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Dissolved organic carbon composition and reactivity in arctic Canadian lakes

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Master thesis, 30 credits, in *Physical Geography and Ecosystem Science*

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Abstract

Freshwater systems are active components of the global carbon cycle and contribute to global CO_2 emissions. Freshwater studies in the Arctic are underrepresented, especially regarding lakes and their Dissolved Organic Carbon (DOC) reactivity and composition dynamics. DOC is a main part of DOM (dissolved organic matter), which drives processes central to carbon cycling, influences chemical and biological characteristics, for example bacterial production (BP) and bacterial respiration (BR), and lake DOC is usually comprised of allochthonous (terrestrial) and autochthonous (algal) sources. Within DOM, CDOM (coloured DOM) and FDOM (fluorescent DOM) are key components, which can impact productivity and act as indicators of DOC composition. The remote area of Churchill Canada is an arctic region with an abundance of lakes, and this study aimed to investigate the relationships between microbial DOC reactivity and DOC composition in these lakes, and to improve our understanding of the influence of environmental properties, and potential CO_2 emissions. In this study, we used documented methods to investigate DOC in 54 lake samples, primarily including dark laboratory incubations over a 28-day period. BP and BR were measured using leucine uptake and dissolved oxygen concentrations respectively, while DOC composition was investigated using fluorescent spectroscopy and PARAFAC. Three fluorescent components were identified: C1 (Terrestrial humic), C2 (Marine/microbial humic), and C3 (algal protein-like). Weak relationships between BP, BR, and the components were found, while lake area proved to be a control on DOC amount and variations in reactivity and composition. As expected CDOM and FDOM showed net production, although this varied, as C3 had the most production especially in low CDOM lakes. pCO_2 (partial pressure of CO_2) and potential CO_2 emissions were linked partially to BR, and DOC, but potentially photoreactivity is important. The results from this study show that the role of Arctic lakes remains highly variable and is closely linked to site specific conditions. Further analysis of these and other lakes, across different environmental and hydrological conditions are suggested, to form a clearer view of the role of Arctic lakes in the carbon cycle, and the complex relationship between DOC reactivity and composition.

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Hypothesis 1: a) Protein-like components will correlate positively with bacterial production. b) Protein-like components will correlate positively with DOC loss.

Hypothesis 2: a) Humic-like components will correlate negatively with bacterial production. b) Humic-like components will correlate negatively with DOC loss.

Hypothesis 3: DOC, CDOM, bacterial respiration, and humic-like components will correlate positively with pCO_2.

1. Introduction

Inland freshwaters have become a focus within global carbon cycle research, due to a shift from thinking of them only as a passive transporter of carbon from land to sea and instead as a transport of carbon along with active carbon exchange with the atmosphere and sediment (de Wit et al, 2018). Of particular importance is the magnitude of carbon that is processed and transported from inland waters, including outgassing to the atmosphere at around 1.85 ± 0.5 Pg CO_2, and storage in sediments at around 0.15 ± 0.1 Pg of carbon (Tranvik et al, 2018, Regnier et al., 2022). Lakes in particular account for only 0.30 Pg CO_2 emissions, but this estimate poorly considers the CO_2 release from sensitive high latitude Arctic lakes (Karlsson et al, 2021).

Despite the known sensitivity of the Arctic to climate change, which impacts limnological processes and carbon cycling (Sobek et al, 2014), the Arctic is still under-represented in Arctic ecosystem studies, with 31% of the studies to date centred around only two study sites, Abisko (Sweden) and Toolik Lake (USA), contributing to scientific bias (Metcalfe et al, 2018). Furthermore, studies on lake DOC (Dissolved Organic Carbon) composition and reactivity, including the potential multiple sources of DOC, deduced through optical and fluorescent properties, and the potential of the DOC to support metabolic processes such as bacterial production and respiration, are limited in Arctic lakes.

The remote area of Churchill Canada is an Arctic region with a high proportion of lakes, situated on continuous permafrost, and previous research has focused mainly on climate change, methane, and lake-ice thickness (Rouse et al, 1995., Duguay et al, 2002., Duguay and Lafleur, 2003., Macrae et al, 2004., Macrae et al, 2014). These clear water lakes are still relatively under researched, especially regarding DOC composition and reactivity. Therefore, a study on these lakes could improve our understanding of how bacterial processes relate to DOC and lake characteristics in Arctic Canada, and to reduce the scientific bias of Arctic studies by selecting an under-studied geographical region.

Dissolved organic matter (DOM) is a complex mixture found in nature which is used and incorporated into different aquatic systems (Larn et al, 2007., Mostovaya et al, 2016., Bastidas Navarro et al, 2022). DOM is measured in carbon units, so within this project DOM and DOC are used synonymously, as we are focusing on dissolved carbon and its analysis rather than all DOM molecules. This should be considered as, for example, fluorescence analysis reveals composition features based on all DOM, but this is referred to as DOC composition in our study.

DOC is a main part of DOM and is a key variable that drives processes central to carbon cycling (Toming et al, 2020). DOC has a strong influence on chemical and biological characteristics and usually represents the largest pool of carbon in the water column (Seekell et al, 2018). However, the amount of DOC in lakes, and its characteristics, vary regionally, due to the

differences in e.g., climate, carbon input, properties of lake and catchment (Smith et al, 2018). Lake area in particular has control over the concentration of DOC, for example because smaller lakes with shorter water residence times are expected to have relatively higher concentrations of DOC and higher reactivity (Evans et al, 2017., Staehr et al, 2012).

DOM has two main sources: terrestrial (allochthonous) and algal-derived (autochthonous). Loading of allochthonous DOM has been linked to lake browning, caused by high amounts of CDOM (Coloured dissolved organic matter), and can lead to decreases in lake productivity, and increases in respiration (Berggren et al, 2020). FDOM (fluorescent dissolved organic matter) is a component of DOM which fluoresces when irradiated by blue and ultraviolet light (Stedmon and Bro, 2008). Fluorescence spectroscopy can be used to identify humic (usually allochthonous) and protein-like (usually autochthonous) components within FDOM, and amounts of CDOM (Cammack et al, 2004., Berggren et al, 2020., Blanchet et al, 2022).

Dark laboratory incubations of lake samples can be used to record bacterial production, bacterial respiration, and fluorescence data and allow comparisons. Berggren et al (2020) found, from 101 subarctic lake samples in Sweden, that FDOM and CDOM were net produced across 28-day dark incubations, with higher FDOM production in lower CDOM lakes. Furthermore, the relationship between components and reactivity was complicated, but bacterial production and DOC loss was found to positively correlate with protein-like components, while humic-like components were negatively correlated (Berggren et al, 2020).

The pCO_2 (the partial pressure of CO_2) in lakes is a key driver of the carbon flux across the lake surface, affected by internal and external processes, and is tied to lake CO_2 emissions (Sobek et al, 2005). pCO_2 has been found to correlate well with DOC levels, as DOC acts as a substrate for microbial respiration, increasing CO_2 saturation in lakes (Sobek et al, 2005). Furthermore, humic-like FDOM components have been found to correlate well with pCO_2, which can be linked to the input of allochthonous DOC (Zhang et al, 2023). Therefore, allochthonous DOC, linked with higher CDOM and humic-like components, should positively correlate with pCO_2 and bacterial respiration.

With our study we can investigate and confirm, with dark incubations of arctic clearwater lakes, the complex relationships between reactivity and fluorescence, DOC and pCO_2, which has previously been reported in subarctic lakes.

1.1. Project aim, and hypotheses

Aim: To define the relationships between microbial DOC reactivity and DOC composition in lakes of the Churchill region of arctic Canada, and to further link DOC reactivity and composition to variations in pCO_2 and lake characteristics.

Hypotheses:

Hypotheses 1, 2 and 4 are chosen based on a previous study on dark incubations of sub-arctic lake samples within Sweden, which demonstrated the production of CDOM and FDOM, with FDOM components showing positive and negative relationships with bacterial production and DOC loss (Berggren et al, 2020). Whereas hypothesis 3 is proposed based on multiple lake studies, as with higher amounts of allochthonous DOC we can expect higher CDOM and bacterial respiration, increasing pCO_2 levels (Sobek et al, 2005., Zhang et al, 2023).

1. a) Protein-like components will correlate positively with bacterial production.
   b) Protein-like components will correlate positively with DOC loss.
2. a) Humic-like components will correlate negatively with bacterial production.
   b) Humic-like components will correlate negatively with DOC loss.
3. DOC, CDOM, bacterial respiration, and humic-like components will correlate positively with pCO_2.
4. CDOM and FDOM will be net produced over the incubation period, with more clear water lakes having higher production of FDOM.

2. Literature overview and theoretical background

2.1. Global Carbon Cycle and Inland waters

Carbon is an important element, essential to life and accounting for ~50% of the dry weight of living organisms (Ussiri and Lal, 2017). Carbon is cycled between different reservoirs and pathways, such as oceans, land, and the atmosphere, and undergoes constant chemical, physical and biological processes (Falkowski et al, 2000., Ussiri and Lal, 2017). The human and natural interactions with the carbon cycle and atmospheric warming have been actively researched, with a greater understanding in more recent years, especially with more advanced data and models (Arrhenius, 1896., Crisp et al, 2022).

Outdated conceptualisations of the global carbon cycle did not consider inland waters as important reactive transport pathways for carbon cycling (Cole et al, 2007), but more recent literature has recognised their importance, as carbon can be transported to the atmosphere and sediments while transitioning through inland waters (Regnier et al, 2022). This has been represented in recent carbon cycle conceptualisations (Fig. 1).

Lakes, for example, have been considered occasionally in early literature, for example to evaluate how much organic carbon could be accumulated in lakes and reservoirs, which was thought as potentially an important part of the global carbon cycle and budget (Mulholland and Elwood, 1982). Some of the main reasons that freshwater systems have been largely underestimated are due to, e.g., how little of the terrestrial surface they cover, around 3.7% of the non-glaciated surface (Verpoorter et al, 2014., Harkort and Duan, 2023), and limited availability of freshwater measurements.

Inland waters exist in various forms, which includes lakes, swamps, rivers, reservoirs, wetlands, and ponds. Together they are a source of freshwater resources and provide a breath of ecosystem services important for ecology and humans (Harkort and Duan, 2023). Inland waters have been recognised as important hot spots for carbon, and have a key role in global biogeochemical cycles, as they are very active areas of organic matter degradation, compared to marine and terrestrial systems (Catalán et al, 2015). One of the most important advancements for how we view inland waters was the shift from thinking of them as passive pathways to an active pipe, originally proposed by Cole et al. 2007 (Fig. 2). Since then, more in depth studies following this thinking have continued to be published, and our understanding of inland waters in the biogeosphere and carbon cycle has continued to improve, but there is still more that can be discerned (Tranvik et al, 2018). Within inland waters, lakes could be considered to be the most important component and are strongly tied to climate and environmental change (Shang et al, 2023). Nonetheless, views differ regarding this importance, as studies have shown that rivers are larger CO_2 emitters globally (Raymond et al, 2013).

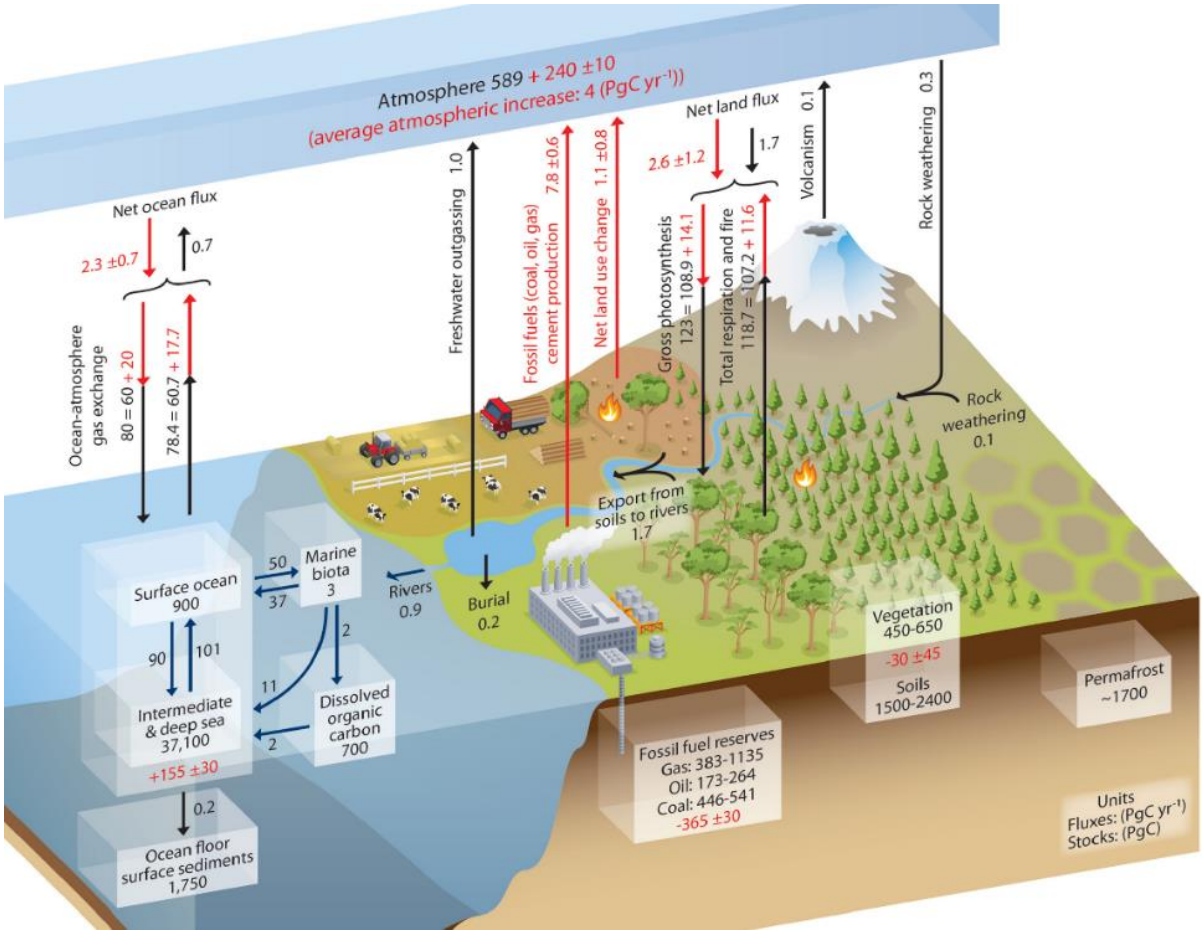

Fig. 1. Graphic of the global carbon cycle. Black arrows represent pools and fluxes before the industrial era (Pg C). Red represents anthropogenic average flux changes from 2000-2009. Graphic from chapter 6, Fig. 6.1, in IPCC AR5 (2014).

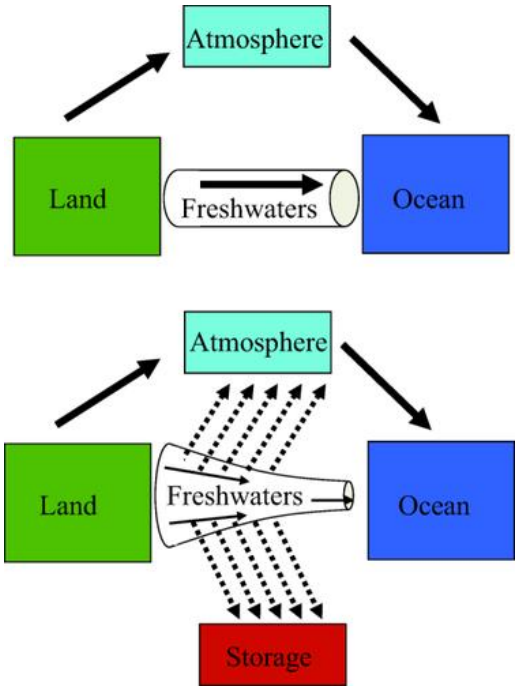

Fig. 2. Graphic showing the previously thought passive pipe for inland waters on top, and the more recently recognised active funnel on the bottom. Arrows represent transport or fluxes of carbon (Cole et al, 2007).

2.2. The Arctic and lakes

The Arctic can be defined as the part of the Earth which lies above the Arctic Circle (66.6°N), or as having mean surface temperatures less than 10°C (McGurie et al, 2009). For example, Canada can experience tundra environmental conditions in areas as low as 54°N, such as the area just south of the Hudson Bay (Schindler and Smol, 2006). The Arctic terrestrial ecosystem contributes around 25% of the total vegetated surface on earth, and contains approximately 33% of earth's terrestrial carbon, including around 40% of close-surface labile carbon in soil (McGuire et al, 2009). Arctic environmental changes are the most rapid on Earth, and due to climate change, the Arctics condition is fluctuating rapidly, shifting vegetation, impacting terrestrial movement of carbon, and lake productivity (Sobek et al, 2014., Bruhwiler et al, 2021). Increased uptake of carbon and CO_2 in the Arctic has been argued, but alternatively increased respiration and CO_2 emissions have been suggested (Bruhwiler et al, 2021).

Lakes are more common in the Arctic than other regions on Earth (Elder et al, 2018). They account for up to 50% of the surface within high latitude regions (Riordan et al, 2006), and in some Arctic regions these ponds and lakes can make up almost 90% of the land surface area (Wang et al, 2016). These lakes can vary from deep stratified lakes with anoxic water, to shallow and nutrient rich ponds in tundra (Vincent et al, 2013). Small shallow lakes, with lower water residence times, are far more abundant and if considered together they still add up to a very significant total volume (Vincent et al, 2013., Christensen et al, 2007), and act as areas of high aquatic carbon cycling (Evans et al, 2017). Smaller lakes are also less exposed to wind, receive less sunlight, contain more algal biomass than large lakes, and have been observed to emit significant amounts of terrestrially derived carbon through CO_2 emissions (Staehr et al, 2012). Thus, most small lakes are thought to be overall heterotrophic or net sources of CO_2 (McGuire et al, 2009).

All lakes in the Arctic experience more extreme changes in solar radiation compared to lower latitudes (Vincent et al, 2013). Low temperatures across the year have a key control on all ecological, chemical, and biochemical processes and reactions within lakes and in their catchments (Vincent et al, 2013). Further decreasing impacts on soil weathering processes, which reduces the amount of nutrients exported from catchments into lakes, leading to mainly oligotrophic states with limited algal biomass (Vincent et al, 2013). Also, Arctic lakes have very limited periods of no ice coverage during the summer, further limiting algal productivity across the year (Chételat et al, 2010). This seasonal freezing and thawing of lake ice has an important effect on biogeochemical and hydrological processes (Duguay et al, 2002).

Microbial communities and bacteria are key components of Arctic lakes, sediments, and soils, and drive biogeochemical cycles within lakes (Wang et al, 2016). Lakes in the Arctic are also at risk of climate change, as with changes in the availability of DOC and nutrients, phytoplankton production could be enhanced while algal production could be reduced, which

will affect lakes in different ways depending on how the lakes are structured currently (Chételat et al, 2010).

2.3. Dissolved Organic Matter

DOM molecular composition is very dynamic and variable, representing its process time within lakes, its source, and biological reactivity (Cory et al, 2007). A large part of DOM is usually humic substances, which are medium to high weighted fulvic and humic acids, and the smaller part of DOM is usually low weight compounds, for example amino acids (Blanchet et al, 2022). Lakes and rivers which contain high amounts of DOM can have a brown or yellow colour since ultraviolet (UV) light is absorbed by DOM (Evans et al, 2005). Microbial activity in lakes is very important in association with DOM, as bacteria uses DOM as an energy source, and microbial degradation of DOM can induce biogeochemical and ecological effects from aquatic metabolism changes (Tranvik, 1992., Guillemette et al, 2013). Microbial degradation in freshwater systems differs depending on, water residence time, nutrient concentrations, light availability, dissolved ions, and DOM characteristics (Bastidas Navarro et al, 2022).

2.4. DOC reactivity

DOC turnover is linked to the bioavailability of its' different constituents and can cover short (minutes) to long (millenia) timescales (Del Giorgio and Davis, 2003). The DOC in unproductive lakes is usually composed mostly by DOC originating from terrestrial production in the catchment (Jansson et al, 2008), whereas the autochthonous DOC can only contribute a small amount of the total DOC (Jansson et al, 2008). When DOC is confined from freshwater inputs, for example in a laboratory, the bioavailability or the proportion of DOC which can be microbially processed, usually declines as the present microbial communities consume the most labile DOC first (Koehler et al, 2012), with the lability of the DOC referring to the DOC which is removed by bacteria over time (Del Giorgio and Davis 2003). By studying the decay of DOC within lake samples, the degradation of more labile (easier to degrade) DOC and more recalcitrant DOC (difficult to degrade) can be identified (Kritzberg et al, 2004). This is linked to bacterial consumption of both autochthonous and allochthonous DOC, as the lability and the chemical properties of these sources can differ, nutrient usage and bacterial availability can change, with the destination of the processed and unprocessed DOC varying (Bastidas Navarro et al, 2022).

Decomposition of DOC is influenced by intrinsic properties (aromaticity, size, complexity), DOC concentrations, photochemical reactions, and extrinsic factors (nutrients, temperature, oxygen, microbial composition) (Bastviken et al, 2004., Koehler et al, 2012). For example, in some 26 clearwater lakes, 14% of the DOC was microbially mineralised over 2 weeks, compared to 2% in brownwater lakes (Søndergaard and Middelboe, 1995). In eutrophic lakes, autochthonous DOC will be more dominant, whereas oligotrophic lakes are more dominated

by the loading of allochthonous DOC which has a large role in lake communities (Smith and Prairie, 2004). Furthermore, the impact of increasing DOC concentrations in lakes can vary, as it has been found that primary production can be increased as DOC protects phytoplankton from UV radiation and increases dissolved CO_2 through photochemical and bacterial processes (Lapierre et al, 2013), but with increasing DOC we can find decreasing primary productivity in boreal lakes, while increasing in arctic lakes (Seekell et al, 2015). Also, concentrations and DOC processes vary in different sized lakes, but this can depend on the catchment area to lake surface area ratio, as smaller lakes could receive similar amounts of allochthonous DOC as large lakes but resulting in larger concentrations in smaller lakes (Staehr et al, 2012). Furthermore, within these high DOC small lakes, we expect less penetration of light, higher planktonic respiration with lower planktonic production, and overall higher ecosystem respiration as allochthonous DOC is mineralised (Staehr et al, 2012., Seekell et al, 2015). Also, with less residence time in small lakes, there is less microbial and photochemical degradation, with small lakes also producing autochthonous DOC to some extent but overall being dominated more by the input of allochthonous DOC (Staehr et al, 2012).

DOC is transformed in three main ways; photochemical reactions from sunlight, biological reactions, and flocculation, which are all controlled by the chemical composition of the DOC (the intrinsic factor), but also by the physical and biological (extrinsic) factors (Anderson et al, 2019). For example, high temperatures can increase DOC breakdown, production of bacterial biomass (secondary production), and carbon mineralisation (Del Giorgio and Cole, 1998., Pomeroy and Wiebe, 2001., Adams et al, 2010). DOC reactivity in controlled laboratory conditions usually refers to the photochemical or biological mineralisation rates, which is evaluated over time, and is different to the labile properties of DOC, as that refers to the different reactivity potential within DOC (Berggren et al, 2022). DOC reactivity relies on both the intrinsic and extrinsic factors, for example photoreactive DOC won't be degraded in a laboratory when there is no sunlight, or biologically reactive DOC won't be affected in a nutrient poor water sample (Smith and Prairie, 2004., Berggren et al, 2022). One way in which DOC is represented is through a reactivity continuum model, which assumes that the DOC contains an infinite amount of reactive components and describes DOC decomposition using decay coefficients (Mostovaya et al, 2016). The reactivity of DOC can decrease as a result of travel time from the field to the lab, and further with storage time before experiments begin, due to some removal of bioavailable compounds (Catalán et al, 2016), which should be considered.

Bacterial Production (BP) is supported by allochthonous DOC in lakes, and therefore has an effect on the overall composition and productivity of lake food webs (Blomqvist et al, 2001). Although, autochthonous DOC has been considered as the main source for BP too (Kritzberg et al, 2005). With increasing allochthonous DOC, lakes become more undersaturated with oxygen and net heterotrophic, and Bacterial Respiration (BR) can become dominating in these

unproductive net heterotrophic lakes, meaning they become sources of CO_2 rather than sinks (Jansson et al, 2008). Sub-arctic lake studies have found BP to be highly variable across different lakes, for example due to nutrient concentrations (Karlsson et al, 2002., Berggren et al, 2020). One way of representing the production and respiration rates of DOC in lakes is by combining BP and BR to calculate Bacterial Growth Efficiency (BGE) (Equation 1), which gives an overview of the extent to which DOC is assimilated by bacteria (Del Giorgio and Cole, 1998). DOC either becomes bacterial biomass, represented by BP, or respired to inorganic carbon, represented by BR (Kritzberg et al, 2005). The part of the DOC which supports bacterial growth (the BGE) controls the relative magnitude of BR and BP (Kritzberg et al, 2005). For example, Arctic Fjords have been found to have low BGE from 7% to 10% (Paulsen et al, 2017).

Equation 1 BGE calculation, (Del Giorgio and Cole, 1998)

$$BGE = BP/[BP + BR]$$

2.5. DOC composition dynamics – CDOM and FDOM

The optical properties of DOM are based on their chemical composition (Stedmon et al, 2003). Chromophoric dissolved organic matter (CDOM), represents the coloured portion of DOC, and can influence primary productivity, reduce light availability, but can lower UV stress (Jiang et al, 2012). CDOM is a fraction of DOC which absorbs UV and visible light, which depending on its concentration within a lake, can negatively impact primary production, but positively impact secondary production by bacteria (Zhang et al, 2007). Freshwater systems, such as lakes, obtain a significant amount of CDOM from their terrestrial surrounding and catchment, with many lakes showing increasing amounts of CDOM and browner water colour in recent decades, linked to climate change and local environmental changes in hydrology and deposition (Bastidas Navarro et al, 2022). In lakes, CDOM or water colour is typically measured using the absorbance coefficients at wavelengths 254 nm or 440 nm (Fasching et al., 2014). With increasing CDOM, there are changes in biological reactivity and composition of DOM (Berggren et al, 2020). Exposure to sunlight and oxidation of aromatic compounds can change CDOM, leading to reduced aromaticity and humifaction (Jiang et al, 2012). The relationship between aromaticity and photoreactivity is commonly deduced from the specific UV absorption (SUVA) (Berggren et al, 2022). However, some studies have found that SUVA and photoreactivity correlated weakly (Cory et al, 2013) and could be linked to extrinsic controls for example pH (Panneer Selvam et al, 2019).

Northern lakes are usually oligotrophic, and are influenced by DOC and its components, which is imported into lakes from their terrestrial surroundings (Thrane et al, 2014). Lakes containing high DOC and CDOM have production which is light-limited, but this can vary depending on

the area and depth of the lake (Seekell et al, 2015). Studies performed in boreal, and sub arctic regions have shown that with increased CDOM from the terrestrial land surface, lake composition changes from mostly fast-cycled protein-like components to majority slow-cycled humic-like components (Guillemette and del Giorgio, 2011., Koehler et al, 2012). The interactions between bacteria and different DOM components can be complicated, for example, one study on sub-arctic lakes found that with differing amounts of terrestrial CDOM, there are trends in in-lake microbial production of further CDOM and fluorescent DOM (Berggren et al, 2020).

A further fraction of CDOM is what fluoresces and known as fluorescent DOM (FDOM) (Coble et al, 1990), and fluorescence measurements can be used to describe these fluorescent parts (Stedmon and Markager, 2005a). Within lake environments, optically active FDOM and CDOM are more stable compared to non-coloured and fast cycled DOM (Berggren et al, 2020). Also, they can contain significant amounts of reactive compounds, important for carbon cycling through microbial degradation (Lapierre et al, 2013). Previously, measurements of fluorescence were used to deduce the transport of DOM from terrestrial sources into rivers or lakes all the way to the ocean (Stedmon and Markager, 2005a).

In clear water Arctic lakes FDOM has been found to be net produced by microbes, due to relatively low terrestrial control compared to DOM from algal sources, which includes both humic and protein like components (Berggren et al, 2020). Protein like components have been shown to be not very reactive, in laboratory conditions, as they have increased while other more labile DOC has been consumed (Berggren et al, 2020). CDOM can be produced over time as well, likely from bacterial sources, while other non-coloured DOM is consumed (Berggren et al, 2020). Subarctic clear-water lakes, for example in Sweden, can have high reactive DOC, bacterial production per DOC, and FDOM production, specifically with the protein-like fluorescent components (Berggren et al, 2020).

2.6. Fluorescence spectroscopy applications

Fluorescence and absorption spectroscopy have been used for many years now as a way to describe DOM and DOC, and an important part of this research is excitation-emissions matrices (EEMs), which specifically contain information regarding the FDOM subfraction, such as, source, chemistry and reactivity (Stubbins et al, 2014). Earlier research used a peak picking technique to reveal this information from different wavelengths (Stubbins et al, 2014). To try and explain excitation and fluorescence simply, we can think of how when light is absorbed by a molecule, an electron becomes “excited” and moves into an empty orbital ring, but the extent to which the electron is excited varies, with differing excited states (Stedmon et al, 2003). This produces broad peaks which can be seen in absorption spectra. After the excitation process, the molecule becomes “relaxed” and drops to the lowest excited state, then other processes including fluorescence compete for a full relaxation to the base “ground” state,

with fluorescence emission wavelength measured as the difference of energy between the ground state and the lowest excited state (Stedmon et al, 2003).

More modern techniques using EEM spectroscopy have demonstrated how different fluorescent components can be separated and identified, for example identifying anthropogenic and terrestrial components (Stedmon and Markager, 2005a). Fortunately, recent developments have allowed us to analyse the full breath of information contained within EEMs to study fluorescent components found in DOC within lakes (Shang et al, 2023). This is done using parallel factor (PARAFAC) analysis, which has been developed as a superior and advantageous technique, compared to more traditional methods (Stedmon and Markager, 2005a., Stedmon and Markager, 2005b). PARAFAC can be used to dissect EEMs of various complexities into individual and insightful fluorescent components (Stedmon et al, 2003).

There are two major types of components which have been found to fluoresce: humic like components which have a more blue fluorescence and protein like components which have a more UV fluorescence (Coble, 1996). Components are identified through their excitation and emissions wavelength peaks, for example Coble (1996) identified 5 fluorescent components in their study, firstly 2 protein-like components: Peak B ex/em of 275/310, and Peak T ex/em of 275/340. 2 humic-like components; Peak A ex/em of 260/380-460, and Peak C ex/em of 350/420-480. Finally, a marine humic-like component Peak M ex/em of 312/380-420. Each component exhibited distinguishable maximum excitation and emission values, for example the protein-like components had low excitation combined with low emission, while the humic-like components had varying excitation combined with high emission. However, in more recent literature Peak M has been identified as a mixture of marine or aquatic humic-like, and of an autochthonous and microbial source (Stubbins et al, 2014).

Therefore, if we want to find out the different concentrations of the complex fractions of DOC, fluorescence spectroscopy can be used to identify compounds which fluoresce at specific emission wavelengths with an intensity which is proportional to their respective concentrations (Cammack et al, 2004). Furthermore, changes in unique fluorescent components can be identified within lake water samples, allowing further insight into dynamics of DOC composition in specific study areas, and facilitating comparisons between different data sets and observed components (Stedmon and Markager, 2005a., Stubbins et al, 2014).

3. Methods

3.1. Sampling and Study Site

54 lake samples from 53 lakes in Churchill Canada, taken in August 2022, were collected with 60ml syringes (flushed 3 times with the lake water) around 10cm below the surface. Samples were stored in cooling boxes before being transferred to a fridge at <4°C, after which they were syringe filtered (whatmann syringe filters 0.7μm) into flushed, with de-ionised water, 60ml HDPE bottles. Samples were supplied by Post Doc researcher Geert Hensgens from Vrije Universiteit Amsterdam. Average latitude and longitude for these samples was 58.7 N, and 93.8 W, with specific sample sites found in Table S1. Environmental measurements were taken and provided by Geert Hensgens, this included the following variables: pH, pCO_2, Electrical Conductivity (EC), lake size and coordinates where each sample was taken. Also, samples were sent to be analysed for DOC concentration. The samples were transported from Churchill to Lund University, while kept at low temperatures in a cool box, and then stored in a cold room at Lund University.

The study site for this project is in the Hudson Bay Lowland close east of Churchill in Canada (Fig. 3). This region has an Arctic Climate but is also influenced by the close proximity of the Hudson Bay, with a mean annual air temperature of -6.9°C, a mean air temperature of 12°C in July, and mean air temperature of -27°C in January (Macrae et al, 2014). The total annual precipitation is around 420 mm, with around 55% falling as rain during May to September, and around 45% falling as a snow during October to May (Duguay and Lafleur, 2003).

The land is very flat with a gentle slope of 1 m km^{-1} (Winter and Woo, 1990, as cited in Macrae et al, 2014), and has been going through isostatic rebound of around 1m $century^{-1}$ since glacial retreat (Macrae et al, 2014). Glacial movement and deposits are responsible for the relief, with all soils underlain by marine silt or clays (Duguay and Lafleur, 2003). The combination of flatness and clayey/silty sediment limits drainage and has led to the development of peatland (Kuhry, 2008). Therefore, the topsoil is dominated by peat, with a thickness of 100-500m (Duguay and Lafleur, 2003).

Most of our study area has underlying permafrost, with active layer depths reaching around 1m (Duguay and Lafleur, 2003., Dyke and Sladen, 2010), and includes some permafrost features such as patterned ground, and palsas (Duguay and Lafleur, 2003). There are three environmental zones in this area; open forest, forest-tundra, and tundra (Duguay and Lafleur, 2003), and overall consists of ponds and shallow lakes which occupy around 40-50% of the surface (Macrae et al, 2014). These water bodies are mostly present in the tundra zone (~32%), followed by forest-tundra (~24%), and then open forest (~7%), with percentages representing how much surface is occupied (Lafleur et al, 1997). The distribution of sampled lakes is shown in Fig. 4.

Fig. 3. Picture shows the study area of Churchill in relation to Canada and the US (Macrae et al, 2014).

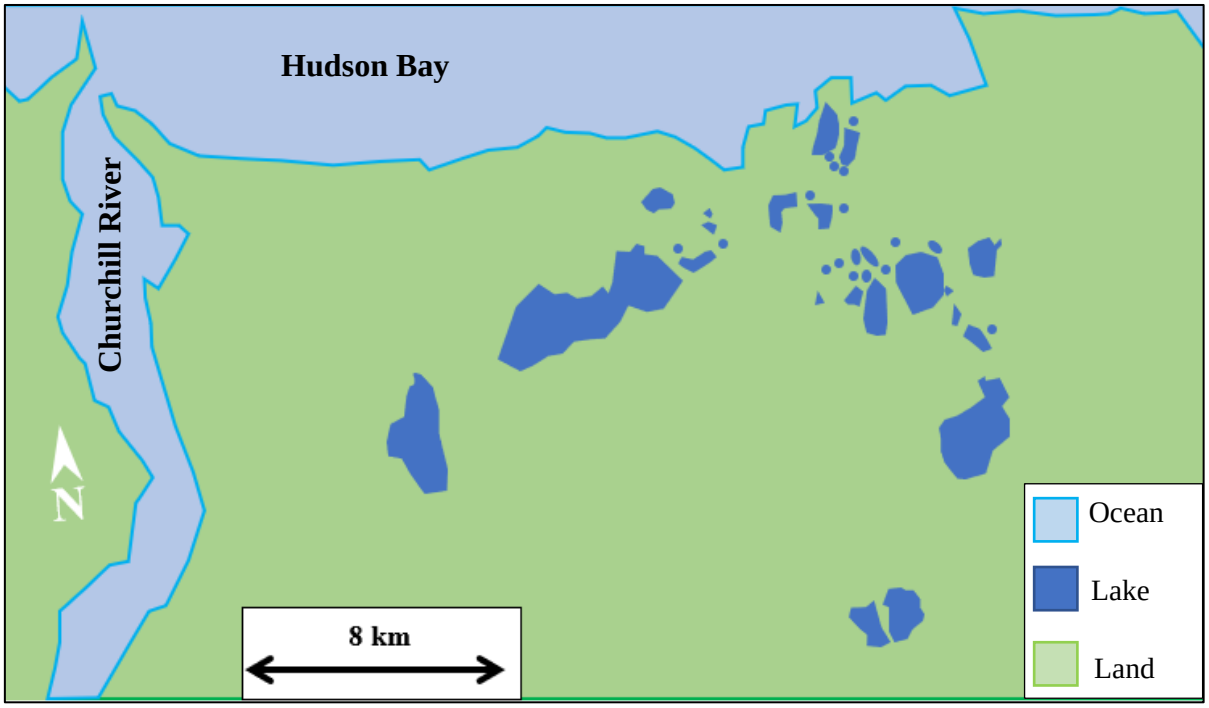

Fig. 4. Map showing distribution of the 53 lakes, with 54 samples collected in August, size can be seen to vary, and some lakes are more grouped than others, and distance to Hudson Bay varies. Max and min latitude of the lakes are 58.77 N and 58.63 N, while max and min longitude are 94.05 W and 93.75 W. Note some lakes are too small to be represented but lie within close proximity of other lakes shown.

3.2. Experimental setup

To remove nutrient amount as a factor which might affect our results, concentrations of 50 microgram per litre of phosphorus (as phosphate) and 500 micrograms per litre of nitrogen (as ammonium nitrate) were added to each sample prior to any of the following experiments. Our study used an incubation length of 28 days, with day 0 acting as the starting date for each experiment.

3.3. Fluorescence

Fluorescence data was generated to support analysis of FDOM and absorbance variables within each lake sample, to give insight into the dynamics of DOC composition in these lakes, related to their origin (Stubbins et al, 2014).

Fluorescence was measured using an optical spectrometer called the HORIBA Aqualog. Aqualog samples were measured at the start of the incubation, day 0, and at the end of the incubation on day 28. Therefore, one set of data was generated for the beginning and end of the incubation period.

Prior to loading of samples, the Aqualog machine was validated following standard procedures (Hansen et al, 2018), for example, a pure water standard was used for validation scans. Aqualog scans were taken in a 1 cm quartz cuvette, with 1 second integration time, with emission and excitation wavelengths ranging from 230nm to 800nm. An injector was used alongside the Aqualog to extract the water from each sample and run through the Aqualog to obtain the absorbance spectra and fluorescence EEMs from each water sample, which were then extracted. Samples were loaded into the injector in two trays with 12 samples each, with each tray containing 1 blank sample of purified Milli-Q water.

All EEMs were corrected by subtracting the blank Milli-Q water scans. Once every sample had been run through the Aqualog, fluorescence and absorbance files were exported using the Aqualog software, ready to be analysed within MATLAB.

3.4. PARAFAC – Fluorescent components

PARAFAC was used to analyse the fluorescence and absorbance files within MATLAB, using the drEEM toolbox and instructions by Murphy et al (2013). From these files, EEMs were extracted, corrected and consolidated into one structure. The EEMS were then smoothed to remove first and second order Raman and Rayleigh scattering, by analysing the spectral variance across all EEMs. Raman is inelastic scatter, while Rayleigh is elastic scatter, caused by photons bouncing around and scattering, some of which ends in the detector, fortunately this scatter was predictable and was simple to remove.

Then the main PARAFAC analysis was done, to detect each component throughout the full set of water samples, combining both data sets from day 0 and day 28. For the dataset, 3 to 5 components were attempted to be extracted. Before going further, the models generated were inspected using an outlier test, and core consistency diagnostic, which indicated the variation of the model and if the model was problematic.

The dataset was then split into different smaller sets for split analysis, to test if similar components could be found across each smaller data set, and then validated using split validation. Split analysis for 3 to 5 components was attempted on a normalised and

unnormalized dataset, since with this dataset which includes the same samples twice, but from different stages of incubation, normalisation could have improved the number of components to be found. Only 3 fluorescent components were finally validated and extracted, all of which could be found on the OpenFluor library (www.openfluor.org) (Murphy et al, 2014). The model was listed with the name "Churchill, Canada lakes" on the site, and all emission and excitation loadings can be seen.

The component fmax (the maximum intensity of each component) values were then extracted to get absolute values of each fluorescent component, along with absorbance at 254nm which gave a representation of DOC concentration (Brandsetter et al, 1996). The Humification index (HIX) which corresponded to extent of humification, and biological index (BIX) which corresponded to biological activity, were extracted (Catalán et al, 2013). The spectral slope between 254nm and 365nm, and the ratio of absorbance between 254nm and 365nm were extracted as they can indicate molecular weight and biodegradability (Berggren et al, 2007). Also, CDOM at 440nm absorbance, used as a reference wavelength, and baseline corrected by subtracting 690nm absorbance, was calculated using Equation 2, to give an estimation of the amount of CDOM within each sample. $SUVA_{254}$ was calculated using Equation 3, which gives an idea of aromaticity (Berggren et al, 2022)

$$CDOM_{440nm} = (a440 - a690) * \ln(10) \quad \text{(Equation 2)}$$

$$SUVA_{254nm} = \frac{(a254 - a690)}{DOC(mgCL^{-1})} \quad \text{(Equation 3)}$$

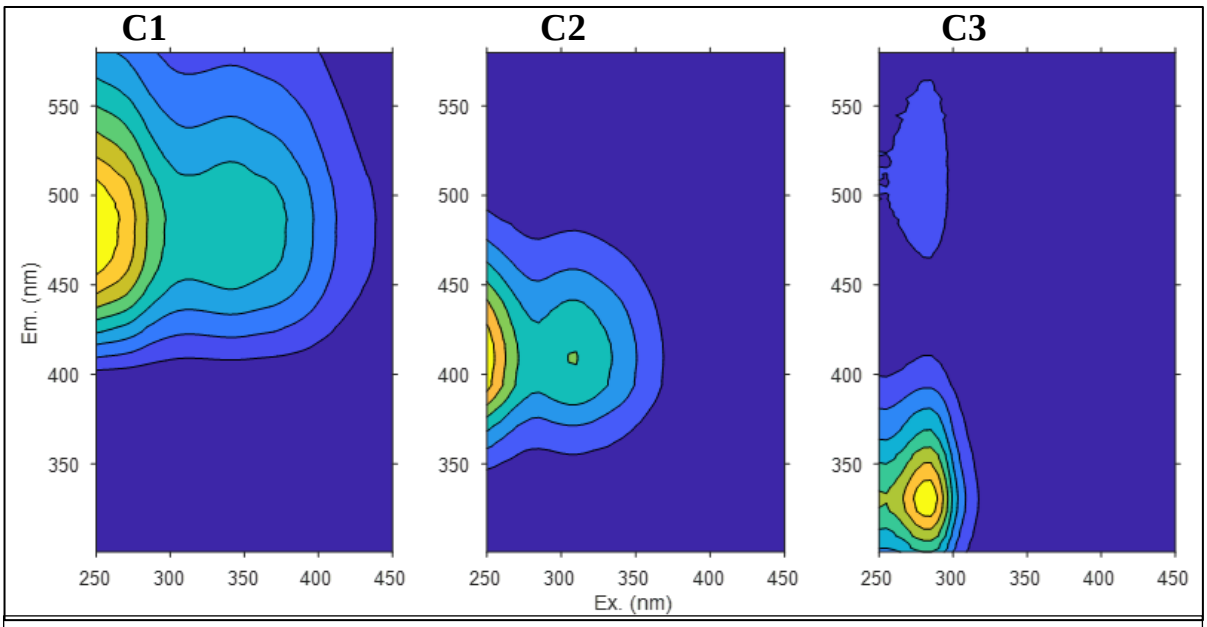

Fig. 5, Shows the PARAFAC model output of the three fluorescent components as excitation emission plots, including C1 (Humic-like, peak C), C2 (Marine humic-like, peak M), and C3 (protein-like, peak T) (Coble, 1996). Produced from Churchill, Canada, lake samples taken in August, including start and end of 28-day dark incubation.

The three components identified (Fig. 5) were interpreted to be derived from different origins (Coble, 1996), with C1 representing a humic-like terrestrial component, C2 representing a humic-like marine component, while C3 was protein-like and microbially derived (Fig. 5). C1 had an ex/em of 340/332-486, C2 had an ex/em of 310/410, and C3 had an ex/em of 285/330. More information on the spectral signatures can be found in Figure S1.

3.5. Bacterial Production – Leucine uptake

Bacterial production was measured on water samples at room temperature over the 28-day incubation period, using the 3H-leucine technique, as demonstrated by Smith and Azam (1992). Measurements done at day 1, 3, 7, 14, 28, and one set of Blanks.

To prepare each batch of samples, leucine was added with a final concentration of 40 nM, then 5% of 100% trichloroacetic acid (TCA) after 60 minutes. This is done to determine leucine incorporation, and the TCA was used to terminate the incubation. Then, on the specific day (1,3,7, etc), samples were centrifuged for 10 minutes at 14,000 rotations per minute (RPM), drained and refilled with 5% TCA, repeated twice, after final centrifuge scintillation cocktail was added, and samples were left to sit for day and finally they were ready to be put in the scintillation counter.

Samples were centrifuged in order to push the bacteria into one space on the back side of the tube, to form a bacterial pellet, which the TCA then rinses, before the scintillation counter measures the radioactivity in each sample (Soares et al, 2017).

The leucine uptake was then converted into bacterial production (Simon and Azam, 1989). CPM (Counts per minute) values were exported from the scintillation counter and using excel (Table 1), CPM was converted to DPM (Disintegrations per minute) using equation 4, and then corrected by subtracting the DPM from the blank values for each sample. Then, the uptake rate of radioactivity per litre and time was calculated using equation 5, which was then converted to leucine uptake rate using equation 6. Finally, Bacterial Production was calculated by converting the leucine uptake rate, using equation 7.

Table 1, parameters p1-p6 used to convert data output from scintillation counter to bacterial production measurements (Kirchman, 2001).

Parameter	Value	Description
Counter efficiency p1	0.71	Machine efficiency on scintillation counter.
Tube volume (l) p2	0.0012	Volume of scintillation tube.
DPM/Ci p3	2.22E+12	Unit conversion factor used to convert DPM to Ci.
Specific activity (Ci/mmol) p4	123.8	Activity of leucine isotope used.
Conversion factor (kg/mol) p5	1.55	Factor used to convert isotope uptake to bacterial production.
Isotopic dilution p6	2	Factor to which leucine synthesis continues when suppressed by bacteria.

$$DPM = CPM/p1 \quad \text{(Equation 4)}$$

$$Ci\ L^{-1}\ d^{-1} = (DPM(corrected)/1(hour))/(p2*p3) \quad \text{(Equation 5)}$$

$$nmol\ L^{-1}\ d^{-1} = p6*1000000*\ Ci\ L^{-1}\ d^{-1}/p4 \quad \text{(Equation 6)}$$

$$\mu g\ C\ L^{-1}\ d^{-1} = nmol\ L^{-1}\ d^{-1}*p5 \quad \text{(Equation 7)}$$

Integrated rate values for BP from day 1 to 7, day 1 to 14, and day 1 to 28, were calculated by averaging the values between each time step multiplied by the number of days, and then summing up all those values and dividing by the total amount of days to get a new integrated value. Otherwise known as the trapezoid rule (Berggren et al, 2020).

3.6. Bacterial Respiration – Dissolved oxygen, and BGE

Dissolved oxygen measurements were carried out in dark conditions, in a climate chamber set to a constant 20°C. Firstly, the 5ml samples were put into sensor vials sealed with butyl rubber septa caps, with each sample having 3 repeats, on top of Sensor Dish Reader (SDR) plates. Each vial was filled to the top with sample water and sealed, making sure there were no air bubbles left inside. In total 9 SDR plates were used with 216 vials, including 9 blank vials, with 3 placed on plate 1, plate 4, and plate 7. Measurements were then taken using software every 2 hours.

Once the incubation period was over, the oxygen values were exported for each sample, the 9 blank samples were then isolated and an average was calculated for each time step, the rest of the samples were corrected by subtracting the change in the average blank value. However, the initial oxygen readings were too erratic and fluctuated significantly, even in the blanks, that the first day of data had to be cut, and day 0 was changed to be the new data start point. Next, since each sample had 3 repeats, these repeats were then averaged into one value for each sample per every 2 hours. From this, 7-day, 14-day, and 28-day BR ug/L/d, was calculated as the slope of oxygen concentration vs time.

The BR data was combined with BP to calculate bacterial growth efficiency (BGE). This was done using equation 1 as mentioned in section 2.6. BGE showed the extent of how the DOC was assimilated by the bacteria in each sample (Del Giorgio and Cole, 1998). With higher values of BGE corresponding to more efficient use of DOC for growth rather than respiration.

3.7. DOC decay

DOC decay was inferred using the DOC measurements taken for each sample, and then decreasing the DOC based on the decline in recorded O_2 data, assuming that one mol DOC lost is equivalent to each mol lost of O_2 (a respiratory quotient 1.0). This was further used to fit the DOC decay to an exponential decay curve in MATLAB using the fitnlm function.

Furthermore, the decay coefficient k values were calculated using a reactivity continuum model within R. By using the initial DOC starting value and the recorded oxygen data, to convert the oxygen data to percent carbon, and modelled how much of the labile DOC was consumed throughout the incubation period. k values, representing decay rate, for day 1, 3, 7, 14, and 28 were extracted and compared with composition and reactivity variables. k values were important as they show how reactivity changes over time, and how labile the DOC in each sample is compared to each other (Mostovaya et al, 2016).

3.8. Comparative and explorative analysis

Microsoft Excel was initially used to consolidate and explore the data, including correlation graphs and to test each dataset for skew, with any sets of data with too high skew > 2, were log10 transformed to make a more normally distributed dataset. From this, the significance of lake area was focussed on, and so the data was split into different sized lake groups, to aid in the results and discussion. Lake groupings were picked based on the range and distribution of the lake areas, small lakes were defined as 0-10,000m^2, medium lakes as 10,000-100,000m^2, and large lakes as 100,000-1,000,000m^2. In total there were 18 small lakes, 20 medium lakes, and 15 large lakes. Some further graphs were then made in RStudio 2022.12.0, to illustrate chosen correlations along with lake size groups.

All variables, including: BR, BP, BGE, pH, log10area, Electrical Conductivity (EC), pCO_2, water temperature, BR/DOC, BP/DOC, DOC, C1%, C2%, C3%, C1 R.U., C2 R.U., C3 R.U.,

SUVA, CDOM, Abs254, Spectral slope, and K decay values, were correlated with each other using the pairs.panel function within RStudio 2022.12.0. This was done for all data sets at day 1, day 28, and the delta or difference between day 28 and day 1. Apart from the environmental data which only has one set of values from the field. Additionally, Principal Component Analysis (PCA) was used to explore the data more, making PCAs for component (HIX, BIX, Abs254, Spectral Slope, C1, C2, C3, SUVA), reactivity (BR, BP, BGE), and environmental related variables (pH, CDOM, log10 area, DOC, EC). Further correlations were done using a modified chart.Correlation function with package "PerformanceAnalytics" installed. Furthermore, residual analysis was done in RStudio and Excel as well to explore the data further, specifically with data expected to have significant correlations but did not, for example pCO_2.

4. Results

This section is split into two sections, the first focusing on overall and more general results, while the second section focuses more on the proposed hypotheses.

4.1. General results

Across the different sized lakes, some patterns were identified with different variables from the start of the incubation, as well as on site measurements, for example DOC, CDOM, pH, BP, BGE, SUVA, the percentage, and absolute components (apart from C3% or the protein-like component), all showed decreasing mean values from small to large lake groups. Whereas C3%, and modelled k, showed increasing mean values. However, variables, such as BR, pCO_2, and EC showed initial mean increases from small to medium lakes, but then decreased for the larger lakes (Table 2). Out of the 3 fluorescent components, C2 had the highest concentration representing nearly 50% of the FDOM, followed by C1 and C3.

Relationships between different key variables were expressed through a correlation matrix (Fig. 6), the most significant correlations were found between DOC, CDOM, each fluorescent component and lake (log10) area. This showed with smaller lake area, there was increased concentrations of DOC, CDOM, fluorescent components, and pCO_2. BR showed low significant corelations with C1, C2, CDOM, DOC, and lake area, while BP showed a low significant correlation with C3 and DOC. For both BR and BP, these correlations were quite weak, as R^2 values were under 0.2, but p-values were under 0.05. The relationship between BP and DOC loss, and between EC, pCO_2 and DOC, was also tested but was found to have no significant correlation (Fig. S2, Fig. S3, Fig. S4).

Table 2. Min to max values and means of key data variables, retrieved from 54 arctic lake samples in August from Canada, along with data generated from start of incubation and laboratory analysis of fluorescence, BP, and BR. Data is separated into 3 different lake size groups as seen in the column heading. Modelled k represents the decay coefficient from applied exponential DOC decay curve.

Table 2	Small (0-10^4) m^2		Medium (10^4 -10^5) m^2		Large (10^5 -10^6) m^2	
Variable	Range	Mean	Range	Mean	Range	Mean
DOC (mg L^{-1})	5.7-12.5	8.0	3.0-8.2	5.8	2.4-7.7	5.1
CDOM (m^{-1})	0.6-4.2	1.8	0.4-2.1	1.0	0.2-1.2	0.7
pH	7.9-9.4	8.6	7.7-9.1	8.5	7.6-9.5	8.4
EC (µs/cm)	140-1227	514.7	98-3029	669.9	162-1362	465.3
pCO_2 (ppm)	216.2-1253.4	586.7	114.6-1168.5	591.2	232.3-534.5	367.4
BR (µg L^{-1} d^{-1})	43.9-129.7	82.5	62.2-153.8	103.9	50.7-123.5	100.9
BP (µg C $L^{-1}d^{-1}$)	0.21-16.6	7.2	2.4-14.5	5.8	1.5-10.5	5.9
BGE %	0.18-18.0	8.6	2.2-18.4	5.6	1.3-8.8	5.5
C1 %	21.2-38.3	30.8	19.1-37.1	28.0	16.9-29.0	24.1
C2 %	42.8-50.9	47.8	37.8-51.4	45.8	33.7-49.0	44.1
C3 %	12.1-36.0	21.5	15.0-43.1	26.3	22.2-49.5	31.8
C1 R.U.	0.26-1.7	0.85	0.16-1.2	0.50	0.07-0.63	0.32
C2 R.U.	0.52-2.2	1.3	0.32-1.9	0.80	0.14-1.2	0.58
C3 R.U.	0.39-0.81	0.53	0.26-0.61	0.41	0.20-0.59	0.37
SUVA ($L\ mg^{-1}m^{-1}$)	1.8-4.5	3.2	1.6-4.2	2.8	1.6-3.1	2.5
Modelled k	0.01-0.05	0.03	0.02-0.13	0.05	0.04-0.13	0.06

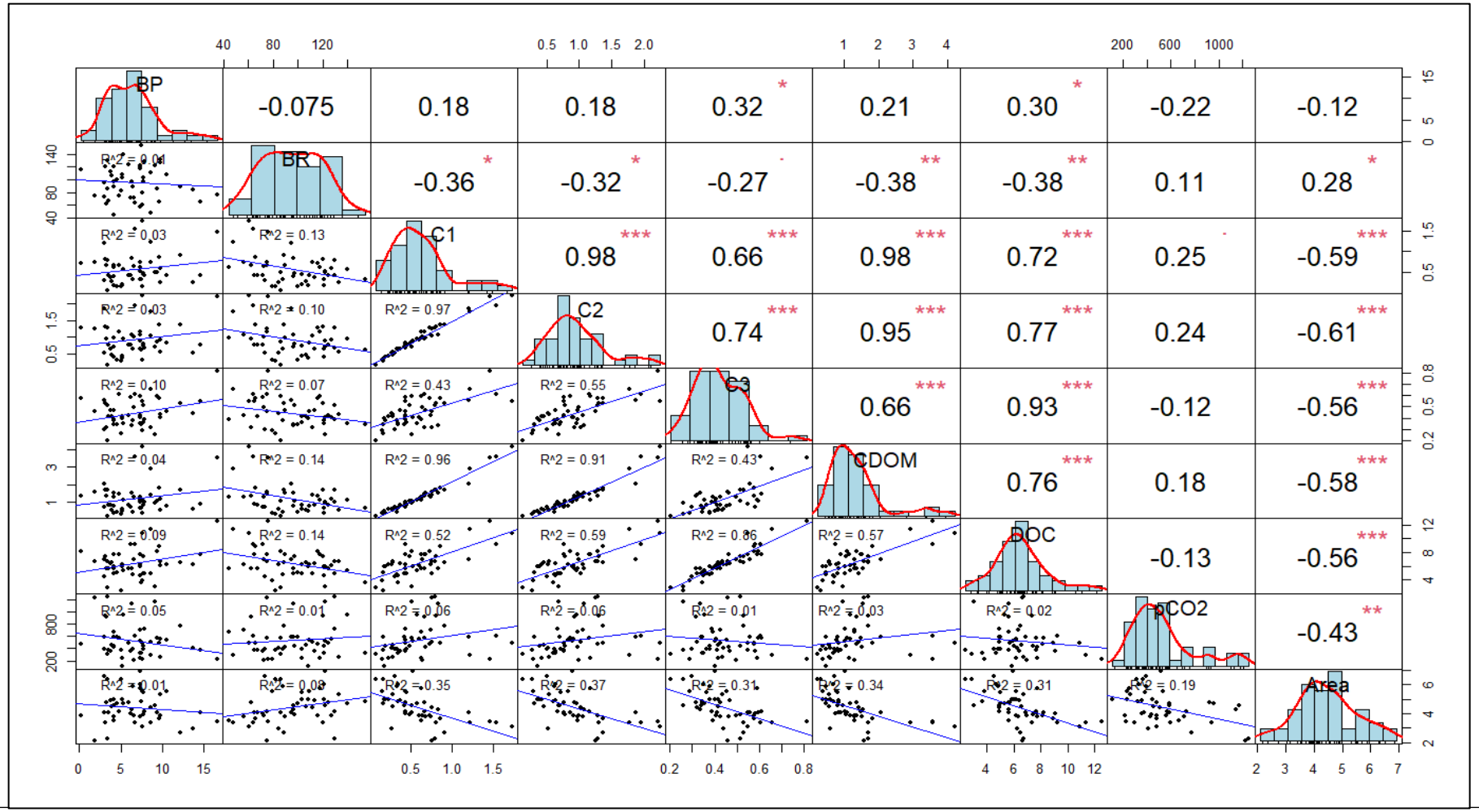

Fig. 6. Correlation matrix. Variables, from start of incubation, correlated are, BP (µg C $L^{-1}d^{-1}$), BR (ug L^{-1} d^{-1}), C1, C2, C3 (R.U.), CDOM (m^{-1}), DOC (mg L^{-1}), pCO_2 (ppm), and log 10 area (m^2). Histograms for each variable are down the diagonal, while Pearson r correlations are shown in the top right, with the significance level denoted by symbols: p-values (0.001, 0.01, 0.05, 0.1) = symbols ("***", "**", "*", "."). In the bottom left are scatter graphs, with regression lines and R^2 values. Data generated from 54 Canadian artic lake samples.

4.2. Hypotheses

Hypothesis 1: a) Protein-like components will correlate positively with bacterial production. b) Protein-like components will correlate positively with DOC loss.

Overall BP and absolute C3 showed a slight positive correlation at the start of the incubation period (Fig. 7A). There was some seperation between the different lake size groupings, for example the smaller lakes generally had higher amounts of C3, but showed the highest range of BP. Compared to the large lakes, which had the lowest concentraritons of C3, and the lowest range of BP values. The medium lakes has C3 values mostly around 0.3 to 0.4, with most having a similar range in BP to the large lakes, apart from one sample at around 15 BP. Furthermore, DOC loss correlated against C3 absolute change showed no signifcant correlation (Fig. 7B).

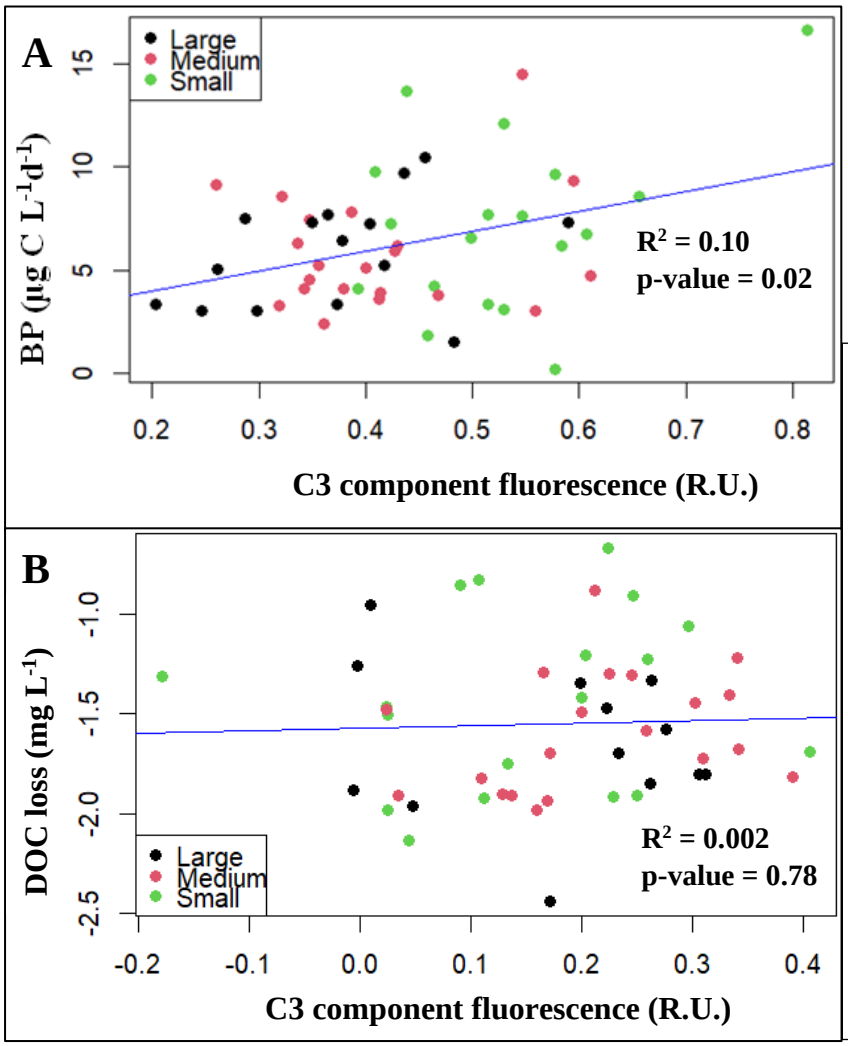

Fig. 7. A) Scatter graph correlating BP and C3 from start of dark incubation, separated into different lake sizes. Blue line is a regression line. B) scatter graph correlating DOC loss over 28-day incubation with C3 component change over 28-day incubation. Blue line is a regression line. Data generated from 54 Canadian arctic lake samples taken in August.

Hypothesis 2: a) Humic-like components will correlate negatively with bacterial production. b) Humic-like components will correlate negatively with DOC loss.

BP had no significant correlation with both C1 and C2 components, although there was some separation between the different lake sizes, as the small lakes showed the largest amounts of both humic-components, followed by the medium and then large (Fig. 8A). There were weak significant correlations between DOC loss and humic-like component change over the incubation period, although slightly more significant for C1. The small lakes showed the smallest loss of DOC, and mostly the largest losses, apart from 2 large lakes which had the largest DOC lost. Furthermore, the medium and large lakes showed similar ranges of DOC lost, apart from the previously mentioned 2 large lakes (Fig. 8B).

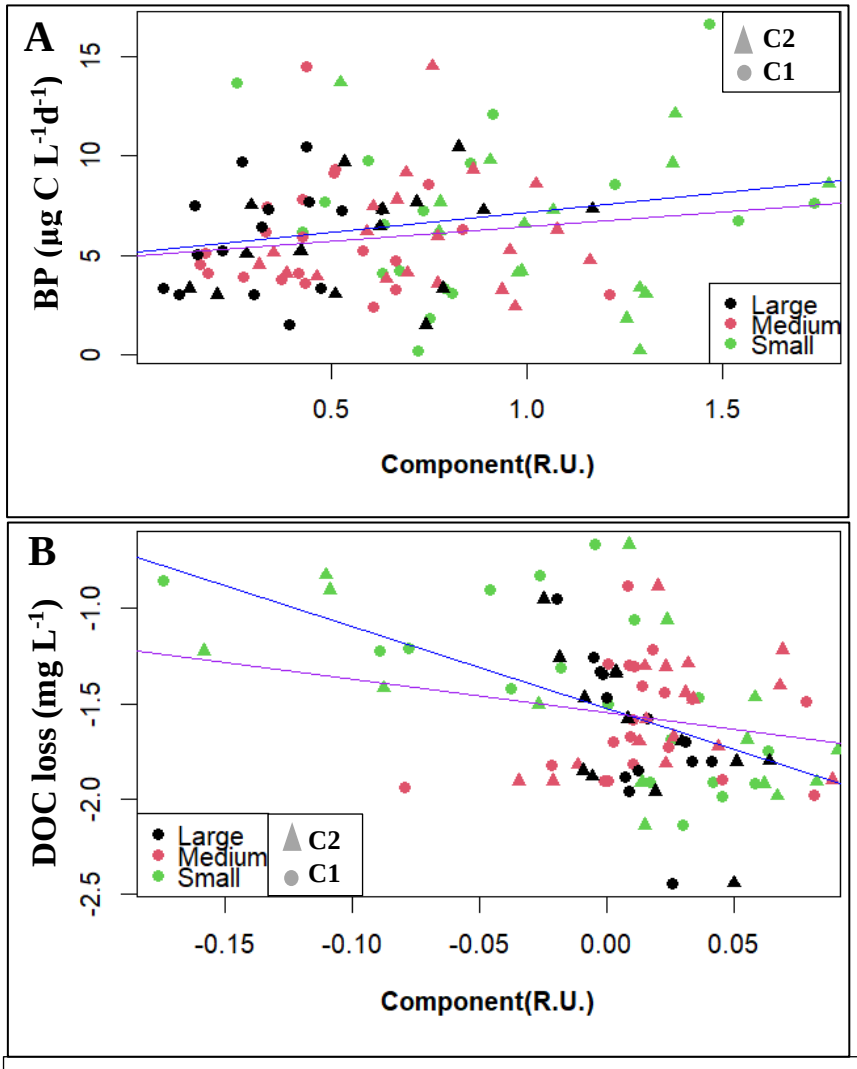

Fig. 8. A shows BP correlated against components C1 and C2, while B shows DOC loss correlated against C1 and C2 change over 28-day incubation. In both, the triangle symbol represents C2, while the circle symbol represents C1, and the purple regression line is tied to C2, while the blue regression is tied to C1. For A, both regression lines have a R^2 of 0.03, and both with a p-value of 0.21. For B, C1 has an R^2 of 0.23 and p-value of 0.0004, while C2 has an R^2 of 0.17 and p-value of 0.003. Data generated from 54 Canadian arctic lake samples taken in August.

Hypothesis 3: DOC, CDOM, bacterial respiration, and humic-like components will correlate positively with pCO2.

pCO_2 and log 10 of lake area showed a small significant negative correlation (Fig. 9A), and the residual generated from this had the highest significant correlation with DOC (Fig. 9B), meaning DOC was responsible for any variation in pCO_2 not explained by area. The residual generated from Fig. 9B (residual 2), had high significant correlations with all three fluorescent components, but especially with the C3 component (Fig. 9C).

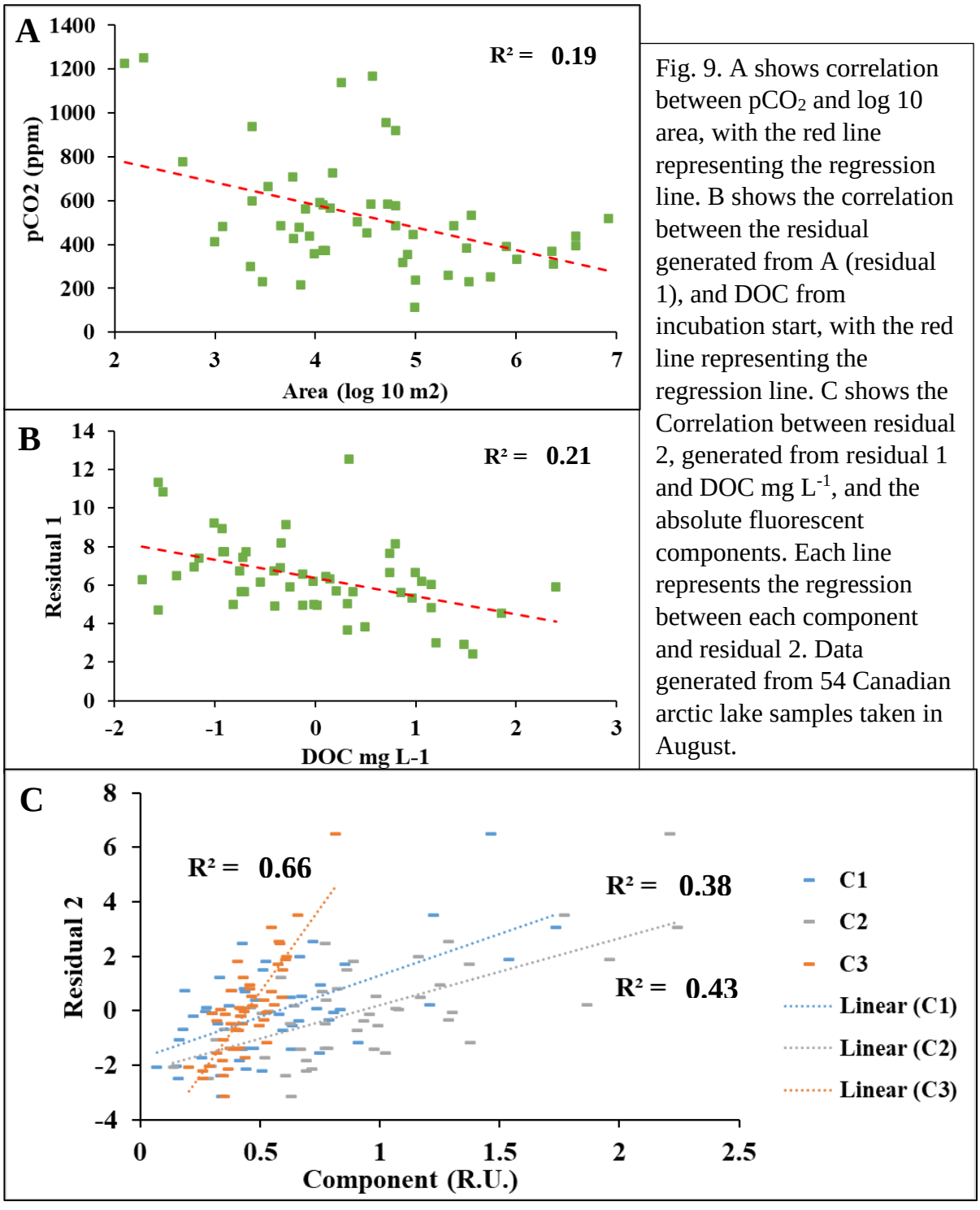

Fig. 9. A shows correlation between pCO_2 and log 10 area, with the red line representing the regression line. B shows the correlation between the residual generated from A (residual 1), and DOC from incubation start, with the red line representing the regression line. C shows the Correlation between residual 2, generated from residual 1 and DOC mg L^{-1}, and the absolute fluorescent components. Each line represents the regression between each component and residual 2. Data generated from 54 Canadian arctic lake samples taken in August.

Further exploring the relationship between pCO_2 and other variables, a PCA was performed on fluorescence variables, which revealed two PCAs responsible for 92.9% of the variance (Fig. 10). PC1 was related to increasing trends in C1, C2, C3, absorbance 254nm, HIX and SUVA, and negatively in BIX, spectral slope, and absorbance ratio 254/365nm. While for PC2, decreasing HIX and SUVA was related, and increasing amounts of the other variables, especially for C3 were related to PC2. Some variation in the individual samples was found between the different lake groups, with small lakes following trends with C1, C2, C3, and absorbance at 254nm, while the medium and large lakes showed links with HIX and SUVA in one direction and the spectral slope, absorbance ratio, and BIX in the opposite direction.

The relationship between the component PCA with pCO_2, BR, DOC and BGE was also explored (Fig. 10). DOC correlated positively with both PC1 and PC2, BGE correlated only with PC2. While pCO_2 and BR/DOC correlated negatively with PC2, and pCO_2 positively with PC1. DOC and BGE followed similar trends to each component and absorbance at 254nm, while pCO_2, and to a less extent BR/DOC, followed similar trends to HIX and SUVA. Therefore, through residual and PCA analysis, pCO_2 was found to relate primarily to DOC concentration, DOC composition, and partially BR, but also with SUVA and HIX.

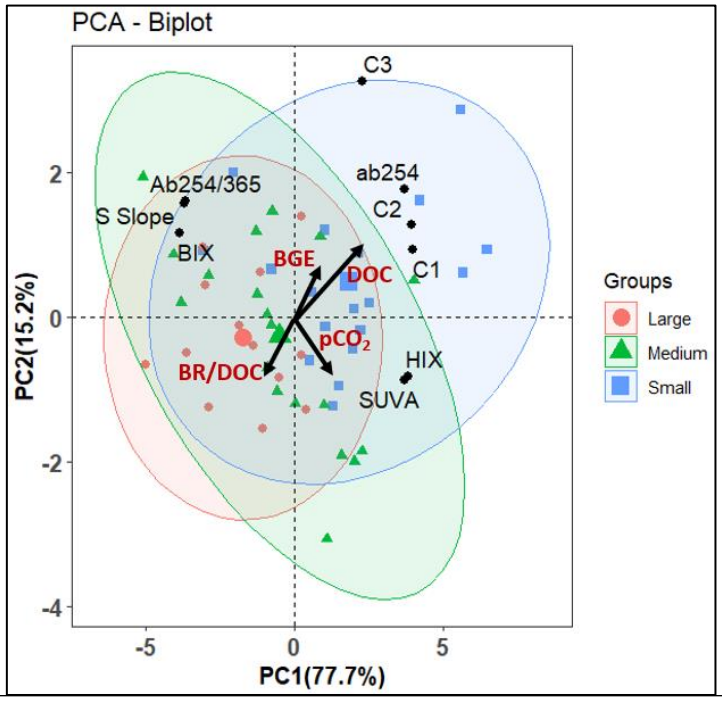

Fig. 10. A PCA Biplot showing loadings of variables with black dots and text, these are on a scale between 0 and 1. The coloured symbols represent the individual scores of each sample and are sorted by lake size. With each ellipses representing the distribution of each lake size group. The black arrows and accompanying red labels represent the correlations between each variable and the PCA scores. Data generated from 54 Canadian arctic lake samples taken in August.
PC1: DOC (r 0.59, p 7.9e-6), BGE (r 0.30, p 0.04), pCO_2 (r 0.40, p 0.005), BR/DOC (r -0.42, p 0.002). PC2: DOC (r 0.75, p 3.6e-10), BGE (r 0.37, p 0.009), pCO_2 (r -0.49, p 0.0003), BR/DOC (r -0.57, p 1.4e-5).

Hypothesis 4: CDOM and FDOM will be net produced over the incubation period.

Over the dark 28-day incubation period, both C1 and C2 showed a mixture of increases and decreases, while C3 showed a net increase over all 3 groups of lakes (Fig. 11A). The small lakes showed the largest range of increase and decrease for all components, but especially the largest decrease in C2. The medium lakes and large lakes showed similar results for each component, apart from that all C3 was net produced in medium lakes, and the large lakes had the lowest ranges in all three components.

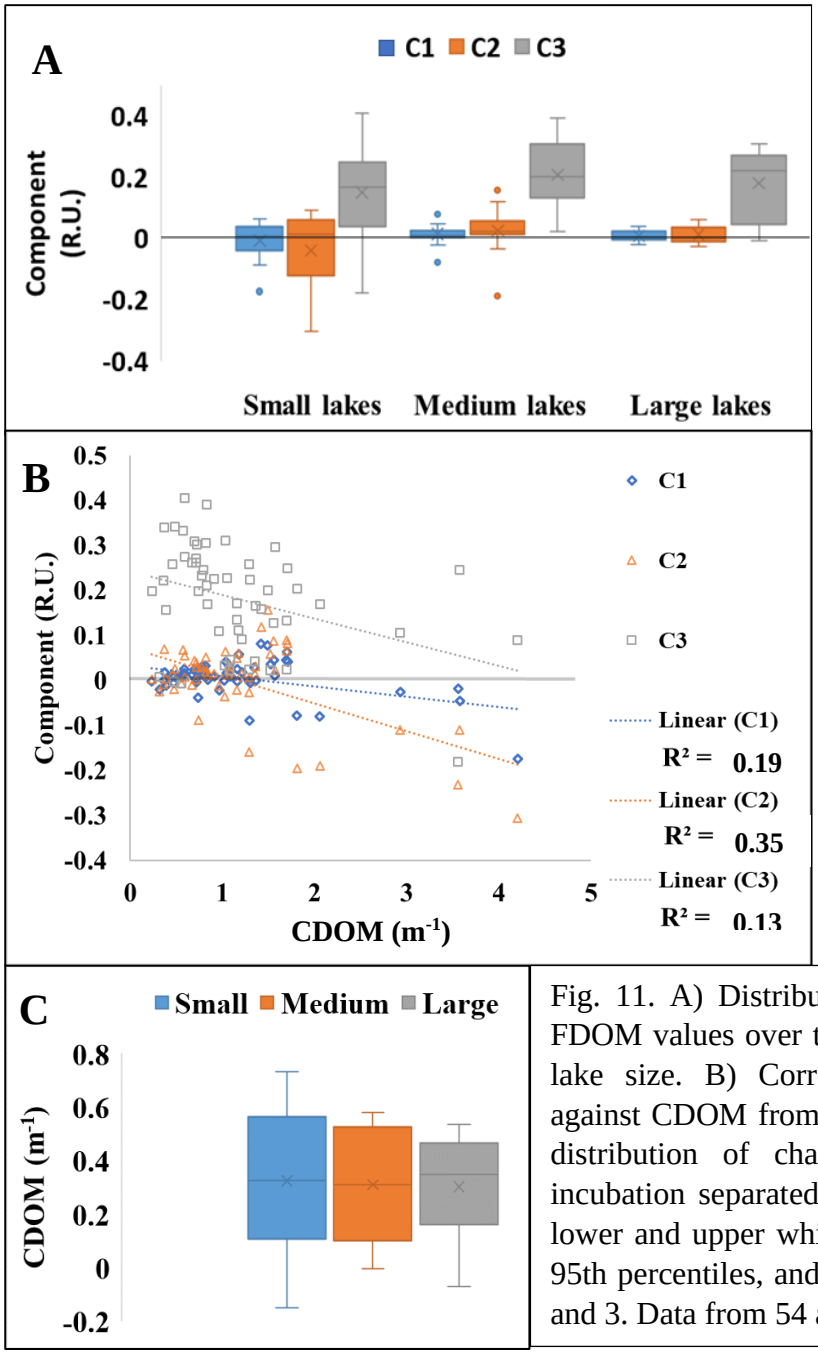

Fig. 11. A) Distribution of change in absolute FDOM values over the incubation, separated by lake size. B) Correlation of FDOM change against CDOM from start of incubation. C) The distribution of change in CDOM over the incubation separated by lake size. In A and B, lower and upper whiskers represent the 5th and 95th percentiles, and boxes represent quartiles 2 and 3. Data from 54 arctic Canada lake samples.

CDOM showed overall a net increase over the incubation, and there was a slight decline in the distribution of CDOM from small to large lakes (Fig. 11C). Each component showed higher

production with lower CDOM levels, especially C3, with a loose shift to consumption with CDOM >2 for C1 and C2, although many C1 and C2 samples show consumption with CDOM<2 (Fig. 11B).

5. Discussion

5.1. Overview

Initially in our study we could see that the environmental lake conditions varied significantly between different sized lakes, and importantly DOC and pCO_2 values varied as well. Looking further into the dynamics of bacterial reactivity and DOC composition, has further revealed significant variations in both areas across the whole dataset. However, the relationships between the fluorescent components or the composition, and bacterial reactivity have proven to be complicated and not direct.

5.2. Hypotheses

This study proposed 4 hypotheses, chosen based on previous arctic and subarctic lake studies, which found complicated dynamics in DOC reactivity and composition, and how they relate to variations in pCO_2. In this section each hypothesis will be explored regarding the results found, and if each hypothesis was found to be met, or if the results differed from what was expected, with thoughts on why this could be.

5.2.1 Hypothesis 1

"a) Protein-like components will correlate positively with bacterial production" "b) Protein-like components will correlate positively with DOC loss."

Hypothesis 1a was met while 1b was not. This backs up the recent study by Berggren et al (2020), in which the protein-like component had a positive relationship also with bacterial production. However, unlike in the Berggren et al (2020) study, no significant correlation was found between the protein-like component and DOC loss over the incubation. This could suggest that in our incubation study, the C3 protein-like component is produced through bacterial production but does not act as a reliable indicator of the more reactive and labile carbon, which was consumed. Therefore, the protein-like FDOM has shown to have a low bio-lability as it was produced the most readily, while DOC was clearly consumed, even though the C3 fluorescent peak T has been described as the most bio-labile FDOM (Stubbins et al, 2014). Even though the results from this study differ from Berggren et al (2020), there is still motivation to support their view that the relationship between FDOM and reactivity is not direct (Berggren et al, 2020), as if we only had data regarding DOC composition which showed

a significant protein like-component, this would not be enough to deduce that we would expect DOC loss to be higher with higher amounts of the reactive protein-like.

It can be assumed that bacteria can transform DOM, either directly into FDOM or as a by-product through consumption of labile DOC, but any tendency to transform DOM rather than to fully consume DOM, will lead to a dampening of the relationship between DOC loss and bacterial reactivity (Mazoyer et al, 2022). This could be the case in our study, or at the very least another factor adding to the complexity of the relationship between FDOM and DOC loss. Interestingly, Mazoyer et al (2022) also found with the dark incubation of bacteria in lake samples, an overall increase in BP over an 18-day incubation period, which supports our BP result in our study, as BP continued to increase for many samples up until day 14. Contrary to Berggren et al (2020), which found BP to generally decrease past day 7 of dark incubation.

5.2.2. Hypothesis 2

"a) Humic-like components will correlate negatively with bacterial production." "b) Humic-like components will correlate negatively with DOC loss."

Hypothesis 2a was not met, but 2b was. Both humic-like components showed no relationship with bacterial production but did show a slight negative relationship with DOC loss. Furthermore, C1 can be linked to terrestrial origins while C2 can be linked to marine/microbial origins, so perhaps the relationship with bacterial production and these external components is not closely linked, especially with low CDOM levels, since Berggren et al (2020) found with high CDOM levels the humic-like components were consumed to a greater extent. This links into hypothesis 4 and lake area, as the small lakes show the highest CDOM levels along with the greatest humic loss. Also, apart from some samples from smaller lakes which showed increased humic component reduction with DOC loss, most samples had very little change in the humic components, while DOC loss varied significantly. One explanation for the difference found in the strength of this relationship, compared to Berggren et al (2020), could be differences in flocculation within the sample bottles, as this can be a source of DOC loss during incubations, which might affect these relationships (Mazoyer et al, 2022). Another explanation could be the difference in contribution of the humic like components, as the lakes in this study were dominated by the marine/microbial humic like, whereas the Berggren et al (2020) study had a higher proportion of the UV humic-like, which has been found to have a low bioavailability compared to the marine humic-like (Stubbins et al, 2014). However, it has already been shown in the previous section the relationship between assumed bioavailability and DOC loss does not seem to be straightforward, and so perhaps there are further complicated differences between humic-like components that are not yet understood.

5.2.3. Hypothesis 3

"DOC, CDOM, bacterial respiration, and humic-like components will correlate positively with pCO_2."

Hypothesis 3 was initially seen to not be met, as all factors proposed had no significant correlations. However, with further residual analysis, we see the main variation in pCO_2 is explained by the log area of each lake, and the remaining variation is explained by DOC and the fluorescent components, particularly C3. Therefore, with smaller lake area we have higher amounts of pCO_2, but this potentially depends equally on the exact composition of the DOC, and the amount of DOC. Furthermore, using a PCA and Pearson regressions, we find that pCO_2 is related to increasing trends in HIX and SUVA, which are related to aromaticity, and both pCO_2 and BR/DOC correlated similarly with the second principal component, related to negative changes in HIX and SUVA, suggesting some contribution of BR towards overall pCO_2. Since we know aromaticity is an intrinsic factor affecting degradation of DOC (Koehler et al, 2012) and SUVA has been linked to trends in photoreactivity, perhaps for these lakes pCO_2 has a greater link to photodegradation of DOC and CDOM, rather than BR, which might only account for a smaller fraction of the pCO_2 values collected.

5.2.4. Hypothesis 4

"CDOM and FDOM will be net produced over the incubation period, with more clear water lakes having higher production of FDOM."

This hypothesis was found to be true, adding to the rare examples of CDOM and FDOM production in incubations (Berggren et al, 2020). This further demonstrates the function of microbial production of CDOM, even only at small levels, which would imply over a longer time CDOM levels could be significantly increased, and at threshold levels could interfere further with bacterial processes. With FDOM, overall, there was a net production, but this importantly varied between the humic-like and protein-like components. This links back into hypothesis 1 and 2, where we find that, through BP, these lakes are mainly producing the protein-like component, rather than the humic-like component. It was also shown that clear water lakes, or lakes with less CDOM, had the highest production of FDOM, although this varied between each component, with the protein-like showing the most significant production of FDOM. This is similar to the result found by Berggren et al (2020), as we also see the continued production of the protein-like component at higher CDOM levels, although our lakes went to a CDOM max of 4.2 m^{-1} whereas the Berggren et al (2020) study had ranges up until 10 m^{-1}. Following the trend in protein-like production against CDOM, it could be assumed that, with increasing terrestrial DOC, CDOM values higher than 4 or 5 m^{-1} might then induce protein-like component loss. This contradicts the positive relationship found between CDOM

and C3 at the start of incubation, but this increase of C3 with increasing CDOM is most likely a product of lower lake areas having increased CDOM and increased C3 concentrations.

A recent study also using dark incubations found the production of FDOM but could not directly link bacteria to DOC and CDOM loss (Mazoyer et al, 2022). The main outcome they found was the high importance of sunlight in CDOM degradation, and as a key factor influencing CO_2 emission and burial of carbon. FDOM production was linked with BP over dark incubations, but CDOM was found to not increase, contrary to our study and Berggren et al (2020). Exploring this comparison, the Mazoyer et al (2022) study was performed on a thermokarst lake with samples collected at late winter, whereas our study had lake samples collected during the summer and not strictly from thermokarst lakes. Perhaps CDOM was observed to not increase in the Mazoyer et al (2022) study as the lakes had already been in dark conditions for several months, potentially producing CDOM throughout and reaching a threshold amount, along with anoxia, where CDOM is no longer produced, or consumed at the same rate as production. Whereas for our study, samples were collected in August, where the lakes will have already been exposed to sunlight for months, leading to the photodegradation of CDOM. But once the samples were confined in dark conditions, this gave the chance of the microbial community to continue to produce CDOM more than consume or transform CDOM.

Another example of differing results is where CDOM and DOC has been found to correlate weakly in the North American Arctic, implying in-lake DOC is comprised from autochthonous sources rather than allochthonous, although this was not the case for the most northern lakes (Kurek et al, 2023). This study concludes that it is a matter of hydrological connectivity which controls DOC composition, with an expectation of isolated lakes to rely more on autochthonous DOC, while lakes with greater connection to the landscape and catchment will have a higher proportion of allochthonous DOC (Kurek et al, 2023). Since our study area had high fractions of allochthonous DOC, related to higher amounts of CDOM, our lakes are most likely not isolated from the catchment.

5.3. The influence of lake area on composition and reactivity

In recent papers, lake area has not been of a main focus, or has not been found to correlate well with composition or reactivity factors (Berggren et al, 2020). However, for our study significant correlations between area, components, DOC, CDOM, and pCO_2 have been observed. As Staehr et al (2012) suggests, our study finds higher DOC and CDOM in smaller lakes, and higher amounts of allochthonous DOC as observed through the humic-like components. However, BR was found to have a low positive correlation with lake area, which is the opposite to what would be expected. Nonetheless, looking closely at the correlation between these variables, the large variation in BR values with lake size makes it difficult to trust the validity of this correlation, and perhaps if lake depth was factored into this relationship, we may see a clearer trend.

Throughout the results for hypothesis 1, 2, and 3, we see trends in different lake area sizes, mainly that the smaller lakes all show the most variability, with some of the highest and lowest values in BP, DOC loss, CDOM, and FDOM production, while the largest lakes had the most stable spread of values with the same factors. It has been previously suggested that small lakes will have more variable biological and chemical components, due to sensitivity of initial conditions and less exchange of organisms from external sources, promoting smaller food chains, less species, and less competition between species (Staehr et al, 2012). This could help explain the variability seen in our study with the smaller lakes, as potentially our small lakes are showing their variable biological and chemical nature.

5.4. Arctic lake pCO_2 and CO_2 emission

CO_2 emissions from these lakes is linked to multiple factors, both directly and indirectly, as BR has been shown to correlate slightly with pCO_2, there is also an expected influence by the amount of DOC and the composition of the DOC (Sobek et al, 2005), and the size of lakes with small lakes expected to emit higher amounts of CO_2 (Staehr et al, 2012). However, a recent study has found that concentration of DOC has no apparent drive on the ratio between CO_2 and O_2 in Arctic lakes, as well as no trend between DOC concentration and CO_2 saturation, regardless of catchment characteristics (Allesson et al, 2022). This study found electrical conductivity as a main factor influencing lake CO_2, sourced from mineral soils rather than DOC mineralisation in the lakes, and for a select few high Arctic lakes conductivity was related to distance to the sea, with closer lakes having higher production due to vegetation and birds (Allesson et al, 2022). They conclude that within their high- and sub-Arctic lakes, that CO_2 is mainly from allochthonous sources, or more specifically through groundwater flow (Allesson et al, 2022). However, in our study electrical conductivity had no significant relationship with pCO_2 and DOC, suggesting groundwater flow or mineral soils to have a limited impact on pCO_2 and CO_2 emissions in our study area. Therefore, when approaching an analysis of Arctic Lake emissions in general, there is an importance of understanding all possible pathways of DOC input into lake systems, as lakes sharing many characteristics may still vary considerably as sources of CO_2.

Following on from section 5.2.3., as our data shows connections between pCO_2 and aromaticity with a limited relationship with BR, pCO_2 and ultimately CO_2 emissions from our Churchill lakes should show a greater relationship with photoreactivity in the field or through light incubations. Also, a factor not investigated in our study is levels of Dissolved Inorganic Carbon (DIC), and its relationship with DOC and CO_2 emissions. For example, DIC and CO_2 has been found to be unrelated to DOC within Arctic and subarctic lakes, suggested due to external DIC input controlling productivity separate to allochthonous DOC input (Puts et al, 2022). Therefore, perhaps amounts of DIC input into our study area would yield a better relationship with pCO_2, which is something that could be investigated further.

5.5. Limitations

If sample water collected could have been larger, this would have allowed for more extensive repeats of bacterial production, respiration, and fluorescence measurements. Further reinforcing the results, and regarding fluorescence, with a larger dataset more fluorescent components could be revealed, allowing further analysis and discussion. Furthermore, since our study has been on small water samples, in dark controlled laboratory conditions, it is not strictly indicative of the magnitude of processes and dynamics of the lakes in the natural environment, but this still allows for an increase in our understanding of key relationships between DOC variables. For example, the effect of photo degradation was not measured in our study with light incubations, whereas in the lakes themselves photo degradation will play a role in transforming DOM and DOC, to perhaps a large extent.

5.6. Implications and outlook

Arctic clear water lakes in Churchill Canada are important examples of the complexity of DOC composition, and the complicated relationship with bacterial reactivity. However, DOC composition alone is not sufficient to understand the large variation in lake reactivity across this site, at least with samples taking during the summer. Our study area would further benefit from analysis of the microbial community, to further investigate the cause behind the large variation of reactivity and composition in lakes with small areas, while larger lakes have less variation. We propose that within small lakes containing higher allochthonous and humic-like DOC, greater photodegradation and mineralisation should occur in the field, producing higher emissions of CO_2. This could be tested through light incubations to account for photo degradation processes, and to further study samples taken from across the year to get a clearer picture of seasonal dynamics.

Our study suggests that Arctic lakes in the global carbon cycle remains complex, as across these clear water lakes we find clear differences in pCO_2, with the small lakes having the higher pCO_2 concentrations. As small lakes are the most abundant in the Arctic (Vincent et al, 2013), any future environmental disruption, for example from climate change, increasing permafrost thaw, precipitation, and movement of terrestrial and soil carbon into the aquatic system, could lead to wide increases in CO_2 emissions. However, the complexities of the environment and ecosystems and their response to climate can be nonlinear, as with changing DOC, DOC composition, and temperature, microbial respiration will likely have a nonlinear response, complicating predictions of future emissions and lake reactivity dynamics (Saros et al, 2022). The period of ice-coverage could also decrease, promoting greater production as the ice-free period increases, allowing continued input of DOC. Therefore, the role of Arctic lakes in the future global carbon budget is difficult to predict based on this study.

Considering lake area had an important role in influencing many factors in these lakes, including reactivity, composition, DOC and pCO_2, this can have significant implications on future lake dynamics. Since, lake area is tied closely to water residence time, with small lakes having shorter water residence times, high DOC input, and reactivity (Evans et al.2017., Staehr et al, 2012), and the Arctic is expected to experience rapid climate changes (Bruhwiler et al, 2021). If these lakes are exposed to greater precipitation or less precipitation, this would change the input of DOC, while changing water residence times and the hydrology between these lakes and the catchment. If the larger lakes in Churchill change to shorter residence times, due to precipitation increase, they could exhibit similar high, but fluctuating, reactivity as the small lakes. Potentially leading to greater pCO_2 concentration, and CO_2 emissions. On of the other hand, decreases in precipitation could lead to higher water residence times (Cardille et al, 2009) and less input of DOC, decreasing reactivity and CO_2 emissions. Regardless, both scenarios will influence the patterns between lake area and DOC factors found in this study, so any potential future studies in this field site may not necessarily find the same conclusions.

DOC reactivity in these lakes is highly variable, affected by both intrinsic and extrinsic factors. However, incubations of lake samples are still useful in understanding how DOC reactivity in lakes varies both in laboratory conditions and in the real environment. There is evidence of turnover, decomposition, and transformation of DOC in these lakes, adding to the view of the dynamic nature of inland waters and lakes as hotspots of carbon. Furthermore, this project further demonstrates the usefulness of fluorescent spectroscopy and PARAFAC, in identifying CDOM and fluorescent components, allowing in depth comparisons and discussion. Comparing composition with reactivity reveals patterns in DOC dynamics, and with an increasing number of studies focused on this area, there is an opportunity to improve our understanding of DOC in lakes, across different regions and environmental conditions.

6. Conclusion

We found that microbial DOC reactivity and DOC composition had weak relationships in lakes of the Churchill region of arctic Canada, but we identified the net production of FDOM and CDOM in dark incubations. Clear water lakes had the highest FDOM production, especially with the C3 protein-like component. Lake area proved to be an overarching influence of lake dynamics, as the smaller lakes showed the highest CDOM values, protein-like component production, humic-like component consumption, and largest BP variation. Furthermore, pCO_2 concentration was shown to be larger in small lakes, linked partially to BR, while DOC composition and amount was found to influence pCO_2 to an unconfirmed extent.

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Appendix

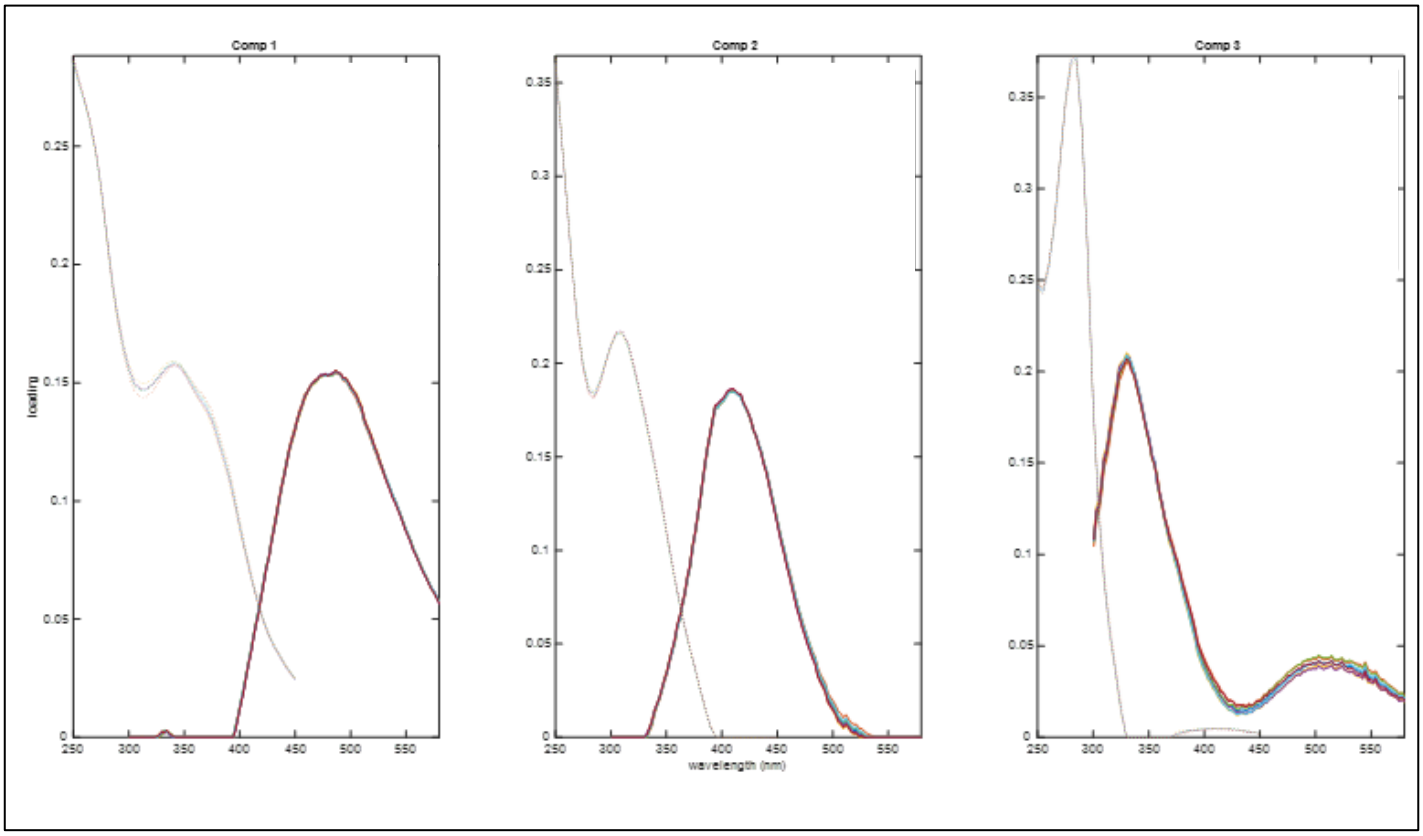

Figure S1. Spectral signatures of the 3 fluorescent PARAFAC (Parallel factor analysis) components identified. The dashes line represents the excitation spectra, while the dotted line represents the emission spectra, with each line representing the different splits of the data used to analyse and validate the 3 components. The plots themselves represent the split-half validation of the model, and the close similarity between the loadings on the y axis with the excitation and emission spectra validates that a three component PARAFAC model fits the dataset.

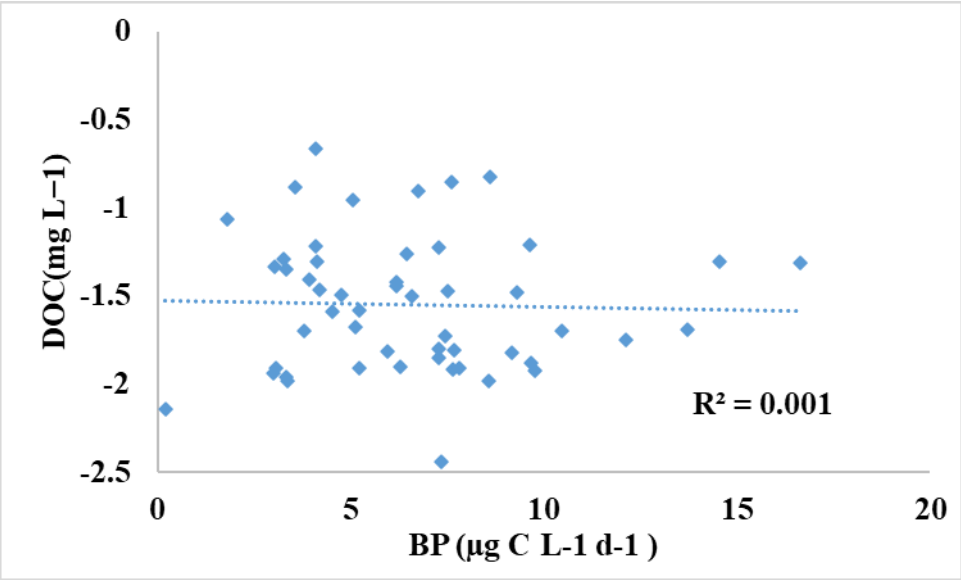

Figure S2. Correlation between Dissolved Organic Carbon loss over 28-day incubation against Bacterial Production from start of incubation, with the blue line showing the linear trend. Data generated from 54 lake samples from Churchill Canada taken in August 2022.

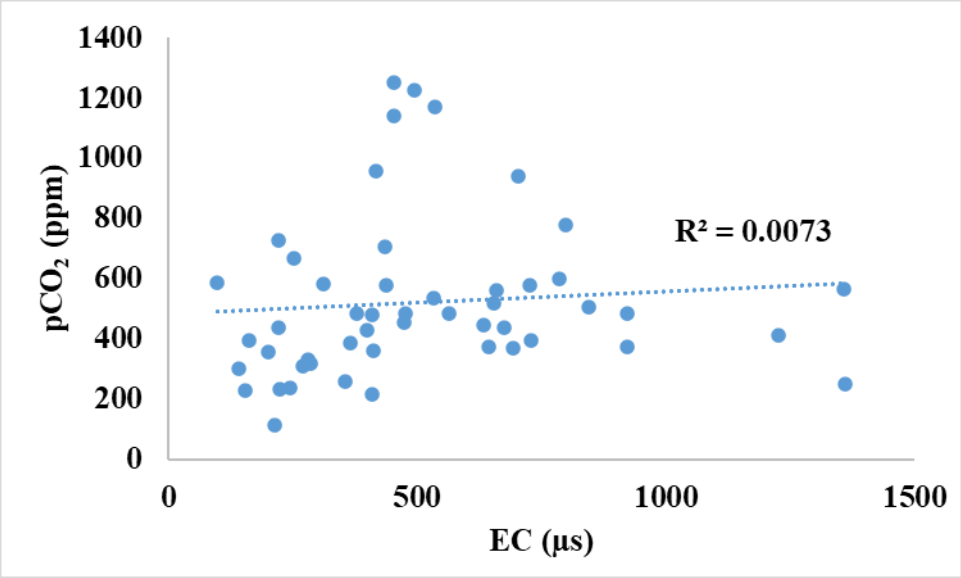

Figure S3. Correlation between partial pressure of CO_2 against Electrical Conductivity, with the blue line showing the linear trend. Data generated from 54 lake samples from Churchill Canada

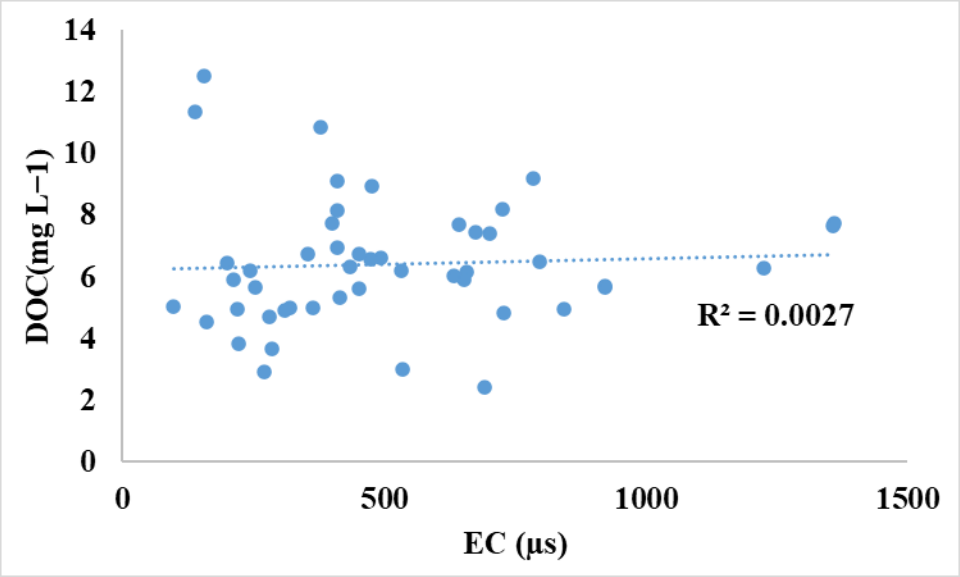

Figure S4. Correlation between Dissolved Organic Carbon at start of incubation against Electrical Conductivity, with the blue line showing the linear trend. Data generated from 54 lake samples from Churchill Canada taken in August 2022.

Table S1. Table showing the 54 lake samples from 53 lakes (as 36 is an extra sample from the same lake as 35), with their respective latitude and longitudes of their exact sampling point.

Sample number	Latitude (N)	**LONGITUDE (W)**
1	58.753	93.82303
2	58.75335	93.8208
3	58.75541	93.82069
4	58.75654	93.8243
5	58.75594	93.82724
6	58.75661	93.82774
7	58.75775	93.82731
8	58.76534	93.81715
9	58.76535	93.81548
10	58.76687	93.81101
11	58.75912	93.82306
12	58.73266	93.89581
13	58.73557	93.88643

14	58.73505	93.88554
15	58.73357	93.88887
16	58.73793	93.90935
17	58.73039	93.77856
18	58.72521	93.77226
19	58.72261	93.77155
20	58.71747	93.77209
21	58.7089562	93.758119
22	58.7072303	93.7638882
23	58.7079573	93.7610618
24	58.7185845	93.8368364
25	58.7185845	93.8368364
26	58.72837	93.82946
27	58.7272396	93.8277536
28	58.73018	93.82849
29	58.72678	93.83517
30	58.70616	94.0528
31	58.7446733	93.8918622
32	58.69233	93.74934
33	58.6274751	93.8191325
34	58.7328972	93.7944307
35	58.7277979	93.8174881
36	58.7277979	93.8174881
37	58.7253075	93.80916
38	58.7256627	93.8064618
39	58.7219182	93.8078823
40	58.7210842	93.8147974
41	58.720628	93.8148026
42	58.722847	93.8157314
43	58.7233468	93.8172174

44	58.7408003	93.822205
45	58.7410079	93.8219244
46	58.74322	93.83139
47	58.74502	93.83664
48	58.7443576	93.8354468
49	58.7462118	93.8439718
50	58.7406943	93.8227753
51	58.74152	93.8236
52	58.75231	93.91719
53	58.72939	93.91559
54	58.6274751	93.8191325