater ecosystems. *Global Change Biology*, *22*(2), 613–626. https://doi.org/10.1111/gcb.13094

Wilson, H. F., & Xenopoulos, M. A. (2009). Effects of agricultural land use on the composition of fluvial dissolved organic matter. *Nature Geoscience*, *2*(1), Article 1. https://doi.org/10.1038/ngeo391

Winslow, L., Read, J., Woolway, R., Brentrup, J., Leach, T., Zwart, J., Albers, S., & Collinge, D. (2019). *rLakeAnalyzer: Lake Physics Tools* (1.11.4.1) [R package]. https://cran.r-project.org/web/packages/rLakeAnalyzer/index.html

Xenopoulos, M. A., Lodge, D. M., Frentress, J., Kreps, T. A., Bridgham, S. D., Grossman, E., & Jackson, C. J. (2003). Regional comparisons of watershed determinants of dissolved organic carbon in temperate lakes from the Upper Great Lakes region and selected regions globally. *Limnology and Oceanography*, *48*(6), 2321–2334. https://doi.org/10.4319/lo.2003.48.6.2321

Yamashita, Y., Maie, N., Briceño, H., & Jaffé, R. (2010). Optical characterization of dissolved organic matter in tropical rivers of the Guayana Shield, Venezuela. *Journal of Geophysical Research: Biogeosciences*, *115*(G1). https://doi.org/10.1029/2009JG000987

Zhang, J., Hudson, J., Neal, R., Sereda, J., Clair, T., Turner, M., Jeffries, D., Dillon, P., Molot, L., Somers, K., & Hesslein, R. (2010). Long-term patterns of dissolved organic carbon in lakes across eastern Canada: Evidence of a pronounced climate effect. *Limnology and Oceanography*, *55*(1), 30–42. https://doi.org/10.4319/lo.2010.55.1.0030

Zhang, Y., Liu, X., Wang, M., & Qin, B. (2013). Compositional differences of chromophoric dissolved organic matter derived from phytoplankton and macrophytes. *Organic Geochemistry*, *55*, 26–37. https://doi.org/10.1016/j.orggeochem.2012.11.007

Zhang, Z., Meng, J., Chen, Z., Zhou, S., Zhang, T., Chen, Z., Liu, Y., & Cui, J. (2023). Response of dissolved organic matter to thermal stratification and environmental indication: The case of Gangnan Reservoir. *Science of The Total Environment*, *868*, 161615. https://doi.org/10.1016/j.scitotenv.2023.161615

Zhou, Y., Hiller, C., Andersson, S., Jakobsson, E., Zhou, L., Hawkes, J. A., Kothawala, D. N., & Tranvik, L. J. (2023). Selective Exclusion of Aromatic Organic Carbon During Lake Ice Formation. *Geophysical Research Letters*, *50*(4), e2022GL101414. https://doi.org/10.1029/2022GL101414

Zsolnay, A., Baigar, E., Jimenez, M., Steinweg, B., & Saccomandi, F. (1999). Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. *Chemosphere*, *38*(1), 45–50. https://doi.org/10.1016/S0045-6535(98)00166-0

Appendices

Table A1. Measurements for average DOC concentration; proportion of lake perimeter with wetlands (PW); proportion of wetlands in catchment (PWC); total area of wetlands in catchment (TWAC); lake area (LA); catchment area (CA); lake volume (LV); lake volume over catchment area, a proxy for WRT (LV/CA); and mean slope (MS).

Lake	DOC (mgL^{-1})	PW	PWC	TWAC (m^2)	LA (m^2)	CA (m^2)	LV (m^3)	LV/CA	MS (%)
L114	7.49	0.07	0.01	3000	120243	402757	180027	0.45	8.47
L223	5.25	0	0.03	63000	275640	2285360	1916317	0.84	8.55
L224	4.53	0.04	0.05	38000	261581	728418	3066671	4.21	9.18
L227	12.53	0	0.01	3000	54953	293047	259439	0.89	8.28
L239	8.31	0.02	0.04	128000	541374	3345626	6169179	1.84	8.91
L304	9.17	0	0.03	5000	37502	203498	120704	0.59	8.51
L373	5.04	0.02	0.01	4000	273816	403183	3107474	7.71	7.39
L378	8.07	0.06	0.02	27000	251579	1185421	1996479	1.68	9.22
L442	7.53	0	0.11	172000	154377	1631623	1352175	0.83	8.10
L626	5.33	0.01	0.02	81000	261326	740000	1900317	2.57	7.50

Table A2. Pearson correlation coefficients for delta values (first minus second sampling) for DOC, some DOM indices, and various lake and watershed variables; proportion of lake perimeter with wetlands (PW), proportion of catchment area with wetlands (PWC), lake area (LA), change in stratification strength (ΔSS), water residence time (WRT), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP).

	PW	PWC	LA	ΔSS	WRT	MS	DOC	TDN	TDP
Epilimnion									
ΔDOC	0.28	0.35	0.20	-0.62	-0.49	0.22	-0.57	-0.23	-0.26
ΔS_R	0.52	0.24	0.00	-0.34	-0.29	0.53	-0.14	0.00	-0.01
$\Delta SUVA_{254}$	-0.63	-0.17	-0.44	**0.72***	0.51	-0.32	0.58	0.28	0.32
ΔC1	-0.33	-0.19	**-0.94*****	**0.74***	0.55	-0.45	0.30	0.43	0.49
ΔC7	-0.09	0.05	0.57	-0.28	-0.24	0.48	0.27	-0.05	-0.07
ΔPC1	-0.42	-0.32	**0.67***	**-0.75***	-0.56	0.62	-0.19	-0.32	-0.35
Hypolimnion									
ΔDOC	**0.71***	-0.46	0.50	-0.26	-0.17	0.45	0.08	0.39	0.50
ΔS_R	0.12	-0.44	0.43	**-0.74***	**-0.71***	-0.07	0.19	0.37	0.48
$\Delta SUVA_{254}$	-0.01	0.41	-0.33	0.62	0.59	0.47	0.20	0.05	-0.02
ΔC1	-0.21	-0.17	0.16	-0.48	-0.47	**-0.69***	-0.31	-0.27	-0.26
ΔC7	0.27	-0.07	0.52	-0.62	-0.58	0.53	0.59	0.37	0.41
ΔPC1	0.46	-0.33	0.65	-0.25	-0.15	0.40	0.00	0.35	0.45

Note: *, **, and *** indicate if a correlation is significant at $p<0.05$, 0.01, and 0.001, respectively.

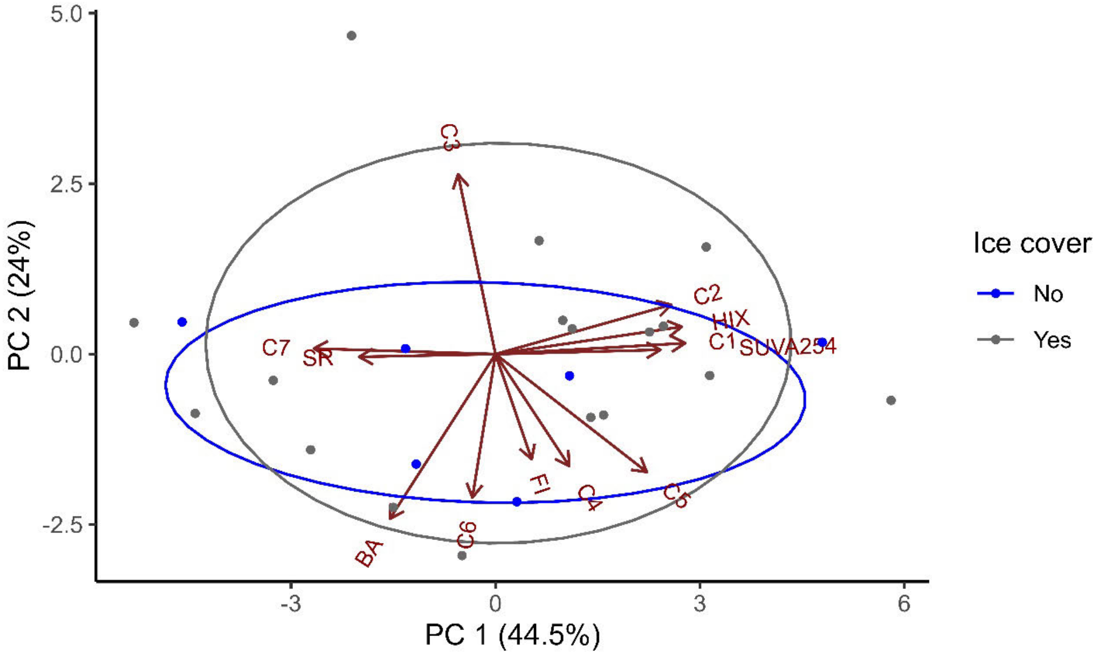

Figure A1. PCA of various DOM composition indices for sites with ice cover (n=21) vs sites without ice cover (n=6).