

UNIVERSIDADE DE LISBOA
INSTITUTO SUPERIOR TÉCNICO

**Seasonal Variability of Thermokarst Lake Biogeochemical
Processes along a Permafrost Gradient**

Diogo Tavares Antão Folhas Ferreira

Supervisor: Doctor João Alfredo Vieira Canário

Co-Supervisors: Doctor Raoul-Marie Couture

Doctor Isabelle Laurion

Thesis approved in public session to obtain the PhD Degree in

Chemistry

Juri final classification: **Pass with Distinction and Honour**

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“...the only simplicity to be trusted is the simplicity to be found on the far side of complexity.” — **Alfred North Whitehead**

"The operation of nature consists of splitting the united or uniting the divided;
this is the eternal movement of systole and diastole of the heartbeat."

– **Goethe**

Resumo

A degradação do pergelissolo nas latitudes setentrionais está a intensificar a mobilização de matéria orgânica, nutrientes e contaminantes para lagos de termocarso, potenciando entre outros, a emissão de gases com efeito de estufa.

Apesar de existirem estudos relacionados com a dinâmica deste lagos, uma abordagem holística que relacione processos biogeoquímicos, regimes sazonais e o tipo de pergelissolo nestes sistemas como um todo, é ainda inexistente.

Consequentemente, este trabalho investiga as dinâmicas sazonais da matéria orgânica natural (NOM) e das espécies aceitadoras de eletrões (TEAs) em lagos de termocarso localizados em zonas de pergelissolo distintas: esporádico, descontínuo e contínuo.

A abordagem conjunta da coluna de água e dos sedimentos permitiu uma perspectiva holística da biogeoquímica lacustre, explorando as interações bentónicas e pelágicas e identificando os mecanismos subjacentes.

A integração de múltiplas perspetivas — caracterização da matéria orgânica dissolvida (DOM): espectroscopias de UV-Vis, fluorescência e FTIR; quantificação de TEAs; a análise de assinaturas isotópicas ($\delta^{13}\text{C}$) e rácios C/N — possibilitou uma melhor compreensão destes lagos como sistemas biogeoquímicos integrados.

Os resultados demonstraram que as alterações na biogeoquímica de lagos termocársicos são moldadas pela interação entre o contexto ecológico e os processos internos. Ciclos sazonais de congelação e fusão controlam as dinâmicas redox e mecanismos de degradação de DOM.

Em permafrost esporádico, a conectividade hidrológica favorece estratificação e o aporte de NOM terrestre, produzindo sistemas ácidos e ricos em DOM cromófora (CDOM). Lagos em pergelissolo descontínuo apresentam características transicionais, enquanto os em pergelissolo contínuo se distinguem por baixos níveis de CDOM e intensa atividade microbiana bentónica. Adicionalmente, em lagos com baixo CDOM a atividade bentónica considerável, a assimilação autotrófica de carbono derivado de metano nos sedimentos superficiais sugere um mecanismo de retenção.

Enquadrar mecanisticamente a biogeoquímica de lagos termocársicos é essencial, dada a dificuldade em extrapolar tendências em sistemas com elevada diversidade limnológica.

Palavras-chave: lago termocarso; pergelisolo; matéria orgânica natural; espécies aceitadoras de electrões; biogeoquímica

Abstract

Permafrost degradation across northern latitudes is accelerating the mobilization of organic matter, nutrients and contaminants into thermokarst lakes, reshaping carbon cycling and driving greenhouse gas production. However, integrated perspectives linking seasonal regimes and permafrost gradients to mechanistic changes in lake biogeochemistry remain limited.

This thesis investigates seasonal dynamics of natural organic matter (NOM) and terminal electron acceptors (TEAs) across thermokarst lakes in sporadic, discontinuous, and continuous permafrost zones. Studying both the water column and sediments aimed to provide a detailed holistic approach to lake biogeochemistry, engaging with benthic-pelagic dynamics and revealing underlying mechanisms.

A multi-perspective approach integrating water column dynamics — through DOM quality (UV-Vis, Fluorescence and FTIR), TEAs, and hydrochemical parameters — and sediment dynamics — via $\delta^{13}\text{C}$ signatures, C/N ratios, FTIR analysis, and Fe and Mn concentrations — captured lake functioning as a unified system.

Overarching shifts in thermokarst lake biogeochemistry across permafrost zones are shaped by both landscape context and internal processing. Across regions, seasonal freeze-thaw cycles govern redox dynamics, as well as the oxic photochemical breakdown of DOM and anoxic condensation into humified structures.

In sporadic permafrost regions, strong hydrological connectivity enhances terrestrial inputs, yielding CDOM-rich, acidic and redox-stratified systems. Conversely, discontinuous permafrost lakes exhibit broadly transitional characteristics, with moderate CDOM levels and dynamic benthic-pelagic coupling. Lakes in continuous permafrost exhibit low CDOM with high sediment oxygen demand, driven by intense benthic microbial dynamics and reduced soil-water connectivity. In low-CDOM, well-oxygenated lakes, autotroph-driven assimilation of methane-derived carbon into surface sediments indicates a potential internal carbon-sink pathway.

These findings provide insight into thermokarst lake biogeochemistry and shifting mechanisms across permafrost landscapes. Limnodiversity limits the transferability of direct observations, highlighting the need for a mechanistic framework. This work refines conceptual models and underscores the importance of understanding thermokarst dynamics for future carbon–climate feedback estimates.

Keywords: Thermokarst lake, permafrost, natural organic matter, terminal electron acceptors, biogeochemistry

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List of Acronyms

ALD – Active layer detachment

AOM – Anaerobic oxidation of methane

BFC – Benthic flux chamber

BIX – Biological index

CDOM – Chromophoric dissolved organic matter

CEN – Centre d'études nordiques

CORCONDIA – Core consistency diagnostic

CRAM – Carboxyl-rich alicyclic molecules

CRM – Certified reference material

DBP – Disinfection by-product

DO – Dissolved oxygen

DOC – Dissolved organic carbon

DOM – Dissolved organic matter

EEM – Excitation-emission matrix

ESI – Electrospray ionization

EPS – Extracellular polymeric substances

FDOM – Fluorescent dissolved organic matter

FI – Fluorescence index

FTC – Freeze-thaw cycle

FTIR – Fourier-transform infrared

FT-ICR-MS – Fourier-transform ion-cyclotron resonance mass spectrometry

GC – Gas chromatography

GC-MS – Gas chromatography mass spectrometry

GEM – Greenland ecosystem monitoring

GHG – Greenhouse gases

HI – Humification index (FTIR)

HIX – Humification index (Fluorescence)

HILIC – Hydrophilic interaction liquid chromatography

HMW – High molecular weight

HPLC – High-performance liquid chromatography

HRMS – High-Resolution Mass Spectrometry

HSQC – Heteronuclear Single Quantum Correlation

ICP-OES – Inductively coupled plasma optical emission spectroscopy

IEC – Ion-exchange chromatography

LC – Liquid chromatography

LC-MS – Liquid chromatography mass spectrometry

LMW – Low molecular weight

MAAT – Mean annual air temperature

MDLT – Material derived from linear terpenoids

MS – Mass spectrometry

MW – Molecular weight

NMDS – Non-metric multidimensional scaling

NOM – Natural organic matter

NPLC – Normal-phase liquid chromatography

OC – Organic carbon

ORP – Oxidation-reduction potential

PAR – Photosynthetically active radiation

PARAFAC – Parallel factor analysis

PC – Principal component

PCA – Principal component analysis

PERMANOVA – Permutational multivariate analysis of variance

PERMDISP – Permutational analysis of multivariate dispersions

POM – Particulate organic matter

Py-GC – Pyrolysis gas chromatography

RPLC – Reverse-phase liquid chromatography

SAS — Sasapimakwananisikw

SEC — Size-exclusion chromatography

SOD – Sediment oxygen demand

SOM – Soil organic matter

SPC – Specific conductance

SPE – Solid-phase extraction

S_R – Slope ratio

SUVA₂₅₄ – Specific ultraviolet absorbance at 254 nm

SWI – Sediment-water interface

TE – Trace element

TEA – Terminal electron acceptors

TEP – Transparent exopolymer particles

UF – Ultrafiltration

UV-Vis – Ultraviolet-Visible spectroscopy

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I. General Introduction and Thesis Outline

I.1. Context and Rationale

Climate warming is transforming Arctic and subarctic landscapes more rapidly than any other place on Earth. In recent decades, air temperatures across northern latitudes have risen between 2.5 and 4 times faster than the global mean (Rantanen et al., 2022; Lee and Romero, 2023), a phenomenon known as Arctic amplification. Among other effects, this accelerated warming is driving widespread permafrost degradation (Smith et al., 2022), fundamentally altering hydrological regimes (Walvoord and Kurylyk, 2016; Jin et al., 2022), vegetation and active layer thickness (Magnússon et al., 2020; Heijmans et al., 2022) and enhanced fluxes of greenhouse gases (GHG) (Schuur et al., 2008; Natali et al., 2021; Christensen et al., 2023).

Terrestrial permafrost underlies approximately 15% of the Northern Hemisphere's land surface (Obu et al., 2019), storing between 1330 and 1580 Pg of organic carbon (1 Pg C = 10^{15} g C) (Schuur et al., 2015). Its distribution varies widely and is classified by estimated coverage into continuous (90-100 %), discontinuous (50-90%), sporadic (10-50%), and isolated (<10%) (French, 2017; Vincent et al., 2017). As warming deepens the thaw in ice-rich permafrost, the melting of ground ice leads to subsidence and ground collapse, producing irregular depressions known as thermokarst. These depressions often fill with water forming thermokarst lakes, landscape features widespread across permafrost regions like in Siberia, Canada and Alaska (Kokelj and Jorgenson, 2013; Manasypov et al., 2014; Vonk et al., 2015; Farquharson et al., 2016; Bouchard et al., 2020).

As permafrost thaws, large quantities of previously frozen natural organic matter (NOM), nutrients and trace elements are mobilized into these emerging aquatic systems. Thermokarst lakes thereby become active biogeochemical hotspots, characterized by intense NOM decomposition and GHG production (Laurion et al., 2010; Matveev et al., 2016; Walter Anthony et al., 2016; Mu et al., 2023), as well as microbially driven cycling of contaminants, namely mercury (Malcata Martins et al., 2025). Therefore, understanding their role in regulating NOM and nutrient cycling is essential for predicting the consequences of permafrost thaw on Arctic ecosystems and global climate feedbacks.

I.2. Research Problem and Knowledge Gaps

Over recent decades, thermokarst lakes have become key subjects of research interest in Arctic biogeochemistry due to their central roles in NOM cycling and elevated GHG production across permafrost landscapes (Laurion et al., 2010; Walter Anthony et al., 2016; Serikova et al., 2019; Heslop et al., 2020; Jongejans et al., 2021).

Research indicates that lake GHG fluxes often reflect lake ontogeny: younger thermokarst lakes typically exhibit higher rates of GHG emission (Turetsky et al., 2020; Gerera et al., 2025), characterized by higher DOC, higher molecular weight DOM and pronounced terrestrial humic signatures (Peura et al., 2020). As lakes mature, their waters tend to stratify and DOC pools decline due to photodegradation and microbial mineralization (Manasypov et al., 2014; Peura et al., 2020). However, observed biogeochemical differences among thermokarst lakes are highly dependent on local factors such as hydrological connectivity, permafrost continuity, soil composition and vegetation (Vonk et al., 2015). In turn, this limits broad extrapolation of research findings in thermokarst lake systems to other Arctic and subarctic domains.

Thermokarst lakes form through distinct mechanisms across permafrost zones. In sporadic and discontinuous permafrost zones, they typically originate from the collapse of ice-rich peat plateaus or palsas (Luoto and Seppälä, 2003; Sjöberg et al., 2013; Martin et al., 2021; Olefeldt et al., 2021). In continuous permafrost zones, these lakes develop primarily from ice-wedge polygon melt (Czudek and Demek, 1970; Bouchard et al., 2016). The formative contrasts profoundly influence water column properties, sediment accumulation and degradation patterns, and the extent of hydrological connectivity, thereby shaping lake biogeochemistry.

Moreover, thermokarst lakes are highly heterogeneous even within the same landscape. Variability in permafrost coverage, ground ice content, soil type (organic vs mineral), topography, and vegetation cover produce strong spatial contrasts and exert profound control over lake chemistry (Vonk et al., 2015; Freitas et al., 2024). As previously discussed, this intra-site heterogeneity hinders efforts to infer broad-scale trends. As a result, findings from one region often lack transferability to be applied to other Arctic regions, resulting in a fundamental limitation of current thermokarst lake research. In addition, lake morphometry further influences existing in-lake biogeochemical regimes,

determining the susceptibility of the waterbody to wind-mixing or to develop stratification.

One way of addressing these limitations requires integrative studies that include water column and sediment biogeochemistry across geographic and seasonal gradients. While biogeochemical processes in thermokarst lakes are established individually (i.e. DOM photodegradation, TEAs cycling, redox zonation, autotrophic carbon fixation, etc.), integrated, cross-regional biogeochemical investigations coupling pelagic and benthic processes remain scarce, and in certain permafrost landscapes (e.g. Greenland) non-existent.

Vertical profiling of DOM quality, terminal electron acceptors (TEA) cycling, hydrochemical properties, and sediment NOM composition may reveal key underlying mechanisms, providing transferable insight into thermokarst lake dynamics across landscapes and permafrost zones. Despite advances, comprehensive approaches targeting both water column and sediment chemistry, particularly in a comparative context across permafrost gradients are rare in the literature.

In this context, there exist considerable knowledge gaps related to thermokarst lake biogeochemistry across permafrost regions, including:

- Seasonal dynamics of NOM and TEA species across permafrost zones;
- Ontogeny and morphometry-drive shifts in biogeochemical mechanisms;
- Differences in thermokarst lake functioning across permafrost regions;
- Influence of relative dominance of benthic versus pelagic processes in lake chemistry;

A multi-perspective approach integrating water column dynamics — through DOM quality, terminal electron acceptors (TEAs), and physicochemical parameters — and sediment dynamics — via $\delta^{13}\text{C}$ signatures, C/N ratios, FTIR analysis, and Fe and Mn concentrations — may provide a broad overview of lake as a unified system. Comparing this empirically obtained ecological “snapshot” in lakes from the same and across different permafrost zones enables interpretation of shifts in underlying biogeochemical mechanisms.

I.3. Research Questions and Objectives

As previously mentioned, the primary aims in this research work focused on detecting overarching trends along the permafrost gradient, as well as the identification of underlying biogeochemical mechanisms influencing thermokarst lake dynamics.

Building on these research needs, this study seeks to address the following key research questions:

- 1) How do biogeochemical properties and natural organic matter (NOM) dynamics vary among thermokarst lakes located across distinct permafrost zones (sporadic, discontinuous and continuous)?
- 2) How does morphometry and ontogeny influence NOM dynamics and chemical properties in thermokarst lakes?
- 3) What seasonally induced shifts occur in thermokarst lake dynamics, and how do these shifts vary across different permafrost regions?
- 4) How do permafrost landscape characteristics shape biogeochemical processes and control the relative influence of internal vs external inputs in thermokarst lakes.
- 5) What do sedimentary carbon isotope signatures ($\delta^{13}\text{C}$) reveal about carbon fixation pathways and methanotrophic activity in thermokarst lakes, and how do these sediment-based insights inform our understanding of lake-scale carbon cycling?

To address these questions, the following research objectives were established:

1. Evaluate contrasts in thermokarst lake biogeochemistry across distinct permafrost zones (sporadic, discontinuous, and continuous), elucidating how permafrost landscape context drives chemical and ecological differences.
2. Study the influence of morphometry and ontogeny on thermokarst lake functioning, integrating water column and sedimentary evidence.

3. Assess the magnitude and nature of seasonally induced biogeochemical shifts in thermokarst lakes and determine how these seasonal patterns differ across contrasting permafrost landscapes.
4. Investigate sediment degradation patterns and explore potential carbon fixation pathways (using $\delta^{13}\text{C}$ isotopic signatures) across thermokarst lakes from across a permafrost gradient.

Employing a multi-perspectival strategy of studying hydrochemical, DOM and TEA dynamics in the water column, coupled with sediment characterization, will provide an integrated perspective aiming to elucidate these research questions.

I.4. Personal Motivation

From a personal perspective, I am strongly motivated to pursue these research goals. During my Master's thesis, which focused on the structural characterization of DOM in thermokarst lakes, I became acquainted with the significant environmental risks these systems pose in terms of GHG emissions. My understanding of climate change in Arctic and subarctic regions deepened further when I served as Executive Secretary for the international scientific project "Terrestrial Multidisciplinary distributed Observatories for the Study of Arctic Connections" (T-MOSAIC). This experience broadened my perspective on the far-reaching implications of accelerated permafrost thaw in the context of global climate change.

These profound consequences highlight the urgent need for closer collaboration between scientists, communicators and policy makers. Addressing such challenges will likely demand both difficult systemic economic adjustments and social solutions to mitigate a challenge of this scale.

The opportunity to investigate the environmental controls that regulate biogeochemical mechanisms in thermokarst lakes — particularly those governing NOM dynamics — was therefore not only an intellectually compelling endeavor, but also a personally meaningful one.

I.5. Outline of the Thesis

This thesis investigates shifts in thermokarst lake biogeochemistry, focusing on natural organic matter (NOM) and terminal electron acceptors (TEAs) across a permafrost gradient.

Each studied lake was analyzed as an integrated system of water column and sediments, within which mechanistic processes leave measurable imprints. In this context, the thesis addresses different facets of diverging thermokarst lake systems within comparable permafrost zones. This thesis is organized into the following chapters:

Chapter I. Brief Introduction

This chapter provides a brief introduction to thermokarst lake systems and the primary scopes of research interest in their functioning. The main research questions and objectives of the present work, as well as the novelty of the approach are explored.

Chapter II. Natural organic matter dynamics in permafrost peatlands: Critical overview of recent findings and characterization tools

This chapter provides a state-of-the-art peer-reviewed literature review of analytical chemistry techniques used in NOM and DOM characterization and their relevance in studying environmental systems, particularly in permafrost landscapes. The review covers general TEA dynamics in NOM decomposition, establishing the conceptual framework for investigating NOM quality as a proxy of biogeochemical changes in natural systems in Arctic and subarctic regions. Although some of the techniques described in this chapter were not utilized during this work, the objective was to provide a more complete overview of the potentialities of each technique and their application.

Chapter III. Seasonal organic matter dynamics in thermokarst lakes of a degrading palsa-rich sporadic permafrost zone (Nunavik, Canada)

This chapter examines the effects of seasonality and ontogeny on thermokarst lake dynamics in a waterlogged peatland located in a sporadic permafrost region, near Kuujjuarapik-Whapmagoostui, Nunavik, Québec, Canada. Three thermokarst lakes at different developmental stages were sampled, including vertical water column profiles, sediments and marginal soil samples. Analyses included TEA concentrations (i.e. nitrate, Fe, Mn, sulfate), DOM quality (i.e.

CDOM, FDOM) and physicochemical parameters for the water column. Isotopic $\delta^{13}\text{C}$ signatures, C/N ratios, FTIR analysis and Fe and Mn concentrations were used to study different sediment layers.

Chapter IV. Seasonal biogeochemistry contrasts in palsa- and lithalsa-origin thermokarst lakes in a discontinuous permafrost zone

This chapter evaluates the effects of seasonality and lake origin (palsa- versus lithalsa-derived) on thermokarst lake dynamics in a transition zone (between boreal forest and tundra) of a discontinuous permafrost zone, near Kangiqsualujjuaq, Nunavik, Québec, Canada. Similar sampling methodologies to Chapter 2 were employed. Comparative analyses highlighted how changes in origin impact lake chemistry and seasonality-driven shifts, with emphasis on TEA availability, DOM quality and the effects of primary producers in pelagic-benthic dynamics.

Chapter V. Emerging thermokarst lakes amplify benthic activity and carbon mobilization in a continuous permafrost landscape

This chapter investigates two recently formed thermokarst lakes in the rapidly evolving tundra of Zackenberg Valley, Greenland, located in a continuous permafrost zone. Thermokarst lakes were sampled only in summer, with similar characterization in water column and sediments as in Chapters II and III. Additionally, a benthic flux chamber experiment was performed at the sediment-water interface to evaluate sediment oxygen demand rates, shifts in redox conditions, and both shared and contrasting biogeochemical mechanisms between lakes.

Chapter VI. General discussion, conclusions and future work

This chapter synthesizes the main findings across the case studies, integrating environmental controls and overarching thermokarst dynamics along the permafrost gradient. It includes a subsection with general statistical tests conducted across sample groups and potential relationships between variables in thermokarst lake sediments. Furthermore, given the GHG production potential in thermokarst lake systems, a conceptual model of a mechanism of carbon fixation within thermokarst lakes is introduced and discussed in the context of the study sites.

Chapter VII. *Supplementary Material*

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II. Natural Organic Matter Dynamics in Permafrost Peatlands: Critical Overview of Recent Findings and Characterization Tools

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II.1. Thawing of permafrost in northern landscapes

Although temperatures have risen globally in recent decades, climate warming has been far more accentuated in Arctic and subarctic regions than in lower latitude areas (Bekryaev et al., 2010; Hinzman et al., 2013), with median air temperatures rising 2.5 to 4 times faster than the global average (Rantanen et al., 2022; Lee and Romero, 2023). These changes have raised significant concerns among climate scientists, prompting a wide range of environmental research to investigate the impacts and processes triggered by such rapid climate shifts. One of the primary concerns for Arctic researchers is the consequences of thawing terrestrial permafrost on ecosystem functioning and greenhouse gas emissions.

Terrestrial permafrost spans approximately 14 million square kilometers, representing about 15% of the Northern Hemisphere's land area (Obu et al., 2019), and is thought to store a carbon pool ranging from 1330 to 1580 Pg C (1 Pg C = 10^{15} g C) (Schuur et al., 2015). The spatial distribution of permafrost across northern landscapes varies significantly, with regions categorized based on the percentage of permafrost coverage: continuous (90-100%), discontinuous (50-90%), sporadic (10-50%), and isolated (<10%) (Vonk et al., 2015; French, 2017; Vincent et al., 2017). The thickness of permafrost varies greatly, ranging from less than 1 meter to several hundred meters (Vincent et al., 2017). As air temperatures rise in the Arctic, permafrost temperatures have also increased over the past decades (Romanovsky et al., 2010; Biskaborn et al., 2019), a trend that is expected to continue. Discontinuous permafrost zones in the Arctic and Subarctic have been particularly affected, with mean annual ground temperatures now exceeding -2 °C (Romanovsky et al., 2010). These conditions are characteristic of many organic-rich northern permafrost peatlands (Hugelius et al., 2014; Schuur et al., 2015; Payandi-Rolland et al., 2021), making them particularly vulnerable to climate-induced landscape changes, such as the emergence of numerous small thermokarst lakes and the drainage of larger, older lakes (Pokrovsky et al., 2016). The thawing of ice-rich permafrost leads to the formation of thermokarst, which acts as a pulse disturbance, while thawing of permafrost with lower ground-ice content generally results in a more gradual, press disturbance (Fig. II.1) (Grosse et al., 2011).

Permafrost warming is widespread across Arctic and subarctic regions (Biskaborn et al., 2019), with typical warming rates less than 0.3 °C per decade in the Subarctic and of up to 1 °C per decade in colder, high-latitude Arctic permafrost (Smith et al., 2022). Despite

thermokarst processes represent localized disturbances to the ground thermal regime, their widespread occurrence is expected to affect wider areas (Grosse et al., 2013), with thermokarst lakes covering 200,000 to 350,000 km² of surface water during the warm season (Grosse et al., 2013; Olefeldt et al., 2016). As permafrost thaws, the mobilized natural organic matter (NOM) becomes available for biogeochemical transformation, converting thermokarst lakes into significant sources of greenhouse gases (GHG) such as CO₂ and CH₄ (Zimov et al., 1997; Vonk et al., 2015; Matveev et al., 2019). Anaerobic conditions in thermokarst lake sediments and bottom waters foster microbial degradation of permafrost-derived NOM, leading to methane production and its subsequent release into the atmosphere. Despite the recognition that thermokarst lakes contribute to the atmospheric carbon budget, little is known about the temporal variability of these emissions and the preferential sources of carbon that fuel them (Bouchard et al., 2015; Schneider von Deimling et al., 2015).



Figure II.1. Sporadic permafrost palsa peatland in Whapmagoostui-Kuujuarapik, Québec, Canada. (Credit: João Canário)

Thermokarst lakes can differ significantly in their limnology (Wrona et al., 2016), geomorphology (Olefeldt et al., 2016), GHG emission rates (Laurion et al., 2010; Wik et al., 2016), as well as in the optical properties of dissolved organic matter (DOM) (Watanabe et al., 2011; Wauthy et al., 2018), highlighting the influence of the region in which they form. While thermokarst lakes in ice-rich yedoma regions have been extensively studied (Zimov et al., 1997; Grosse et al., 2013; Heslop et al., 2015), research

on thermokarst lakes on organic-rich peatlands remains limited, despite the high carbon content of lakes in these areas (Hugelius et al., 2014; Vonk et al., 2015) and their potential to act as hotspots for methane emissions (Matveev et al., 2016, 2019; Wik et al., 2016).

Northern peatland soils have two key characteristics: a high-water content and substantial quantities of NOM. When these soils are flooded, microbial activity on NOM mineralization leads to rapid oxygen consumption, and eventually chemical oxidation, with terminal electron acceptors (TEAs) such as NO_3^- , Mn (IV), Fe (III), and SO_4^{2-} used during anaerobic respiration (Philben et al., 2020). These process results in changes to the oxidation states of the involved chemical species. The dynamics of microbial degradation of NOM in anoxic soils are closely tied to the effects of vegetation and TEAs. For instance, a reduction in TEAs due to increased respiration could trigger an increase in soil CH_4 production (Murray et al., 2019; Philben et al., 2020).

Soil degradation rates can increase up to four times in the presence of plant roots, a phenomenon known as the rhizosphere priming effect (Keuper et al., 2020). In permafrost soils, rhizosphere priming promotes a surge in soil respiration, which in turn leads to a reduction in soil carbon stocks and an increase in GHG production (Keuper et al., 2020). Plant communities that enhance oxygenation within the rhizosphere are known to create oxidizing conditions belowground, characterized by high redox potentials. This process results in higher $\text{CO}_2\text{:CH}_4$ ratios in pore water, which suggests an environment favoring methanotrophy (Rupp et al., 2019).

Climate-driven vegetation shifts in northern peatlands play a crucial role in influencing both the chemical composition of soil organic matter (SOM) and the evolving biogeochemistry of these ecosystems. As vegetation transitions from palsas to fens and then to *Sphagnum*-rich bogs, this shift would condition certain NOM pools become progressively less bioavailable (Wilson et al., 2022). It has been proposed that the higher carbohydrate content in bog peat results from low pH values that inhibit microbial degradation, while aromatic and lignin-like compounds are consumed in palsa environments but tend to accumulate under anaerobic conditions in both fens and bogs (Wilson et al., 2022).

Accumulation of organic matter (OM) in peatland soils is driven by complex interactions among water content, soil temperature, vegetation, and the subsequent production, composition, and degradation of litter (Vonk et al., 2015; Treat et al., 2016; Gałka et al., 2017; Lyons and Lindo, 2020). Bogs and fens are widespread across northern peatland landscapes, with the transition of fens into acidic bogs typically associated with the

gradual colonization of *Sphagnum* species, while fens are generally dominated by vascular macrophytes, such as *Carex* sedges (Scheffer et al., 2001). Litter from *Sphagnum* mosses has higher C:N ratios than *Carex* litter (Scheffer et al., 2001; Palozzi and Lindo, 2017; Lyons and Lindo, 2020), suggesting that *Sphagnum* litter contribute more recalcitrant OM to peat soils, due to slower microbial processing, whereas *Carex* sedges produce litter of higher quality for microbial decomposition. In boreal peatlands, litter inputs in *Carex*-rich fens have been linked to a fivefold increase in microbial biomass and twice the rate of basal respiration compared to *Sphagnum*-rich fens, which also exhibit lower peat moisture (Lyons and Lindo, 2020). This distinction is further supported by findings that DOM from *Sphagnum*-dominated sites display higher aromaticity, O:C content, and average molecular weight (MW), while the absence of *Sphagnum* is associated with greater aliphatic content and higher microbially-driven DOM (Tahvanainen and Haraguchi, 2013; Hodgkins et al., 2016). The low pH of *Sphagnum*-dominated sites may inhibit phenol oxidase activity, leading to the accumulation of aromatic compounds (Tahvanainen and Haraguchi, 2013; Hodgkins et al., 2016), reinforcing the recalcitrant nature of *Sphagnum*-derived DOM and its role in these ecosystems. Additionally, vascular macrophytes in thermokarst lakes have been shown to bioaccumulate inorganic contaminants, as well as macro- and micronutrients, namely Fe and Mn (particularly in the upper organic layer of sediments), suggesting a phytoremediation function in these species (Manasypov et al., 2021).

Seasonal temperature changes play a critical role in regulating the thickness of ice cover and the exposure of surface waters to atmospheric oxygen. Warmer temperatures shorten ice-cover duration and extend the period of oxygen exchange at the water-atmosphere interface (Vonk et al., 2015). At the same time, increased temperatures accelerate permafrost thaw and promote the erosion of surrounding permafrost soils into thermokarst lakes. The highest rates of methane emissions have been observed at the edges of thermokarst lakes (Heslop et al., 2015).

During the open water season, DOM undergoes two main processes in the water column: photodegradation and biodegradation, particularly driven by aerobic respiration in surface waters, where there is sufficient light and oxygen. However, DOM photodegradation in Arctic thermokarst lakes has been shown to primarily act as a catalyst for microbial activity by enabling the photolysis of recalcitrant, terrestrially derived DOM into smaller, more labile compounds that are bioavailable for microbial transformation (Laurion and Mladenov, 2013). Photodegradation also contributes to the release of volatile organic

carbon directly to the atmosphere (Fillion et al., 2024). It is important to note that even in oxygenated strata of the lake, methanogenesis can still occur (Bogard et al., 2014).

Winter observations in peatland thermokarst lakes have indicated substantial accumulation of CH₄ and CO₂ beneath the ice, suggesting potential for significant spring emissions once the surface ice melts (Matveev et al., 2019). However, GHG release from thermokarst lakes is influenced by mixing regimes, with deeper lakes exhibiting a very strong stratification. In these lakes, methane concentrations increase at the bottom during the summer, with most being released in the fall (Matveev et al., 2019). The geomorphological characteristics of thermokarst lakes control their mixing regime that directly influences NOM degradation and GHG production and consumption. Seasonal changes also affect microbial communities in thermokarst lakes in terms of both activity and abundance. In the warm season, microbial communities are primarily engaged in phototrophy and aerobic metabolism (including oxygen respiration, and oxidation of methane, methanol and H₂S), combined with the degradation of both particulate and photodegraded NOM. Additionally, CO₂ emissions may be amplified due to methane oxidation (Laurion et al., 2010), with methanotrophic microbial communities in the upper water column acting as a biofilter that reduces CH₄ emissions (Crevecoeur et al., 2017). During the winter season, microbial activity shifts towards anaerobic pathways, such as methanogenesis, which favor the reduction of TEAs, thereby enhancing the degradation potential of recalcitrant NOM (Vigneron et al., 2019).

The importance of transition periods on biogeochemical processes — particularly for the transport of DOM and inorganic moieties from surrounding soils — has been emphasized in thermokarst lakes (Manasypov et al., 2015; Pokrovsky et al., 2020). Payandi-Rolland et al. (2021) conducted an experiment to evaluate the effects of freeze-thaw cycles (FTC) on leachates from soil and vegetation compared to various surface waters. Although they found only minor changes in dissolved organic carbon (DOC) levels and DOM quality, concentrations of elements such as Fe, Al, P, Mn and As increased in leachates. In contrast, in thermokarst lake waters, experimental FTC led to a reduction in aromatic structures and Fe concentrations, suggesting the degradation of humic moieties into smaller, more labile aliphatic groups (Pokrovsky et al., 2018). Notably, Fe-DOM complexes have been shown to account for the majority of dissolved Fe in organic-rich aquatic systems, where they are subject to both photodegradation and biodegradation, resulting in the formation of low molecular weight (LMW) DOM (Kügler et al., 2019; Oleinikova et al., 2019). The structural composition of the DOM fraction that forms these

Fe-DOM complexes is crucial, as it determines whether the resulting organometallic colloids are labile or stable, thereby influencing microbially-driven redox processes (Kügler et al., 2019).

The dynamics of NOM in northern thermokarst lakes are governed by complex interactions among a wide range of variables, and a comprehensive understanding of these processes can only be achieved through multidisciplinary approaches that consider these lakes as integrated systems. Nevertheless, the biogeochemical behavior of NOM in these systems is strongly influenced by its chemical composition and the presence of oxidant species (oxygen and TEAs), which serve as key drivers for microbial mineralization of the organic fraction. In this review, we aim to provide: (i) a brief overview of the biogeochemical processes associated with the four main TEAs in aquatic systems — nitrate, iron, manganese, and sulfate — and (ii) an overview of the optical and structural characterization of NOM in natural systems, along with its environmental significance. The goal is to highlight the importance of integrating the study of redox-sensitive species and processes with the analysis of NOM quality, in order to better understand the dynamics of thermokarst systems.

II.2. Terminal electron acceptors in NOM degradation

In aquatic systems, redox processes influence the composition and quality of natural waters by not only mobilizing or immobilizing existing toxicants but also governing the degradation and production of both organic and inorganic redox byproducts (McMahon and Chapelle, 2008; McMahon et al., 2011). Microbial communities that drive redox processes often compete for chemical moieties acting as electron donors. This competition favors the most energy-efficient redox pathways, suggesting that processes involving highly efficient electron donors and acceptors are more likely to have a competitive advantage (Fig. II.2) (McMahon and Chapelle, 2008).

	Respiration Process	Schematic Reaction	ΔG^0
Oxygen Status ↑ Oxidic ↓ Sub-Oxidic ↓ Anoxic	Aerobic Respiration	$\text{CH}_2\text{O} + \text{O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O}$	- 402
	Nitrate Reduction	$5\text{CH}_2\text{O} + 4\text{NO}_3^- \rightarrow 2\text{N}_2 + 4\text{HCO}_3^- + \text{CO}_2 + 3\text{H}_2\text{O}$	- 359
	Manganese Reduction	$\text{CH}_2\text{O} + 3\text{CO}_2 + \text{H}_2\text{O} + 2\text{MnO}_2 \rightarrow 2\text{Mn}^{2+} + 4\text{HCO}_3^- + \text{CO}_2$	-385
	Iron Reduction	$\text{CH}_2\text{O} + 7\text{CO}_2 + 4\text{Fe}(\text{OH})_3 \rightarrow 4\text{Fe}^{2+} + 8\text{HCO}_3^- + 3\text{H}_2\text{O} + \text{CO}_2$	-241
	Sulphate Reduction	$\text{CH}_2\text{O} + \text{SO}_4^{2-} \rightarrow \text{H}_2\text{S} + 2\text{HCO}_3^- + \text{CO}_2$	- 43.8
	Methanogenesis	$\text{CH}_2\text{O} \rightarrow \text{CH}_4 + \text{CO}_2$	- 19.9

Figure II.2. Distribution of common terminal electron accepting processes and their relative contribution to overall C oxidation across redox gradients, and the Gibbs free energy yield (ΔG^0) of each electron accepting process. Data from McMahon and Chapelle (2008); McMahon et al. (2011); Keiluweit et al. (2016). CH_2O represents a simplified formula for NOM.

The use of dissolved oxygen (O_2) generates the greatest energy per mole of oxidized organic carbon (OC) compared to other commonly available electron acceptor species, making it the preferential electron acceptor by microbial communities for respiration (McMahon and Chapelle, 2008; McMahon et al., 2011). When an aquatic system is isolated from the atmosphere, the rapid consumption of remaining dissolved O_2 leads to suboxic or anoxic conditions, forcing microbial communities to shift towards alternative redox processes. In these conditions, electron acceptors like nitrate (NO_3^-), Mn (IV), Fe(III), and sulfate (SO_4^{2-}) are utilized. The preferential order of electron acceptor usage, based on energy efficiency, follows this sequence: $\text{O}_2 > \text{NO}_3^- > \text{Mn}^{4+} > \text{Fe}^{3+} > \text{SO}_4^{2-} > \text{CO}_2$. Their use results in the reduction of these species, such as NO_2^- , Mn(II), Fe(II), H_2S , and CH_4 (Davison, 1993; McMahon et al., 2011; Herndon et al., 2020b).

Changes in drainage and precipitation will lead to greater fluctuations in water levels, which in turn will cause more variable redox conditions in northern wetlands (Herndon et al., 2020b). These changes will also result in a more heterogeneous redox distribution in soils, as thermoerosion gullies promote the formation of adjacent oxidic and anoxic soil fractions, each exhibiting distinctly different redox conditions within these organic-rich soils (Abbott and Jones, 2015). Naturally, the dynamics of NOM transformations in these

systems are closely linked to the biogeochemical cycling of the electron acceptor species present and the redox processes in which they are involved.

Although O₂ and CH₄ availability mainly control aerobic CH₄ oxidation (Hornibrook et al., 2009; Lofton et al., 2014), the presence of TEAs in oxygen-depleted systems may also provide substrates for methanotrophic communities (Kits et al., 2015; Perryman et al., 2020). Perryman et al. (2020) found methane oxidation rates to be consistently higher in flooded fens than in palsas and bogs within permafrost peatlands. They observed increases in methane oxidation accompanied by decreases in redox potential, with the highest CH₄ oxidation occurring at sites with low redox potential and a high water table.

II.2.1 Nitrate reduction

Suboxic or anoxic conditions promote microbial denitrification, leading to nitrate consumption within the ecosystem and reducing the availability of nitrate for plant primary production and carbon storage (Herndon et al., 2020b). While the highest concentrations of nitrate are generally found in well-drained, oxic soils, soils with greater moisture and suboxic/anoxic conditions tend to have higher levels of ammonium (NH₄⁺) (Heikoop et al., 2015; Zhu et al., 2016). Despite the low nitrate concentrations in anaerobic, organic-rich Arctic peat soils (Lipson et al., 2010, 2012), rising ground temperatures and thawing permafrost are expected to increase nitrogen bioavailability in these systems (Herndon et al., 2020b). The low levels of nitrate in Arctic peatlands are further supported by the fact that plant communities in these regions preferentially use amino acids as a nitrogen source, rather than NO₃⁻ or NH₄⁺ (Persson et al., 2003; Herndon et al., 2020b), and by the generally low *in situ* rates of denitrification in Arctic soils. However, *Sphagnum* mosses in northern landscapes have been shown to effectively retain NO₃⁻ in peatlands (Blodau et al., 2006). As thermokarst processes evolve and water contact shifts from organic-rich peat soils to mineral soils, an increase in nitrate concentrations is anticipated, either due to nitrification processes or reduced NO₃⁻ uptake (Harms and Jones Jr., 2012; Louiseize et al., 2014).

II.2.2 Manganese and iron reduction

Redox-sensitive metals like manganese and iron are fundamental micronutrients that play a critical role in regulating biological production in aquatic systems (Boyd and Ellwood,

2010; Tagliabue et al., 2017). They govern the bioavailability of micronutrients and trace elements (Ingri et al., 2011; Herndon et al., 2020a; Leclerc et al., 2021), and, to some extent, can even physically protect certain fractions of NOM from decomposition (Kleber et al., 2005; Riedel et al., 2013). In their reduced forms, Mn^{2+} and Fe^{2+} can be found dissolved in natural waters, while their oxidized forms (Mn^{4+} , Mn^{3+} and Fe^{3+}) tend to form less soluble oxides (Lipson et al., 2010; Schroth et al., 2015) or organo-metallic colloids (Pokrovsky et al., 2011, 2016; Herndon et al., 2017), particularly in the case of iron.

Iron and manganese oxides, hydroxides, and oxyhydroxides, commonly found in benthic environments (Lipson et al., 2010; Herbert et al., 2020), can be utilized as TEAs by microbial communities to oxidize NOM in the absence of more thermodynamically favorable oxidants (Lipson et al., 2010; Magen et al., 2011). The relative abundance of redox-sensitive metal oxides in sediments in turn can influence the extent of NOM degradation in benthic environments, as the dissimilatory reduction of solid-phase Fe and Mn oxides under anoxic conditions drives NOM degradation and CO_2 production and release (Lipson et al., 2010; Magen et al., 2011). Under reducing conditions, Mn and Fe oxyhydroxides in the sediments are reduced and redissolved into their ionic forms, allowing them to migrate through diffusion in the water column or sediment pore waters (Davison, 1993; Ingri et al., 2011; Herndon et al., 2015). An upward migration of these species results in the reprecipitation of Mn(IV) and Fe(III) oxyhydroxides at the oxic top benthic layer (Herndon et al., 2015, 2017), while downward migration facilitates the formation of iron sulphides (FeS and FeS_2) (Davison, 1993; Ingri et al., 2011). It is important to note that Fe^{2+} oxidation by O_2 at circumneutral pH is extremely rapid, whereas the oxidation of Mn^{2+} is much slower (Madison et al., 2013). The widespread presence of microbial Fe mats in sediments in Arctic and subarctic permafrost regions further suggests that the accumulation of Fe oxides at redox interfaces is common in iron-rich environments (typically sourced from mineral soils or deep groundwater) in northern landscapes (Herndon et al., 2015, 2017, 2020b). Additionally, as temperatures rise, soil moisture in Arctic wetlands is expected to decrease (Hinzman et al., 2013), leading to more oxic conditions and increased DOM degradation, which in turn may promote greater precipitation of Fe oxides in sediments (Herndon et al., 2017).

The presence of total Fe and Mn in solid-phase profiles of subarctic lake sediments has been shown to decrease substantially just below the sediment-water interface (SWI), likely due to the accumulation of Fe and Mn (oxy)hydroxides. In contrast, dissolved Fe

and Mn profiles in porewater display an inverse trend, with concentrations of both metals increasing with depth, a result of the reduction and redissolution of these sedimentary oxides (Ingri et al., 2011; Leclerc et al., 2021).

Although research on this topic remains limited, permafrost-affected soils in Northern Siberia have been shown to contain high concentrations of both iron and manganese (Antcibor et al., 2014), with rapid thaw phenomena also associated with elevated Fe concentrations (Loiko et al., 2017). Once mobilized from soils, these iron-rich organic complexes undergo transformation as NOM decomposes, fueling the release and subsequent reaggregation of nanoparticulate Fe oxyhydroxides into larger colloids (Pokrovsky et al., 2011, 2016). In fact, the DOC/Fe composition of colloids tends to be higher at the onset of permafrost thaw (depressions ponds), indicating the presence of more organic complexes (Pokrovsky et al., 2016). The dynamics and impacts of iron-organic matter complexes in northern ecosystems are still not fully understood. While some research suggests that Fe-DOM associations are resistant to microbial degradation, other studies indicate that Fe-DOM complexation may actually accelerate microbial-mediated redox processes in fens (Kügler et al., 2019) and peat leachates (Oleinikova et al., 2017).

Despite the thermodynamic favorability for Mn redox utilization, microbial Mn reduction can be restricted by low concentrations of Mn oxides and their shallow depth distribution (Thamdrup, 2000). In contrast, Fe biogeochemistry, due to its greater abundance, plays a more prominent role in permafrost-affected systems.

II.2.3 Sulfate reduction

The redox cycling of sulfur, similarly to that of Fe and Mn, is closely linked to NOM dynamics, directly influencing its degradation and storage in permafrost regions (Herndon et al., 2020b). Microbial respiration that mediates sulfur redox cycling relies on sulfate (SO_4^{2-}) as an electron acceptor to oxidize NOM, leading to the formation of CO_2 and sulfide (as hydrogen sulfide or mono-/polysulfides) under anoxic conditions. Low concentrations of dissolved sulfate in saturated Arctic soils (Lipson et al., 2010; Newman et al., 2015) suggest that available sulfate may be consumed in oxygen-depleted soils (Lipson et al., 2013).

Iron-rich environments can significantly influence sulfur dynamics, not only by affecting the extent of iron-mediated microbial reduction compared to sulfate reduction (Lipson et

al., 2010, 2013) but also due to the capacity to produce and accumulate precipitated iron sulfides (FeS) during sulfate reduction in non-aerated permafrost soils (Herndon et al., 2017, 2020a). These sulfide minerals, when exposed to oxic conditions, can be oxidized back to sulfate, as observed in streams draining from permafrost watersheds (Stutter and Billett, 2003; Calmels et al., 2007; Toohey et al., 2016). Increased sulfate reduction may also intensify competition between the newly formed sulfides and existing phosphate for Fe-binding, ultimately impacting phosphorus cycling and bioavailability in the ecosystem (Herndon et al., 2020a).

As permafrost thaw progresses, erosion phenomena become more pronounced, and the active layer gradually deepens into the soil mineral layer, exposing sulfide minerals to weathering and oxidation (Calmels et al., 2007). This leads to an increase in sulfate concentrations, as observed in streams as permafrost degradation advances (Stutter and Billett, 2003; Keller et al., 2010; Toohey et al., 2016). Greater thaw depth and erosion can amplify the exposure of sulfides to weathering and oxidation, providing a sulfate source for sulfate-reducing microbial communities to facilitate the anaerobic respiration of NOM (Herndon et al., 2020b).

Sulfate concentration gradients have been observed between different types of lakes, with forest and forest-tundra thermokarst lakes exhibiting low SO_4^{2-} concentrations, while tundra lakes and particularly glacial lakes display higher sulfate levels (Huang et al., 2020). Although oligotrophic lakes generally have relatively low SO_4^{2-} concentrations, these levels tend to increase under oxic conditions. However, most sulfate utilization by microbial respiration occurs in lakes that are more organic-rich and anoxic, ultimately favoring the conversion of existing SO_4^{2-} to hydrogen sulfide (Holmer and Storkholm, 2001).

II.3. Natural organic matter in permafrost aquatic environments

Natural organic matter (NOM) is a heterogeneous complex mixture of chemical moieties with a wide range of reactivities, MWs, and solubilities. The chemical composition of NOM can vary significantly depending on its source or origin, as well as the types of biotic and abiotic processes it has undergone, which ultimately leads to a highly diverse pool of organic structures. By definition, the NOM fraction that passes through a $0.45 \mu\text{m}$

or 0.2 μm pore size filter is referred to as DOM (dissolved), while the retained fraction is termed particulate organic matter (POM). Although this distinction is important when investigating the chemical properties of DOM in a sample, it is worth noting that the separation between DOM and POM is an operational one, leaving colloids in the dissolved fraction. Colloids are an important component of the water column, and special attention has been given to this fraction in thermokarst lake environments. Pokrovsky et al. (2011) demonstrated that colloid concentrations have a pronounced effect on trace element (TE) fractionation, with some TEs bonding to organic carbon colloids, which plays a key role in their distribution across different fractions. More recently, Manasypov et al. (2023) conducted a pilot study showing that colloidal fractions of alkali and alkaline-earth metals decreased with an increase in lake size, likely due to lower DOC concentration in larger lakes. However, they noted the need to further research, particularly to assess the seasonality of colloid concentration and properties in these environments.

NOM is a pervasive component of natural systems and plays an integral role in aquatic environments. A significant portion of NOM has been categorized as humic substances, especially in peat and soil organic matter, which are generally described as macromolecular structures of refractory nature and high molecular weight (HMW) (Thurman, 1985; Sillanpää et al., 2015). Humic substances are conventionally divided into humic acids (insoluble at low pH), fulvic acids (soluble at any pH), and humins (insoluble), and they are thought to play a crucial role in the availability of plant nutrients in soils, particularly P, Fe and Cu (Gerke, 2018). However, the use of the term “humic” has been a subject of debate, with some soil scientists advocating for the “soil continuum model”. This model conceptualizes SOM as a continuous spectrum of organic moieties at various stages of degradation and of differing sizes, a perspective that has generated both support and criticism within the scientific community (Lehmann and Kleber, 2015; Weber et al., 2018; Baveye and Wander, 2019).

As mentioned previously, the formation and chemical composition of SOM is highly site-specific, influenced by factors such as climate, hydrological regimes, nutrient sources, and vegetation. For instance, SOM in palsa surface peat differs significantly from peat SOM in wetter environments. In palsa areas, the dry surface peat allows for aerobic degradation of SOM, while the flooding of peatlands creates anaerobic conditions (Philben et al., 2020). Although anaerobic conditions can facilitate the oxidation of SOM by redox-sensitive TEAs (Philben et al., 2020), decomposition in peatlands mainly occurs

in the DOM fraction rather than directly in the peat itself (Chanton et al., 2008; Hodgkins et al., 2016). Water-saturated peat SOM tends to have a higher aliphatic content compared to drained peat SOM, as anaerobic conditions may favor the preservation of aliphatic moieties over others (Heller et al., 2015).

The thermokarst processes occurring in Arctic and subarctic thaw ponds, namely the erosion of surrounding soils (Heslop et al., 2015; Deshpande et al., 2017), may result in the mobilization of substantial amounts of terrestrial SOM into aquatic systems. This influx of SOM can lead to a rapid degradation of the NOM fraction in these environments (Deshpande et al., 2017; Wauthy et al., 2018). Therefore, peatlands may shift from acting as carbon sinks to carbon sources. Once SOM from peatland soils is exported to aquatic systems, both POM and DOM fractions become exposed to photodegradation and biodegradation processes (Ward and Cory, 2016; Lin et al., 2021).

Although bacterial transformation has traditionally been considered the primary control of DOM exported from terrestrial to aquatic systems, photodegradation has been shown to account for 70 to 95% of DOM transformations in the water column of Arctic freshwater systems (Cory et al., 2014; Ward and Cory, 2016). Aromatic structures in DOM, known for their light-absorbing properties, are key players in initiating photochemical transformations, which degrade these complex structures into smaller, more labile aliphatic LMW DOM. This process not only alters the chemical composition of DOM but also contributes to the loss of DOM color (Cory et al., 2007; Helms et al., 2008; Mann et al., 2012; Ward and Cory, 2016).

In the case of thermokarst lakes in northern peatlands, the main sources of NOM are terrestrially derived DOM from the surrounding soils (Wauthy et al., 2018). Given the typically high aromaticity of this input, it is expected that the DOM entering aquatic systems will undergo significant photochemical transformation. This process breaks down aromatic compounds into more labile and aliphatic forms, making the DOM more bioavailable for microbial consumption. However, this influx of POM and colored DOM increases the turbidity of the water, which in turn significantly attenuates light penetration throughout the water column (Watanabe et al., 2011). This attenuation reduces the availability of light for autotrophic production, shifting the balance of the system in favor of heterotrophic production (Forsström et al., 2013; Roiha et al., 2015; Deshpande et al., 2017; Wauthy et al., 2018).

The mobilization of NOM and nutrients from surrounding peatland soils into thermokarst lakes also create favorable conditions for the development of free-living and particle-

based bacterial communities (Deshpande et al., 2016). As a result, labile moieties — whether originating from surrounding vegetation or produced as byproducts of photodegradation — become available for microbial degradation within the water column.

II.4. Brief overview of DOM extraction methods

Given the complexity of NOM processes in natural systems, it is crucial to employ a diverse range of analytical tools to assess its properties and chemistry. This is essential for understanding the changes in its chemical composition and the biogeochemical processes driving those changes. In this work, we aim to highlight the significance of various characterization methods for NOM samples from northern regions. We will also explore how shifts in the overall chemical composition can inform us about the underlying biogeochemical processes at play. To provide context, we will briefly outline the most common isolation methods for DOM and the fractionation techniques used to separate complex NOM fractions before further analysis.

The two main isolation methods for DOM samples are solid-phase extraction (SPE) and ultrafiltration (UF), each utilizing distinct approaches to extract DOM. UF is a purely physical process that separates DOM based on molecular size, while SPE depends on chemical interactions between the selected sorbent and the DOM. These differences in methodology result in compositional variations within the extracted DOM (Schwede-Thomas et al., 2005; Esteves et al., 2007; Mopper et al., 2007). Common UF methods for isolating DOM by size include tangential-flow and cross-flow UF, both of which can isolate LMW and HMW fractions (Mopper et al., 2007). Typically, a membrane with a MW-cutoff of 1-10 kDa is used in UF, and the isolated colloidal NOM or HMW-DOM represents the fraction obtained with reported efficiencies for these methods ranging from 20 to 90% of the total DOM (Hedges et al., 2000; Kaiser¹ and Sulzberger, 2004; Kaiser et al., 2004; Schwede-Thomas et al., 2005). In some cases, the <1 kDa fraction is collected to explore the characteristics of smaller DOM components (Schwede-Thomas et al., 2005).

For SPE of DOM, three main types of sorbents are typically used: polymeric resins (e.g. XADTM, PPL, HLB), alkyl- and aryl-silicas (e.g. C18-functionalized silica), and ion-exchange sorbents. Hydrophobic sorbents, in particular, often require pre-activation by

passing polar solvents such as methanol or acetonitrile through the sorbent material. Additionally, acidifying the DOM sample prior to extraction is a common practice to enhance the recovery of phenolic and carboxylic-rich compounds, given their sorption is more efficient at lower pH levels. However, two main issues are associated with SPE methods: (1) the potential leaching of material from the sorbent, which can contaminate the extracted DOM sample, and (2) the effects of sample acidification, which may alter the composition of DOM (Dalzell et al., 2009; Pace et al., 2012; Sandron et al., 2015). Since different sorbents exhibit varying affinities for isolating specific classes of DOM compounds, it is important to recognize that no single SPE method can fully extract all components of DOM from a given sample. Nevertheless, the cost-effectiveness, high extraction efficiency, and relatively short processing times of SPE, along with the ability to selectively target specific DOM fractions by choosing appropriate sorbents, make it a widely used and practical technique despite its limitations (Green et al., 2014; Li et al., 2017). Sorbents such as PPL, C18, and HLB resins are known to capture a broad range of DOM moieties in a single extraction (Dittmar et al., 2008; Perminova et al., 2014; Raeke et al., 2016; Li et al., 2017), and several studies have compared the impact of different SPE sorbents on DOM composition (Minor et al., 2014; Sandron et al., 2015; Li et al., 2017; Yang et al., 2017). For instance, Li et al. (2017) found C18 resin preferentially extracted more saturated DOM components, characterized by higher H/C and lower O/C ratios, compared to PPL. In contrast, HLB resins retained DOM with a higher O/C ratio than the C18 extracts. While both UF and SPE typically involve freeze-drying the extracted DOM before storage, Sandron et al. (2015) cautioned that rapid freezing could cause structural damage to labile DOM fractions due to the formation of large ice crystals, which may alter the sample's integrity.

Given the complexity of DOM extracts, fractionation methods are employed to separate it into more homogeneous subgroups, facilitating both chemical and structural characterization of its components. Liquid chromatography (LC) is preferred over gas chromatography (GC) in DOM fractionation studies, mainly because DOM consists of HMW, polar, and non-volatile compounds that are generally unsuitable for GC (Mopper et al., 2007). Additionally, LC offers a diverse range of mobile and stationary phases that can separate DOM based on various chemical properties (Minor et al., 2014). High-performance liquid chromatography (HPLC) methods encompass a variety of specific modes, each designed to promote DOM separation based on specific properties such as size, polarity, or charge. In some cases, multi-dimensional approaches are employed,

using more than one separation mode. Some of the most widely used chromatographic techniques for DOM include size-exclusion chromatography (SEC), hydrophilic interaction liquid chromatography (HILIC), ion-exchange chromatography (IEC), normal-phase liquid chromatography (NPLC), and reverse-phase liquid chromatography (RPLC). These methods are often coupled with high-resolution mass spectrometry (HRMS), UV-Visible and fluorescence spectroscopy, or nuclear magnetic resonance (NMR) to characterize the separated fractions. This underscores the importance of selecting the appropriate chromatographic technique for effective separation before analysis. While this review does not focus on DOM separation methods per se, it remains a crucial consideration when conducting DOM characterization studies. Sandron et al. (2015) provided a critical and comprehensive review of DOM separation techniques, covering both liquid and gas chromatography methods, as well as electrophoretic approaches, and addressing the challenges of DOM separation within the context of carbon cycling research in environmental sciences.

II.5. Optical properties of DOM

The optical properties of DOM have proven to be powerful tools for investigating its chemical composition and biogeochemical dynamics. A sizeable fraction of the DOM pool, known as chromophoric DOM (CDOM), absorbs light efficiently in the blue and ultraviolet wavelengths (Stedmon and Nelson, 2015). Within the CDOM fraction, certain moieties, particularly those with rigid structures like aromatic compounds, can absorb light and emit it as fluorescence (FDOM) as they transition from an excited state to a ground state (Cory and Kaplan, 2012; Stedmon and Nelson, 2015). Larger aromatic fluorophores tend to exhibit smaller energetic differences between the excited and ground states, resulting in emission at longer wavelengths, allowing their recognition.

Photodegradation and biodegradation are the primary transformation processes that alter DOM in natural systems, leading to changes in its chemical composition, which are reflected in the fluorescence and absorbance spectra of DOM (Hansen et al., 2016; Kellerman et al., 2018; McCallister et al., 2018). By employing absorption and fluorescence spectroscopy, researchers can monitor these changes to infer DOM origins and the biogeochemical processes responsible for its transformation. These optical methods are widely used in environmental biogeochemical research, providing valuable

insights into DOM dynamics along natural gradients (Gandois et al., 2019; Grunert et al., 2021) or within specific ecosystems (Laurion and Mladenov, 2013; Roiha et al., 2015; Wauthy et al., 2018).

UV-Visible and fluorescence spectroscopy have emerged as complementary and essential tools for characterizing DOM in environmental systems. UV-Visible spectroscopy is a fast, cost-effective method for analyzing the bulk CDOM fraction, providing insights into key DOM properties such as aromaticity and average MW (Weishaar et al., 2003; Helms et al., 2008). Due to its simplicity in sample preparation, high throughput, and widespread availability of spectrophotometers in research institutions and environmental laboratories, UV-Visible spectroscopy is commonly used in large-scale environmental monitoring and time-series studies across diverse aquatic ecosystems, from freshwater to marine (Coble, 1996; Søndergaard et al., 2003; Coble et al., 2014; Stedmon and Nelson, 2015).

On the other hand, molecular fluorescence spectroscopy offers much higher sensitivity, enabling the detection and characterization of specific fluorophores within the FDOM fraction (Coble, 1996; Coble et al., 2014). This sensitivity is particularly valuable for analyzing low-concentration samples and provides detailed information on the source, composition, and dynamics of DOM in aquatic systems (Murphy et al., 2013). The multi-dimensional nature of fluorescence data, especially in the form of Excitation-Emission Matrices (EEMs), allows for advanced analytical techniques like Parallel Factor Analysis (PARAFAC), which aids in identifying components and tracing their sources (Stedmon and Bro, 2008).

Together, these techniques have greatly advanced our understanding of DOM properties and their environmental significance. While UV-Visible spectroscopy provides foundational insights into bulk DOM properties, fluorescence spectroscopy offers deeper analysis into DOM composition and transformations. Combining both methods provides a powerful analytical toolkit that has substantially contributed to understanding DOM dynamics, from molecular processes to global carbon cycling (Laurion and Mladenov, 2013; Hansen et al., 2016; Massicotte et al., 2017; Mazoyer et al., 2022).

However, both techniques have limitations. They are sensitive to inner filter effects, light scattering, and interference from inorganic species like Fe (Poulin et al., 2014). Moreover, while these methods offer valuable insights into DOM properties, neither technique provide specific structural or functional details about the DOM mixture. Fluorescence

measurements, in particular, can be affected by quenching effects from metal ions and generally require longer analysis time compared to UV-Visible spectroscopy.

II.5.1 Absorption spectroscopy

UV-Visible spectroscopy has been widely used to characterize CDOM in natural aquatic systems, offering valuable insights into ongoing biogeochemical processes (Forsström et al., 2013; Laurion and Mladenov, 2013; Roiha et al., 2015; Peura et al., 2020). In this section, we focus primarily on the key indicators for DOM chemical composition and their application to research in permafrost-affected systems.

Specific absorption coefficients (a) are commonly employed to quantify CDOM and as proxies for its chemical composition. For instance, a_{250} is often used as a proxy for allochthonous DOM (Tranvik and Bertilsson, 2001; Santos et al., 2016), a_{320} is a general indicator of CDOM quantity in experiments (Laurion et al., 2021; Mazoyer et al., 2022) and a_{350} is associated with lignin and DOC content (Johnston et al., 2019). The shape of the absorption spectrum of CDOM provides additional insight into its chemical composition, and many studies have focused on variations in spectral slopes at specific wavelength intervals to further characterize DOM (Stedmon and Nelson, 2015).

Environmental research into both marine and freshwater systems frequently employs the specific UV absorbance at 254 nm normalized to DOC concentration ($SUVA_{254}$), which correlates with DOM aromaticity and MW (Weishaar et al., 2003; Kellerman et al., 2018; Ward and Cory, 2020). Absorption ratios at specific wavelengths also serve as indicators for CDOM quality. For example, the $E_2:E_3$ ratio (250 to 365 nm) is inversely correlated with DOM molecular size (Guo and Chorover, 2003; Santos et al., 2016), $E_2:E_4$ (254 to 436 nm) estimates the relative composition of autochthonous versus terrestrial DOM (Jaffé et al., 2004; Li and Hur, 2017), and $E_3:E_4$ (330 to 400 nm) correlates negatively with humification (Erlandsson et al., 2012; Li and Hur, 2017). While longer-wavelengths absorption ratios, namely 400-499 nm relative to 600-699 nm, are also used to assess humification degree and composting (Ikeya and Watanabe, 2003), their reliability can be compromised due to poor signal-to-noise ratios at higher wavelengths (Helms et al., 2008).

Helms et al. (2008) focused on spectral slopes (S) at shorter wavelengths, namely 275-295 nm ($S_{275-295}$) and 350-400 nm ($S_{350-400}$), and introduced the slope ratio (S_R) between these spectral regions. Both $S_{275-295}$ and S_R are negatively correlated with DOM MW

(Ward and Cory, 2015, 2016). Loiselle et al. (2009) expanded on spectral slope variation by using the absorption spectral slope curve (S_λ) to track changes in DOM and associated biogeochemical processes (Laurion and Mladenov, 2013; Roiha et al., 2015; Li and Hur, 2017). The maxima of spectral slopes can also aid in DOM characterization, with specific slopes such as S_{382} linked to terrestrial moieties, and S_{289} associated with algal-derived carbon (Galgani et al., 2011; Roiha et al., 2015; Wauthy et al., 2018). However, high concentrations of Fe in DOM samples can reduce absorption spectral slopes, complicating the interpretation of CDOM in environments with varying Fe concentrations (Xiao et al., 2013). To address this, Poulin et al. (2014) proposed corrections for absorption spectra to minimize the effects of Fe interference. Additionally, nitrate has been shown to interfere with DOM absorption spectra (in the range 220–302 nm), affecting absorption coefficients and $S_{275-295}$ values (Li and Hur, 2017).

As previously discussed, photodegradation of DOM induces changes to its chemical composition, particularly the preferential transformation of aromatic moieties (Cory et al., 2007, 2014). This process leads to a loss of aromaticity and a decrease in DOM MW (Helms et al., 2008; Ward and Cory, 2016). Photodegradation results in the reduction of CDOM, especially at longer wavelengths, which is reflected in higher $S_{275-295}$ and S_R values and lower $SUVA_{254}$ values (Ward and Cory, 2016; Lee et al., 2018; Xu et al., 2018; Nevalainen et al., 2020; Mazoyer et al., 2022). After photodegradation, increases in bacterial respiration and autochthonous DOM are often observed, suggesting the formation of more labile DOM components (Ward and Cory, 2015, 2020). In contrast, microbial degradation typically increases $SUVA_{254}$ and decreases $S_{275-295}$ values, favoring the transformation of non-aromatic structures or other labile DOM moieties into more aromatic components (Hansen et al., 2016; Lee et al., 2018; Xu et al., 2018). Higher E_2/E_3 values have also been associated with increased contributions from LMW autochthonous DOM (Bowen et al., 2020).

These optical indicators are valuable for tracing DOM sources and understanding the underlying biogeochemical processes in natural systems. However, interpreting these indicators can be complex, as they reflect averaged properties of a diverse mixture of molecules. For instance, during spring freshet, aquatic systems with strong terrestrial DOM inputs show significant increases in S_R values when the DOM undergoes photodegradation, indicating a reduction in MW (Helms et al., 2008; Ward and Cory, 2016; Gareis and Lesack, 2018). Terrestrial CDOM typically displays S_R values below 1, while photodegraded terrestrial CDOM and more labile DOM exhibit $S_R > 1$ (Guéguen

and Cuss, 2011). Notably, as photodegradation progresses, biodegradation tends to further decrease S_R values (Bowen et al., 2020). Ward & Cory (2016) investigated the effect of photodegradation on DOM leached from organic mat soils and permafrost DOM, finding a 1.5-fold greater increase in S_R values for organic mat DOM compared to permafrost DOM. However, when light-normalized, the increase was notably higher in permafrost DOM.

Roiha et al. (2015) examined the seasonal effects on DOM composition in heterotrophic subarctic thermokarst ponds, using absorption spectroscopy to monitor changes in CDOM properties. Summer DOM showed higher aromaticity (higher $SUVA_{254}$ and a_{320}) and greater abundance of algal-derived DOM in surface waters (higher S_{289} values, particularly in the most transparent pond), along with stronger terrestrial inputs in bottom waters (higher S_{382} values). In contrast, winter DOM showed lower aromaticity and CDOM, despite higher DOC concentrations. The idea of a stronger presence of DOM-Fe complexes during the summer has also been proposed (Roiha et al., 2015). Thermokarst ponds in subarctic peatland regions typically show higher a_{320} (or a_{440}) values, along with higher $SUVA_{254}$, and lower S_{289} and S_R values compared to bedrock catchments and tundra ponds unaffected by thermokarst processes (Wauthy et al., 2018). In Arctic rivers during spring freshet, increased terrestrial inputs from soil also lead to higher $SUVA_{254}$ and lower S_R values (Mann et al., 2012; Lin et al., 2021). This suggests that permafrost thawing mobilizes substantial amounts of terrestrial DOM from surrounding soils.

II.5.2 Fluorescence spectroscopy

Fluorescence spectroscopy has emerged as a powerful tool for investigating composition and biogeochemical dynamics of DOM in natural systems (Cory et al., 2007; Wauthy et al., 2018; Lin et al., 2021). This technique relies on the excitation of fluorophores to a singlet state, followed by the measurement of emitted light as they return to the ground state (Lakowicz, 2006; Coble et al., 2014). The resulting data is often presented as an excitation-emission matrix (EEM), where the fluorescence intensity is plotted across a range of emission wavelengths for corresponding excitation wavelengths. Specific regions within the EEM are associated to distinct types of fluorophores (Coble, 1996; Stedmon et al., 2003). To ensure reliable measurements, it is crucial to apply certain corrections, such as inner filter adjustments, spectral calibration, and intensity normalization.

Fluorescence EEMs of natural waters typically display two main types of signals: humic-like and protein-like (Coble et al., 2014; Hansen et al., 2016). Protein-like signals are generally associated to amino acids in proteins, namely tyrosine and tryptophan. Humic-like signals, on the other hand, are more prevalent and are commonly linked to terrestrial-derived fluorophores in DOM, although specific compounds within this group remain unidentified (Stedmon et al., 2003; Murphy et al., 2013). Interestingly, dark incubation of pond water has revealed microbial production of humic-like fluorophores, suggesting that the traditional classification of these compounds may need to be revisited (Laurion et al., 2021; Mazoyer et al., 2022). Fluorescence peaks obtained through PARAFAC analysis are typically labeled with either a letter or a number. In marine and coastal waters, the fluorescence peaks most commonly associated with humic substances are designated as peaks A, C, and M, while the primary protein-like fluorescence peaks are referred to as peaks B and T (Fig. II.3) (Coble, 1996; Coble et al., 2014). Freshwater studies more frequently assign numerical labels to these peaks, although due to variations in the PARAFAC models used and the number of components extracted (from 4 to 8), these numerical labels often do not align across different studies, making comparison challenging.

The use of fluorescence intensity ratios from specific peaks in EEMs has proven to be a valuable approach for gaining insights into changes in the source, composition, and dynamics of FDOM in aquatic systems (Hansen et al., 2016; Coble et al., 2014). Among the most commonly used intensity-ratio indices are the Fluorescence Index (FI), calculated as the ratio of emission at 470 nm to 520 nm, with excitation at 370 nm. This index is useful for distinguishing between microbial and terrestrial contributions to the DOM mixture (McKnight et al., 2001; Cory et al., 2010). Another widely employed index is the Humification Index (HIX), which is calculated by dividing the area under the emission wavelengths 435–480 nm by the sum of the areas of emission wavelengths 300–345 nm and 435–480 nm, with excitation at 254 nm. HIX serves as an indicator of the extent of humification in the DOM pool (Zsolnay et al., 1999; Ohno, 2002). Other fluorescence-based indices, such as the Biological Index (BIX) (Huguet et al., 2009), Freshness Index (β/α) (Wilson and Xenopoulos, 2009), and peak ratios like C/T and A/T (Hansen et al., 2016), have also been employed to characterize DOM, focusing primarily on the relative contributions of fresh, autochthonous versus terrestrial, allochthonous sources (Fig. II.3).

Due to their differing chemical compositions, DOM from various sources exhibit distinct behaviors when subjected to microbial transformation and photodegradation. For example, photodegradation of DOM in freshwaters has been shown to reduce overall fluorescence, particularly affecting humic-like fluorophores (Coble et al., 2014; Mazoyer et al., 2022). During spring freshet, higher discharges lead to increased humic-like fluorescence and generally lower FI values, reflecting the leaching of terrestrial DOM from soil organic layers (Lin et al., 2021; Mann et al., 2012). These natural processes result in variations in DOM composition across different aquatic ecosystems, as reflected in their absorbance and fluorescence spectra. For instance, High Arctic ponds have been found to exhibit higher BIX and lower HIX values compared to High Arctic rivers, likely due to greater contributions of labile DOM derived from thawing permafrost (Wang et al., 2018).

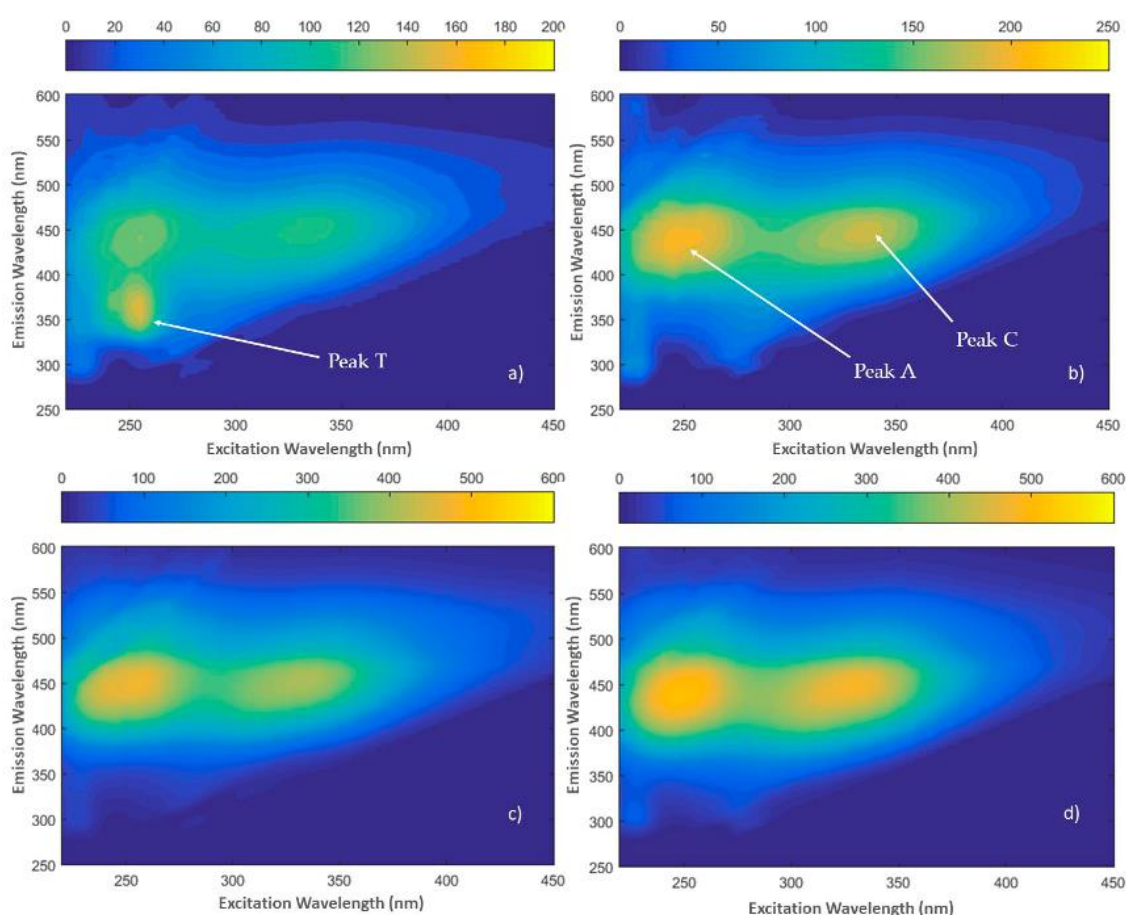


Figure II.3. Typical fluorescence excitation–emission matrices (EEMs) of water samples from two thermokarst lakes in Nunavik (Canada) after acidification, showing the presence of the A, C and T peaks. (a) Lake 1 surface water; (b) Lake 1 bottom waters; (c) Lake 2 surface waters; (d) Lake 2 bottom (Data Folhas, D. *unpublished*)

Biodegradation of DOM from different sources has been shown to reduce DOC concentrations, with algal-derived DOM being more susceptible to degradation compared to soil-derived DOM (Hansen et al., 2016; Lee et al., 2018). Biodegradation of soil-derived DOM typically result in minimal changes to HIX, FI and BIX values. In contrast, incubation experiments on plant leachates and algal-derived DOM have reportedly resulted in higher FI and BIX values, as well as an increase in HIX, likely due to the formation of more highly conjugated organic structures during microbial transformation (Hansen et al., 2016; Lee et al., 2018). Additionally, microbial degradation consistently leads to the removal of protein-like fluorescence components from DOM samples (Hansen et al., 2016; Lee et al., 2018).

Photodegradation impacts on DOC concentrations have varied, with some studies reporting only slight decreases (below 3%) in plant, algae and soil-derived DOM (Hansen et al., 2016), while others have observed more pronounced reductions as in Suwannee River Fulvic Acid (SRFA; 22% decrease) (Hur et al., 2011). However, studies on the effects of photodegradation on DOM composition from different sources have produced contradictory results. For instance, Lee et al. (2018) noted a more pronounced decrease in HIX values for SRFA (a more terrestrial sample) than for algae-derived DOM, and higher increases in FI and BIX values for algal-derived DOM compared to SRFA. On the other hand, Hansen et al. (2016) found a stronger reduction in HIX values of plant and algae-derived DOM compared to soil leachates, as well as a decrease in FI values across all DOM types. These contrasting findings likely stem from different experimental conditions, where Hansen et al. focused on microbial consumption of the labile fraction during the experiment, leading to lower FI values (Hansen et al., 2016), while Lee et al. (2018) focused solely on photodegradation, which primarily removed aromatic moieties and increased FI values. Additionally, photodegradation preferentially removes humic-like fluorophores over protein-like fluorophores, supporting the idea of a photochemical removal of humic substances (Du et al., 2016; Lee et al., 2018).

Both photodegradation and biodegradation processes in natural systems often mask each other's signals, complicating the direct use of optical indices for source discrimination. These processes require careful consideration when interpreting fluorescence data (Hansen et al., 2016; Du et al., 2016; Lee et al., 2018). Challenges in controlling bacterial abundance during experiments on photochemical degradation may also contribute to conflicting results, as bacteria are not easily removed by simple filtration (Mazoyer et al., 2022).

The role of particulate organic matter (POM) in DOM composition is often overlooked in studies of OM biogeochemistry. After irradiation, losses of POM can occur, as it absorbs light and undergoes photo-transformation processes, similar to DOM. This results in the photo-dissolution of POM and increases in DOC values (Hu et al., 2022). Photo-released DOM from POM is typically enriched in humic-like substances, as the process involves both the photoproduction and photodegradation of DOM in aquatic systems (Hu et al., 2020). The chemical composition and sources of POM strongly influence the composition of newly produced DOM. Photo-released DOM from soils is reportedly rich in humic-like components, while DOM from litter tends to be dominated by protein-like components (Lee et al., 2019). Additionally, the concurrent increase of humic-like components and metal ions such as Fe^{3+} and NO_3^- suggests their role in the formation of hydroxyl radicals, which drive the photoproduction of DOM (Hu et al., 2020, 2022). Photo-released DOM is often positively correlated with reducible Fe oxides and NOM-Fe complexes in POM, as well as photo-dissolved Fe. The dominant mechanism for this transformation is thought to be the photochemical degradation of NOM-Fe into dissolved Fe and DOM (Hu et al., 2021).

To extract more detailed information from EEMs, researchers have turned to statistical approaches such as PARAFAC, which decomposes EEMs into individual fluorescence components (Bro, 1997; Stedmon and Bro, 2008). This method not only provides a more thorough characterization of DOM fluorescence but also allows for the tracking of specific DOM fractions in natural systems (Stedmon and Markager, 2005; Murphy et al., 2013). However, because PARAFAC fits fluorescence data into a model, humic-like components are often expressed with dual peaks at both shorter and longer emission wavelengths (Stedmon et al., 2003; Coble et al., 2014). For example, research on the Yukon River has revealed insights into the microbial production of humic substances (which are optically indistinguishable from terrestrial sources) and their relationship with DOM size (Grunert et al., 2021). Another study also highlighted the seasonal dynamics of HMW-DOM and LWM-DOM in the Yukon River, providing valuable information on the complex interactions between these two fractions (Lin et al., 2021).

Studies on thermokarst lakes using PARAFAC have further provided insights into DOM sources, biogeochemistry, and lake ontogeny. Younger thermokarst lakes exhibit stronger terrestrial DOM inputs and increased microbial degradation of complex DOM moieties, while more mature lakes show biodegradation potential mainly in deeper, anoxic waters (Peura et al., 2020). This suggests that microbial communities adapt to environmental

conditions as lakes progress through succession. The relative impact of NOM inputs on DOM composition in permafrost thermokarst lakes has revealed that terrestrial contributions are much more significant than those from phytoplankton or benthic sources, possibly due to soil erosion in the catchment area (Deshpande et al., 2017; Peura et al., 2020). In winter, DOM collected under the ice cover of a thermokarst lake in a subarctic peatland showed high photoreactivity, with the bacterial community strongly stimulated by sunlight as more labile compounds are produced (Mazoyer et al., 2022).

II.6. Structural and chemical composition of NOM

Although optical tools have proven effective for assessing the biogeochemical processes of DOM, they primarily rely on the behavior of CDOM and FDOM pools, often overlooking the role of non-chromophoric DOM moieties in these processes. As such, a more comprehensive understanding of DOM composition requires structural characterization of the functional groups within NOM, which can provide insights into its sources and the biogeochemical processes at play. However, given the heterogeneous nature and structurally complex nature of NOM, such characterization remains a challenging task (Sillanpää et al., 2015).

Fourier-transform Infrared spectroscopy (FTIR) is a widely used technique that measures the absorption of infrared light by chemical bonds, producing spectra that allow for the identification of functional groups within NOM and the detection of specific compounds. Despite its versatility, FTIR spectra often exhibit significant peak overlap, which complicates the interpretation of complex NOM mixtures.

On the other hand, Nuclear Magnetic Resonance (NMR) spectroscopy focuses on the response of atomic nuclei to a magnetic field, offering valuable information on the molecular structure of NOM. While 1D NMR experiments (such as ^1H -NMR and ^{13}C -NMR) can also suffer from peak overlap, 2D NMR techniques—such as heteronuclear single quantum correlation (HSQC)—allow for the correlation of specific carbon atoms with their directly attached protons, enhancing the clarity of structural data.

Both FTIR and NMR have complementary strengths: FTIR offers rapid, non-destructive, and cost-effective analysis, with minimal sample preparation and broad applicability to a wide range of sample types. However, FTIR's limited structural resolution and peak overlap in complex mixtures can make spectral interpretation challenging. In contrast,

NMR spectroscopy provides higher resolution and a more detailed structural characterization of NOM, facilitating the quantitative analysis of various molecular components. The ability of NMR to focus on specific nuclei (e.g., ^1H , ^{13}C , ^{15}N) allows researchers to explore internuclear relationships within the NOM mixture through multidimensional experiments, offering deeper insights into its composition (Hertkorn et al., 2013). However, NMR requires larger sample quantities, more extensive sample preparation, and specialized expertise, making it a more expensive and time-consuming technique compared to FTIR (Mopper et al., 2007; Minor et al., 2014). When possible, the integration of both techniques can provide a more comprehensive characterization of NOM leveraging the advantages of both methods.

Mass spectrometry (MS) offers significant advantages for NOM characterization by providing high sensitivity and precision in identifying the molecular mass and composition of NOM compounds, as well as the relative abundance of different species (Mopper et al., 2007). MS, when coupled with chromatographic separation methods, enables high-throughput analysis of large numbers of samples, making it particularly valuable in environmental studies. However, analyzing complex, heterogeneous NOM mixtures with MS can be challenging due to extensive fragmentation during analysis, which can obscure compound identification. Moreover, careful optimization is required to detect both low and highly abundant compounds, while minimizing matrix effects and contamination interference. Ionization efficiency is another critical factor, as certain NOM components may not ionize effectively, leading to the loss of specific fractions during analysis. While MS excels at identifying molecular formulas and characterizing compound composition, it provides limited insight into the functional groups and molecular interactions within NOM. In contrast, FTIR and NMR provide detailed structural information, making them complementary techniques to MS. In permafrost research, the combined use of MS and FTIR or NMR has proven invaluable in elucidating the biogeochemical processes at work in complex environmental systems (Pautler et al., 2010; Hodgkins et al., 2014; Mann et al., 2015; Ward and Cory, 2015; Grewer et al., 2016; Wilson et al., 2022).

II.6.1 Fourier-transform infrared spectroscopy

Characterization using FTIR spectroscopy is based on the absorption of infrared light by the chemical bonds within NOM, with the absorbed energy corresponding to the

vibrational frequencies of those bonds, producing an absorption spectrum. This spectrum can then be used to identify organic functional groups within the NOM sample. Unlike other methods, FTIR allows for the study of bulk samples, thus avoiding potential alterations in chemical composition that may occur during sample extraction processes (Schmidt et al., 2011). This feature makes FTIR particularly valuable for investigating NOM from soils and sediments. Although spectral overlap can present challenges in interpretation, FTIR spectra provide valuable insights into specific functional groups, such as alkyl and carboxyl groups, which can reveal information about ongoing biogeochemical processes [105]. As such, FTIR spectroscopy has become a widely employed tool in the study of SOM and the factors influencing its chemical composition (Hodgkins et al., 2014, 2018; Heller et al., 2015; Wilson et al., 2022).

For example, undrained peatlands in temperate regions are typically rich in aliphatic compounds, which are likely preserved under anaerobic conditions. In contrast, increased drainage leads to a reduction in C-H bonds (2920 and 2860 cm^{-1}) associated with alkyl groups, and an increase in C=O absorption (1700 and 1610 cm^{-1}) linked to carboxyl groups, suggesting aerobic degradation processes (Heller et al., 2015; Harris et al., 2023). Additionally, peat from fens tends to exhibit lower C-H absorption compared to peat bogs, indicating a lower degree of degradation in the nutrient-poor ombrotrophic bogs when compared to the more nutrient-rich fens (Fig. II.4) (Heller et al., 2015).

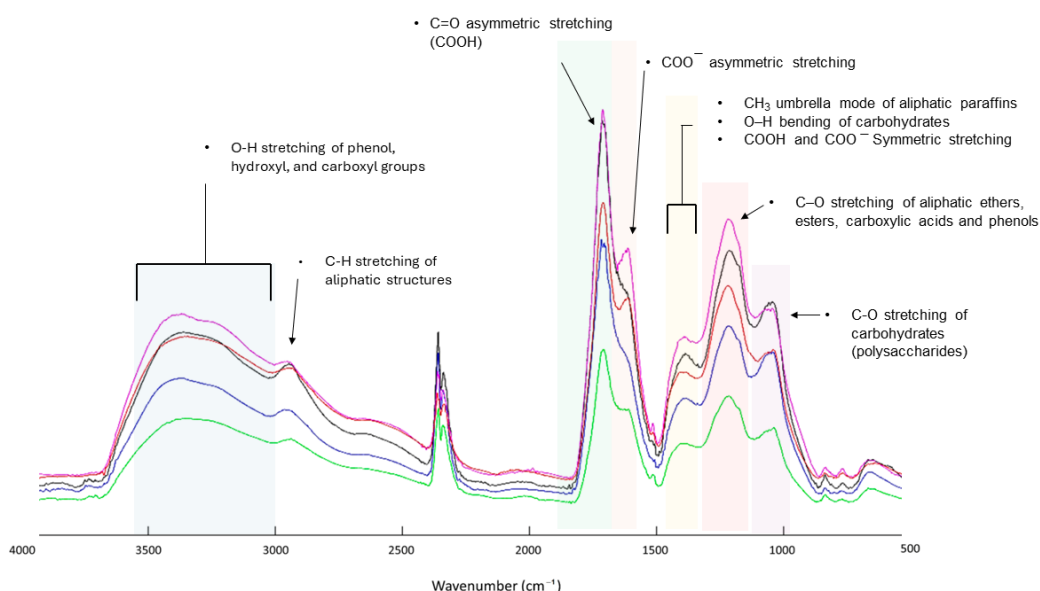


Figure II.4. Typical Fourier-transform infrared spectroscopy–attenuated total reflectance (FTIR-ATR) spectra of PPL-DOM water samples from thermokarst lakes in Nunavik, Canada. Black: lake 1 bottom

water; Red: lake 3 middle water; Blue: lake 1 surface water; Green: lake 2 bottom water; Magenta: lake 2 surface water; Red: lake 3 middle water (Folhas, D., *unpublished data*).

Peat soils from different latitudes exhibit contrasts in their SOM composition, primarily driven by climatic factors. In lower-latitude regions, upper peat layers typically show higher aromatic content (~ 1500 and ~ 1600 cm^{-1} bands) and lower carbohydrate content (~ 1030 cm^{-1}), compared to near-surface peat from northern regions, despite a noticeable decline in carbohydrate content with depth (Hodgkins et al., 2018). In northern regions, colder temperatures and seasonally frozen soils result in slower peat decomposition, whereas at lower latitudes, the high aromatic content and reduced oxidation state of peat contributes to its more recalcitrant nature, despite warmer temperatures (Hodgkins et al., 2018).

Changes in peat soils along a thaw progression, from collapsed palsa to *Sphagnum*-dominated bog to bog with *Sphagnum* and Sedges and further to Sedge-dominated fens, suggest a loss of carboxylic acid bands (often due to higher pH, keeping these moieties as undetected carboxylate anions) and a decrease in aromatic structures and oxygen content. This progression is accompanied by a gradual shift towards LMW DOM and increased microbial DOM (Hodgkins et al., 2014).

Furthermore, GHG production along this thaw progression suggests increased CO_2 and CH_4 production, particularly the latter (Hodgkins et al., 2014). Depth variations in SOM composition are especially pronounced in fen peat compared to bogs or collapsed palsas. Fen surface peat tends to have higher polysaccharide content (primarily cellulose from plant material), and lower lignin, aromatic, and aliphatic content than deeper, more humified peat layers (Hodgkins et al., 2014).

A study by Wilson et al. (2022) in a permafrost thaw gradient (Palsa $\rightarrow$ Bog $\rightarrow$ Fen) observed proximity in chemical composition between palsa peat SOM and lichen NOM, bog peat SOM and *Sphagnum*-derived NOM, and fen peat SOM and *Eriophorum*-derived NOM. The fen peat showed stronger aromatic and protein-like peaks and lower carboxylic content, while bog peat had a higher carbohydrate and lower aliphatic content and waxy lipids, suggesting an accumulation of polysaccharides (i.e. carbohydrates) in *Sphagnum*-dominated peat. To assess degradation differences between samples and sites, specific spectral ratios comparisons are used, such as 2920 cm^{-1} : 1030 cm^{-1} (alkyl C: O-alkyl C), 1630 cm^{-1} : 1030 cm^{-1} (aromatic C: O-alkyl C), and 1515 : 1030 cm^{-1} (lignin

residue: O-alkyl C), since O-alkyl-C moieties are reliable proxies for soil decomposition rate (Tfaily et al., 2014; Harris et al., 2023).

In their 2019 study, Liu et al. (2019) explored the photodegradation of algal DOM and macrophyte DOM (water chestnut), finding that biodegradation dominated the removal of DOM, while photodegradation played a smaller role in both cases. Algal DOM (*Chlorella*) was mainly composed of polysaccharides, proteins, and phenols, with small contributions from humic substances. Both light and dark experiments led to similar spectral changes, primarily the loss of polysaccharides (1061 cm^{-1}) and proteins, phenols and alcohols (3274 cm^{-1}). Similarly, macrophyte DOM also consisted mainly of polysaccharides, proteins, and phenols, with minimal humic substances. Under both light and dark conditions, the spectral data showed a reduction in polysaccharides and proteins, phenols and alcohols. Additionally, the peak at 1579 cm^{-1} increased and exhibited a blue shift, suggesting the production of carboxylic moieties likely due to the synthesis of proteins during incubation.

Kim et al. (2017) investigated the chemical changes in soil-derived DOM ($<0.2\text{ }\mu\text{m}$) and colloidal NOM ($0.2\text{--}1.2\text{ }\mu\text{m}$) under controlled freeze-thaw conditions. Their finding showed that freeze-thaw cycles promoted the consumption of dissolved polysaccharides (or silicates) (1049 cm^{-1}) and the production of aliphatic alcohols (1111 cm^{-1}), while warming conditions led to the opposite trend, actively promoting the humification of DOM.

Heffernan et al., (2020) studied the long-term effects of permafrost thaw on peatland soils in a sporadic and discontinuous permafrost region. They assessed the degree of peat humification using peak ratios between the 1630 cm^{-1} and 1090 cm^{-1} bands to quantify the relative abundance of aromatic and carboxylate moieties compared to polysaccharides (Broder et al., 2012; Harris et al., 2023). A higher aromatic/polysaccharide ratio was associated with increased peat humification. In young and mature bogs, deep peat layers exhibited low potential enzyme activities due to their highly humified nature, which reflected the stability of older peat following permafrost thaw. In contrast, near-surface peat in young bogs displayed higher potential enzyme activity compared to mature bogs. This difference was likely due to variations in the SOM quality between the sites (likely driven by differences in vegetation), rather than promoted by oxygen availability (Heffernan et al., 2021).

II.6.2 Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance (NMR) spectroscopy has been a vital tool to investigate the chemical composition of NOM in a wide range of environments (Hertkorn et al., 2006; Normand et al., 2017; Wang et al., 2018; Gagné et al., 2020; Heslop et al., 2021). In this section we will not explore the differences between liquid-state and solid-state NMR, rather focusing on the application of NMR for structural characterization of NOM mixtures (Fig. II.5). The two primary NMR experiments conducted for NOM characterization are ^1H NMR and ^{13}C NMR. The ^1H NMR spectra, given the abundance in protons, can provide information on structural proton distribution within NOM samples, with certain spectral regions associated with specific types of structural groups (Ma et al., 2001; Hertkorn et al., 2006; Sillanpää et al., 2015). The chemical shift range of δ_{H} : 0.0-1.6 ppm is associated with protons on methyl and methylene groups, also referred to as materials derived from linear terpenoids (MDLT); δ_{H} : 1.6-3.2 ppm comprises the protons of methyl and methylene carbons alpha to aromatic, carboxy and carbonyl groups, also described as a fraction containing carboxyl-rich alicyclic molecules (CRAM); δ_{H} : 3.2-4.3 ppm, associated with protons in hydroxyl, ether and esters, as well as protons in methyl, methylene and methyne groups directly-bonded to N and O (i.e. carbohydrates and peptides); δ_{H} : 6.0-8.5 ppm, generally linked to aromatic protons from unsaturated structures like quinones, phenols, oxygen-rich heteroaromatics (Woods et al., 2012; Wang et al., 2018; Heslop et al., 2021).

Similarly, the ^{13}C NMR spectra of NOM tends to display specific chemical shift regions associated with different functional groups. In SOM samples the chemical shift range of δ_{C} : 0-50 ppm is linked to saturated aliphatics like waxy lipids (alkyl C); δ_{C} : 50-75 ppm, associated with methoxyl groups; δ_{C} : 75-112 ppm, encompasses the range associated with carbohydrates (RC-OH) and RC-OR moieties; δ_{C} : 112-145 ppm is typically linked to aromatic structures; δ_{C} : 145-163 ppm, however is associated with phenolic groups; and finally the δ_{C} : 163-180 ppm range is related to carboxylic, carbonyl, amine and ester carbons. Applications of ^{13}C NMR techniques have included studies from plant litter to peat organic matter composition, as well as compositional changes across a gradient and in depth (Tfaily et al., 2014; Ejarque and Abakumov, 2016; Gagné et al., 2020).

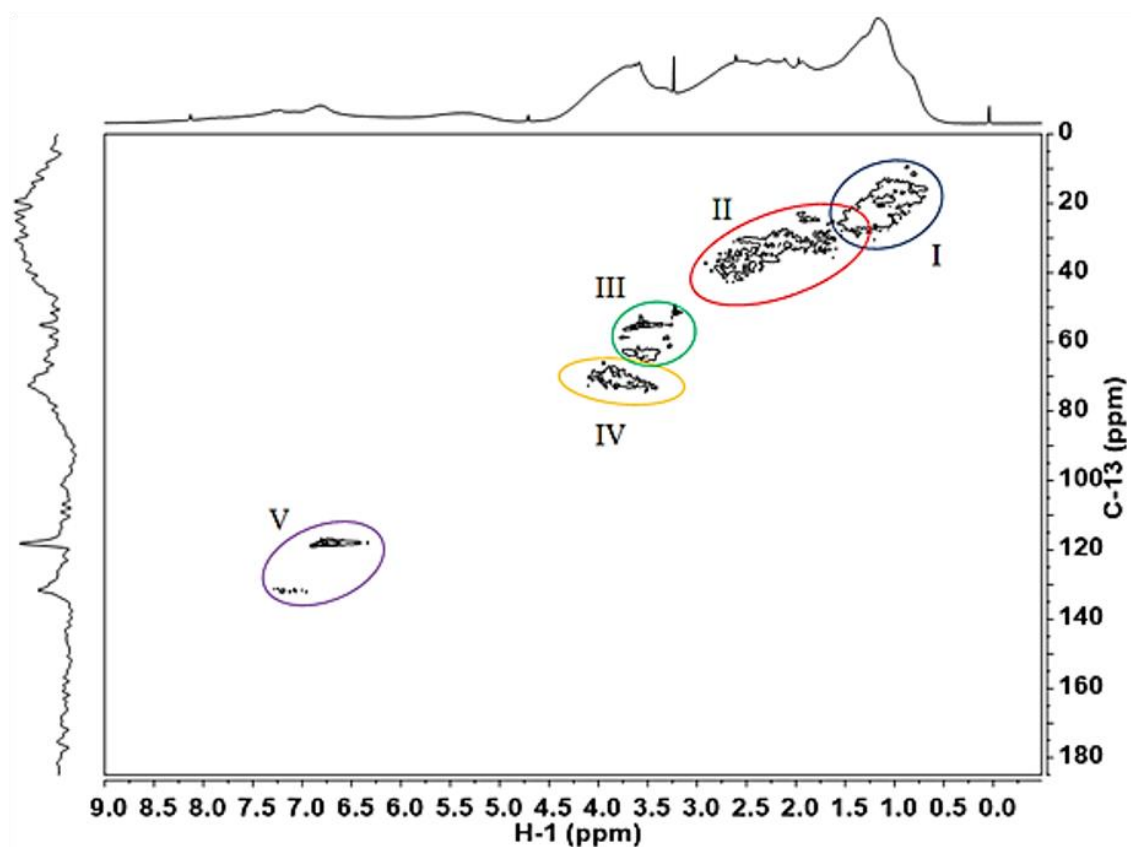


Figure II.5. ^1H - ^{13}C HSQC NMR spectra of PPL-DOM surface thermokarst lake water. Five specific spectral regions are highlighted in the spectrum: Region I (blue): Aliphatic moieties; Region II (red): CRAM fraction; Region III (green): ethers and esters; Region IV (yellow): carbohydrate moieties; Region V (purple): aromatic molecules – Data from Folhas et al., (2020).

Given the severe overlap in one-dimensional ^1H and ^{13}C NMR spectra of NOM due to its highly heterogeneous and complex nature, employing multidimensional NMR techniques can provide additional help in structural elucidation, ultimately being more appropriate for NOM characterization (Deshmukh et al., 2007; Sillanpää et al., 2015). Although NMR can be performed as liquid-state, most NOM characterization is conducted using solid-state NMR spectroscopy, especially in the study of SOM (Preston, 1996). The high content of SOM in soil samples can allow for several ^{13}C experiments developed in recent years, namely the use of spectral editing techniques on overlapping peaks, ultimately facilitating the interpretation of one-dimensional NMR spectra (Mao et al., 2011, 2017; Sillanpää et al., 2015). Combination of NMR spectroscopy with other types of characterization, such as elemental composition measurements, fluorescence or FTIR spectroscopy, is widely used to gain a better understanding of the characteristics of NOM (Heller et al., 2015; Wang et al., 2018; Gagné et al., 2020; Heslop et al., 2021).

Permafrost disturbances like active layer detachment slides (ALD) in Arctic rivers and ponds have been shown to promote the release of labile moieties, ultimately prompting microbial transformation of previously inaccessible SOM pools (Pautler et al., 2010; Abbott et al., 2015). Nevertheless, while ALDs in northern aquatic systems have been shown to increase the input of carbohydrate content in DOM even 1 year after the disturbance (Woods et al., 2011), over time these labile components are depleted and accompanied by an increase in CRAM and MDLT moieties increase, suggesting that while ALD impacts are not long-term, they promote microbial degradation (Wang et al., 2018). This is further substantiated by Heslop et al. (2021) verifying DOM from ponds affected by ALDs displayed lower DOC and higher autochthonous DOM compared to unaffected ponds, where DOM composition was more heavily influenced by allochthonous sources (i.e. modern vegetation).

Wang et al. (2018) explored how ALDs affect the biogeochemistry and composition of DOM from Arctic ponds and rivers. While all DOM samples were mainly dominated by MDLT (aliphatics), CRAM (carboxylic moieties) and carbohydrate structures, ALD-disturbed ponds displayed significantly older ^{14}C DOC ages, increased CRAM structures and lower carbohydrate content. Arctic ponds showed higher autochthonous contributions and lower MDLT content than arctic rivers, with disturbed ponds displaying more oxidized structures, indicative of stimulated microbial activity. Using diffusion edited ^1H NMR experiments, DOM macromolecular aggregates have been found to be more enriched in carbohydrates and reduced in both CRAM and MDLT structures, suggesting the latter are predominantly smaller molecules (Wang et al., 2018).

Gagné et al. (2020) explored the seasonal differences in DOM composition from thermokarst lakes in Alaska, as well the leachates from active layer soils and Yedoma permafrost to assess their impact on the lakes. While winter DOM in the least active lake seemed to showcase a greater content in substituted aromatics, there was an uptick in the aliphatic and the overall aromatic content in the summer. However, this seasonal variation in DOM composition was minimal in the much more active Goldstream lake, with the high O-alkyl content in the lake being attributed to inputs from the carbohydrate-rich active layer rather than permafrost. Additionally, isolates from older permafrost seemed to exhibit stronger carbohydrate/O-alkyl C (^{13}C : 60-90 ppm) and anomeric C (^{13}C : 110 ppm) signals, while the youngest permafrost displayed the highest aromatic and aliphatic content, as well as showing the most photoreactivity and photolability.

Increased carbohydrate content in soils has been linked with undisturbed permafrost, indicating that a lower carbohydrate content prompts an increase in microbial activity in exposed permafrost as a result from active layer detachment (Pautler et al., 2010).

Tendentially, SOM composition in Arctic soils is thought to showcase a less decomposed character (Ejarque et al., 2016) heavily reflecting the chemical composition of the precursor material and their organic contributions. Therefore, different vegetation communities will produce changes to SOM quality, for instance, palsa live vegetation has been shown to produce more alkyl moieties, while *Sphagnum* and fen vegetation inputs are more enriched in O-alkyl structures, leading to accumulation of carbohydrates in inundated soils (Normand et al., 2017). In this sense, soils in northern regions have been described as enriched in aliphatic structures, especially O-alkyl and alkyl moieties, while showing a relatively lower aromatic character (Paré and Bedard-Haughn, 2013; Schuur et al., 2015; Ejarque and Abakumov, 2016; Normand et al., 2017). Elevated alkyl content in SOM is linked with the persistence of lipids (leaf cutins and root suberins), or as the byproduct of microbial degradation of labile moieties in the soil (Clemente et al., 2013). Paré and Bedard-Haughn, (2013) found organic surface soils in subarctic and low Canadian Arctic regions to be more enriched in labile SOM than high Arctic soils, revealing higher O-alkyl content and lower quantity of aromatic moieties, attributed to potential higher contributions from lignin-like compounds from tall shrubs at lower latitude regions compared to the high Arctic, reinforcing the notion that vegetation type heavily influences SOM composition and lability, since greater SOM lability was found in organic surface soils rather than mineral surface soils, which displayed higher content in alkyl and aromatic moieties.

Intending to further assess the influence of vegetation on SOM along a thaw gradient (palsa → *Sphagnum* → fens), Normand et al., (2017) utilized FTIR and ¹³C NMR spectroscopy to discern contrasts in SOM composition with depth along the gradient. In all three environments, increases in soil depth had little influence on aromatic, carboxyl and methoxyl groups, unlike O-alkyl and alkyl moieties. While surface soils seemed to indicate a high O-alkyl with low alkyl content in all samples, their variations with depth are significantly different, with palsa soil profiles showing the most pronounced decline in O-alkyl moieties accompanied by an increase in alkyl structures, suggesting aerobic degradation of labile SOM and preservation of alkyl groups. However, the dominance of O-alkyl moieties in *Sphagnum* and fen soils remained at depth, until an increase in alkyl moieties in *Sphagnum* soil (15 cm depth) and fen soil (25 cm depth) indicated an increase

in degradation of SOM like palsa soils. The shift from O-alkyl to alkyl structures (possibly due to earlier aerobic microbial degradation and subsequent preservation of alkyl structures) combined with the similarity in C/N ratios and $\delta^{15}\text{N}$ soil profiles in palsa, *Sphagnum* and fen soils at progressively greater depths, suggested a shift in both vegetation communities and hydraulic conditions with the passage of time.

Soils from Gydan Peninsula, Russia, showed great regional and vertical homogeneity, particularly in aromatic and carboxyl-carbonyl groups, while most variability was found in aliphatic structures, namely methoxyl, O-alkyl and alkyl content (Ejarque et al., 2016). Utilizing both FTIR and ^{13}C NMR spectroscopy, Pedersen et al., (2011) found that drained thermokarst lake basins in Alaska to be enriched in O-alkyl structures at the surface, while deeper layers show an increase in the alkyl/O-alkyl ratio accompanied by an increase in aromatic content, suggesting the degradation of carbohydrates with depth and preservation of alkyl structures.

Examining leached DOM composition from the organic mat (active layer) and permafrost soil layer, Ward and Cory, (2015) observed significant differences between both DOM pools. Shallow organic mat DOM had greater aromaticity, higher MW and was more oxidized and of unsaturated character compared to deeper permafrost DOM, which is in accordance with literature from Arctic and subarctic soils (Tfaily et al., 2013; Hodgkins et al., 2014). Organic mat DOM displayed greater aromatic content, with an aromatic to aliphatic content ratio 29% higher than permafrost DOM, as well as greater content in oxygen-containing functional groups (i.e. aldehydes, ketones and carboxyl moieties) and a lower carbohydrate content than permafrost DOM (Ward and Cory, 2015). Conservative estimates showed no difference in bacterial respiration rates between organic mat DOM and permafrost DOM, suggesting these shifts in DOM composition may not directly produce shifts in bacterial respiration (Ward and Cory, 2015).

Expanding on the effects of complete and partial photodegradation on organic mat DOM and permafrost DOM, Ward and Cory, (2016) found that despite their composition differences, similar moieties in both DOM samples underwent photodegradation. Aromatic content decreased after sunlight exposure, with aromatic to aliphatic ratios decreasing in organic mat DOM (15%) and permafrost DOM (39%) as expected, given the role light-absorbing aromatic structures play in initiating photochemical reactions. Photo-exposure also precipitated the decrease in oxygen-containing functional groups like aldehyde, ketone and carboxyl moieties, as well as an increase in carbohydrate content. Ward & Cory, (2016) further pointed towards the importance of

photodegradation of carboxyl moieties in the complete degradation of DOM to CO₂, possibly accounting for 37% and 89% of CO₂ produced by organic mat DOM and permafrost DOM, respectively. Also, despite the decrease in carboxyl content under sunlight exposure, the decrease of aldehyde and ketone moieties under photodegradation may prompt increases in carboxyl content (Xie et al., 2004), ultimately likely fueling an increase in CO₂ production. However, a high iron content in freshwaters in the Arctic may catalyze DOM photodecarboxylation, leading to a higher CO₂ production than O₂ consumption (Ward and Cory, 2020). Despite having lower aromatic content, permafrost DOM showed more proneness to photodegradation compared to organic mat DOM (attributed to a greater susceptibility to partial photooxidation), likely due to a lower phenolic content in permafrost DOM, since phenolic moieties are generally associated with aromatic lignin and tannin-like compounds which have been known to quench reactive oxygen species (Aeschbacher et al., 2012).

Incubation experiments on algal DOM and macrophyte DOM have shown initial decreases in O-alkyl content accompanied by the increase in alkyl moieties on a first instance, reflecting the initial consumption of polysaccharides and gradual accumulation of waxy lipids, followed by an inversion of this trend once labile moieties were completely removed; overall, photo-exposure did not promote the degradation of O-alkyl and alkyl moieties (Liu et al., 2019). Light exposure also promoted the removal of aromatic and phenolic moieties, while dark incubations resulted in the production of aromatic and a decrease in phenolic content (although less accentuated than under photodegradation), indicating the phenolic susceptibility to both biodegradation and photodegradation (Liu et al., 2019).

II.6.3. Fourier-transform ion cyclotron mass spectrometry (FT-ICR MS)

Mass spectrometry (MS) techniques have had an important role in NOM characterization studies, providing insights on molecular composition across various environmental systems. By analyzing fragmentation patterns and mass-to-charge ratios (m/z), MS techniques enable the identification of a broad range of compounds and key components in the NOM mixture such as lipids, proteins, carbohydrates and humic substances. In most studies, separation techniques are coupled with MS to improve selectivity and resolution, namely LC-MS, GC-MS or Pyrolysis-GC-MS, as previously mentioned.

Ionization techniques play a crucial role in MS analysis of NOM, with electrospray ionization (ESI) having emerged as the most widely used method for NOM studies due to its ability to efficiently ionize polar, water-soluble compounds and detect a broad range of molecular size fractions. ESI is particularly effective for analyzing DOM in aquatic systems, enabling detailed molecular formula assignments (Patriarca et al., 2020). For more refractory and HMW components in macromolecules, common in humic substances (Lu et al., 2000; Zhao et al., 2012; Fuse et al., 2016) and soil organic matter (Eshetu et al., 2012; Chen et al., 2020) comprised of moieties too large for LC and GC, application of Pyrolysis-gas chromatography (Py-GC) has been widely successful in environmental studies (Picó and Barceló, 2020). Subjecting the sample to controlled thermal degradation, induces bond cleavage and generates smaller, more readily analyzable fragments, particularly useful for humic substances and larger macromolecules.

Although several mass spectrometry approaches have been employed to permafrost environmental studies (Weiss and Kaal, 2018; Allain et al., 2024; Fillion et al., 2024), Fourier-transform ion cyclotron (FT-ICR) MS has emerged as the MS technique most utilized in recent research on NOM characterization and DOM dynamics. Since the main goal of this review is to showcase recent research on NOM characterization in the context of permafrost peatland research and DOM dynamics, we will be focusing primarily on research conducted using FT-ICR MS, rather than studies employing other MS techniques. Moreover, it is not the aim of this study to go into depth on ionization techniques and separation techniques (briefly expanded on section 3 of this paper) commonly applied in MS experiments.

The introduction of FT-ICR MS has provided significant advancements in characterization of complex organic samples, with the advantage of providing high resolution data and using non-destructive ion sources like electrospray ionization (ESI), emerging as one of the main analytical techniques for NOM characterization (Nebbioso and Piccolo, 2013; Bahureksa et al., 2021). Given the enhanced detection provided by FT-ICR MS, data is commonly displayed in either Kendrick mass plots, and Van Krevelen diagrams, relating the retrieved O/C and H/C ratios of the dataset. Although online coupling of FT-ICR MS to LC systems is difficult, off-line use of HPLC techniques and FT-ICR MS has been shown to provide valuable insights (Liu et al., 2011). In this section, we focused on recent research employing FT-ICR MS, rather than studies utilizing non-ICR methods, to investigate dissolved organic matter (DOM) dynamics and permafrost peatland ecosystems. This approach highlights the types of information

obtained and emphasizes the contributions of FT-ICR MS to advancing permafrost research, considering the popularity of the technique in recent times.

In the previously mentioned Ward et al. (2015) study, employing FT-ICR MS not only showed a higher average MW in organic mat DOM leachates compared to permafrost DOM leachates, but also the distinction between unique structures between the two leachates, with unique organic mat DOM being more oxidized and less saturated (higher O/C and lower H/C ratios) than permafrost DOM. Herzprung et al. (2017) studying the DOM leaching of rewetted peatlands compared to pristine peatlands, found the former to contain more oxygen-rich and relatively unsaturated compounds, likely from polyphenol and tannin-like structures.

MacDonald et al., (2021) characterized DOM sequential extraction leachates from permafrost endmembers (tills, diamicton, lacustrine, peat and Yedoma) in the western Canadian Arctic and compared them to active layer soils from peatlands, riparian and hilltop landscapes. Consistent with previous studies, active layer leachates showed greater proportion of aromatic and condensed aromatic structures, with lower aliphatic content relative to permafrost endmembers. Additionally, a decrease in aromatic structures with an increase in aliphatic moieties were observed from diamicton → lacustrine → Yedoma → peat → till leachates in water-extractable organic matter.

Wei et al., (2023) observed lower photodegradation potential in sediment DOM, followed by surface water DOM and extracellular algal DOM, with intracellular algal DOM showing the highest photolability. In all samples, photoirradiation produced higher O/C ratios, indicating the formation of oxygen-containing functional groups. Lipids, lignin and tannin-like moieties in algal DOM displayed higher photolability, while phenolic and carboxyl structures in surface water DOM and sediment DOM were shown to resist photodegradation. Interestingly, unlike in surface and sediment DOM, phenolic and carboxylic moieties from algal DOM were strongly photoreactive, suggesting DOM origin plays a role in the reactivity of functional groups.

Researching composition shifts in lake DOM quality across a soil gradient (permafrost, seasonally frozen ground, short-time frozen ground and non-frozen ground), Wen et al. (2024) observed a clear increase in molecular diversity along the warming gradient. Moreover, permafrost lakes showed lower MW and aromaticity, higher content in labile aliphatic moieties ($H/C \geq 1.5$) and $E_2:E_3$, while lakes in warmer regions were richer in polyphenols, overall displaying opposite characteristics.

Liu et al. (2020) studied DOM from macrophytes, algae, sediments and freshwater samples in eutrophic lakes finding autochthonous DOM inputs to be enriched in lipids (56.8%) and lignin (15.0%), while macrophyte inputs were particularly comprised of lignin (50.6%) and tannin (17.4%) moieties. The strong presence of both lignin (75.4–79.8%), and CHOS/N structures in lake Taihu pointed towards the presence of anthropogenic material and terrestrial inputs, while DOM from lake Dianchi was shown to be rich in lipids (43.8%) and lignin (40.9%), indicating a more autochthonous nature. Focusing on composition changes between humic acid and fulvic acid extracted from yedoma and alas (thermokarst deposit formed upon permafrost thaw) cores, Zhrebker et al. (2019) found increases in CHO and nitrogen-containing groups in humic acids from yedoma with depth, while humic acids were enriched in condensed tannins with decreased aliphatic content. The compositional changes supported the notion that thawing yedoma permafrost exposes labile NOM to intense biodegradation, gradually producing and accumulating more humified structures. Fulvic acids appeared to be predominantly comprised of oxygen-rich unsaturated compounds while humic acids were mainly saturated and aromatic moieties.

Also studying Yedoma permafrost transformations, Carneiro Barreto et al. (2024) performed incubations on Yedoma DOM in oxic and anoxic conditions to assess reactivity and degradation processes. Ultimately, anoxic conditions promoted the degradation of protein-like compounds, while lipids increased and lignin structures remained constant, resulting in a loss of 33% of the initial carbon pool as gases over 21 days of incubation. Comparatively, oxic conditions produced increases in lignin compounds while lipids and protein moieties underwent degradation, with an estimated loss of 58% of the original carbon pool. Additionally, exploring the nominal oxidation state of carbon (NOSC) in both instances, Carneiro Barreto et al. (2024) observed a shift in anoxic conditions of NOSC to lower values for lignin, protein and lipids over the incubation period, indicating the preferential microbial consumption of oxygen-rich moieties like amino acids, carboxylic acids and proteins. A critical review on the use and potential of FT-ICR MS on SOM characterization was produced by Bahureksa et al. (2021), focusing on the steps including collection and processing, to analysis and data interpretation of SOM composition. Despite reinforcing the unparalleled ability of FT-ICR MS for the characterization of highly complex mixtures, Bahureksa et al. (2021) remark that the use of complementary techniques to both constrain and reinforce FT-ICR

MS data is often necessary to interpret the resulting complex datasets, namely UV-visible absorption and fluorescence spectroscopy.

II.7. Synthesis and Future Work

Thawing permafrost in northern regions mobilizes large quantities of NOM and contaminants, disrupts existing hydrological regimes, and alters vegetation communities. These changes result in environmental challenges, including the production and release of GHG from both thermokarst lakes and soils, as well as the speciation of contaminants along trophic chains.

Despite NOM's complex and heterogeneous nature, several chemical characterization techniques, as discussed in this review, offer valuable insights into compositional changes. These techniques help identify potential sources and the biogeochemical processes that drive transformations along aquatic continuums and over time. Within NOM, the DOM fraction is particularly reactive and mobile, acting both as a by-product of microbial degradation and as a substrate for further microbial processing. DOM plays a dual role in the environment: it facilitates the mobilization of metals and nutrients while also acting as a bottleneck that regulates the bioavailability of contaminants (Bolan et al., 2011). In this context, DOM serves as an indicator for tracking biogeochemical shifts and their extent in natural ecosystems.

The presence of TEAs in permafrost thermokarst lakes and soils is crucial for the anoxic oxidation of NOM, which initiates new degradation pathways, shifting both redox conditions and NOM composition. These processes contribute to the production of GHGs.

As demonstrated throughout this review, employing a combination of analytical techniques has proven instrumental in unraveling the complex dynamics of NOM. The most effective approaches typically integrate optical spectroscopy methods (UV-visible and fluorescence), structural spectroscopy techniques (such as FTIR and NMR), and mass spectrometry (e.g., FT-ICR MS) (Ward and Cory, 2015; Hodgkins et al., 2016; Herzsprung et al., 2017; Normand et al., 2017; Wang et al., 2018; Liu et al., 2020; Miranda et al., 2020; MacDonald et al., 2021; Wei et al., 2023; Wen et al., 2024). A well-planned research strategy that selects complementary techniques to comprehensively analyze NOM samples is crucial for generating meaningful insights.

Biogeochemical processes have a profound influence on NOM composition, providing site-specific details on the dynamics of natural systems. However, interpreting these changes requires a solid understanding of redox conditions and the temporal evolution of environmental processes, especially in permafrost regions. It is important to recognize that NOM characterization typically represents the chemical composition of a sample at a specific point in time, necessitating careful interpretation in light of the dynamic nature of these systems.

For example, when studying permafrost thermokarst lakes, a focus on DOM characterization of the water column will not provide a full understanding of the system's dynamics. It is essential to consider the interplay between freeze-thaw cycles, physicochemical parameters, TEAs, and DOM, as well as the seasonal contributions from surrounding soil and sediments, to gain a holistic view of a thermokarst system.

Research into NOM composition in permafrost regions should pursue various avenues, depending on specific environmental challenges and research goals. There are two main scientific approaches: (i) characterize NOM at specific study sites to document the current state of the ecosystem, and (ii) use biogeochemical experiments in a controlled environment to investigate how simulated biogeochemical dynamics affect NOM cycling. Pertaining to NOM characterization studies in permafrost peatlands, the following research areas could be pursued:

1. Assess changes in DOM composition and reactivity in thermokarst ponds across different permafrost regions;
2. Characterize SOM and mobilized DOM from permafrost peatlands to nearby waterbodies, particularly in relation to issues faced by nearby indigenous communities. These include the evaluation of the risk associated with the formation of disinfection byproducts (DBPs) in drinking water supplied to communities, as DOM acts as a precursor for these regulated byproducts.
3. Investigate the effects of permafrost thaw on NOM composition and its influence on the methylation of contaminants, notably of mercury. This has important implications for both wildlife and nearby human populations.
4. Explore the links between emerging vegetation (Arctic greening), underlying SOM quality, thaw progression, and potential GHG production along environmental gradients. This could provide insights into the feasibility of using remote sensing as a proxy for underlying SOM dynamics.

5. Conduct longitudinal studies of NOM composition across well-documented sites, permafrost gradients, and bioclimatic subzones to understand the links between biogeochemical shifts and NOM transformations.

Several environmental phenomena pose risks and are worthy of exploration in controlled experimental settings:

6. Examine how freeze-thaw cycle affects the quality of NOM mobilized from soils into aquatic systems over time, particularly focusing on the photodegradation potential of mobilized DOM in response to the shortening winters associated to climate change.
7. Quantify the conversion of DOM into CO₂ and CH₄ under oxic (light-exposed) and anoxic (dark) conditions, simulating seasonal effects observed in thermokarst lakes.
8. Investigate SOM transformation in anoxic conditions to gauge soil respiration potential for GHG production in different permafrost peatland regions. Assess the primary TEAs used during these transformations and their impact on SOM quality.

As global temperatures rise in Arctic and subarctic regions, permafrost continues to thaw rapidly, especially in sporadic and discontinuous permafrost zones. Although continuous permafrost zones thaw at a slower rate, the melting of ice (ice lenses and/or ice-wedges) in subarctic peatland soils can quickly alter local dynamics (Liljedahl et al., 2016). Over the past few decades, thaw ponds, bogs, and fens have emerged in these regions (Bhiry et al., 2011; Chasmer and Hopkinson, 2017), and complete permafrost loss in boreal western Canada is projected by the end of this century (Olefeldt et al., 2021).

The mobilization of NOM due to permafrost thaw and an assessment of its composition is also increasingly relevant for protecting the water quality of Indigenous communities' drinking water. The browning of surface waters, caused by the increasing release of NOM to natural aquatic systems as permafrost thaw and vegetation progresses, makes surface waters in the North vulnerable to microbiological and chemical contamination. Many of these systems are currently under boil-water advisories that have lasted for years. Chlorination, commonly used to inactivate pathogens, can lead to the formation of undesirable DBPs, including regulated trihalomethanes and haloacetic acids, both of which are linked to chronic health risks (some being carcinogenic) (Rodriguez et al.,

2004; Villanueva et al., 2007; Stalter et al., 2016). Sub-chronic health risks associated to DBPs remain insufficiently understood, and specific NOM components contributing to DBP formation are still unclear. Monitoring and controlling DBP levels, in correlation with NOM concentration and quality, is crucial to mitigate health risks. Analytical techniques play an essential role in advancing this understanding and ensuring safe water access.

Permafrost degradation is driving irreversible environmental shifts, reshaping northern landscapes and ecosystems. With these shifts come substantial increases in NOM mobilization, impacting fluxes of GHGs and contaminants. Understanding the evolving trends in permafrost peatlands and their biogeochemistry is essential for building accurate models that predict the larger-scale implications of these changes. Given the important role of NOM in various environmental processes, studying NOM dynamics in permafrost regions appears crucial for understanding and addressing the broader environmental challenges these regions will face.

II. References

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III. Seasonal organic matter dynamics in thermokarst lakes of a degrading palsa- rich sporadic permafrost zone (Nunavik, Canada)

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III.1. Introduction

Over the last decades, Arctic and subarctic regions have experienced much faster rates of warming (Bekryaev et al., 2010; Hinzman et al., 2013), with median air temperatures increasing 2.5 to 4 times more rapidly than the global average (Rantanen et al., 2022; Lee and Romero, 2023). Terrestrial permafrost underlies around 15% of the Northern Hemisphere landmass (Obu et al., 2019). Northern peatlands represent a key component of this region's carbon reservoir, storing 415 ± 150 Pg C and accounting for ~80% of the total global peatland carbon stock, with nearly half of this stock located in permafrost-affected zones (Hugelius et al., 2020). As temperatures rise, northern permafrost peatlands become increasingly vulnerable to climate change. One of the consequences of this environmental shift is the formation of thermokarst lakes (Romanovsky et al., 2010; Kokelj and Jorgenson, 2013; Pokrovsky et al., 2016). These lakes have been the subject of extensive biogeochemical and microbial research (Crevecoeur et al., 2015; Matveev et al., 2016; Przytulska et al., 2016; Folhas et al., 2020; Laberge-Carignan et al., 2024). The mobilization of natural organic matter (NOM) and nutrients into these aquatic systems stimulates microbial activity, driving the production of greenhouse gases (GHG) such as CO₂ and CH₄ (Zimov et al., 1997; Vonk et al., 2015; Matveev et al., 2019), and influencing the cycling of contaminants like mercury (Schaefer et al., 2020).

Thermokarst lakes vary greatly in their limnology and geomorphology (Olefeldt et al., 2016; Wrona et al., 2016; Freitas et al., 2024) but also in their GHG production (Laurion et al., 2010; Matveev et al., 2019; Voigt et al., 2017; Prėskienis et al., 2021) and dissolved organic matter (DOM) optical properties (Watanabe et al., 2011; Wauthy et al., 2018, Laurion et al., 2021). Dissolved organic carbon (DOC) concentrations in thermokarst lakes vary widely, typically ranging from 2 to 60 mg C L⁻¹ (Peura et al., 2020; Manasypov et al., 2015; Stolpmann et al., 2024). The chromophoric fraction of DOM (CDOM) efficiently absorbs light, especially in the ultraviolet and blue regions of the spectrum (Stedmon and Nelson, 2015). The subset of CDOM that fluoresces upon irradiation is referred to as fluorescent DOM (FDOM) (Cory and Kaplan, 2012; Coble et al., 2014; Stedmon and Nelson, 2015). Complementary study of CDOM and FDOM fractions in natural aquatic systems have been particularly useful for investigating biogeochemical dynamics such as photodegradation and microbial processing of DOM (Hansen et al., 2016; Roiha et al., 2015; Wauthy et al., 2018; Gandois et al., 2019; Grunert et al., 2021; Mazoyer et al., 2022).

Shallow waterbodies near palsas – peat mounds with a permanently frozen core in discontinuous and sporadic permafrost zones – are typically younger and strongly influenced by inputs of NOM and nutrients, particularly nitrogen, mobilized from degrading palsas (Fuchs et al., 2019; Leroy et al., 2025). In contrast, older lakes primarily receive inputs from deeper peat layers and surrounding terrestrial vegetation (Roiha et al., 2015; Peura et al., 2020). This mobilization arises from hydrological connectivity between peatland soils and aquatic systems, which facilitates the export of soil carbon into nearby waterbodies (Prijac et al., 2023). Additionally, microbial communities in younger thermokarst lakes show a higher abundance of genes related to organic matter degradation, with both DOM quality and the potential for microbial carbon cycling changing across lake ontogeny (Peura et al., 2020). Leroy et al. (2025) noted that the strong connectivity between emerging thermokarst lakes and surrounding soils plays a key role in shaping their microbial communities. In contrast, microbial communities in stratified mature lakes are less influenced by soil inputs and are instead governed by the distinct environmental conditions within the lake's stratified layers.

Thermokarst lake biogeochemistry exhibits significant seasonal variation, driven by fluctuations in photosynthetic activity (both terrestrial and aquatic) and two major drivers: light and dissolved oxygen (DO). In northern peatlands, terrestrial primary production generally increases over time as vegetation shifts toward a greater abundance of vascular plants relative to mosses (Blodau, 2002; Wieder, 2006). The accumulation of organic carbon in peatland soils results from the imbalance between net primary production and the rate of soil decomposition (Craft et al., 2008; Blodau, 2002). In aquatic systems, primary production is favored in clear, shallow waters that receive inputs of both DOM and nutrients, whereas deeper, more colored lakes typically exhibit suppressed primary production (Roiha et al., 2015; Vonk et al., 2015). Light availability in the water column is also strongly controlled by seasonal changes in incoming solar irradiance, as well as the presence of ice and snow cover.

The role of light in shaping DOM composition has been studied in several northern lakes and ponds (Cory et al., 2014; Hansen et al., 2016; Laurion et al., 2021; Mazoyer et al., 2022). For example, Mazoyer et al. (2022) examined the photodegradation of late-winter DOM in a mature thermokarst lake and found that, despite its recalcitrant nature, the DOM exhibited high photolability. This exposure to spring sunlight apparently led to significant DOC losses through both photomineralization and photoflocculation.

Moreover, reactive oxygen species (ROS) are produced through photochemical reactions involving DOM (Scully et al., 2003; Waggoner et al., 2017; Grunert et al., 2021), and via iron redox cycling (Huang et al., 2020). During winter however, incoming irradiance declines sharply, and the formation of ice and snow cover results in complete darkness in the water column, while also preventing gas exchange with the atmosphere. These conditions rapidly deplete DO, prompting microbial communities to shift toward alternative redox processes. In the absence of oxygen, microbes utilize terminal electron acceptors (TEAs) such as nitrate (NO_3^-), manganese (Mn(IV)), iron (Fe(III)), and sulfate (SO_4^{2-}) (McMahon et al., 2011; Herndon et al., 2020; Perryman et al., 2020). A comprehensive understanding of thermokarst lake biogeochemistry and DOM dynamics must consider the role of TEAs in sustaining microbial activity under anoxic environments.

Thermokarst lake morphometry is strongly influenced by initial palsa size and ground ice availability. These morphometric divergences can have a profound effect on lake dynamics — for instance, lakes with larger surface areas tend to exhibit enhanced wind-mixing effects, promoting changes to lake functioning relative to lakes with smaller surface areas.

To capture a multi-perspective snapshot of thermokarst lake dynamics, we combined several analytical approaches. UV-Visible absorbance (CDOM) and fluorescence spectroscopy (FDOM) were used to characterize vertical patterns in DOM quality. CDOM captured broad shifts in DOM transformation, while FDOM resolved specific fluorophore groups linked to biogeochemical processes. Measurements of TEAs aimed to identify dominant redox regimes, revealing vertical and inter-lake gradients. A similar suite of analysis was applied to sediments, with FTIR spectroscopy to characterize changes in functional groups, C/N ratios and $\delta^{13}\text{C}$ signatures provided indicators of NOM degradation and source inputs, while total Fe and Mn concentrations showed accumulation patterns.

Thermokarst lakes exhibit considerable heterogeneity both within and across permafrost landscapes, limiting the transferability of site-specific findings. However, elucidating the underlying biogeochemical mechanisms provides a more generalizable framework for understanding thermokarst lake dynamics. By examining whether these mechanisms vary with lake ontogeny and morphometry, we can distinguish between lake-intrinsic

characteristics and broader biogeochemical principles governing thermokarst functioning.

In this study, we investigated the seasonality of DOM in thermokarst lakes at different stages of development and with diverging morphometry, located in a sporadic permafrost region of Subarctic Canada. Specifically, we aimed to address the following questions: (1) How do DOM dynamics in the water column of thermokarst lakes differ between summer and winter? (2) What seasonal patterns and biogeochemical processes govern NOM dynamics in the sediments and sediment porewaters of these lakes? (3) What are the key similarities and differences in the biogeochemical processes and characteristics among thermokarst lakes with varying ontogeny and morphometry? This framework allows us to link DOM quality, redox patterns, and sediment NOM characteristics to biogeochemical mechanisms and their variation across lake ontogeny, morphometry, and seasonality in thermokarst lakes.

III.2. Materials and Methods

III.2.1. Study sites and sampling

In this study we selected three thermokarst lakes (SAS1B, SAS1A, and SAS2A; Fig. III.1) in peatlands of the Sasapimakwananisikw (SAS) River Valley, near the communities of Whapmagoostui-Kuujuarapik, in Nunavik, Québec, Canada. The SAS valley lies within the sporadic permafrost zone, which experiences a wide range of seasonal temperature variations. The mean annual air temperature (MAAT) for the period from June 2021 to May 2022 in Kuujuarapik, Québec, Canada was -2.4 °C, based on daily mean air temperature (Environment and Climate Change Canada, 2025). The average mean air temperature was 12.5 °C in August 2021, and -14.7 °C in March 2022, corresponding to the two field sampling periods. The organic-rich soils in the region are

underlain by a marine clay layer that was deposited more than 5000 years ago, prior to the recession of the ancient Tyrell Sea (Bhiry et al., 2011).



Figure III.1. Photograph of the three thermokarst lakes sampled in the SAS region during summer.

Although the SAS study sites include waterbodies ranging from shallow ponds to deeper lakes, we refer to all three as 'lakes' throughout this study for simplicity, following previous studies (Matveev et al., 2016; Leroy et al., 2025).

SAS1B was directly connected to a thawing and eroding palsa, indicating that it is in an early stage of development. Approximately 45 meters away, SAS1A was slightly deeper and nine times larger in surface area than SAS1B (Table III.1) and may still be influenced by the adjacent thawing palsa. SAS1A is at a more advanced ontogenic stage due to its larger surface area, likely resulting from lateral expansion as its margins eroded (Bouchard et al., 2016). On the opposite side of the SAS River, SAS2A is classified as a mature lake based on its greater distance from the nearest palsa, its maximum depth, and its well-defined vertical stratification.

Table III.1. Surface area, maximum depth, geographical coordinates and distance from the nearest palsa for the three studied thermokarst lakes.

Lakes	Surface Area	Lake Depth	Coordinates	Distance from Palsa
SAS1B	175 m ²	140 cm	55°13' 08.3 N, 77°42' 29.6 W	Connected to the palsa
SAS1A	1580 m ²	180 cm	55°13' 07.7 N, 77°42' 28.4 W	Approximately 45 m
SAS2A	196 m ²	280 cm	55°13' 35.6 N, 77°41' 49.1 W	Approximately 75 m

Summer sampling (samples labeled SUM21) was conducted by boat at the center of each lake on August 14-15, 2021. Late winter sampling (labeled WIN22) took place from March 8-13, 2022, by drilling through the ice. To minimize sediment resuspension – given the lake's stagnant conditions and high concentrations of particulate matter near the bottom – we limited water sampling to maximum depths of 75 cm in SAS1B, 150 cm in

SAS1A, and 225 cm in SAS2A. Temperature, conductivity, dissolved oxygen, and pH were measured along the water column using an EXO1 multiparameter probe (YSI Inc.). While physicochemical variables were recorded at 25 cm intervals during winter, this depth resolution could not be maintained during summer sampling.

Water samples were collected every 25 to 50 cm using a peristaltic pump (Manning MSP250 fluid pump, Cole-Parmer Canada) during winter, and a horizontal 4 L Van Dorn bottle during summer. Water samples for DOC quantification, as well as CDOM and FDOM characterization, were filtered through 0.22 μm polyether sulfone membrane filters (VWR) using 60 mL rubberless syringes and preserved in amber glass vials previously acid-washed and burned (500°C, 4h) to remove any organic contaminants. During winter sampling, an additional DOM fraction was collected by filtering water through 0.45 μm Macherey-Nagel syringe filters (CHROMAFIL Xtra PA-45/25). Larger DOM samples (2.5 L), for solid-phase extraction and structural characterization, were also collected in both seasons from the surface, intermediate depth, and bottom of each lake, and filtered through 0.45 μm Durapore polyvinylidene fluoride membrane filters. For total Fe and Mn analysis, water samples were collected into a Teflon bottle containing 4% Merck suprapur® nitric acid (HNO_3). For Fe(II) analysis, the samples were transferred into 5 mL Falcon tubes containing ferrozine (Viollier et al., 2000). For anion measurements (NO_3^- , SO_4^{2-}), vials were purged with N_2 gas and water samples were injected through a rubber septum, maintaining anoxic conditions. Field blanks were done for all analyzed parameters. All measurements for major elements in water samples exhibited RSD below 10%, with both DOC and sulfide measurements showing RSD values below 12%.

Surface soil samples were collected from the margins of each lake using decontaminated and sterilized cups, then frozen at -20°C and freeze-dried prior to analysis. Sediment cores were collected, right after the water sampling and in the same position, using a 9-cm diameter gravity corer equipped with a core catcher (Uwitec). Each recovered core was sectioned into four parts, frozen at -20°C, and subsequently freeze-dried. Sediment porewaters were extracted using a corer tube with pre-drilled holes at 1 cm intervals, into which Rhizon lysimeters (Rhizosphere) were inserted. From the collected porewaters, concentrations of dissolved Fe and Mn, nutrients (NO_3^- and SO_4^{2-}), and total DOC were measured, as previously reported in Laberge-Carignan et al. (2024). Here we additionally report new data on FDOM, analyzed using Parallel factor analysis (PARAFAC), as

described below. During winter sampling at SAS1B, porewater retrieval was not possible. Additionally, as most of the water column was frozen at the sampling location – which was likely not at the lake’s deepest point, given that ice thickness is generally less one meter – we were only able to collect a sample from a single depth. As a result, winter data from SAS1B were excluded from our analysis, except for FTIR spectra of DOM and sediment characterization.

III.2.2. DOM Characterization

Absorbance and fluorescence excitation emission matrices (EEMs) were obtained in an Aqualog spectrofluorometer (Horiba Scientific, USA). CDOM absorbance spectra (200-700 nm, 1 nm resolution) were measured in 1 cm quartz cuvettes and blank-corrected with ultrapure Milli-Q water ($18.2 \text{ M}\Omega\cdot\text{cm}$ at 25°C , $\text{DOC} < 2 \text{ ppb}$). SUVA_{254} values (proxy for DOM aromaticity) were calculated according to Weishaar et al., (2003), and corrected for iron interferences (Poulin et al., 2014). The slope ratio (S_R), the ratio between spectral slopes between 275-295 nm and 350- 400 nm, was calculated as an indicator of DOM molecular weight (Helms et al., 2008; Li and Hur, 2017). The absorption coefficient at 320 nm was used as a proxy for CDOM content, while slope curves at 289 nm (S_{289}) and 382 nm (S_{382}) were used to distinguish algal-derived and a terrestrial-derived DOM fractions (Loiselle et al., 2009; Roiha et al., 2015). Second-derivative UV-Visible absorbance spectra were calculated using a Savitzky-Golay filter (polynomial order: 3; 21-point window) in OriginPro (v. 2023b). Interpolations of physicochemical parameters in the water column were also conducted in OriginPro.

Fluorescence EEMs were acquired with an excitation range of 240-600 nm in 2 nm increments, with an emission range of 250-800 nm in 2.5 nm increments. For every 10 samples, a new Milli-Q blank was analyzed and used for Raman and Rayleigh scattering corrections. Inner-filter effect correction and 0-1 normalization were performed for every EEM, as per the Aqualog Manual (Aqualog® Operation Manual, rev. K, 2019).

Parallel factor analysis (PARAFAC) was conducted in SOLO (Eigenvector Research Inc., Manson, DC, USA). A non-negativity constraint was applied, and primary and secondary Rayleigh scatter were set to missing values. A five component PARAFAC model (99.7% fit) was generated using 219 samples of natural DOM samples collected across Arctic and Subarctic lakes as part of our field campaigns. Sum of square residuals, spectral loadings,

core consistency diagnosis (CORCONDIA) and split-half analysis were used to validate the PARAFAC components (Bro, 1997; Murphy et al., 2013). Split-half validation (74.7%) indicated moderate model stability, while the CORCONDIA value of 53, although lower than ideal, was consistent with expectations for EEMs derived from complex natural DOM samples (Bro, 1997). We adopted a cautious interpretation of PARAFAC results, integrating them with UV-Visible and FTIR data to provide a more comprehensive understanding of biogeochemical dynamics (Folhas et al., 2025). PARAFAC component emission and excitation loadings and split-half validation results are present in Fig. VII.I.6, VII.I.7, and VII.I.8 of the Supplementary Information. The PARAFAC model was generated following the proposed workflow in the Aqualog Manual (Aqualog® Operation Manual, rev. K, 2019).

FTIR spectra were collected using a Perkin Elmer Spectrum Two spectrophotometer equipped with an attenuated total reflectance module. Spectra were acquired over the 500-4000 cm^{-1} range at 1 cm^{-1} resolution, with sixteen scans/sample recorded, averaged, and corrected against air as background. DOM extracts were obtained from larger water samples (2.5L) using solid-phase extraction (SPE) with styrene-divinylbenzene polymer (PPL) cartridges, following the method described by Dittmar et al. (2008). FTIR peak identification and characterization of SPE-DOM samples (Fig. VII.I.10) is presented in Supplementary Information (Supplementary Note SN.VII.I.2).

III.2.3. Soil and sediment characterization

Sediment core samples were divided into four 5-cm thick sections: 0-5 cm, 5-10 cm, 10-15 cm, and 15-20 cm. This sectioning was applied to all sediments except for SAS1B winter, which was divided into smaller sections: 0-3 cm, 3-6 cm, 6-9 cm, and 9-12 cm. Soil and sediment sections were then homogenized using an agate mortar, freeze-dried, and stored in glass containers protected from light.

A portion of soil and sediment samples were digested using a 1:3 mixture of aqua regia (prepared as a 3:1 ratio of $\text{HCl}:\text{HNO}_3$) and hydrofluoric acid (HF), with all reagents being of Merck Suprapur® grade. Digestion was carried out at 100°C for 1 hour. After digestion, a boric acid (H_3BO_3) solution was added to complex residual HF, and the samples were subsequently diluted 10:1 with Milli-Q water containing 2% HNO_3 prior to analysis. These samples were analyzed by atomic absorption spectrometry (Thermo

Scientific - SOLAAR S Series AA spectrometer). To assess digestion recovery, three certified reference materials were used: MESS-4 (Marine Sediment Certified Reference Material, NRC, Canada), CRM-227, and BCR-60 (Aquatic Plant *Lagarosiphon major*).

A different portion of soil and sediments was kept for FTIR analysis, with spectral acquisition performed using the same equipment and scanning conditions as for DOM samples.

To remove the inorganic carbon fraction (mainly carbonates), samples were homogenized and treated with 0.1M HCl for 2 hours. The treated samples (organic fraction) were then washed several times with Milli-Q water and freeze-dried until analysis. The $\delta^{13}\text{C}$ signatures, along with %C and %N content in both total carbon and organic sediment samples, were measured using a EuroVector Elemental Analyzer coupled to a Nu Horizon isotope ratio mass spectrometer (Water Quality Center, Trent University, Canada). $\delta^{13}\text{C}$ values were calibrated relative to the Vienna Pee Dee Belemnite (VPDB) standard using a three-point calibration curve anchored by USGS40 (glutamic acid; $\delta^{13}\text{C} = -26.39\text{‰}$, %C = 40.82, %N = 9.52), USGS62 ($\delta^{13}\text{C} = -14.79\text{‰}$, %C = 49.48, %N = 28.85), and USGS63 ($\delta^{13}\text{C} = -1.17\text{‰}$, %C = 49.50, %N = 28.90). USGS40 was also used to calibrate elemental compositions for %C and %N determinations.

III.2.4. Statistics and data processing

The slope curves at 289 nm (S_{289}) and 382 nm (S_{382}), as well as S_R values were calculated using the CDOM R package (Massicotte and Markager, 2016) in RStudio (version 2023.06.2-561). Spearman's rank correlations were computed for porewater variables, with significance set at $p < 0.05$.

Two principal component analyses (PCAs) were conducted on RStudio. The first PCA aimed to identify patterns in water column dynamics among lakes and seasons, while the second PCA focused on differences in sediment porewater dynamics among lakes during summer. Measurements of physicochemical properties were interpolated to the same depths as the other variables to generate consistent depth profiles for the water column PCA.

Permutational Multivariate Analysis of Variance (PERMANOVA: Euclidean distance, 999 permutations) tested for variance explained by lake and seasonal groups, followed by

permutational analysis of multivariate dispersions (PERMDISP) to assess group dispersions; both used the vegan package (Dixon, 2003; Brückner and Heethoff, 2017). Bonferroni-corrected pairwise comparisons were conducted to assess statistical differences between groups.

Nonmetric multidimensional scaling (NMDS) analysis was performed using the same variables selected for the porewater PCA, to visualize multivariate similarity between sample groups with acceptable stress values. Although Bray-Curtis dissimilarity is commonly used in ecological studies, we utilized Euclidean distance, as the porewater variables represent continuous environmental measurements. All variables were Z-Score normalized prior to statistical analysis.

III.3. Results

III.3.1. Physicochemical properties of the water column

Physicochemical properties showed marked seasonal and ontogenic variability across thermokarst lakes. During summer, the shallower lakes SAS1B and SAS1A, exhibited higher mean temperatures (10.0 °C and 12.2 °C, respectively) relative to SAS2A (6.5 °C). SAS1B showed a gradual thermal decline with depth, whereas SAS1A and SAS2A displayed distinct thermal stratification, despite limited vertical resolution (Fig. VII.I.1). In winter, temperature inversions were observed, with bottom waters in SAS1A and SAS2A up to 3.3 °C warmer than waters under the ice. Epilimnion, metalimnion, and hypolimnion layers were identified from temperature, conductance and oxygen profiles, and are shown in Fig. VII.I.1. Specific conductance (SPC) increased with depth in summer across all lakes, peaking near 300 $\mu\text{S cm}^{-1}$ in SAS2A compared to SAS1A and SAS1B ($< 200 \mu\text{S cm}^{-1}$). Winter conductance in SAS1A remained stable ($\sim 150 \mu\text{S cm}^{-1}$), while SAS2A showed a bottom increase less pronounced than in summer.

Seasonal contrasts were prominent in pH and DO profiles (Fig. VII.I.1 and VII.I.2). Summer DO saturations peaked at surface while remaining below air-equilibrium (47%, 64%, 54% in SAS1B, SAS1A and SAS2A, respectively), decreasing sharply with depth. In winter, DO was uniformly low, averaging 0.7% in SAS1A and 0.3% in SAS2A. Summer pH profiles varied: SAS1A's upper layer averaged pH 6.3 and declined with

depth (pH 5.8), while SAS2A showed more acidic waters (pH 5.3) in the upper layers, with pH values increasing towards the bottom (Fig. VII.I.2).

III.3.2. Terminal electron acceptors concentrations in the water column

Dissolved Fe and Mn concentrations showed distinct seasonal and vertical patterns across the three lakes. SAS1B showed the lowest summer Fe concentrations (1.3 to 3.6 mg L⁻¹) and Mn ranged 24 to 28 µg L⁻¹, with no winter data. In SAS1A, summer dissolved Fe varied from 0.4 to 9.3 mg L⁻¹, remaining below 1 mg L⁻¹ in the upper 100 cm (Fig. VII.I.3), while dissolved Mn remained ~10 µg L⁻¹ in the upper layers before rising to 61–80 µg L⁻¹ at depth (Fig. VII.I.4). In winter, concentrations of dissolved Fe and Mn increased and stabilized in the 13–14 mg L⁻¹, and 0.24–0.26 mg L⁻¹ range, respectively.

SAS2A had summer dissolved Fe range from 0.5 to 7.4 mg L⁻¹, similarly low near the surface and increasing with depth (Fig. VII.I.3), with dissolved Mn exhibiting a similar pattern, lower near the surface (6.5 to 7.5 µg L⁻¹) and rising toward the bottom (27 µg L⁻¹). In winter, concentrations of dissolved Fe and Mn were lowest in upper waters with sharp increases near bottom, ranging from 4.1 to 17.7 mg L⁻¹ for Fe and 12 to 67 µg L⁻¹ for Mn. During winter, Fe(II) accounted for 85–89% of total dissolved Fe in SAS1A and 44–79% in SAS2A (Fig. VII.I.23).

Sulfate (SO₄²⁻) concentrations remained below 0.6 mg L⁻¹ across all lakes and seasons and tended to decrease with lake ontogeny (Fig. VII.I.4). Nitrate (NO₃⁻) showed a similar trend, with higher concentrations in SAS1B and SAS1A compared to SAS2A during summer (Fig. VII.I.4). Also, nitrate concentrations were higher in SAS1A than in SAS2A during winter.

III.3.3. Dissolved organic carbon content and quality

Summer DOC ranged from 25–31 mg L⁻¹ in SAS1B (peaking at 61 mg L⁻¹ at 50 cm), 14–31 mg L⁻¹ in SAS1A, and 13–21 mg L⁻¹ in SAS2A (spiking to 40–57 mg L⁻¹ in the hypolimnion). Winter DOC increased by 95% in SAS1A (35–56 mg L⁻¹), and 72% in SAS2A (26–59 mg L⁻¹).

During summer, SUVA₂₅₄ was lower in SAS1B ($3.9 \pm 1.7 \text{ L mg C}^{-1} \text{ m}^{-1}$) and SAS1A ($4.3 \pm 1.9 \text{ mg C}^{-1} \text{ m}^{-1}$) than SAS2A ($9.9 \pm 3.4 \text{ L mg C}^{-1} \text{ m}^{-1}$; Fig. III.2). In SAS2A, SUVA₂₅₄ values spiked at 50 cm, remaining elevated throughout the metalimnion. Winter values in SAS2A dropped by 63%, indicating reduced aromaticity relative to summer.

During summer, a_{320} values (Fig. VII.I.20) ranged between 112-185 m^{-1} in SAS1B (higher near surface). SAS1A displayed lower a_{320} values in the first 100 cm (45-120 m^{-1}), increasing near the bottom (213-307 m^{-1}). SAS2A had the highest a_{320} values, gradually increasing to the metalimnion (202-217 m^{-1}) and the hypolimnion (315-434 m^{-1}). In winter, a_{320} rose in SAS1A ($288 \pm 44 \text{ m}^{-1}$), and decreased in SAS2A ($180 \pm 24 \text{ m}^{-1}$), spiking at bottom (545 m^{-1}).

S_R values were stable in the SAS1B water column (0.68-0.72), declining near the bottom (0.41). In SAS1A and SAS2A, S_R peaked sharply at 50 cm (1.80 and 1.53, respectively; Fig. III.2) and declined with depth. Winter S_R was lower overall, remaining stable in SAS1A (0.57), and gradually declining with depth in SAS2A (0.66 to 0.58).

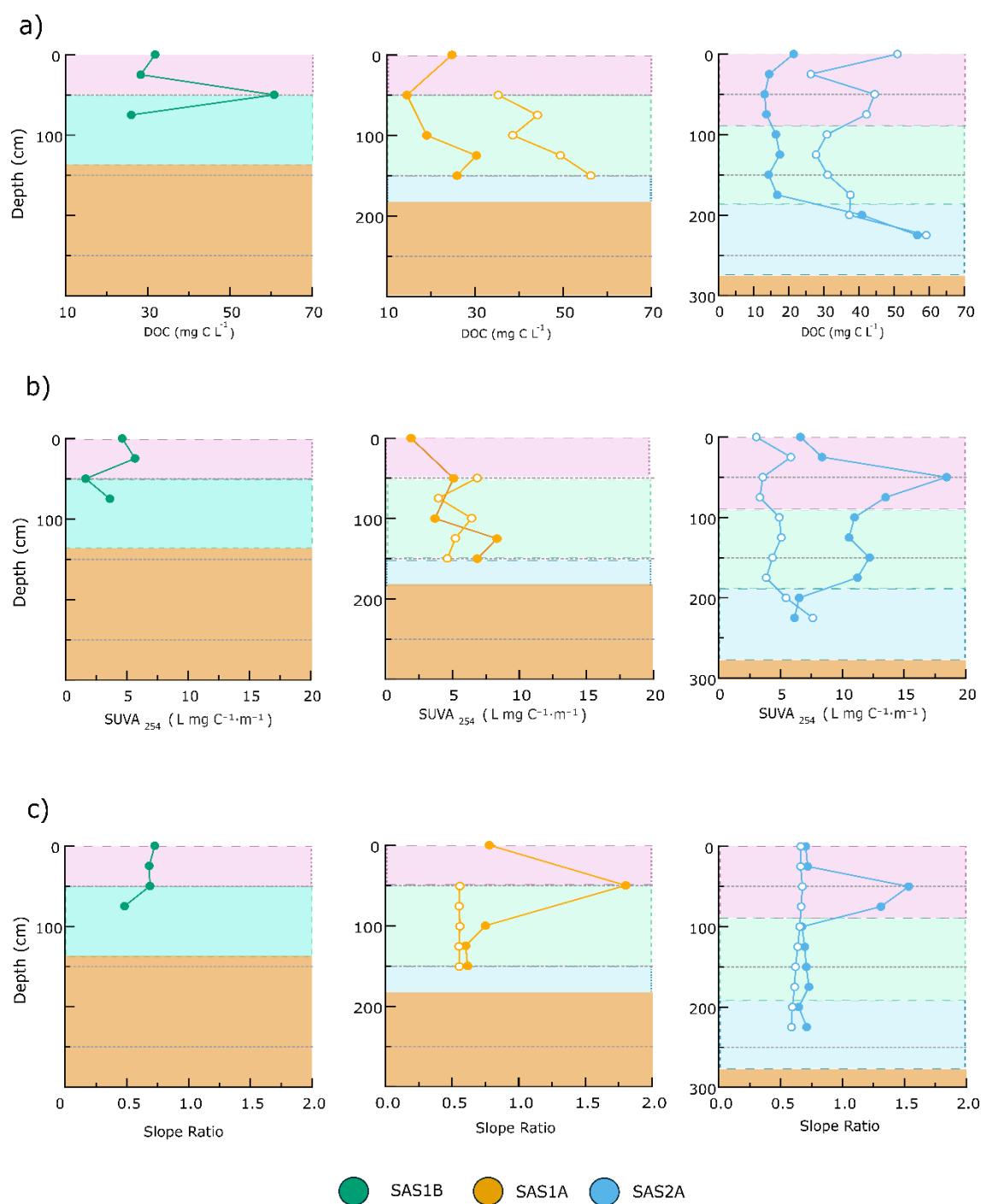


Figure III.2. Summer (solid circles) and winter (open circles) vertical profiles in SAS1B, SAS1A, SAS2A of: a) Dissolved organic carbon (DOC) concentrations (mg L^{-1}); Specific ultraviolet absorbance at 254 nm (SUVA_{254}); c) Slope ratio (S_R).

The PARAFAC model identified five components (EEMs in Fig. VII.I.5), categorized following Pacoureau et al. (2025) as humic-like terrestrial (HT), humic-like microbial (HM), and protein-like (P). HT1, HT2 and HM1 have two excitation maxima, while P1

and P2 have one (Table III.2). Component details are provided in Supplementary Information (Supplementary Note SN.VII.I.1).

Table III.2. Identification of five PARAFAC components and their assignment based on the literature (provided in SN.VII.I.1). For components with dual peaks, primary peak (PP) and secondary peak (SP) are identified.

Component	Excitation _{max}	Emission _{max}	Assignment
HT1	250 nm (PP)/ 340 nm (SP)	450 nm	Humic-like terrestrial
HT2	265 nm (PP)/ 400 nm (SP)	520 nm	Humic-like terrestrial
HM1	240 nm (PP)/ 310 nm (SP)	400 nm	Humic-like microbial
P2	275 nm	295 nm	Tyrosine-like
P1	280 nm	330 nm	Tryptophan-like

Relative fluorescence (% of each component) profiles of PARAFAC components revealed clear vertical and seasonal patterns across lakes (Fig. III.3 and VII.I.9). In SAS1B, summer FDOM components showed minimal variation along the water column, with P1 consistently absent. In SAS1A, the surface showed a P1 spike and HT1 decrease, with P2 remaining stable in the first 100 cm of the water column, before declining to the bottom. In SAS2A during summer, protein-like FDOM was absent from the epilimnion. P1 showed a peak at 125 cm, followed by a decrease and a secondary increase near the bottom (Fig. III.3). HT2 showed a strong negative correlation with water column depth ($r = -0.87$; $p < 0.003$). During winter, FDOM profiles in SAS2A showed reduced vertical variation, with HM1 increasing and HT1 decreasing gradually toward the bottom (Fig. VII.I.9). During winter, P1 remained more abundant than P2 in both lakes.

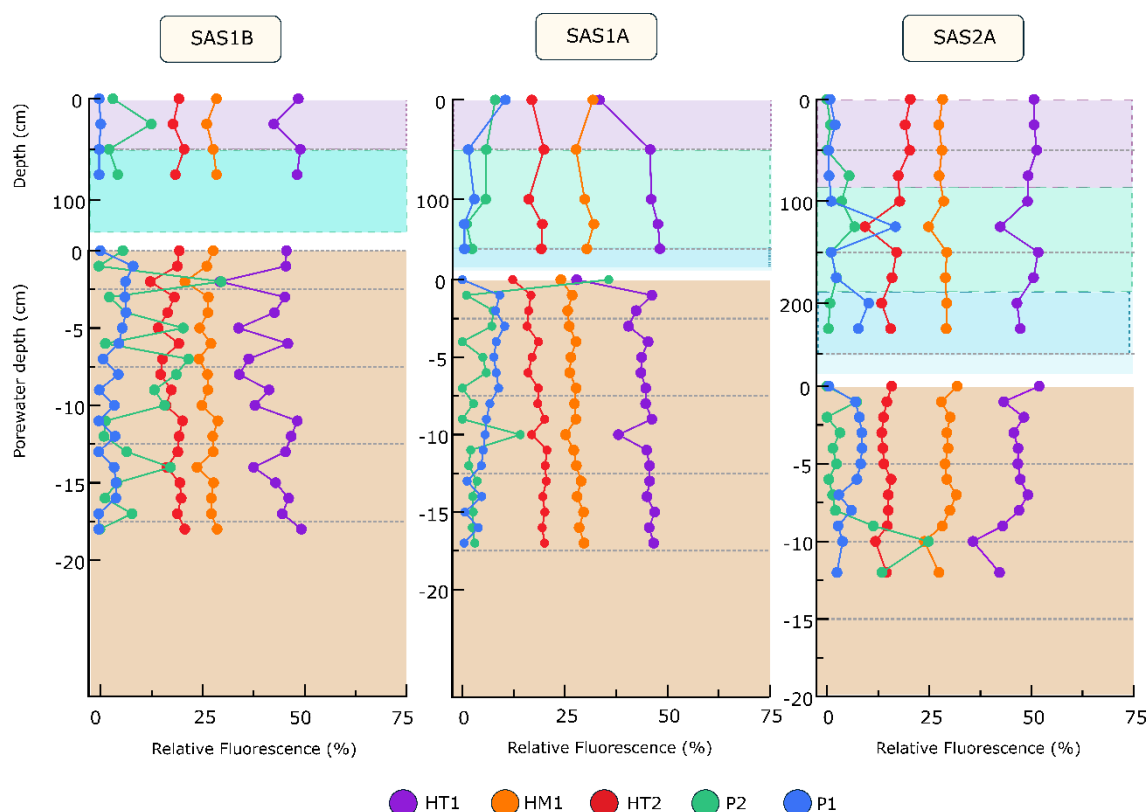


Figure III.3. Variation in PARAFAC components as a function of depth (in cm) in the water column (blue background, water column depth from the surface) and sediment porewater (orange background, negative depths from the water-sediment interface) during summer in SAS.

III.3.4. Principal component analysis of water columns

An exploratory PCA of water column properties (DO, pH, SPC, Fe, Mn, SO_4^{2-} , NO_3^- , S_R , SUVA_{254} , a_{320} , S_{382} , S_{289}) across lakes and seasons revealed patterns in biogeochemical processes and environmental conditions. The first three principal components explained 71.1% of cumulative variance (PC1: 35.4%; PC2: 21.5%; PC3: 14.2%; loadings in Table VII.I.5).

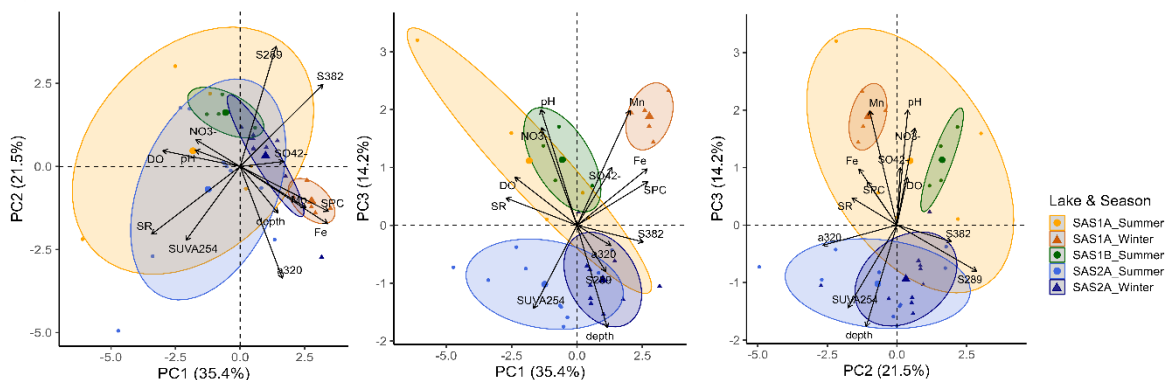


Figure III.4. Principal component analysis biplots of water column samples across lakes and seasons (3 PCs: 71.1% cumulative variance). Each point corresponds to an individual water column sample. Ellipses indicate 68% confidence interval around the centroid of each group, illustrating the multivariate dispersion and group separation.

PC1 reflected oxic-anoxic gradients with SPC, S_{382} , dissolved Fe and Mn (positive) separate from DO, SUVA₂₅₄, S_R (negative). PC2 contrasted S_{289} and S_{382} in DOM (positive) from a_{320} , SUVA₂₅₄, and S_R (negative), suggesting a DOM molecular weight gradient. PC3 separated pH and TEAs (NO_3^- and Mn; positive) with depth and SUVA₂₅₄ (negative), suggesting biogeochemical changes linked to ontogenic succession.

Seasonally, summer waters in SAS1A and SAS2A displayed broader ellipses, indicating greater water column heterogeneity, while winter samples clustered tightly. SAS1B aligned with positive PC2 and PC3 scores (elevated nitrate concentrations), while SAS1A aligned with positive PC3 and partial negative PC1 scores (pH, DO, nitrate and S_R), and SAS2A aligned with negative PC3 scores (SUVA₂₅₄ and a_{320}). Winter SAS1A and SAS2A showed both positive PC1 scores (SPC, dissolved Fe and S_{382}) reflecting anoxic conditions, though SAS1A also aligned with positive PC3 (pH and Mn), whereas SAS2A aligned with negative PC3 scores (Fig. III.4).

PERMANOVA showed lake and season explained 43.8% of observed variance ($p = 0.001$), with no significant group dispersion differences (PERMDISP: $p = 0.344$). Post-hoc pairwise comparisons with Bonferroni corrections (Table VII.I.6) revealed significant seasonal differences in SAS2A ($R^2=0.21$; p -adjusted = 0.01), and between-lake differences in winter ($R^2=0.44$; p -adjusted = 0.01).

III.3.5. Isotopic $\delta^{13}\text{C}$ signatures and C/N ratios in soils and sediments

The $\delta^{13}\text{C}$ signatures and C/N ratios of soils and sediments were analyzed to assess carbon sources and processing. For soils, $\delta^{13}\text{C}$ signatures were consistent with the range for C3 terrestrial plants (Lamb et al., 2006), with $\delta^{13}\text{C}$ and C/N ratios being similar between total carbon and the organic fraction; therefore, analyses focused on the organic fraction. C/N ratios varied widely in soils, with SAS2A soils exhibiting the highest ratios, indicating carbon accumulation in peat soils, likely reflecting lower decomposition rates (Table VII.I.3).

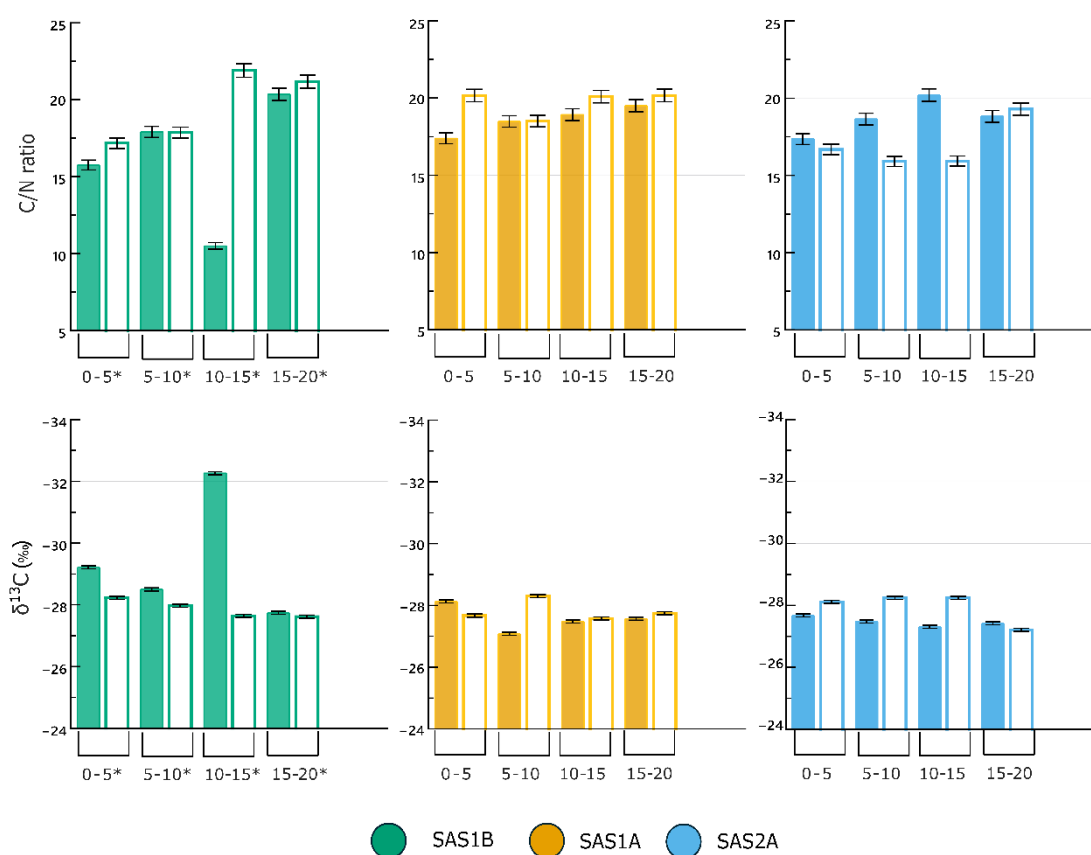


Figure III.5. Seasonal distribution of C/N ratios and $\delta^{13}\text{C}$ signatures in the organic fraction of sediment layers in winter (light shade color) and summer (dark shade color). Green: SAS1B; Yellow: SAS1A; Blue: SAS2A. *Winter samples correspond to 0-3; 3-6; 6-9; 9-12 cm in SAS1B sediments.

Sediment $\delta^{13}\text{C}$ and C/N ratios were generally consistent between total and organic fractions, except in two SAS1B layers (summer: 10-15 cm; winter: 9-12 cm; see Table VII.I.1). Across all three lakes, seasonal and depth-dependent patterns emerged (Fig. III.5). SAS1B showed pronounced seasonal shifts, with 20% lower C/N ratios and more depleted $\delta^{13}\text{C}$ signatures in summer relative to winter, suggesting stronger seasonal

variation in carbon sources or processing at this site compared to SAS1A and SAS2A. SAS1A exhibited modest seasonal change (6% higher C/N ratios on average in winter) with stable $\delta^{13}\text{C}$ signatures, while C/N ratios increased with depth during summer. SAS2A showed a 13% decrease in C/N ratio and shift towards more ^{13}C -depleted signatures in winter, particularly in the upper 15 cm, consistent with increased autochthonous sources and enhanced microbial degradation during winter. Furthermore, SAS2A had the lowest carbon content (in weight percentage, wt%) across both seasons (Table VII.I.4).

III.3.6. Metal contents in soils and sediments

In surrounding soils (Fig. VII.I.12), SAS2A exhibited the lowest Fe and Mn concentrations, consistent with long-term depletion from repeated freeze-thaw mobilization. Soils from SAS1A and SAS1B showed greater within-site variability at the lake margins.

Total Fe and Mn content in SAS lake sediments showed distinct seasonal patterns (Fig. VII.I.11). In both SAS1A and SAS1B, Fe concentrations were higher in summer than in winter, with increases of 25-39% in SAS1A and 30-44% in SAS1B, indicating Fe accumulation during the warmer months. In SAS1B, this was especially marked at the surface, where Fe reached 19 g kg^{-1} in the 0-5 cm layer, 43% higher than in the 5-10 cm layer. In contrast, SAS2A sediments showed increasing Fe and Mn concentrations with depth during summer. However, in winter, Fe levels rose in the upper 10 cm of SAS2A sediments relative to summer, suggesting surface mobilization.

Mn behaved differently than Fe, particularly in SAS1A, where concentrations increased by 8-49% in winter compared to summer, especially in deeper layers. This pattern points to distinct redox controls and potentially different sources. Mn levels in SAS1A also consistently exceeded those in SAS1B and SAS2A.

III.3.7. Fourier-transform infrared spectroscopy in soils and sediments

The FTIR spectra of the organic-rich sediments (Fig. III.6) generally exhibited the following features: (1) a band centered at $1070\text{-}1030 \text{ cm}^{-1}$; (2) a broad band centered at 1225 cm^{-1} region; (3) a broad band in the $1370\text{-}1460 \text{ cm}^{-1}$ range; (4) a broad band centered

at 1630 cm^{-1} with a shoulder in the $1550\text{-}1520\text{ cm}^{-1}$ region; (5) a small shoulder-like band at 1730 cm^{-1} (6) two well-defined peaks at 2918 and 2850 cm^{-1} ; and (7) a large broad band at $3000\text{-}3400\text{ cm}^{-1}$. Detailed FTIR spectra of winter and summer sediment samples for lakes SAS1B, SAS1A and SAS2A are provided in Supplementary Information, Fig. VII.I.13, VII.I.14, and VII.I.15, respectively.

Asymmetric Si-O-Si stretching vibrations can overlap with the polysaccharide band (Ellerbrock et al., 2022). A shoulder at 955 cm^{-1} in near-surface sediments, can indicate Si-O stretching (Ritz et al., 2014), while the band at 800 cm^{-1} has been linked to the presence of biogenic silica (Petrovskii et al., 2016). Functional group attributions for organic FTIR signals are detailed in Table VII.I.7.

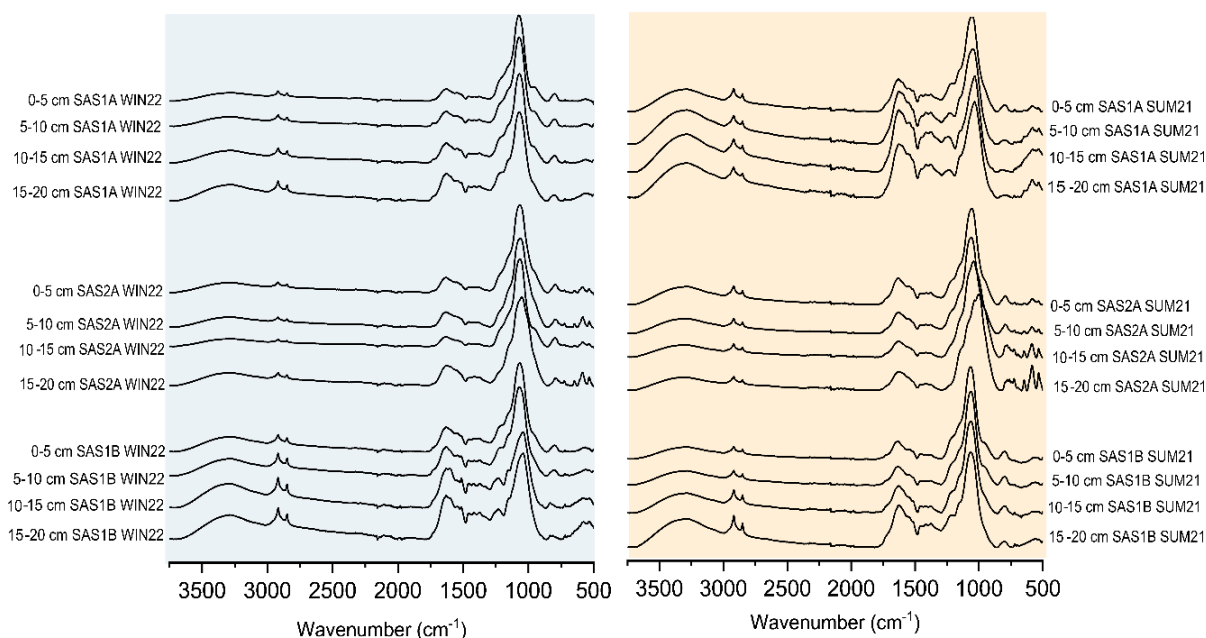


Figure III.6. FTIR spectra of sediment samples at 0-5, 5-10, 10-15 and 15-20 cm depth during winter (left) and summer (right) for SAS1A, SAS2A and SAS1B.

The seasonal effects on the FTIR spectra of SAS1B sediments differ from those in other SAS lakes. In deeper summer SAS1B sediments, increases in the broad $3400\text{-}3000\text{ cm}^{-1}$ band, the 2920 and 2850 cm^{-1} peaks, and the 1226 and 1155 cm^{-1} bands, indicate the increase in hydroxyl groups, aliphatic structures (i.e. waxy lipids, fatty acids), aliphatic esters and possibly phenolic moieties with depth. The Si-O band at 955 cm^{-1} intensifies toward surface sediment layers. In winter, similar trends occur at depth, although more pronounced. Specifically, increases in 2918 , 2850 , 1515 , and 1226 cm^{-1} bands, the polysaccharide band shifts to lower wavenumbers and 1460 , 1417 , and 1380 cm^{-1} bands

become more defined at greater depths. Unlike SAS1A and SAS2A, winter SAS1B sediments show higher content in waxy lipids/fatty acids, aromatic structures, and aliphatic esters than in summer.

In SAS1A sediments, the polysaccharide band shifted to lower wavenumbers in summer relative to winter, with increases in 2918, 2850, 1630 and 1226 cm^{-1} (well-defined peak at depth) bands, plus enhanced hydroxyl band stretching (3400-3000 cm^{-1}), indicating a higher presence of waxy lipids, carboxylic acids, and oxygen-rich functional groups during the warmer season. The 954 cm^{-1} band emerged during winter and increased in intensity toward the surface in SAS1A.

In summer SAS2A sediments, the polysaccharide band shifted to lower wavenumbers, the 1733, 1630 and 1226 cm^{-1} bands decreased with depth, indicating reduced carboxylic acids and possibly phenolic structures at greater depths. Winter SAS2A sediments exhibited decreased 3400-3000 cm^{-1} , 2920 and 2850 cm^{-1} bands, loss of definition in 1460, 1417, and 1380 cm^{-1} bands. Coupled with the 950 cm^{-1} band in deeper sediment layers suggests losses of oxygenated functional groups and aliphatic compounds.

Winter sediments in SAS1A and SAS2A showed lower humification indices (HI) than their summer counterparts (Table VII.I.2), opposite to SAS1B. Higher HI indicate greater aerobic decomposability (Harris et al., 2023), though the 1630 cm^{-1} region includes overlapping signals (aromatic C=C, amide I C=O, and carboxylate C-O) requiring careful interpretation.

III.3.8. Sediment porewater dynamics

In summer, SAS1B porewaters showed elevated and variable P1 signals relative to SAS1A and SAS2A, suggesting active transformation of labile NOM (Fig. III.3). SAS1A exhibited a pronounced P1 peak at the sediment-water interface, while SAS2A displayed limited FDOM variation and lower S_R (Fig. VII.I.17). In both SAS1A and SAS2A, winter porewaters exhibited lower S_R compared to summer, indicating the presence of high molecular weight DOM (Fig. VII.I.19). A PCA of summer sediment porewater properties (SUVA_{254} , a_{320} , S_R , DOC, %HT1, %HM1, %HT2, %P1, %P2, SO_4^{2-} , and NO_3^-) revealed patterns in sediment biogeochemistry (Fig. III.7).

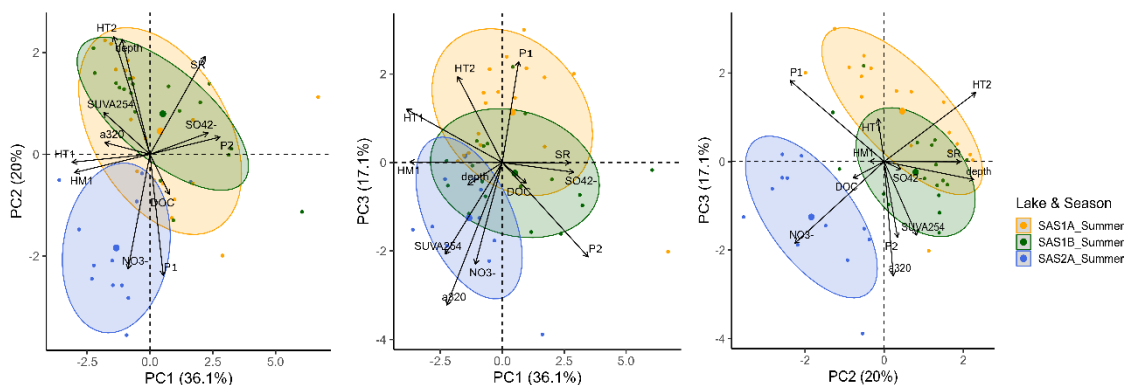


Figure III.7. Principal component analysis biplots of sediment porewaters across lakes and seasons (3 PCs: 73.2% cumulative variance). Each point corresponds to an individual porewater sample. Ellipses indicate 68% confidence interval around the centroid of each group, illustrating the multivariate dispersion and group separation.

The first three principal components explained 73.2% of cumulative variance (PC1: 36.1%; PC2: 20.0%; PC3: 17.1%; loadings in Table VII.I.5). PC1 separated sulfate-rich environments with higher S_R and P2 (positive), from humic-like, aromatic, CDOM-rich porewaters (HT1, HM1, a_{320} , SUVA₂₅₄ — negative). PC2 contrasted depth-related processes separating depth, S_R and HT2 (positive) from more nitrate-rich environments with P1 enrichment (negative). PC3 opposed increases in P1, HT2 and HT1 (positive) from nitrate-enriched, high aromatic, CDOM-rich with increased P2 signals in (NO_3^- , P2, a_{320} , and SUVA₂₅₄ — negative). PCA revealed distinct porewater biogeochemical patterns: SAS1B aligned with positive PC2 (higher SO_4^{2-} , S_R and P2); SAS1A showed positive PC3 while shifting to more positive PC2 (elevated HT2, P1, and S_R); SAS2A aligned with negative loadings in all PC1, PC2 and PC3 (higher a_{320} and NO_3^-) indicating CDOM-rich, humic DOM in nitrate-rich conditions.

NMDS analysis revealed distinct biogeochemical signatures between lakes during summer (stress = 0.149), with SAS1A occupying an intermediate position between SAS2A and SAS1B, suggesting a transitional state (Fig. VII.I.16). PERMANOVA showed lake groupings explained 42.7% of observed variance ($p = 0.001$), with PERMDISP ($p = 0.33$) indicating differences arose from mean centroid differences. Pairwise comparisons (Bonferroni corrected) indicated significant divergences between sediment porewaters (Table VII.I.6).

III.4. Discussion

III.4.1. DOM dynamics among lakes

During open water conditions, all thermokarst lakes exhibited strong thermal stratification, likely influencing microbial activity and DOM cycling (Davidson and Janssens, 2006; Hu et al., 2024). Across all thermokarst lakes, photochemical breakdown of large, recalcitrant DOM mobilized from surrounding peat soils produced smaller aromatic DOM structures, as described by Hu et al. (2023). This photolysis of larger recalcitrant moieties also likely increased CDOM content in the lakes. However, lake morphometry profoundly shaped the patterns produced by this mechanism.

In the younger SAS1B, this mechanism is evident at the surface, where waters showed increased CDOM and DOM aromaticity (a_{320} and SUVA₂₅₄ values) while deeper waters displayed higher molecular weight DOM (lowest S_R values). The absence of P1 (tryptophan-like FDOM) and rapid DO decline throughout the water column suggests the rapid consumption of labile DOM.

However in SAS1A, the shallow depth and larger surface area (8 times larger than SAS2A and 9 times larger than SAS1B) likely promoted wind-driven mixing during summer, in turn enhancing photochemical degradation of DOM at surface — illustrated by decreased CDOM and DOM aromaticity. Reactive oxygen species (ROS) produced by the interaction of sunlight, CDOM and molecular oxygen (Cory et al., 2010) drive photobleaching in well-mixed surface waters (Scully et al., 2003). Increased nutrient availability and elevated temperatures, combined with enhanced photobleaching, may support primary production at the surface (Beardall and Raven, 2013; Visser et al., 2016; Vasker et al., 2021). This is consistent with increases in protein-like fluorescence (P1 at surface, and P2 in the first 100 cm) and elevated pH through CO₂ uptake (Gerardi and Lytle, 2015). Deeper waters in SAS1A, however, remain enriched in CDOM and aromatic structures, likely from sediment inputs, potentially stabilizing the dissolved Fe concentrations (Maloney et al., 2005; Xiao et al., 2013).

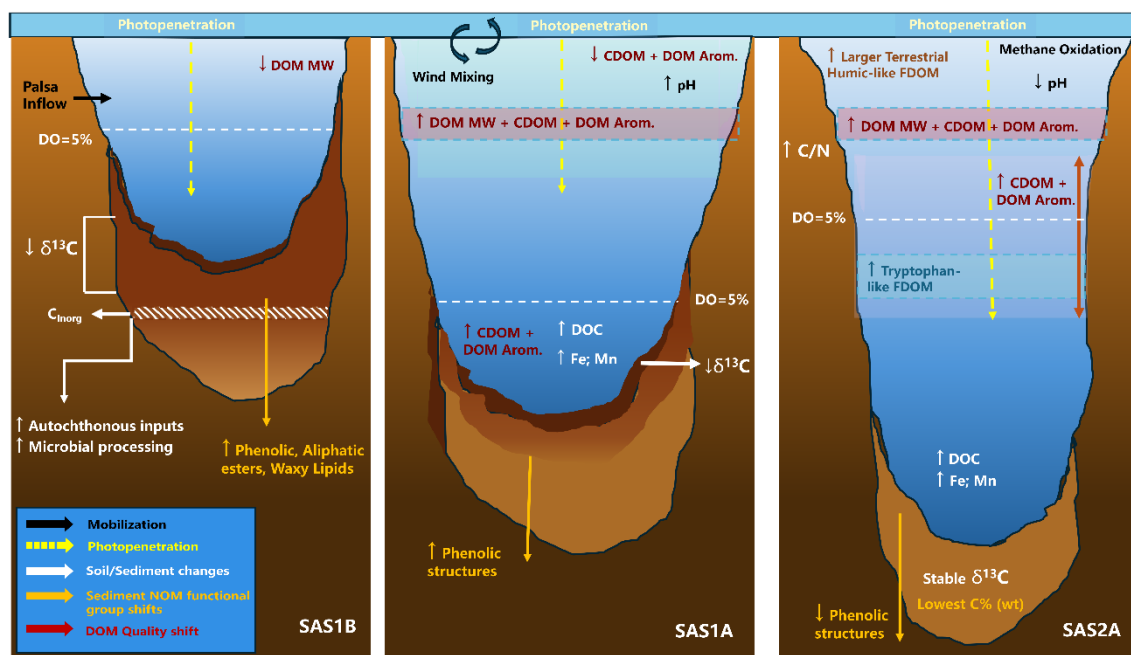


Figure III. 8. Conceptual diagram of summer biogeochemical processes across the three studied thermokarst lakes at different stages of ontogenic development in a subarctic peatland. The figure illustrates vertical gradients in DO consumption (white dashed line), DOM composition, molecular weight (MW), and pH in the water column, as well as C/N, $\delta^{13}C$ and FTIR-derived functional groups in the sediment profile. Arrows denote increase/decreases ($\uparrow/\downarrow$), while yellow dashed lines represent the extent of light penetration. Yellow dashed arrow: light penetration extent. Yellow solid arrow: sediment NOM functional group changes. White solid arrow: changes in soil/sediment sources/processes. Red solid two-way arrow: region of DOM quality shifts.

In the deeper, stratified SAS2A, the same photochemical breakdown mechanism operated, but stratification fundamentally altered its vertical distribution and intensity. In the SAS2A epilimnion, there are signs of a greater extent of microbial and bacterial processing, leading to the depletion of labile DOM — decline of protein-like FDOM (P1 and P2) and increase in terrestrial humic-like component HT2. Surface waters do not exhibit the same photobleaching patterns observed in SAS1A, since the smaller surface area limits wind-mixing effects. Moreover, conditions are notably more acidic in the epilimnion of SAS2A, consistent with the accumulation of dissolved CO_2 . This may reflect methane oxidation and associated CO_2 production, a possibility previously reported in this lake (Crevecoeur et al., 2017; Vigneron et al., 2019), or the formation and accumulation of organic acids. Metalimnetic conditions are profoundly shaped by: (i) photodegradation of larger recalcitrant DOM into smaller aromatic structures; (ii) the interplay between primary producers and grazers. CDOM content and DOM aromaticity increases in the metalimnion, which may partially arise from photoproduct aromatic

DOM from the breakdown of larger recalcitrant structures. Increased light penetration coupled with Fe availability may create optimal conditions for metalimnetic primary production (Schuback et al., 2015; Dengg et al., 2022; Swanner et al., 2022) and heterotrophic activity — potential origin of P1 (tryptophan-like FDOM) increase at 125 cm depth. Wauthy et al., (2020) showed phytoplankton biomass peaked near this region (150 cm depth), with zooplankton accumulating in the lower epilimnion to metalimnion region of SAS2A during summer. Similar to other SAS lakes, the hypolimnion accumulated CDOM and increased dissolved Fe and DOC concentrations from sediment inputs and reductive dissolution (Davison, 1993; Chadwick et al., 2006; Munger et al., 2016), while DOM aromaticity decreased relative to the metalimnion.

III.4.2. Unexpected shift in S_R in summer

A clear change in DOM molecular weight (increase in S_R) is observed at 50 cm in SAS1A ($S_R = 1.8$) and between 50–75 cm in SAS2A ($S_R = 1.3$ – 1.5), relative to surface values (SAS1A: $S_R = 0.78$; SAS2A: $S_R = 0.70$; Fig. III.2). This shift to higher S_R coincides with a strong decrease in both S_{289} and S_{382} slopes (Fig. VII.I.21), with S_{382} showing a more pronounced decrease relative to S_{289} .

Concurrently, this region is marked by increased DOM aromaticity (higher $SUVA_{254}$) and CDOM content (higher a_{320}), along with enhanced absorbance in the photosynthetically active radiation (PAR) range (400–700 nm). Second-derivative UV-visible spectra (Fig. VII.I.22) reveals the presence of chromophores absorbing at longer wavelengths in these samples, likely derived from pigments (Squier et al., 2004; Lozovik et al., 2007; Röttgers and Koch, 2012) or humic structures.

The distinct accumulation of aromatic DOM and CDOM occurred at 50 cm depth in both SAS1A and SAS2A, represents an intermediate zone of DOM transformation, likely comprising a photochemically-produced pool. At this depth, photochemical breakdown of larger recalcitrant particulate and colloidal organic matter ($>0.2 \mu\text{m}$) produced smaller aromatic structures that passed through the $0.2 \mu\text{m}$ filter. With sufficient light penetration to drive the photochemical breakdown of larger recalcitrant structures, this zone represents optimal conditions for DOM photochemical processing. The accumulation of these photoproduct aromatic compounds before further degradation or removal creates a transient pool distinct from surface waters.

Additionally, we note the photochemically-produced aromatic DOM at 50 cm may be further modified by heterotrophic activity. Localized primary production near surface — likely supported by CDOM-mediated UV shielding (Arrigo and Brown, 1996; Goldman et al., 2013; Häder et al., 2014; Strandberg et al., 2023) — may generate autochthonous DOM and fuel zooplankton activity (Kissman et al., 2017; Hernández-León et al., 2020), contributing to the pool being processed in this range. Zooplankton biomass peaks at the oxic-anoxic interface in these highly heterotrophic systems (Wauthy et al., 2020) and is known to enhance PAR absorbance (Williamson et al., 2007) and CDOM production (Steinberg et al., 2004). Nevertheless, we expect the primary driver of the accumulation of high molecular weight DOM and aromatic structures to be the photochemical breakdown of larger recalcitrant DOM, with heterotrophic processing potentially amplifying CDOM concentrations at this depth.

III.4.3. Seasonal trends in DOM

Seasonal variations in ice cover and oxygen availability fundamentally separate the dynamics of thermokarst lake functioning into two distinct regimes: (i) winter anoxia favoring TEAs redox-driven DOM processing; (ii) open water oxic photochemical and heterotrophic pathways of DOM processing. These environmental controls on oxygen, temperature, and light availability drive shifts in redox dynamics, DOM composition and dissolved Fe distribution patterns, with lake-specific variations reflecting differences in morphometry.

Under winter anoxic conditions, DOM processing is driven by TEAs due to the absence of DO in the water column. The shift in redox conditions promotes both the redissolution of Fe and the release of reactive terrestrial DOM from lake sediments (Lau et al., 2024) — illustrated by lower S_R and higher Fe and DOC concentrations — with water column profiles becoming more homogeneous compared to summer. Additionally, %Fe(II) correlated positively with HT1 during winter throughout the water column in SAS2A (Spearman's $\rho = 0.915$; $p\text{-value} < 0.001$). This likely reflects redox recycling of suspended Fe(III) particulate matter along the water column, whereby reduction promotes the release of Fe(II) and previously sorbed terrestrial humic-like DOM (HT1) (Peter et al., 2016; Lau et al., 2024). Although often correlated, high molecular weight DOM does not always coincide with elevated $SUVA_{254}$ (Korak & McKay, 2024). Freeze-concentration effects

under-ice water column are expected to have contributed partially to higher DOC concentrations. Production and accumulation of CO₂ in winter waters from DOM mineralization led to more acidic pH values, consistent with total dissolved gas profiles (Fig. VII.I.18) and previous studies (Matveev et al., 2019).

Both lakes display the same underlying redox-driven mechanisms, however differences in mixing may have contributed to the emergence of lake-specific patterns. Namely the increase of CDOM and dissolved Fe concentrations near the bottom in SAS2A, while SAS1A displayed a more homogeneous water column. Notably, both lakes displayed the preservation of protein-like FDOM due to diminished microbial processing at low temperatures (Bouvet et al., 2025), with P1 consistently higher than P2 (Fig. VII.I.9). Conversely, Mazoyer et al., (2022) found no protein-like FDOM in late winter DOM from SAS2A. This discrepancy may be due to consumption of labile protein moieties during the period of four months between sampling and the experimental setup, addressed in the study.

Our observations indicate that anoxic winter conditions enable the simultaneous occurrence of three distinct processes: (i) preservation of labile DOM fractions (protein-like FDOM) due to low microbial rates; (ii) mobilization of recalcitrant terrigenous material (high molecular weight aromatic DOM) through Fe(III) reduction-driven desorption; (iii) accumulation of dissolved CO₂ from NOM mineralization.

Spring thaw represents a critical transition when inherited winter conditions — low pH, elevated Fe and DOC with high molecular weight — interact with light to drive photochemical DOM mineralization. We note that in spring thaw, thermokarst lakes are also expected to receive spring freshet from surrounding soils providing inputs of NOM, Fe and other nutrients into the lake. Lower pH significantly enhances the rate of Fe-stimulated photochemical DOM degradation (Anesio and Granéli, 2003; Gu et al., 2017). Given that SAS2A showed significantly lower pH than SAS1A during winter, Fe-driven photochemical DOM mineralization may be more pronounced in SAS2A. We emphasize that direct measurements of photochemical rates or photochemical yields were not obtained in this study, nor did we research spring thaw effects. Our inferences are based on the chemical pre-conditions established during winter sampling (low pH, elevated Fe) and the existing literature of photochemical interactions between DOM and Fe at low pH.

In summer, surface and deeper waters follow starkly divergent characteristics, divided by thermal stratification and light attenuation by CDOM and particulate organic matter (POM). In the oxic epilimnion, light-driven photochemical reactions dominate DOM processing, while anoxic deeper waters are governed by redox-controlled transformations mediated by TEAs.

Surface water photochemistry is fundamentally constrained by CDOM and POM. High POM and CDOM concentrations attenuated light with depth, creating a self-limiting photochemical regime where DOM photodegradation becomes substrate-rich but light limited in the near-surface layer, particularly in SAS2A where reduced surface area and limited wind mixing enhance stratification. Despite this light limitation, photochemical fragmentation of terrestrial high molecular weight DOM ($>0.2 \mu\text{m}$) produces lower molecular weight aromatic compounds, as evidenced by elevated SUVA_{254} and CDOM (a_{320}) in the metalimnion. These photochemically-produced aromatics subsequently fuel heterotrophic dynamics of specialized communities adapted to using them as substrate.

The persistent depletion of dissolved Fe in the epilimnion and metalimnion suggests an active Fe removal mechanism. Photochemical degradation of high molecular weight aromatic DOM may drive the aggregation of dissolved Fe(III) into colloidal particles that settle to deeper waters—a process documented as photoflocculation in terrestrial-dominated freshwater systems (Helms et al., 2013; Shammi et al., 2017). In contrast, anoxic deeper waters are characterized by TEA-driven DOM processing. The rapid DO decline in the water column, especially pronounced in SAS1B, reflects intense heterotrophic respiration. In anoxic bottom waters, reducing conditions mobilize previously complexed dissolved Fe and sorbed terrestrial DOM from sediments, creating a distinct gradient.

III.4.4. Ontogenic trends in thermokarst lakes

Thermokarst lake ontogeny fundamentally reshapes biogeochemical functioning through systematic changes in substrate supply. As young lakes mature, terrigenous inputs shift from labile, nutrient-rich sources to increasingly recalcitrant, nutrient-poor sources. Increased hydrological connectivity facilitates the mobilization of NOM and nutrients from surrounding soils into thermokarst lakes (Koch et al., 2018; Wauthy et al., 2018; Prijac et al., 2023). The three study lakes represent distinct ontogenic stages: SAS1B and

SAS1A as emerging/developing systems, and SAS2A as mature. This progression drives coupled changes in nutrients, DOM composition and sediment processing.

The ontogenic stage heavily influences input quality, with young and developing lakes receiving more nutrient-rich material, reflected in elevated summer nitrate concentrations — likely arising from increased inputs of ammonium and organic nitrogen species from surrounding soils. Mature lake SAS2A, however, is characterized by recalcitrant soils — elevated C/N ratios (Table VII.I.3) — and decreased Fe and Mn concentrations — indicative of depletion over successive freeze-thaw cycles (Payandi-Rolland et al., 2021), consistent with the region's prolonged permafrost thaw (Bhiry et al., 2011). The anaerobic peat soil conditions in waterlogged peatlands promote Fe reduction (Patzner et al., 2020), and when depleted promote the accumulation of recalcitrant NOM. In turn, the mobilized NOM inputs from soils to the lake become increasingly recalcitrant.

Young SAS1B shows the signs of being the most microbially active thermokarst lake during summer — evidenced by the most accentuated DO decline, extensive sediment NOM processing and absence in P1 throughout the water column. Tryptophan-like FDOM (P1) has been associated with biodegradable DOM fractions (Cammack et al., 2004; Berggren et al., 2019), and its depletion indicates accelerated DOM processing of labile moieties. Hydrological inputs from thawing palsas likely fuel benthic processes in SAS1B to a greater extent than in SAS1A and SAS2A.

In mature SAS2A, a smaller surface area and deeper stratification, combined with greater inputs of recalcitrant NOM from surrounding peat soils, likely enhance the photochemical production of aromatic structures (Hu et al., 2023; Zhou et al., 2024) — evidenced by increased SUVA₂₅₄ (DOM aromaticity) and a_{320} (CDOM). This change in DOM composition shaped bacterial communities and existing metabolisms, as high aromatic DOM shifts bacterial communities toward deterministic assembly (Zhou et al., 2021). Vigneron et al. (2019) found the metalimnion of SAS2A was dominated by Betaproteobacteria (Burkholderiales), comprising 27% of the community during summer, a community often associated with elevated DOM aromaticity and CDOM content (Roiha et al., 2016; Kang et al., 2023). While we did not measure microbial communities directly, our observations converge with established literature, suggesting a shift in DOM processing mechanisms in mature thermokarst lakes relative to younger systems, promoted by both changes in NOM inputs and bacterial community assembly.

In contrast, SUVA₂₅₄ values for SAS1A and SAS1B during summer were within the range previously reported for subarctic thermokarst lakes (Wauthy et al., 2018; Peura et al., 2020; Shirokova et al., 2020; Zabelina et al., 2021; Kurashev et al., 2024). However, we note that in the case of SAS1A, lake morphometry could have contributed to enhanced photobleaching, producing decreased DOM aromaticity at surface. Clearly isolating effects arising from morphometry and from ontogeny is challenging since they are intrinsically linked. However, by focusing on variables linked to the evolution of the system, including the water-soil interaction — inputs of nitrogen species and more recalcitrant DOM and POM inputs — we can identify factors more closely associated with the lake maturation stage.

III.4.5. Insights from sediment and riparian soil composition

Sediments act as NOM processors in thermokarst lakes, with compositional and degradation patterns reflecting the ontogenic stage of each lake. Surrounding soils, waterlogged and reducing beneath permafrost-affected landscapes, mobilize both Fe and Mn, as well as NOM into lakes through successive freeze-thaw cycles and spring freshet events, with input composition reflecting soil age and thaw history. The depositing of NOM from surrounding soils to lake bottom create a seasonal input of fresh NOM to lake sediments.

Lake sediments were deposited as organic-rich gyttja (humic muds) as described by Bouchard et al. (2011), displaying distinct seasonal contrasts in processing. Both SAS1A and SAS1B sediments showed higher Fe content in summer than winter, reflecting immobilization and precipitation in Fe-POM complexes and Fe(oxy)hydroxides during open water conditions (Laberge-Carignan et al., 2024). In contrast, the mature SAS2A exhibited increasing Fe concentrations with depth during summer, indicating enrichment in deeper sediment layers relative to recent sediment deposits. This pattern likely reflects higher Fe inputs during earlier stages of sediment deposition, similar to those observed in SAS1A and SAS1B, whereas more recent inputs from currently Fe-depleted surrounding soils result in lower Fe concentrations in surface sediments.

Summer porewater biogeochemistry reflected enhanced benthic processing: increased P1 (labile, tryptophan-like FDOM) toward the sediment surface and increasing HT2 (terrestrial humic-like FDOM) with depth. Across all lakes, P1 decreased significantly

with increasing depth (Spearman's $\rho = -0.54$; p -value <0.001), whereas HT2 increased with depth ($\rho = 0.47$; p -value <0.001). This depth-dependent pattern indicates enhanced production of labile, biodegradable DOM in upper sediment layers during summer, likely driven by higher temperatures and intensified heterotrophic degradation of NOM substrates. Laberge-Carignan et al. (2024) reported higher Fe and Mn concentrations in winter SAS porewaters, confirming that seasonal redox-driven mobilization beneath the sediment-water interface increases during anoxic winter conditions.

The emerging SAS1B displayed the most pronounced seasonal sediment NOM variation. Summer sediments showed signs of intense benthic processing: more depleted $\delta^{13}\text{C}$ signatures, lower C/N ratios, and decreases in hydroxyl, aliphatic, phenolic, and carboxylic moieties in sediments. The presence of Si-O bands in degraded sediment layers suggests microbially mediated amorphous silicate precipitation (Osorio-Rodriguez et al., 2023), a secondary mineral formation pathway.

In contrast, winter sediments in SAS1B accumulated aromatic structures, waxy lipids, aliphatic esters, and recalcitrant NOM (higher C/N, hydrogen index, and enriched $\delta^{13}\text{C}$ signatures), reflecting the accumulation of refractory compounds resistant to winter microbial degradation. The isotopic offset between total and organic carbon fractions (summer: 10-15 cm; winter: 9-12 cm) suggests the presence of inorganic carbonates. These authigenic carbonates likely originate from methanotrophic activity (Marlow et al., 2014; Akam et al., 2020; Argentino et al., 2022), consistent with the high proportion of methanotrophs detected in bottom waters of SAS1B (Crevecoeur et al., 2015). The presence of active methanotrophy indicates that anaerobic methane oxidation (AOM), coupled with assimilation by autotrophs into sediment organic matter, is likely to represent an active pathway in the emerging SAS1B. The primary $\delta^{13}\text{C}$ signature across all sediments is aligned with decaying C3 terrestrial plant matter (Lamb et al., 2006), indicating the bulk sediment $\delta^{13}\text{C}$ signal reflects the predominantly terrestrial origin of the organic matter substrate. The $\delta^{13}\text{C}$ signatures (Sobek et al., 2014; Osburn et al., 2019) and C/N ratios (Pedersen et al., 2011; Krickov et al., 2018; Manasypov et al., 2022) were within the reported ranges for Arctic lake sediments. Conflicting mechanisms drive $\delta^{13}\text{C}$ shifts during microbial NOM degradation: $\delta^{13}\text{C}$ enrichment may arise from preferential ^{12}C consumption (Ågren et al., 1996; Wang et al., 2015), while depletion can result from loss of ^{13}C -enriched labile NOM (Meisel et al., 2021). Microbial methanogenesis produces ^{13}C -depleted methane (Whiticar, 1999; Conrad & Claus, 2005),

while subsequent anaerobic methane oxidation (AOM) with assimilation by autotrophs into sediment NOM (Kellermann et al., 2012; Nowak et al., 2015) may contribute to depleted $\delta^{13}\text{C}$ NOM signatures. Given the documented GHG production in SAS lakes (Matveev et al., 2016; Matveev et al., 2019), depleted $\delta^{13}\text{C}$ signatures likely reflects assimilation of recycled methane-derived carbon, acting as a secondary source modifying the bulk terrestrial signature.

SAS1A sediments in summer showed a more processed surface layer ($\delta^{13}\text{C}$ depletion, C/N decrease) with recalcitrant NOM accumulation (higher C/N, increased phenolic and carboxylic structures) in deeper layers, consistent with a top-down degradation pattern. Summer porewater biogeochemistry in SAS1A occupied an intermediate position between SAS1B and SAS2A in multivariate ordination space (NMDS), mirroring the ontogenic gradient. Winter sediments in SAS1A showed accumulation of recalcitrant NOM (higher C/N, lower hydrogen index), with decreases in hydroxyl, phenolic, and carboxylic groups, indicating the consumption of nitrogen-rich compounds (Wakeham, 2002) and oxygen-rich functional groups (Carneiro Barreto et al., 2024).

In contrast to SAS1A and SAS1B, where recalcitrant NOM accumulates in winter, mature SAS2A exhibited a distinct pattern reflecting its advanced state of degradation. Summer sediment processing in SAS2A showed limited vertical patterns, while sediment porewaters showed elevated CDOM and nitrate concentrations, indicated active benthic mineralization. Critically, during winter, SAS2A sediments exhibit signs of more extensive degradation rather than accumulation of recalcitrant NOM compared to SAS1A and SAS1B. This is reflected by the decreased C/N ratios, depleted $\delta^{13}\text{C}$ signatures, along with decreases in oxygen-containing functional groups (hydroxyl, phenolic, carboxylic structures). The ontogenic progression of thermokarst lakes is reflected in sediment NOM processing. Younger and developing lakes engage in the degradation of labile nitrogen-containing functional groups and accumulation of recalcitrant NOM during winter (along with consumption of oxygen-rich functional groups). In contrast, mature SAS2A exhibits a transition in degradation pathways toward the utilization of recalcitrant NOM pools.

III.5. Conclusion

This study investigated the seasonal biogeochemistry of thermokarst lakes in a subarctic peatland region and their shifts with ontogeny. Specifically, we asked:

- 1) How do DOM dynamics in the water column of permafrost thermokarst lakes vary between summer and winter?
- 2) What seasonal patterns and biogeochemical processes govern NOM dynamics in the sediments and sediment porewaters of these thermokarst lakes?
- 3) What are the key similarities and differences in the biogeochemical processes and characteristics among permafrost thermokarst lakes with different ontogeny and morphometry?

Our findings reveal that thermokarst lake biogeochemistry in waterlogged peatlands is organized by three primary controls: oxygen availability (seasonal), substrate supply quality (ontogenic) and lake morphometry (structural).

Light exposure and oxygen availability seasonally divides thermokarst lake functioning into two distinct biogeochemical regimes. Winter anoxia fuels TEA-driven redox cycling, mobilizing dissolved Fe and terrestrial DOM from sediments, simultaneously accumulating dissolved CO₂ and preserving tyrosine- and tryptophan-like FDOM in the water column. Summer oxic conditions shift processing toward photochemical breakdown of large recalcitrant structures producing smaller aromatic DOM. Photoflocculation of DOM and Fe in upper waters in summer likely creates an Fe removal mechanism from the water column. These seasonal transitions create a fundamental control on thermokarst lake dynamics in these landscapes.

Ontogeny controls the quality of the substrates entering the thermokarst lakes. Younger systems receive fresher and nutrient-rich inputs, supporting the consumption of labile DOM and nutrient cycling. Mature lakes support the utilization of increasingly recalcitrant, aromatic DOM through substrate-adapted bacterial communities. Winter sediments in mature lakes show evidence of sustained degradation of recalcitrant structures, even under anoxic conditions, suggesting an adaptation to available substrates, contrary to accumulation patterns observed in younger systems.

Lake morphometry profoundly modulates the impact of these biogeochemical mechanisms on thermokarst lake dynamics. Large, shallow lakes likely experience enhanced wind-driven mixing, contributing to enhanced photobleaching at surface. Deeper, more stratified systems concentrate different biogeochemical processes in specific depth zones: dissolved CO₂ accumulation near surface; photoproduction of aromatic DOM and protein-like FDOM production in the metalimnion; hypolimnion dominated by sediment inputs. In both systems however, the lower epilimnion represented a zone of photochemical production of CDOM and aromatic DOM. These morphometric controls indicate that the same fundamental mechanisms exhibit contrasting patterns depending on thermokarst lake structure.

These controls — oxygen availability, ontogenic substrate progression, and lake morphometry — fundamentally shape DOM dynamics in thermokarst lake systems in waterlogged peatlands. While young lake functioning is fueled by labile and nutrient-rich inputs, mature systems become enriched in aromatic DOM and develop substrate-specific bacterial communities. Understanding their interplay and biogeochemical mechanisms is critical for establishing reliable models for carbon dynamics in permafrost landscapes.

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IV. Seasonal
biogeochemistry contrasts in
palsa- and lithalsa-origin
thermokarst lakes in a
discontinuous permafrost
zone

IV.1. Introduction

Over the last decades, average global air temperatures have been gradually increased, particularly in Arctic and subarctic regions (Hinzman et al., 2013; Rantanen et al., 2022; Lee and Romero, 2023), driving increases in ground temperature (Aalto et al., 2018; Biskaborn et al., 2019). The increase in air and soil temperatures fuels the thawing and degradation of the underlying permafrost (Romanovsky et al., 2010; Aalto et al., 2018; Biskaborn et al., 2019). Terrestrial permafrost is estimated to span across 15% of the Northern Hemisphere's land area (Obu et al., 2019), storing a carbon pool within the 1330 to 1580 Pg C (1 Pg C = 10¹⁵ g C) range (Schuur et al., 2015).

The spatial distribution of permafrost in northern landscapes changes considerably, and regions are often categorized based on permafrost coverage percentage: continuous (90-100%), discontinuous (50-90%), sporadic (10-50%) and isolated (<10%) (Vonk et al., 2015; French, 2017; Vincent et al., 2017). Mean annual ground temperatures in discontinuous permafrost zones have been reported to reach warming rates of 0.20 0.10 °C dec⁻¹ (Biskaborn et al., 2019).

In ice-rich sporadic and discontinuous permafrost zones, the development of permafrost features such as palsas (organic peat mounds) and lithalsas (mineral soil mounds) is commonly observed. Vegetation cover plays a key role by influencing thermal insulation during summer and modulating snow cover thickness in winter, thereby affecting ground temperatures (Zoltai, 1995; Grünberg et al., 2020; Heijmans et al., 2022). Reduced or irregular snow cover permits colder air temperatures to penetrate deeper into the soil, promoting frost penetration. In water-rich soils, cryosuction and ice segregation processes lead to the formation of segregated ice lenses at depth, causing ground heave and the gradual creation of a mound (Thomas et al., 2009; Pissart, 2013). Palsas and lithalsas primarily differ in their internal composition, which is strongly controlled by the nature of the surface deposits they develop within, with palsas characterized by an organic cover and lithalsas by a predominantly mineral substrate (Pissart, 2013).

Thawing of palsas and lithalsas promotes ground subsidence, which in turn drives the formation of thermokarst lakes, mobilizing dissolved organic matter (DOM), contaminants and nutrients into these newly formed aquatic systems (Vonk et al., 2015; Folhas et al., 2025; MacDonald et al., 2025). This mobilization stimulates microbial

activity, making thermokarst lakes into highly dynamic systems, driving the production of greenhouse gases (GHG) (Zimov et al., 1997; Matveev et al., 2018, 2019) and influencing the cycling of contaminants such as mercury (Schaefer et al., 2020; Malcata Martins et al., 2025). However, depending on the region and landscape in which they form, thermokarst lakes can differ substantially in their limnology (Wrona et al., 2016), GHG production (Laurion et al., 2010; Matveev et al., 2019) and DOM optical properties (Watanabe et al., 2011; Wauthy et al., 2018; Kurashev et al., 2024). For instance, in waterlogged peatlands the hydrological connectivity between peat soils and thermokarst lakes facilitates the export of soil organic matter into the lake (Prijac et al., 2023) and considerably shapes microbial communities in emerging systems (Leroy et al., 2025).

An important point to make is that thermokarst lakes are highly heterogeneous even within the same landscape. Variability in permafrost coverage, soil type, topography, vegetation and ground ice are regional factors of profound influence to thermokarst lake dynamics (Vonk et al., 2015). This heterogeneity hinders the transferability of direct interpretations on thermokarst lake research and efforts to infer broad-scale trends in thermokarst lakes from local observations. Therefore, an approach focusing on identifying biogeochemical mechanisms influencing lake dynamics may provide transferable insights relating to thermokarst lake system functioning.

Along a boreal forest-tundra transect in Nunavik, Canada, Freitas et al., 2024) reported higher limnodensity and limnodiversity in the discontinuous permafrost zone, where closely spaced palsa- and lithalsa-origin thermokarst lakes showed widely contrasting characteristics.

Thermokarst lakes of palsa-origin are dark-colored due to high concentrations of CDOM, with sunlight being absorbed strongly near lake surface, driving high stratification and anoxic conditions throughout the water column (Matveev et al., 2018; Freitas et al., 2024). Reportedly these lakes are strong emitters of both CO₂ and CH₄ (Matveev et al., 2018). In turn, lithalsa-origin thermokarst lakes are typically opaque and more varied in color, ranging from blue-green to white and light brown, receiving higher inputs of inorganic particulate matter (i.e. silts and clays) (Pissart, 2013; Coulombe et al., 2016; Freitas et al., 2024).

However, it has been established that there isn't a sharp boundary separating palsas and lithalsas, but rather a continuous spectrum of mound types (Pissart, 2013). This would suggest a range of hybrid characteristics in mounds between “pure organic palsas” and “pure mineral lithalsas”. For clarity of identification, the term palsa is abandoned as soon as the mound no longer exhibits a peat cover (Pissart, 2013).

Seasonality strongly influences biogeochemical dynamics in thermokarst lakes. During open water season, the exposure to sunlight and availability of dissolved oxygen (DO) play a significant role in photosynthetic activity and in shaping both DOM and redox dynamics (Cory et al., 2014; Hansen et al., 2016; Laurion et al., 2021). Once temperatures drop, an ice cover develops at the surface of the lake and pelagic conditions are separated from the atmosphere, with DO becoming rapidly consumed. In oxygen depleted conditions, microbial communities are forced to shift towards alternative terminal electron acceptors (TEAs) like nitrate (NO_3^-), Mn, Fe, and sulfate (SO_4^{2-}) (McMahon et al., 2011; Herndon et al., 2020; Perryman et al., 2020). Moreover, photochemical processes in the water column are significantly decreased due to the presence of both snow and ice cover, promoting the accumulation of recalcitrant, photolabile DOM (Mazoyer et al., 2022). In open water conditions, DO is usually available in the water column but depleted in deeper sediments, driving microbial activity to rely on TEAs to fuel benthic processes. Thus, mobilizing TEAs into the water column, serving as indicators of dominant redox cycling dynamics in thermokarst lake sediments (Arndt et al., 2013).

Natural organic matter (NOM), including DOM, plays a pivotal role in the biogeochemistry of thermokarst lakes, as microbial activity relies on the availability of labile NOM pools to use as substrate (Kurashev et al., 2024). It is expected that lakes formed from palsas and lithalsas will exhibit contrasting NOM dynamics in water column and sediment, despite limited literature on their different functioning. Several analytical approaches can be taken to explore these differing biogeochemical regimes, namely stable carbon isotope ratios ($\delta^{13}\text{C}$) in sediments, useful tracers for evaluating source contributions and transformation pathways of sediment NOM in thermokarst lakes (Wauthy et al., 2018; Wauthy and Rautio, 2020).

In this study, we investigate the seasonal dynamics of two thermokarst lakes — one formed from a palsa and the other from a lithalsa — located in the transition zone between a boreal forest and tundra environment in a discontinuous permafrost region.

Our approach involved a dual focus, with comprehensive characterization of both water column and sediment layers, enabling an integrated understanding of the thermokarst lake system as a whole. We address two main research questions:

- (1) How does seasonality influence the overarching biogeochemical dynamics of DOM, TEAs and physicochemical parameters in these thermokarst lakes?
- (2) How do the biogeochemical profiles of these two lakes differ, and what are the underlying reasons?

Understanding how seasonal changes and lake origin drive contrasts in thermokarst lake biogeochemistry provides essential insights and lays the groundwork for scaling these findings to assess their broader impacts on GHG dynamics and contaminant cycling.

IV.2. Materials and Methods

IV.2.1. Sampling and study sites

The study sites were situated in the Canadian subarctic, in an open glacial valley in the southeastern sector of the Ungava Bay, Nunavik (58°45'N, 66°0'W). Fluvio-marine sediments were deposited in the valley by incursions of the d'Iberville Sea into the coastal areas of the Ungava Bay during the deglaciation stage. The landscape in the valley is characterized by the presence of palsas, lithalsas and several thermokarst lakes, while vegetation was mainly comprised of mosses, lichens, herbaceous vegetation, shrubs (e.g. *Salix*, *Betula*).

Two thermokarst lakes were selected, lakes KANG1 and KANG2, with the former originating from the degradation of a lithalsa, and the latter from a palsa. Information on lake geographic coordinates and surface area is shown in Table IV.1. The surroundings of lake KANG1 exhibited smaller vegetation, comprised of lichens, herbaceous vegetation and small *Salix* shrubs. In contrast, the margins of KANG2 showed particularly dense shrub distribution (both *Salix* and *Betula*). Additionally, we note that the waters of KANG1 do not appear to be opaque and milky as observed in other lithalsa-formed thermokarst lakes.

Table IV.1. Identification of the thermokarst lakes, respective coordinates, surface area and origin.

<i>Lake</i>	<i>Coordinates</i>	<i>Surface area (m²)</i>	<i>Origin</i>
KANG1	58.757207°N, -66.009280°W	874	Lithalsa
KANG2	58.758626°N, -66.014306°W	794	Palsa

Landscape comparison between aerial photographs from 2022 (credits to Diana Martins) and 1960, reveal KANG2 is the more recently formed thermokarst lake (Fig. IV.1 and IV.2).

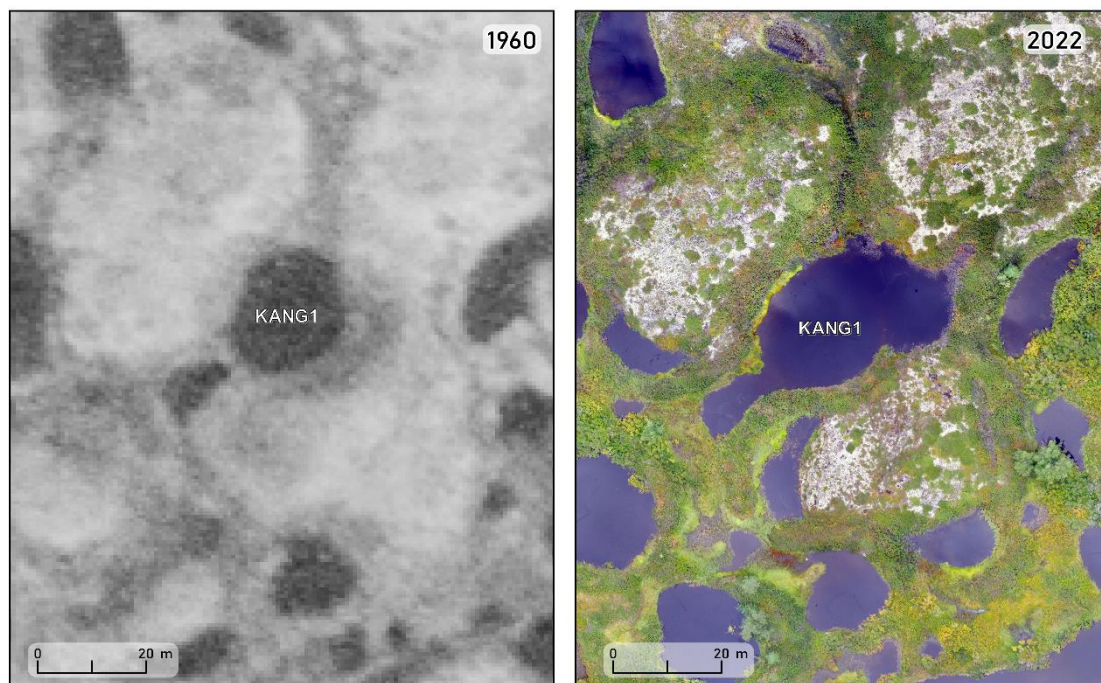


Figure IV.1. Evolution of lake KANG1 in aerial photographs from 1960 (left) and 2022 (right) – credits: Diana Martins.

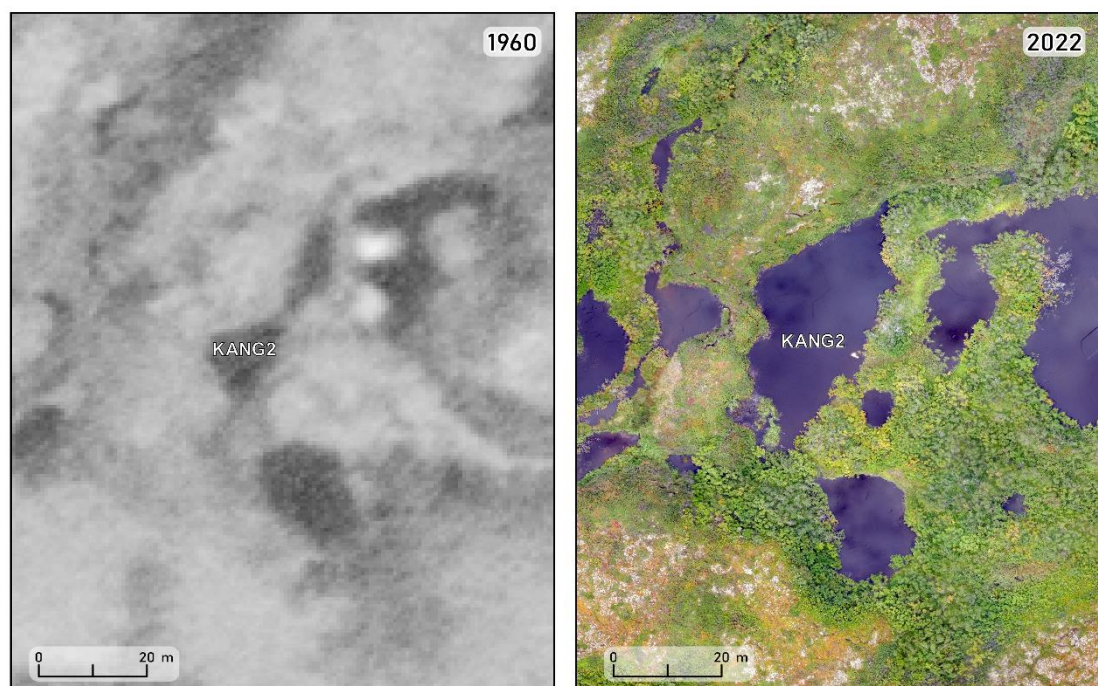


Figure IV.2. Evolution of lake KANG1 in aerial photographs from 1960 (left) and 2022 (right) – credits: Diana Martins.

Winter sampling took place during March 4th to 6th 2023, while summer sampling occurred during September 20th to 22nd 2023. Water samples were collected at the center of each lake using an inflatable boat, sampling every 25 cm depth using a portable peristaltic pump (12VDC Advanced, Royal Eijkelpamp). Considering the differences in depth profiles of KANG2 between seasons, we should consider that summer and winter sampling did not occur at the same part of the lake.

Mean annual air temperature (MAAT) was -1.9 °C, calculated for the continuous 12-month period from September 26, 2022, to September 26, 2023. MAAT was calculated based on hourly temperature records obtained from the Creep climate station (Nordicana D; doi:10.5885/45264SL-AF7FDA53F9E44927), which is the closest monitoring site to our study area (Sarrazin and Allard, 2024) .

Dissolved organic carbon (DOC) quantification and DOM characterization (UV-Vis and Fluorescence spectroscopy) samples were filtered at 0.22 µm polyether sulfone membrane filters (VWR) and 0.45 µm Whatman® Puradisc syringe filter and stored in decontaminated amber vials.

For total Fe and Mn analysis, water samples were collected into a Teflon bottle containing 1% Merck suprapur® nitric acid (HNO_3) and filtered at 0.22 and 0.45 μm . For Fe(II) analysis, water samples were delivered into 5 mL Falcon tubes containing ferrozine (Viollier et al., 2000). Concentrations of nitrate (NO_3^-), ammonia (NH_4^+), sulfate (SO_4^{2-}), sulfide (S^{2-}) and chloride (Cl^-) were measured in unfiltered water samples. Samples for total sulfide measurements (S^{2-}) were collected into vials previously purged with N_2 gas and water samples were injected through a rubber septum, maintaining anoxic conditions. We note that some DOM samples from summer (0.2 μm fraction) were broken during transportation. Therefore, we decided to focus primarily on the 0.45 μm fraction to compare the effects of seasonality in DOM dynamics in the water column. All water samples were stored and kept at 4°C until analysis.

Measurements of physicochemical parameters such as pH, dissolved oxygen (DO), temperature, oxidation-reduction potential (ORP), and specific conductance (SPC) were obtained using a EXO1 multiparameter probe (YSI Inc.). Chlorophyll concentrations were measured using a YSI EXO Total Algae PC Smart Sensor, which measures total chlorophyll content. Moreover, a large 2.5L water sample was collected near surface, at medium depth and bottom to a previously decontaminated amber bottle in each lake for solid-phase extraction and DOM characterization.

A manual metallic sediment corer was used to collect a sediment core. Upon sampling, the sediment cores were sectioned into 4 cm intervals, then kept frozen and freeze-dried prior to analysis.

IV.2.2. Chemical analysis in water column

Total dissolved Mn and Fe in water samples were measured using an ICP-OES (Perkin Elmer ICP OES Optima 2000DV), and anion (NO_3^- , NH_4^+ , SO_4^{2-} , Cl^-) concentrations were measured using ion chromatography (Dionex ICS-1500). The dissolved Fe(II) fraction was determined using the Ferrozine method (Viollier et al., 2000). Total sulfide (S^{2-}) concentrations were measured according to an adaptation of the method described by Guenther et al. (Guenther et al., 2001). DOC concentrations were measured in a Vario TOC-Cube TOC analyzer (Elementar). The relative standard deviation (RSD) for water column measurements remained below 5% — NO_3^- : 1.4%; NH_4^+ : 4.5%; SO_4^{2-} : 1.5%; S^{2-} : 3.4%; Fe: 2.1%; Mn: 1.8%.

UV-Visible absorbance spectra and fluorescence excitation emission matrices (EEMs) were acquired in an Aqualog spectrofluorometer (Horiba Scientific). Absorbance spectra (200-700 nm, 1 nm resolution) were measured in quartz cuvettes (1 cm pathlength) and blank-corrected with ultrapure Milli-Q water (18,2 M Ω ·cm at 25 °C, DOC < 2 ppb).

The slope ratio (S_R), the spectral slope between 275-295 nm divided by the slope at 350-400 nm, was calculated and used as an indicator of DOM molecular weight (Helms et al., 2008; Li and Hur, 2017). $SUVA_{254}$ (proxy of DOM aromatic content) was calculated as described by Weishaar et al. (Weishaar et al., 2003). Absorption coefficient at 320 nm was used as a proxy for chromophoric DOM (CDOM) content (Laurion and Mladenov, 2013; Mazoyer et al., 2022).

Fluorescence EEMs were obtained using an excitation range of 240-600 nm with 2 nm increments, and an emission range of 250-800 nm with 2.5 nm increments. A new Milli-Q blank was acquired and used every 10 samples. Inner-filter effect correction and 0-1 normalization were performed for every EEM, following the Aqualog Manual (HORIBA, 2019).

Parallel factor analysis (PARAFAC) was performed using the commercial platform SOLO (Eigenvector Research Inc., Manson, DC, USA). Non-negativity constraint was applied, and primary and secondary Rayleigh scatter set to missing values. A five component PARAFAC model (99.7% fit) was generated using 219 DOM samples collected across Arctic and subarctic lakes in previous field campaigns. Spectral loadings, core consistency diagnosis (CORCONDIA) and split-half analysis were used to validate the PARAFAC components (Bro, 1997; Murphy et al., 2013). Split-half validation (74.7%) indicated moderate model stability and the CORCONDIA value of 53 was consistent with expectations for EEMs from environmental samples (Bro, 1997).

PARAFAC component loadings and split-half validation results are shown in Fig. VII.I.6, VII.I.7, and VII.I.8 in Supplementary Information chapter (VII). PARAFAC model was generated following the workflow in the Aqualog Manual (HORIBA, 2019).

DOM was concentrated from 2.5L water samples using solid-phase extraction (SPE) with styrene-divinylbenzene polymer (PPL) cartridges, using the method described by Dittmar et al. (Dittmar et al., 2008). Fourier-transform infrared spectroscopy (FTIR) was used to characterize SPE-DOM samples. FTIR spectra were acquired in a Perkin Elmer Spectrum Two spectrophotometer equipped with an attenuated total reflectance module. Spectra

were measured over the 500-4000 cm^{-1} range with 1 cm^{-1} resolution, and sixteen scans/sample recorded, averaged, and corrected against air as background.

IV.2.3. Sediment chemical analysis

Frozen sediment sections were freeze-dried and homogenized prior to analysis, with different portions being taken for measurement of total Fe and Mn concentrations, FTIR analysis, C/N ratios and $\delta^{13}\text{C}$ signatures.

FTIR analysis of sediment layers were performed using the same equipment and scanning conditions as for SPE-DOM samples. Upon baseline correction, the ratio of 1630/1060 cm^{-1} was calculated, commonly described in literature as the humification index (HI), assessing the proportion of aromatic and carboxylate structures relative to polysaccharides (Broder et al., 2012; Heffernan et al., 2020).

Total concentrations of Fe and Mn in sediment samples were determined following an acid digestion with a 1:3 mixture of aqua regia ($\text{HCl}:\text{HNO}_3$, 3:1 v/v) and hydrofluoric acid (HF). All reagents used were Merck Suprapur® grade.

The digestions were carried out at 100°C for the duration of 1h. Upon digestion, a boric acid (H_3BO_3) was added to complex residual HF. Samples were diluted 10:1 with Milli-Q water (with 2% HNO_3), before analysis by inductively coupled plasma optical emission spectroscopy (ICP-OES) with an Optima 2000 DV ICP-OES Spectrometer (PerkinElmer). Two certified reference materials were used to assess the accuracy of our method: MESS-4 (Marine Sediment Certified Reference Material, NRC, Canada), and BCR-60 (Aquatic Plant *Lagarosiphon major*).

The C/N ratios and $\delta^{13}\text{C}$ signatures in sediment layers were measured for both the total carbon and for the organic fraction. To remove the inorganic carbon fraction, samples were treated with 0.1M HCl for 2 h, then washed several times with Milli-Q water and freeze-dried until analysis. The $\delta^{13}\text{C}$ signatures, %C and %N content were measured using a EuroVector Elemental Analyzer coupled to a Nu Horizon isotope ratio mass spectrometer (Water Quality Center, Trent University, Canada). Isotopic $\delta^{13}\text{C}$ signatures were calibrated relative to Vienna Pee Dee Belemnite (VPDB) standard using a three-point calibration curve anchored by USGS40 (glutamic acid; $\delta^{13}\text{C} = -26.39$ ‰, %C = 40.82, %N = 9.52), USGS62 ($\delta^{13}\text{C} = -14.79$ ‰, %C = 49.48, %N = 28.85), and USGS63

($\delta^{13}\text{C} = -1.17 \text{ ‰}$, $\% \text{C} = 49.50$, $\% \text{N} = 28.90$). USGS40 was also used to calibrate elemental compositions for $\% \text{C}$ and $\% \text{N}$ determinations.

IV.3. Results

IV.3.1. Physicochemical parameters

Physicochemical properties in the water column showed marked seasonal variability across thermokarst lakes. Water column temperatures ranged from 7.1-6.4 °C, decreasing to the bottom in KANG1, with the same pattern being observed in KANG2 between 7.3 and 6.0 °C during summer. In winter, temperatures remained relatively stable with depth in KANG1 (decreased from 0.5 to 0.2 °C), while in KANG2 a slight temperature inversion was observed (0.2 to 1.0 °C) (Fig. VII.II.1).

Summer dissolved oxygen (DO) profiles showed gradual decrease with depth in both lakes, with KANG1 decreasing from 91% to 86%, and KANG2 decreasing from 85% to 81%. In winter, surface waters showed relatively high DO (KANG1: 17%; KANG2: 43%) due to mixing induced oxygenation upon drilling through the ice cover. The winter DO decrease with depth in both lakes, although more abruptly in KANG2, with higher DO near the surface arising likely due to mixing immediately after ice drilling.

During summer, specific conductance (SPC) decreased slightly with depth in both lakes, with KANG2 (177 to 165 $\mu\text{S cm}^{-1}$) displaying SPC approximately three times higher than KANG1 (58 to 50 $\mu\text{S cm}^{-1}$). In winter, SPC values were considerably higher in both lakes, ranging in the 273-270 $\mu\text{S cm}^{-1}$ and 262-267 $\mu\text{S cm}^{-1}$ in KANG1 and KANG2, respectively.

Oxidation-reduction potential (ORP) varied significantly between lakes, especially in winter. During summer ORP ranged in 163-159 mV in KANG1 and 142-136 mV in KANG2, while in winter ORP shifted to -83 to -132 mV in KANG1 and -92 to -210 mV in KANG2, both decreasing with depth. KANG2 displayed more reducing conditions during winter compared to KANG1 (Fig. IV.4).

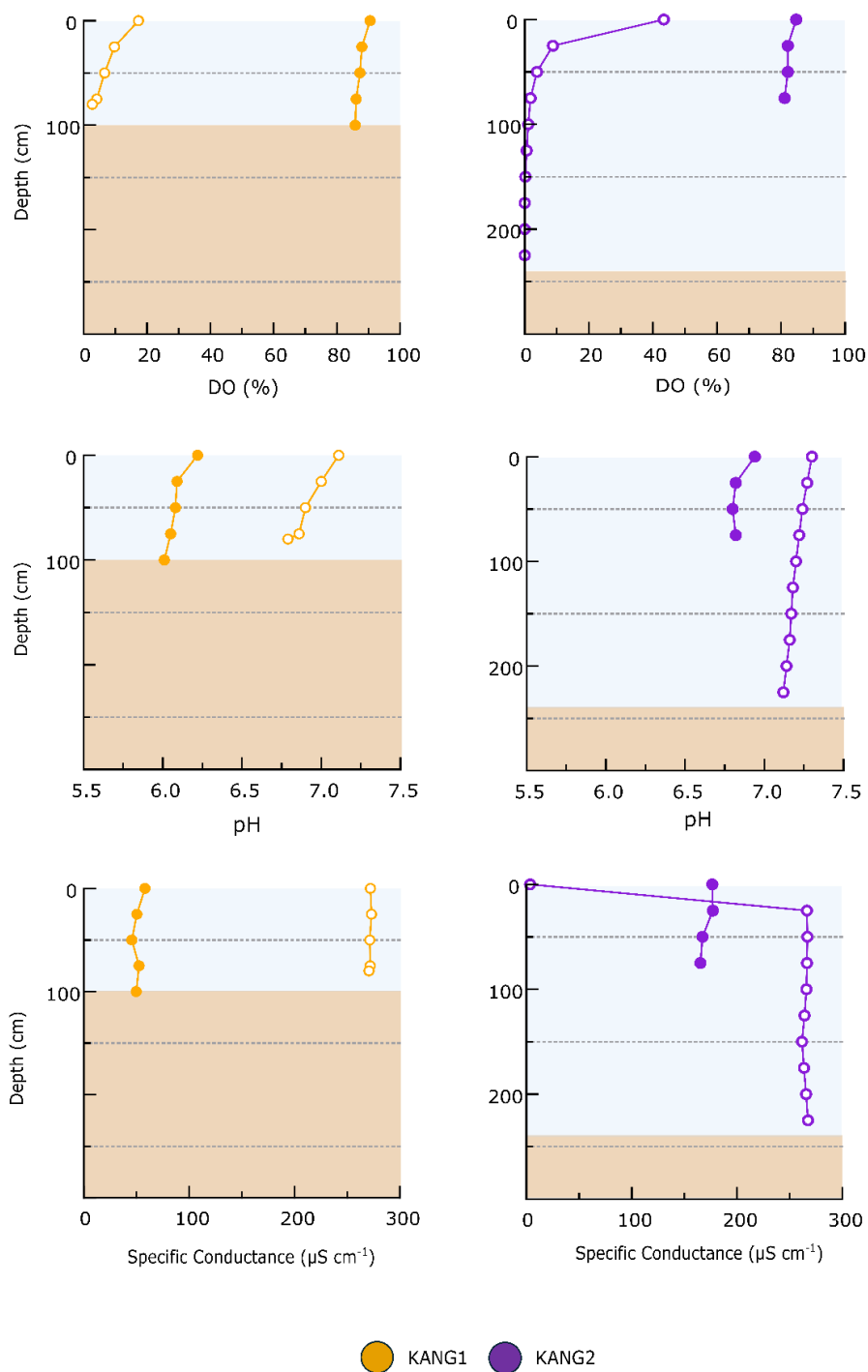


Figure IV.3. Seasonal vertical profiles of dissolved oxygen (DO), pH and specific conductance (SPC) in KANG1 and KANG2. Open circles represent winter measurements; closed circles indicate summer data.

Summer pH values were more acidic than during winter in KANG1 and KANG2. In late summer, pH values show slight decreases with depth in KANG1 (6.2 to 6.0) and KANG2 (6.9 to 6.8). During winter, a similar trend of pH decrease with depth was observed in KANG1 (7.1 to 6.8) and KANG2 (7.3 to 7.1).

Chlorophyll probe measurements showed increased chlorophyll presence towards the bottom in KANG2 during summer (11 to 167 $\mu\text{g L}^{-1}$) relative to KANG1 (3 to 6 $\mu\text{g L}^{-1}$) (Fig. IV.4). However, in winter waters, chlorophyll was more abundant in KANG1 (59 to 32 $\mu\text{g L}^{-1}$) than KANG2 ($\sim 5 \mu\text{g L}^{-1}$).

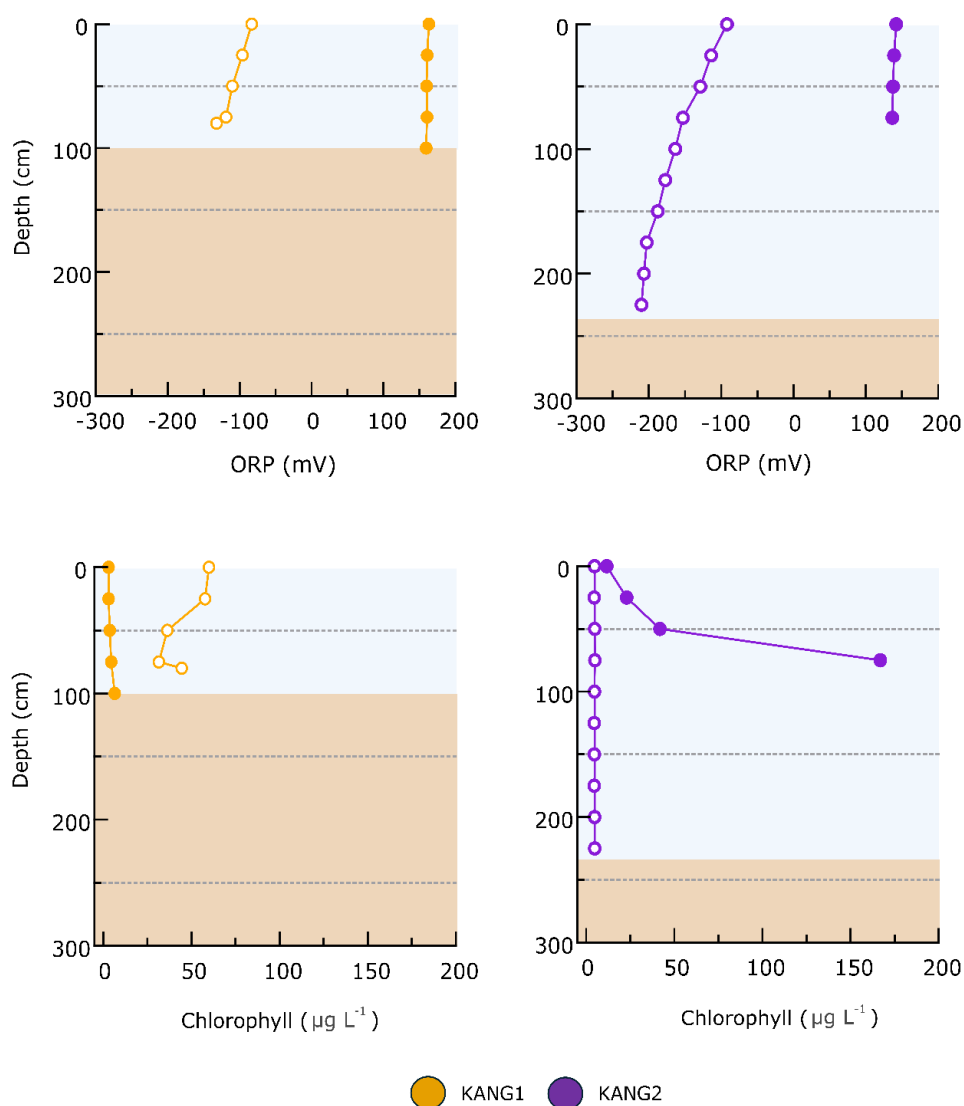


Figure IV.4. Seasonal vertical profiles of oxidation-reduction potential (ORP) and total chlorophyll measurements in KANG1 and KANG2. Open circles represent winter measurements; closed circles indicate summer data.

In both lakes, temperature, pH, SPC and DO profiles do not reveal a clear stratification into epilimnion, metalimnion and hypolimnion layers during summer.

IV.3.2. Terminal electron acceptors

During summer, dissolved Fe concentrations remained in the 16.1-15.8 mg L⁻¹ range in KANG1 showing little variation, and between 30.1-25.2 mg L⁻¹ in KANG2 showing a decrease to the bottom. In winter, dissolved Fe concentrations ranged between 19.0-12.6 mg L⁻¹ in KANG1, and 6.0—5.6 mg L⁻¹ in KANG2. The proportion of Fe(II) during summer ranges from 23 to 45% in KANG1, and 56 to 64% in KANG2. During winter, Fe(II) proportion shifts to 30-47% in KANG1 and 28-36% in KANG2.

Dissolved Mn concentrations in summer remain relatively stable in the water column of both lakes, ranging between 5.4-5.3 mg L⁻¹ and 6.3-5.8 mg L⁻¹ in KANG1 and KANG2, respectively. In winter, dissolved Mn concentrations decrease to 0.34-0.24 mg L⁻¹ in KANG1 and 0.53-0.50 mg L⁻¹ in KANG2.

Nitrate concentrations during summer oscillated between 0.4 and 0.8 mg L⁻¹ in KANG1, remaining below detection limit in KANG2. During winter, nitrate concentrations remained below detection limit in both sites.

Sulfate concentrations in summer ranged between 10.2 and 8.5 mg L⁻¹ in KANG1, while KANG2 showed concentrations ranging 18.3-16.8 mg L⁻¹. During winter, sulfate concentrations in KANG1 were 12.9-12.0 mg L⁻¹ and 20.9-23.8 mg L⁻¹ in KANG2.

IV.3.3. DOM quality

DOC concentrations showed notable differences not only seasonally, but particularly between the 0.45 and 0.2 µm filtered fractions during winter. In summer, KANG1 showed DOC concentrations of 7.3-11.2 mg C L⁻¹, with KANG2 displaying 5.9-12.2 mg C L⁻¹. DOC concentrations in the 0.2 µm fraction differed only slightly from the 0.45 µm during summer.

DOC concentrations (0.45 µm) in winter range between 26.2-24.3 mg C L⁻¹ and 12.5-18.8 mg C L⁻¹ in KANG1 and KANG2, respectively. However, in the 0.2 µm fraction DOC ranges shift to 41.0-62.3 mg C L⁻¹ in KANG1 and 63.5-95.3 mg C L⁻¹ in KANG2. This represents a relative increase of DOC in the 0.2 µm of 86% for KANG1 and 366% for KANG2. Bacteria have been shown to pass through the 0.45 µm filter (Wang et al.,

2007), likely engaging in DOM processing and leading to lower DOC concentrations in 0.45 μm samples.

In late summer, SUVA_{254} values in KANG1 decrease moderately with depth, ranging from 3.9 to 2.7, while KANG2 shows slightly higher SUVA_{254} values (3.4-4.1). During winter, SUVA_{254} values increased to 8.2-9.1 and 5.7-8 ranges in KANG1 and KANG2, respectively. We note that considering the discrepancy between DOC at 0.45 and 0.2 μm , SUVA_{254} values at 0.45 are strongly influenced. The SUVA_{254} and DOC profiles (0.45 μm fraction) are shown in Fig. VII.II.4 in Supplementary Information.

The slope ratio (S_R) was oscillated between 0.79 and 0.95 in KANG1 and 0.75-0.80 in KANG2 during summer. In winter, S_R values shifted to 0.49-0.50 in KANG1 and 0.58-0.60 in KANG2. KANG1 exhibited the biggest seasonal amplitude of S_R compared to KANG2 (Fig. IV.4).

The a_{320} values in summer were 30-33 m^{-1} in KANG1 and 26-44 m^{-1} in KANG2. During winter, a_{320} values range between 290-273 m^{-1} and 121-131 m^{-1} in KANG1 and KANG2, respectively (Fig. IV.4).

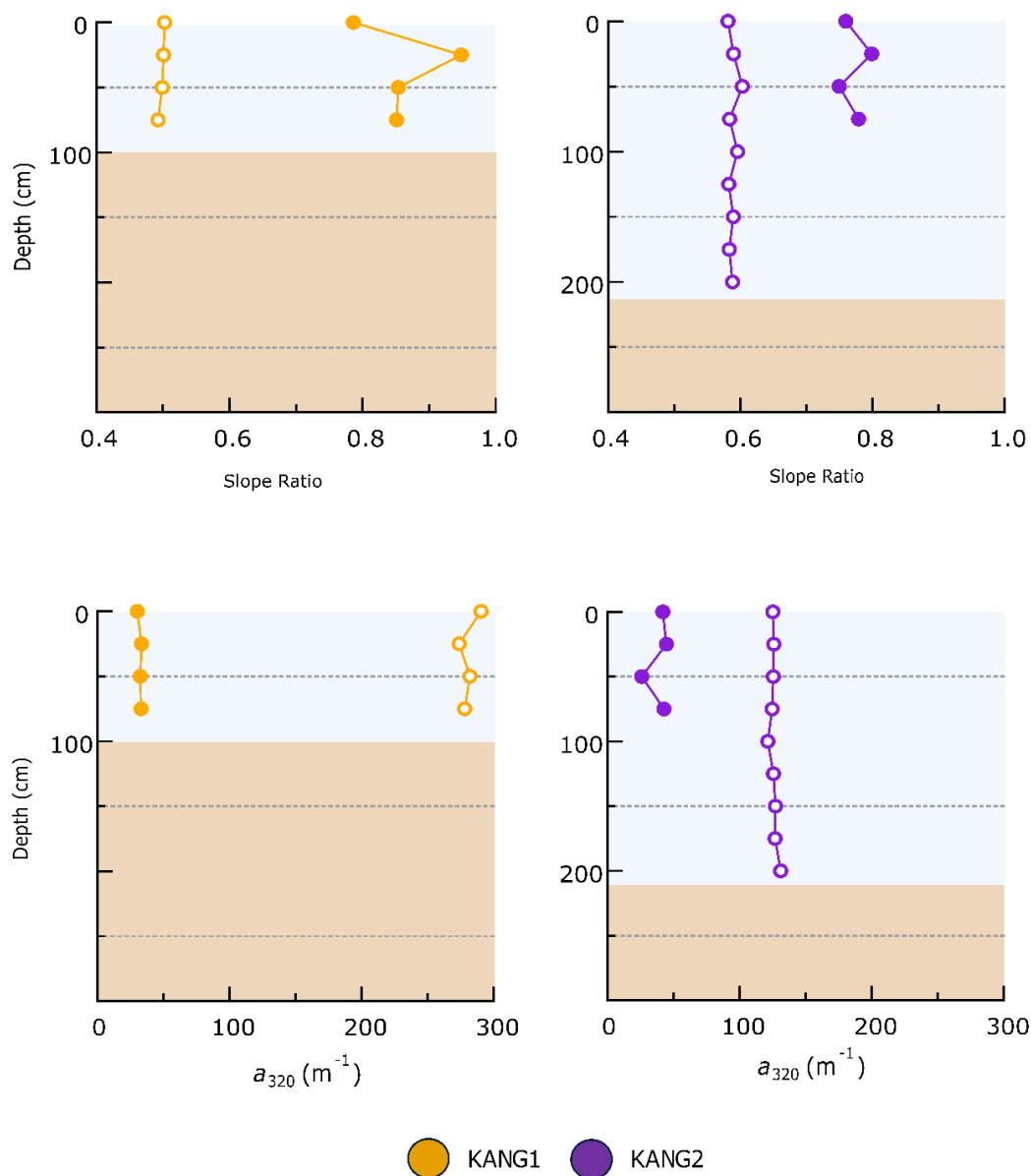


Figure IV.5. Seasonal vertical profiles of slope ratio (S_R) and a_{320} (CDOM proxy) in KANG1 and KANG2. Open circles represent winter measurements; closed circles indicate summer data.

The PARAFAC model identified five components (EEMs in Fig. VII.I.5), categorized following (Pacoureau et al., 2025) as humic-like terrestrial (HT), humic-like microbial (HM), and protein-like (P). HT1, HT2 and HM1 have two excitation maxima, while P1 and P2 have one (Table IV.2). PARAFAC component details are provided in Supplementary Information (Supplementary Note SN.VII.I.1).

Table IV.2. Identification of five PARAFAC components and their assignment based on the literature (provided in SN.VII.I.1).

Component	Excitation _{max}	Emission _{max}	Assignment
HT1	250 nm/ 340 nm	450 nm	Humic-like terrestrial
HT2	265 nm/ 400 nm	520 nm	Humic-like terrestrial
HM1	240 nm/ 310 nm	400 nm	Humic-like microbial
P2	275 nm	295 nm	Tyrosine-like
P1	280 nm	330 nm	Tryptophan-like

During late summer, PARAFAC component profile in KANG1 showed a gradual increase in P2 and decrease in HM1 with depth. We also note an increase in P1 and a decrease in HT1 at 25 cm. Lake KANG2 shows little variation in PARAFAC component distribution along the water column profile compared to KANG1 during summer.

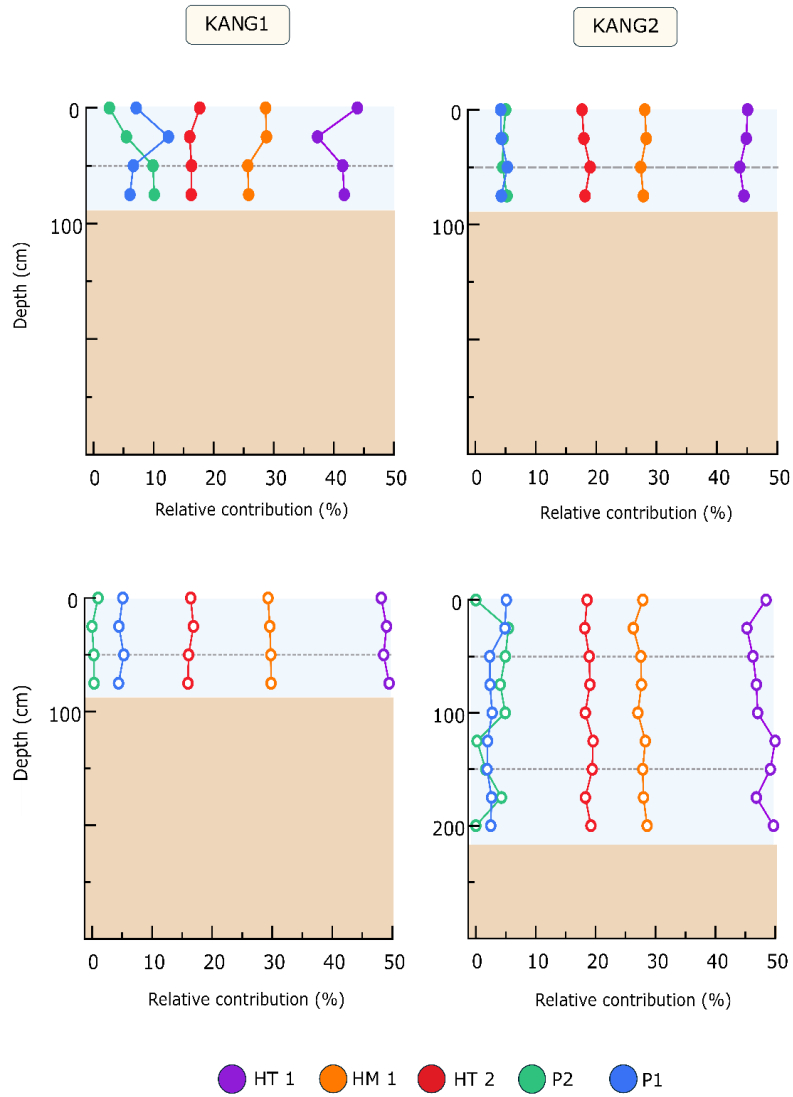


Figure IV.6. Seasonal vertical profiles of relative contributions (%) of PARAFAC components in KANG1 and KANG2. Open circles represent winter measurements; closed circles indicate summer data.

In winter waters, the opposite trend was observed as KANG1 showed little variability in PARAFAC component distribution along the water column. KANG2 showed an increase in P1 closer to the surface (under ice), with P2 increasing after (25-100 cm). At 125 cm P2 decreased, accompanied by an increase in HT1 and HM1, and at 175 cm depth, P2 increased with HT1 and HM1 decreasing.

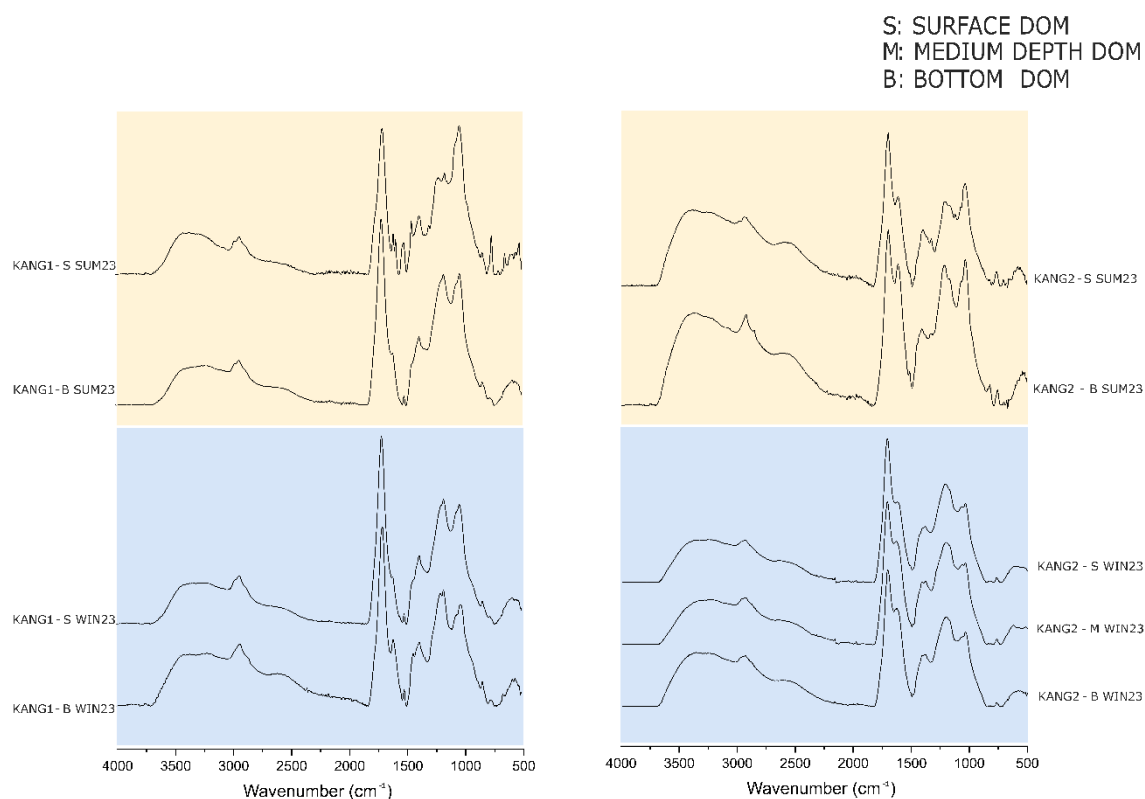


Figure IV.7. FTIR spectra of SPE-DOM samples from surface (S), medium depth (M) and bottom (B) in KANG1 and KANG2 during summer (SUM23) and winter (WIN23).

All SPE-DOM FTIR spectra exhibited general similarities in main features: (1) band centered in $\sim 1030\text{ cm}^{-1}$; (2) band centered near $\sim 1210\text{ cm}^{-1}$; (3) band situated in the $\sim 1380\text{--}1400\text{ cm}^{-1}$; (4) band centered around $\sim 1610\text{ cm}^{-1}$; (5) band located at $\sim 1700\text{ cm}^{-1}$; (6) broad band in the $\sim 2500\text{--}2700\text{ cm}^{-1}$ range; (7) band centered at 2920 cm^{-1} ; (8) broad band in the $3000\text{--}3400\text{ cm}^{-1}$ region. The FTIR band assignments are shown in Table VII.I.7 in Supplementary Information.

FTIR spectra of DOM in KANG1 changed in surface relative to bottom during summer. Surface DOM showed the increase in FTIR bands at 760 cm^{-1} , 1220 cm^{-1} , 1300 cm^{-1} , 1451 cm^{-1} , 1587 cm^{-1} and increase in the bands at 1520 and 1607 cm^{-1} . This indicates an increase in aromatic lignin, polysaccharide, alkene structures and deprotonated carboxylic acids near the surface.

In KANG2, surface DOM showed an increase in the bands at 1036 cm^{-1} , 1119 cm^{-1} , 1326 cm^{-1} , and a decrease in 822 cm^{-1} , 1515 cm^{-1} , 1613 cm^{-1} , 2850 and 2920 cm^{-1} . This indicates an increase in polysaccharides, with decreases in aromatic lignin, aliphatic moieties and potentially carboxylic acids. Comparatively, summer DOM showed higher

presence of carboxylic structures (higher bands at 2500-2700 and 1607 cm^{-1}) in KANG2 relative to KANG1.

Spectral differences were less pronounced with depth during winter in both lakes, particularly in the case of KANG2. During winter, bottom waters in KANG1 showed an increase in a shoulder-like band at 1068 cm^{-1} and 1204 cm^{-1} , and bands at 1436 cm^{-1} , 1609 cm^{-1} and 2500-2700 cm^{-1} . This suggests an increase in carboxylic acids, aliphatic esters and carbohydrates, likely arose from inputs from sediment processing.

IV.3.4. Sediment characterization:

The $\delta^{13}\text{C}$ signatures and C/N ratios of both total and organic carbon fractions were measured in KANG1 and KANG2 sediment. Generally, $\delta^{13}\text{C}$ values did not differ significantly between the total carbon and organic fractions. Hereafter, we focused on $\delta^{13}\text{C}$ signatures and C/N ratios of the organic fraction of lake sediments (Fig. IV.8).

In KANG1, summer sediments showed lower C/N ratios (decreased 8% on average) and slightly more depleted $\delta^{13}\text{C}$ signatures relative to winter (Fig. IV.8). In both seasons, C/N ratios increased with sediment depth at this site.

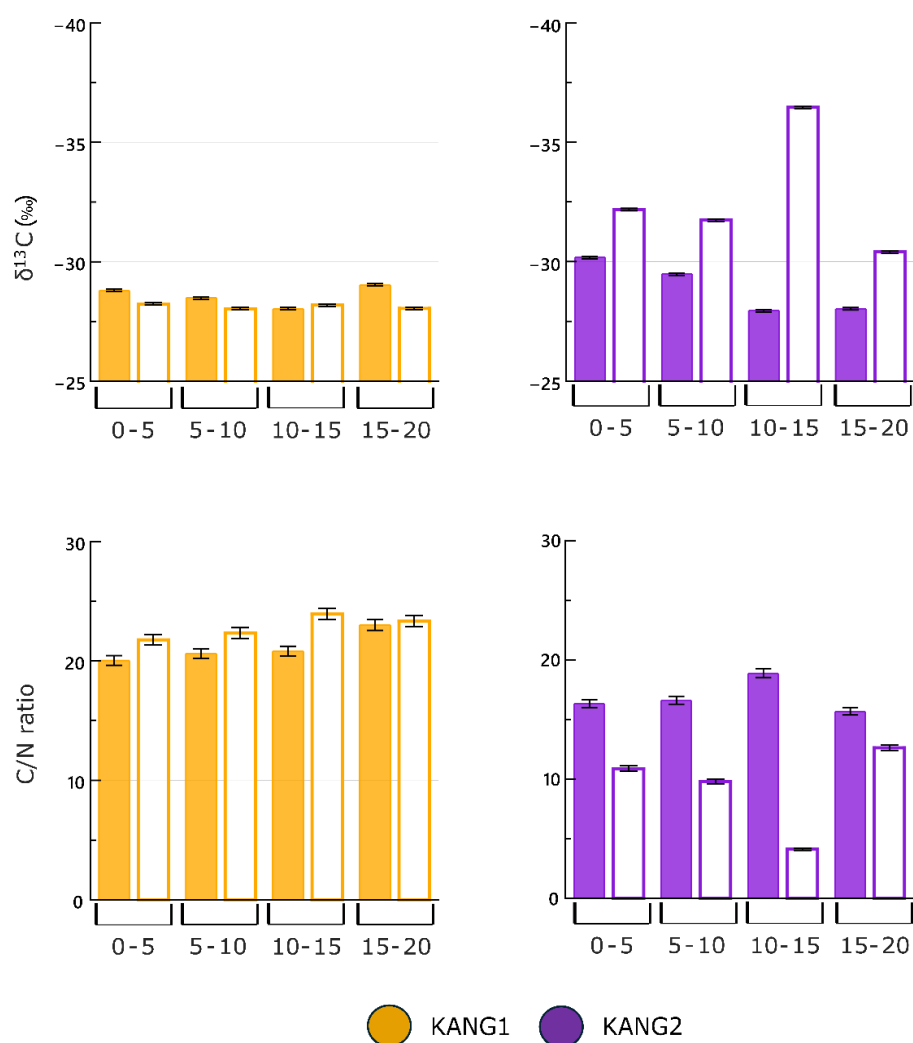


Figure IV.8. Seasonal distribution of C/N ratios and $\delta^{13}\text{C}$ signatures in sediment layers in KANG1 and KANG2: 0-5 cm; 5-10 cm; 10-15 cm; 15-20 cm. Open bars represent winter data; closed bars indicate summer measurements.

In contrast, KANG2 sediments showed a marked decrease in C/N ratios during winter compared to summer (on average by 44.6%), along with more negative $\delta^{13}\text{C}$ signatures. KANG2 sediments therefore exhibited an opposite seasonal pattern compared to KANG1. During summer, KANG2 upper sediments displayed lighter $\delta^{13}\text{C}$ signatures, suggesting strong biogeochemical processing of the natural organic matter (NOM) pool in upper sediments. Comparatively, C/N ratios in KANG2 sediments were consistently lower than KANG1, with generally lighter $\delta^{13}\text{C}$ signatures.

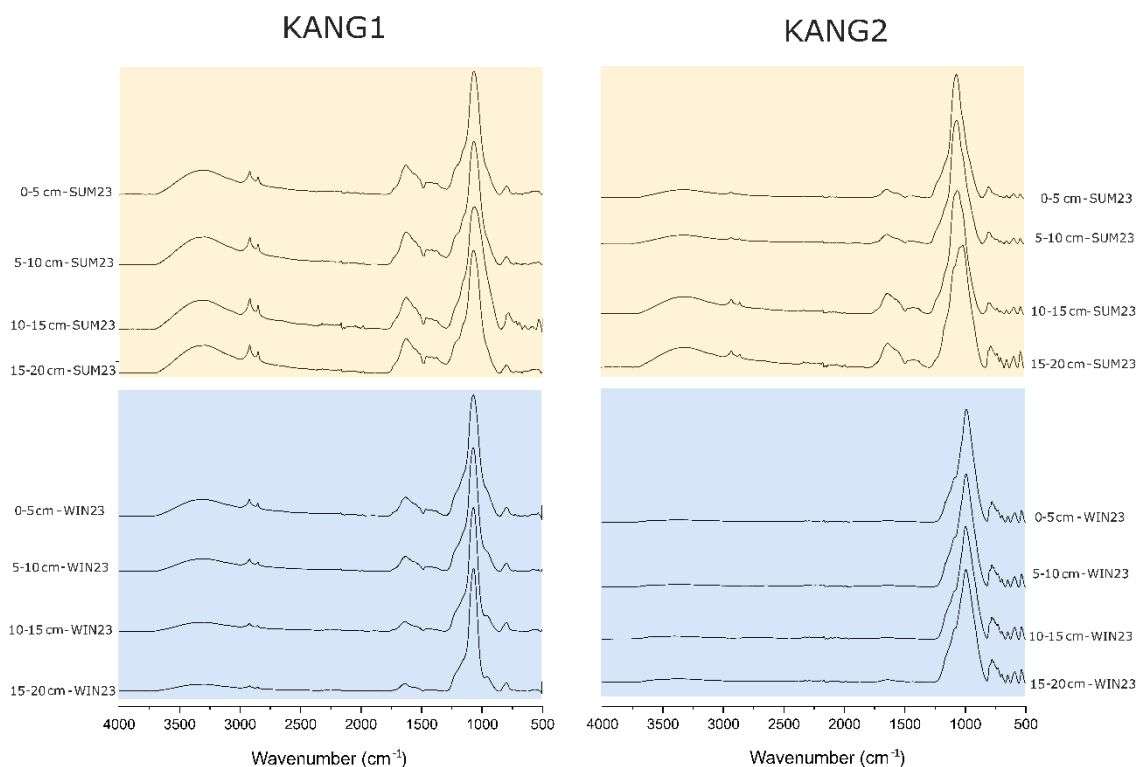


Figure IV.9. FTIR spectra of sediment layers in KANG1 and KANG2 during summer (SUM23) and winter (WIN23): 0-5 cm; 5-10 cm; 10-15 cm; 15-20 cm.

All sediment FTIR spectra exhibited general similarities in main features: (1) band centered at 1066 cm^{-1} ; (2) a shoulder-like band at 1220 cm^{-1} ; (3) band situated in the $1380\text{--}1450\text{ cm}^{-1}$ region; (4) a shoulder-like band at 1546 cm^{-1} ; (5) a band centered at 1630 cm^{-1} ; (7) dual peaks at 2850 and 2918 cm^{-1} ; (7) a broad band in the $3000\text{--}3400\text{ cm}^{-1}$ region. The FTIR band assignments are shown in Table VII.I.7 in Supplementary Information.

Clear seasonal spectral differences were observed in KANG1 and KANG2 sediments, with increased presence of organic functional groups during summer relative to winter.

During summer, KANG1 sediments exhibit little spectral changes with depth. In winter however, deeper sediment layers are more degraded, with decreases in the bands at $3000\text{--}3400$, 2918 , 2850 , and 1630 cm^{-1} , while showing the emergence of a band near 950 cm^{-1} . This indicates the degradation of NOM pools containing hydroxyl, aliphatic and aromatic structures in deeper sediments during winter.

In summer, the upper layers of KANG2 sediments show a decrease in the bands located at $3000\text{--}3400$, 2918 , 2850 , 1630 , and 1546 cm^{-1} . This consumption of hydroxyl, aliphatic, aromatic and amide II structures in upper sediments, suggests a greater degree of

processing. During winter however, the spectral signatures in KANG2 sediments were depleted in hydroxyl, aliphatic, aromatic and amide moieties, with a strong broad band of polysaccharide structures (Fig. IV.9). This suggests sediment NOM has been strongly processed during winter, to a far greater extent than in KANG1.

Humification index (HI), the spectral ratio between 1630/1060 cm^{-1} (aromatics and carboxylate/polysaccharides), were consistently higher in summer sediments relative to winter (Fig. VII.II.6). Furthermore, HI values in KANG1 sediments were higher than in KANG2 in both seasons. While summer sediments exhibited no clear depth-related patterns in KANG1, winter sediments showed a gradual HI decline toward deeper layers. This trend inverted for KANG2, as summer sediments showed a decline in HI values toward surface layers, while winter sediments showed no clear pattern.

Total Fe and Mn concentrations in KANG1 and KANG2 showed distinct seasonal trends (Fig. IV.10). During summer, KANG1 sediments showed greater Fe and Mn concentrations in the upper sediments, although no clear gradient was observed. The sediment Fe concentrations ranged 8000-14000 mg kg^{-1} and Mn concentrations between 68 and 99 mg kg^{-1} in KANG1. In winter however, the accumulation of both Fe and Mn in surface sediments was clear, with a gradual decrease in concentration of both with depth (Fe: 21000 to 9000 mg kg^{-1} ; Mn: 122 to 54 mg kg^{-1}). We note also that although upper KANG1 sediments contained the highest Fe and Mn in both seasons, the concentrations were highest in winter.

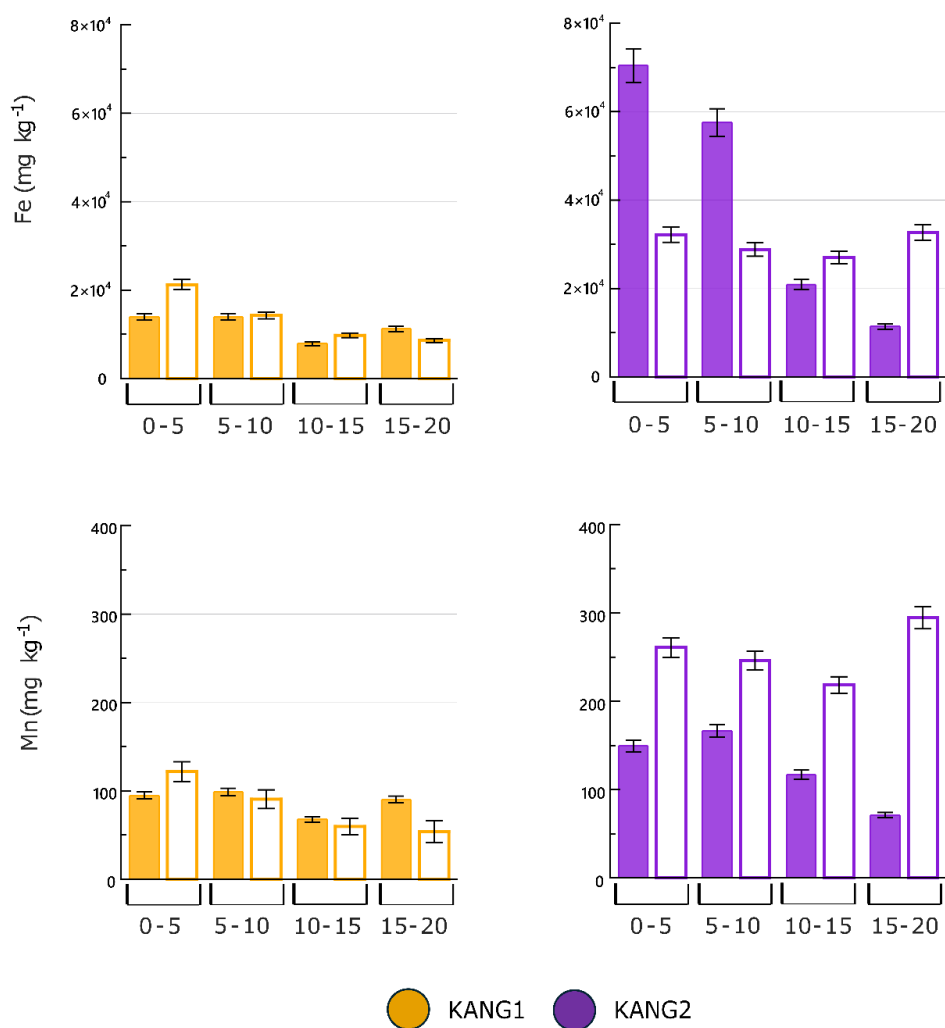


Figure IV. 10. Seasonal Fe and Mn concentrations in sediment layers of KANG1 and KANG2: 0-5 cm; 5-10 cm; 10-15 cm; 15-20 cm. Open bars represent winter measurements; closed bars represent summer data.

In summer, KANG2 sediments showed an accumulation of Fe in surface sediments, and declining with depth, decreasing from 70000 to 11000 mg kg⁻¹. Fe concentrations in KANG2 surface sediments reached approximately five times the concentrations observed in KANG1 surface sediments (during summer). Mn concentrations were highest in the near upper sediments, declining towards deeper sediments in summer, ranging between 71 and 167 mg kg⁻¹. During winter, KANG2 sediments showed Fe concentrations oscillating between 27000 and 33000 mg kg⁻¹, and Mn concentrations varied between 218 and 295 mg kg⁻¹.

IV.4. Discussion:

IV.4.1. Seasonal shifts in overarching biogeochemical dynamics

Seasonality exerts a strong influence in redox, solute concentrations and DOM quality in both thermokarst lakes. In winter, more negative ORP values signal enhanced reduction in the anoxic water column, and specific conductance (SPC) increases, likely due to freeze effects influencing changes in solute concentration. Unlike thermokarst lakes in sporadic permafrost zones (Vigneron et al., 2019; Malcata Martins et al., 2025), pH values were higher in winter than summer. This may reflect the accumulation of CO₂ from CH₄ oxidation and respiration during oxic summer conditions, leading to more acidic pH values. The increase in pH in winter, might be associated with the removal of CO₂ likely due to carbonate buffering (Zolkos and Tank, 2020) and reduction to CH₄ by hydrogenotrophic methanogenesis (Heslop et al., 2020; C. Mu et al., 2025).

Winter conditions were associated with more condensed, aromatic DOM and higher CDOM compared to late summer. This likely results from both the sediment release of labile terrestrial DOM and humification processes in the water column. Seasonal shifts (summer to winter) in CDOM content (a_{320}) and DOM molecular weight (S_R) were more pronounced in KANG1 — average S_R : 0.86 to 0.50; average a_{320} : 32 to 281 m⁻¹ — compared to KANG2 — average S_R : 0.77 to 0.59; average a_{320} : 39 to 126 m⁻¹. Winter waters in KANG1 showed higher CDOM and higher molecular weight DOM (more condensed structures) with lower DOC (at 0.2 µm) than KANG2.

Interestingly, chlorophyll profiles revealed contrasting seasonal trends between lakes. During summer, maximum chlorophyll concentrations were exceptionally higher in KANG2 (164 µg L⁻¹) compared to KANG1 (6 µg L⁻¹), with the chlorophyll maxima occurring near sediment-water interface, indicating near-bottom primary production. While total chlorophyll concentrations in KANG1 align with a mesotrophic aquatic system, KANG2 was more in line with a hypertrophic system, with nutrient, light and substrate abundance. Although research in thermokarst lakes in subarctic regions generally focuses on specific types of chlorophyll (i.e. Chl *a*; Chl *b*) (Daniels et al., 2015; Przytulska et al., 2016), their ranges seem to align more closely with KANG1. However, during winter conditions, chlorophyll concentrations increased in KANG1 — on average 11 times higher than in summer — despite reduced light availability, possibly due to

inhibited zooplankton grazing (Randelhoff et al., 2025) combined with partial light penetration from heterogeneous ice and snow cover.

Surprisingly, dissolved Fe and Mn were lower in winter than summer despite reducing conditions, while sulfate was slightly higher. We expect this general decrease arose from the gradual precipitation of Fe and Mn. Under strong anoxia, precipitation of Fe as sulfide minerals, following reduction of sulfate to sulfide in the sediments would gradually remove Fe(II) from the water column (Audry et al., 2011). In summer, active benthic processes in deeper sediment layers promoted reductive dissolution of Fe and Mn oxides and their release into the water column, leading to higher concentrations.

Winter sediments in both lakes were noticeably more degraded than in summer. Lake KANG2 exhibited the most extensively processed sediments, reflected in the low HI, C/N along with a strong shift to lighter $\delta^{13}\text{C}$ signatures in all sediment layers. Concurrently, the FTIR spectra showed a relative decrease in aromatic and carboxylic structures. Moreover, winter KANG2 sediments showed very limited NOM substrate, indicating an advanced stage of sediment processing. The change in isotopic signatures in KANG2 winter sediments arose from the incorporation of CO_2 produced from methane oxidation into sediment NOM by autotrophs, leading to depleted $\delta^{13}\text{C}$ signatures (Wauthy et al., 2018). Notably, winter KANG1 sediments were more recalcitrant in deeper layers (higher C/N, lower HI), indicating increased benthic activity at depth.

Although previous research has associated higher HI values with more humified samples and lower HI with less decomposed samples (Broder et al., 2012; Heffernan et al., 2020), sediment samples in this study with lower HI exhibit lower organic carbon content and FTIR spectra characteristic of more advanced decomposition. This apparent contradiction may reflect the unique biogeochemical dynamics of thermokarst lakes, which are recognized greenhouse gas hotspots where methanogenic communities actively degrade lignin-derived aromatics under anaerobic conditions (Kato et al., 2015). Unlike static peat systems where conventional HI application was established, the dynamic benthic processes in thermokarst lakes likely alter the relationship between HI values and degradation extent.

Comparatively, summer KANG1 sediments did not exhibit clear depth-related decomposition patterns, showing consistently higher HI and lower C/N than in winter. In

contrast, summer KANG2 sediments showed a clear degradation pattern toward the surface (lower HI, lower C/N) with lighter $\delta^{13}\text{C}$ signatures.

Collectively, these patterns highlight that seasonality not only alters pelagic redox conditions and primary production but also reshapes benthic-pelagic dynamics of release and sequestration, namely regarding NOM and TEAs cycling.

IV.4.2. Inter-lake biogeochemical contrasts in late summer

During late summer, clear differences emerge in the biogeochemistry of the two thermokarst lakes. In KANG2, higher chlorophyll concentrations indicate a greater abundance of primary producers, which likely underlies several differences in water column and sediment dynamics. Although DO% levels are only slightly lower at the bottom of KANG2 (81%) than in KANG1 (85%), the greater presence of phytoplankton and the circumneutral pH suggest photosynthetic activity is offsetting the oxygen depletion from sediment respiration while simultaneously consuming CO_2 . This masking of increased benthic activity may also explain the higher specific conductance (SPC) observed in KANG2, reflecting higher concentrations of TEAs and other ions mobilized from anoxic sediment layers.

In active thermokarst lake sediments, degradation of NOM drives the production of GHG (Matveev et al., 2019; Jongejans et al., 2021). The increased phytoplankton abundance in KANG2 facilitates the aerobic oxidation of CH_4 into CO_2 , effectively acting as a biofilter due to oxygen production (Milucka et al., 2015; Schorn et al., 2024).

Microbial methanogenesis produces ^{13}C -depleted signatures in methane (Whiticar, 1999; Conrad and Claus, 2005). Once methane undergoes oxidation to CO_2 , it becomes available to be assimilated by autotrophs into sediment NOM (Kellermann et al., 2012; Nowak et al., 2015), contributing to lighter $\delta^{13}\text{C}$ signatures.

In KANG2 sediments, this shift in isotopic signatures was accompanied by lower C/N ratios and lower HI in surface sediments during summer. The elevated concentrations of Fe and Mn in surface sediments likely reflect enhanced precipitation of Fe and Mn oxides under redox conditions favorable to metal oxide formation, having been previously mobilized from deeper sediment layers under more reducing conditions.

Comparatively, KANG1 was a less active site than KANG2 during summer, likely due to their differing origin. The reduced phytoplankton biomass in KANG1 likely limited CO₂ uptake via photosynthesis, enabling the accumulation of CO₂ in the water column, resulting in more acidic conditions (Fig. IV.10). In contrast to KANG2, primary production in KANG1 is unlikely to offset oxygen consumption from sediments; therefore, the higher DO% observed is more likely a consequence of lower sediment activity rather than photosynthetic production. Sediment profiles showed little evidence of pronounced early diagenesis in the upper 15 cm, with minimal variation in HI, C/N (both higher than KANG2) and $\delta^{13}\text{C}$ isotopic values (heavier signatures than KANG2). Moreover, DOM in KANG1 was characterized by slightly lower CDOM content, reduced aromaticity, and smaller average molecular weight DOM compared to KANG2, suggesting less condensed DOM inputs from benthic processing. While dissolved Fe and Mn concentrations were lower than in KANG2, they nevertheless remained relatively high for the open-water season when compared to other Arctic and subarctic sites (Manasypov et al., 2015; Laberge-Carignan et al., 2024).

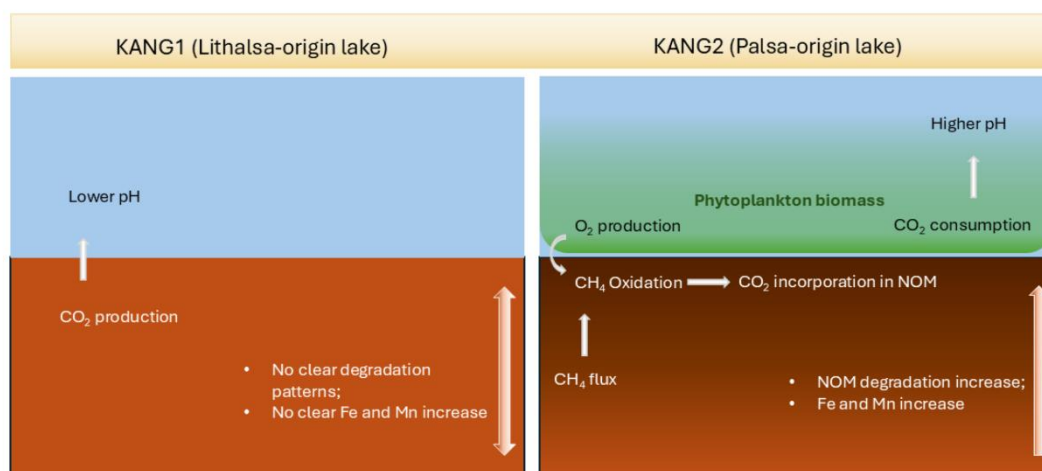


Figure IV. 11. Schematic representation of contrasting phytoplankton dynamics in both thermokarst lake systems and its effects on benthic and pelagic conditions.

The abundance of phytoplankton in KANG2 in late summer was largely attributed to their differing origin — KANG1: formed from lithalsa; KANG2: formed from palsa — and the age of the lakes. A younger, more dynamic natural system is expected to provide benthic inputs of labile DOM and nutrients to the water column, promoting phytoplankton development. The depletion of nitrate and ammonium below detection limits in KANG2

reflects the active nitrogen uptake by phytoplankton communities and is consistent with high productivity. While phosphorous compounds were not measured, they may also have been an important limiting factor influencing phytoplankton biomass development. The contrasts between KANG1 and KANG2 emphasize the role of lake-specific productivity regimes in pelagic and benthic dynamics during open water season.

IV.4.3. Inter-lake biogeochemical contrasts in winter:

The profiles of DOM quality indicators, TEAs, physicochemical properties in the water column, and sediment characteristics represent a biogeochemical “snapshot” of the thermokarst system at a given time. Interpreting the effects and extent of both current and past processes is essential for extracting meaningful insights into specific dynamics.

Sediments in KANG2 showed distinct geochemical characteristics compared to KANG1, including more ^{13}C -depleted isotopic signatures, lower C/N and HI values and an overall decline in available NOM substrate. We hypothesize that elevated microbial activity in KANG2 sediments during winter led to more extensive degradation of NOM, resulting in substantially lower NOM substrate relative to KANG1 sediments. This is similarly reflected in the elevated DOC concentrations at 0.2 μm in KANG2 ($47 \pm 10 \text{ mg C L}^{-1}$) compared to KANG1 ($79 \pm 9 \text{ mg C L}^{-1}$). The substantial shift in isotopic $\delta^{13}\text{C}$ signatures can be explained by the deposition of senescent phytoplankton biomass. Observations from summer indicate intense methane oxidation to CO_2 — exhibiting lighter $\delta^{13}\text{C}$ signatures — and elevated phytoplankton biomass in the lower water column of KANG2. We postulate that phytoplankton communities would assimilate CO_2 with lighter $\delta^{13}\text{C}$ signatures into their biomass (Wauthy and Rautio, 2020), which upon senescence and sedimentation, contributes to the ^{13}C -depletion in KANG2 sediments during winter.

The presence of higher CDOM and higher molecular weight DOM in KANG1 compared to KANG2 in winter, reflects the accumulation of more condensed DOM structures likely arising increased terrestrial DOM inputs from the sediment (Lau et al., 2024). Since KANG1 sediments were still abundant in NOM substrate, their DOM inputs are expected to be terrestrial in origin. In the case of KANG2, the extent of sediment degradation denotes intense past benthic activity, depleting NOM substrate and retaining organic pools produced by autotrophs with lighter $\delta^{13}\text{C}$ signatures. Nevertheless, redox conditions are still more reducing than in KANG1. This could potentiate enhanced DOM

degradation in the water column, breaking down CDOM and larger condensed DOM structures (lower a_{320} and slightly higher S_R) relative to KANG1.

The higher chlorophyll content in KANG1 relative to KANG2 likely arises from reduced grazing pressure (Randelhoff et al., 2025). The relative availability of dissolved CO₂ and fresh organic inputs from the sediment can also favor increased chlorophyll accumulation in KANG1, despite limited light conditions.

Additionally, the slightly higher pH in KANG2 (7.2 ± 0.1) relative to KANG1 (6.9 ± 0.1) could align with a higher proportion of methanogenesis and decreased presence of CO₂. Hydrogenotrophic methanogenesis relies on existing CO₂ to convert to CH₄ (Humez et al., 2016; in 't Zandt et al., 2020; M. Mu et al., 2025), consistent with the more reducing conditions (ORP) observed in KANG2.

IV.4.4. Final Considerations for DOC Measurement in Dynamic Arctic Lake Systems

We noted that during summer the DOC difference between 0.45 and 0.2 μm fractions ranged between 25 and 27%. In winter conditions the 0.45 μm fraction averaged a DOC decrease of 46% in KANG1 and 79% in KANG2 relative to the 0.2 μm fraction. This indicates a significantly higher DOC degradation potential in KANG2, either due to a greater bacterial/microbial activity or greater passage through 0.45 μm filters. This rapid transformation of DOC in winter points to an elevated microbial activity favored by anoxic conditions, likely influenced by seasonal microbial and bacterial community shifts. It has been reported that bacteria can pass through samples filtered at 0.45 μm (Hasegawa et al., 2003; Wang et al., 2007), allowing for DOC degradation in filtered samples, likely explaining the observed differences. Furthermore, winter sediments in KANG2 are depleted in organic content, aligning with the proposed notion of enhanced NOM processing in this lake.

This is an important methodological concern that should be taken into consideration when researching DOM dynamics in highly dynamic natural systems like thermokarst lakes. Since bacteria passing 0.45 μm filters can continue degrading DOC, concentration measurements in especially active sites will be prone to biases. Considering DOM optical indices such as SUVA₂₅₄ rely on DOC normalization, these biases can profoundly affect

interpretability of data, compromising conclusions about DOM quality. Comparative profiles for DOC concentrations and SUVA₂₅₄ values in winter waters in 0.45 and 0.2 μm fractions can be found in Fig. VII.II.5.

IV.5. Conclusion:

This study revealed pronounced seasonal shifts and inter-lake contrasts in thermokarst lake biogeochemistry in a discontinuous permafrost zone. Differences in lake origin and age contributed significantly to contrasts in inter-lake primary production and microbial activity. Most notably, we observed a pronounced seasonal cycle: open water (oxic) conditions promoted photochemical breakdown of DOM, while anoxic conditions facilitated the accumulation of larger, more condensed DOM structures.

In summer, phytoplankton abundance was notoriously elevated in the younger thermokarst lake formed from a palsa, exerting a strong control in water column conditions and enhancing the two-step process of methane oxidation $\rightarrow$ incorporation of CO_2 into sediment NOM by autotrophs. Despite elevated DO in the water column in late summer, the presence of high dissolved Fe and Mn concentrations is suggesting active benthic processing in both lakes. Winter conditions promoted Fe and Mn precipitation despite reducing conditions, increases in CDOM and DOM molecular weight, along with a shift to higher pH values attributed to hydrogenotrophic methanogenesis. Interestingly, the lithalsa-formed thermokarst lake showed high winter chlorophyll levels, while the palsa-formed thermokarst lake showed extensive degradation in the sediments.

These findings emphasize the complex interplay of biogeochemical processes in permafrost-affected aquatic systems, highlighting their dynamic nature and potential role in continued GHG emissions across northern latitudes. Furthermore, the methodological considerations regarding DOC filtration and microbial/bacterial degradation are particularly important in highly active natural systems. Building on this work, future research should focus on microbial community distinctions between palsa-formed and lithalsa-formed thermokarst lakes, monitoring GHG measurements and fluxes, and establishing broader spatial comparisons with similar systems in other discontinuous permafrost zones. The comprehensive characterization of thermokarst lake dynamics is a crucial foundation for developing models to assess regional and global impacts of these rapidly evolving ecosystems.

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V. Emerging thermokarst lakes amplify benthic activity and carbon mobilization in a continuous permafrost landscape

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V.1. Introduction

Climate warming in the Northern Regions is strongly accentuated relative to the global average, with mean air temperatures rising more than twice the global rate (Rantanen et al. 2022; Lee and Romero 2023). This warming drives accelerated permafrost thaw (Smith et al. 2022), profoundly reshaping Arctic landscapes by altering hydrological regimes (Walvoord and Kurylyk 2016; Jin et al. 2022), triggering vegetation succession (Magnússon et al. 2020; Heijmans et al. 2022), and increasing the production and release of greenhouse gases (GHG) (Schuur et al. 2008; Natali et al. 2021).

One of the landscape features arising from permafrost thawing is the formation of thermokarst lakes. As permafrost thaws, it mobilizes dissolved organic matter (DOM), nutrients and contaminants into these newly formed aquatic systems, which in turn become reactive hotspots not only for GHG production (Laurion et al. 2010; Matveev et al. 2016; Walter Anthony et al. 2016; Mu et al. 2023) but also altering biogeochemical processes of contaminants like mercury (Malcata Martins et al. 2025).

The genesis of these lakes varies across the permafrost regions. In sporadic and discontinuous permafrost zones, thawing of ice-rich palsas and peat plateaus is a key process in thermokarst lake formation (Luoto and Seppälä 2003; Sjöberg et al. 2013; Martin et al. 2021; Olefeldt et al. 2021). In contrast, continuous permafrost regions are often characterized by ground patterns with ice wedges, forming polygonal networks. Over time, freeze-thaw cycles cause depressions in the center of polygons (low-center polygons) (Czudek and Demek 1970).

In polygonal tundra landscapes, thermokarst lake formation is commonly initiated by degradation of ice wedges, causing subsidence along polygon troughs and altering local hydrology. As troughs widen and deepen, water accumulates along polygon boundaries and forms small ponds. Continued thaw of ground ice may lead to the progressive subsidence of the polygon interior, allowing the expansion and coalescence of the adjacent ponds into larger thermokarst lakes. Over time, these shallow lakes deepen as the underlying permafrost continues to thaw and develop laterally through thermal erosional processes (Czudek and Demek 1970; Bouchard et al. 2016).

Thermokarst lakes formed in organic-rich landscapes can exhibit high dissolved organic carbon (DOC) concentrations because of the direct contact and mobilization between water and organic soil horizons (Vonk et al. 2015). The mobilization of DOM into lakes,

as well as the DOM quality in sediment porewaters, is strongly influenced by the permafrost regime. In sporadic and discontinuous permafrost regions, active leaching from peat and soil porewaters into lakes occurs and is associated with lower-quality DOM in sediment (Kurashev et al. 2024).

Comparisons of a set of thermokarst lakes in the high Arctic (continuous permafrost) and subarctic regions revealed that although all were methane-supersaturated, Arctic lakes could be undersaturated in carbon dioxide (CO₂), reflecting the influence of colonizing vegetation. In contrast, the sampled turbid subarctic lakes were consistently CO₂-saturated (Breton et al. 2009; Laurion et al. 2010). Laurion et al. (2010) further reported that Arctic lakes exhibit higher pH, lower chromophoric dissolved organic matter (CDOM) content, and reduced suspended solids compared to those in the subarctic region. However, it should be noted that generalizations cannot be drawn from this limited set of studied lakes, as regional specificities exert a major influence on their biogeochemistry.

The biogeochemistry of thermokarst lakes evolves markedly as they transition from newly formed to mature systems, reflecting progressive changes in substrate availability and organic matter reactivity following permafrost thawing. Younger thermokarst lakes exhibit elevated methane production rates owing to the availability of labile substrates (Turetsky et al. 2020; Gerera et al. 2025). They typically display higher DOC concentrations and are enriched in high-molecular-weight DOM with strong terrestrial humic signatures (Peura et al. 2020). Newly formed lakes are also more acidic and metal-rich, often showing elevated Fe and Al concentrations (Manasypov et al. 2014). In contrast, more mature thermokarst lakes are generally stratified, showing progressive DOC losses driven by photochemical degradation and heterotrophic mineralization (Manasypov et al. 2014; Peura et al. 2020). Nevertheless, the ontogenic contrasts in lake biogeochemistry reported in these studies are profoundly influenced by specific regional factors (i.e., hydrological connectivity, permafrost continuity, and soil composition), which limit the direct transferability of these findings to other Arctic and subarctic regions.

In aquatic systems, redox processes govern the production and degradation of Natural Organic Matter (NOM) pools, with microbial communities competing for the most energy-efficient redox pathways (McMahon and Chapelle 2008). The use of dissolved oxygen (DO) generates the most energy per mole of oxidized organic carbon, making it

the preferential electron acceptor for respiration by microbial communities. When oxygen is unavailable, microbial communities are forced to shift towards alternative terminal electron acceptors (TEAs), such as nitrate (NO_3^-), Mn(IV), Fe(III), and sulfate (SO_4^{2-}) (McMahon and Chapelle 2008; McMahon et al. 2011). The preferential order of electron acceptor usage based on energy efficiency is $\text{O}_2 > \text{NO}_3^- > \text{Mn}^{4+} > \text{Fe}^{3+} > \text{SO}_4^{2-} > \text{CO}_2$.

In thermokarst lakes during summer, DO is generally available in the pelagic zone but is depleted in deeper sediments. This drives the reliance of microbial communities to rely on TEAs to fuel benthic respiration. Consequently, TEAs are mobilized into the water column, serving as indicators of dominant redox cycling dynamics, NOM degradation pathways (Arndt et al. 2013) and the lake maturation stage (Audry et al. 2011). Different TEAs produce different degradation efficiencies in specific sediment NOM pools (Mu et al. 2022).

The Zackenberg Valley in northeastern Greenland is located in a continuous permafrost zone and exhibits characteristics of an early-stage permafrost peatland (Christensen et al. 2023). While most northern peatlands date back an average of four to five thousand years (Gorham 1991), vegetated wet tundra landscapes in Zackenberg only emerged during the Little Ice Age (fourteenth to eighteenth century) (Christensen et al. 2023). Nevertheless, the current methane fluxes during the growing season in some Zackenberg fens are of similar intensity to those from developed peatlands at lower latitudes (Mastepanov et al. 2013). Approximately one-third of the lowland area in Zackenberg is poorly drained, with depressions that accumulate water from snowmelt during the summer (Westermann et al. 2015). Consistently, dry and wet zones show stable methane fluxes, whereas transition zones (with varying dry and wet conditions) exhibit highly variable methane fluxes (Scheller et al. 2021). Similarly, differences in substrate (Ström et al. 2003), vegetation cover, primary production (Joabsson and Christensen 2001; Ström et al. 2012), grazing (Falk et al. 2014, 2015), and water table depth (Tagesson et al. 2013) have all been shown to affect methane production.

Fouché et al. (2025) suggested that increased rainfall intensity and rising temperatures could accelerate thermal erosion, leading to the formation of more thermokarst features such as retrogressive thaw slumps in the Zackenberg Valley. Projections for the valley's landscape evolution include an increase in active layer thickness and ground temperatures of up to 5 °C by 2100 (Westermann et al. 2015), with a positive correlation observed between temperatures and methane fluxes (Geng et al. 2019). Christensen et al. (2023)

proposed that early-stage peat soil formation in high Arctic tundra landscapes, triggered by permafrost thaw, drives a rapid shift from a carbon sink to carbon source dynamics due to elevated methane emissions during initial peat development.

In this study, we present the first comprehensive biogeochemical characterization of DOM and terminal electron acceptors (TEAs) in thermokarst lakes in Zackenberg Valley, Northeast Greenland. We addressed the following questions: (1) How do biogeochemical dynamics in recently formed thermokarst lakes within continuous permafrost regions shift over time? (2) How do benthic dynamics differ between the two thermokarst lakes in a rapidly evolving permafrost landscape during the early stages of peatland formation?

To the best of our knowledge, no previous research has integrated the water column and sediment biogeochemical dynamics in thermokarst lakes in this region. More broadly, integrated approaches to studying dynamic natural systems in permafrost regions recently affected by thawing remain scarce. By combining detailed vertical profiling, sediment layer analysis, and benthic flux chamber experimental measurements, our work offers novel insights into the interplay between the water column and sediment processes during the early stages of thermokarst lake development in continuous permafrost regions.

V.2. Materials and Methods

V.2.1. Sampling and Study site

The Zackenberg Valley, Northeast Greenland, is situated within a continuous permafrost zone, with an estimated permafrost thickness of 200-300 m and an active layer thickness ranging from 40 to 65 cm (Christiansen et al. 2008). This region exhibits significant periglacial activity (Elberling et al. 2004, 2008).

Fieldwork sampling took place from July 19 to August 3 of 2023, focusing on two previously selected thermokarst lakes in the main valley east of Zackenberg River (Fig. V.1): ZAC1 (74°28'40.2"N, 20°33'11.0"W) and ZAC2 (74°28'53.7"N, 20°31'16.9"W). The formation of large-scale ice-wedge polygons has been observed in the eastern part of the Zackenberg lowland near the study area (Christiansen et al. 2008). During the fieldwork period, mean daily air temperatures were 4.6 ± 2.5 °C, with an average monthly air temperature of 6.2 ± 1.9 °C between July 15 and August 15, 2023 (Greenland Ecosystem Monitoring, 2025).



Figure V.1. Map of the study sites (ZAC1 and ZAC2) in the Zackenberg valley, and its broader location within Greenland.

Although both lakes (Fig. V.2) are believed to be of recent origin, they represent different stages of the thermokarst development. ZAC1 exhibited a deeper central water column, consistent with a more advanced stage of lake maturation than ZAC2. In contrast, ZAC2 is an emerging thermokarst lake, with a nearby pond actively merging into the main basin as permafrost thaw continues. It should be noted that both lakes presented geomorphologic characteristics of aquatic systems resulting from permafrost thaw. Lake ZAC1 emerges in a low-center polygon landscape, occupying a clear depression surrounded by noticeably elevated margins. Lake ZAC2 however, is at a developmental stage where the polygon troughs have begun to deepen into ponds, while the polygon interior has not yet subsided. This configuration results in two ponds gradually moving toward coalescence into a larger thermokarst lake.

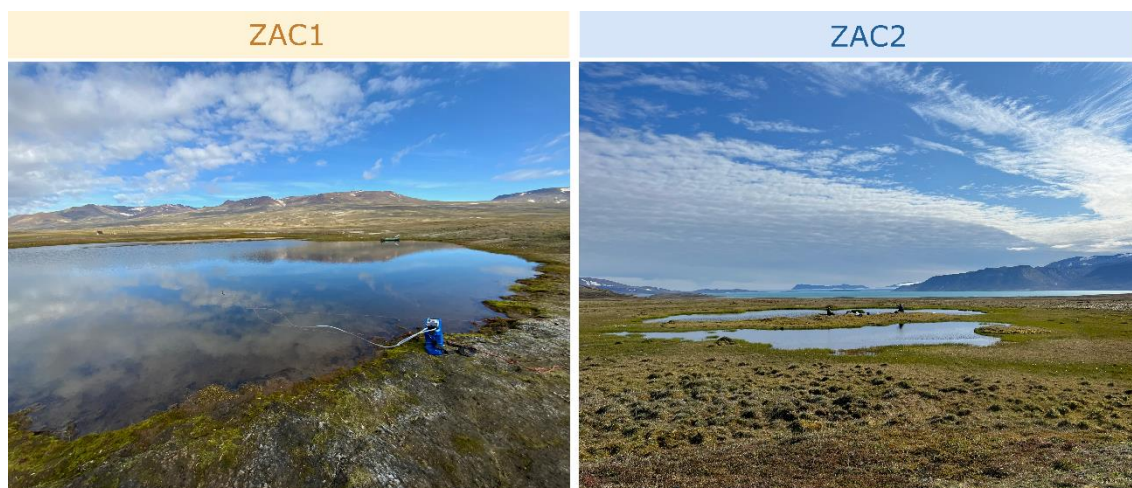


Figure V.2. Photograph of the two sampled thermokarst lakes (ZAC1 and ZAC2) in the Zackenberg valley, Greenland.

Water samples were collected, between 11 a.m. and 12 noon, from the center of each lake using an inflatable boat, with sampling conducted at 25 cm intervals throughout the water column using a portable peristaltic pump (12VDC Advanced, Royal Eijkelpkamp). For the quantification of DOC, manganese (Mn), and iron (Fe), as well as for DOM characterization via UV-Vis and Fluorescence spectroscopy, samples were filtered using 0.2 μm polyether sulfone membrane filters and a 0.45 μm Whatman® Puradisc syringe filter. Filters with two different pore sizes were selected to evaluate potential differences associated with the passage of bacterial communities through the 0.45 μm filter and to assess how DOM composition varied between the resulting fractions. However, because 0.22 μm -filtered samples could not be obtained for the benthic flux chamber in ZAC2, the 0.45 μm fraction was used as the primary analytical reference to maintain methodological consistency throughout the study. For total Fe and Mn analyses, water was collected in Teflon bottles containing 1% Merck Suprapur® nitric acid (HNO_3). Fe(II) concentrations were measured by collecting samples in 5 mL Falcon tubes containing ferrozine reagent (Viollier et al. 2000). Unfiltered water samples were also collected for nitrate (NO_3^-), ammonium (NH_4^+), sulfate (SO_4^{2-}), sulfide (S^{2-}), and chloride (Cl^-) quantification. For the total S^{2-} measurements, water samples were injected through a rubber septum into vials previously purged with N_2 gas to maintain anoxic conditions.

In situ measurements of pH, dissolved oxygen saturation (DO, %), temperature ($^{\circ}\text{C}$), and oxidation-reduction potential (ORP, mV) were conducted using a Mettler Toledo SevenGo DuoPro portable logger equipped with a pH electrode (InLab Expert Go-ISM),

redox electrode (LE510), and DO sensor (InLab 605-ISM). All parameters were measured right after the sample collection. Additionally, a large surface water sample (2.5 L) was collected from each lake in pre-cleaned amber bottles for solid-phase extraction and DOM characterization.

A manual metallic sediment corer was used to collect sediment cores approximately 5 m from the shoreline, in the same area where the water samples were collected. Immediately after collection, the cores were sealed at both ends, transported to the laboratory at Zackenberg Research Station, and sectioned for analysis. Sediment cores were typically divided into 4 cm intervals. For ZAC1, sections were 0-4 cm, 4-8 cm, 8-12 cm, and 12-17 cm. For ZAC2, the core was divided into 0-4 cm, 4-8 cm, and 8-12 cm, as deeper layers were completely frozen and could not be sampled. Sediment sections were frozen and freeze-dried prior to analysis. Water samples were stored at 4°C until processing.

V.2.2. Benthic flux chamber experiment

A benthic flux chamber (BFC, Fig. V.3) was deployed in both lakes to assess biogeochemical changes at the sediment-water interface and to quantify solute fluxes between the two compartments. The BFC was constructed from transparent acrylic with a rectangular base covering a sediment area of 0.24 m² and a total internal volume of 0.072 m³. The chamber design included a small opening at the top to allow air bubbles to escape and to allow lake water to enter during deployment. The stirring device was operated at a constant speed of 12 rpm to ensure homogeneous mixing of the water inside the chamber while avoiding sediment resuspension. A preliminary experiment, in which several rotation speeds were tested, was conducted in the same lake but at a location distant from the actual sampling site.

Water samples were collected from T0 to T7 (Table VII.III.1) over a 6-hour period using a portable peristaltic pump (12VDC Advanced, Royal Ejkelkamp) and Teflon tubing. T0 corresponded to the start of the experiment, with T1 collected 30 min later (acclimatization period) and T2 collected 60 min after T0. From T2 to T7, samples were taken at 60-minutes intervals. At each time point (T0-T7), physicochemical parameters — including temperature (°C), pH, ORP (mV), and DO (%) — were measured using the equipment described in section 2.1 and at the exact moment of collection of each sample. Water samples were also collected for the analysis of DOM properties, DOC

concentrations, and TEAs (dissolved Fe, Mn, Fe(II), NO_3^- , NH_4^+ , SO_4^{2-} , and S^{2-}) following the same protocols used for the water column profiles.

All measurements of DOC and TEAs in the benthic flux chamber experiment were corrected to account for the dilution effects resulting from the cumulative removal of approximately 2 L of water at each sampling time. Given that the chamber height was 30 cm, we used the water column conditions measured 25 cm above the sediment (ZAC1:100 cm; ZAC2:25 cm) to estimate and apply concentration corrections for DOC and TEAs. All the samples were stored at 4°C until analysis.

To capture potential rapid changes in the Sediment Water Interface (SWI) conditions after chamber deployment, the first post-deployment sample (T1) was collected at 30 min, rather than the standard 60 min used for the rest of the BFC experiment. This shorter interval was selected to provide greater time resolution in the early phase of the experiment, where shifts in SWI biogeochemistry were hypothesized to be the most pronounced.

Table V.1. Defined T0 through T7 and their respective sampling times during the BFC experiment in ZAC1 and ZAC2.

T	T0	T1	T2	T3	T4	T5	T6	T7
Time (min)	0	30	60	120	180	240	300	360

Sediment oxygen demand (SOD) rates were calculated using linear regression applied to the linearly decreasing phase of the DO curve, following the approach described by Utley et al. (2008). This method avoids periods in which the DO levels approach zero, ensuring more reliable rate estimates. For each flux chamber deployment, T0 was defined as the initial DO measurement, and the termination point was set at the time when DO saturation dropped below 5%, maintaining consistency across the experiments. The SOD values reported in this study were not corrected for the oxygen demand of the overlying water.

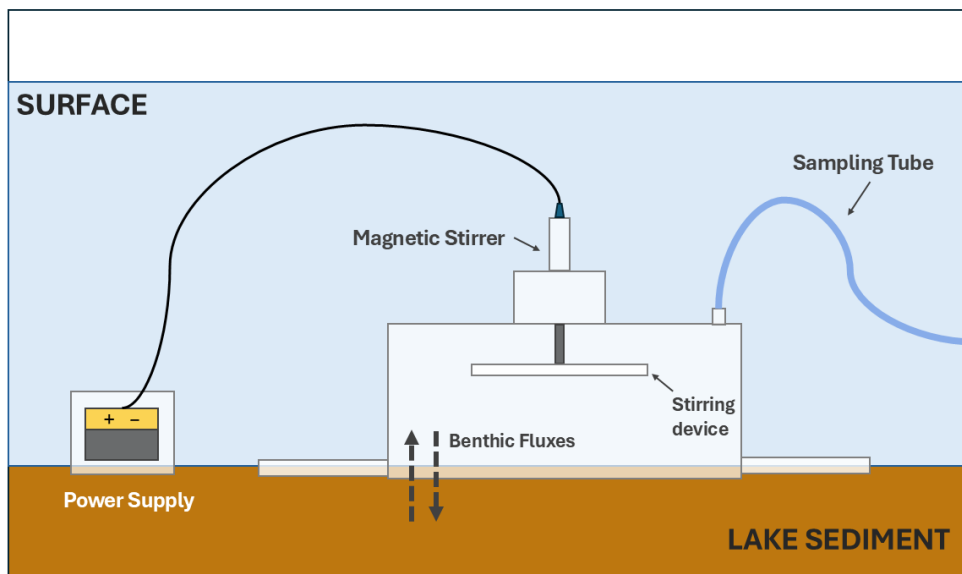


Figure V. 3. Schematic representation of key components of the benthic flux chamber system.

V.2.3. Chemical analysis on water samples

The Fe(II) fraction was determined using the ferrozine method (Viollier et al. 2000). The total dissolved Mn and Fe in the water samples were directly measured using ICP-OES (Perkin Elmer ICP OES Optima 2000DV), while ion (NO_3^- , NH_4^+ , SO_4^{2-} , Cl^-) concentrations were measured using ion chromatography (Dionex ICS-1500). Total S^{2-} concentration was measured according to an adaptation of the method described by Guenther et al. (2001). DOC measurements were acquired using a Vario TOC-Cube TOC analyzer (Elementar).

UV-visible absorbance spectra and fluorescence excitation emission matrices (EEMs) were obtained using an Aqualog spectrofluorometer (Horiba Scientific). Absorbance spectra (200-700 nm, 1 nm resolution) were measured in a quartz cuvette (1 cm pathlength) and blank-corrected with ultrapure Milli-Q water ($18.2 \text{ M}\Omega \cdot \text{cm}$ at 25°C , $\text{DOC} < 2 \text{ ppb}$). The slope ratio (S_R), the spectral slope between 275-295 nm divided by the slope at 350-400 nm, was calculated as an indicator of DOM molecular weight (Helms et al. 2008; Li and Hur 2017). Spectral slope curves at 289 nm (S_{289}) and 382 nm (S_{382}) were measured to assess algal-derived and terrestrial-derived DOM fractions (Loiselle et al. 2009; Roiha et al. 2015). SUVA_{254} (a proxy of DOM aromatic content) was calculated

as described by Weishaar et al. (2003). The absorption coefficient at 320 nm was used as a proxy for the CDOM content.

Fluorescence excitation-emission matrices (EEMs) were acquired in the excitation range of 240-600 nm with 2 nm increments, and an emission range of 250-800 nm with 2.5 nm increments. A new Milli-Q blank was acquired every 10 samples. Inner-filter effect correction and 0-1 normalization were performed for every EEM following the Aqualog Manual (HORIBA, 2019, rev. K).

Parallel factor analysis (PARAFAC) was conducted using the commercial platform SOLO (Eigenvector Research Inc., Manson, DC, USA), following the workflow in the Aqualog Manual (HORIBA, 2019, rev. K). A non-negativity constraint was applied, and the primary and secondary Rayleigh scatterers were set as missing values. A five-component PARAFAC model (99.7% fit) was generated using 219 DOM samples collected across the Arctic and subarctic lakes in previous field campaigns. Spectral loadings, core consistency diagnosis (CORCONDIA), and split-half analysis were employed to validate PARAFAC components (Bro 1997; Murphy et al. 2013). The split-half validation (74.7%) suggested that the model was moderately stable. The CORCONDIA was 53, which is consistent with expectations for EEMs of environmental samples (Bro 1997). The PARAFAC component loadings and split-half validation results are shown in Supplementary Fig. VII.I.6, VII.I.7, and VII.I.8.

DOM was concentrated from 2.5 L water samples using solid-phase extraction (SPE) with styrene-divinylbenzene polymer (PPL) cartridges, using the method described by Dittmar et al. (2008). The retrieved SPE-DOM samples were characterized using Fourier-transform infrared spectroscopy (FTIR). FTIR spectra were obtained using a PerkinElmer Spectrum Two spectrophotometer equipped with an attenuated total reflectance module. Spectra were measured over the 500-4000 cm^{-1} range with 1 cm^{-1} resolution, and 16 scans/sample were recorded, averaged, and corrected against air as the background.

V.2.4. Chemical analysis on lake sediments

The frozen sediment samples were freeze-dried and homogenized prior to analysis. From the homogenized sediments, portions were taken for the determination of total Fe and Mn concentrations, FTIR analysis, C/N ratios, and $\delta^{13}\text{C}$ signatures.

For total Fe and Mn measurements, sediment aliquots were digested in a 1:3 mixture of aqua regia (prepared at a 3:1 v/v ratio of HCl:HNO₃) and hydrofluoric acid (HF); all reagents were Merck Suprapur® grade. The digestion was performed at 100°C for 1h. After digestion, boric acid (H₃BO₃) was added to the complex residual HF to prevent fluoride precipitation. Samples were diluted 10:1 with Milli-Q water containing 2% HNO₃ prior to ICP-OES analysis, as described above. Quality control measures included blanks and two certified reference materials (CRM): MESS-4 (Marine Sediment CRM, NRC, Canada) and BCR-60 (Aquatic Plant *Lagarosiphon major*). The measured concentrations of the reference materials had relative standard deviations below 6%, which agreed with the certified values for Fe and Mn.

FTIR analysis of the homogenized sediment layers was conducted using the same equipment and scanning conditions as those used for the DOM samples.

C/N ratios and $\delta^{13}\text{C}$ signatures were measured for both the total carbon fraction and the organic carbon fraction of the sediments. Inorganic carbon was removed by treating samples with 0.1M HCl for 2 h, followed by several Milli-Q water rinses and freeze-drying prior to analysis. $\delta^{13}\text{C}$, %C, and %N were measured using a EuroVector Elemental Analyzer coupled to a Nu Horizon isotope-ratio mass spectrometer (Water Quality Center, Trent University, Canada). $\delta^{13}\text{C}$ values were calibrated relative to the Vienna Pee Dee Belemnite (VPDB) standard using a three-point calibration anchored by USGS40 (glutamic acid: $\delta^{13}\text{C} = -26.39$ ‰, %C = 40.82, %N = 9.52), USGS62 ($\delta^{13}\text{C} = -14.79$ ‰, %C = 49.48, %N = 28.85), and USGS63 ($\delta^{13}\text{C} = -1.17$ ‰, %C = 49.50, %N = 28.90). USGS40 was also used to calibrate elemental compositions for %C and %N determinations.

V.2.5. Statistics and data processing

The slope curves at 289 nm (S_{289}), 382 nm (S_{382}), and S_R values were calculated using the CDOM R package (Massicotte and Markager 2016) in RStudio (version 2023.06.2-561). Principal component analysis (PCA) was conducted in RStudio to identify the differing biogeochemical patterns between the two lakes in the benthic flux chamber experiment. Permutational Multivariate Analysis of Variance (PERMANOVA; Euclidean distance, 999 permutations) was used to test for variance explained by the lake, followed by permutational analysis of multivariate dispersions (PERMDISP) to assess group

dispersions the vegan package (Dixon 2003; Brückner and Heethoff 2017). All variables were Z-score normalized prior to statistical analysis.

V.3. Results

V.3.1. Vertical profiles of physicochemical parameters

Temperature profiles showed gradual increases from surface to bottom in both ponds, with values rising from 4.3 to 5.1 °C in ZAC1 and from 6.8 to 7.4 °C in ZAC2 (Fig. V.4). The DO saturation (%DO) profile of ZAC1 displayed a moderate decline from 63% at the surface to 39% at the bottom. However, ZAC2 exhibited an accelerated decrease from 65% at the surface to 12% at the bottom.

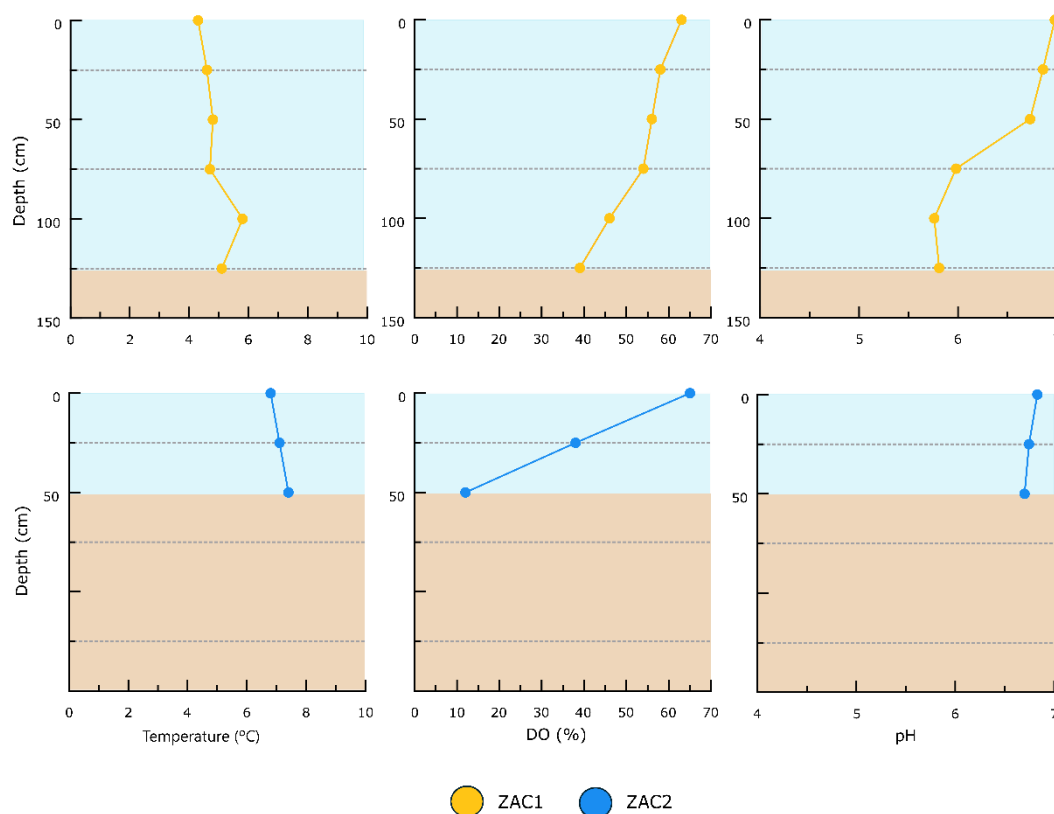


Figure V.4. Vertical profiles of temperature (°C), DO (%) and pH in both thermokarst lakes. The blue section represents the water column, and the brown section represents the lake sediment.

The pH profiles of ZAC1 and ZAC2 also differed (Fig. V.4). In ZAC1, the surface (upper 50 cm) showed higher pH values (6.7-6.8) while the transition to deeper waters was

marked by a shift to lower pH (5.7-5.9). However, the pH values in ZAC2 remained stable along the water column (6.7-6.8).

In ZAC1, the oxidation-reduction potential (ORP) decreased from 286 to 226 mV in the upper 25 cm, followed by a more gradual decline to 207.8 mV at the bottom. The ORP in ZAC2 decreased steadily from 290.3 to 223.2 mV with depth (Fig. VII.III.2).

No thermal stratification was observed; therefore, we refrained from defining clear epilimnion, metalimnion, or hypolimnion layers in ZAC1 (Fig. V.4). The same occurred for shallow ZAC2 (50 cm depth), where wind mixing was likely to occur to a greater extent.

V.3.2. Terminal electron acceptor profiles

Total dissolved Fe concentrations in ZAC1 ranged from 2.3 to 2.7 mg L⁻¹ throughout the water column, with a local increase to 4.0 mg L⁻¹ at 25 cm depth (Fig. V.5). In ZAC2, the total Fe concentrations were consistently higher, increasing with depth from 7.2 to 12 mg L⁻¹ (Fig. V.5). The average total dissolved Fe concentration in ZAC2 is more than three times higher than that in ZAC1. The Fe(II) levels in ZAC1 ranged from 0.31 to 0.55 mg L⁻¹, representing 11–22% of total dissolved Fe. In ZAC2, Fe(II) concentrations ranged from 1.3 to 3.0 mg L⁻¹, corresponding to 12–34% of the total dissolved Fe (Fig. VII.III.6),

Total dissolved Mn concentrations increased with depth in both lakes, ranging from 2.3 to 16.1 µg L⁻¹ in ZAC1 and from 5.6 to 32.0 µg L⁻¹ in ZAC2, two-times higher than the levels obtained in ZAC1 (Fig. V.5).

The nitrate concentrations showed contrasting trends among the lakes. In ZAC1, NO₃⁻ remained below the detection limits throughout the water column, except for a localized peak of 2.04 mg L⁻¹ at 100 cm (Fig. V.5). Ammonium was similarly undetectable in the upper half of the profile, with increases to 1.56 and 1.87 mg L⁻¹ at 75 and 125 cm, respectively. In ZAC2, both NO₃⁻ (5.4 mg L⁻¹), and NH₄⁺ (1.2 mg L⁻¹) were elevated at the surface and decreased sharply with depth.

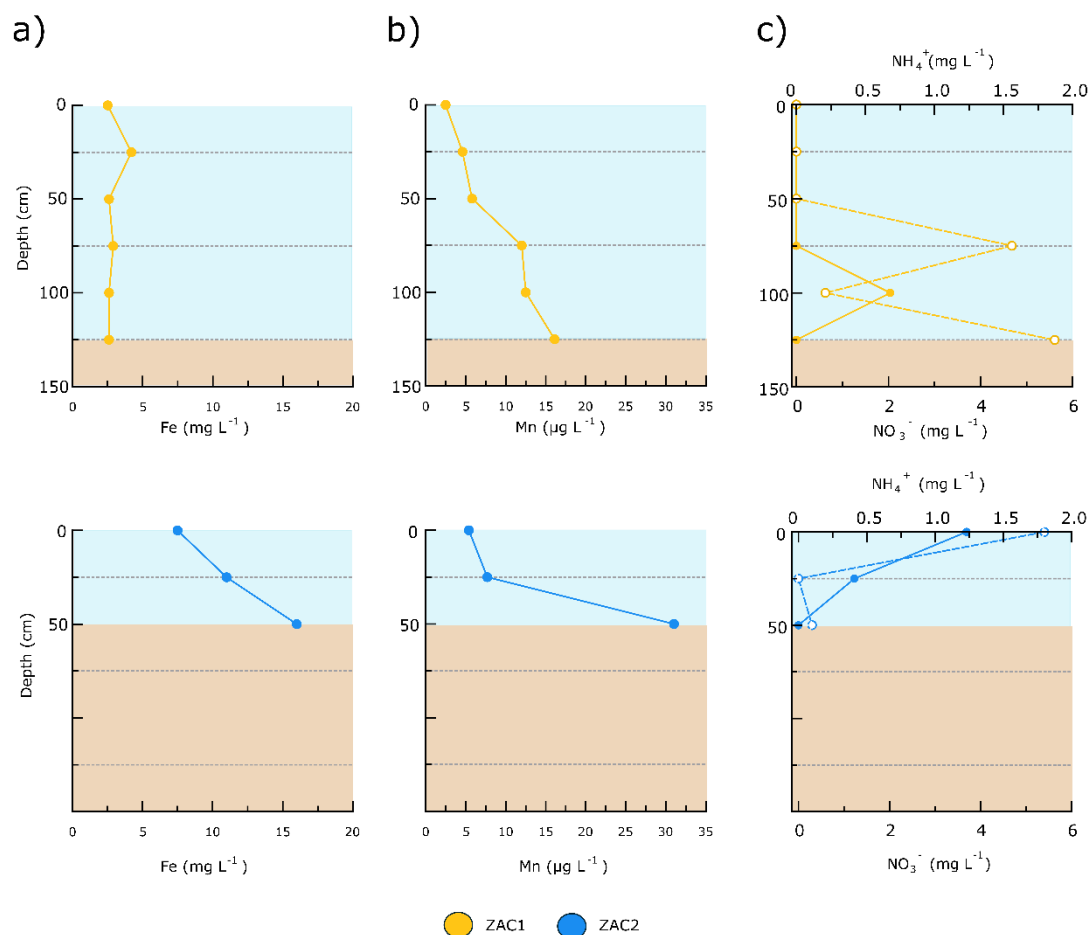


Figure V.5. Vertical profiles of: a) dissolved Fe; b) dissolved Mn; c) nitrate (NO_3^-) (closed circles) and ammonium (NH_4^+) (open circles) in the two lakes.

Sulfate concentrations in ZAC1 were relatively stable throughout the profile, ranging only from 13.5 to 14.2 mg L^{-1} . In contrast, S^{2-} gradually increases with depth (Fig. VII.III.6) but remained low overall (0–0.54 $\mu\text{g L}^{-1}$). In ZAC2, SO_4^{2-} was highest at the surface (12.6 mg L^{-1}), declining to 9.6–9.4 mg L^{-1} with depth. The sulfide concentration rose to 6.4 $\mu\text{g L}^{-1}$ at 25 cm and decreased to 1.3 mg L^{-1} at the bottom (Fig. VII.III.6). Interestingly, while the SO_4^{2-} levels were of the same order of magnitude in both lakes, the S^{2-} concentrations were up to 12 times higher in ZAC2 than in ZAC1.

V.3.3. Dissolved organic matter

The DOC concentrations in ZAC1 fluctuated between 3.9 to 6.4 mg L^{-1} along the water column, with ZAC2 showing higher DOC values in the surface (6.3–7.7 mg L^{-1}) compared

to the bottom (4.3 mg L^{-1}). Notably, DOC concentrations were higher in the $0.2 \mu\text{m}$ fraction than in the $0.45 \mu\text{m}$ fraction in both lakes (on average: 27% in ZAC1 and 54% in ZAC2). The variations in the SUVA_{254} , a_{320} and S_R vertical profiles in ZAC1 and ZAC2 are illustrated in Fig 5.

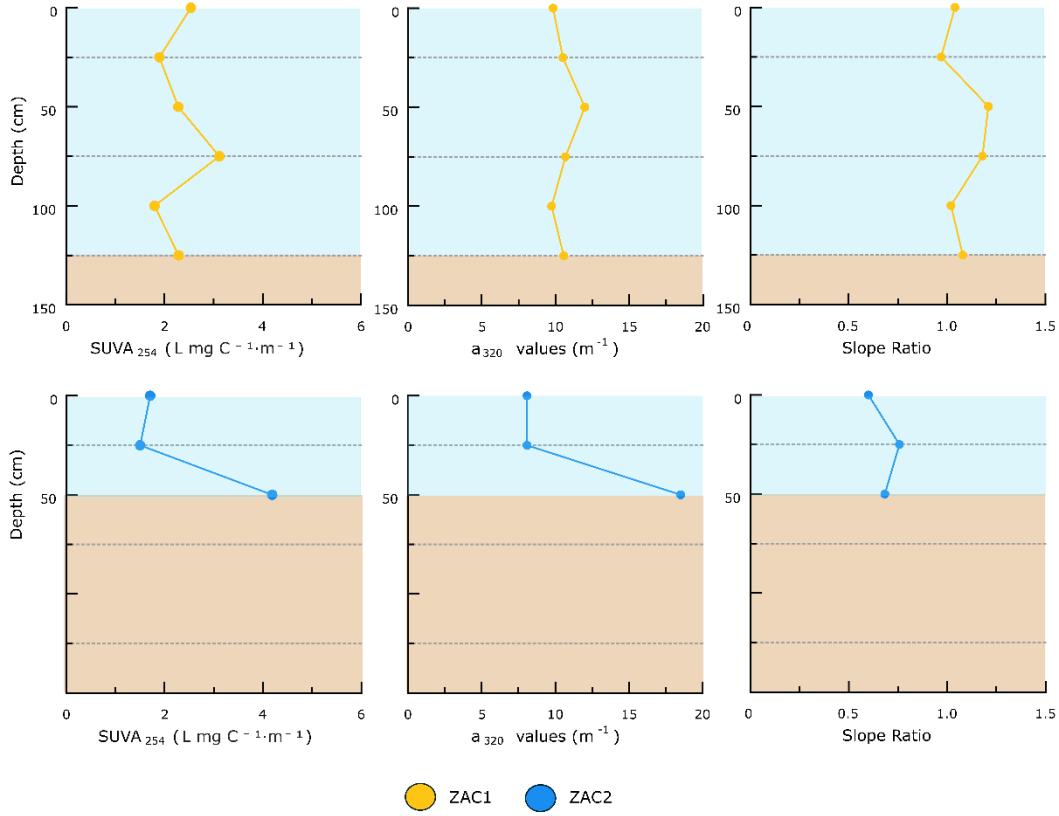


Figure V.6. Vertical profiles of SUVA_{254} , a_{320} , and slope ratio (S_R) in the water column of ZAC1 and ZAC2.

The SUVA_{254} values in ZAC1 oscillate between 1.80 and $3.12 \text{ L mg C}^{-1} \text{ m}^{-1}$ along the water column, while ZAC2 shows a stark increase at the bottom ($4.18 \text{ L mg C}^{-1} \text{ m}^{-1}$) quickly diminishing towards the surface (1.50 - $1.71 \text{ L mg C}^{-1} \text{ m}^{-1}$). The a_{320} values showed little depth variation for ZAC1, ranging between 9 - 12 m^{-1} throughout the water column, while ZAC2 showed a clear increase towards the bottom (from 8 to 18 m^{-1}). The S_R values differed significantly between lakes, with ZAC1 displaying a higher S_R , ranging from 0.97 to 1.21 . ZAC2, however, displayed lower S_R values between 0.60 to 0.76 , suggesting an overall increase in the DOM molecular weight relative to ZAC1.

The generated PARAFAC model identified five fluorescent components (EEMs in Fig. VII.I.5), categorized according to Pacoureau et al. (2025) as humic-like terrestrial (HT),

humic-like microbial (HM), or protein-like (P). The PARAFAC component details are provided in the Supplementary Note VII.I.1 (SN.VII.I.1). The general PARAFAC component identifications are provided in Table V.2, and the vertical distribution profiles of the PARAFAC components (% contribution of each component) in the water column are shown in Fig. V.7.

Table V.2. Identification of five PARAFAC components and their assignment based on the literature (provided as Supplementary Note in SN.VII.I.1).

Component	Excitation_{max}	Emission_{max}	Assignment
HT1	250 nm/ 340 nm	450 nm	Humic-like terrestrial
HT2	265 nm/ 400 nm	520 nm	Humic-like terrestrial
HM1	240 nm/ 310 nm	400 nm	Humic-like microbial
P2	275 nm	295 nm	Tyrosine-like
P1	280 nm	330 nm	Tryptophan-like

Vertical profiles of the relative contribution of PARAFAC components (% of each component) reflected the biogeochemical patterns imprinted in the FDOM in both lakes (Fig. V.7).

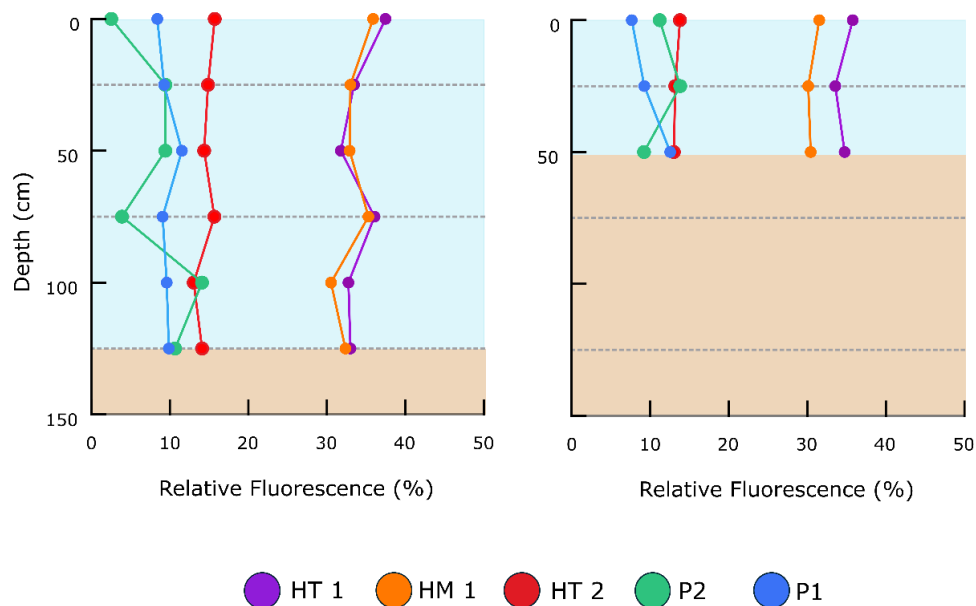


Figure V.7. Variation of PARAFAC components (% of each component) throughout the water column of ZAC1 and ZAC2.

In ZAC1, we observed an increase in P2 from the surface to the bottom, while the other components exhibited limited variation along the water column. We note that in the 0.2 μm DOM fraction of ZAC1, the vertical profile showed a near absence of P2 (Fig. VII.III.5). ZAC2 displays a slight increase in P1 towards the bottom.

The FTIR spectra of the SPE-DOM samples from ZAC1 and ZAC2 showed notable differences in their functional groups (Fig. V.8). The identification and FTIR peak assignments are listed in Table VII.I.7.

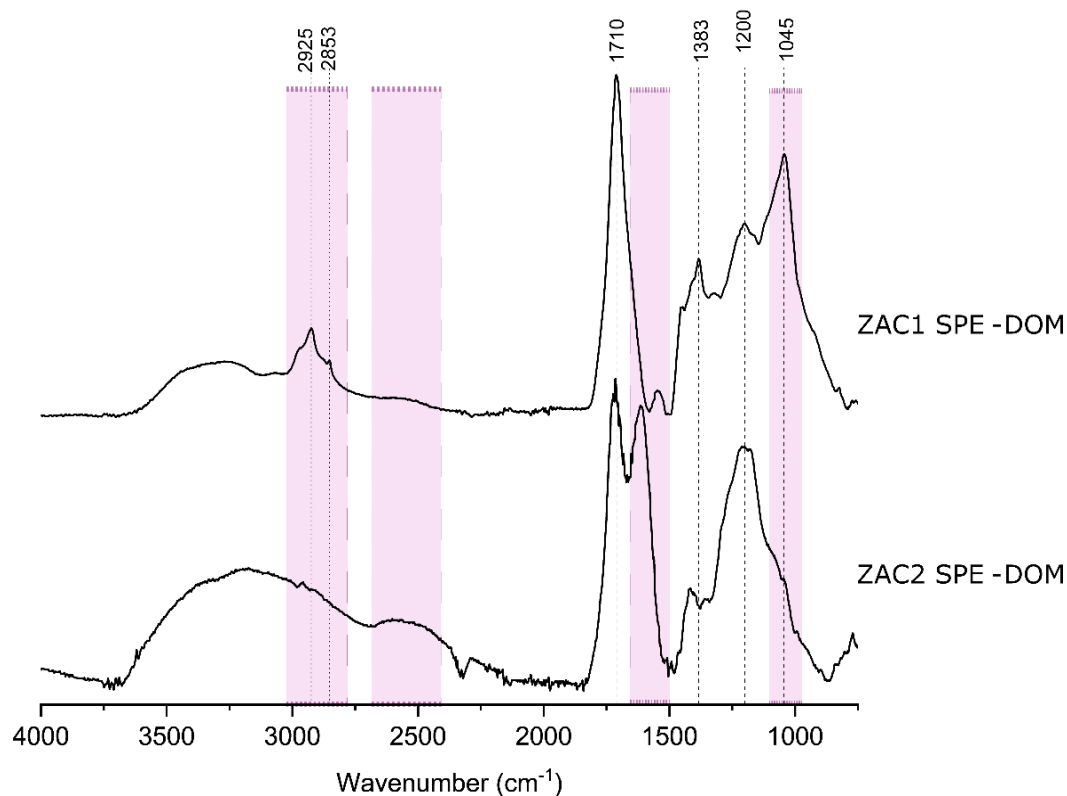


Figure V.8. FTIR spectra of SPE-DOM water samples from ZAC1 and ZAC2. Main spectral differences between DOM samples are highlighted in magenta.

The SPE-DOM from ZAC1 showed peaks at 1045, 1200, and 1383 cm^{-1} , a shoulder-like peak at 1450 cm^{-1} , a small band at 1545 and 1710 cm^{-1} , dual peaks at 2853 and 2925 cm^{-1} , and a broad band in the 3000-3400 cm^{-1} range. The SPE-DOM FTIR spectra from ZAC2 showed a small shoulder-like band at 1045 cm^{-1} , a band at 1200 cm^{-1} , a band at 1420 cm^{-1} , a strong band at 1613 and 1710 cm^{-1} , a broad band between 2500-2700 cm^{-1} , and a broad band in the 3000-3400 cm^{-1} range.

ZAC1 showed an increased polysaccharide content, greater amide II presence, and more aliphatic signatures relative to ZAC2 SPE-DOM. However, ZAC2 SPE-DOM displayed higher contributions from carboxylic acids and functional groups associated with cellulose and lignin compounds, denoting more terrestrial contributions than ZAC1.

V.3.4. Sediment characterization

The $\delta^{13}\text{C}$ signatures and C/N ratios (Fig. V.9) of both the total and organic carbon fractions were analyzed in the sediments. The $\delta^{13}\text{C}$ signatures were generally consistent between the total and organic fractions across all sediment samples, except for the deepest ZAC1 sediment layer. However, the C/N ratios of the organic fraction of ZAC1 sediments were slightly higher than those of the total carbon fraction, reflecting the removal of other inorganic compounds that affect the C/N ratio of the HCl-treated samples.

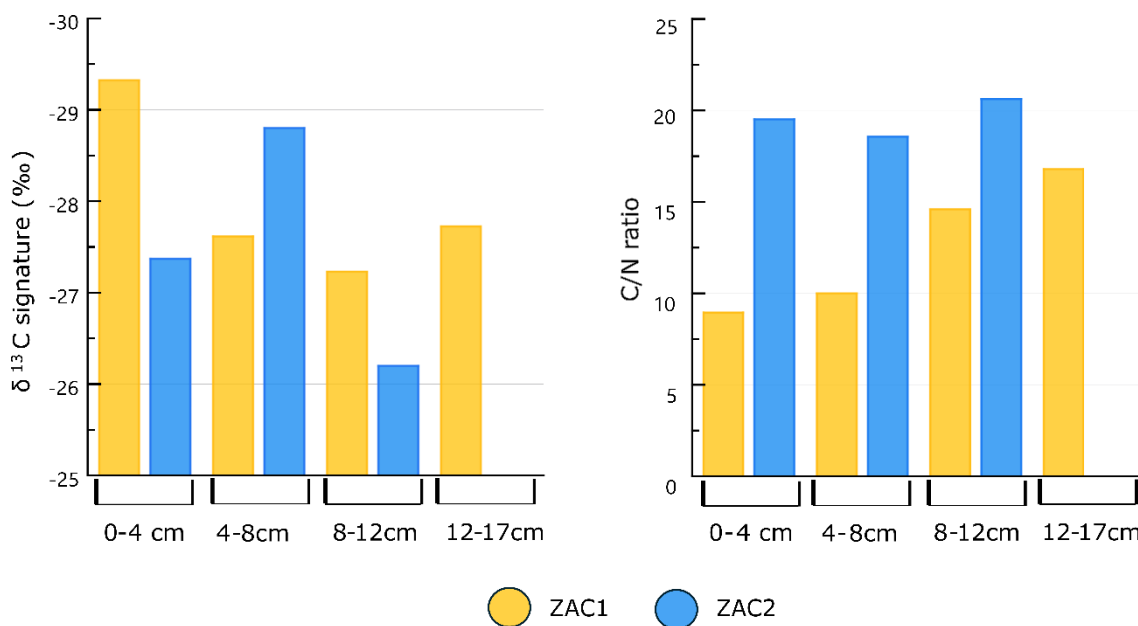


Figure V.9. Isotopic $\delta^{13}\text{C}$ signatures (left) and C/N ratios (right) of lacustrine sediment layers of ZAC1 and ZAC2.

Comparatively, the ZAC1 sediments showed lower C/N ratios than the ZAC2 sediments (Fig. V.9), indicating a greater degree of degradation, which is also supported by the lower carbon weight percentage (wt%) shown in Table VII.III.2. In ZAC1 sediments, the C/N ratios increased with depth, with lighter $\delta^{13}\text{C}$ signatures occurring in the surface sediments. Additionally, in the ZAC2 sediments, the lowest C/N ratios displayed the most ^{13}C -depleted signatures.

The differences in C/N ratios of sediment layers between lakes mirrored the differences observed in their FTIR spectra. ZAC1 sediments showed a decrease in FTIR signals associated with organic functional groups, particularly in the upper sediments, compared to ZAC2 sediments.

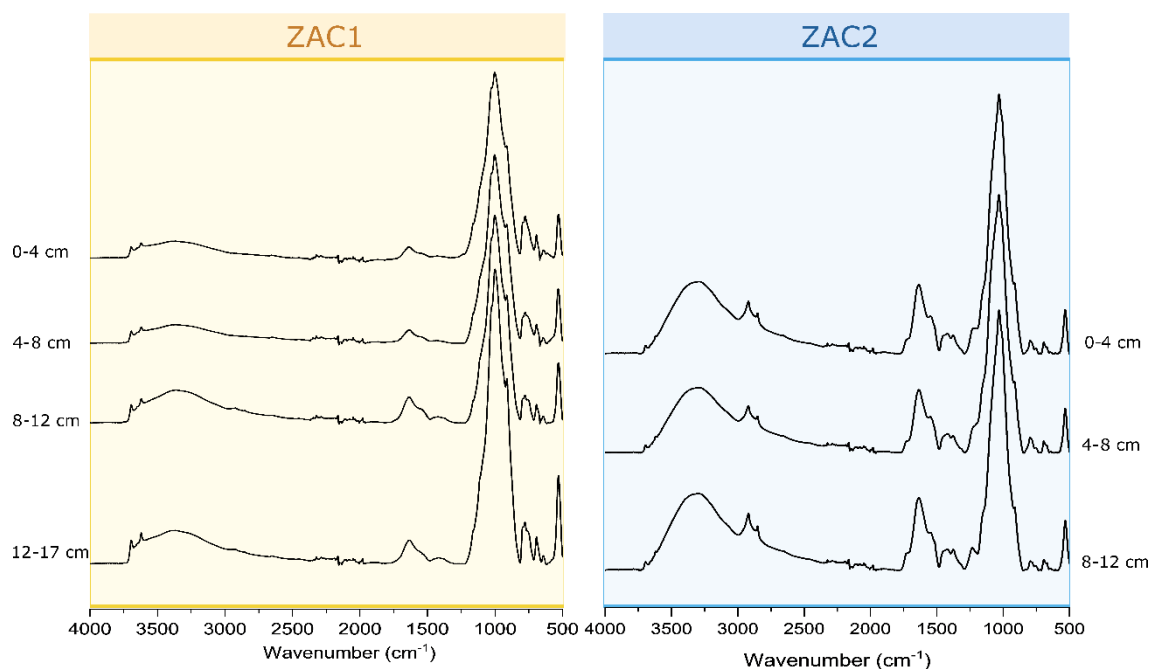


Figure V.10. FTIR spectra of sediment layers of ZAC1 (left) and ZAC2 (right) lakes.

The ZAC1 sediments showed peaks at 531 cm^{-1} , attributed to the substitution of Al- for Fe- in the structure of hematite (Kosmas et al. 1986; Salama et al. 2015), with peaks at 691 , 778 , and 796 cm^{-1} indicating the presence of quartz minerals (Reig et al. 2002; Mei et al. 2022). The shoulder-like peak at 754 cm^{-1} was attributed to the hydroxyl translation vibrations linked with kaolinite (Frost and Johansson 1998), similar to the peaks at 912 cm^{-1} (Al-O-H) (Salama et al. 2015; Sánchez-Sánchez et al. 2019), and the 3619 and 3693 cm^{-1} (O-H stretching) were also linked with kaolinite (Frost et al. 2002; Mei et al. 2022). A strong band centered at 1002 cm^{-1} likely arises from the Si-O vibrations of silica minerals (Petrovskii et al. 2016) and C-O stretching of polysaccharides (Abdulla et al. 2010; Beć et al. 2020; Maxson et al. 2021).

Deeper sediments showed the emergence of a band in the $1375\text{-}1455\text{ cm}^{-1}$ region, along with bands at 1542 , 1635 , and a broad band at $3000\text{-}3400\text{ cm}^{-1}$. This increase in amide, carbohydrate, and phenolic structures indicates a greater presence of organic substrates in deeper sediment layers (Fig. V.10).

Compared to ZAC1, ZAC2 sediments displayed FTIR bands of organic functional groups, indicating a greater abundance of natural organic matter (NOM). In general, the FTIR spectra of the ZAC2 sediments showed the following: (1) broad band centered at 1030 cm^{-1} ; (2) shoulder-like peak at 1230 cm^{-1} ; (3) broad band covering 1376 to 1455 cm^{-1} ; (4) shoulder-like peak at 1546 cm^{-1} ; (5) band centered at 1640 cm^{-1} ; (6) band centered

at 1730 cm^{-1} ; (7) dual peak at 2918 and 2850 cm^{-1} ; and (8) broad band at $3400\text{--}3000\text{ cm}^{-1}$. The FTIR peak assignments for the organic functional groups in the sediments are listed in Table VII.I.7.

ZAC2 sediments showed increases in polysaccharides, cellulose, amide I and II, hydroxyl groups, and aliphatic structures from waxy lipids. The strong presence of both cellulose and amide structures indicates contributions from the plant material and algae (Maxson et al. 2021). Despite the abundance of NOM signals, hematite (532 cm^{-1}), quartz minerals (692 , 778 , and 796 cm^{-1}), and kaolinite (750 , 914 , 3619 , and 3693 cm^{-1}) were detected in the ZAC2 sediments.

V.3.5. Total Fe and Mn content in sediments

The iron content in ZAC1 sediments increased with depth (Fig. VII.III.11), with the 8–12 cm layer showing a 72.7% increase relative to the 0–4 cm layer (21.2 g kg^{-1}). In contrast, Fe content in ZAC2 sediments increased in the upper layers (0–8 cm), ranging from 22.3 to 24.0 g kg^{-1} , before decreasing to 13.6 g kg^{-1} in the 8–12 cm layer.

ZAC1 sediments consistently exhibited higher Mn content than ZAC2 sediments. Similar to Fe, the Mn concentrations in ZAC1 increased with depth, with the 8–12 cm layer showing a 119% increase relative to the 0–4 cm layer (163 mg kg^{-1}). In ZAC2, the Mn content remained stable at 105 mg kg^{-1} in the upper layers (0–4 and 4–8 cm) before declining slightly to 93 mg kg^{-1} at 8–12 cm (Fig. VII.III.11).

V.3.6. Benthic Flux Chamber data

The benthic flux chamber (BFC) experiments in ZAC1 and ZAC2 revealed differences in the sediment water interface (SWI) biogeochemistry between both lakes from T0 to T7 (Table V.1). Average BFC measurements revealed that ZAC1 generally exhibited higher S_R values and elevated DOC, SO_4^{2-} , NH_4^+ and Cl^- concentrations, with considerably higher pH values compared to ZAC2. Conversely, in the BFC experiment, ZAC2 was characterized by higher dissolved Fe, Mn, and S^{2-} concentrations, increased SUVA_{254} values, and notably more acidic pH. Additionally, ZAC2 showed a sharp decline in DO (from 23% to 1% within the first 30 min) and more negative ORP values (ranging from -

93 to -303 mV between T2 and T7) (Fig. V.11). During the course of the BFC experiment, both lakes exhibited a gradual decrease in Fe(II) percentage, with ZAC1 experiencing a 90% decrease and ZAC2 a 48% decrease. No consistent trends were observed in DOM composition throughout the BFC experiment in either lake.

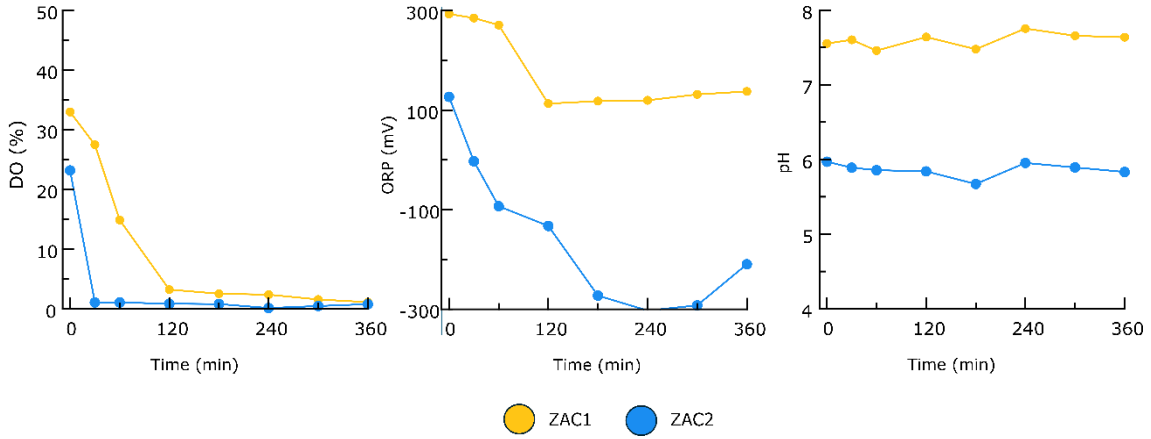


Figure V.11. Benthic flux chamber measurements of DO (%), ORP (mV) and pH in ZAC1 and ZAC2.

The hourly SOD rate measured in ZAC1 was $0.52 \text{ g O}_2 \text{ m}^{-2} \text{ h}^{-1}$, while ZAC2 exhibited a higher rate of $1.5 \text{ g O}_2 \text{ m}^{-2} \text{ h}^{-1}$. Corresponding daily SOD rates were 12.4 and $36.0 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ for ZAC1 and ZAC2, respectively. The SOD rate in ZAC2 sediments was nearly three times higher than that measured in ZAC1 sediments.

An exploratory PCA (Fig. V.12) was conducted on benthic flux chamber samples from ZAC1 and ZAC2 collected across the entire duration of the BFC experiment (T0 to T7). The analysis aimed to identify the overall contrasts between the two lakes in benthic biogeochemical variables (pH, ORP, DO, DOC, S_R , $SUVA_{254}$, a_{320} , Fe, Mn, NO_3^- , SO_4^{2-} , Cl, NH_4^+ and S^{2-}) and capture dominant patterns influencing benthic dynamics in each lake. The first three principal components (PCs) explained 85.2% of the cumulative variance (PC1: 61.6%; PC2:12.6%; PC3:11.0%). The positive and negative variable loadings for each PC are presented in Table VII.III.3.

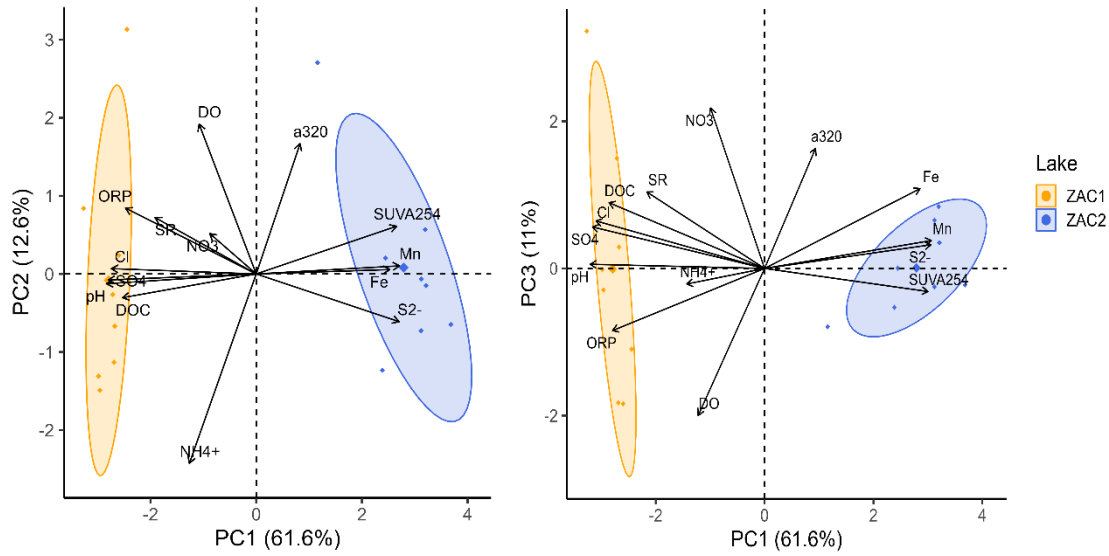


Figure V.12. Principal component analysis (PCA) biplots of measured variables in BFC experiment in ZAC1 and ZAC2 — biplot of PC1 (x-axis) and PC2 (y-axis) (left) and biplot of PC1 (x-axis) and PC3 (y-axis) (right). Ellipses were generated around group centroids with a 68% confidence interval.

PC1 separated dissolved Fe, Mn, S^{2-} , and $SUVA_{254}$ (positive loadings) from the ORP, pH, Cl^- , DOC, and SO_4^{2-} concentrations (negative loadings). A gradient from more Fe- and Mn-rich environments with higher DOM aromaticity to more alkaline and oxidizing conditions with increases in DOC and SO_4^{2-} concentrations. PC2 separated water conditions with higher a_{320} and DO values (positive loading) from environments rich in NH_4^+ (negative loading). This PC reflects a transition from more oxygenated conditions with higher CDOM content towards anoxic environments with increased NH_4^+ concentrations, likely from NO_3^- reduction. PC3 divided environments with higher a_{320} , NO_3^- , S_R , Fe, and DOC (positive loadings) from those with higher DO conditions (negative loadings). This gradient reveals a separation between environments containing higher CDOM and lower molecular weight DOM, with higher DOC, dissolved Fe, and NO_3^- availability under more oxic conditions.

PCA further illustrates the division between ZAC1 and ZAC2 samples described above, with ZAC1 BFC samples aligned with negative PC1 loadings (i.e., pH, DOC, Cl^- , SO_4^{2-} and ORP) and ZAC2 BFC samples with positive PC1 loadings (i.e., Fe, Mn S^{2-} , and $SUVA_{254}$). PERMANOVA indicated that lake groupings explained 67.4% of the observed variance (F-stat= 28.9; $p = 0.001$) with PERMDISP (F-stat= 2.11; $p = 0.176$),

supporting the assertion that these differences arose from mean centroid differences between lake groups.

V.4. Discussion

V.4.1. Thermokarst lake development stage and general dynamics

Both thermokarst lakes are expected to have recently formed, with researchers at the Zackenberg Research Station observing the ZAC1 form in recent years. In ZAC2, the ongoing aggregation of smaller adjacent pond into a larger lake is clearly observable (Fig. V.2) and consistent with early-stage thermokarst lake development. Given the shallower depth and lower Cl^- concentrations (expected lower contributions from deeper NaCl deposits) and, overall, less degraded sediments, we expect ZAC2 to be at an earlier stage of development than ZAC1, despite both being recently formed aquatic systems. Nevertheless, the biogeochemical dynamics of both lakes are profoundly affected by the early stage of peatland formation and relatively shallow active layer depth. This likely results in limited hydrological connectivity between the lake and surrounding soils, leading to a reduced influence of peat soil on thermokarst lake dynamics, in contrast to the more developed permafrost peatland landscapes in subarctic regions (Vonk et al. 2015; Prijac et al. 2023).

Moreover, lake dynamics are closely linked with the developmental stage of the aquatic system, as the deeper and more developed ZAC1 exhibits a slight degree of stratification in pH profiles, whereas the younger, shallow ZAC2 is more effectively mixed and tightly coupled to sediment activity through a thin water column. Solar radiation penetrates the clear waters of both ZAC1 and ZAC2, being absorbed by the darker sediments, warming the upper sediment layers and ultimately driving intensified heterotrophic processing. This vertical degradation pattern is clearly exhibited in ZAC1 sediments, as upper layers are more extensively degraded than at depth (lower C/N ratios and decreased organic functional groups signatures in FTIR spectra). Moreover, upper sediments also display a shift to more ^{13}C -depleted signatures. The Zackenberg valley has been widely reported to display elevated methane emissions (Tagesson et al. 2013; Scheller et al. 2021; Christensen et al. 2023). Methanogenesis leads to the production of methane with highly negative $\delta^{13}\text{C}$ signatures (Whiticar 1999; Conrad and Claus 2005), becoming available to

methanotrophic communities and being subsequently incorporated into sediment NOM, producing ^{13}C -depleted signatures (Kellermann et al. 2012; Nowak et al. 2015). This process has been proposed in subarctic thermokarst lakes by Wauthy et al. (2018). Although these vertical sediment patterns are observed in ZAC1, they are not discernible in ZAC2 sediments, likely due to the latter being at an earlier stage of development.

Nevertheless, both lakes exhibit signs of elevated benthic activity (i.e. elevated SOD), with ZAC2 displaying rates approximately 3 times higher than ZAC1, likely due to a greater abundance of labile NOM substrates. This difference is reflected in enhanced mobilization of dissolved Fe, Mn and CDOM to ZAC2 bottom waters. One should note ZAC2 sediments have higher mercury methylation rates, as well as higher Hg and MMHg than ZAC1 sediments (Martins et al. 2025).

Both redox reduction and benthic activity promote mobilization of dissolved Fe and Mn into the water column of ZAC lakes. ZAC1 exhibited stabilized dissolved Fe and a gradual dissolved Mn increase with water column depth, consistent with slower, diffusion-dominated transport. However, ZAC2 displayed a strong enrichment of both in bottom waters, indicating more vigorous sediment–water exchange and rapid upward migration of reduced Fe and Mn driven by redox mobilization from active sediments in the shallow system.

This enhanced sediment activity and substrate availability in ZAC2 sediments is reflected in DOM quality in the water column, promoting a shift to higher average DOM molecular weight and increased presence of carboxylic acids. In contrast, DOM inputs from the degraded sediments with lower benthic activity in ZAC1 are seemingly comprised of lower molecular weight DOM (higher S_R) and higher presence of carbohydrate and aliphatic structures. This divergence in DOM quality may also indicate a greater extent of photochemical processing. Notably, the 0.2 μm fraction exhibited higher DOC concentrations than the 0.45 μm fraction, especially in ZAC2. We attributed this to bacterial degradation of DOM in 0.45 μm filtered samples between sampling until DOC quantification, since bacterial communities of natural freshwater ecosystems have been shown to pass through the 0.45 μm pore size (Wang et al. 2007). In turn, we recognize this may influence SUVA_{254} values in DOM samples filtered at 0.45 μm .

Water column dynamics also exhibited differences between lakes. The deeper ZAC1 exhibited a distinct shift to lower pH in deeper pelagic layers, likely reflecting the

accumulation of dissolved CO₂ near the bottom, along with a moderate DO decline with depth. In the shallow ZAC2, the short distance between surface and sediments, together with high SOD rates, causes rapid DO depletion in bottom waters, steepening redox gradients at the SWI. Consequently, this contributes to increases in dissolved Fe, Mn, CDOM and aromatic DOM near bottom due to redox cycling in anoxic sediment layers.

Permafrost releases large quantities of large NOM and dissolved organic nitrogen, initially degraded to reactive ammonium and then undergoing nitrification (Voigt et al. 2017; Francis et al. 2023). In ZAC1, the decreased mixing and intermediate redox conditions favor such coupled ammonification–nitrification processes in the deeper water column. Elevated NH₄⁺ concentrations at 75 and 125 cm, together with a NO₃⁻ maximum at 100 cm, support this interpretation. In contrast, the shallow ZAC2 promotes nitrification and S²⁻ oxidation to SO₄²⁻ predominantly in surface and upper-water layers, where DO is highest and DOM breakdown (CDOM decrease and increasing spectral slopes *S*₂₈₉ and *S*₃₈₂ towards surface) provides labile N.

The differing stages of thermokarst evolution of the lakes strongly influence their hydrology and consequently their TEA cycling dynamics. Lake ZAC2 remains tightly coupled to sediment processes due to its thin water column. High SOD rates rapidly deplete DO along the water column, promoting strong upward fluxes of reduced Fe and Mn to bottom waters. In contrast, the deeper and more developed ZAC1 shows partial stratification, limiting rapid exchanges between surface and bottom waters and allowing gradients to develop within the water column. Under these conditions, reduced Fe and Mn can diffuse from anoxic sediment layers and be partially re-oxidized in the water column, resulting in more gradual redox cycling. This structure promotes stronger sediment-water coupling in ZAC2, whereas the deeper water column in ZAC1 creates a more buffered environment where redox transformations occur more gradually.

These observations highlight how thermokarst lake evolution modifies lake biogeochemistry in polygonal tundra landscapes. Early-stage systems composed of shallow ponds exhibit strong benthic-pelagic coupling and more elevated SOD as abundant NOM substrates are available for mineralization. As thermokarst lakes mature and deepen, mixing regimes and redox gradients change, promoting processes such as nitrification and CO₂ accumulation in deeper waters. At later stages, progressive sediment degradation reduces the availability of NOM substrates, leading to comparatively lower benthic microbial activity.

V.4.2. Benthic activity and sediment processing

The BFC experiment showed clear similarities and contrasts in biogeochemical patterns between thermokarst lakes at two different stages in their development in the early development stage peatlands in continuous permafrost regions.

The DO and ORP dynamics illustrated more intense benthic activity in ZAC2 sediments than in ZAC1. DO levels dropped to near 1% after 30 min (T1) in ZAC2, whereas ZAC1 exhibited a much more gradual decrease in DO. Redox conditions strongly shifted to a reducing environment in ZAC2 over time (ORP: 127 to -272 mV from T0 to T4), whereas ZAC1 exhibited a less pronounced ORP decrease, never reaching negative values.

Sediment oxygen demand (SOD) rates measured in both lakes are exceptionally high compared to the highest values reported in sediment oxygen consumption studies in Arctic lakes in Alaska – highest SOD: $1.25 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ($39 \text{ mmol m}^{-2} \text{ d}^{-1}$) – (Fortino et al. 2014), and subarctic Québec – $0.49 \pm 0.17 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ – (Deshpande et al. 2017), as well as reported ranges in synthesis papers for marine sediments across the Arctic Ocean (Bourgeois et al. 2017). The elevated water transparency observed in both lakes likely contributes to their increased benthic activity (Fortino et al. 2014), with a strong potential for primary production in the sediment. The high SOD rate in ZAC1 and ZAC2 reinforces the idea that these thermokarst lake sediments are hotspots of intense microbial activity in the Zackenberg Valley with notable potential for GHG production.

The pH ranges widely differed, with ZAC1 remaining at circumneutral pH (ca. 7.5), while ZAC2 showed much more acidic conditions (ca. 5.8). We noted that the bottom waters in ZAC1 showed more acidic pH values than those observed in the BFC experiment. It is possible that in bottom waters, when oxygen is still relatively available, aerobic oxidation of methane produces increased CO_2 concentrations, leading to lower pH values. This process would not occur in the BFC experiment considering the decline in available DO, thereby limiting methane oxidation and maintaining higher pH values. In contrast, ZAC2 showed more acidic conditions in the BFC than in the lake profile. We hypothesized that methane was oxidized to CO_2 in ZAC2 sediments, as large quantities of gas bubbles were released during sediment sampling and the placement of the BFC in ZAC2.

Differences in physicochemical properties were reflected in the TEAs and DOM dynamics between lakes. ZAC1 had higher concentrations of SO_4^{2-} , Cl^- and NH_4^+ . Increased NH_4^+ suggests a stronger consumption of NO_3^- in benthic processes. We postulate that more extensive thaw at depth in ZAC1 sediments causes the dissolution of NaCl from marine deposits (Caning and Rasch, 2000), ultimately leading to higher Cl^- concentrations. In contrast, ZAC2 showed higher Fe and Mn concentrations triggered by the initial shift to anoxic conditions and the availability of Fe and Mn oxides. Despite displaying slightly lower SO_4^{2-} in ZAC2, S^{2-} concentrations were higher than those in ZAC1, arising from sulfate-reducing processes in the organic-rich sediment.

Throughout the BFC experiment, DOM characteristics varied on average between lakes, with ZAC2 showing higher DOM aromaticity, slightly higher DOM molecular weight (higher SUVA_{254} and lower S_R), and higher %HM1 and HT2 than ZAC1. These inputs were consistent with DOM released from enhanced sediment NOM processing.

Nevertheless, certain biogeochemical similarities were observed over time, namely a consistent decrease in the available Fe(II) fraction due to probable immobilization and precipitation into the sediment. This decrease was more pronounced in ZAC1, likely because the Fe reduction processes in the sediment occurred to a lesser extent than in ZAC2. Additionally, Mn concentrations decreased over time in ZAC1, indicating that similar processes may occur for dissolved Mn.

The observed shifts in benthic dynamics between ZAC1, characterized by extensively degraded sediments, and the recently formed ZAC2, illustrate a clear trajectory of thermokarst lake development in early stage peatlands in continuous permafrost regions. In emergent systems, the abundance of NOM substrates and TEAs facilitates rapid biogeochemical transformations of lake sediments under both oxic and anoxic conditions. The intense active degradation of organic-rich sediments promotes GHG production, terrestrial DOM inputs into the water column, and the increased mobilization of TEAs (Davison 1993; Audry et al. 2011; Wauthy and Rautio 2020; Heffernan et al. 2024; Freitas et al. 2025). Dissolved Fe and Mn, mobilized during anoxic sediment processing, may precipitate as oxides/oxyhydroxides upon re-oxygenation or become immobilized in sulfide minerals under reducing conditions (Audry et al. 2011). Over time, as NOM substrates become increasingly depleted, benthic processing shifts to deeper sediment layers. However, the deeper layers are still frozen, given the shallow active layer thickness in the Zackenberg valley. Consequently, benthic microbial activity becomes

progressively attenuated as the thermokarst lake matures. The dynamic processing and shift in conditions within the BFC in both thermokarst lakes was so intense that we were unable to properly quantify the magnitude of benthic fluxes in the SWI. The explanation for this observation could be related to the sediment type in lakes ZAC1 and ZAC2, which consists predominantly of fine-grained mud and therefore exhibits very low porosity. Low porosity influences porewater dynamics and, consequently, solute fluxes. It should also not be ruled out that, because the chamber functions as a semi-enclosed system with limited hydrodynamic exchange at the SWI and minimal mixing with the overlying water column of each lake, it may promote internal processes that override potential diffusive fluxes.

Nevertheless, as Arctic temperatures increase and deeper permafrost layers thaw, benthic activity in thermokarst lake sediments is expected to increase. Additionally, as thermokarst lakes expand through thermal erosional processes, these expansion zones become sites of intensified biogeochemical processing and greenhouse gas (GHG) production (Walter Anthony et al. 2016).

V.5. Conclusion

In summary, this study provides the first biogeochemical assessment of thermokarst lakes in distinct early development stage peatlands in a continuous permafrost zone in Zackenberg, Greenland. Our findings reveal that lakes of different ontogenies display contrasting benthic and water column dynamics, fueled by the availability of NOM substrate in the sediment.

The younger, shallower lake (ZAC2) was characterized by higher microbial activity, rapid shifts in redox conditions, and enhanced processing of sediment NOM. This resulted in increased terrestrial DOM inputs and dissolved Fe and Mn in the water column compared to the more mature aquatic system (ZAC1), with more extensively degraded sediments and reduced benthic activity. Over time, as sediment NOM becomes depleted and active layer constraints persist, benthic activity may attenuate, reflecting the dynamic trajectory of thermokarst lake development.

Given the limited thickness of the active layer in the Zackenberg Valley, hydrological connectivity between the surrounding soils and the lake is diminished, suggesting a

dominant role for benthic dynamics in governing water column processes. As permafrost thaw progresses, both the formation of new thermokarst lakes and the thermal erosional expansion of developing lakes suggest increases in benthic microbial processes with increases in rapid NOM cycling and GHG emissions.

Peat soils in developed permafrost peatlands enhance the thermal insulation of permafrost and actively limit thawing because of their low thermal conductivity (Decharme et al. 2016; Du et al. 2022; Tao et al. 2024). In the Zackenberg Valley, an early stage permafrost peatland with a thin active layer, the lack of peat insulation significantly increases permafrost vulnerability to thawing, aligning with the sink-to-source shift in the combined GHG balance (CO_2 and CH_4) proposed by Christensen et al. (2023). The SOD rates in both thermokarst lakes indicated exceptionally strong microbial activity in lake sediments, acting as a biogeochemical hotspot for NOM degradation and a potentially significant source of GHG emissions.

This baseline understanding enhances our capacity to predict ecosystem responses to ongoing Arctic warming and direct future research towards integrative studies with microbial community assessments and GHG production measurements (direct flux measurements and controlled incubation experiments). As a long-term monitoring site within the Greenland Ecosystem Monitoring (GEM) program, Zackenberg Valley provides a unique opportunity to study thermokarst lake development and its impact on GHG emissions in a region characterized by elevated methane fluxes.

V. References

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VI. General Discussion, Conclusions and Future perspectives

The research conducted in this thesis integrates data from three contrasting permafrost regions (Fig. VI.1): (i) Kuujjuarapik-Whapmagoostui, (Nunavik, Québec, Canada): sporadic permafrost region; (ii) Kangiqsualujjuaq, (Nunavik, Québec, Canada): discontinuous permafrost region; (iii) Zackenberg Valley (northeast Greenland): continuous permafrost region. Seven thermokarst lakes were sampled at multiple depths during both open-water and ice-cover seasons (except for Zackenberg valley). In Kuujjuarapik-Whapmagoostui, a waterlogged peatland, three lakes of distinct ontogeny and morphometry were selected. In Kangiqsualujjuaq, a transition zone between boreal forest and tundra landscape, two lakes with differing origins (palsa vs lithalsa) were studied. Lastly, in the Zackenberg valley, a tundra environment, two recently formed lakes at different stages of maturation were sampled. Comprehensive analyses of dissolved organic matter (DOM) quality, water chemistry and terminal electron acceptors (TEAs) in the water column, along with sediment natural organic matter (NOM) characterization, isotopic $\delta^{13}\text{C}$ signatures and total Fe and Mn concentrations. This multi-regional and seasonal dataset enabled the examination of how catchment composition and permafrost context shape biogeochemical functioning across a permafrost gradient but also addressing inter- and intra-regional variability according to origin, ontogeny and morphometry.



Figure VI.1. Permafrost distribution in higher latitudes and selected sampling sites for fieldwork. — Copyright-holders of this figure are the Arctic Institute and Malte Humpert.

The research presented in this thesis reveals overarching biogeochemical variations in thermokarst lake dynamics across permafrost zones, highlighting complex interactions driven by local landscape heterogeneity, lake morphometry and ontogeny, and the permafrost region. However, direct extrapolations cannot be made from thermokarst systems along the permafrost gradient. Furthermore, lake heterogeneity within the same landscape reflects the heightened intra-site contrasts caused by variability in factors such as organic-mineral content during permafrost formation, vegetation succession and inputs and lake morphometry. Naturally, the profound influence of these factors in thermokarst lake biogeochemistry will limit the direct transferability of research findings from a research study to other Arctic and subarctic regions.

Nevertheless, by providing an ecological “snapshot” of physicochemical properties, NOM (including DOM) and TEAs dynamics in thermokarst lakes, examining their interplay may reveal underlying mechanisms. Identifying and understanding the role these biogeochemical mechanisms involved lake dynamics can provide transferable insights into processes shaping pelagic and benthic dynamics in these dynamic systems. I chose to focus on a more detailed investigation of fewer lakes. While this approach naturally limits the number of lakes that can be sampled due to logistical constraints, generating vertical profiles of these selected variables (at 25 cm resolution) offers a more precise and in-depth understanding of lake dynamics.

This research work aimed to identify overarching trends along a permafrost gradient and identify meaningful biogeochemical mechanisms driving thermokarst lake dynamics, while acknowledging the high limnodiversity within the landscape and the limited number of lakes sampled.

This general discussion and conclusion are organized into four main subsections:

- 1) **A synthesis of thermokarst lake chemistry:** A statistical analysis of water column chemistry differences among sampled lakes during summer and winter, and the relationships between sediment variables.
- 2) **Primary gradient-induced mechanistic shifts in lake chemistry:** linking the permafrost gradient to broader changes in lake biogeochemistry and their impacts on pelagic and benthic dynamics.
- 3) **Carbon fixation mechanisms in thermokarst lakes:** exploring how carbon fixation occurs in thermokarst lakes along the permafrost gradient, tracking

isotopic signatures of methane-derived NOM and examining the role of primary producers.

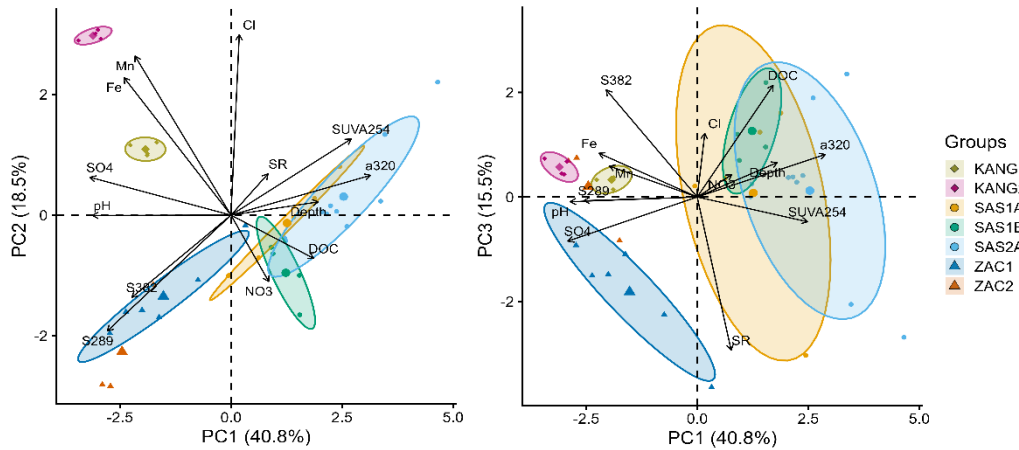
- 4) **Relevance of the present work in thermokarst lake chemistry:** summary of scientific relevance of this research work on thermokarst lake dynamics and functioning.
- 5) **Future research on thermokarst lakes.**

VI.1. Synthesis of thermokarst lake chemistry

To better provide context to the biogeochemical contrasts, water column variables including pH, DOC, a_{320} , SUVA₂₅₄, S_R , S_{289} , S_{382} , dissolved Fe, dissolved Mn, NO_3^- , SO_4^{2-} , and Cl^- concentrations were used to explore differences across the permafrost gradient (Fig. VI.2). Datasets from open-water and winter seasons (same variables used, except for pH) were separated to focus on comparable contrasts in thermokarst lake functioning within the same season. In this sense, I initially synthesized the primary outcomes from all sites of multivariate analysis of water column data in both summer and winter conditions. For that, principal component analysis (PCA) and multivariate statistical tests (PERMANOVA and PERMDISP) were employed.

This approach revealed distinct separation among lake groups supported to some extent by pairwise comparisons (using Bonferroni corrections). These results helped identify the main axis of ecological variability across systems. Additionally, for the winter dataset, an additional lake was included: Big Trail (BT), a Yedoma thermokarst lake in Alaska, Fairbanks. Despite not being able to include this lake in the previous chapters due to sampling constraints, the available data can complement this statistical approach for winter samples. Thus, all variables were measured using the same methodology as in the sporadic, discontinuous and continuous permafrost zone lakes. Moreover, pH was removed from the winter dataset, since it was not measured during BT sampling.

Summer



Winter

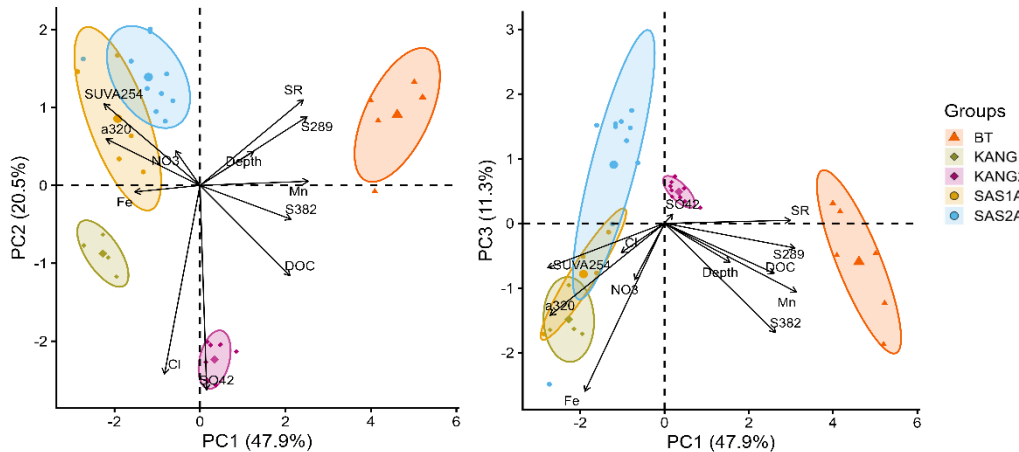


Figure VI.2. Exploratory principal component analysis (PCA) of biogeochemical variables in lake groups during summer and winter, for PC1 vs PC2, and PC1 vs PC3. Ellipses have a 68% confidence interval.

In summer waters, the intra-lake heterogeneity is tendentially higher in SAS1A and SAS2A compared to other lakes. The effects of wind mixing and stratification respectively, profoundly shaped the heterogeneous vertical profiles in both CDOM-rich thermokarst lake systems. Similarly, SAS lakes are primarily separated from ZAC and KANG lakes in multivariate space by CDOM content (a_{320}), DOM aromaticity (SUVA₂₅₄) and DOC concentrations (Fig. VI.2). In turn, increases in variables like pH, sulfate, dissolved Fe, S_{289} and S_{382} drive the separation of ZAC and KANG lakes from the SAS systems. This indicates ZAC and KANG lakes exhibit tendentially smaller DOM structures — reflected by higher slopes S_{289} and S_{382} , especially in ZAC lakes — and higher abundance of dissolved Fe, Mn and sulfate concentrations. In winter waters

however, the statistically significant contrasts among lakes could not be attributed to differences in dispersion arising from intra-lake variability. The BT Lake was clearly separated from all other winter lakes, with higher DOM molecular weight (S_R), S_{289} and S_{382} , along with DOC concentrations. While KANG2 exhibited a slightly intermediate nature, and KANG1 aligned more closely with SAS lakes during winter, with higher CDOM content (a_{320}), DOM aromaticity ($SUVA_{254}$) and dissolved Fe (Fig. VI.2).

In addition to this PCA aimed at overarching biogeochemical characteristics between thermokarst lakes, a different exploratory PCA was conducted between summer and winter datasets, comprised solely of the relative PARAFAC component contributions (Fig. VI.3).

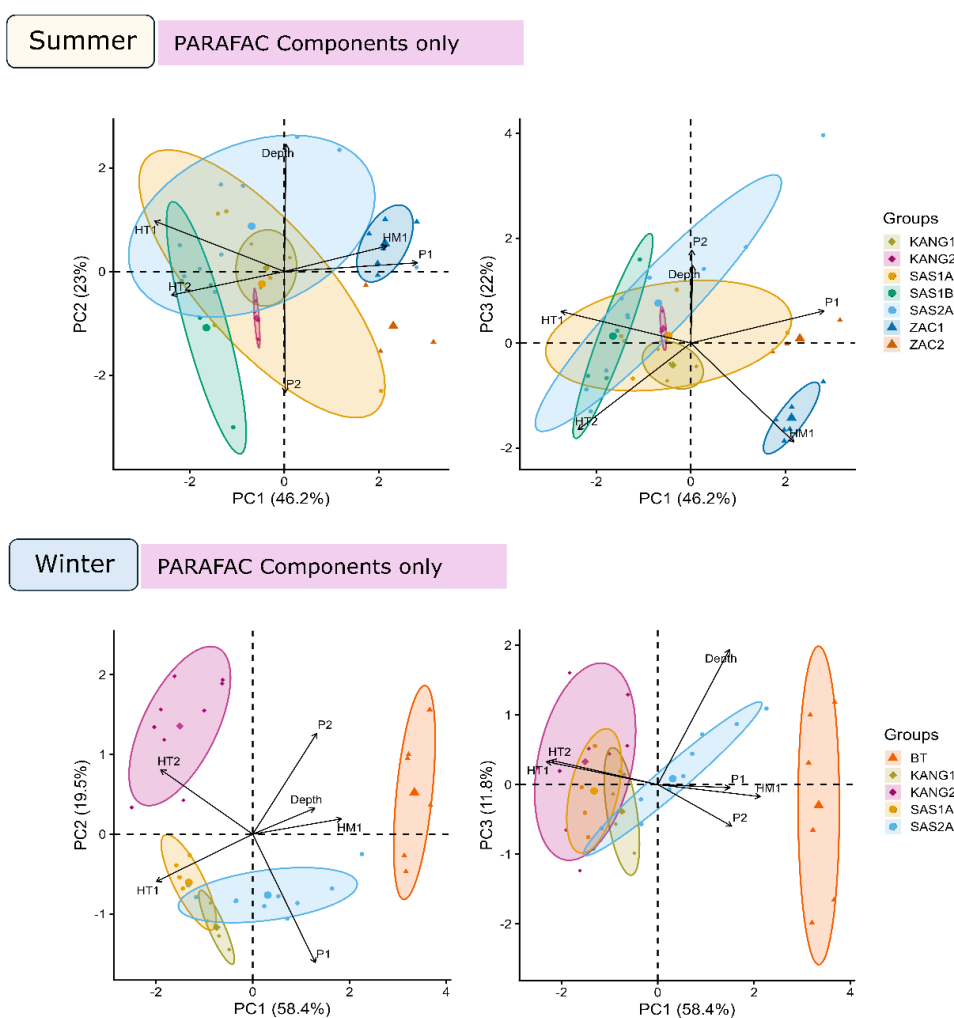


Figure VI.3. Exploratory principal component analysis (PCA) of PARAFAC component distributions across lakes in summer and winter, for PC1 vs PC2, and PC1 vs PC3. Ellipses have a 68% confidence interval.

Components were described as HT (terrestrial humic-like FDOM), HM (microbial humic-like FDOM), P1 (tryptophan-like FDOM) and P2 (tyrosine-like FDOM). In summer, SAS lakes exhibited greater FDOM heterogeneity and elevated presence of terrestrial humic-like components (HT1, HT2), while KANG lakes showed more balanced profiles, reflecting intermediate terrestrial-microbial influence (Fig. VI.3). Lake ZAC1 was distinguished by elevated microbial humic-like (HM1) signals, consistent with in-lake processing. ZAC2 did not have enough datapoints to calculate an ellipse, but the scatterplot was closely situated near ZAC1 in multivariate space. In winter, both SAS and KANG lakes clustered by increased terrestrial humic contributions (HT1, HT2), but with notable variation: KANG2 influenced by higher HT2, and SAS2A elevated in P1, possibly indicating enhanced microbial activity under the ice cover. However, BT was dominated by HM1, P1 and P2 compared to other winter lakes, suggesting differences in lake functioning, potentially aligning with a more active system (Fig. VI.3).

Although a comprehensive study was not conducted in BT lake like in the other lakes, considerable differences in DOM quality and water column biogeochemistry may merit further investigation. The BT Lake showed elevated DOC concentrations ($91 \pm 3 \text{ mg C L}^{-1}$), alongside higher spectral slopes (S_{289} : 0.019; S_{382} : 0.021) and S_R (0.91 ± 0.03), with lower CDOM absorbance (a_{320} : $43 \pm 7 \text{ nm}^{-1}$) compared to all other lakes in winter. In addition to the relative increase in HM1, P1 and P2 compared to other lakes, these optical properties indicate a shift toward lower molecular weight DOM, consistent with accelerated microbial degradation of DOM in the water column into smaller structures (Loiselle et al., 2009; Fasching et al., 2014). This suggests BT is a more biogeochemically active system with enhanced rates of DOM processing relative to other winter lakes and may merit further investigation.

To identify general biogeochemical relationships within thermokarst lake sediments regardless of site, scatterplots were produced for all variable pairings across the combined dataset ($n=47$ sediment samples pooled from all sites and seasons). This exploratory, cross-sample analysis uncovered two main relationships: (i) the humification index (HI) values and the percentage of organic carbon weight (%); (ii) HI values and isotopic $\delta^{13}\text{C}$ signatures. By pooling samples across regions, this approach seeks to detect consistent biogeochemical patterns that may operate universally in thermokarst sediment systems, as opposed to site-specific variability. In literature, HI is commonly employed to assess the humification degree of peat soils using their FTIR spectra, as it related the aromatic

and carboxylate bands ($\sim 1630\text{ cm}^{-1}$) and the polysaccharide band ($\sim 1060\text{ cm}^{-1}$) (Broder et al., 2012; Heffernan et al., 2020; Harris et al., 2023). These studies usually attribute higher HI values to more humified and decomposed peat soils. However, the context in which thermokarst lake sediments are inserted and their biogeochemical processing contrasts considerably from peat soils explored in literature.

The humification index (HI) exhibited a moderate nonlinear reciprocal relationship with the percentage of organic carbon weight (%) ($R^2=0.54$), where organic carbon content plateaus at higher HI ranges, and decreases rapidly with relatively small changes at low HI values (Fig. VI.4). In this context, sediments with lower HI exhibited lower organic carbon content, indicating a more advanced stage of degradation. Contrary to peat dynamics of carbon accumulation and humification in soils where HI was originally developed and employed, the dynamic processes in thermokarst lake sediments likely fundamentally alter the relationship between HI values and the sediment degradation extent.

Moreover, HI also exhibited a moderate reciprocal model fit with $\delta^{13}\text{C}$ signatures, although not quite as strong ($R^2=0.52$) (Fig. VI.4). This suggests that the $\delta^{13}\text{C}$ signature shifts rapidly with low HI values, but it is influenced by additional biogeochemical factors including methane oxidation, likely indicative of the balance between contrasting NOM sources. Despite the limited number of sediment samples, these findings could support a range-dependent nonlinear relationship between NOM degradation extent and the calculated HI based on FTIR spectra in thermokarst lake sediments. Moreover, it is simultaneously possible that a similar relationship links HI values and the $\delta^{13}\text{C}$ signatures in sediments, relating shifts in source contributions.

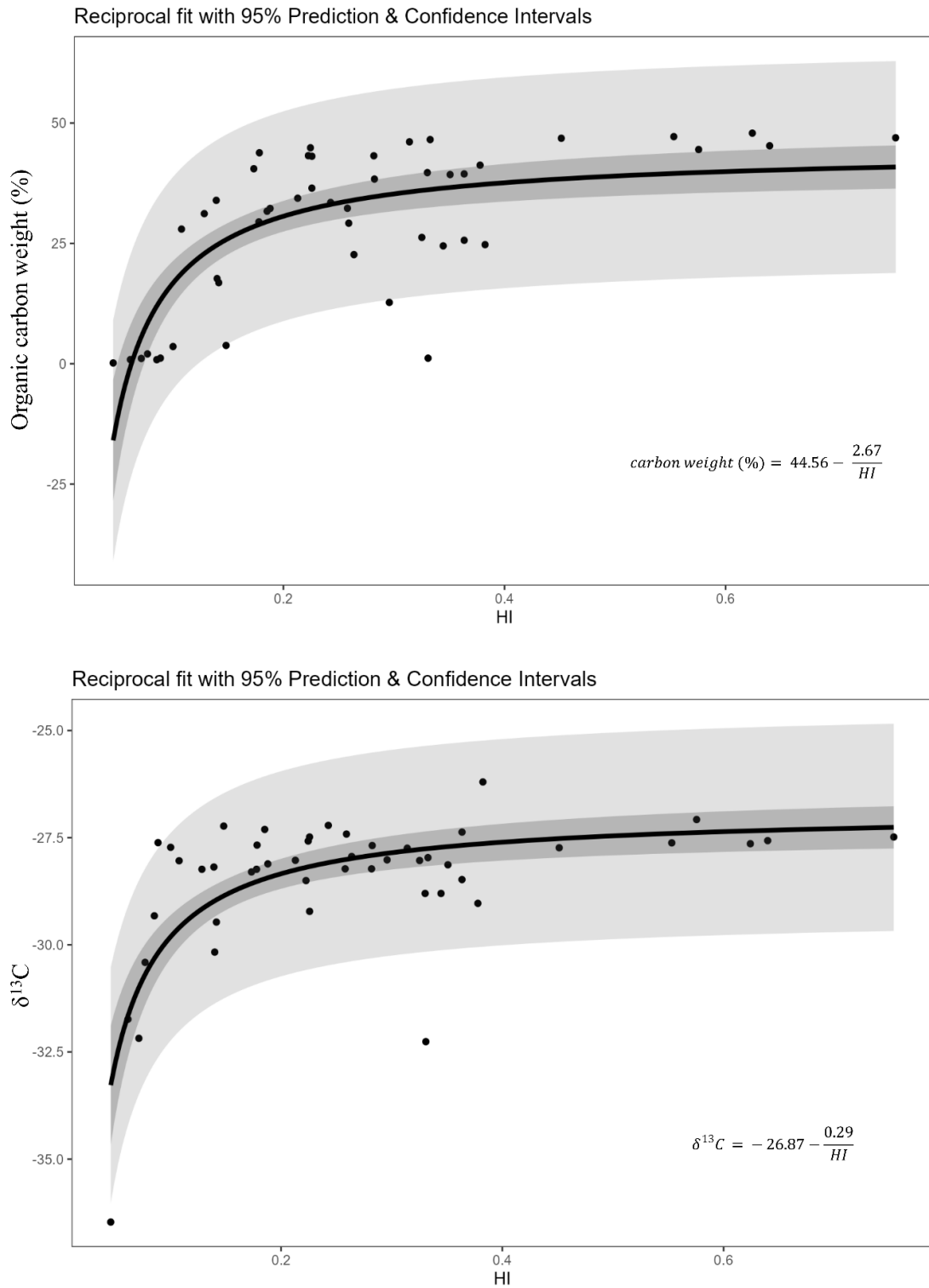


Figure VI.4. Reciprocal model fit and scatterplot of both Carbon weight (%) — HI and δ¹³C — HI. Light gray area defines the predictive interval (95%) and the dark gray area defines the confidence interval (95%).

The gradual depletion of NOM pools of varying lability with increasing degradation extent results in the relative enrichment of recalcitrant NOM structures. This recalcitrant pool likely includes complex aliphatic hydrocarbons (Meyers, 2003) potentially arising

from autochthonous sources (Tornabene, 1981; Zhang et al., 2019), and thus may carry $\delta^{13}\text{C}$ signatures reflecting the carbon fixation mechanism. If autochthonous recalcitrant hydrocarbons contribute significantly to the C-O stretching bands ($\sim 1030\text{ cm}^{-1}$) observed in FTIR spectra, this selective preservation could explain the pronounced $\delta^{13}\text{C}$ shifts at lower HI values. Nevertheless, this interpretation remains speculative without direct measurement of compound-specific $\delta^{13}\text{C}$ values for algal biomarkers.

VI.2. Primary gradient induced mechanistic shifts in lake chemistry

The formation and development of thermokarst lakes across northern permafrost landscapes raises significant concerns regarding their potential for greenhouse gas emissions (GHG), along with nutrient and contaminant release and cycling. Research studies approaching thermokarst lake contrasts along permafrost gradients focus on targeting a larger number of sites (Pokrovsky et al., 2018; Serikova et al., 2019; Manasypov et al., 2020), allowing for a greater sample size to establish correlations along the selected gradients. Serikova et al. (2019) found emissions to be higher during shoulder seasons in colder permafrost-rich landscapes (i.e. discontinuous and continuous permafrost regions). In turn, Pokrovsky et al. (2018) reported the highest dissolved Selenium concentrations in the discontinuous permafrost zone mobilized by recalcitrant DOM, and suggesting two-fold increases in Selenium concentrations in continuous permafrost zones as thaw progresses in the future. Manasypov et al. (2020) observed lakes from continuous permafrost zone exhibited higher Fe, total P and CO_2 , and lower SO_4^{2-} concentrations, while transitional zones showed higher DOC and Mn.

However, this approach limits a more holistic perspective on pelagic-benthic dynamics in thermokarst systems in different permafrost zones. In this sense, an integrated perspective of natural organic matter (NOM) dynamics provides a closer look at biogeochemical differences across Arctic and subarctic regions. The distinct ecosystems in which these lakes form play an important role in shaping the characteristics of thermokarst lakes and determining their dominant ecological processes. It is worth noting that heterogeneity in thermokarst lakes (i.e. limnodiversity) in permafrost landscapes is widespread even across small geographic areas. Contrasts in origin soil types (organic vs mineral), limnodensity hotspots and geomorphology in a permafrost landscape often

dictate considerable differences between lake dynamics within the same region (Freitas et al., 2024).



Figure VI.5. Photographs illustrating the different permafrost landscapes across studied regions. Left: lake SAS2A in Kuujjuarapik-Whapmagoostui; Middle: lake KANG1 in Kangiqsualujuaq; Right: lake ZAC1 in Zackenberg.

The permafrost gradient studied here spans markedly different landscapes (Fig. VI.5), which in turn shapes thermokarst lake formation and biogeochemistry. The sporadic permafrost site is characterized by waterlogged peatlands with strong hydrological connectivity between soils and lakes. The discontinuous permafrost site occupies a transition zone between tundra and boreal forest, while the continuous permafrost site lies in a tundra environment with a thinner active layer, and reduced soil-water connectivity. These landscape contrasts strongly influence the balance between external (i.e. surrounding soils) versus internal (i.e. benthic) controls on lake biogeochemistry.

This work showed that, in the sporadic permafrost site, strong hydrological connectivity promotes high terrestrial DOM inputs, leading to elevated POM, CDOM concentrations and DOM aromaticity. This restricts light penetration and likely confines primary production to surface waters. It should be noted that POM content was not quantified or characterized across lakes, but simply inferred by turbidity and direct observation during sampling. The high organic matter supply fuels microbial activity (mainly heterotrophic) and methane oxidation, resulting in rapid dissolved oxygen (DO) depletion throughout the water column. In SAS lakes, particularly SAS2A, photochemical breakdown of large, recalcitrant DOM produced an increase in smaller aromatic structures and CDOM, as described by Hu et al. (2023). In larger surface lakes (i.e. SAS1A), it is expected enhanced wind-mixing, likely fueling the photochemical mineralization of CDOM (in upper waters) and aromatic DOM to a greater extent.

Dissolved CO₂ is likely to accumulate in these lakes, driving a shift towards lower pH values along with the presence of organic acids arising from peat inputs. This may arise from a convergence of methanotrophic processes and DOM mineralization to CO₂ via biotic degradation. On average, lakes in the sporadic permafrost zone exhibited the lowest pH values, compared with lakes in discontinuous and continuous permafrost regions during the open-water season (Table VI.2).

Across discontinuous and continuous permafrost landscapes, reduced hydrological connectivity limits terrestrial inputs from surrounding soils, with benthic processes having a greater influence in lake dynamics. Here, sediment NOM degradation, redox cycling of TEAs and sediment-water interactions increasingly shape DOM quality and lake chemistry. Thus, the permafrost landscape determines whether the biogeochemistry of thermokarst lakes is dominated by external loadings (terrestrial inputs from waterlogged peat soils) or in-lake processes and sedimentary controls. A schematic diagram of overarching thermokarst lake biogeochemistry along the permafrost gradient is shown in Fig. VI.6.

Both ontogeny-driven and morphometry-driven changes in thermokarst lake dynamics were most notably studied in Kuujjuarapik-Whapmagoostui, the sporadic permafrost landscape in Chapter III. A larger surface area and shallow depth lake system likely receives enhanced wind-mixing, promoting surface photobleaching and nutrient redistribution. In contrast, a deeper lake with smaller surface area becomes more stratified, with specific dynamics in each water column layer. This work suggests emerging lakes will tend to exhibit enhanced sediment microbial activity, while mature thermokarst lakes showed higher CDOM content and DOM aromaticity, as the bacterial communities have shifted towards the consumption of more recalcitrant DOM. Notably, these ontogenic differences were attributed to thermokarst lakes formed in a waterlogged peatland (Kuujjuarapik-Whapmagoostui) and may not translate similarly to other Arctic and subarctic landscapes.

A common seasonal cycle across regions (sporadic and discontinuous zones) was observed for DOM dynamics in thermokarst lakes. In winter anoxia, reducing conditions shift degradation processes and an accumulation of larger condensed DOM structures (noted by decreases in S_R). The average percentual increase of S_R in summer relative to winter reflected this shift in DOM molecular weight, more pronounced in Kangiqsualujjuaq (discontinuous permafrost) lakes. While S_{382} ranged within the -4 to -

6% range in all lakes, S_{289} increased (Table VI.1). Moreover, the CDOM proxy (a_{320}) showed an opposite seasonal trend in SAS2A compared to other sites, where CDOM content increased significantly in winter anoxic conditions (particularly in Kangiqsualujjuaq), whereas SAS2A during summer showed remarkably high CDOM. Paired t-tests revealed the mean seasonal differences in S_R , S_{289} and S_{382} , were statistically significant ($p < 0.05$), and a_{320} was borderline significant ($p = 0.053$).

Table VI.1. Percentual variation between average measurements in summer relative to winter.

	<i>Shifts summer relative to winter</i>			
		S_R	CDOM	S_{289}
<i>Kuujuarapik-Whapmagoostui</i>	SAS1A	19%	-47%	13%
	SAS2A	9%	17%	2%
<i>Kangiqsualujjuaq</i>	KANG1	43%	-90%	44%
	KANG2	32%	-68%	25%

This shift in DOM quality likely results from the processing of released terrestrial labile DOM from lake sediment and humification processes. During summer, photic and oxic conditions promote the photochemical breakdown of larger DOM structures and promote primary production of smaller DOM molecules. Comparatively, the seasonal shift in DOM molecular weight and CDOM content was more accentuated in lakes from the discontinuous permafrost zone, likely due to the decreased terrestrial inputs in the open-water season.

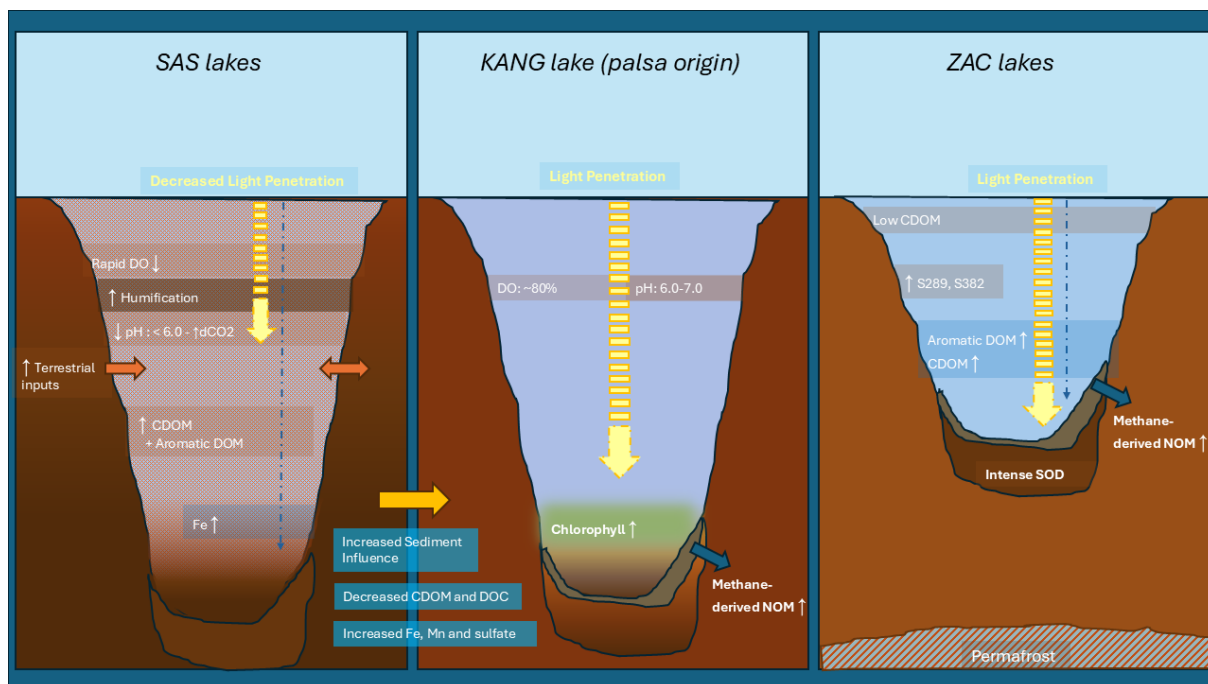


Figure VI.6. General conceptual diagram of observed biogeochemical trends across thermokarst lakes (of organic origin) in sporadic (SAS), discontinuous (KANG) and continuous (ZAC) permafrost zones.

There are, however, certain discrepancies between zones in seasonal pH patterns and TEA dynamics. While studied lakes from the sporadic permafrost zone showed more acidic conditions during winter, lakes from the discontinuous permafrost zone showed higher pH values in winter (circumneutral pH) compared to summer. In this work I hypothesize that in lakes from the waterlogged peatlands (Kuujjuarapik-Whapmagoostui, sporadic permafrost) there is an accumulation of dissolved CO₂ under ice conditions, while in tundra-boreal forest transition zone (discontinuous permafrost) processes like hydrogenotrophic methanogenesis might consume a significant fraction of dissolved CO₂, driving pH values to circumneutral ranges. This could indicate contrasting winter dynamics between lakes from sporadic and discontinuous permafrost zones, possibly reflecting differences in the intensity of microbial activity. It is important to note that these inferences were made solely using pH profiles and lack direct measurements of CO₂ and CH₄ concentrations. Moreover, the presence of *Sphagnum* mosses has been associated with low pH environments, consistent with the characteristics in lakes from Kuujjuarapik-Whapmagoostui, especially in summer (Clymo et al., 1984; Scheffer et al., 2001).

Consistent with the findings of Huang et al. (2020), sulfate concentrations were higher in lakes in tundra and tundra transition environments (Table VI.2). In the waterlogged peatland lakes in Kuujjuarapik-Whapmagoostui, sulfate concentrations were

considerably lower. Moreover, dissolved Fe and Mn concentrations were on average higher in Kangiqsualujjuaq thermokarst lakes compared to lakes from Zackenberg and Kuujjuarapik-Whapmagoostui (Table VI.2).

Table VI.2. Percentual variation between average measurements in summer relative to winter.

		pH	Fe (mg L ⁻¹)	Mn (µg L ⁻¹)	SO ₄ ²⁻ (mg L ⁻¹)
<i>Kuujjuarapik-Whapmagoostui</i>	SAS1B	5.8 ±0.1	2 ±1	26 ±2	0.49 ±0.02
	SAS1A	6.0 ±0.2	3 ±4	34 ±34	0.18 ±0.04
	SAS2A	5.4 ±0.1	2 ±2	12 ±7	0.12 ±0.02
<i>Kangiqsualujjuaq</i>	KANG1	6.1 ±0.1	6 ±1	5450 ±58	9.2 ±0.7
	KANG2	6.9 ±0.1	18 ±2	6200 ±216	17.5 ±0.6
<i>Zackenberg valley</i>	ZAC1	6.4 ±0.6	3 ±1	9 ±5	13.7 ±0.3
	ZAC2	6.8 ±0.1	12 ±4	15 ±14	10.5 ±1.8

In studied thermokarst lakes, DO consumption was consistently higher in the water column of SAS lakes, with the hypolimnion seemingly remaining anoxic year-round, being heavily shaped by TEAs such as nitrate, sulfate, dissolved Fe and Mn. This high DO demand is consistent with the dominance of bacterial heterotrophy in SAS lakes (Creed et al., 2018), contrasting with KANG and ZAC lakes.

These CDOM-rich thermokarst lakes exhibit sharp vertical redox gradients during summer, as stratification keeps oxygenated and anoxic layers strongly separated, with reducing conditions in the hypolimnion reflecting TEA utilization in benthic processes. On the opposite endmember of our gradient in Zackenberg, Greenland, recently formed thermokarst lakes in continuous permafrost tundra landscapes revealed extraordinarily high rates of sediment oxygen demand (SOD). The decreased thickness of the active layer and the thawing of continuous permafrost, along with low CDOM content in the water column, drives the enhanced benthic microbial activity in these systems.

VI.3. Carbon fixation mechanism in thermokarst lakes

Across thermokarst lakes in three permafrost areas, sediment characterization with FTIR spectroscopy, C/N ratios and $\delta^{13}\text{C}$ isotopic signatures, as well as water column properties revealed existing biogeochemical patterns. Naturally, benthic dynamics are intimately linked with overlying water column dynamics.

Creed et al. (2018) proposed in clear, nutrient-poor lakes, modest allochthonous DOM inputs initially boost primary production, but rising concentrations (i.e. browning) reduce light penetration, limiting pelagic phytoplankton and benthic autotrophs, fueling a shift toward heterotrophic bacterial production. In Kangiqsualujjuaq (discontinuous permafrost) and Zackenberg (continuous permafrost), lakes exhibited relatively lower CDOM and turbidity, allowing light to penetrate deeper in the water column. In contrast, SAS lakes in Kuujjuarapik-Whapmagoostui (sporadic permafrost) were consistently CDOM-rich and highly turbid, with limited light penetration. Moreover, benthic activity promotes NOM degradation resulting in CO_2 and methane (CH_4) production. The availability of methane drives the emergence of methanotrophic communities, fueling its conversion to CO_2 . This CO_2 is partially utilized as substrate by primary producers, entering the food web and carrying the ^{13}C -depleted isotopic signature (from methane). The enhanced light availability in low CDOM environments promote autotrophic processes in the upper sediment layer, enabling more phytoplankton growth at depth. The utilization of $\delta^{13}\text{C}$ signatures in lake sediments sheds light on a potential carbon sink mechanism occurring in thermokarst lakes: methane production $\rightarrow$ methane oxidation to CO_2 $\rightarrow$ phytoplankton/autotroph CO_2 fixation $\rightarrow$ carbon sink.

In thermokarst lakes where particulate matter and CDOM are limited and light penetration is enhanced, it is expected that phytoplankton can develop in the upper sediment and deeper layers of the water column, using dissolved CO_2 as substrate for growth. This would leave an isotopic imprint in sediment NOM and likely contribute to higher pH values in the lake. This was observed in the palsa-derived thermokarst lake in Kangiqsualujjuaq (lake KANG2 in Chapter IV) and Zackenberg (lake ZAC1 in Chapter V), where surface sediments are more degraded — lower HI, lower C/N and more negative $\delta^{13}\text{C}$ signatures — while deeper sediments are more recalcitrant, terrestrial NOM. This illustrates a degradation pattern in thermokarst lake sediments. The enhanced

mineralization of NOM pools in surface sediments decreases the organic carbon content, selectively retaining autotrophic recalcitrant NOM from carbon fixation mechanisms.

However, in thermokarst lakes with elevated CDOM and high turbidity like in the SAS lakes in Kuujjuarapik-Whapmagoostui (Chapter III), it is unlikely that phytoplankton can thrive in deeper waters. Considering the limited light penetration, it is expected phytoplankton communities would only be able to develop in the upper layers of the water column. Comparatively, this would promote an accumulation of dissolved CO₂ and a consequent shift to lower pH values in the aquatic system. The isotopic patterns observed — lighter $\delta^{13}\text{C}$ signatures in surface sediments — in the studied discontinuous and continuous permafrost zone thermokarst lake sediments (specifically KANG2 and ZAC1) were not clearly discernible in SAS lakes.

In both KANG2 and ZAC1, enhanced light penetration to the bottom drives autotrophic processes and produces a shift to lighter $\delta^{13}\text{C}$ signatures in more degraded surface sediment layers. In ZAC2 this pattern is not yet discernible given the recency of lake formation.

In contrast, SAS lakes with elevated CDOM and POM restrict primary production to surface waters. The resulting ^{13}C -depleted biomass does not accumulate in sediments forming the pattern observed in KANG2 and ZAC1. However, Gagnon et al. (2024) found POM in SAS1A and SAS2A exhibited more ^{13}C -depleted signatures compared to the colloidal fraction. We hypothesize autotrophic carbon fixation in these systems caused a selective enrichment in the particulate fraction. This enrichment could arise from autotrophic fixation at surface producing ^{13}C -depleted biomass, in turn incorporated by bacterial communities being propagated along the food web. Moreover, primary production byproducts like extracellular polymeric substances (EPS) are prone to photoflocculation (Shammi et al., 2017), which could carry the isotopic signature and incorporate it into POM. Thus, across systems, light regimes determine whether the carbon fixation mechanism takes place in sediments or the water column.

These isotopic patterns match those postulated by Wauthy et al. (2018), who observed lighter $\delta^{13}\text{C}$ signatures linked with methane cycling in zooplankton from thermokarst lakes. However, our ability to properly compare carbon fixation between the three systems is constrained by data gaps. Specifically, chlorophyll measurements are lacking for lakes in sporadic and continuous permafrost zones, as well as $\delta^{13}\text{C}$ signatures of POM

from the different lakes. Nevertheless, further investigating this mechanism and whether there is shift of CO₂ fixation pathway towards POM in CDOM-rich, turbid thermokarst lakes relative to fixation mechanisms in the upper sediment of more transparent, low CDOM lakes merits consideration for future research on carbon cycling.

VI.4. Relevance of the present work in thermokarst lake chemistry

Thermokarst lakes are critical features in permafrost landscapes, yet their heterogeneity across landscapes and within-site limits data transferability across systems. Therefore, this research work focused on the identification of biogeochemical mechanisms driving lake functioning and variation across permafrost zones. This mechanistic understanding is important for establishing models of lake dynamics as permafrost thaws and landscape features evolve, while also reframing empirically observed trends through this mechanistic perspective.

Landscape determines which factors primarily control lake biogeochemistry: waterlogged peatlands (SAS lakes) with elevated hydrological connectivity exhibit enhanced terrestrial inputs, serving as external drivers of lake dynamics, while tundra and tundra-boreal forest transition zones (KANG and ZAC lakes) experience reduced connectivity, allowing for internal controls to dominate.

Consistent with Vonk et al. (2015), we attribute thermal stratification in SAS lakes relative to KANG and ZAC lakes, to CDOM (and POM) surface warming — via solar radiation absorption as the driving mechanism. This profoundly shapes light availability along the water column across different lakes, since light attenuation is much stronger in CDOM and POM-rich systems. Light availability in thermokarst lakes controls where autotrophic communities manage to develop in the aquatic system, in turn shaping whether carbon fixation mechanisms occur in the water column or lake sediments.

Although photochemical processing occurs in open waters in thermokarst lakes, the outcomes depend on substrate quality inputs. In the waterlogged peatland of Kuujjuarapik-Whapmagoostui (SAS lakes), high terrestrial DOM inputs support the photoproduction of aromatic DOM from the breakdown of larger recalcitrant moieties, particularly in SAS2A. Conversely, reduced hydrological connectivity in

Kangiqsualujjuaq limits recalcitrant DOM supply, favoring a different degradative pathway outcome: photochemical processing reduces DOM aromaticity and CDOM content in summer relative to winter.

The effects of landscape and photochemical controls shift lakes toward heterotrophy (in SAS lakes) and towards more algal contributions (in KANG and ZAC), also illustrated by DO profiles (Creed et al., 2018). However, the notable DO decline in ZAC lake profiles arises from extremely high rates of SOD (ZAC1: $12.4 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$; ZAC2: $36.0 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$), reflecting intense benthic activity in the rapidly changing continuous permafrost landscape of the Zackenberg valley.

Morphometry played an important role in DOM dynamics in thermokarst lakes, with larger surface areas likely enhancing wind-mixing and fueling photobleaching at the surface of SAS lakes. Contrastingly, in mature, stratified lakes (with lower surface area) there is an accumulation of CDOM and aromatic DOM in the metalimnion, likely photoproducts from the breakdown of larger recalcitrant structures. In both SAS1A and SAS2A lakes, the lower epilimnion exhibited spectroscopic signatures consistent with photochemical production of aromatic DOM and CDOM: declining spectral slopes (S_{289} and S_{382}), elevated SUVA_{254} and CDOM content (a_{320}). This pattern was unique to these two SAS lakes and aligns with the proposed mechanism by Hu et al. (2023), limited to systems receiving recalcitrant terrestrial peat inputs with high hydrological connectivity.

This illustrates contrasting DOM dynamics at lake surface in SAS relative to KANG and ZAC lakes. The extent to which these differences shape other aspects of lake functioning remains unclear, since processes like the photochemical formation of reactive oxygen species (ROS) is expected to occur and influence CO_2 production and bacterial activity (Scully et al., 2003), and could potentially affect methane oxidation processes and formation of carboxylic acids.

The stark gradient of dissolved Fe concentrations in SAS lakes, in contrast to KANG and ZAC lakes, likely suggests photochemical flocculation as a dominant dissolved Fe removal mechanism. Sunlight-driven interactions between terrestrial DOM and Fe have been known to induce flocculation (Helms et al., 2013; Shammi et al., 2017), potentially enhanced by the presence of EPS released by primary producers. This mechanistic perspective may provide an explanation for previously reported lower Fe concentrations

in thermokarst lakes from sporadic permafrost regions relative to discontinuous and continuous permafrost zones (Manasypov et al., 2020).

Seasonal responses in thermokarst lakes exhibited system-specific dynamics, namely winter pH profiles inverted among lake groups: SAS lakes acidified under ice due to CO₂ accumulation, while KANG lakes shifted toward circumneutral pH, potentially due to hydrogenotrophic methanogenesis consuming available CO₂. This divergence reflects fundamentally different winter degradation pathways among these two lake groups.

Although limited in number, the thermokarst lakes examined here span three distinct permafrost zones and landscape types, enabling the identification of mechanisms and primary environmental drivers controlling lake functioning. By integrating water column chemistry and sediment characterization, this work establishes a mechanistic framework for thermokarst lake dynamics across permafrost landscapes and within the same landscape.

VI.5. Future research directions

Future research on thermokarst lakes should address both small-scale biogeochemical mechanisms within individual systems and broader patterns that determine their regional and global significance. Bridging the two perspectives towards advancing scientific knowledge in these research areas will not only clarify specific biogeochemical mechanisms in thermokarst lakes but also improve models to extrapolate the environmental implications for northern ecosystems and global carbon dynamics. In this context, key directions for future work should include:

- **Influence of ROS on methane oxidation processes and carboxylic acid formation in CDOM-rich lakes**

Investigate how photochemical reactive oxygen species (ROS) formation in CDOM-rich thermokarst lakes enhances methane oxidation in surface waters. Theoretically, increased availability of ROS could limit oxygen demand by heterotrophic communities (van Erk et al., 2023), potentially enhancing methane oxidation in surface waters. Understanding this mechanism and its relative influence to GHG dynamics in thermokarst lakes is key to improving models of methane emissions from permafrost thaw systems. Moreover, ROS interaction with aromatic DOM can enhance oxidative dearomatization, leading to

formation carboxylic acids (Li et al., 2024), potentially serving as substrate to bacterioplankton (Bertilsson and Tranvik, 1998).

- **Carbon fixation pathways and isotopic signatures**

Conduct targeted studies on the assimilation of greenhouse gases (GHG) into particulate organic matter (POM) and sedimentary NOM, using $\delta^{13}\text{C}$ signatures to trace the role of primary producers in carbon fixation within thermokarst lakes. Further investigation into these mechanisms and quantifying their influence in carbon cycling will be essential for future biogeochemical models. Including a ^{14}C datation perspective could further elucidate the carbon pool being utilized in these pathways.

- **Relative contributions of CO_2 and CH_4 across permafrost gradients**

Quantify $\text{CO}_2:\text{CH}_4$ ratios in lakes spanning sporadic, discontinuous, and continuous permafrost zones to assess how permafrost and landscape context shapes GHG balance and potential feedbacks.

- **Sediment incubation experiments**

Controlled incubation studies on GHG production in thermokarst lake sediments have increased in recent years, suggesting key differences in sediment activity between non-Yedoma and Yedoma thermokarst lakes. Further work probing the interplay of sediment NOM quality, GHG production rates and depth profiles — performing incubations on extensively-studied thermokarst lake systems — could provide valuable insight into benthic GHG cycling and its primary controls. The advantages of selecting previously studied systems lie in already having multiple perspectives on lake functioning, helping to better contextualize the research being conducted.

- **Process-based lake models**

Develop mechanistic models that integrate sediment GHG production, water column processing (namely methane oxidation), and surface–atmosphere exchange to evaluate the net GHG balance of thermokarst systems.

- **Scaling to regional and global contributions**

Couple *in situ* GHG emission rates with permafrost distribution data to improve year-round estimates of thermokarst lake contributions to global GHG budgets — including the shoulder seasons (spring and autumn), recognized as periods of critical change in

environmental conditions and GHG fluxes. Accounting for transitional periods is essential for accurately quantifying sink-source dynamics and informing climate models.

Together, these future research directions aim to unravel the complex biogeochemical processes shaping NOM dynamics and GHG potential of thermokarst lakes in Arctic and subarctic regions. As temperatures rise across northern landscapes, understanding how permafrost disturbances drive the transition from carbon sinks to sources — at the individual lake, regional, and global scales — is becoming increasingly urgent. Timely insights from this research will be critical to improving projections of permafrost carbon feedbacks and provide a critical foundation to inform global policy making, climate mitigation, and adaptation strategies.

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VII. Supplementary Information

VII.I. Seasonal organic matter dynamics in thermokarst lakes of a degrading palsa-rich sporadic permafrost zone (Nunavik, Canada)

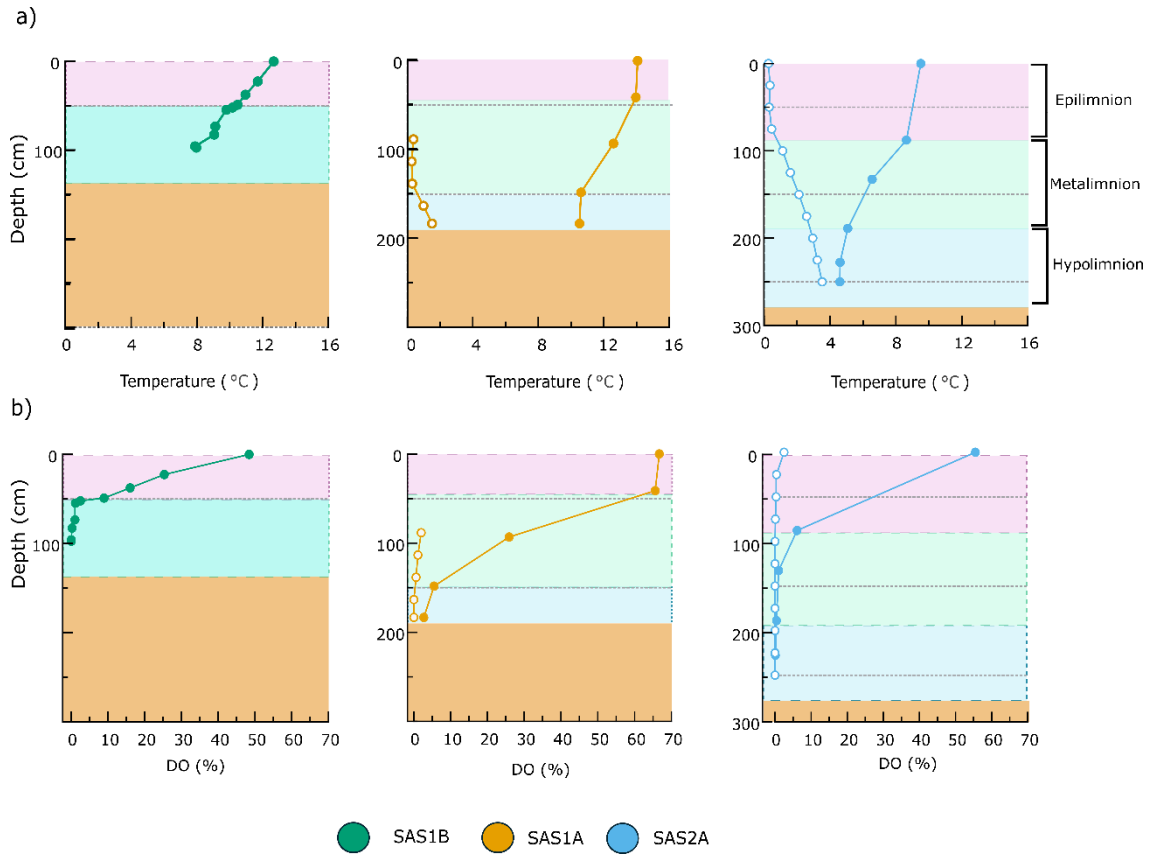


Figure VII.I.1. Vertical profiles of temperature and dissolved oxygen (DO) for summer (solid circles) and winter (open circles) in all three SAS lakes (SAS1B was frozen to the bottom in winter).

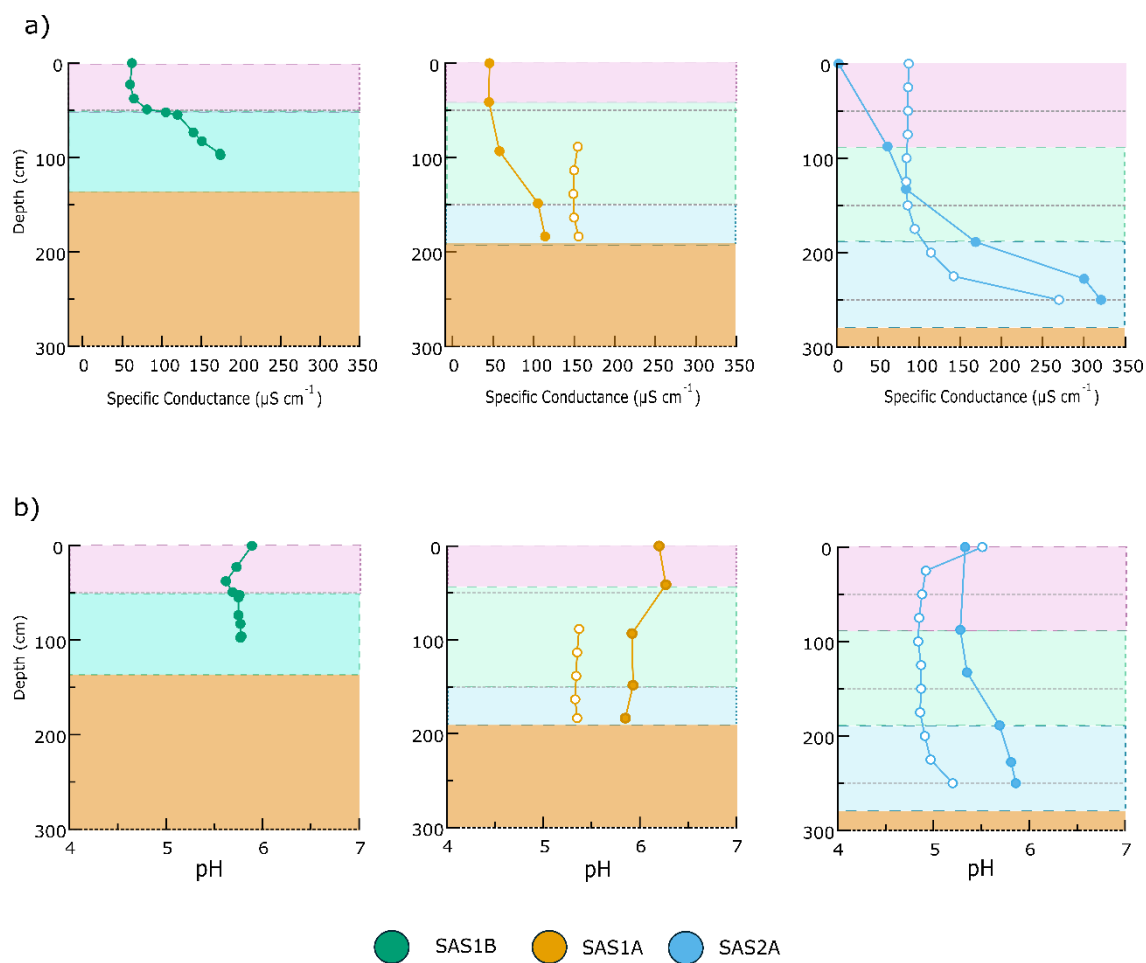


Figure VII.I.2. Vertical profiles of specific conductance (SPC) and pH for summer (solid circles) and winter (open circles) in all three SAS lakes.

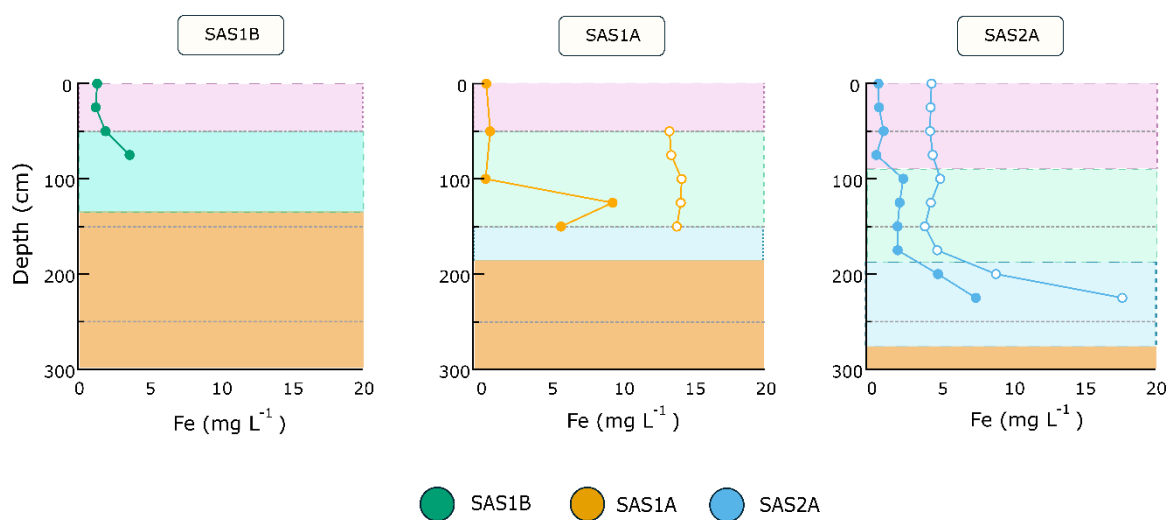


Figure VII.I.3. Dissolved iron (Fe) concentrations (mg L^{-1}) during summer (solid circles) and winter (open circles), in SAS1B, SAS1A and SAS2A.

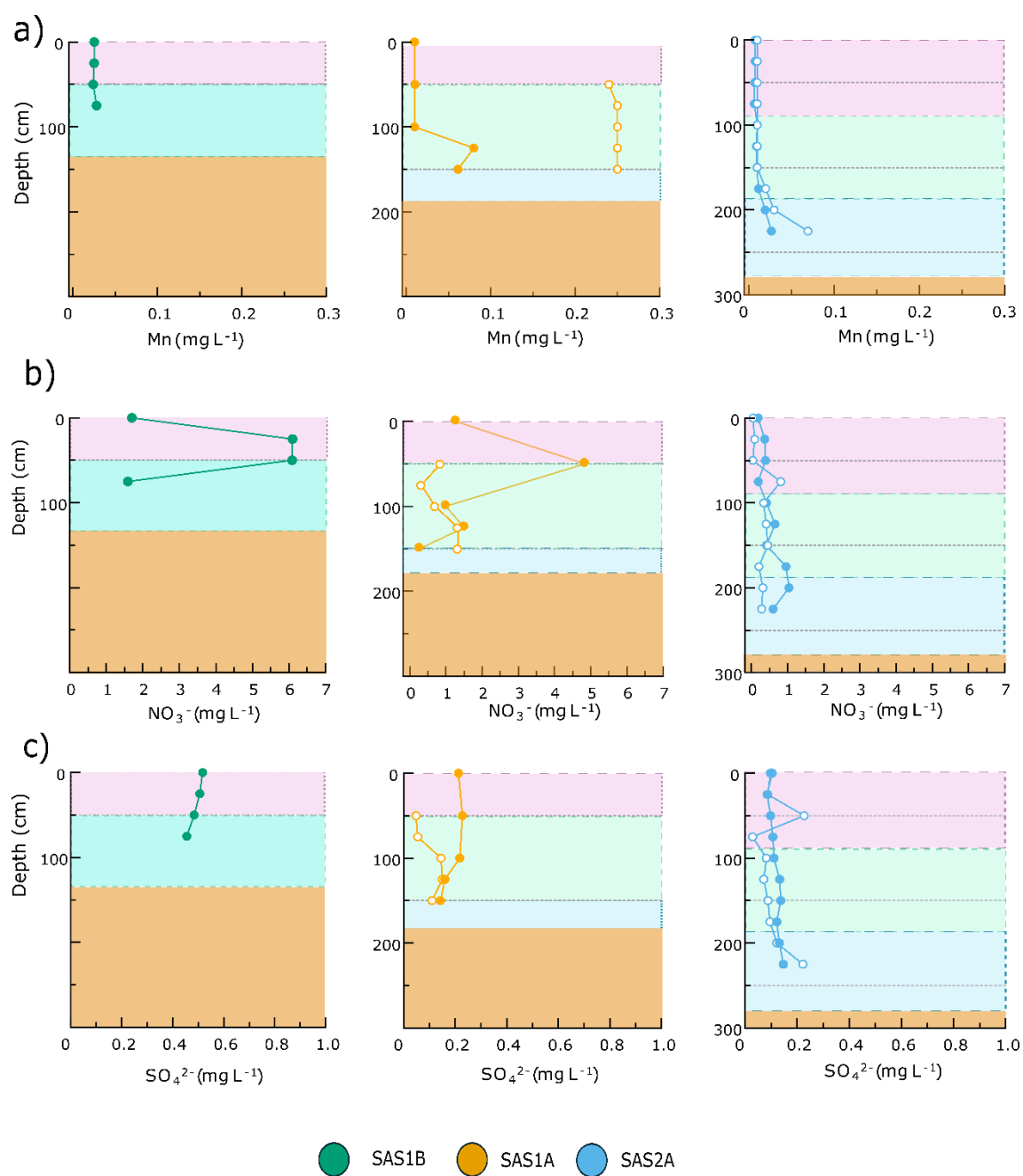


Figure VII.I.4. Summer (closed circle) and winter (open circle) water column profiles of SAS ponds: a) Mn concentrations; b) nitrate (NO_3^-); c) sulfate (SO_4^{2-}).

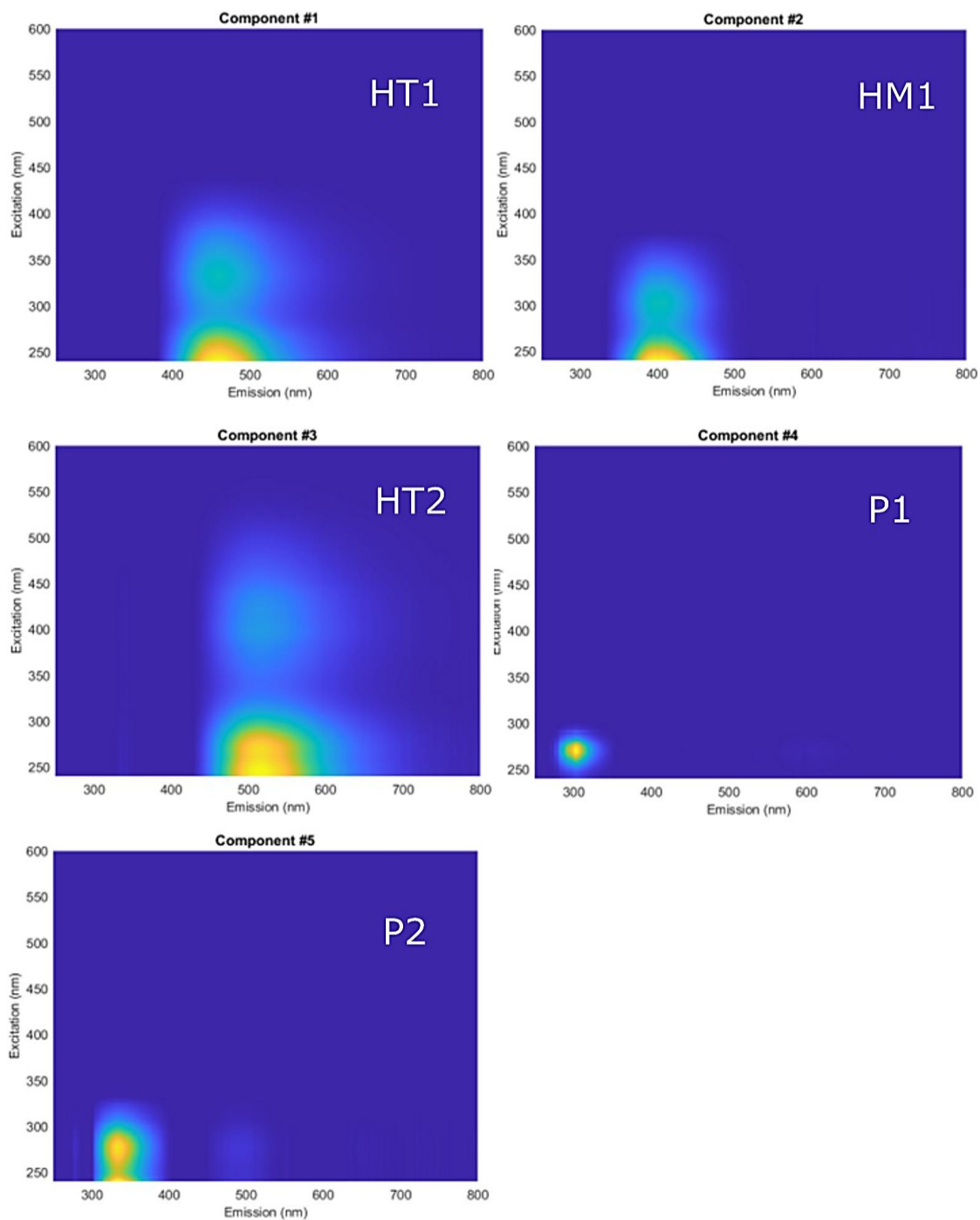


Figure VII.I.5. Excitation Emission Matrices (EEMs) for each of the five fluorescence components identified using PARAFAC analysis.

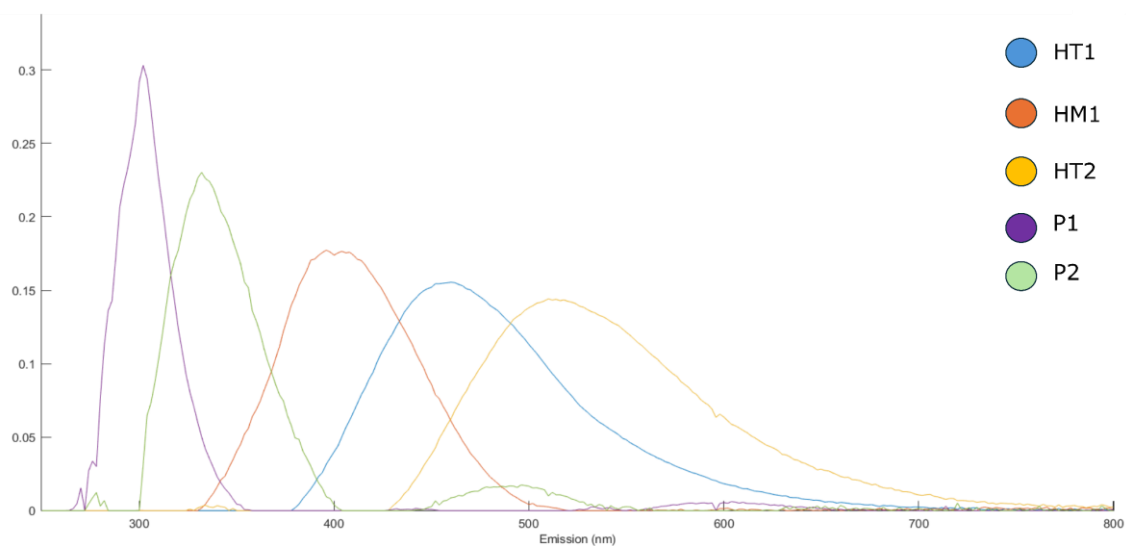


Figure VII.I.6. PARAFAC model component loadings – Emission wavelengths of the five PARAFAC components.

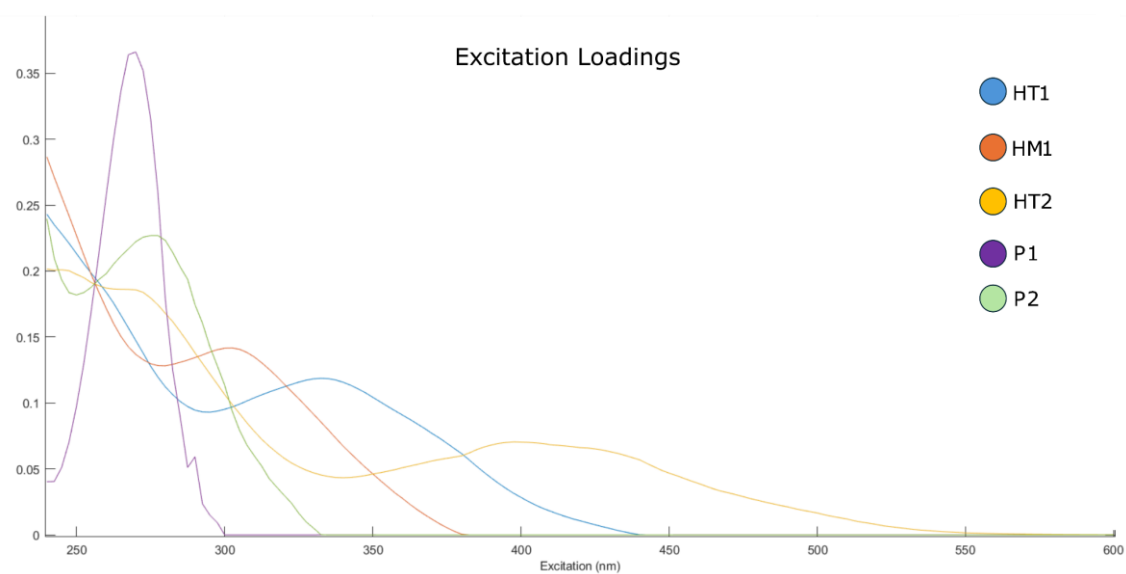


Figure VII.I.7. PARAFAC Model Component loadings – Excitation wavelength of the five components.

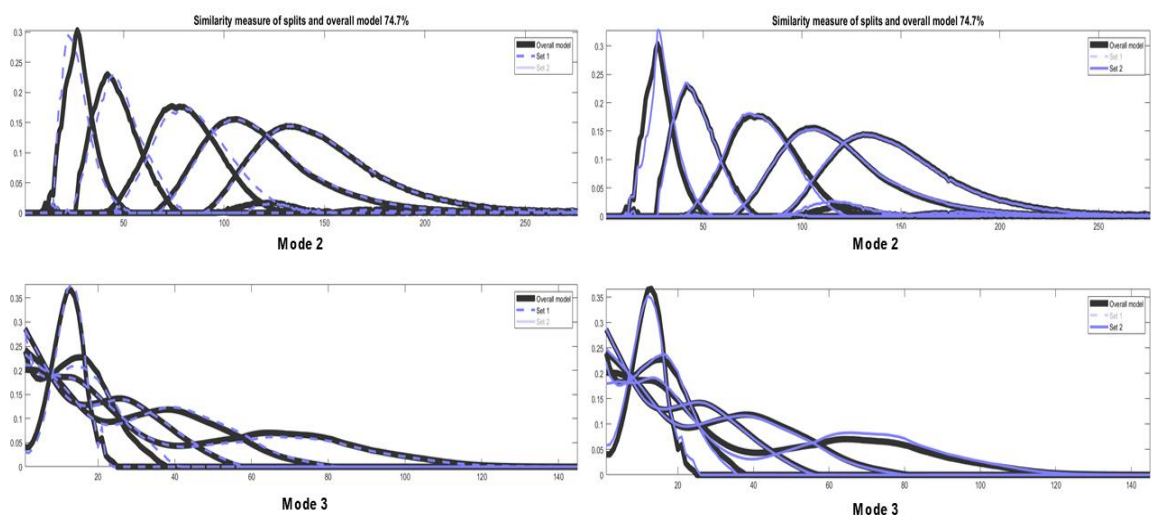


Figure VII.I.8. Split-half validation of the PARAFAC model (74.7%) generated using SOLO Eigenvector.

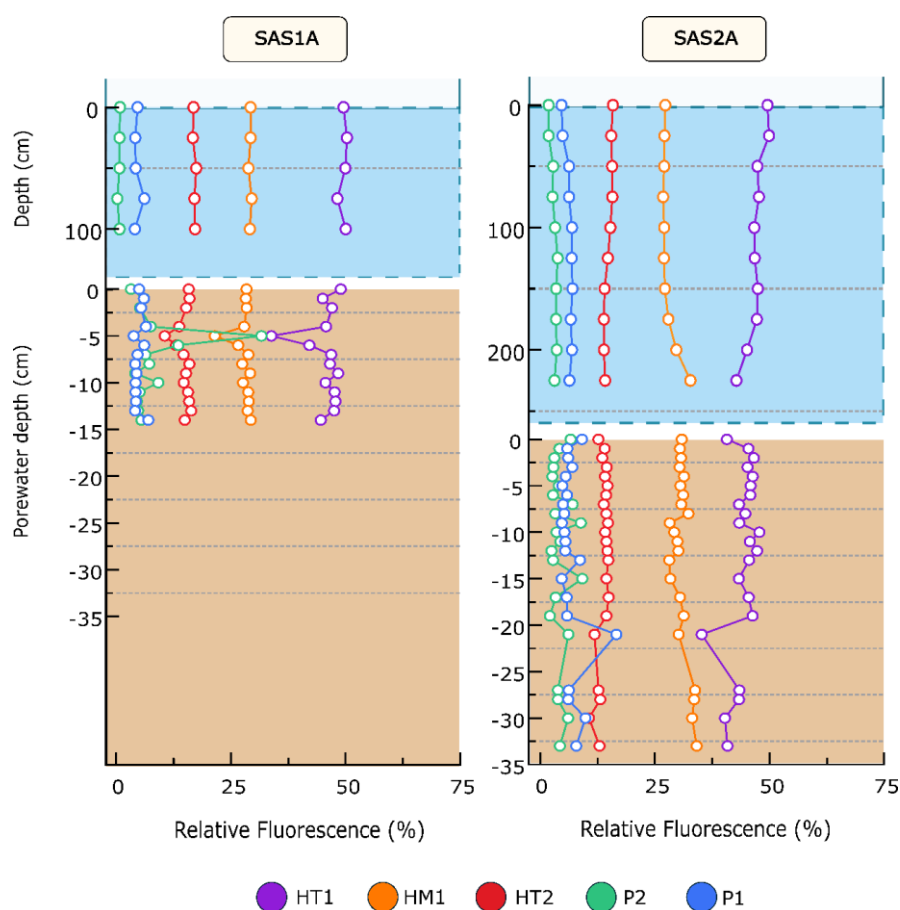


Figure VII.I.9. Variation in PARAFAC components as a function of depth (in cm) in the water column (blue background, water column depth from the surface) and sediment porewater (orange background, negative depths from the water-sediment interface) during winter in SAS1A and SAS2A.

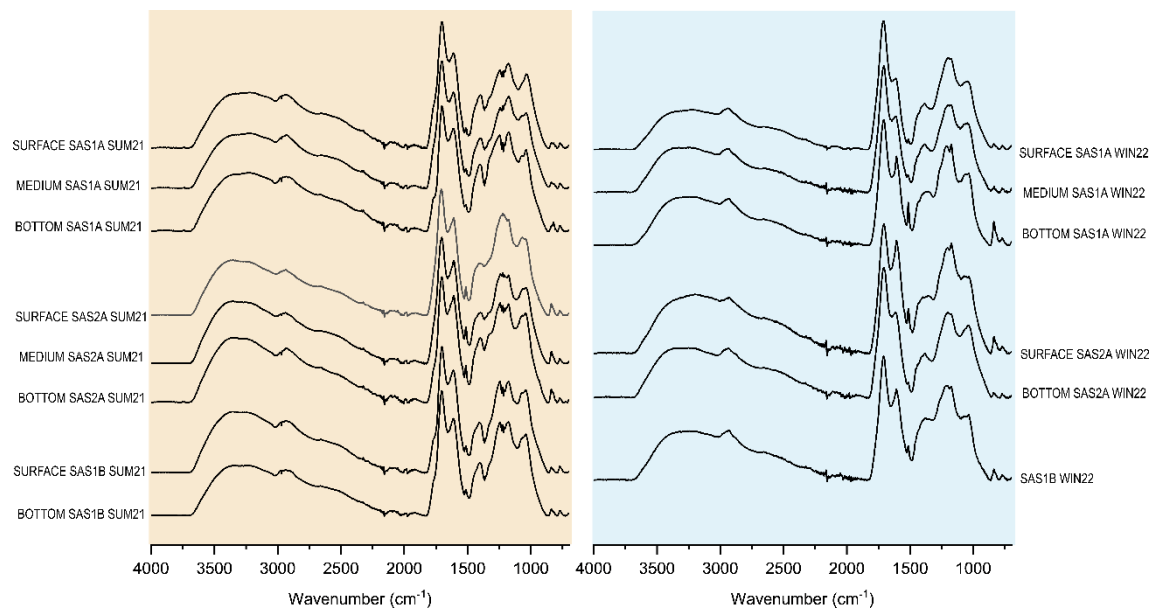


Figure VII.I.10. FTIR spectra of both summer (left) and winter (right) PPL-DOM from SAS ponds.

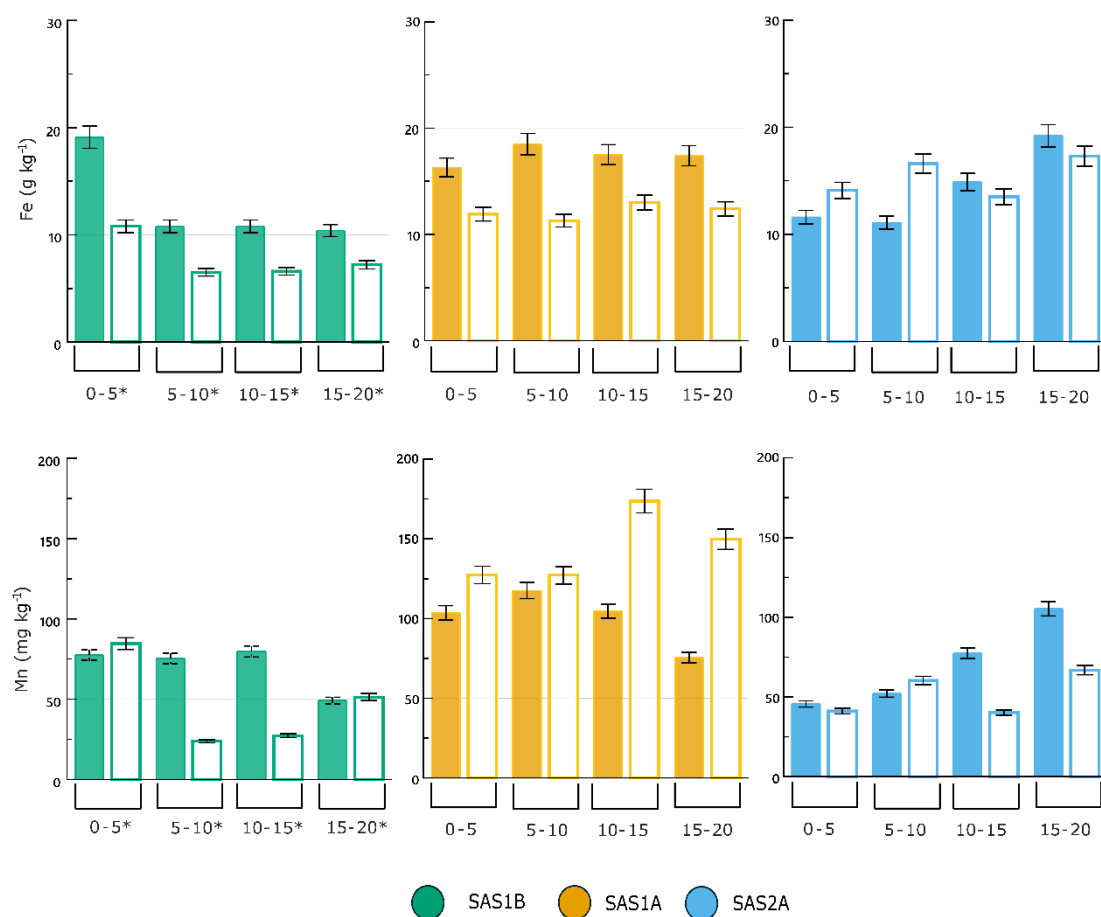


Figure VII.I.11. Total content of SAS sediment sections (0-5 cm; 5-10 cm; 10-15 cm; 15-20 cm) for: a) iron (Fe); b) manganese (Mn) during winter (light shade color) and summer (dark shade color). Green:

SAS1B; Yellow: SAS1A; Blue: SAS2A. *winter samples correspond to 0-3; 3-6; 6-9; 9-12 cm in SAS1B sediments.

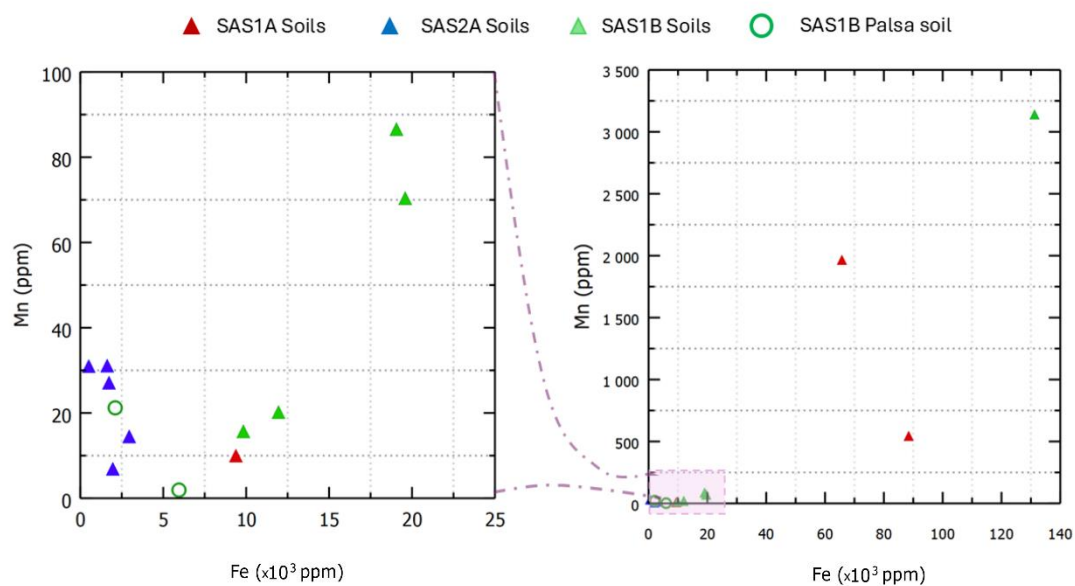


Figure VII.I.12. Total content of SAS soils for iron (Fe) and manganese (Mn) in ppm.

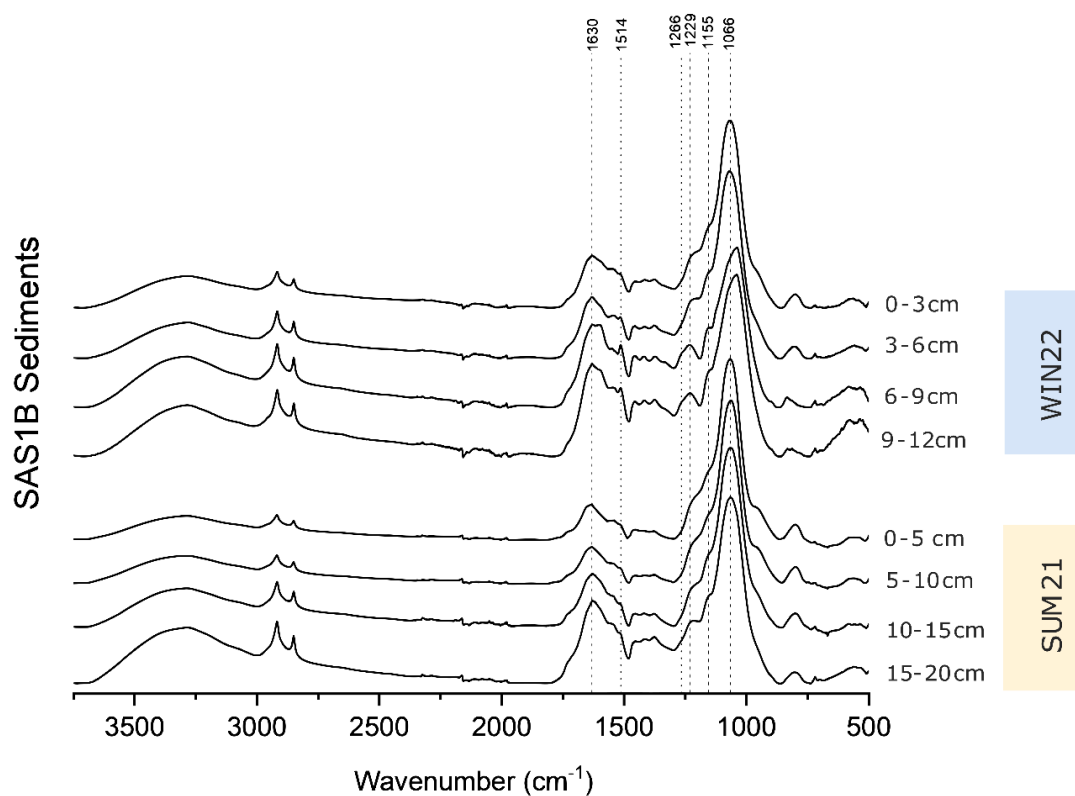


Figure VII.I.13. FTIR spectra of sediment samples at 0-3, 3-6, 6-9 and 9-12 cm depth during winter (WIN22) and at 0-5, 5-10, 10-15 and 15-20 cm depth during summer (SUM21) for SAS1B.

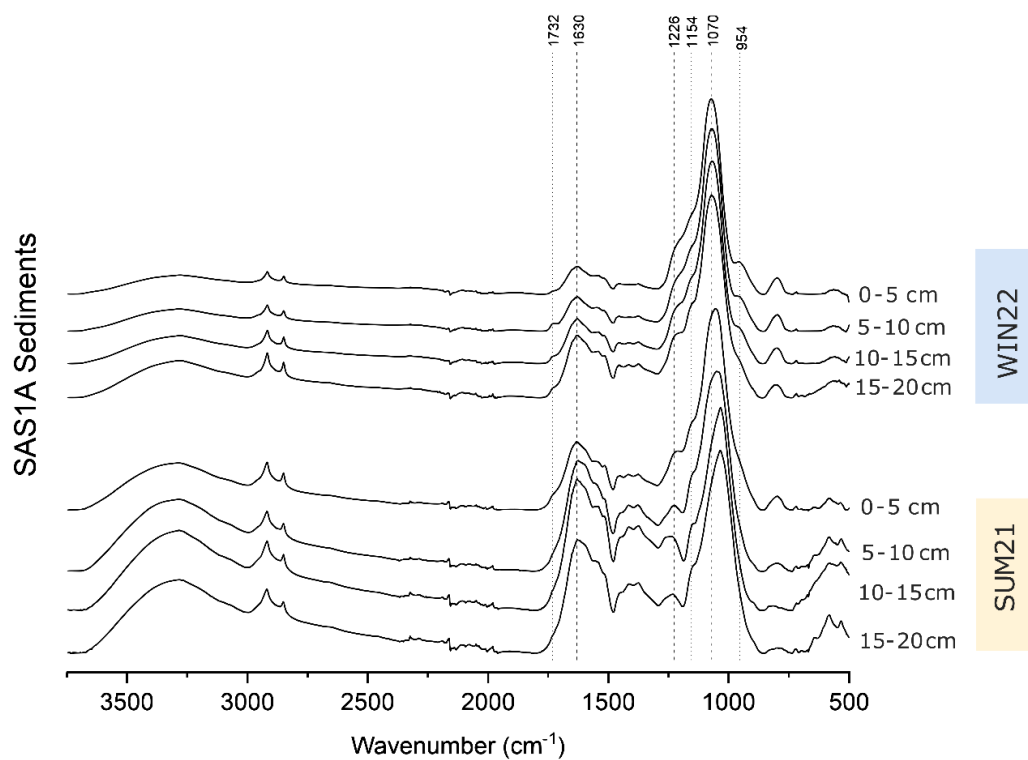


Figure VII.I.14. FTIR spectra of sediment samples at 0-5, 5-10, 10-15 and 15-20 cm depth during winter (WIN22) and summer (SUM21) for SAS1A.

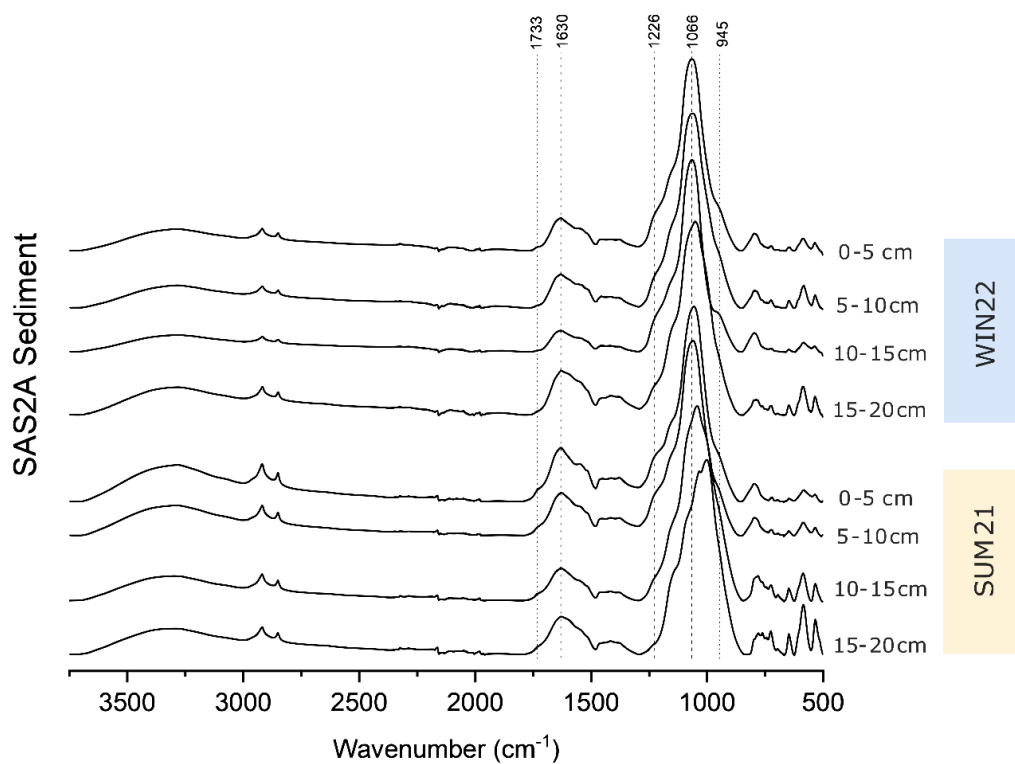


Figure VII.I.15. FTIR spectra of sediment samples at 0-5, 5-10, 10-15 and 15-20 cm depth during winter (WIN22) and summer (SUM21) for SAS2A.

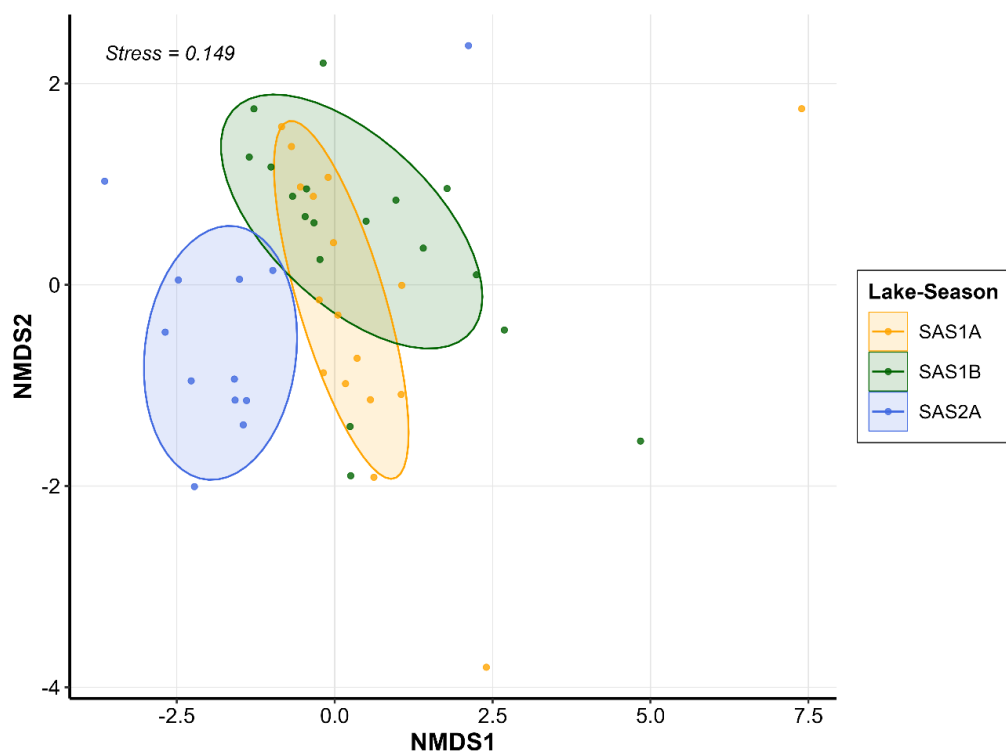


Figure VII.I.16. Non-metric Multidimensional Scaling (NMDS) ordination of SAS sediment porewaters during summer (two-dimensional stress = 0.149). Ellipses used a 68% threshold for confidence interval.

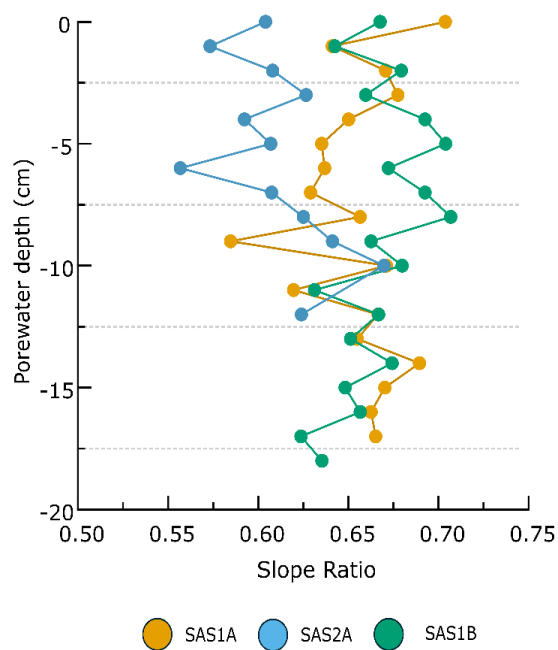


Figure VII.I.17. Slope ratio of CDOM in summer sediment porewater profiles of SAS1A (yellow), SAS1B (green) and SAS2A (blue).

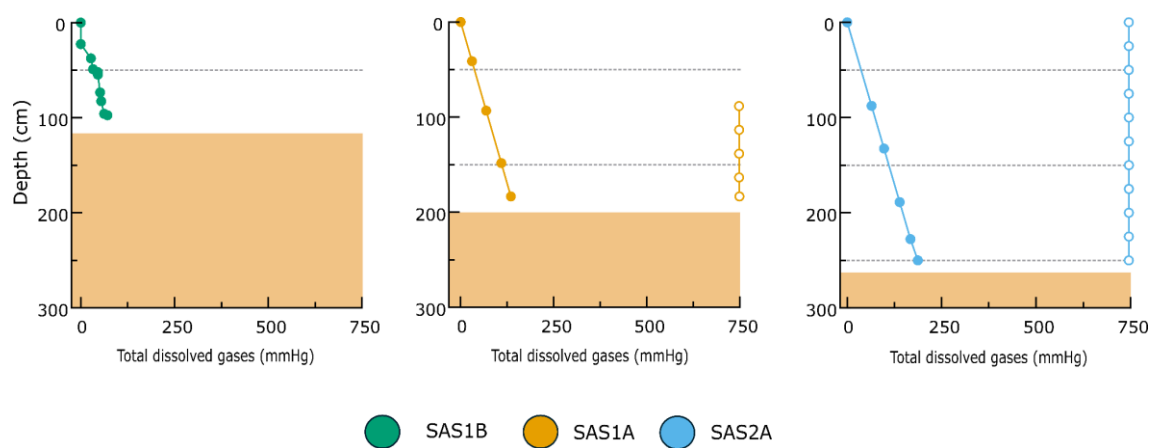


Figure VII.I.18. Vertical profile of total dissolved gases (mmHg) in SAS ponds during summer (solid circles) and winter (open circles).

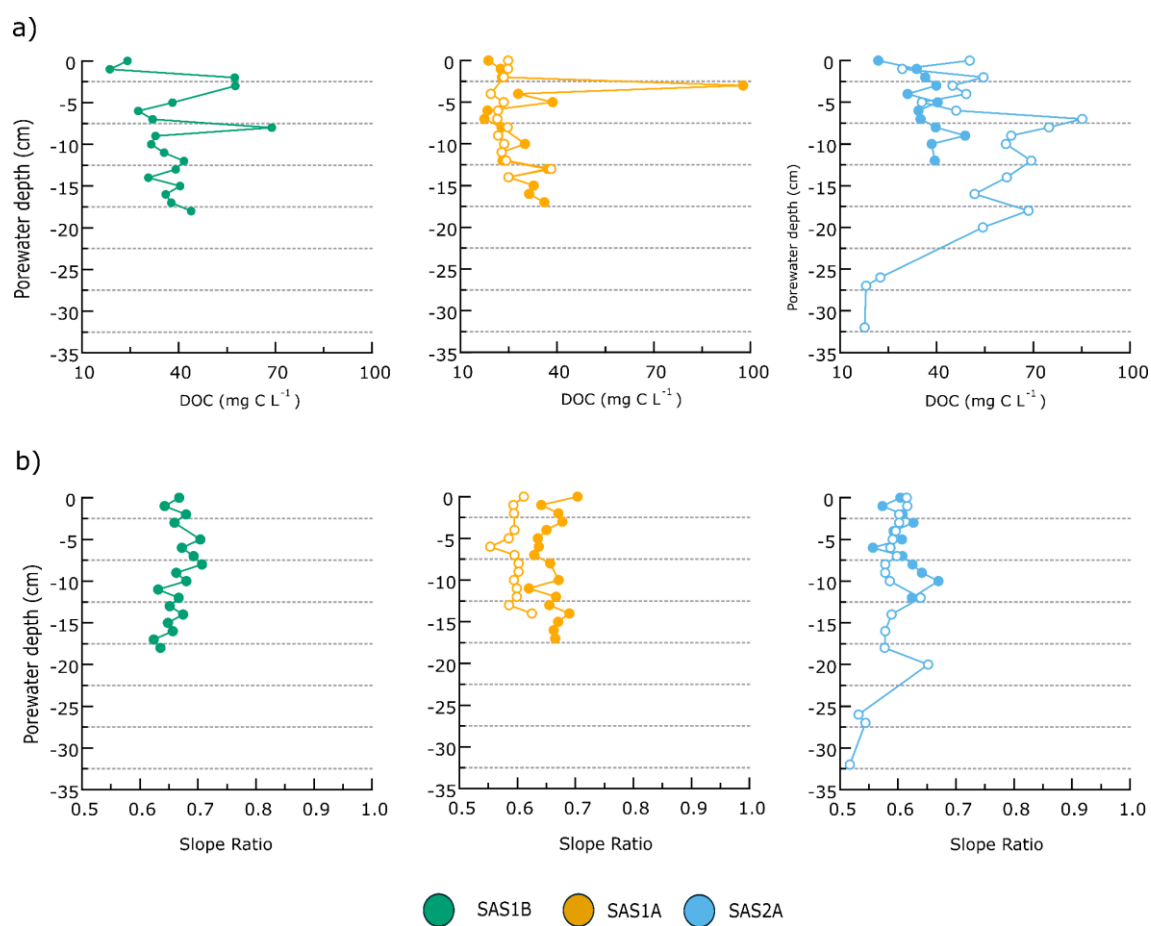


Figure VII.I.19. Sediment porewater profiles during summer (solid circles) and winter (open circles) in SAS1B, SAS1A, and SAS2A for: a) dissolved organic carbon (DOC) concentrations; b) Slope ratio.

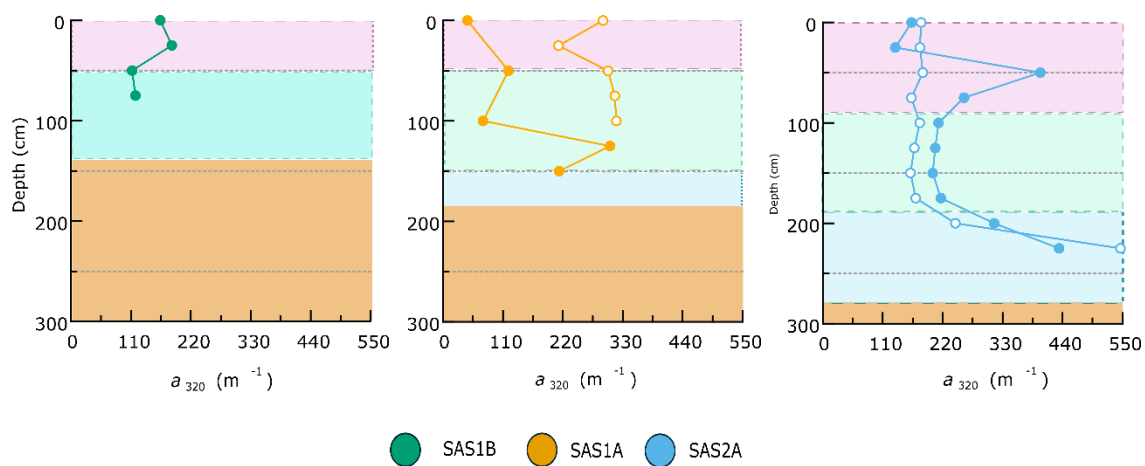


Figure VII.I.20. Vertical profiles of the absorption coefficient at 320 nm (a_{320}) in SAS lakes.

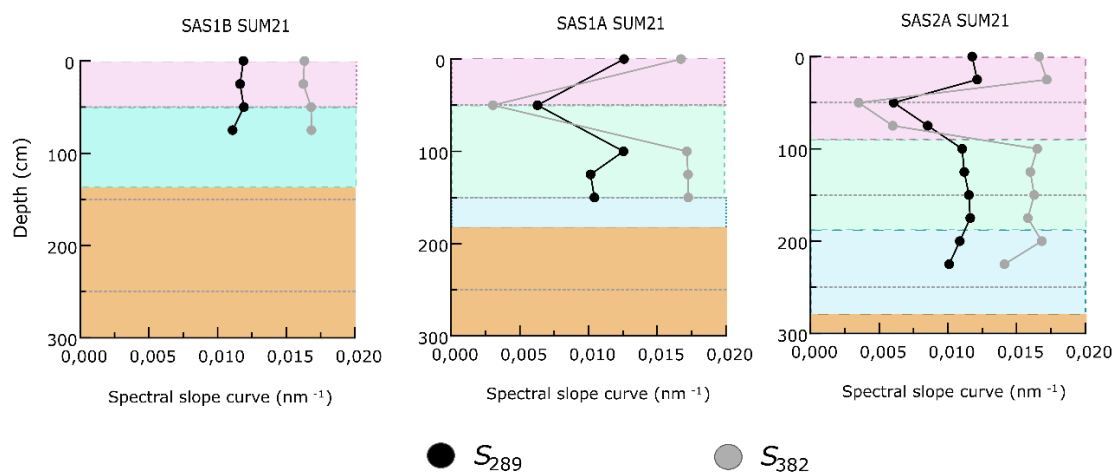


Figure VII.I.21. Vertical profiles of spectral slope curves (S_{289} and S_{382}) in SAS lakes during summer.

Second-derivative of UV-Vis spectra

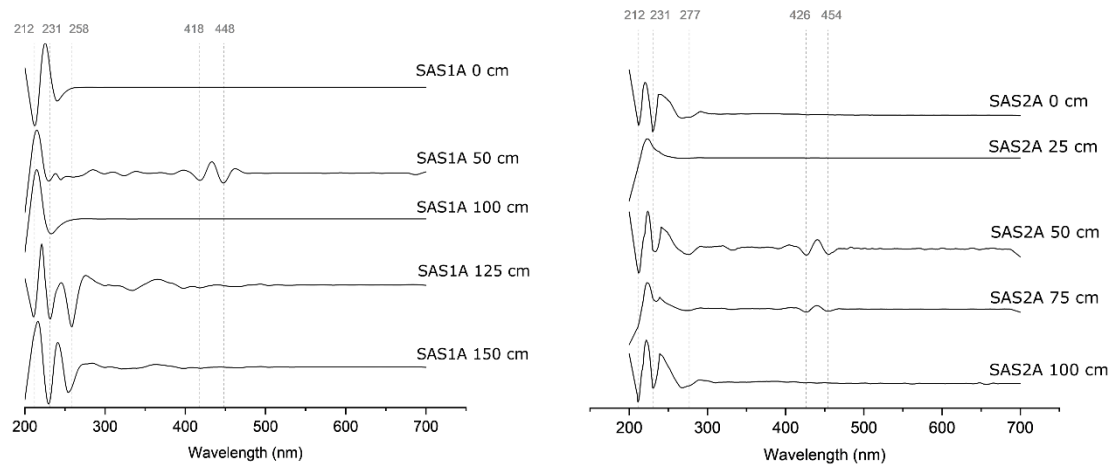


Figure VII.I.22. Second-derivative of UV-Visible spectra in SAS1A and SAS2A (0-100 cm depth) during summer.

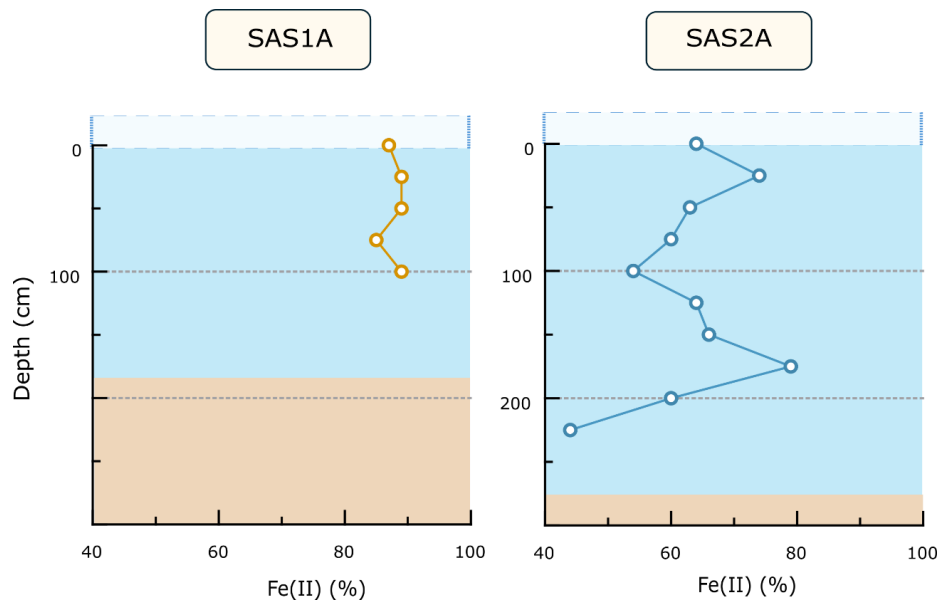


Figure VII.I.23. Vertical profile of Fe(II) percentage in SAS1A and SAS2A during winter.

Table VII.I.1. Seasonal $\delta^{13}\text{C}$ signatures and C/N ratios for both total carbon and organic carbon sediment section samples in all SAS ponds.

Total Carbon Samples						Organic Fraction Samples					
SAS1A						SAS1A					
Section	WIN22		Section	SUM21		Section	WIN22		Section	SUM21	
	$\delta^{13}\text{C}$	C/N		$\delta^{13}\text{C}$	C/N		$\delta^{13}\text{C}$	C/N		$\delta^{13}\text{C}$	C/N
0-5 cm	-27.9	19.8	0-5 cm	-28.2	17.8	0-5 cm	-27.7	20.2	0-5 cm	-28.1	17.4
5-10 cm	-28.4	18.2	5-10 cm	-27.1	18.3	5-10 cm	-28.3	18.5	5-10 cm	-27.1	18.5
10-15 cm	-27.7	20.3	10-15 cm	-27.1	19.7	10-15 cm	-27.6	20.1	10-15 cm	-27.5	18.9
15-20 cm	-27.5	19.2	15-20 cm	-27.5	19.3	15-20 cm	-27.7	20.2	15-20 cm	-27.6	19.5
SAS1B						SAS1B					
Section	WIN22		Section	SUM21		Section	WIN22		Section	SUM21	
	$\delta^{13}\text{C}$	C/N		$\delta^{13}\text{C}$	C/N		$\delta^{13}\text{C}$	C/N		$\delta^{13}\text{C}$	C/N
0-3 cm	-28.3	17.3	0-5 cm	-29.3	16.2	0-3 cm	-28.2	17.2	0-5 cm	-29.2	15.8
3-6 cm	-28.0	18.5	5-10 cm	-28.5	17.6	3-6 cm	-28.0	17.9	5-10 cm	-28.5	17.9
6-9 cm	-27.6	21.6	10-15 cm	-28.1	20.0	6-9 cm	-27.6	21.9	10-15 cm	-32.3	10.5
9-12 cm	-20.2	17.7	15-20 cm	-27.6	18.8	9-12 cm	-27.6	21.2	15-20 cm	-27.7	20.4
SAS2A						SAS2A					
Section	WIN22		Section	SUM21		Section	WIN22		Section	SUM21	
	$\delta^{13}\text{C}$	C/N		$\delta^{13}\text{C}$	C/N		$\delta^{13}\text{C}$	C/N		$\delta^{13}\text{C}$	C/N
0-5 cm	-28.1	17.0	0-5 cm	-27.8	17.5	0-5 cm	-28.1	16.7	0-5 cm	-27.7	17.3
5-10 cm	-28.3	15.9	5-10 cm	-27.3	18.6	5-10 cm	-28.2	15.9	5-10 cm	-27.5	18.6
10-15 cm	-28.3	15.1	10-15 cm	-27.3	18.6	10-15 cm	-28.2	15.9	10-15 cm	-27.3	20.2
15-20 cm	-27.2	19.8	15-20 cm	-27.4	19.1	15-20 cm	-27.2	19.3	15-20 cm	-27.4	18.8

Table VII.I.2. Seasonal variation of Humification Index (1630 cm⁻¹/1066 cm⁻¹) in FTIR spectra of SAS sediments. *For SAS1B WIN22 consider the sediment depth order to be: 0-3, 3-6, 6-9 and 9-12 cm.

Sediment depth (cm)	SAS2A WIN22	SAS2A SUM21	SAS1A WIN22	SAS1A SUM21	SAS1B WIN22*	SAS1B SUM21
0-5	0.19	0.28	0.18	0.35	0.28	0.23
5-10	0.18	0.23	0.17	0.58	0.33	0.22
10-15	0.13	0.19	0.22	0.75	0.62	0.33
15-20	0.24	0.26	0.31	0.64	0.55	0.45

Table VII.I.3. Organic fraction $\delta^{13}\text{C}$ signatures and C/N ratios in SAS riparian soils (n=2 per site).

Soil samples (n=2)	$\delta^{13}\text{C}$ signature	C/N ratio
SAS1A	$-27.7 \pm 0.2 \text{ ‰}$	25.1 ± 11.8
SAS2A	$-27.3 \pm 0.7 \text{ ‰}$	40.2 ± 7.8
SAS1B	$-27.3 \pm 0.2 \text{ ‰}$	22.6 ± 0.1

Table VII.I.4. Weight percentage (wt%) of carbon in the organic fraction of SAS sediments during summer (SUM21) and winter (WIN22). *For SAS1B WIN22 consider the sediment depth order to be: 0-3, 3-6, 6-9 and 9-12 cm.

	SAS2A WIN22	SAS2A SUM21	SAS1A WIN22	SAS1A SUM21	SAS1B WIN22*	SAS1B SUM21
0-5 cm	32.3	38.4	43.8	39.3	43.2	43.1
5-10 cm	29.5	36.5	40.5	44.5	46.6	43.2
10-15 cm	31.2	31.7	44.9	46.9	47.9	1.2
15-20 cm	33.5	29.2	46.1	45.3	47.2	46.8

Table VII.I.5. Principal component analysis (PCA) variable loadings for the three selected principal components in the two performed PCAs: water column and summer sediment porewaters.

Water column				Sediment porewaters (SUM21)			
Variables	PC1	PC2	PC3	Variables	PC1	PC2	PC3
Depth	0.16	-0.2	-0.39	%HT1	-0.45	-0.03	0.2
S_{289}	0.16	0.52	-0.18	%HM1	-0.43	-0.07	0
S_{382}	0.36	0.35	-0.06	%HT2	-0.21	0.45	0.33
S_R	-0.38	-0.29	0.11	%P1	0.4	0.07	-0.36
a_{320}	0.18	-0.48	-0.08	%P2	0.08	-0.46	0.38
SUVA_{254}	-0.23	-0.32	-0.32	Depth	-0.16	0.44	-0.09
pH	-0.19	0.07	0.44	NO_3^-	-0.13	-0.44	-0.39
DO	-0.33	0.07	0.18	SO_4^{2-}	0.33	0.08	-0.04
SPC	0.38	-0.19	0.17	DOC	0.11	-0.15	-0.08
NO_3^-	-0.19	0.12	0.37	S_R	0.32	0.37	0
SO_4^{2-}	0.19	0.02	0.22	a_{320}	-0.26	0.04	-0.54

Mn	0.28	-0.18	0.44	SUVA ₂₅₄	-0.27	0.16	-0.35
Fe	0.38	-0.25	0.22				

Table VIII.I. 6. Pairwise comparisons (Bonferroni corrected) between sample groups in the water column and sediment porewaters (during summer) with significant differences ($p < 0.05$).

	Comparison	R ²	p-adjusted
Water column	SAS2A SUM21 - SAS2A WIN22	0.213	0.01
	SAS2A SUM21 - SAS1A WIN22	0.429	0.02
	SAS1A SUM21 - SAS2A WIN22	0.310	0.01
	SAS1B SUM21 - SAS2A WIN22	0.368	0.05
	SAS2A WIN22 - SAS1A WIN22	0.439	0.01
Sediment Porewaters (SUM21)	SAS2A - SAS1A	0.556	0.003
	SAS2A - SAS1B	0.183	0.03
	SAS1A - SAS1B	0.262	0.006

Table VII.I.7. Identification of FTIR band (wavenumber), assigned molecular vibration signal, compound class and respective reference based on the literature for organic matter samples.

FTIR Band	Signal	Compound class	References
1070-1030 cm ⁻¹	C-O stretch	Carbohydrates (polysaccharides) and aliphatic esters	(Mascarenhas et al., 2000; Brandenburg and Seydel, 2001; Abdulla et al., 2010)
1155 cm ⁻¹	C-O-C stretch	Aliphatic esters	(El Fels et al., 2014; Łucejko et al., 2018)
1175 cm ⁻¹	C-O-C stretching	Ethers and phenols	(Michalska et al., 2001; Nikonenko et al., 2005; Enev et al., 2021; Hong et al., 2021)
1222 cm ⁻¹ and 1211 cm ⁻¹ (1200 cm ⁻¹ region)	C-O stretching	Phenols, esters and carboxylic acids	(Chen et al., 2002; Kim et al., 2006; Rodríguez et al., 2016; Liu et al., 2019; Enev et al., 2021; Gu et al., 2024)
1245 cm ⁻¹	C-C-O stretching	Acetyl esters and polyphenols	(Abdulla et al., 2010; Wen et al., 2011; Jiang et al., 2017)
1270 cm ⁻¹	C-O stretching	Carboxylic acids	(Gressel et al., 1995; Abdulla et al., 2010)
1385 cm ⁻¹	O-H deformation and C-O stretching; COO- stretching; C-H deformation of CH ₃	Phenolic and carboxylic groups; Aliphatic structures	(Senesi et al., 1989; Santos et al., 2009; Abdulla et al., 2010)
1400 cm ⁻¹	C-H symmetrical bending; Symmetric stretching of COO ⁻	Aliphatic structures, and/or phenolic, deprotonated carboxylic groups	(Abdulla et al., 2010; Huang et al., 2018; Liu et al., 2019)

1417 cm ⁻¹	C-H deformation of CH ₂ and CH ₃ ; C-OH deformation	Aliphatic groups	(Abdulla et al., 2010; Margenot et al., 2017; Hansima et al., 2022; Pärnpuu et al., 2022)
1455 cm ⁻¹	C-H bend of CH ₂ and CH ₃	Aliphatic groups (methyl) and lignin	(Ajuong and Redington, 2004; Abdulla et al., 2010; Biyada et al., 2020; Hansima et al., 2022)
1515 cm ⁻¹	C=C stretch in aromatic ring	Aromatic lignin	(Abdulla et al., 2010)
1540-1580 cm ⁻¹	N-H bend	Amide II bonds	(Wang et al., 2013)
1600 cm ⁻¹	Asymmetric stretching of COO ⁻	Deprotonated carboxylic groups	(Abdulla et al., 2010)
1630-1650 cm ⁻¹	C=C stretching;	Conjugated aromatic systems;	(Ma, 2004; Rodríguez et al., 2016; Li et al., 2017)
	C=O stretching	Ketones and amide I	(Kim et al., 2006; Rodríguez and Núñez, 2011; Maxson et al., 2021)
	COO ⁻ asymmetric stretching	Carboxylic groups	(Abdulla et al., 2010; Pernet-Coudrier et al., 2011)
1700-1730 cm ⁻¹	C=O stretching	Carboxylic acids and aliphatic esters	(Stevenson and Goh, 1971; Abdulla et al., 2010; Jiang et al., 2017; Gu et al., 2024)
1770 cm ⁻¹	C=O stretching	γ -lactone esters	(Abdulla et al., 2010)
2500-2700 cm ⁻¹	O-H stretching	Carboxylic acids	(Shevchenko 1963; Cabaniss et al. 1998)
2918 (2930) cm ⁻¹	C-H stretching of CH ₂ and CH ₃	Aliphatic lipids, waxes and fatty acids	(Calderón et al., 2011; Pärnpuu et al., 2022)
2850 cm ⁻¹	C-H stretching of CH ₂ and CH ₃	Aliphatic lipids, waxes and fatty acids	(Calderón et al., 2011; Pärnpuu et al., 2022)
3000-3400	O-H vibrations in hydroxyl groups; N-H stretching	Alcohols, carboxyl groups, and phenols; Amines and amides	(Senesi et al., 1989; Ait Baddi et al., 2004; Madari et al., 2006; Calderón et al., 2011; El Fels et al., 2014; Pärnpuu et al., 2022)

Supplementary Note SN.VII.I.1 - Extended description of Fluorescence PARAFAC Components:

HT1 is characterized as a typical humic-like group of molecules of terrestrial origin (Stedmon and Markager, 2005; Osburn et al., 2012; Coble et al., 2014; Du et al., 2016), primarily derived from the active layer and permafrost soils (Pacoureaux et al., 2025). HT2 exhibits the longest excitation and emission peaks among the identified components, suggesting its association with particularly high molecular weight terrestrial humic substances (Stedmon et al., 2003; Stedmon and Markager, 2005; Coble et al., 2014; Du et al., 2016). Like HT1, HT2 has also been linked to active layer and permafrost sources in Pacoureaux et al. (2025). HM1 represents humic-like compounds modified through microbial transformation and has been commonly identified in marine systems (Stedmon

et al., 2003; Kowalczyk et al., 2010; Coble et al., 2014). The protein-like components P1 and P2 correspond to tyrosine-like and tryptophan-like fluorophores, respectively, and are indicative of biodegradable, freshly microbially-produced FDOM (Osburn et al., 2012; Coble et al., 2014; Fouché et al., 2020). In the context of permafrost peatlands, these protein-like compounds have been associated with both living and decomposing vegetation, particularly in leachates from grasses, mosses, shrubs, and cyanobacterial mats (Pacoureaux et al., 2025).

Supplementary Note SN.VII.I.2 - FTIR Characterization and Peak Assignment of Water Column SPE-DOM:

FTIR spectra were interpreted by first assigning functional groups to specific peaks based on existing literature. Below, we identify the FTIR peaks and contrast their presence and relative abundance among the sites. Generally, the DOM FTIR spectra exhibited the following features: (1) a band centered at 1070-1030 cm^{-1} ; (2) a broad and more intense band in the 1300-1150 cm^{-1} region; (3) a smaller band in the 1370-1460 cm^{-1} range; (4) a peak at 1515 cm^{-1} ; (5) a shoulder-like band at 1600 cm^{-1} next to a band at 1700 cm^{-1} ; (6) a band centered at 2930 cm^{-1} ; and (7) a large broad band at 3000-3400 cm^{-1} . FTIR signatures were compiled along with their respective peak assignments from the literature in Table VII.I.7.

All summer DOM samples (except for surface SAS2A) showed two well-defined peaks at 1222 cm^{-1} and 1211 cm^{-1} . These peaks appear between two broader bands, one with a maximum at 1175 cm^{-1} and one at 1245 cm^{-1} . We note that a small shoulder-like peak at 1336 cm^{-1} was observed and assigned to the presence of polyhydroxylated phenols and C-N stretching of aromatic amines (Anjuman et al., 2023). Additionally, the formation of a band at 1400 cm^{-1} and a shoulder-like band at 1770 cm^{-1} was observed in summer DOM samples, indicating an increase in γ -lactone esters, and possible increases in aliphatic, phenolic, and carboxylic groups.

In contrast, winter DOM showed a broad band at 1200 cm^{-1} , potentially masking the dual peaks observed in summer (1222 and 1211 cm^{-1}), and is typically found in aquatic humic substances (Niemeyer et al., 1992). This broad band was accompanied by a shoulder-like band at 1270 cm^{-1} . Moreover, the 1515 cm^{-1} peak, although present in both summer and winter samples, appeared more pronounced in winter samples.

Summer SAS1A (Figure VII.I.6) shows slight declines in peaks 1770, 1335 and 1070 cm^{-1} towards the surface, along with a slight but incomplete masking of peaks 1222 and 1211 cm^{-1} . These features are indicative of a decrease in γ -lactones esters, aromatic amines, and some polysaccharide structures closer to the surface. Additionally, there is a gradual increase in the presence of aliphatic esters and carboxylic acids toward the upper layer of the water column.

In winter, bottom waters at SAS1A exhibited strong FTIR bands at 1515, 1608, and 1175 cm^{-1} , along with a shoulder at 1270 cm^{-1} , corresponding to carboxylic acids, aromatic moieties, and phenolic structures. Toward the surface, the 1515, 1175, and 1270 cm^{-1} bands weakened, the 1608 cm^{-1} band broadened, and an increase in the 1384 cm^{-1} band was observed, suggesting a relative decrease in aromatic lignin, aliphatic esters, and carboxylic moieties.

Towards the surface in summer SAS2A (Figure. S6), we observe a decrease in the intensities of the 1335, 1770 and 1036 cm^{-1} bands, along with the emergence of a broad band centered at 1200 cm^{-1} , linked to aquatic humic substances. This broad band ultimately masks the 1222 and 1211 cm^{-1} peaks observed deeper in the water column.

This suggests a decrease in aromatic amines, polyhydroxylated phenols, γ -lactone esters, and possibly polysaccharides, as well as an increase in condensed humified structures at the lake surface. In winter, SAS2A displayed an increase in intensities of the 1175, 1267, 1335, 1515 and 1607 cm^{-1} bands, along with a decrease in the 1383 cm^{-1} band in surface waters compared to bottom waters. The broad 1200 cm^{-1} band from summer surface waters became ubiquitous in the water column. These shifts in SAS2A indicate a stronger presence of carboxylic acids, aromatic and phenolic moieties towards the surface, while the bottom shows an increase in more aliphatic structures.

For SAS1B, little compositional change was observed from bottom to surface in summer waters, given its shallow nature, apart from a slight increase at 1770 cm^{-1} . However, winter SAS1B showed a decrease in the polysaccharide band, an increase in the band at 1200 cm^{-1} , a sharpening of the 1600 cm^{-1} band, along with an increase at 2930 cm^{-1} and a widening of the band centered at 1400 cm^{-1} towards lower wavenumbers, while maintaining a relatively noticeable peak at 1175 cm^{-1} . These changes denote the increase in aliphatic compounds (i.e. waxy lipids, fatty acids), aquatic humic substances, carboxylic acids and the degradation of labile polysaccharide structures during winter.

VII.II. Seasonal biogeochemistry contrasts in palsa- and lithalsa-origin thermokarst lakes in a discontinuous permafrost zone

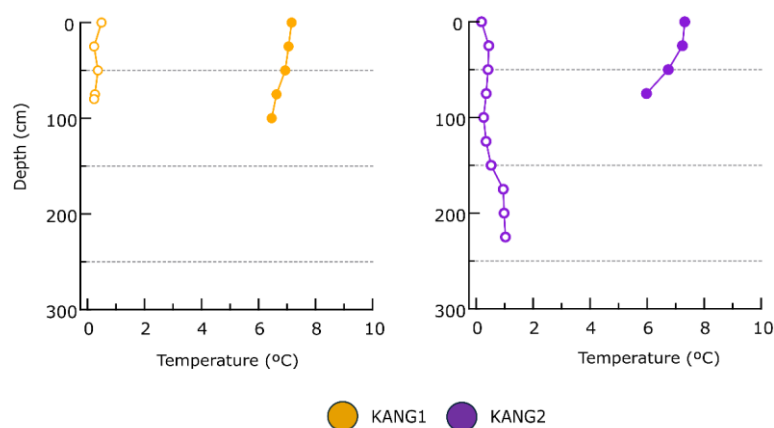


Figure VII.II.1. Temperature profiles in summer and winter in KANG1 and KANG2. Open circles represent winter data; closed circles represent summer measurements.

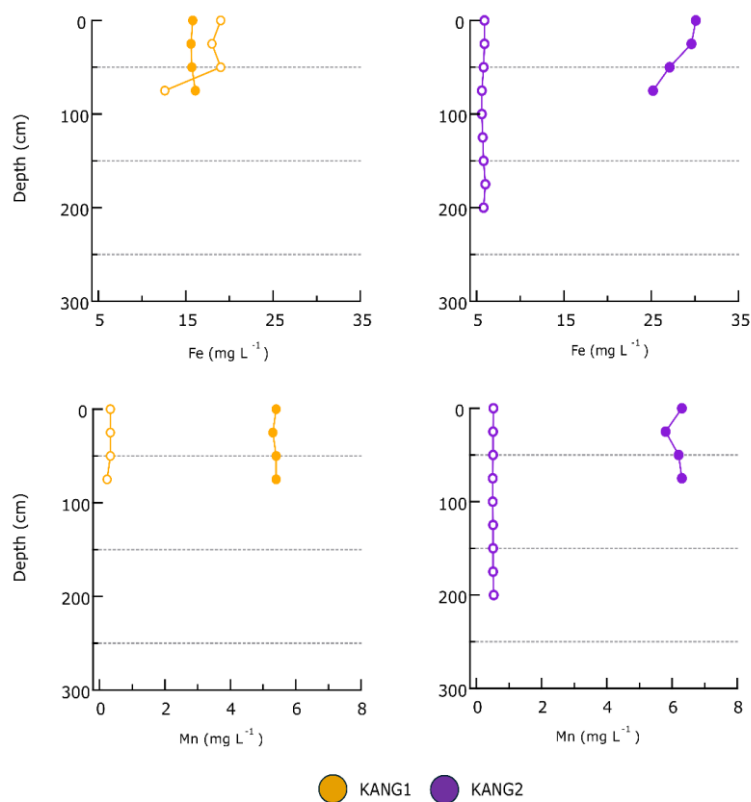


Figure VII.11.2. Dissolved Fe and Mn profiles in summer and winter in KANG1 and KANG2. Open circles represent winter data; closed circles represent summer measurements.

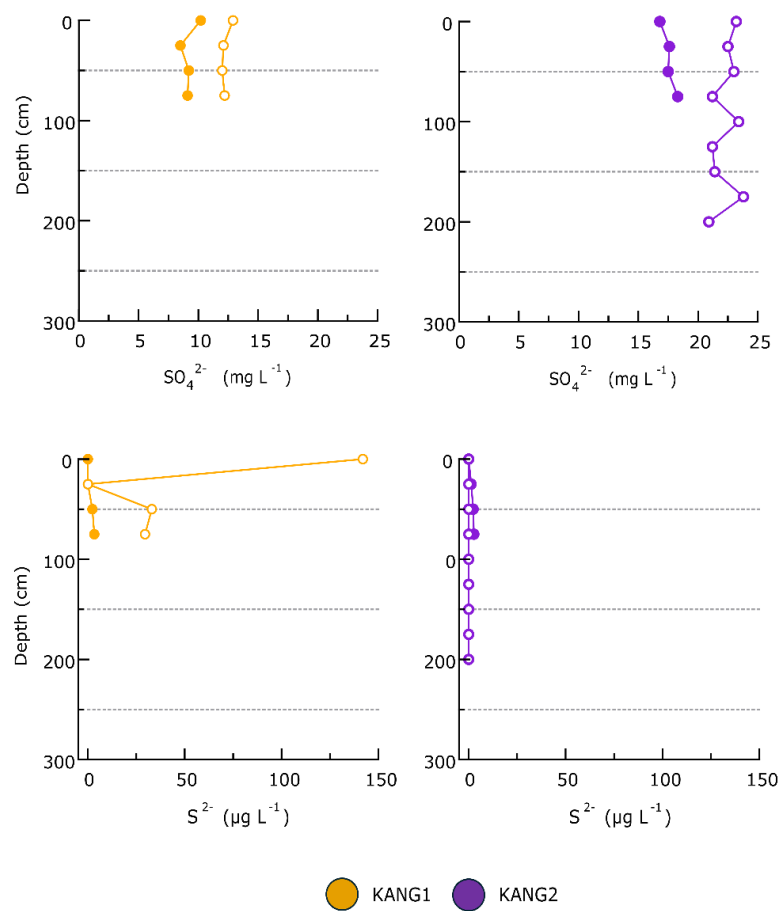


Figure VII.II.3. Dissolved sulfate (SO_4^{2-}) and sulfide (S^{2-}) profiles in summer and winter waters in KANG1 and KANG2. Open circles represent winter data; closed circles represent summer measurements.

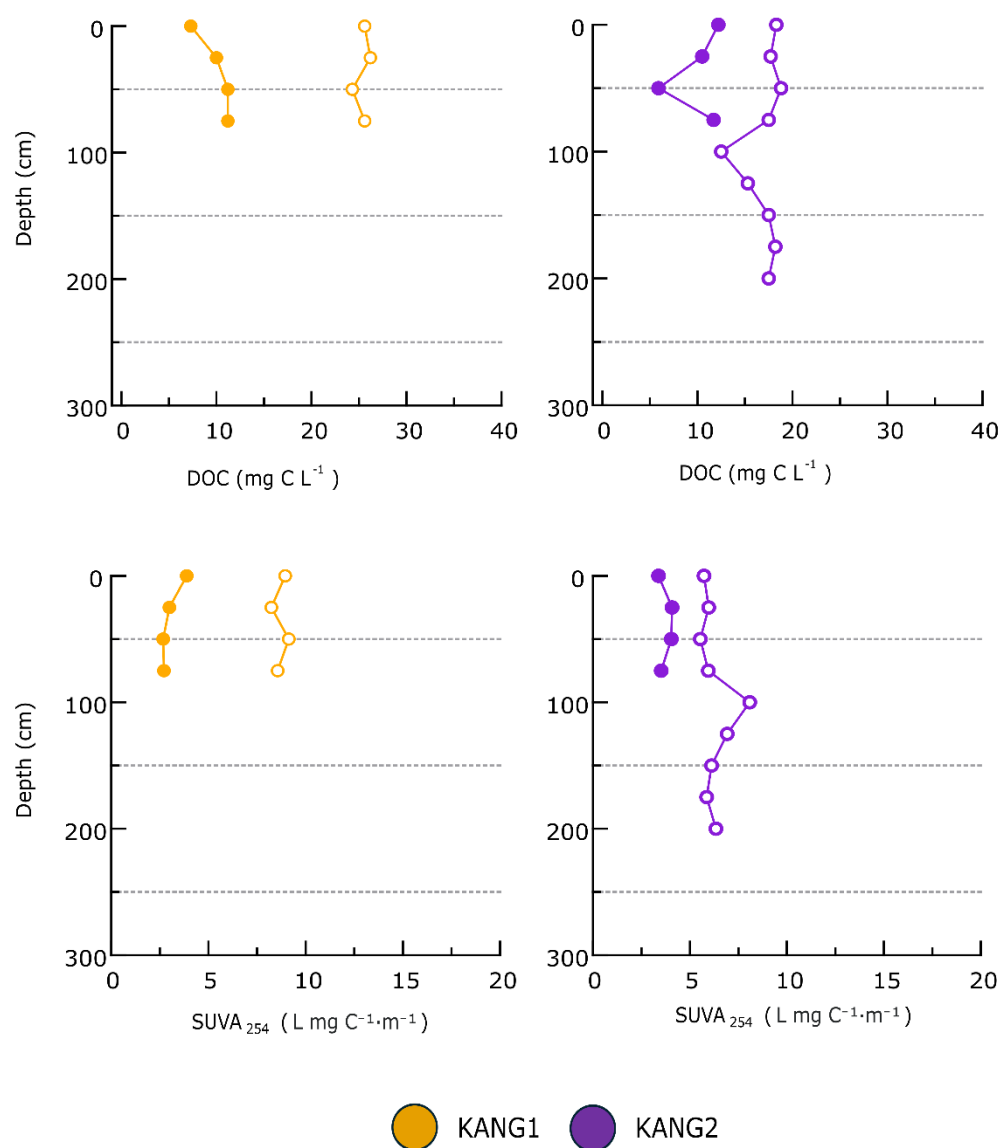


Figure VII.II.4. Dissolved organic carbon (DOC) and specific ultraviolet absorbance (SUVA₂₅₄) profiles in summer and winter waters in KANG1 and KANG2 (0.45 μm fraction). Open circles represent winter data; closed circles represent summer measurements.

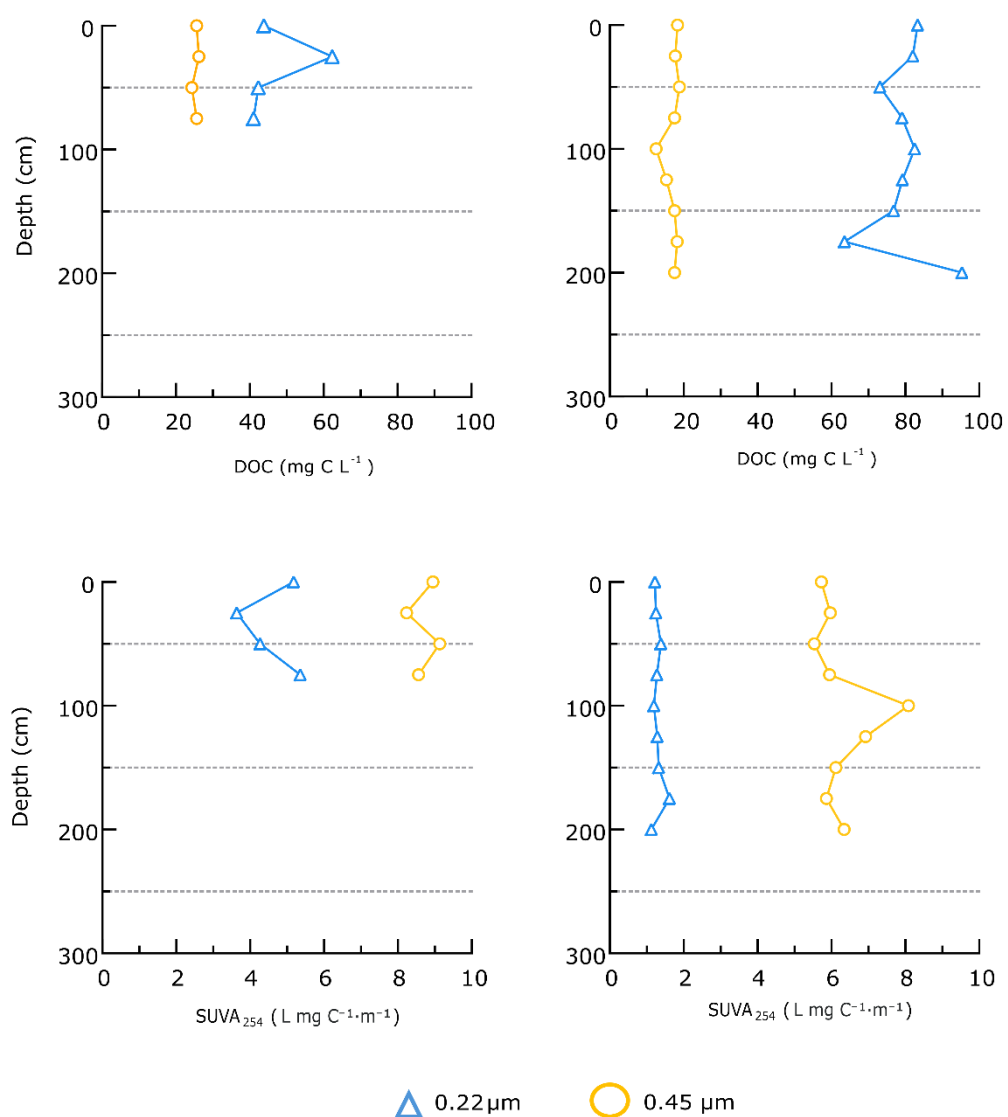


Figure VII.II.5. Comparison of dissolved organic carbon (DOC) and specific ultraviolet absorbance (SUVA₂₅₄) profiles during winter in KANG1 and KANG2 for the 0.2 and 0.45 μm fraction.

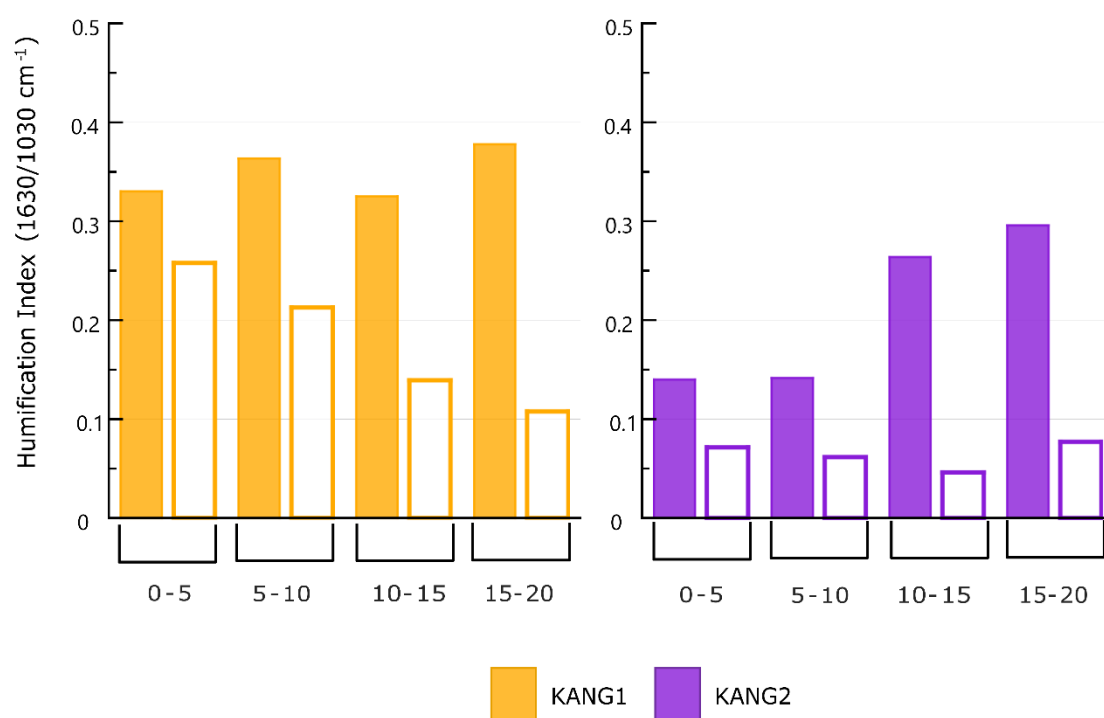


Figure VII.II.6. Seasonal variations in humification index (HI, 1630/1030 cm⁻¹) of sediment layers in KANG1 and KANG2. Open bars represent winter measurements; closed bars represent summer data.

Supplementary Note VII.II.1 (SN.VII.II.1): Winter SUVA₂₅₄ profiles in 0.2 µm fraction (compared to 0.45 µm) in both thermokarst lakes

During winter, SUVA₂₅₄ values in the 0.2 µm fraction ranged between 3.6-5.4 in KANG1, and in the 1.1-1.6 range in KANG2. In contrasts, the DOC reduction observed in the 0.45 µm fraction resulted in elevated SUVA₂₅₄ values ranging within 8.2-9.1 in KANG1, and 5.5-8.1 in KANG2. The pronounced DOC contrast between 0.45 and 0.2 µm fractions highlight substantial cumulative effects on DOC-normalized indicators and require careful consideration.

VII.III. Emerging thermokarst lakes amplify benthic activity and carbon mobilization in a continuous permafrost landscape



Figure VII.III.1. Photograph of the benthic flux chamber system in the margin of the lake (left) and during the experiment (right).

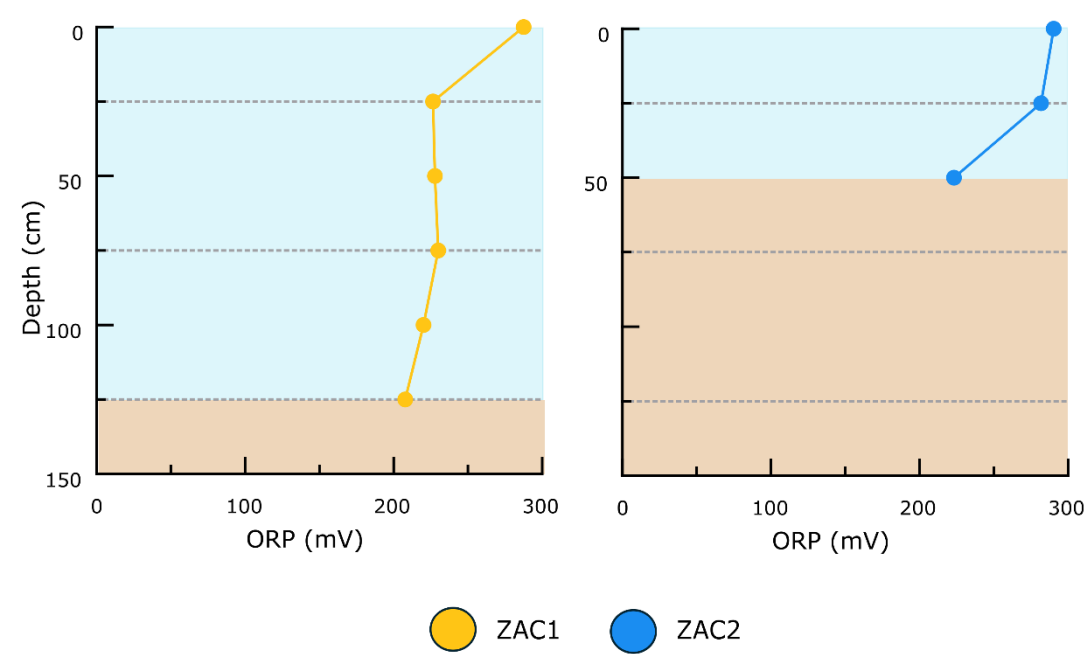


Figure VII.III.2. Vertical profiles of oxidation-reduction potential (ORP) in ZAC1 and ZAC2 lakes.

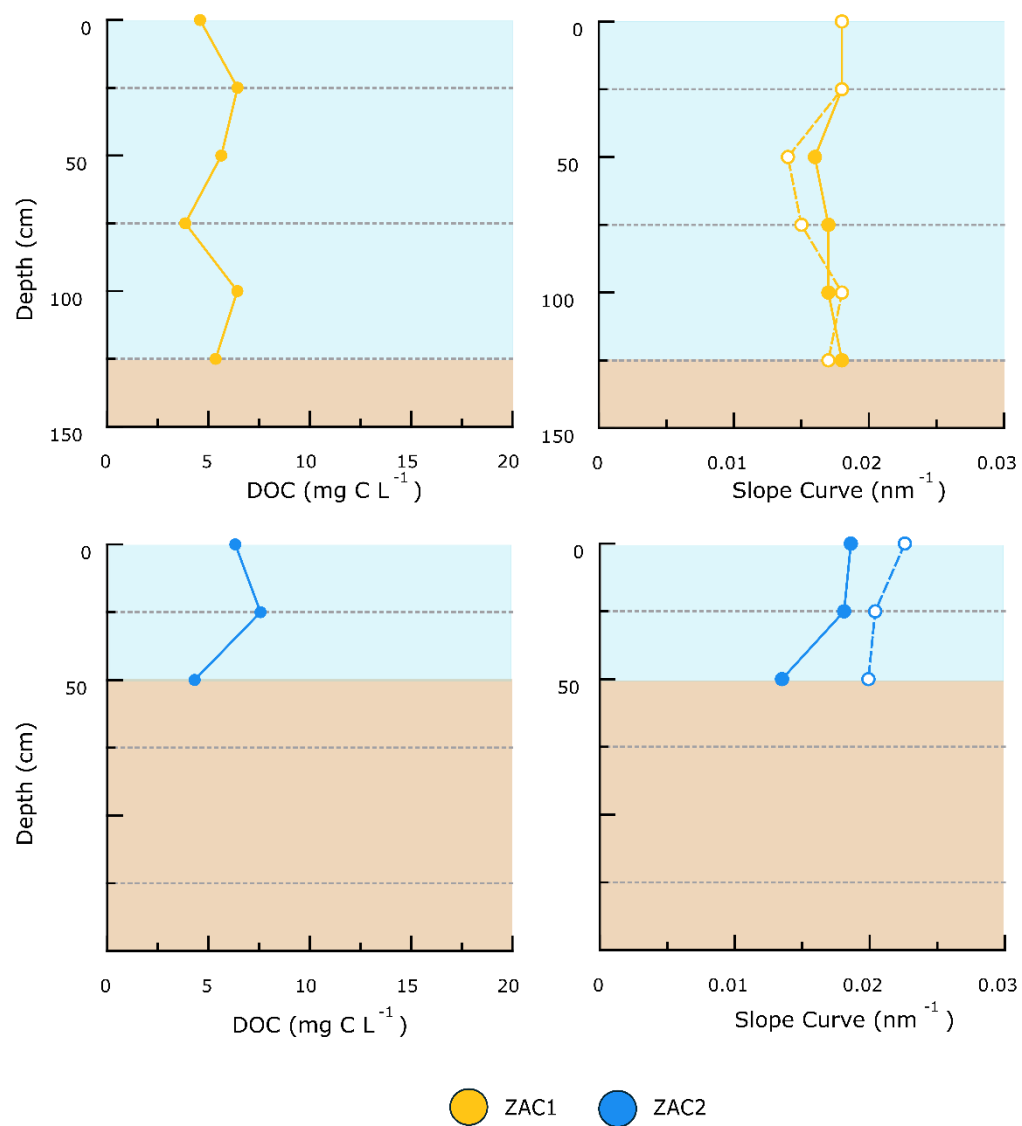


Figure VII.III.3. Vertical profiles (0.45 μm fraction) of DOC concentrations (left), and spectral slope curves S_{289} (closed circles) and S_{382} (open circles) (right) in ZAC1 and ZAC2 lakes.

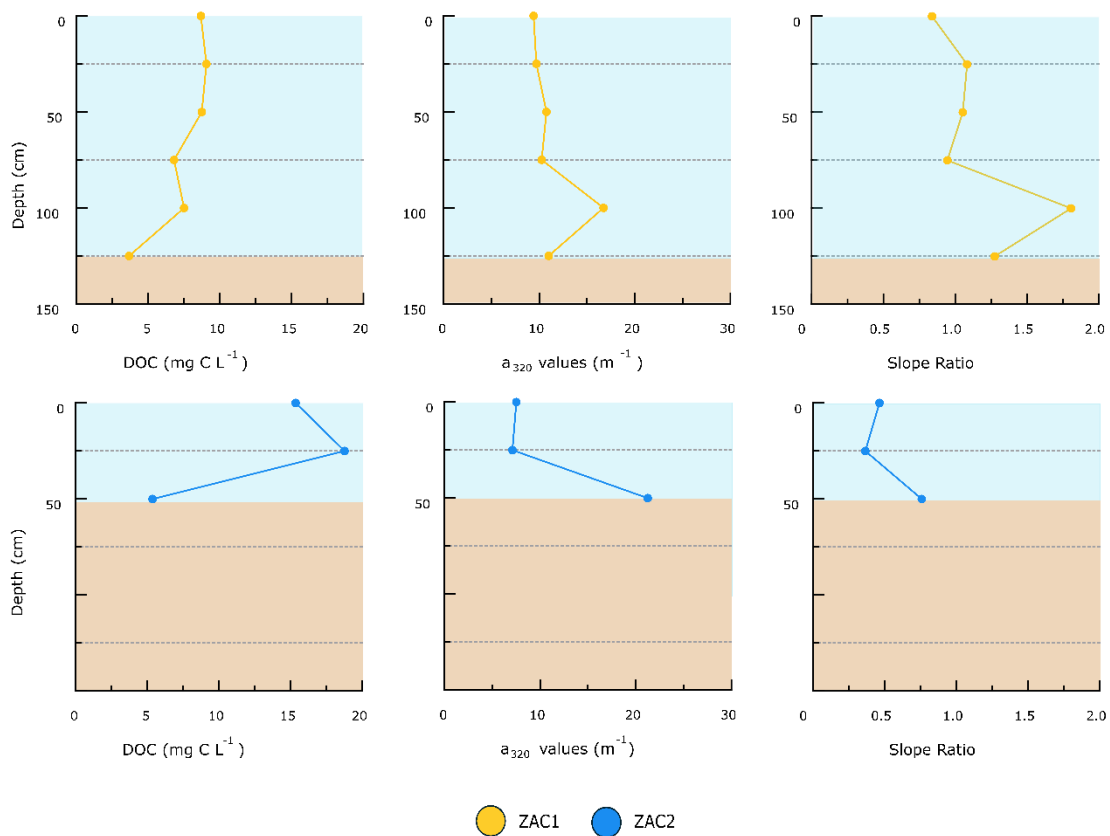


Figure VII.III.4. Vertical profiles (0.2 μm fraction) of DOC concentrations (left), the absorption coefficient at 320 nm (middle), and slope ratio (right) in ZAC1 and ZAC2 lakes.

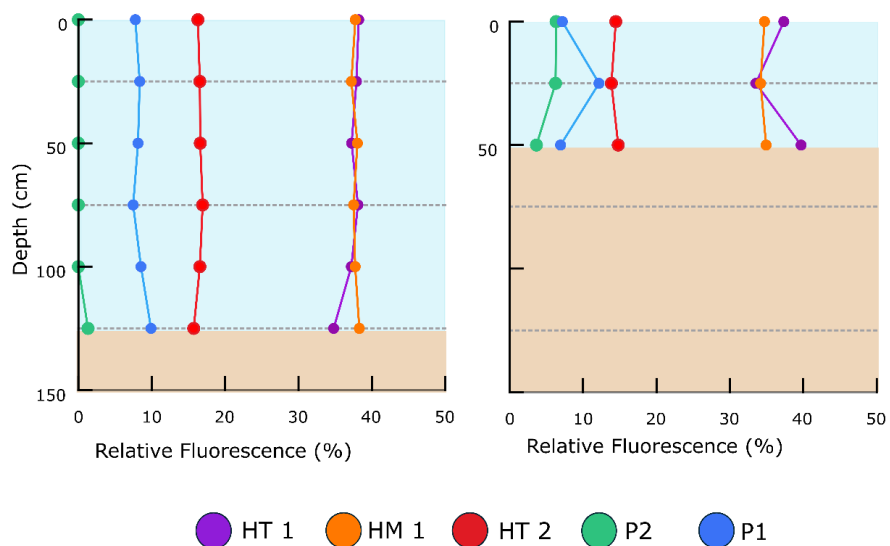


Figure VII.III.5. Vertical profiles (0.2 μm fraction) of PARAFAC component distribution (% of each component) in ZAC1 (left) and ZAC2 (right).

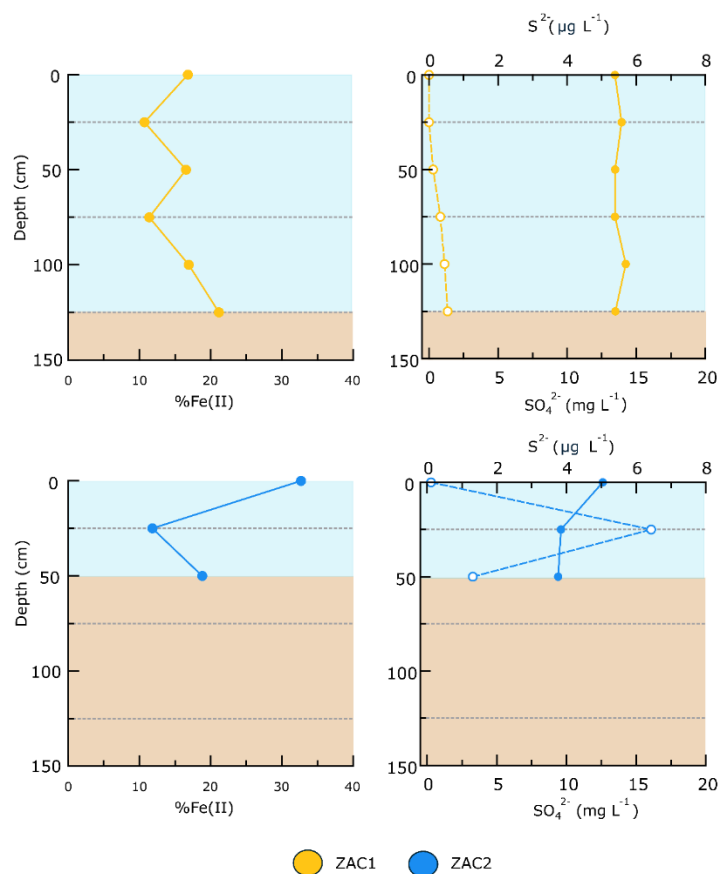


Figure VII.III.6. Vertical profiles (0.45 µm fraction) of distribution of %Fe(II) (left); distribution of sulfate (closed circle) and sulfide (open circle) (right) in ZAC1 and ZAC2.

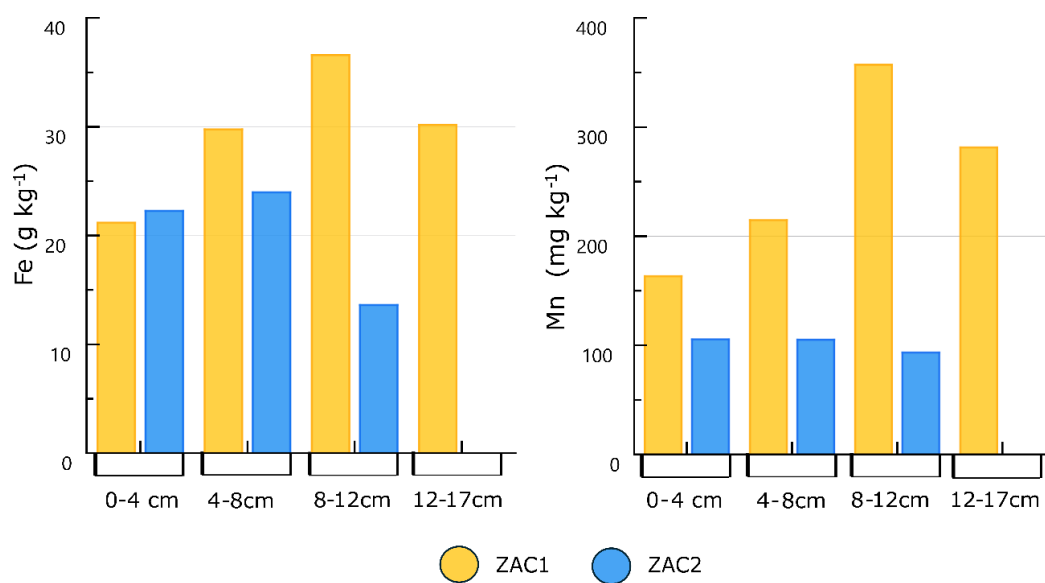


Figure VII.III.7. Sediment layer concentrations of Fe (g kg^{-1}) (left) and Mn (mg kg^{-1}) (right) in ZAC1 and ZAC2.

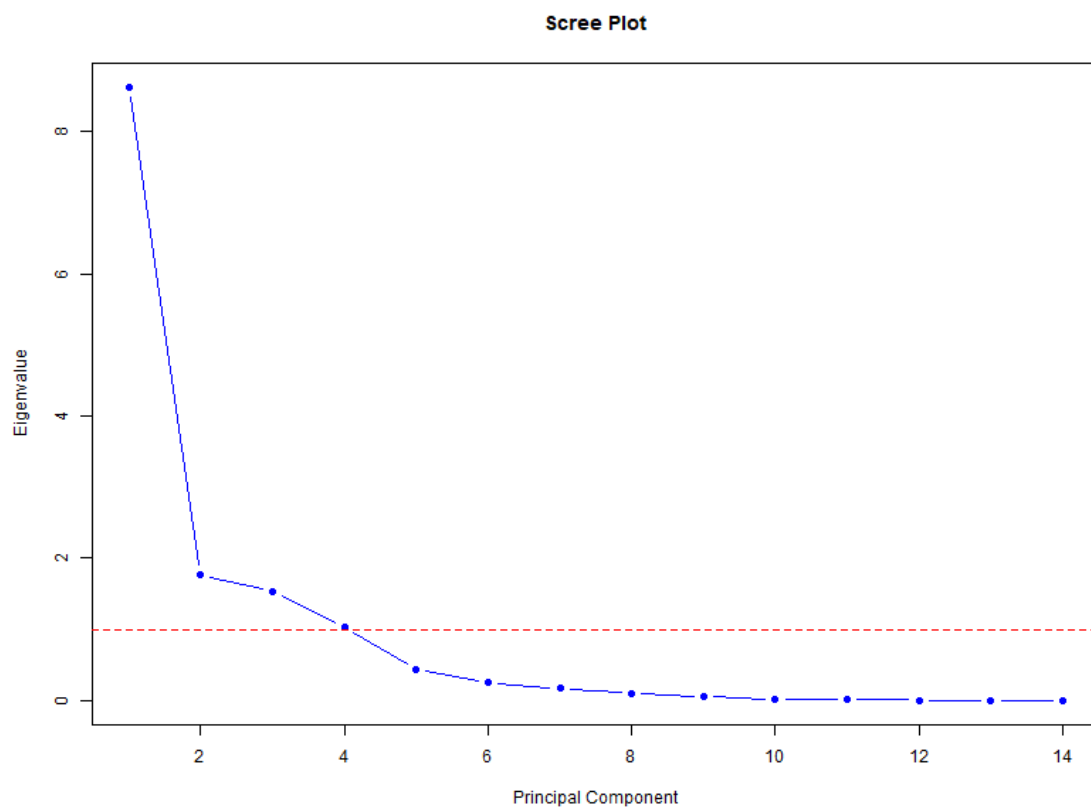


Figure VII.III.8. Scree plot of the principal component analysis conducted on the benthic flux measurements of the two lakes. Although the shoulder emerges at principal component (PC) 2, we considered the PC3 due to its eigenvalue (>1).

Table VII.III.1. Total carbon and organic fraction measurements for $\delta^{13}\text{C}$, C/N, and weight percentage of C and N (%).

$\delta^{13}\text{C}_{\text{VPDB}}$	wt% C	wt% N	C:N _{atomic}	Layer (cm)	Lake		
-29.06	0.90	0.12	8.43	0-4	ZAC1	Total Carbon	
-27.52	1.49	0.17	10.03	4-8			
-27.11	4.56	0.39	13.58	8-12			
-27.13	2.27	0.23	11.66	12-17			
-27.46	26.00	1.58	19.17	0-4	ZAC2		
-29.09	26.12	1.59	19.11	4-8			
-26.18	27.17	1.47	21.53	8-12			
-29.32	0.84	0.11	8.95	0-4	ZAC1	Organic Fraction	
-27.62	1.17	0.12	11.62	4-8			
-27.23	3.80	0.30	14.60	8-12			
-27.72	3.57	0.25	16.80	12-17			
-27.37	25.64	1.53	19.52	0-4	ZAC2		
-28.80	24.49	1.54	18.56	4-8			
-26.20	24.74	1.40	20.62	8-12			

Table VII.III.2. Principal component analysis variable loadings for each principal component (PC).

	PC1	PC2	PC3
pH	-0.33	-0.03	0.01
ORP	-0.29	0.22	-0.21
DO	-0.13	0.50	-0.49
a_{320}	0.10	0.43	0.40
SUVA ₂₅₄	0.31	0.16	-0.08
S _R	-0.23	0.19	0.26
Fe	0.30	0.01	0.27
Mn	0.32	0.03	0.09
SO ₄ ²⁻	-0.33	-0.02	0.14
NO ₃ ⁻	-0.10	0.13	0.54
DOC	-0.30	-0.08	0.22
Cl ⁻	-0.32	0.02	0.16
NH ₄ ⁺	-0.15	-0.63	-0.05
S ²⁻	0.32	-0.16	0.08

Supplementary Note VII.III.1 (SN.VII.III.1): DOM profiles (0.2 μm fraction) in thermokarst lakes

DOC concentrations in ZAC1 decreased with depth between 9.1 to 6.8 mg L^{-1} (0-100 cm depth) and further declined at the bottom (3.7 mg L^{-1}). A similar pattern was observed in ZAC2, although more pronounced. ZAC2 showed higher DOC concentrations in the epilimnion (15.4-18.8 mg L^{-1}), before decreasing to the bottom (5.4 mg L^{-1}).

Similar to the 0.45 μm fraction, the ranges of slope ratio (S_R) were different between lakes. In ZAC1, S_R ranged between 0.83 and 1.08 (0-75 cm depth), spiking at 100 cm (S_R : 1.80) and decreasing slightly to the bottom (S_R : 1.28). ZAC2 showed lower S_R values in the epilimnion (S_R : 0.36-0.46), increasing to the bottom (S_R : 0.75).

SUVA₂₅₄ values in ZAC1 showed a gradual increase towards the bottom (from 1.35 to 3.43), while ZAC2 displayed lower SUVA₂₅₄ in the epilimnion (0.56-0.71) before increasing at the bottom (3.70).

The a_{320} values varied little in the water column in ZAC1 (9.4-10.9 m^{-1}), except for an increase at 100 cm depth (a_{320} : 16.7 m^{-1}). ZAC2 however, showed a clear increase at the bottom (21.2 m^{-1}) relative to the epilimnion.

Supplementary Note VII.III.2 (SN. VII.III.2): Detailed description of Benthic Flux Chamber experiment

Dissolved oxygen levels in ZAC1 showed a gradual decline, going from 33% in T0 to 3.2% in T3 (120 min), slowly decreasing to 1.2% at T7 (360 min). ZAC2 however, decreased from 23.2% in T0 to 1.09% in T1 (30 min), remaining in the 1.1-0.16% range until T7. BFC in ZAC1 showed ubiquitously higher pH values with no clear trend, remaining in the 7.5-7.8 range. ZAC2 showed overall more acidic pH, exhibiting a decrease from 5.9 to 5.6 at T4 (180 min) before rising and stabilizing in the 5.9-5.8 range.

ZAC1 shows continuously higher ORP values than ZAC2 throughout the experiment, with decreasing from 293.3 to 271.2 mV at T2 (60 min), before dropping and stabilizing in the 113.2-137.6 mV range from T3 to T7 (120 to 360 min). ZAC2 however, decreased from 126.8 to -302.7 mV from T0 to T5 (240 min), and then increased towards T7 (-209.6 mV). ZAC1 showed a gradual temperature rise from 9.6 to 13.1 mg L^{-1} (T0 to T7), while ZAC2 temperatures fluctuated between 10.7 and 11.7 across the experiment.

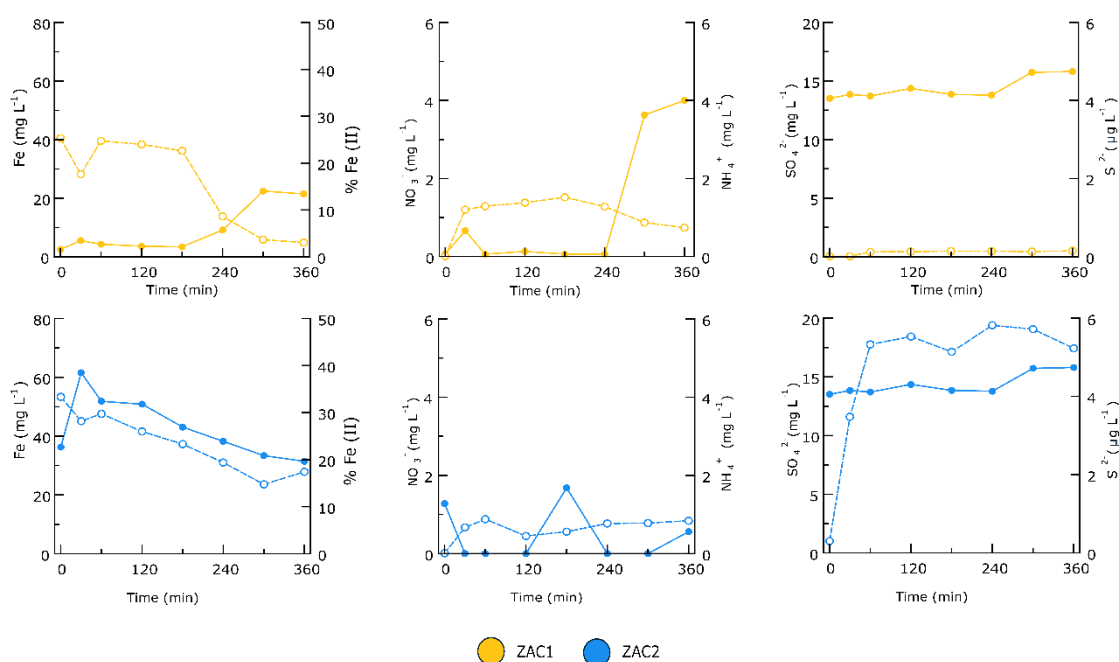


Figure VII.III.9. Benthic flux chamber experiment measurements of: a) Fe (mg L^{-1}) in the left y-axis (closed circles), and Fe(II) percentage in the right y-axis (open circles); b) Nitrate (mg L^{-1}) in the left y-axis (closed circles), and ammonium (mg L^{-1}) in the right y-axis (open circles); c) Sulfate ($\mu\text{g L}^{-1}$) in the left y-axis (closed circles), and sulfide ($\mu\text{g L}^{-1}$) in the right y-axis (open circles).

In ZAC1, total dissolved Fe remained between $2\text{--}6 \text{ mg L}^{-1}$ from T0 to T4 (180 min), with a sharp rise to 23 mg L^{-1} at T6 (300 min). This is likely driven by enhanced reductive dissolution of Fe as redox conditions shift in ZAC1. In contrast, ZAC2 displayed higher Fe concentrations and a more gradual decline. Dissolved Fe increased from 37 to 63 mg L^{-1} at T1 (30 min), then decreased steadily to 32 mg L^{-1} at T7 (360 min). The Fe(II)% in both systems decreased over time, with ZAC2 consistently maintaining higher relative proportions, supporting stronger reducing conditions at the sediment–water interface.

ZAC1 showed an increase in nitrate (0 to 0.66 mg L^{-1}) and ammonium (0 to 1.20 mg L^{-1}) at T1, with nitrate values decreasing after ($0.06\text{--}0.13 \text{ mg L}^{-1}$) between T2 to T5. Ammonium remained elevated to T5 ($1.28\text{--}1.52 \text{ mg L}^{-1}$) before decreasing towards T7 (0.74 mg L^{-1}). Nitrate concentrations spiked in T6 to 3.67 mg L^{-1} . In ZAC2 nitrate concentrations quickly fell from 1.28 mg L^{-1} to 0 , as ammonium increased from 0 to 0.67 mg L^{-1} after 30 min. Nitrate concentrations remained below detection limit from T1 to T3, spiking to 1.72 mg L^{-1} in T4, and decreased back to below detection limit in T5 and

T6, before a slight increase to 0.57 mg L^{-1} at T7. Ammonium concentrations oscillated between 0.88 and 0.45 mg L^{-1} from T2 to T7.

ZAC1 sulfate concentrations seem to gradually rise from 13.5 to 15.8 mg L^{-1} , while sulfide concentrations increase to $0.11 \text{ } \mu\text{g L}^{-1}$ at T2 and stabilized in the $0.12\text{-}0.14 \text{ } \mu\text{g L}^{-1}$ range throughout the experiment. In ZAC2 we observe a sulfate decrease from 10.3 to 9.2 mg L^{-1} in the first 30 min, accompanied by an increase in sulfide values from 0.30 to $3.5 \text{ } \mu\text{g L}^{-1}$. Sulfate concentrations stabilized in the $9.1\text{-}9.8 \text{ mg L}^{-1}$ range and sulfide concentrations in the $5.1\text{-}5.8 \text{ } \mu\text{g L}^{-1}$ range until T7. While sulfate concentrations are higher in ZAC1 ($13.5\text{-}15.8 \text{ mg/L}$ compared to $9.1\text{-}9.8 \text{ mg/L}$), we note ZAC2 displays an approximately 30-fold increase in sulfide concentrations compared to ZAC1.

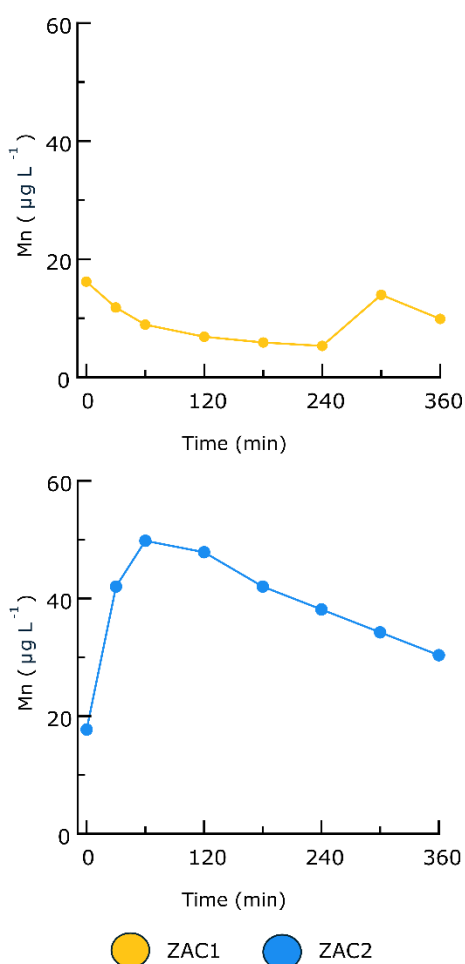
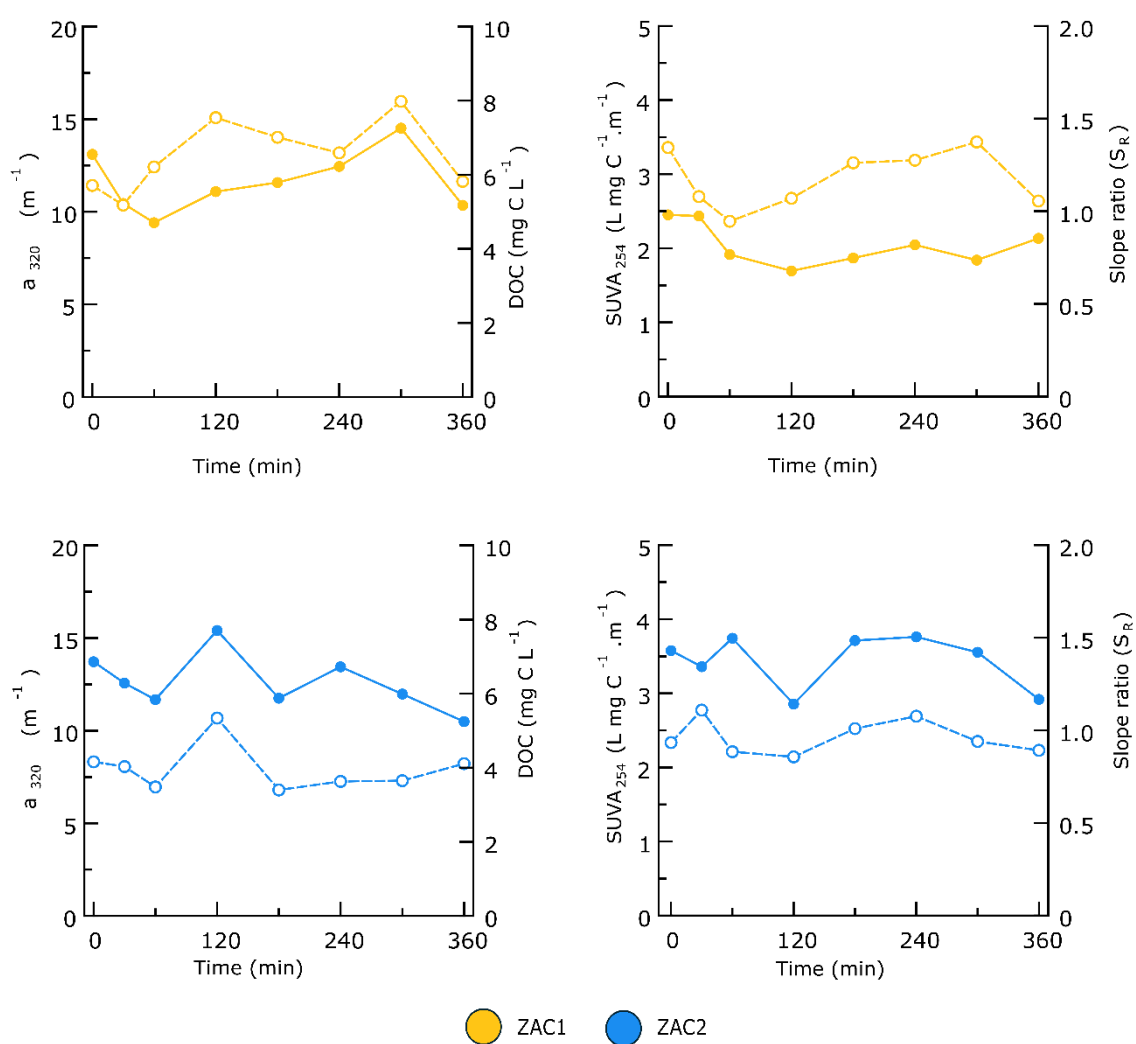


Figure VII.III.10. Benthic flux chamber experiment measurements of dissolved Mn ($\mu\text{g L}^{-1}$) in ZAC1 and ZAC2 from T0 to T7.

In ZAC1, dissolved Mn concentrations declined from 16.2 to 5.3 $\mu\text{g L}^{-1}$ between T0 and T5, followed by a subsequent increase to 14 $\mu\text{g L}^{-1}$ at T6, mirroring the secondary rise observed in total dissolved Fe and nitrate concentrations. This biphasic pattern may reflect an initial removal of dissolved Mn, followed by Mn release or utilization under increasingly reducing conditions. In ZAC2, dissolved Mn increased rapidly within the first 30 minutes, from 18 to 42 $\mu\text{g L}^{-1}$, peaking at 49 $\mu\text{g L}^{-1}$ at T2. Mn concentrations declined steadily to 30 $\mu\text{g L}^{-1}$ by T7. The initial mobilization of Mn is consistent with the rapid redissolution of Mn oxides in the sediments, followed by possible reprecipitation in later stages.



ZAC1 showed a decrease in a_{320} values to T2 (13 to 9 m^{-1}), increasing gradually to T6 (15 m^{-1}) and decreasing at T7 (10 m^{-1}). ZAC2 displayed a slight decrease to T2 (14 to 12 m^{-1}), an increase at T3 (15 m^{-1}), oscillating between 13-11 m^{-1} to T7.

Slope ratios in ZAC1 decreased to T2 (1.3 to 0.9), increased to T6 (1.4) and decreased at T7 (1.1). In ZAC2, slope ratio (S_R) values are lower and oscillated less than ZAC1, with S_R increasing at T1 (0.9 to 1.1), decreasing at T2 (0.9), increasing gradually to T5 (1.1) and decreasing slightly to T7 (0.9).

ZAC1 showed a slight decrease in SUVA_{254} to T3 (2.5 to 1.7) and ranged between 1.8 to 2.1 throughout the BFC experiment. ZAC2 displayed relatively stable SUVA_{254} values from T0 to T2 (3.4-3.7), with a decrease at T3 (2.9), before stabilizing at T4-T6 (3.6-3.8), and decreasing slightly at T7 (2.9).

VII.IV. General Discussion, Conclusions and Future Research

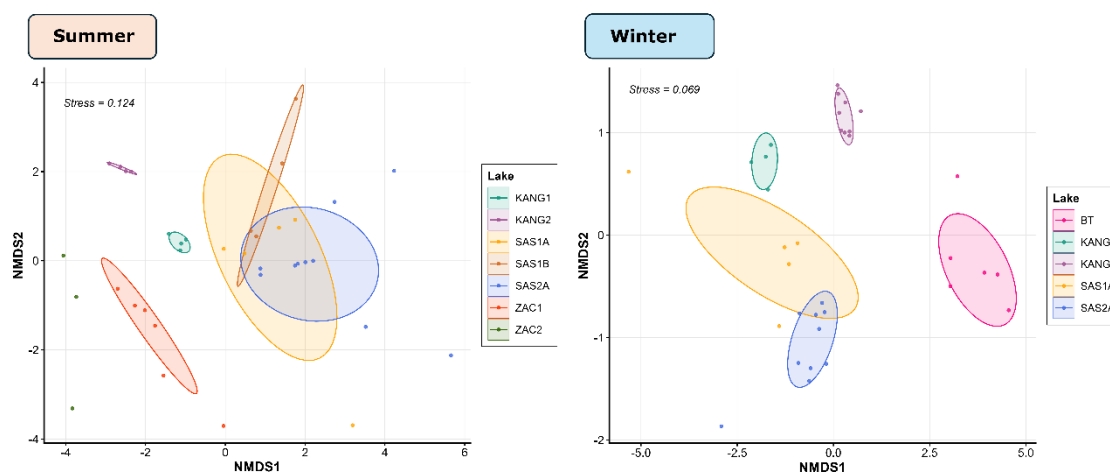


Figure VII.IV.1. Non-metric multidimensional scaling (NMDS) plot of lake groups during summer and winter — overarching biogeochemical parameters: UV-Vis absorbance indices, terminal electron acceptors and pH (only in summer).

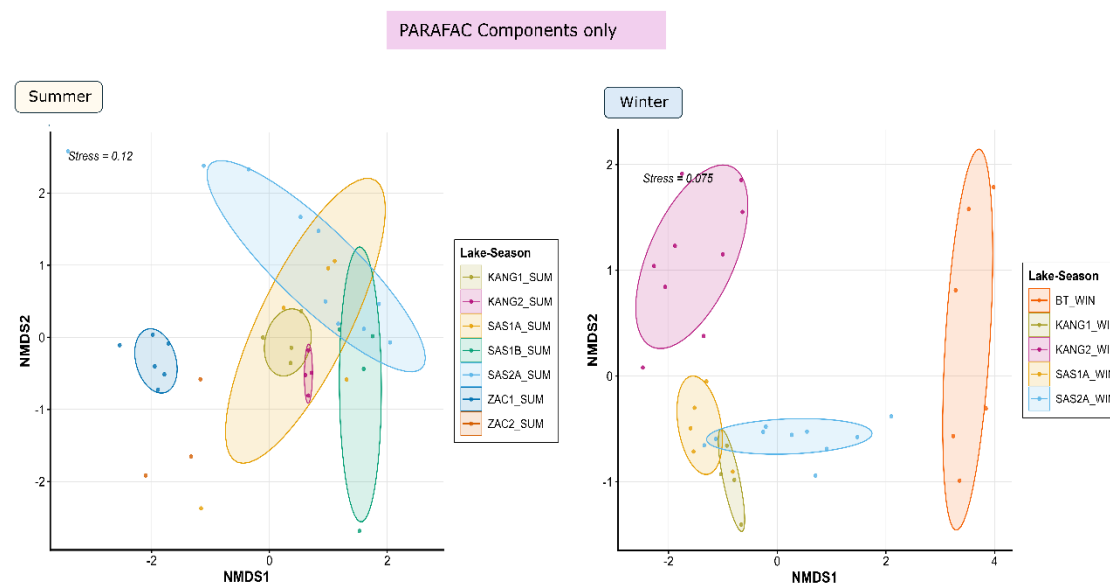


Figure VII.IV.2. Non-metric multidimensional scaling (NMDS) plot of lake groups using only PARAFAC component distribution during summer and winter.

Table VII.IV.1. Principal component analysis (PCA) variable loadings for the first three principal components for summer and winter — overarching biogeochemical parameters.

<i>Summer</i>				<i>Winter</i>			
	PC1	PC2	PC3		PC1	PC2	PC3
Depth	0.226	0.038	0.023	Depth	0.193	0.103	-0.154
S_{289}	-0.358	0.142	-0.263	S_{289}	0.385	0.207	-0.097
S_{382}	-0.262	0.491	-0.011	S_{382}	0.328	-0.101	-0.431
a_{320}	0.386	0.067	0.100	a_{320}	-0.337	0.140	-0.363
SUVA ₂₅₄	0.330	-0.210	0.136	SUVA ₂₅₄	-0.344	0.246	-0.175
S_R	0.076	-0.598	-0.138	S_R	0.371	0.258	0.012
Cl	0.097	0.057	0.664	Cl	-0.127	-0.568	-0.115
NO_3^-	0.097	0.187	-0.243	NO_3^-	-0.088	0.105	-0.222
Mn	-0.277	-0.252	-0.138	Mn	0.389	0.013	-0.272
Fe	-0.245	0.000	0.567	Fe	-0.234	-0.019	-0.664
SO_4^{2-}	-0.378	-0.185	0.162	SO_4^{2-}	0.024	-0.617	0.036
DOC	0.234	0.441	-0.041	DOC	0.324	-0.272	-0.197
pH	-0.368	0.018	0.121				

Table VII.IV.2. Information on the significance of Permutational Multivariate Analysis of Variance (PERMANOVA) and Permutational Multivariate Analysis of Dispersion (PERMDISP) for groups in summer and winter samples — overarching biogeochemical parameters.

Number of Permutations		999		
		R^2	F	p.adj (Pr(>F))
PERMANOVA				
	Summer	0.635	8.419	0.001
	Winter	0.537	8.411	0.001
PERMDISP				
	Summer		2.756	0.033
	Winter		0.732	0.614

Table VII.IV.3. Pairwise comparisons (Bonferroni correction) for lake groups in summer and winter seasons — overarching biogeochemical parameters. * p below 0.1; ** p below 0.05.

Summer						
Lake A		Lake B	F	R^2	p-value	p-value adj.
ZAC1	vs	ZAC2	1.67	0.193	0.182	1.000
ZAC1	vs	SAS2A	21.25	0.603	0.001	0.021**
ZAC1	vs	SAS1A	7.03	0.439	0.007	0.147
ZAC1	vs	SAS1B	20.01	0.714	0.004	0.084*
ZAC1	vs	KANG1	1.45	0.154	0.265	1.000

ZAC1	vs	KANG2	2.80	0.259	0.133	1.000
ZAC2	vs	SAS2A	11.79	0.517	0.003	0.063*
ZAC2	vs	SAS1A	4.21	0.412	0.080	1.000
ZAC2	vs	SAS1B	17.21	0.775	0.030	0.630
ZAC2	vs	KANG1	0.80	0.138	0.348	1.000
ZAC2	vs	KANG2	2.25	0.311	0.153	1.000
SAS2A	vs	SAS1A	2.40	0.156	0.132	1.000
SAS2A	vs	SAS1B	4.08	0.254	0.037	0.777
SAS2A	vs	KANG1	13.70	0.533	0.001	0.021**
SAS2A	vs	KANG2	12.52	0.511	0.002	0.042**
SAS1A	vs	SAS1B	0.57	0.075	0.543	1.000
SAS1A	vs	KANG1	4.46	0.389	0.069	1.000
SAS1A	vs	KANG2	3.88	0.357	0.078	1.000
SAS1B	vs	KANG1	16.66	0.735	0.039	0.819
SAS1B	vs	KANG2	14.00	0.700	0.023	0.483
KANG1	vs	KANG2	0.72	0.107	0.475	1.000

Winter

Lake A		Lake B	F	R2	p.value	p.adj
SAS1A	vs	SAS2A	2.05	0.136	0.128	1.00
SAS1A	vs	KANG1	0.53	0.071	0.566	1.00
SAS1A	vs	KANG2	23.64	0.663	0.001	0.01**
SAS1A	vs	BT	34.96	0.795	0.002	0.02**
SAS2A	vs	KANG1	1.64	0.120	0.191	1.00
SAS2A	vs	KANG2	4.28	0.201	0.005	0.05**
SAS2A	vs	BT	8.56	0.379	0.001	0.01**
KANG1	vs	KANG2	18.85	0.631	0.001	0.01**
KANG1	vs	BT	30.06	0.790	0.004	0.04**
KANG2	vs	BT	6.28	0.326	0.013	0.13

Table VII.IV.4. Principal component analysis (PCA) variable loadings for the first three principal components for summer and winter — PARAFAC components.

<i>Summer</i>				<i>Winter</i>			
	PC1	PC2	PC3		PC1	PC2	PC3
Depth	0.007	0.683	0.421	Depth	0.320	0.141	0.927
HIT1	-0.540	0.271	0.171	HIT1	-0.497	-0.257	0.157
HM1	0.428	0.137	-0.536	HM1	0.459	0.083	-0.083
HT2	-0.468	-0.127	-0.470	HT2	-0.475	0.350	0.166
P2	0.004	-0.650	0.505	P2	0.330	0.547	-0.286
P1	0.553	0.047	0.175	P1	0.323	-0.697	-0.021

Table VII.IV.5. Information on the significance of PERMANOVA and PERMDISP for groups in summer and winter samples — PARAFAC components.

Number of Permutations		999		
		R ²	F	p.adj (Pr(>F))
PERMANOVA				
	Summer	0.310	2.170	0.087
	Winter	0.277	2.776	0.049
PERMDISP				
	Summer		1.842	0.131
	Winter		1.752	0.169

Table VII.IV.6. Information on the Akaike information criterion (AIC) and Bayesian Information Criterion (BIC) values of the reciprocal model fit and linear model fit.

Carbon weight (%) — HI		R ²	0.54	
		AIC	BIC	
	Reciprocal	362.7	368.2	
	Linear	375.8	381.4	
$\delta^{13}\text{C}$ — HI		R ²	0.52	
		AIC	BIC	
	Reciprocal	155.0	160.6	
	Linear	181.0	186.6	

Table VII.IV.7. Table containing the estimate, standard error, t-value and p-value for the two parameters in the reciprocal model produced for the two variable pairings.

$y=a+(b/x)$				
Carbon weight (%) — HI				
	Estimate	Std. Error	t-value	Pr(> t)
<i>a</i>	44.56	2.67	16.67	2.00 x10 ⁻¹⁶
<i>b</i>	-2.78	0.38	-7.24	4.51 x10 ⁻⁰⁹
$\delta^{13}\text{C}$ — HI				
	Estimate	Std. Error	t-value	Pr(> t)
<i>a</i>	-26.87	0.294	-91.52	2.00 x10 ⁻¹⁶
<i>b</i>	-0.295	0.042	-6.98	1.09 x10 ⁻⁸

Supplementary Note VII.IV.1 (SN.VII.IV.1): Principal component analysis across lakes in permafrost zones

An exploratory PCA was conducted on water sample chemistry from thermokarst lakes during summer and winter separately. The selected variables aimed to explore biogeochemical patterns among lakes during summer were: depth, pH, DOC, S_R , $SUVA_{254}$, a_{320} , Fe, Mn, NO_3^- , SO_4^{2-} , Cl^- , S_{289} and S_{382} . In winter, the variables were the same, except for pH.

In the summer PCA, the first three principal components (PCs) explained 71.8% of cumulative variance (PC1: 41.3%; PC2: 16.7%; PC3: 13.8%). Positive and negative variable loadings for each PC are shown in Table VII.IV.1.

PC1 separated $SUVA_{254}$, a_{320} , DOC and depth (positive loadings) from pH, S_{289} , S_{382} , sulfate, dissolved Fe and Mn (negative loadings). A gradient from more acidic environments rich in DOC, CDOM and high DOM aromaticity to conditions with higher dissolved TEAs (Mn, Fe, and sulfate) with higher algal DOM inputs. PC2 separated conditions with higher S_{382} and DOC concentrations (positive loadings) from waters with higher S_R , $SUVA_{254}$ and dissolved Mn (negative loadings). This PC reflects a transition from more DOC-rich environments with increased terrestrially derived DOM (S_{382}), towards lower average molecular weight DOM, higher DOM aromaticity and dissolved Mn. PC3 divided environments with high dissolved Fe and chloride (Cl^-) concentrations (positive loadings) from higher nitrate and S_{289} (negative loadings). This gradient reveals a separation of environments with higher chloride and Fe, from water conditions with higher nitrate concentrations and increased algal derived DOM (S_{289}).

The PCA clearly illustrated the division between SAS lakes and the lakes in Zackenberg and Kangiqsualujjuaq, aligned with positive PC1 loadings largely driven by higher DOC concentrations, DOM aromaticity ($SUVA_{254}$) and CDOM content (a_{320}).

PERMANOVA indicated lake groupings explained 63.5% of the observed variance (F-stat= 8.42; p = 0.001), with PERMDISP (F-stat= 2.76; p = 0.033) indicating that group dispersions also differed, suggesting contrasting heterogeneity within lakes contributed to the observed differences in PERMANOVA, rather than just mean centroid differences. The intra-lake heterogeneity is marked by the increased dispersion in multivariate space, particularly noticeable in SAS1A and SAS2A (Fig. VI.2).

Pairwise comparisons (Bonferroni corrected) were conducted on summer samples, with ZAC1 being statistically different from SAS2A and SAS1B (adjusted $p \leq 0.05$). The Bonferroni correction adjusts for increased multiple comparisons by restricting the significance criteria. This greatly reduces the risk of false positives, however will restrict the significance to the most extremely distinct endmembers. Therefore, pairwise comparisons with adjusted p-values between 0.1 and 0.05 were considered as potentially statistically different systems. In this sense, SAS2A was shown to be potentially dissimilar from ZAC2 and KANG1 (Table VII.IV.3).

In the winter PCA, the first three PCs explained 79.7% of cumulative variance (PC1: 47.9%; PC2: 20.5%; PC3: 11.3%). Positive and negative variable loadings for each PC are shown in Table VII.IV.1.

PC1 separated S_{289} , S_{382} , S_R , DOC and dissolved Mn (positive loadings) from $SUVA_{254}$, a_{320} , dissolved Fe (negative loadings). A gradient from DOC-rich waters with lower average molecular weight DOM, and higher dissolved Mn to conditions with higher CDOM, DOM aromaticity and dissolved Fe. PC2 separated conditions with higher S_{289} , $SUVA_{254}$, S_R (positive loadings) from waters with higher sulfate and chloride concentrations, and higher DOC concentrations (negative loadings). This PC reflects a transition from waters with increased algal derived DOM (S_{289}), lower average molecular weight DOM and higher DOM aromaticity, towards chloride and sulfate-rich waters with high DOC waters. The separation promoted by PC3 was mostly between null loadings and very negative loadings. In this sense, the most negative loadings were nitrate, dissolved Fe, Mn, S_{382} , and a_{320} .

The winter PCA clearly separated the SAS lakes (SAS1A and SAS2A) and KANG1 from BT and KANG2. SAS lakes overlapped partially, indicating higher within-region similarity. KANG1 grouped more closely with SAS lakes than KANG2, likely driven by higher dissolved Fe, DOM aromaticity and CDOM content. Interestingly, BT was distinctly isolated as a group in multivariate space, highlighting potential contrasting dynamics compared to other lakes. Compared to other winter lakes, BT exhibited elevated S_R (0.91 ± 0.03), S_{289} (0.019 ± 0.0004), S_{382} (0.021 ± 0.0004), high DOC (91 ± 20 mg C L⁻¹) and dissolved Mn (2.0 ± 0.2), while exhibiting lower DOM aromaticity ($SUVA_{254}$: 0.67 ± 0.21), CDOM content (a_{320} : 43 ± 7) and dissolved Fe (3.9 ± 4.4).

PERMANOVA indicated winter lake groupings explained 53.7% of the observed variance ($F\text{-stat}=8.41$; $p=0.001$) with PERMDISP not meeting the significance threshold ($F\text{-stat}=0.73$; $p=0.61$), suggesting contrasts among lakes were primarily due to mean centroid differences. Contrary to summer, group dispersions were not overly dissimilar between lake groups due to the increased intra-lake homogeneity in winter under-ice conditions (Table VII.IV.2).

Pairwise comparisons (Bonferroni corrected) in winter lakes, revealed the three lakes SAS1A, SAS2A and KANG1 were statistically distinct from KANG2 and BT lakes (adjusted $p \leq 0.05$). These results suggest KANG2 and BT undergo different winter biogeochemical regimes compared to KANG1 and the two SAS lakes. The high S_{289} range in BT during winter indicates a considerable increase in smaller DOM structures (commonly associated with algal production), despite lower CDOM and higher DOC ranges (Fig. VII.IV.1), suggesting enhanced DOM microbial transformation in the water column. In turn, this may suggest BT could be the most active thermokarst lake during winter.

Supplementary Note VII.IV.2 (SN.VII.IV.2):

An exploratory approach was taken to compare variables from all sediments across sites and seasons to assess whether biogeochemical relationships could be discerned. Two main trends were observed, moderately approximating a reciprocal function: 1) Humification index (HI) — calculated from FTIR spectra — and the percentage of organic carbon weight (%); 2) isotopic $\delta^{13}\text{C}$ signature and HI.

In the first case, the HI exhibited a moderate nonlinear reciprocal relationship with the percentage of organic carbon weight (%) in a sediment sample ($R^2=0.54$). This moderate fit suggests a biogeochemical coupling, where organic carbon content plateaus at higher HI, and decreases rapidly with relatively small changes at low HI in thermokarst lake sediments. In this context, it makes sense to consider samples with lower HI would have been likely to have undergone a greater extent of NOM degradation.

In the second case, the HI showed a moderate reciprocal model fit with $\delta^{13}\text{C}$ signatures ($R^2=0.52$). This suggests that isotopic $\delta^{13}\text{C}$ signatures shift (to lighter signatures) abruptly with low HI values, and plateaus in the terrestrial $\delta^{13}\text{C}$ signature range towards higher HI.

This is likely influenced by contrasts in NOM sources. Biogeochemical pathways of carbon fixation of oxidized methane are likely to be driving shifts to more ^{13}C -depleted signatures.

The reciprocal models captured the overarching relationship between both variable pairings, with the majority of observations falling within the 95% prediction interval. Although the models explain a considerable portion of variance, the variability in the scatterplot likely reflects additional biogeochemical controls not explained by the relationship. Despite their moderate fit and limited sample size ($n=47$), these relationships merit consideration when focusing on benthic processes in dynamic natural systems like thermokarst lakes.

In both reciprocal model fits, the Akaike information criterion (AIC) and Bayesian Information Criterion (BIC) values of the model were lower than the linear model alternatives (Table VII.IV.6). The parameters of the reciprocal model for both fits can be found in Table VI.IV.7, while the scatterplots with their overlain model (including 95% confidence interval and 95% predictive interval) can be found in Fig. VI.4.

VII. References

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