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Causes and consequences of soil carbon mobilization and lake brownification in northern forested landscapes

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Abstract

Global changes are fundamentally changing terrestrial and aquatic ecosystems. Some of the highest rates of global change are in the north, where they are leading to the faster destabilization of forest soil carbon and its mobilization as dissolved organic matter and causing the brownification of lakes. This thesis investigated the causes of soil carbon destabilization and consequences of the mobilized soil carbon to lake food webs. The first finding was that global change increased carbon export from catchments. Increased temperatures and changes in hydrologic connectivity interacted with catchment topography to modulate the timing, magnitude, and fate of soil carbon export. Increased temperatures led to hydrologic disconnectivity that favoured export of soil carbon from carbon-rich wetlands to the atmosphere. However, extreme precipitation events saturated the soils and increased the frequency of periods of hydrologic connectivity from the catchment to the drainage network that led to higher export of carbon to streams, rivers, and lakes. The second finding was that increased carbon content in lakes with an associated shift towards more refractory carbon resulted in lower light availability and larger nutrient pools in lakes. Brownification of clear oligotrophic lakes increased pelagic primary productivity, but favoured cyanobacteria that could adapt to the browner conditions. The third finding was that changes in the biomass and composition of phytoplankton communities altered the carbon transfer and efficiency of lake food webs. The brownification-driven shift towards cyanobacteria prevalence was associated with a decline in phytoplankton quality due to cyanobacteria having a lower content of essential fatty acids. The decline in phytoplankton quality did not impact the essential fatty acid content of primary consumers, but it shifted their reliance from essential fatty acids transferred from phytoplankton, to essential fatty acids transferred through the less-efficient bacterial driven microbial loop. As global change proceeds, further destabilization of soil carbon is likely to stop having a stimulatory effect on lake production by alleviating nutrient limitation, to having an inhibitory effect by creating light limitation. Once lakes pass this threshold, the declines in the productivity and transfer of essential fatty acids to higher trophic levels will place food webs at great risk.

Keywords

Carbon, dissolved organic matter, brownification, primary production, cyanobacteria, aquatic food webs, essential fatty acids.

Co-Authorship Statement

Oscar E. Senar (OES) and Irena F. Creed developed the ideas and hypotheses for this thesis. IFC provided the funding through a Natural Sciences and Engineering Research Grant. This thesis contains three manuscripts.

Chapter 2 was published in *Journal of Geophysical Research: Biogeosciences* (reprint permission in Appendix C). OES was the first author and the co-authors were Kara L. Webster (KLW) –Canadian Forest Services–, and IFC. OES, KLW, and IFC contributed to the synthesis of ideas. KLW and IFC provided the original data; and OES conducted all statistical analysis. All authors contributed to the preparation of the manuscript.

Chapter 3 will be submitted as a manuscript for publication. OES will be the first author and IFC will be a co-author. OES and IFC contributed to the synthesis of ideas. OES designed and conducted the fieldwork, laboratory, and geographic information systems (GIS) analysis. Both authors contributed to the study design, data analysis and preparation of the manuscript.

Chapter 4 was submitted as a manuscript for publication to the journal *Freshwater Biology*. OES is the first author, and IFC, Ursula Strandberg (US) and Michael T. Arts (MTA) –postdoctoral fellow and professor at Ryerson University, respectively– are co-authors. OES and IFC developed the original idea and hypothesis of the manuscript, and US and MTA conducted the laboratory analyses. All authors will collaborate on editing the final manuscript prior to publication.

Quan surts per fer el viatge cap a Ítaca,
has de pregar que el camí sigui llarg

A Inma, Ángel, i les Vicentiques

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List of Abbreviations

C	Carbon
CO_2	Carbon dioxide
CyanoHABs	Cyanobacteria harmful algal blooms
DHA	Docosahexaenoic acid
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid
Fe	Iron
FI	Fluorescence index
LC-SFA	Long-chain fatty acids
N	Nitrogen
NPP	Net primary production
P	Phosphorus
PC	Phycocyanin
PCA	Principal component analysis
PLSR	Partial least squares regression
POC	Particulate organic carbon

PUFA	Polyunsaturated fatty acids
SOC	Soil organic carbon
SFA	Saturated fatty acids
SUVA	Specific UV absorbance
TCU	True colour units
TLW	Turkey lakes watershed
TN	Total nitrogen
TP	Total phosphorus
WTD	Water table depth

Chapter 1

1 Introduction

1.1 Problem Statement

Carbon (C) is a defining element of the Anthropocene, an era characterized by global changes in atmospheric, climatic, and hydrologic conditions driven by human actions on Earth (Lewis & Maslin, 2015). These changes are triggering alterations in the stability of C pools and fluxes between ecosystems (Raupach & Canadell, 2010). Anthropocene-associated changes are being observed globally, but northern ecosystems are experiencing the fastest rates of change (Smith et al., 2015). Lakes in particular have been identified as sentinels of change as they integrate atmospheric, terrestrial, and aquatic processes (Adrian et al., 2009; Williamson et al., 2009). The destabilization of soil C pools is resulting in increases of terrestrial dissolved organic matter (DOM) loads from the surrounding catchments to lakes at northern latitudes (Monteith et al., 2007; Creed et al., 2018). This results in brownification of northern lakes (Kritzberg & Erkström, 2012). Brownification drives changes in phytoplankton community composition, favouring organisms that are able to thrive in low-light and low-nutrient availability conditions (Jones, 1998). In fact, brownification has been linked to declines in phytoplankton diversity and the formation of cyanobacteria harmful algal blooms (cyanoHABs) in oligotrophic systems (Sorichetti et al., 2014; Urrutia-Cordero et al., 2017). Furthermore, these changes in phytoplankton community composition are changing C transfer and efficiency along aquatic food webs (Karlsson et al., 2009; Finstad et al., 2014), potentially altering the capacity of lakes to provide aquatic ecosystem functions and associated services. This thesis examines the drivers and consequences of brownification in northern lakes, linking brownification-associated changes in lakes to the increased prevalence of cyanobacteria, and the potential consequences of the increased prevalence of cyanobacteria on food webs.

1.2 Scientific Justification

1.2.1 Carbon Cycling in the Anthropocene

Carbon cycling alterations are driving changes in terrestrial and aquatic ecosystems (Walther et al., 2002). The lithosphere is the largest C pool on Earth (Falkowski et al., 2000; Figure 1.1), with over 75 million Pg C (1 Pg = 1 Gt = 10^9 tonnes = 10^{12} Kg). The extraction of hydrocarbons from the Earth's crust and their combustion combined with emissions from deforestation and land use changes average 10.7 Pg C y^{-1} during the last decade (Le Quéré et al., 2017).

Oceans are the second largest C reservoirs, storing 38,200 Pg C (Cox et al., 2000) as inorganic C (CO_2 and bicarbonate and carbonate ions), and dissolved and particulate organic C (DOC, POC; Post et al., 1990). From all three C fractions, the inorganic C is in equilibrium with atmospheric CO_2 concentrations, and projected increases in atmospheric CO_2 will result in further increases in oceanic C content (Cox et al., 2000).

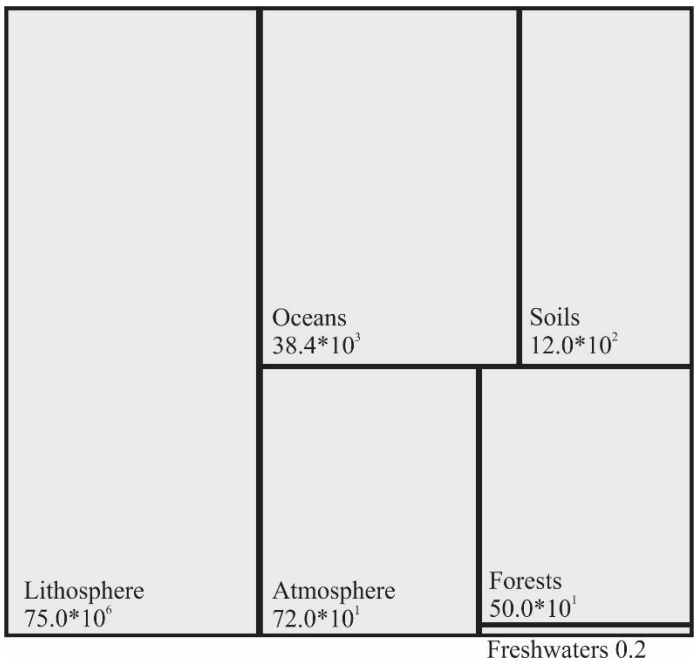

Figure 1.1. Global C pools in Pg C (data sources referenced in text). Box sizes are proportional to the (log-scaled) content of C in each pool.

Forests store over 1,200 Pg C, and over two-thirds of their total C is contained in their soils (Cox et al., 2000; Dixon et al., 1994). Given that photosynthesis is the major C flux (estimated between 150 – 175 Pg C y^{-1}; Welp et al., 2011), and greater than the total forest respiration (a flux approximately equal to 98 Pg C y^{-1}), forests are considered C sinks (Bond-Lamberty & Thomson, 2010). However, increases in air temperatures are enhancing soil microbial metabolism at a fast rate, and studies suggests that the stability of forest soils may be compromised, shifting from C sinks to C sources by 2100 (Cox et al., 2000). The shift of forest soils from C sinks to sources will result in further increases of atmospheric concentrations of C greenhouse gases, intensifying the alterations in climatic and hydrologic patterns and consequently in global C cycling (Kirtman et al., 2013).

Soil respiration is the major C export flux from forest soils (Davidson et al., 2006). Soil heterotrophic respiration is controlled by environmental conditions (soil temperature and moisture) and characteristics of the C pools (quantity and quality; Webster et al., 2014; Lecki & Creed, 2016). Both, soil conditions and C pools are further controlled by landscape topography (Webster et al., 2008). For example, wetlands and ecotones (i.e., transition zones that are intermittently wet) store large quantities of C, compared to uplands (Webster et al., 2008). Soil temperature and moisture directly influence soil microbial metabolism (Kang et al., 2003). While soil temperature is strongly correlated to air temperature, soil moisture is controlled by precipitation and the capacity of soil to retain water (Lohse et al., 2009), and can inhibit microbial respiration by inducing water limitation (low moisture) or air limitation (high moisture). In addition, the availability of soil C in the different landscape positions is modulated by the C sorption capacity of soils (Kaiser et al., 1996).

Besides exporting C to the atmosphere, soils also export C to the aquatic network. However, this flux (estimated at 1.9 Pg C y^{-1} globally; Cole et al., 2007) is smaller than the atmospheric one, and is often neglected from global C budgets (Räike et al., 2012). Freshwaters have been considered ‘passive pipes’ that transport C from terrestrial ecosystems to oceans (Cole et al., 2007), even though it is estimated that lake sediments store 820 Pg C (Einsele et al., 2001). Despite being a relatively small flux, the export of

C from soils as POC and DOC plays a significant role on aquatic ecosystem functioning (Battin et al., 2008). C fluxes from terrestrial to aquatic ecosystems are increasingly driven by a combination of atmospheric, climatic, and hydrologic mechanisms (Solomon et al., 2015). The process of increasing terrestrial organic subsidies to aquatic systems is known as brownification and is characterized by changes in water colour associated to terrestrial DOC (Williamson et al., 2015).

Hydrologic connectivity plays an important role in driving the partitioning of the total soil C export between its atmospheric or aquatic fates (Pacific et al., 2010; Riveros-Iregui, 2012). Hydrologic connectivity is the transfer of matter and energy mediated by water fluxes between landscape positions (Turnbull et al., 2008; Bracken et al., 2013). There is a direct link between hydrologic connectivity and aquatic C export. Besides this, changes in hydrologic connectivity control atmospheric C export by regulating soil moisture (Bracken et al., 2013).

Besides the intensification of hydrologic cycling (Huntington, 2006), the mobilization of terrestrial C to the northern aquatic ecosystem increases with the observed increases in temperature (Freeman et al., 2001; Kirtman et al., 2013) and longer growing seasons (Jeganathan et al., 2014; Creed et al., 2015). In addition, warmer and longer growing seasons enhance terrestrial net primary production (NPP), resulting in larger soil C pools (Jansson et al., 2008). Land cover influences DOM loads, which are higher from forested than agricultural lands (Kritzberg, 2017), and higher from coniferous than deciduous forests (although current trends are leading to greater deciduous than coniferous forest; Sittaro et al., 2017). Finally, the recovery from acidification reported in Europe and North America (Stoddard et al., 1996) further promotes brownification, as higher soil pH reduces soil C adsorption capacity and increasing its mobility (Monteith et al., 2007; Ekström et al., 2011). All these mechanisms have been identified as major drivers of brownification of freshwater systems (Creed et al., 2018; Figure 1.2).

The shift from C sink to C source results in an increase in atmospheric C export and the associated increase in atmospheric CO_2 that contributes to further warming (i.e., a

positive feedback) (Davidson & Janssens, 2006). It also results in an increase in aquatic C export that contributes to brownification of lakes, leading to alterations in aquatic primary production which in turn effect the stability and nutritional quality of aquatic food webs (Williamson et al., 2015; Creed et al., 2018).

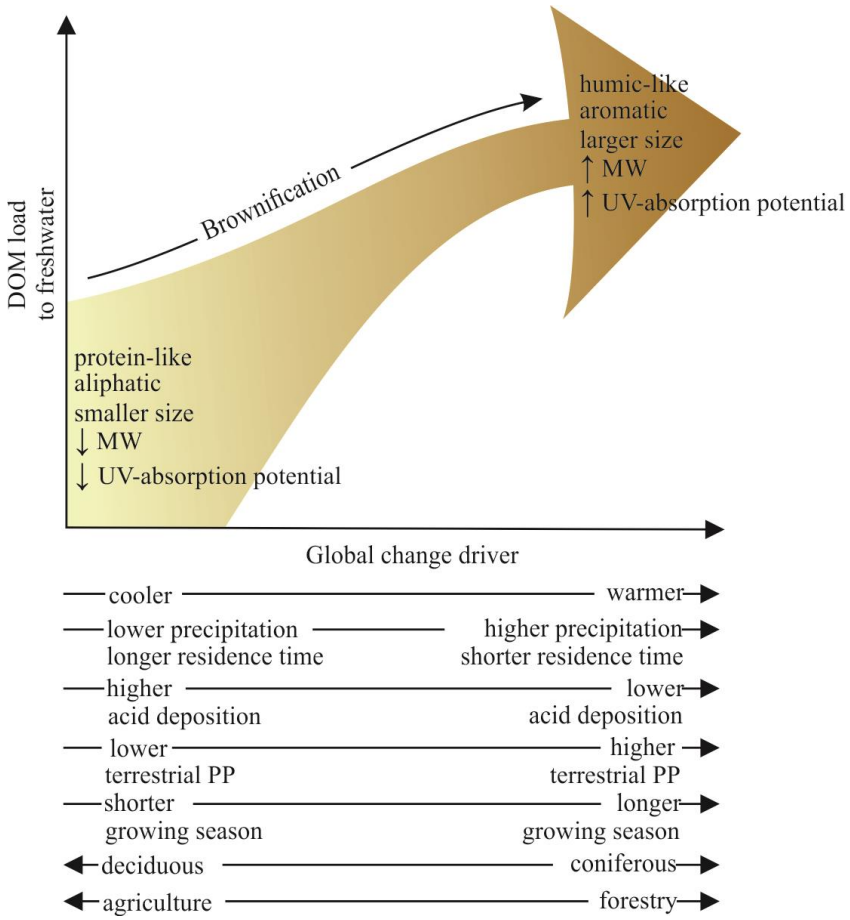

Figure 1.2. Drivers of brownification and associated changes in lake DOM quality (MW = molecular weight, PP = primary production) (Adapted from Creed et al., 2018).

1.2.2 The brown new world: Consequences of brownification in northern lakes

Dissolved organic matter is a mixture of heterogeneous compounds from biological origin defined as the fraction of organic particles that pass through a filter between 0.7 and 0.2 µm (being the most common reported fraction the one that passes through a 0.45µm filter; Xu & Guo, 2017). DOM composition depends on its origin and

the physical, chemical, and biological transformations it undergoes from source to lake (Kang & Mitchell, 2013). Both DOM quantity and quality are important in determining its role in aquatic ecosystems (Fellman et al., 2010). Generally, autochthonous (in-lake produced) DOM is labile (i.e., protein-like, aliphatic, low molecular weight) while allochthonous (externally produced) DOM is more refractory (i.e., humic-like, aromatic, high molecular weight). Allochthonous DOM is generally the dominant fraction in the DOM pool of lakes (Berggren et al., 2014). Quantification of DOM is measured as dissolved organic carbon (DOC) concentration, and its characterization has been traditionally based on fractionation by acidic functionality, hydrophobic character, and molecular weight (McKnight et al., 2003). Key advances in DOM characterization are substituting the traditional separation methods for more advanced separation and characterization techniques like mass spectrometry (e.g., Fourier-transform ion cyclotron resonance-mass spectrometry; Woods et al., 2011) and chromatography (e.g., high-performance liquid chromatography, high-performance size exclusion chromatography, and hydrophilic interaction chromatography; Woods et al., 2011) that provide greater chemical resolution (Woods et al., 2011). Among the new techniques, spectrophotometry and absorbance are frequently used for being relatively cheap and simple analyses that are highly sensitive and widely used (McKnight et al., 2001; Woods et al., 2011).

Due to differences in composition between autochthonous and allochthonous DOM, an increase in terrestrial DOM subsidies is not only associated with increases in DOC, but also shifts in DOM quality towards more refractory DOM (Creed et al., 2018). Brownification takes its name from the changes in water colour driven by allochthonous DOM inputs, richer in chromophores than autochthonous DOM. In addition, iron (Fe) complexes DOM (especially in DOM originating in wetlands) increasing water colour (Kritzberg & Erkstrom, 2012). As a consequence of the increasing water colour, brownification is associated with decreases in light penetration and shallower euphotic zones (Karlsson et al., 2009; Figure 1.3). The reduction of light penetration in the water column decreases photosynthetic active radiation, potentially inducing light limitation to primary producers (Karlsson et al., 2009). In addition, the lower penetration of solar radiation creates a shallower and warmer epilimnion with a more stable thermocline (i.e., greater differences in the temperature between epilimnion and hypolimnion; Houser,

2006). The shallower epilimnion results in a greater proportion of nutrients stored in the hypolimnion, where most of primary producers cannot access them (Fee et al., 1996).

Dissolved organic matter contains C and other macronutrients (nitrogen (N) and phosphorus (P)) and micronutrients (Fe) (Findlay, 2003). In addition to its role as a nutrient vector, DOM maintains P and Fe in solution preventing them from precipitating in the oxic conditions of the epilimnion (Jones, 1998). For this reason, the organic fraction of P and Fe can represent a major proportion of the total nutrient pool (Findlay, 2003). DOM quality modulates nutrient speciation and bioavailability (Findlay, 2003), and consequently an increase in nutrient inputs is not always associated with an increase in the pool of available nutrients. Increasing DOM aromaticity is associated with lower nutrient bioavailability, but also with higher photolability (i.e., susceptibility to be photo-mineralized; Moran & Zepp, 1997). Photo-mineralization of aromatic DOM results in the formation of aliphatic organic and inorganic C compounds, increasing nutrient availability (Bertilsson & Tranvik, 2000; Jones, 1998).

Brownification induces changes in primary production due to changes in physical and chemical conditions of lakes (Finstad et al., 2014; Solomon et al., 2015; Creed et al., 2018). In clear oligotrophic systems, allochthonous DOM inputs may induce shifts in the trophic status of lakes by supplying nutrients and supporting phytoplankton growth (Seekell et al., 2015). However, in dark humic lakes, increases in DOM inputs will limit primary production by reducing the availability of photosynthetic active radiation (Thrane et al., 2014). The composition of the phytoplankton community in lakes with low versus high allochthonous DOM inputs will be influenced by nutrient and light availability conditions and the species-specific strategies that enable them to adapt to these changes and outcompete other taxa.

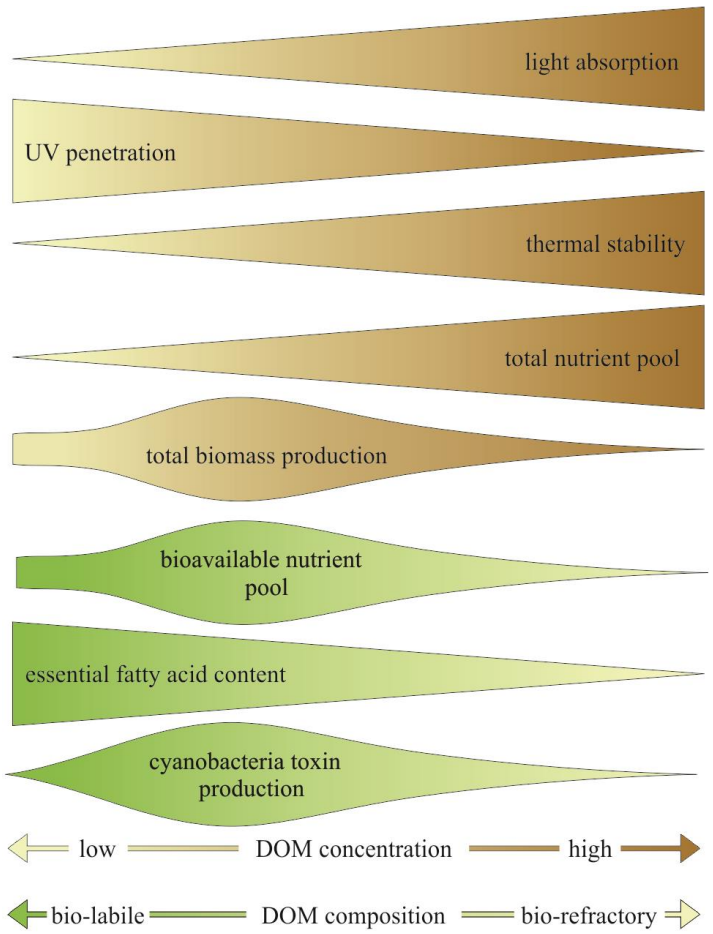

Figure 1.3. Effects of DOM in freshwater systems (Adapted from Creed et al., 2018).

1.2.3 Endless forms most beautiful: Adaptive strategies of cyanobacteria to brownification

The combined effects of brownification and warming have in fact been associated with decreases in phytoplankton diversity and increases in cyanobacteria growth (Urrutia-Cordero et al., 2017). Among all phytoplankton species, cyanobacteria often receive more attention due to their harmful effects on human health and well-being (Paerl et al., 2001). Cyanobacteria, also referred to as blue-green algae, are a diverse group of prokaryotic autotrophs composed of over 3000 species (Guiry, 2012; Nabout et al., 2013). Their characteristic blue-green coloration is given by their accessory pigments, the phycobiliproteins phycocyanin (PC) and phycoerythrin (PE), that allow them to photosynthesize in a broader wavelength range than those photoautotrophs with only

chlorophyll (chl). The dominant paradigm is that cyanobacteria blooms are triggered by excess macronutrients (P and N), a paradigm based on the ground-breaking experiments in the Ontario Experimental Lakes Area carried out in the 1970s (Schindler, 1977). In these experiments, cyanobacteria responded to whole-lake N and P additions by increasing their growth and forming a bloom despite low C concentrations (Schindler & Fee, 2011). A reduction in the formation of cyanoHABs in eutrophic (nutrient-rich) lakes has been associated with a reduction of P (and to a lesser extent N) loads from agricultural and residential areas to lakes (Sterner, 2008; Paerl et al., 2016). However, the formation of cyanoHABs in oligotrophic (nutrient-poor) lakes is an emerging problem. A new lens on the dominant macronutrient limitation paradigm is that macronutrients, rather than being externally loaded to lakes is being released from sediments (i.e., internal loading) (Paerl et al., 2011). An alternative to the macronutrient limitation paradigm is that micronutrients (like Fe) are also being released from the sediments and are promoting cyanobacteria growth (Molot et al., 2014).

The rise in cyanoHABs may also be associated with climate change, including browner waters (Creed et al., 2018; Urrutia-Cordero et al., 2017), increases in water temperatures (Paerl & Huisman, 2008), and a lengthening of the ice-free season (Kanoshina et al., 2003). Accessory pigments give cyanobacteria a competitive advantage against other phytoplankton groups under low-light availability (Pattanaik et al., 2010). Besides their ability to adapt to low light conditions, other competitive advantages that cyanobacteria have include the following. Higher temperature optima confer them with faster growth rates in the warmer conditions of the epilimnion of a brownifying lake (Paerl & Huisman, 2008; O'Neil et al., 2012). Gas vesicles allow them to access deeper areas temporarily, away from the euphotic zone to areas with higher nutrient concentrations (e.g., *Aphanizomenon sp.*; Carey et al., 2012). Luxury P uptake and storage provides a P source under low P availability conditions (Paerl & Otten, 2013), a process that is enhanced due to their small surface area to volume ratio (Finkel et al., 2010). Greater P loads that result in declines of N:P ratios favour those species able to fix N (Chaffin & Bridgeman, 2014). Production of organic ligands allows them to scavenge Fe from DOM complexes (e.g. *Dolichospermum sp.*; Wilhelm & Trick, 1994). Finally,

mixotrophy, allows them to access C under low-light conditions and macro- and micro-nutrients adsorbed to DOM (Jones, 1998; Deininger et al., 2017).

CyanoHABs can impact benthic primary production by increasing water turbidity and reducing light penetration. In addition, the rapid production of biomass enhances bacterial degradation, which can cause anoxia and in extreme cases fish kills (Smith et al., 1999; O'Neil et al., 2012). Of even greater concern is the role of cyanobacteria as neurotoxin and hepatotoxin producers (Paerl et al., 2001). Microcystins, the most common cyanotoxins in freshwaters, are hepatotoxins that target the liver and pose risk to animal and human health (Carmichael, 2001; O'Neil et al., 2012).

The consequences of brownification, therefore, go beyond changes in physical and chemical characteristics of lakes (Creed et al., 2018). Changes in primary production (both biomass and composition) will likely translate into changes in the quantity and quality of basal resources for pelagic food webs.

1.2.4 Consequences of brownification for lake food webs

Dissolved organic matter is a nutrient source for primary producers, but also for heterotrophs and mixotrophs (Hessen et al., 1990). An increase in allochthonous DOM inputs provides basal resources for bacteria thereby promoting heterotrophic growth (Tranvik, 1992). The transfer of C to higher trophic levels through the heterotrophic pathway is less efficient than the autotrophic pathway due to the energy lost in the two additional trophic transfers (ciliates, flagellates) prior to reaching the zooplankton level (Jones, 1998). Despite this, the heterotrophic food web can constitute a major C transfer pathway in dystrophic or ultra-oligotrophic lakes where primary production is minimal (Craig et al., 2005; Premke et al., 2010).

Increases in allochthonous DOM inputs can drive a shift from algal to bacterial production (Kissman et al., 2017). However, heterotrophic-based food webs may lack the essential nutrients to support secondary growth (Faithfull et al., 2011; Wenzel et al., 2012). Autochthonous resources contain essential fatty acids (EFAs) that are necessary for consumers' growth and reproduction and are only produced by phytoplankton (Lau et

al., 2014). EFAs determine energy transfer efficiency (Müller-Navarra et al., 2000), and are preferentially retained by consumers and progressively enriched (Persson & Vrede, 2006). Among EFAs, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) are structurally vital for cell membranes functionally vital for reproduction and growth in consumers (Sargent et al., 1995; Parrish, 2009). Therefore, while it may constitute an alternative C source, the extent to which allochthonous DOM subsides can support aquatic food webs is debated (Brett et al., 2017).

While the EPA and DHA content in phytoplankton has proven to be essential to support zooplankton production (Brett et al., 2009), food webs studies based on stable isotope analyses identify high reliance (> 80%) of zooplankton on allochthonous DOM subsidies in low productivity lakes (Cole et al., 2006, 2011). Further research investigating C sources and aquatic food webs concluded that a combination of both autochthonous and allochthonous DOM support zooplankton production (Wilkinson et al., 2013) as long as sufficient EFAs from phytoplankton are available (Tanentzap et al., 2017). In fact, it has been observed that an increase in autochthonous DOM may prompt the uptake of allochthonous DOM (Guillemette et al., 2016), or vice versa (Grieve et al., 2018). However, when autochthonous DOM is in low supply, consumers may allocate EFA to anabolic, rather than catabolic processes, in order to survive periods of scarcity (Wetzel et al., 1995).

Brownification can also alter the production and pools of EFA in autochthonous resources by driving changes in the composition of the phytoplankton community (Strandberg et al., 2015; Taipale et al., 2016). Among phytoplankton, most eukaryotes (except for green algae) produce relatively high concentrations of EPA and DHA (Lau et al., 2014). However, cyanobacteria are considered poor quality resources due to their low content in EPA and DHA. It is for this reason that brownification-driven changes in phytoplankton communities have been associated with a decrease in EFA pools in lakes (Taipale et al., 2014, 2016). In addition, increases in water temperature, CO_2 concentrations, and nutrient availability (all of them indirect consequences of brownification) have been associated with lower EFA production and content in

phytoplankton (Guschina & Hardwood, 2009; Hixson & Arts, 2016). However, brownification can also prevent EFA damage from light (Harwood, 1998).

Brownification affects C transfer pathways, basal resource quantity, and basal resource quality, and therefore the nutritional quality of aquatic food webs. The shifts in EFA availability and transfer have direct consequences on the nutritional content of top predators (i.e., fish; Taipale et al., 2018), and their quality for human consumption (Hixson et al., 2015).

1.3 Goals and Hypotheses

Brownification has been suggested to promote cyanobacteria growth in oligotrophic lakes due to the ability of cyanobacteria species to adapt to brownification-driven alterations in lakes. However, studies that integrate multiple stressors of brownification (e.g., light, nutrients, temperature) are not common, and often focus on DOM quantity while overlooking the effects of changing DOM quality (Creed et al., 2018). The goal of this study is to identify drivers of brownification and the consequences of changing DOM quantity and quality for primary production and food webs in temperate oligotrophic lakes. The objectives of this thesis are: (1) to identify the hydrologic drivers of soil C mobilization and transport to aquatic systems; (2) to identify the effects of brownification on lake primary production; and (3) to describe the consequences of brownification-associated changes on the nutritional quality of pelagic food webs. I explore these objectives through three hypotheses:

H1. Hydrologic connectivity drives the magnitude and partitioning of soil C export to the atmospheric and aquatic fates, favoring aquatic C export in wet years when the landscape becomes hydrologically connected.

H2. Nutrient inputs associated with brownification drive the shift from oligo- to meso- and ultimately eutrophic conditions, increasing lake primary production, and favouring cyanobacteria through shifts towards more refractory DOM quality.

H3. Brownification-driven shifts in primary producer biomass and community composition will result in larger fatty acid pools in oligotrophic lakes; however, the

proportion of EFAs will decrease due to EFA-poor cyanobacteria dominance. Consequently, the transfer of EFA to consumers will decrease, shifting their reliance from autotrophic to heterotrophic and terrestrial C sources.

Findings from this thesis will improve our knowledge on the role of DOM quality (rather than just quantity) on modulating primary production and food web efficiency. In addition, this thesis will provide support for explanations for the rise in cyanobacteria growth and cyanoHABs formation alternative to the current paradigms of N and P triggered cyanoHABs.

1.4 Thesis Organization

The structure of this thesis follows the integrated article format and consists of three manuscripts (chapters 2 to 4) that explore each one of the three hypotheses. The introduction (Chapter 1) sets the theoretical background of brownification, its causes, consequences, and associated changes in DOM quantity and quality. The first manuscript (Chapter 2) investigates the role of hydrologic connectivity on the magnitude and partitioning of soil C export along a topographic gradient in a five-year time-series collected in a small temperate catchment in central Ontario. The second manuscript (Chapter 3) researches the effects of DOM quantity and quality in lake physical and chemical conditions, and consequently on phytoplankton and cyanobacteria biomass in 71 lakes across central Ontario. The third manuscript (Chapter 4) investigates the brownification-associated changes in the pools and transfer of EFA from primary producers to zooplankton (cladocerans and copepods) in a subset of lakes studied in the previous chapter. Findings from the manuscripts are summarized in the last chapter (Chapter 5), which additionally identifies main conclusions, scientific significance of these conclusions, and future research directions.

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Chapter 2

2 Catchment-scale shifts in the magnitude and partitioning of carbon export in response to changing hydrologic connectivity in a northern hardwood forest

2.1 Introduction

World forest soils are major carbon (C) pools storing 1255 Pg C (Cox et al., 2000). While soils are considered C sinks, destabilization of soil organic carbon (SOC) stores due to a changing climate is occurring around the globe (Bellamy et al., 2005). A key question that arises is which fate of destabilized SOC, aquatic or atmospheric, will become more dominant under a new climate regime. Destabilized soil C mobilized in hydrologic flows transfers C to downstream aquatic ecosystems as particulate or dissolved forms of C. Destabilized C that is mineralized by soil microbes transfers C to the atmosphere as carbon dioxide (CO_2) or methane (CH_4). Acceleration of either or both of these export pathways decreases the C sequestration potential of forest soils, raising substantial doubts whether soils will continue to function as stable stores for C (Bellamy et al., 2005; Schulze & Freibauer, 2005).

Atmospheric export of SOC that is respired is considered to be the second most important C flux in forests, following gross primary production (Davidson et al., 2006a). Forests constitute a net sink of atmospheric CO_2 given that CO_2 emissions through respiration are more than offset by photosynthetic C fixation. However, respiration is thought to be more sensitive to changing temperatures than photosynthesis, resulting in predicted declines in forest sink strength (Davidson & Janssens, 2006). CO_2 emissions through respiration includes autotrophic and heterotrophic sources and has been estimated at 98 ± 12 Pg C y^{-1} globally (Bond-Lamberty & Thomson, 2010). Heterotrophic sources are controlled by microbes influenced by environmental conditions, primarily soil temperature and moisture, C availability and C quality (Lecki & Creed, 2016; Webster et al., 2014). Climatic shifts towards warmer conditions enhance rates of heterotrophic respiration (Kirschbaum, 1995), but simultaneous changes in soil moisture can limit microbial respiration by reducing the diffusion of DOC (low soil

moisture) or oxygen (high soil moisture) to aerobic microbes (Lohse et al., 2009). The timing of these changes in environmental conditions is equally important. For example, an increase in temperature in winter may be more important than increases during the summer when heterotrophic activity is supported by freshly fallen litter (Baldrian et al., 2013). Alternatively, a decrease in temperature in winter can create soil frost that promotes breakdown of SOC and, if the frost is followed by consistent snow cover that insulates soils, an increase in heterotrophic activity (Brooks et al., 1997; Brooks & Williams, 1999; Brooks et al., 2011). Similarly, a larger proportion of precipitation falling in summer can increase respiration by relieving microbial water-limitation, but is insufficient to alleviate plant water stress that suppresses plant productivity (Stielstra et al., 2015).

Aquatic C export represents a smaller proportion of total export compared to atmospheric C export; it has generally been neglected in global C stock and flow estimates (Räike et al., 2012), but is still an important C flux from forest ecosystems (Öquist et al., 2014). Aquatic organic C export can occur as particulate organic C (POC) or dissolved organic C (DOC), but DOC represents the major fraction of the total C export and the most important fraction for aquatic ecosystems (Battin et al., 2008). DOC from forests is a heterogeneous mixture of complex organic compounds of various origins such as root exudates, plant litter (e.g., leaves, woody debris), peat and recalcitrant soil organic matter (Kalbitz et al., 2000). Increases in DOC concentration and flux in streams have been observed in recent years, although the proposed mechanisms for this increase vary (e.g., Tranvik & Jansson, 2002; Roulet & Moore, 2006; Creed et al. 2018). Shifts in forest cover (Kritzberg et al., 2017, Sittaro et al., 2010) and productivity (Lepistö et al., 2014) driven by rising temperatures and longer growing season (Jeganathan et al., 2014) could increase the SOC pools and its transformations. In addition, declines in atmospheric acid deposition (Evans et al., 2006; Monteith et al., 2007; Sawicka et al., 2016) and changes in precipitation and soil moisture that influence the frequency, magnitude and timing of hydrologic events, increase soil DOC solubility and may result in mobilization or retention of DOC (de Wit et al., 2016; Raymond & Saiers, 2010). While the effects of individual factors on DOC production and mobilization have been described, the generalized increase in DOC subsidies in aquatic

system is most likely driven by complex interactions between these drivers (Creed et al., 2018).

Changes in both temperature and precipitation influence hydrologic connectivity, the water-mediated transfer of matter and energy between different landscape positions (Bracken et al., 2013; Turnbull et al., 2008), which also plays a major role on SOC export. Within northern hardwood forests, there is spatial heterogeneity among topographic features in soil C stores (Creed et al., 2002; Webster et al., 2011), soil porewater DOC (Creed et al., 2013) and soil environmental factors (Webster et al., 2008a, b), which in turn leads to spatial heterogeneity in source areas for the precursors of DOC and CO_2 hotspots (Creed et al., 2013; Webster et al., 2008a, b). The potential C export from a catchment is determined by the diversity of landscape units, their slopes, and how they are spatially linked (Bracken et al., 2013; Turnbull et al., 2008). It is also determined by changes in discharge generating events (e.g., snowmelt, storms) that generate connections between the landscape units (Okin et al., 2015). During wet periods the areas further upslope in the catchment are more hydrologically connected to the stream and during dry periods source areas within the catchment are hydrologically isolated (Jencso et al., 2009). Inter-annual variability in meteorological conditions may alter these patterns in hydrologic connectivity, influencing DOC export (McGlynn et al., 2003; Pacific et al., 2010).

This paper examines how inter-annual variability in meteorological conditions affects the magnitude and partitioning of C export pathways to aquatic or atmospheric fates. We hypothesize an overall similar magnitude of C export among years, but that hydrologic connectivity determines whether aquatic versus atmospheric C export pathways dominate. We predict that during wet years, landscapes become hydrologically connected, favoring aquatic fate over atmospheric fate, and that during dry years, landscapes become hydrologically disconnected, effectively trapping precursors of CO_2 (Holden, 2005) and thus favoring atmospheric fate. Temperature may further complicate the partitioning of C, as increased temperature raises the rate of biological reactions including decomposition of SOC. To test this hypothesis, we pose the following question: how do changes in meteorological conditions affect hydrologic connections within catchments and, in turn, the magnitude and partitioning between aquatic and atmospheric

fates of C export from the catchment? We test this hypothesis in a catchment within a long-term ecological monitoring watershed in the Great Lakes-St. Lawrence forest region (Rowe, 1972) of northeastern Ontario over a five-year period from 2006 to 2010 during which substantial variability in meteorological conditions were observed.

2.2 Methods

2.2.1 Study Area

The Turkey Lakes Watershed (TLW) is a 10.5 km^2 experimental watershed centered at 47º03'N and 84º25'W approximately 60 km north of Sault Ste. Marie in the Algoma Highlands of northeastern Ontario in the Great Lakes-St. Lawrence forest region (Rowe, 1972; Figure 2.1). For over 35 years, federal government agencies (Natural Resources Canada and Environment and Climate Change Canada) have been monitoring TLW catchments, streams, and a chain of five lakes that drain into Norberg Creek, then Batchawana River, and ultimately Lake Superior. The focus of this study is on Catchment 38 (c38), a 6.3 ha first order catchment within the TLW which contains uplands draining into a large swamp (representing 20% of the catchment area) which in turn drains into a stream (Figure 2.1).

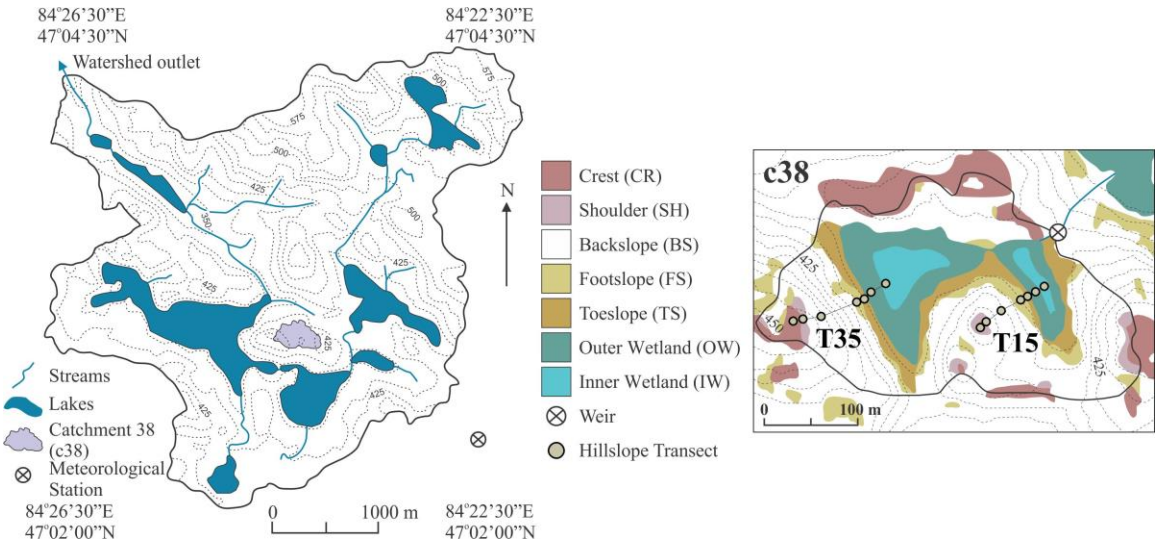

Figure 2.1. The Turkey Lakes Watershed centered at 47°'03' N and 84°'25' W with the location of catchment C38 highlighted. C38 catchment (right) divided into topographic positions and two sampling transects.

The TLW is positioned leeward to Lake Superior and consequently the continental climate is strongly influenced by the lake. Over the climate record from 1982 to 2010, average annual temperature from the Algoma station of the Canadian Air and Precipitation Monitoring Network (CAPMON) located just outside the watershed (47°02'06"N, 84°22'52"W) was 4.6 °C and average total annual precipitation was 1189 mm y^{-1}. A snowpack typically persists from late-November through to early April. Peak discharge events occur in late May following snowmelt and again in late September to early October, coinciding with autumn storms.

The TLW rests on Precambrian silicate greenstone formed from metamorphosed basalt, with small outcrops of felsic igneous rock near Batchawana Lake and Little Turkey Lake (Jeffries et al., 1988). The overall relief of the TLW is 390 m, from 630 m above sea level at the top of Batchawana Mountain to 240 m above sea level at the outlet to the Batchawana River. The topography is strongly influenced by the bedrock and contains rugged slopes which terminate in depressions that may be hydrologically connected or disconnected from the drainage systems. This has formed topographic features that can be classified as uplands (frequently dry), ecotone (intermittently wet) and wetlands (frequently wet). The overlaying soils are composed of a two-component till ranging in depth from < 1 m in uplands to 2 m in lowlands, and up to 70 m in bedrock

faults. Due to shallow soil depths and the low permeability of the lower basal tills, snowmelt and rainstorm water infiltrate the surface soils and move laterally along the interface between the upper ablation till and the lower basal till or bedrock.

The TLW is covered by an uneven-aged northern hardwood forest. This forest is dominated (90%) by sugar maple (*Acer saccharum* Marsh.) with occurrences of white pine (*Pinus strobes* L.), white spruce (*Picea glauca* Moench Voss.), ironwood (*Ostrya virginiana* (Mill.) K. Koch) and yellow birch (*Betula alleghaniensis* Britton) (Wickware & Cowell, 1985). Uplands have a relatively uniform stand density (904 stems ha^{-1}), dominant height (20.5 m), diameter at breast height (15.3 cm) and mean basal area (25.1 m^2 ha^{-1}) (Jeffries et al., 1988). In wetlands, stand density increases and dominant height decreases. The sparse understories of upland stands are dominated (≥ 95%) by saplings and seedlings of sugar maple as well as a variety of herbs and ferns. Wetland stands contain a mixture of black ash (*Fraxinus nigra* Marsh.), eastern white cedar (*Thuja occidentalis* L.), red maple (*Acer rubrum* L.), balsam fir (*Abies balsamea* (L.) Mill.), yellow birch (*Betula alleghaniensis* Britt.), and tamarack (*Larix laricina* (DuRoi) K. Koch.) (Wickware & Cowell, 1985). Wetland understories are composed of the seedlings and saplings of overstory trees and various ferns and herbs.

Digital terrain analyses on a LiDAR-derived digital elevation model (5 m resolution) were conducted to discretize c38 into seven topographic features along a hillslope gradient (Webster et al., 2011). Based on the map of the discretization of these topographic features (Figure 2.1), two transects, one shallow (T15) and one steep (T35) were established on northerly hillslopes. Plots were established along both transects in upland features [crest (CR), shoulder (SH), and backslope (BS)], ecotone features [footslope (FS), and toeslope (TS)] and wetland features [the perimeter of the wetland referred to as outer wetland (OW) and the centre of the wetland referred to as inner wetland (IW)]. At each plot, environmental conditions (soil temperature and moisture), atmospheric C fluxes and soil C pools were sampled.

2.2.2 Aquatic Carbon Export

Stream DOC samples were collected weekly to biweekly throughout the summers and winters and daily during spring melt and autumn storms from 2006 to 2010. Grab samples were collected in high density polyethylene bottles as water flowed over the V-notch weir (Figure 2.1). These samples were filtered through 0.45 μm mixed cellulose ester membrane filters (GN-6, Pall Gelman Science, Ann Arbor, Michigan) and analyzed for DOC and dissolved inorganic C (DIC) within 48 hrs on an AA3 Autoanalyzer (Seal Analytical). DIC was excluded from the analysis as it represented a small proportion of the total annual dissolved C export during the study period (ranging from 6.48 kg DIC ha^{-1} to 14.99 kg DIC ha^{-1} in 2006 and 2008, respectively).

Stream daily discharge (mm d^{-1}) was measured at the c38 outlet from 1982 to 2010. Discharge was determined from continuous recordings of stream stage at the V-notch weir and converted to discharge from established stage-discharge relationships. Stream DOC flux was derived from measurements of discharge and the concentration of DOC in the discharge in samples collected throughout the year. Daily concentrations of DOC in discharge were generated by linear interpolation between sampling dates. Flux of total dissolved C (kg C ha^{-1}) was calculated as the product of daily discharge and the daily concentration of DOC in discharge and summed for the water year before being normalized by catchment area (6.3 ha). Water table depth (WTD; cm) in the wetland was calculated as the average of the outer wetland WTD measurements collected in each transect using a water level logger (WT-HR Water Height Data Logger, TruTrack Inc.). Measurements were taken hourly and averaged to produce daily WTD.

2.2.3 Atmospheric Carbon Export

Daily soil CO_2 efflux (μmol m^{-2} d^{-1}) was calculated for each of seven features on both the shallow (T15) and steep (T35) slope transects using the empirical model developed by Lecki & Creed (2016). Chamber-based measurements of CO_2 fluxes collected during the same time period from the same study area were used to create this model. In order to predominantly capture heterotrophic soil respiration, chambers were located away from tree roots and vegetation was removed from the collars. CO_2 was

sampled every 2-3 weeks during the summer period, at weekly intervals during the early and late growing season, and at daily intervals during the spring melt in April (Equation 1, (adj. $r^2 = 0.72$, $p < 0.05$):

$$\begin{aligned} Daily\ CO_2\ efflux \\ &= \exp(-1.5983 + 0.1603T + 0.0519M - 0.0008M^2 \\ &- 0.0019C_{FFL} - 0.0006C_{LFH} + 0.0006C_{Ah} + 0.0001C_{Ae} \\ &- 0.05SC_{Ah} - 0.1001SC_{Ae}) \end{aligned} \quad [1]$$

where T is soil temperature (˚C), M is soil moisture (%volume), C is carbon pool content (g C m^{-2}) and SC is carbon sorption capacity (mol m^{-2}), with subscripts referring to C pools within freshly fallen leaves (FFL) and the litter fibric-humic (LFH) soil layers, and the eluviated Ae and organic rich Ah horizons (as defined in the Canadian System of Soil Classification; Canadian Agricultural Services Coordinating Committee & Soil Classification Working Group, 1998).

Soil temperature and moisture were recorded using Campbell Scientific CR 10X data loggers as mean hourly values and then averaged for each day. Soil temperature was measured with a thermocouple wire (Type T Omega FF-T-24-TWSH) embedded into a copper tube with epoxy. Soil moisture was measured with a Campbell Scientific CS616 Water Content Reflectometer (WCR, Campbell Scientific Canada Corp., Edmonton, AB). These sensors were placed at a depth of 5 cm below the surface of the mineral soil. Soil moisture was converted to volumetric water content through calibration equations provided by the manufacturer for measurements taken in upland positions, and by Yoshikawa et al. (2004) for measurements in wetland soils. Regressions between soil temperature and moisture data from the same topographic positions of the other transect were built to interpolate missing data in the snow-free season (April 1 to November 30). Linear interpolation was used to infill temperature and moisture for those days in which neither transect had available data for certain features. Soil temperature and moisture for the snow season (December 1 to March 31) were assumed constant and the average between the last and first day of the preceding and following snow-free seasons.

Soil organic C pools were measured at each topographic feature along the hillslope transect by sampling the organic and mineral soil pools prior to the formation of the snowpack in 2006 and 2008 (see Appendix A, Table A1) (Webster et al., 2008a). FFL samples (n = 5) were collected prior to the development of a snowpack on 30 cm × 30 cm mesh placed on the surface of forest floor prior to leaf fall. LFH samples (n = 3) at each position were collected by cutting 15.5 cm × 15.5 cm blocks into the forest floor and measuring the height of the LFH block so that the total volume could be calculated. At the upland features, A horizon samples (n = 3) were collected for chemistry using an open-sided sampler (40 cm × 4.4 cm I.D.) and for bulk density using an AMS split core sampler (32 cm × 4.8 cm I.D.) with slide hammer attachment (AMS Inc., American Falls ID, USA). At the wetland features, peat (0-10 cm below LFH layer) samples (n = 3) were collected for chemistry and for bulk density using a Jeglum sampler (7.6 cm × 7.6 cm × 50 cm) (Jeglum et al., 1992). All soil samples were placed in labelled plastic bags and transported from the field to the laboratory in coolers. Once in the laboratory, samples to estimate bulk density were oven dried at 60 °C (for FFL, LFH and peat) or 105 °C (for A horizon) until constant weight. Stones > 2 mm in diameter were removed and weighed to correct for coarse fragment content. Soil samples for chemistry were air dried at 25 °C and then sieved (2 mm). The samples were analyzed for total C using a Carlo-Erba NA2000 analyzer at the Great Lakes Forestry Centre. Some of the samples were analyzed for total organic C using loss on ignition at 500 °C (Kalra & Maynard, 1991), and these analyses indicated that there was little to no inorganic C in the samples as the concentration of total C and total organic C were not significantly different. This finding is supported by previous literature, which indicates that the content of calcium carbonate ($CaCO_3$) in the TLW soil ranges from 0 to 2% (Jeffries et al., 1988). SOC pools in FFL were calculated by multiplying organic C concentrations by litter mass. SOC pools in LFH and the A horizon or peat were calculated by multiplying the organic C concentration by bulk density (g m^{-2}) and then by depth of each horizon (m).

Soil DOC can be sorbed onto iron (Fe) and aluminum (Al) oxyhydroxides in mineral soils via ligand exchange (Kaiser et al., 1996). Sorption capacity was determined by the concentration of Fe oxyhydroxides from dithionite-citrate-bicarbonate (DCB) extraction and Al oxyhydroxides produced from ammonium oxalate (AO) extraction

(Shaw, 2001) as described in Creed et al. (2013). The concentrations of Fe and Al oxyhydroxides were determined using inductively coupled plasma atomic emission spectroscopy. Sorption capacity was calculated as the sum of Al_{AO} (Al extracted using AO) and Fe_D (Fe extracted using DCB). Potential saturation of the sorption capacity was estimated by the atomic ratio of SOC to sorption capacity (after Kaiser & Zech, 1998) (see Appendix A, Table A2).

Carbon export from each topographic feature was calculated by averaging modeled CO_2 values from both transects from each topographic feature and multiplying the averaged CO_2 by its area within the catchment. The total area of each topographic feature within the catchment was 0.35 ha (5.5%) CR; 0.32 ha (5.0%) SH, 3.15 ha (49.7%) BS; 0.31 ha (4.9%) FS; 0.63 ha (9.9%) TS; 0.92 ha (1.6%) OW; and 0.66 ha (10.5%) IW. The topographic features were then grouped into three major positions of upland, ecotone and wetland. Atmospheric C export for upland was the sum CR, SH, and BS, for ecotone was the sum of FS and TS, and for wetland was the sum of OW and IW. The atmospheric C export for the catchment (kg C ha^{-1}) was the sum of the seven topographic feature exports (or three position exports).

2.2.4 Determination of hydrologic connectivity periods

Water years from June 1 to May 31 were defined for the analysis of C export fluxes in order to avoid splitting the spring snowmelt or fall storms hydrologic connectivity periods into two consecutive water years. WTD and discharge were used to determine periods of hydrologic connectivity (fall storms and spring snowmelt) and disconnectivity (summer and winter) within the catchment (Figure 2.2). Hydrologic connectivity was defined as snow-free periods during which WTD was greater than 0 cm (i.e., inundated) and discharge was greater than a low-flow threshold of 0.5 mm d^{-1} (Figure 2.2a, 2.2b). The 0.5 mm d^{-1} discharge threshold was selected based on the relationship between discharge and WTD – a lower discharge threshold would include days in which WTD would be below the surface, while a higher discharge threshold would exclude days with WTD < 0 cm. The snow-free period was defined as the period between snowpack disappearance and appearance, estimated from snow cover data from National Aeronautics and Space Administration's Northern Hemisphere EASE-Grid 2.0

Weekly Snow Cover and Sea Ice Extent dataset (version 4) (Creed et al., 2015). Seven-day intervals that met the WTD and discharge criteria in each day were classified as hydrologically connected (Figure 2.2c). Hydrologically connected (fall storms and spring snowmelt) and disconnected (summer) periods were homogenized by removing trailing discontinuous periods (Figure 2.2.d).

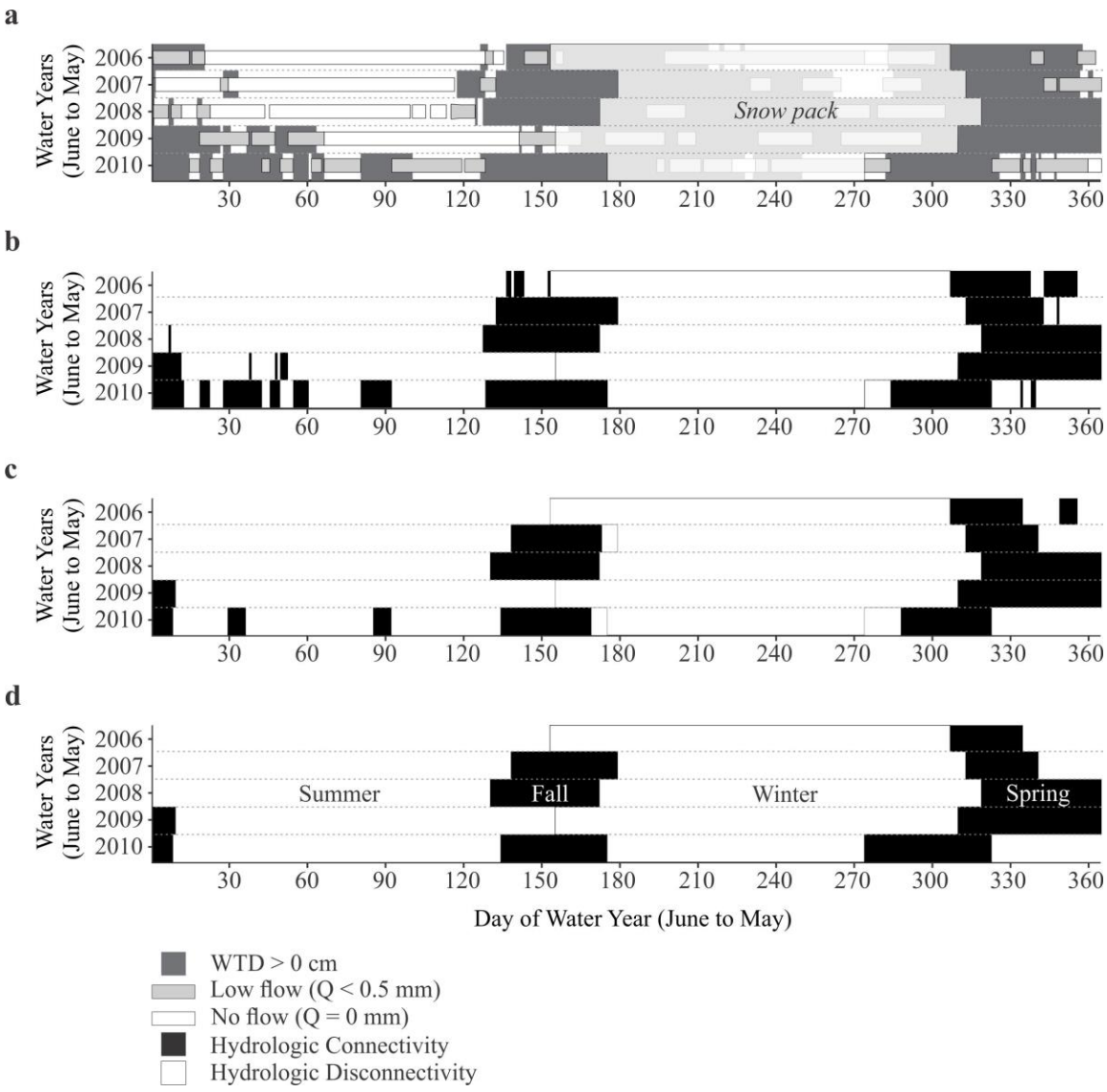

Figure 2.2. Definition of hydrologic connectivity periods in four steps. (a) Identification of days with WTD > 0 cm (black), low-flow days (discharge < 0.5 mm d^{-1}) (grey bars), no-flow days (discharge = 0 mm d^{-1}) (white bars), and snowpack period (grey area). (b) Days that met WTD and discharge hydrologic connectivity criteria. (c) Seven-day periods that met WTD and discharge hydrologic connectivity criteria in

each day. (d) Final hydrologic connectivity (fall storms and spring snowmelt) and disconnectivity (summer and winter) periods.

Determination of the timing and duration of hydrologic connectivity periods between the entire time series from 1982 to 2010 was based on the discharge criterion alone due to the lack of WTD data. However, using the discharge criterion alone classified 98% of the days in the 2006 to 2010 period compared to the combination of the WTD and discharge criteria.

2.2.5 Statistical Analysis

One-way ANOVA on ranks followed by Dunn's post hoc pairwise comparison tests were used to detect significant differences in temperature, discharge, and atmospheric and aquatic C export among seasons. Linear regression analyses were used to identify potential relationships between climatic parameters (annual and snow-free discharge, soil moisture, and air temperature) and annual and snow-free aquatic and atmospheric C export. All data in the regression analysis passed tests for normality and constant variance and did not require transformation. All ANOVA on ranks, post hoc pairwise comparison tests and regression analyses were performed in Sigma Plot 12.0 (Systat Software, San Jose, CA). Long-term (1982-2010) temporal trends of temperature, discharge and duration of the hydrologic seasons (as defined by the state of hydrologic connectivity) were analyzed using the non-parametric Mann-Kendall trend test using the package 'Kendall' in R (v 3.3.1) (McLeod, 2011; R Core Team, 2017).

2.3 Results

2.3.1 Seasonal Trends

The division of the water year based on states of hydrologic connectivity resulted in four hydrologic periods in most of the years (Figure 2.2). There were two periods of hydrologic connectivity characterized by high discharge (fall storms and spring snowmelt), and two periods of hydrologic disconnectivity characterized by low discharge (summer and winter). While there were no significant differences between the median temperatures during the fall storms (7.3°C) and the spring snowmelt (7.1°C), median daily discharge during the spring snowmelt (1.69 mm d^{-1}) was significantly larger than in

any other period, including fall storms (median discharge = 1.0 mm d^{-1}; Table 2.1). Among hydrologic disconnected periods, the summer period was warmest (median daily temperature = 16.4°C) with lower flow (median discharge = 0.02 mm d^{-1}) than any other hydrologic period, while the winter period was coldest (median temperature = -6.2°C), with slightly more discharge than the summer period (median discharge = 0.46 mm d^{-1}).

The differences in temperature and discharge among seasons were coupled with differences in aquatic and atmospheric C export. Aquatic C export was highest during hydrologic connectivity periods during the fall storm period (median daily stream DOC concentration = 0.19 kg DOC ha^{-1} d^{-1}) and spring snowmelt period (0.21 kg DOC ha^{-1} d^{-1}), and was lowest during the hydrologically disconnected periods during the summer (0.00 kg DOC ha^{-1} d^{-1}) and winter (0.06 kg DOC ha^{-1} d^{-1}) (Table 2.1). In contrast, atmospheric C export displayed an annual peak during the summer (42.8 kg C-CO_2 ha^{-1} d^{-1}) and an annual low during the winter (6.6 kg CO_2-C ha^{-1} d^{-1}), with higher atmospheric C export during the fall storms (18.7 kg C-CO_2 ha^{-1} d^{-1}) compared to the spring snowmelt (9.9 kg C-CO_2 ha^{-1} d^{-1}; Table 2.1). Results from two-way ANOVA on ranks identified significant differences in atmospheric C export among seasons and positions, with the ecotone being the largest source of atmospheric C export among all seasons, and the wetland being the smallest source of atmospheric C export (Table 2.1).

Table 2.1. Summary results from (1) one-way ANOVA on ranks and post-hoc pairwise Dunn's test for temperature, discharge, DOC export and catchment CO_2 efflux between hydrological connectivity periods, and (2) two-way ANOVA on ranks and post-hoc pairwise Holm-Sidak test for CO_2 efflux between hydrological periods and positions. Uppercase letters represent significant differences between hydrological connectivity periods, and lowercase letters represent significant differences between positions.

Hydrologic connectivity periods	Temperature (°C)	Discharge (mm d^{-1})	Aquatic C export (kg DOC ha^{-1} d^{-1})	Atmospheric C export (kg CO_2-C ha^{-1} d^{-1})			
				Catchment	Wetland	Ecotone	Upland
Summer							
Median	16.40	0.02	0.00	42.82	28.48	61.72	43.03
25%	12.80	0.00	0.00	31.78	18.79	44.46	31.31
75%	19.10	0.28	0.05	54.80	40.35	77.57	56.26
Sig. Dif	**A**	**A**	**A**	**A**	**Aa**	**Ab**	**Ac**
Fall Storms							
Median	7.30	1.00	0.19	18.70	9.83	27.24	20.46
25%	3.20	0.16	0.03	10.85	5.48	16.51	11.73
75%	12.10	2.28	0.41	31.61	19.61	44.99	33.18
Sig. Dif	**B**	**B**	**B**	**B**	**Ba**	**Bb**	**Bc**
Winter							
Median	-6.24	0.46	0.06	6.56	3.29	10.76	6.72
25%	-10.60	0.23	0.03	6.26	3.28	9.40	6.65
75%	-1.50	0.90	0.12	6.62	4.07	11.38	6.95
Sig. Dif	**C**	**C**	**C**	**C**	**Ca**	**Cb**	**Cc**
Spring Snowmelt							
Median	7.05	1.69	0.21	9.92	4.95	12.93	10.66
25%	3.20	0.89	0.11	7.08	3.28	10.31	7.93
75%	10.65	5.75	0.53	15.55	4.07	21.03	17.44
Sig. Dif	**B**	**D**	**D**	**D**	**Da**	**Db**	**Dc**

2.3.2 Annual Trends

Carbon export in the 5-year period (2006 to 2010) was dominated by atmospheric C export (Figure 2.3), approximately two orders of magnitude larger than aquatic C

export. Catchment atmospheric C export during the 2006 to 2010 period differed between positions: the ecotone presented the largest export and the smallest variability among years, whereas the upland and wetland positions showed an inverse relationship with increases in atmospheric C export in the wetland corresponding to decreases from the upland and vice versa. During this 5-year period, there was an increase in the contribution to catchment atmospheric C export from the upland (from 29% in 2006 to 38% in 2010) and a decrease from the wetland (shifting from 25% contribution in 2006 to 16% in 2010).

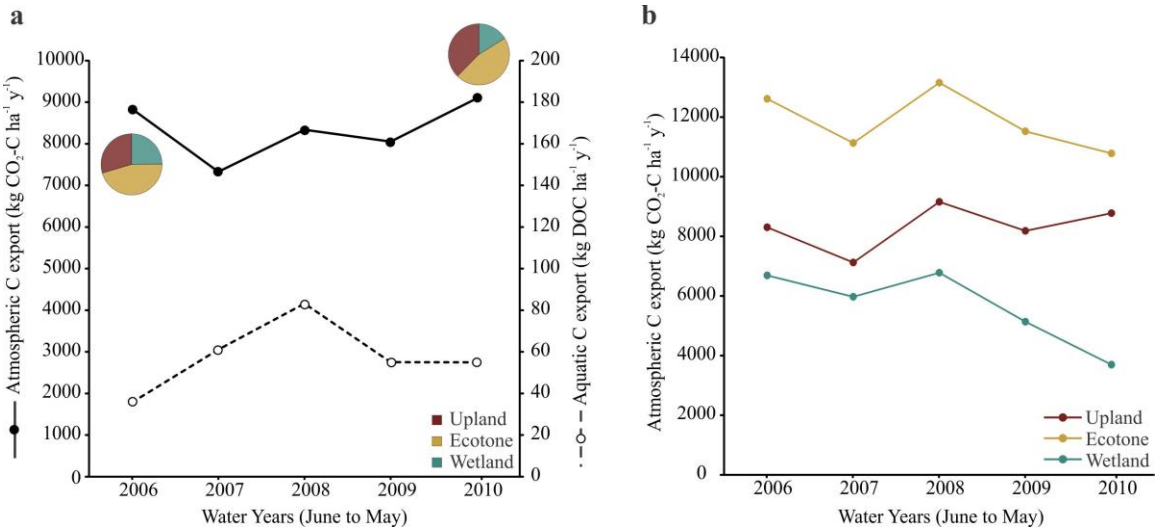

Figure 2.3. (a) Catchment-integrated annual C export (measured as kg C ha^{-1} y^{-1}) into the aquatic (dashed line, right axis) and atmospheric fates (solid line, left axis), and (b) separation of the atmospheric C export according to topographic positions. Pie charts represent contributions of each topographic position in the whole-catchment atmospheric C export for 2006 and 2010.

Catchment aquatic C export was a function of discharge at both annual (r^2 = 0.89, p = 0.02) and snow-free timescales (r^2 = 0.84, p = 0.05; Figure 2.4a); as discharge was used in the calculation of this export flux. Annual atmospheric C export (from the catchment or any of the positions) was not significantly correlated to either discharge or air temperature (Figure 2.4a, 2.4c). However, snow-free season atmospheric C export (which represented between 86% and 90% of the annual atmospheric C export in the catchment) was linearly related to air temperature during the snow-free season in the wetland (r^2 = 0.75, p = 0.05) (Figure 2.4c). Average soil moisture was negatively correlated to atmospheric C export from the ecotone at annual (r^2 = 0.96, p = 0.03) and

snow-free timescales ($r^2 = 0.80$, $p = 0.40$), and to atmospheric C export from the wetland annually ($r^2 = 0.66$, $p = 0.096$; Figure 2.4b).

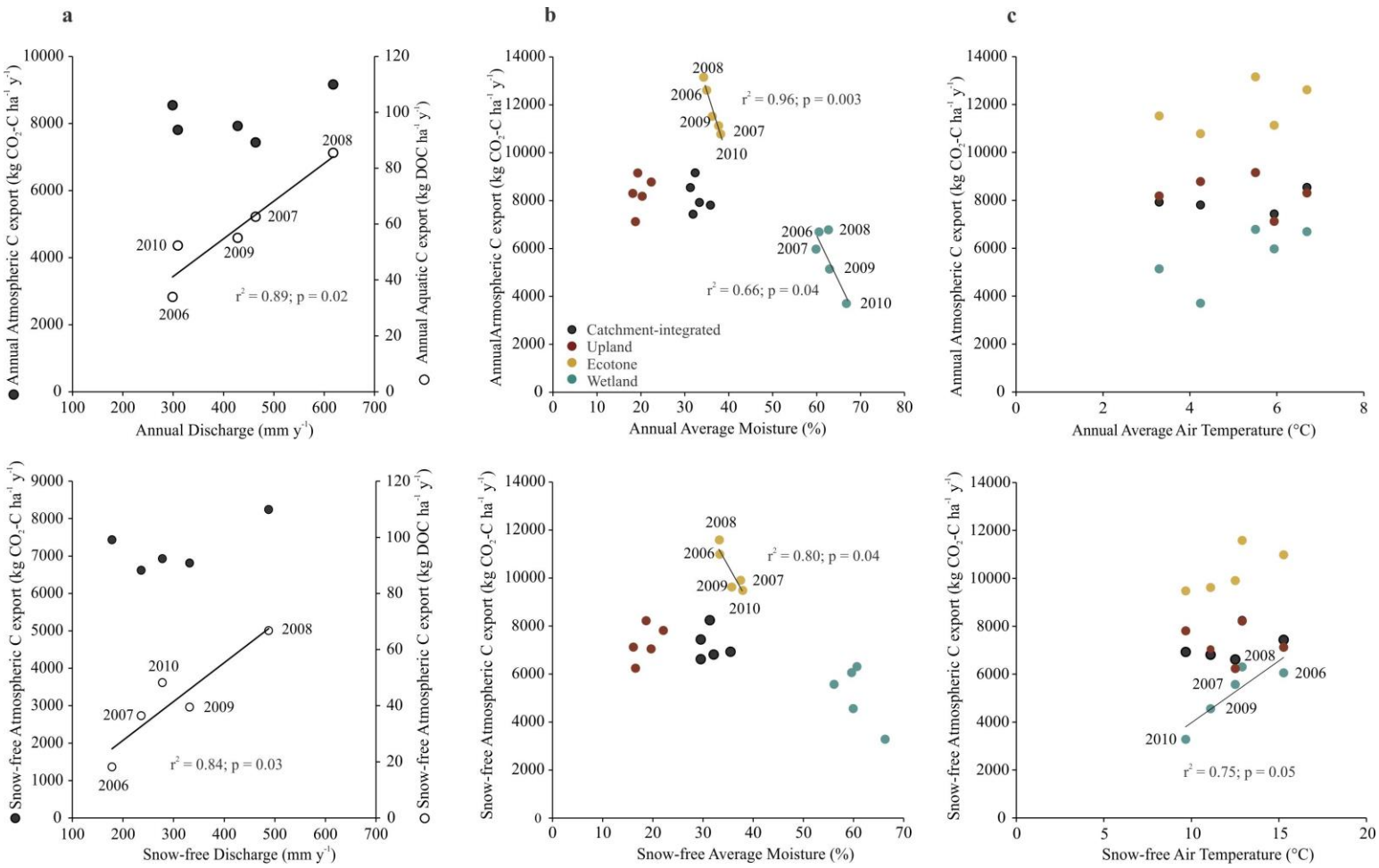

Figure 2.4. (a) Relationship between discharge and atmospheric C export (left axis) and aquatic C export (right axis), (b) relationship between soil moisture and atmospheric C export, and (c) relationship between air temperature and atmospheric C. Top panels display annual values and bottom panels display snow-free period values. Lines represent significant correlations.

2.3.3 Long-Term Trends

Long-term trends in meteorological data showed a shift towards warmer (average annual temperature $\tau = 0.31$, $p = 0.02$) and drier conditions (annual discharge $\tau = -0.41$, $p = 0.002$). There were also changes to the timing, duration and magnitude of discharge of the hydrologic periods (Figure 2.5). While the snowpack disappeared earlier in the year ($\tau = -0.34$, $p = 0.01$) without significant changes in its appearance or duration, the duration and discharge of the fall storms periods slightly declined ($\tau = -0.24$, $p = 0.07$ and $\tau = -0.25$, $p = 0.07$ respectively). In addition, both the beginning and the end of the spring snowmelt shifted earlier in the year ($\tau = -0.32$, $p = 0.02$ and $\tau = -0.24$, $p = 0.05$ respectively), without significant changes in its duration or discharge. The earlier spring and the shorter fall lead to a lengthening of the summer period ($\tau = 0.46$, $p < 0.01$).

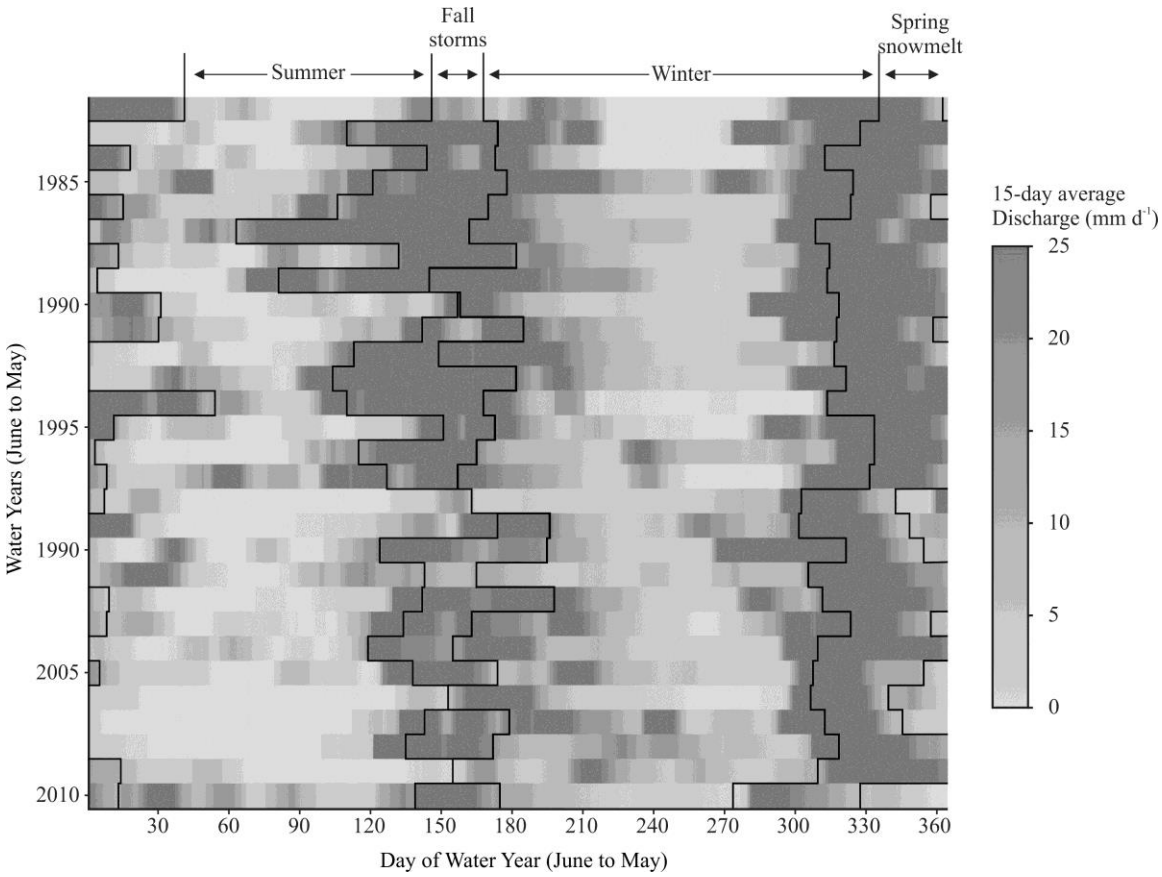

Figure 2.5. Daily discharge (15-day moving average) from 1982 to 2010, and division of the water years into four hydrologic periods.

Long-term trends in the environmental drivers of snow-free aquatic and atmospheric C export display opposite trends (Figure 2.6). While annual discharge (correlated with aquatic C export) presented a negative trend (τ = -0.23, p = 0.09), air temperature (correlated with atmospheric C export from the wetland) increased (τ = 0.23, p = 0.08).

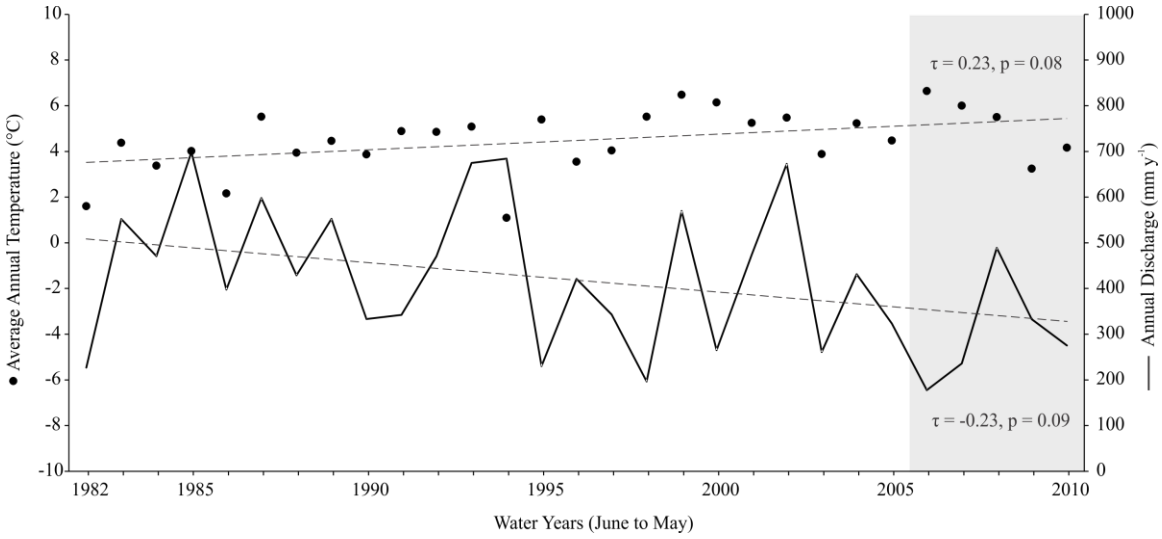

Figure 2.6. Annual average air temperature (°C; circles, left axis) from Algoma CAPMON station, and annual stream discharge (mm d^{-1}) from c38 (solid line, right axis) from 1982 to 2010. Grey area represents 2006 to 2010 study period.

2.4 Discussion

Forests across the world are major C sinks, storing over two-thirds of their C pool in the soil (Dixon et al., 1994). Temperate forests account for approximately 30% of the global organic C sink, but SOC input and export fluxes may be compromised by changes in hydrologic patterns (Pan et al., 2011). In temperate forested catchments, hydrologic connectivity controls the magnitude and distribution of soil moisture, the depth of the water table in wetlands, and the discharge to the stream. These hydrologic characteristics interact with soil C pools and enhance or suppress soil microbial respiration, directly influencing the mobilization of C into the drainage network (Jencso et al., 2009, Lohse et al., 2009). This study considered the role of hydrologic connectivity, which controls soil moisture and is reflected by changes in stream discharge, in mediating the export of SOC,

identifying the extent to which hydrologic connectivity controls the magnitude and partitioning of C export to the atmosphere and aquatic network.

2.4.1 Aquatic Carbon Export

Hydrologic connectivity is directly related to the export of SOC into the aquatic network (Eimers et al., 2008; Jencso et al., 2009; Stieglitz et al., 2003). In northern hardwood forests, the seasonal effects of hydrologic connectivity result in an annual discharge pattern with two peaks – one during spring melt and another during fall storms (Table 2.1). Aquatic C export is highest during these two peak flow periods when the catchment is hydrologically connected along the upland-ecotone-wetland-stream continuum, and lowest during periods of hydrologic disconnectivity when only the wetland contributes discharge to the stream (Table 2.1).

Seasonality does not only drive the hydrological export of soil organic compounds, but it also controls the breakdown of soil organic material and therefore the production of DOC precursors (Clark et al., 2010). Soil microbial respiration decreases after the fall storms responding to the decrease in temperatures but increases again after the formation of the snow pack, which insulates the soil (Dawson et al., 2008). The accumulation of dissolved organic compounds in soil resulting from winter breakdown of soil organic material and its subsequent mobilization could explain the spring peak in annual aquatic C export (Table 2.1). However, while the winter hydrologic disconnectivity period could contribute to the peak in spring snowmelt aquatic C export, the lower aquatic C export during the fall storms period (Table 2.1) could indicate that the summer disconnectivity period may not play a considerable role in aquatic C export. Even though increasing temperatures have been shown to enhance C cycling rates (Davidson & Janssens, 2006), the magnitude and partitioning of total C export could be further modulated by hydrologic connectivity. Results from c38 indicate that lower precipitation rates will lead to forests prone to more droughts and therefore to greater summer hydrologic disconnectivity and subsequently less aquatic C export. On the other hand, stable or higher precipitation rates will lead to forests prone to more saturated and inundated soils that hydrologically connect the uplands-ecotones-wetlands to the stream, potentially leading to larger aquatic C export (Dawson et al., 2008), as shown in the

strong correlation between discharge and aquatic C export (Figure 2.4a). Aquatic C export is therefore driven by "interruptions" in hydrologic connectivity, and these interruptions are expected to become more frequent due to changing hydrologic regimes (Jensen et al., 2003). An intensification of the hydrologic cycle contributes to prolonged periods of hydrologic disconnectivity during droughts interrupted with bursts of hydrologic connectivity during intense storms (Figure 2.5, Creed et al., 2015).

This interrupted hydrologic connectivity is likely to have consequences on the physical and chemical environments of downstream lakes. Increased DOC inputs into northern lakes have been observed in a process known as brownification (Monteith et al., 2007), but an intensification of the hydrologic cycle may eventually lead to decreased DOC inputs into these lakes, with consequences to the sustainability of the lake food webs (Solomon et al., 2015). In aquatic systems, DOC acts as a light screen and a nutrient vector that supplies macro- (nitrogen and phosphorus) and micronutrients (e.g., Fe) that are essential for primary producers (Jones, 1998). Therefore, a reduction of aquatic C export from forest soils could also lead to a reduction in primary production in lakes by limiting the supply of nutrients to primary producers and inducing the oligotrophication process (Dillon & Molot, 1997). Therefore, although relatively small compared to atmospheric C export, shifts in aquatic C loads could have major impacts to primary production in downstream ecosystems (Creed et al., 2018).

2.4.2 Atmospheric Carbon Export

Hydrologic connectivity also influences the spatial and temporal patterns of moisture within the landscape (Bracken et al., 2013), which further modulates soil microbial respiration by inducing water-limitation in low moisture retention areas and oxygen-limitation in water-saturated areas (Lohse et al., 2009). Soil temperature, soil moisture, and C supply are important determinants of soil microbial respiration (Davidson et al., 2006b, Lohse et al., 2009; Riveros-Iregui et al., 2012; Webster et al., 2009). Among these determinants, soil temperature and moisture are more sensitive to changes in meteorological conditions at daily to annual time scales (Stielstra et al., 2015). Since no differences in soil temperature among positions were detected in c38 (Lecki & Creed, 2016), spatial differences in atmospheric C export could be explained by

topography-driven differences in soil moisture retention (Webster et al., 2008a). Previous studies have identified the role of topography on soil microbial respiration (Lecki & Creed, 2016). Even though soil temperature and moisture were identified as the major drivers of atmospheric C export, both soil C distribution and temperature were identified to be more relevant predictors of soil microbial respiration in steeper slopes compared to gentle slopes.

The ecotone is the major atmospheric C export source and the least variable atmospheric C export source among years of varying water availability (soil moisture) (Table 2.1, Figure 2.4b). While the efficiency of the ecotone as a CO_2 source has already been described (Webster et al., 2008a), this study provides evidence of the effects of changing hydrologic conditions on atmospheric export from this area. During the snow-free period, atmospheric C export from the ecotone is suppressed with higher soil moisture (Figure 2.4b, bottom). The same effect of increasing soil moisture suppressing atmospheric C export is observed during the snow-free period in the ecotone and wetland (Figure 2.4b, top). This process reflects rises in the water table, which saturates surface soils in lowlands (ecotone and wetland), leading to reducing conditions that limit aerobic respiration (Lecki & Creed, 2016; Smith et al., 2003). While the ecotone presents a more diverse microbial community (Du et al., 2015), with different microbial populations thriving during drier and wetter conditions (Goldman et al., 2017), increasing soil moisture could induce oxygen limitation and suppress overall atmospheric C export from this area (Figure 2.4c).

Even though temperature is a major driver of soil respiration in the wetland (Figure 2.4c), soil moisture could explain the divergent trends in atmospheric C export from the upland and wetland over the 2006 to 2010 period (Figure 2.3b) and the changes in the contribution of each position to whole catchment atmospheric C export from year to year (Figure 2.3a). An increase in soil moisture, caused by the rise of the water table during wet years, could induce oxygen limitation in the aerobic microbial communities in the wetland, reducing atmospheric C export from this position. It is for this reason that, while temperature is a major driver of annual atmospheric C export, soil moisture conditions, especially in the lower areas of the catchment (ecotone and wetland), could

act as an escalating/supressing factor on the potential effect of temperature on soil microbial respiration at the catchment level.

Changes in the character of hydrologic connectivity (i.e., decreasing magnitude during spring melt, increasing length of summer drought, and intensification of fall storms) increase the complexity in predicting the magnitude and partitioning of C export, especially due to the diverse effects on the mechanisms that drive soil microbial respiration.

2.4.3 Long-Term Trends in Carbon Cycling

The relationship between soil environmental conditions and soil microbial respiration has been previously described (e.g., Pacific et al., 2009; Chang et al., 2014). However, the study of this relationship at larger spatial and temporal scales is essential to understand implications of climate change on catchment C budgets. Historical trends in c38 displayed a decrease in stream discharge and an increase in snow-free cumulative temperature (Figure 2.6). These changes are expected to have contrasting effects on aquatic and atmospheric C export from different landscape positions. In addition to these changes, the lengthening of the summer hydrologic disconnectivity period and the shift to more dynamic periods of hydrologic connectivity characterized by earlier spring melt and more frequent and intense fall storms (Creed et al., 2015) will further influence C budgets. The lengthening of the summer hydrologic disconnectivity period combined with the decrease in annual discharge (Figure 2.5, Figure 2.6) suggest a greater likelihood of longer and more intense droughts, with implications for soil moisture availability. Changes in the timing and intensity of droughts, snowmelts, and storms directly influenced the timing, magnitude and partitioning of C export. Even though the decrease in stream discharge could have resulted in a reduction of aquatic C export, the lengthening of the summer hydrologic disconnectivity period and the increase of temperatures could have promoted microbial respiration and subsequently atmospheric C export during this period, especially from the wetland, increasing overall C export. The decrease in aquatic C export associated with longer droughts and disconnectivity of the landscape from the drainage network (Figure 2.5) has been observed in other forested catchments experiencing drought (Savage & Davidson, 2001; Schindler et al., 1997). The

study area was more prone to droughts, subsequently hydrologically disconnecting the uplands from the drainage network. However, the increase in temperatures compensated for the effect of droughts by increasing atmospheric C export, especially from the wetlands. In catchments dominated by lowlands, droughts could increase atmospheric C export from the wetlands due to a deepening of the water table (Smith et al., 2003) by relieving the oxygen-limited respiration in these saturated soils (Figure 2.5). A shift in landscape atmospheric C sources could be expected at a longer term if droughts continue lengthening, potentially locking soil C in the upland while releasing it from the wetland. However, in the TLW, the large organic deposits in the wetlands are characterized by a low C to nitrogen ratio indicating low organic matter quality that could hinder the increase in atmospheric C export when water table depth is lowered (Webster et al., 2014).

In the TLW, soil inputs of plant biomass through leaf turnover have been reported to be relatively constant at 3817 kg phytomass ha^{-1} y^{-1} (Morrison and Foster, 2001) or 1832 kg C ha^{-1} y^{-1}, assuming a 48 % C content in leaf biomass (Morrison, 1990). The belowground inputs through fine root biomass are estimated to be 1253 kg C ha^{-1} y^{-1}, resulting from the attributed 2506 kg C ha^{-1} y^{-1} fixation rate (Morrison et al., 1993) and the 50 % y^{-1} fine root turnover rate (Majdi et al., 2005). Therefore, total C inputs to soil through leaf and fine root turnover are 3085 kg C ha^{-1} y^{-1}. Given that our modelled atmospheric C export includes both autotrophic and heterotrophic respiration, and that the ratio between these CO_2 sources has been estimated to be 50:50 (Hanson et al., 2000), atmospheric C export from degradation of leaf and fine root inputs ranges in the 5-year study period between 3718 kg C ha^{-1} y^{-1} in 2007 and 4581 in 2008 kg C ha^{-1} y^{-1}. These results indicate that c38 is a C source exporting between 121 and 148 % of the soil C inputs (see Appendix B). With long-term trends indicating an increase in temperatures, we hypothesize that atmospheric C export from the wetland will increase. In addition to this increase in C export, droughts will induce a shift in landscape C sources, as export in the upland decreases due to the lengthening of summer droughts that will reduce soil moisture, limiting microbial respiration.

In the future, other factors that influence soil C pools will interact with the effects of changing hydrologic connectivity and soil moisture, and the trends in C export may

not hold. Lengthening of the growing season (Zhou et al., 2003) together with increasing atmospheric CO_2 concentrations (Huntington, 2006) will stimulate forest primary production and increase soil C inputs (Guay et al., 2014). In addition, further reductions in acidic atmospheric pollutants and recovery from acidification will mobilize SOC, favoring its transport into aquatic systems (Evans et al., 2005; Evans et al., 2012; Roulet & Moore, 2006).

2.5 Conclusions

Hydrologic connectivity plays a major role in controlling the magnitude and partitioning of C export to aquatic versus atmospheric fates within northern hardwood forests. The efficiency of different topographic positions at exporting C into the atmosphere varies, being highest (and least variable) in the ecotone, intermediate in the upland, and lowest in the wetland. Interruptions in hydrologic connectivity suppress catchment aquatic C export and the associated decrease in soil moisture promotes atmospheric C export from the wetland and ecotone. In contrast, more continuous hydrologic connectivity simultaneously promotes aquatic C export and suppresses atmospheric C export from ecotones and wetlands. Climate change-driven alterations in hydrologic patterns (i.e., timing, duration and intensity of droughts and storms) will define future trends in the magnitude and fate of C export from each topographic position. Long-term trends suggest a continued increase in temperature coupled with prolonged periods of hydrologic disconnectivity interrupted by bursts of hydrologic connectivity. These changes will induce an initial increase in catchment C export driven by greater atmospheric C export from the ecotones and wetlands, but an eventual decrease in atmospheric C export as the forest becomes water limited. Throughout, there will be a decrease in aquatic C export that will have consequences for downstream surface waters.

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Chapter 3

3 Catchments fuel cyanobacteria growth in shallow lakes on forested landscapes

3.1 Introduction

Northern ecosystems, of all global ecosystems, are the most sensitive to the accelerating rates of global change (Smith et al., 2015). Changes in temperature, hydrology, and atmospheric deposition are altering the terrestrial-aquatic linkages and disturbing major carbon (C) pools (Creed et al., 2018) in forest soils, wetlands (including cryptic wetlands, Creed et al., 2003), and streams (including streams incognito, Bishop et al., 2008). Due to their capacity to integrate atmospheric, terrestrial, and aquatic processes, lakes have been described as sentinels of change (Williamson et al., 2008; Williamson et al., 2016), and may provide early warning signs of alterations to C cycling. Even though C fluxes from soils to the atmosphere are generally larger, inputs of terrestrially-produced organic C to aquatic systems as dissolved organic matter (DOM) function as a major source of energy and nutrients (Jones et al., 1998; Cole et al., 2007).

Increases in DOM loads into lakes, a process known as brownification, have been observed in the boreal and temperate regions of Europe and North America, and are driven by changes in the pools and mobilization of soil organic C (Monteith et al., 2007; Kritzberg & Erkström, 2012). Current trends of increasing atmospheric carbon dioxide, the associated rise in air temperature, and the lengthening of the growing season are contributing to increased forest net primary production (Jansson et al., 2008; Kirtman et al., 2013; Jeganathan et al., 2014), and therefore inputs of C into the soil (Finstad et al., 2016). These alterations, coupled with a decrease in atmospheric acid deposition that reduces soil C adsorption capacity (Stoddard et al., 1999; Kalbitz et al., 2000; SanClements et al., 2012), and changes in hydrologic connectivity (Huntington, 2006; Creed et al., 2015; Senar et al., 2018), are contributing to the increased transport of soil organic C into lakes as DOM (deWit et al., 2016). Besides changes in DOM quantity, brownification is also associated with shifts in the quality of DOM, as allochthonous (i.e., externally produced) DOM is generally more refractory and characterized by higher

molecular weight aromatic compounds (McKnight et al., 2003), compared to the more labile autochthonous DOM (i.e., produced in-lake) derived from the decay of aquatic biomass (mostly phytoplankton) (Bertilsson & Jones, 2003).

Reports of cyanobacteria harmful algal blooms (cyanoHABs) have been on the rise (Winter et al., 2011; Pick, 2016) and are occurring simultaneously to the brownification of northern lakes (Dillon & Molot, 1997; Keller et al., 2008). An algal bloom is generally defined as an episode of phytoplankton growth characterized by the dominance (> 50%) of a single species (Molot et al., 2014). However, there is no general agreement on what constitutes an algal bloom (Carvalho et al., 2013), especially given that even small numbers of cells of toxin-producing phytoplankton can have harmful effects on aquatic ecosystems (Smayda, 1997). Even without forming blooms, cyanobacteria can have negative effects on ecosystems, as they are nutritionally poor – lacking essential fatty acids – and therefore constitute low-quality resources for consumers (Brett & Müller-Navarra, 1997). In addition, many species of cyanobacteria can produce toxins that affect water supplies, accumulate in food webs, and affect the human health and well-being of communities that depend on these resources (Rantala et al., 2004; Calteau et al., 2014).

While cyanobacteria growth and dominance have been associated with nutrient-rich eutrophic waters, many nutrient poor oligotrophic temperate lakes are now experiencing a rise in cyanobacteria growth and bloom reports, suggesting other drivers may be behind these episodes (LeBlanc et al., 2008; Callieri et al., 2014). Widespread increases in temperature and changes in solar irradiance have been described as major drivers of phytoplankton growth and bloom formation (Davis et al., 2009; Paerl & Huisman, 2008; Paerl & Paul, 2012. However, these trends do not explain differences among blooming and non-blooming neighboring systems. Changes associated with brownification—which are ultimately dependent on catchment and lake characteristics—have been linked to shifts in phytoplankton community structure (Sterner et al., 1992), reduced phytoplankton diversity (Urrutia-Cordero et al., 2017), and greater cyanobacteria growth and dominance in lakes (Sorichetti et al., 2014, 2016; Creed et al., 2018).

Brownification-driven changes in aquatic ecosystems are complex. High concentrations of allochthonous DOM entering aquatic systems alter the underwater light environment (Jones, 1998). The resulting decrease in visible and UV light penetration in the water column (Jones, 1992) contributes to the formation of shallower and warmer epilimnion and euphotic zone (Houser, 2006; Porcal et al., 2009). In shallow, non-stratified lakes, this reduction in light availability limits benthic primary production and prompts a shift from benthic (macrophytes and periphyton) to pelagic (phytoplankton) primary production (Brothers et al., 2014; Vasconcelos et al., 2018). In deeper, stratified lakes, a shallower thermocline could result in a greater loss of nutrients to the hypolimnion, which are inaccessible to most pelagic primary producers (Fee et al., 1996). Allochthonous DOM inputs also drive changes in the total and bioavailable pools of nutrients (Findlay, 2003). In addition to containing C, an energetic resource for heterotrophic and mixotrophic organisms (Battin et al., 2008), allochthonous DOM is a vector of macronutrients (nitrogen (N) and phosphorus (P)), micronutrients (e.g., iron (Fe)) (Maranger & Pullin, 2003; Qualls & Richardson, 2003; Ged & Boyer, 2013). However, even though DOM may serve as a nutrient vector, the bioavailable fraction of these nutrients may not increase, as refractory DOM binds more strongly to them (Findlay, 2003; Creed et al., 2018). In addition to the DOM-bound allochthonous nutrients, P, Fe, manganese (Mn), and sulphur (S) stored in the sediments may be mobilized in stratified lakes, given that additional organic inputs support hypolimnetic bacterial respiration leading to hypoxia (Nürnberg & Shaw, 1999; Wetzel, 2001; Matthews et al., 2008).

These brownification-driven changes in light and nutrient availability may cause shifts in phytoplankton biomass and community composition (Sterner et al., 1992), benefiting those species that can adapt to browning conditions. In early brownification stages, moderate inputs of allochthonous DOM (up to about 10 mg dissolved organic C L^{-1}) could increase primary production in clear oligotrophic lakes through the supply of nutrients (Seekell et al., 2015). However, the reduction of light at larger DOM concentrations could limit productivity in shallow and deeper lakes (Brothers et al., 2014; Seekell et al., 2015). Relative to the eukaryotic counterparts, cyanobacteria are equipped with numerous adaptations that confer them with a competitive advantage under the low

light and nutrient conditions associated with brownification (Creed et al., 2018). For example, in addition to chlorophyll, a major photosynthetic pigment found in all major phytoplankton classes, cyanobacteria possess phycobiliproteins—accessory pigments that absorb in the orange spectrum (Oliver & Ganf, 2000). The presence of phycobiliproteins is advantageous under browning conditions, as DOM more strongly absorbs blue and green wavelengths as opposed to longer wavelengths (i.e., orange and red). Apart from this adaptation, specific cyanobacteria groups have gas vesicles that allow them to regulate their buoyancy, allowing them to lower themselves to more nutrient-rich areas below the euphotic zone (Carey et al., 2012), that other photoautotrophs cannot access. Given the aromatic nature of allochthonous DOM, nutrients (like N, P, and Fe) bound to organic complexes may not be readily available to primary producers (Jones, 1998). Inputs of N-rich DOM may initially trigger primary production, but subsequently limit it after depletion of the organic N pools, favoring N-fixing cyanobacteria (Rolff et al., 2007). Certain cyanobacteria groups (e.g., *Dolichospermum sp.*) can scavenge Fe from DOM complexes by synthesizing siderophores (Fe-binding ligands) (Sorichetti et al., 2014, 2016; Molot et al., 2014). Furthermore, cyanobacteria with mixotrophic capacities (e.g., *Cyanothece sp.*) have been observed to consume organic compounds (Jones, 1998), or small heterotrophs (Jones, 2000; Bergström et al., 2003; Deininger et al., 2017), not only as a C source under light-limiting conditions, but also to incorporate N and P when their bioavailable inorganic forms are scarce.

In this paper, we examine the link between the observed trends of brownification and cyanobacteria growth in lakes in central Ontario. Given that human development in the forested landscapes of the northern temperate zone in eastern Canada is restricted to sparse and low-impact activities, we hypothesized that global change-driven shifts in DOM quantity and quality in the northern temperate biome are contributing to the increasing cyanobacteria growth. We predicted that brownification-driven nutrient inputs will induce the shift from oligo- to meso- and eutrophic conditions increasing lake primary production. However, the shift to more refractory DOM will select for cyanobacteria that can adapt to the reduced light and nutrient availability conditions. We tested this hypothesis in a comparative study by sampling a set of 71 lakes in central Ontario that represented gradients of lake depth, DOM quantity and quality, and nutrient

concentrations. The sampled ranges in DOM quantity and quality allowed us to swap time for space to describe the consequences of brownification on lake environmental conditions and phytoplankton growth.

3.2 Methods

3.2.1 Study lakes

Lakes (71) were selected in the Ontario northern temperate biome on the Precambrian Shield. The lakes are underlain with metasedimentary rocks and the soils are generally shallow tills and acidic podzolic soils (Chapman & Putnam, 1973; Figure 3.1). All lakes are located in the north temperate climatic zone (Palmer et al., 2011), with a mean annual temperature of 5.2 °C and a mean annual precipitation 832 mm (based on data collected between 1981 and 2010 from the Muskoka Airport meteorological; weatheroffice.gc.ca.). The landscape is forested, dominated by mixed hardwoods, including sugar maple (*Acer saccharum* Marsh.), yellow birch (*Betula lutea* F. Michx.), and beech (*Fagus grandifolia* Ehrh.) with a sparse presence of white pine (*Pinus strobus* L.), balsam fir (*Abies balsamea* (L.) Mill.), and eastern hemlock (*Tsuga canadensis* (L.) Carr.) (Perera et al., 2011). Lakes were selected to represent gradients of dissolved organic carbon (DOC) and total phosphorus (TP).

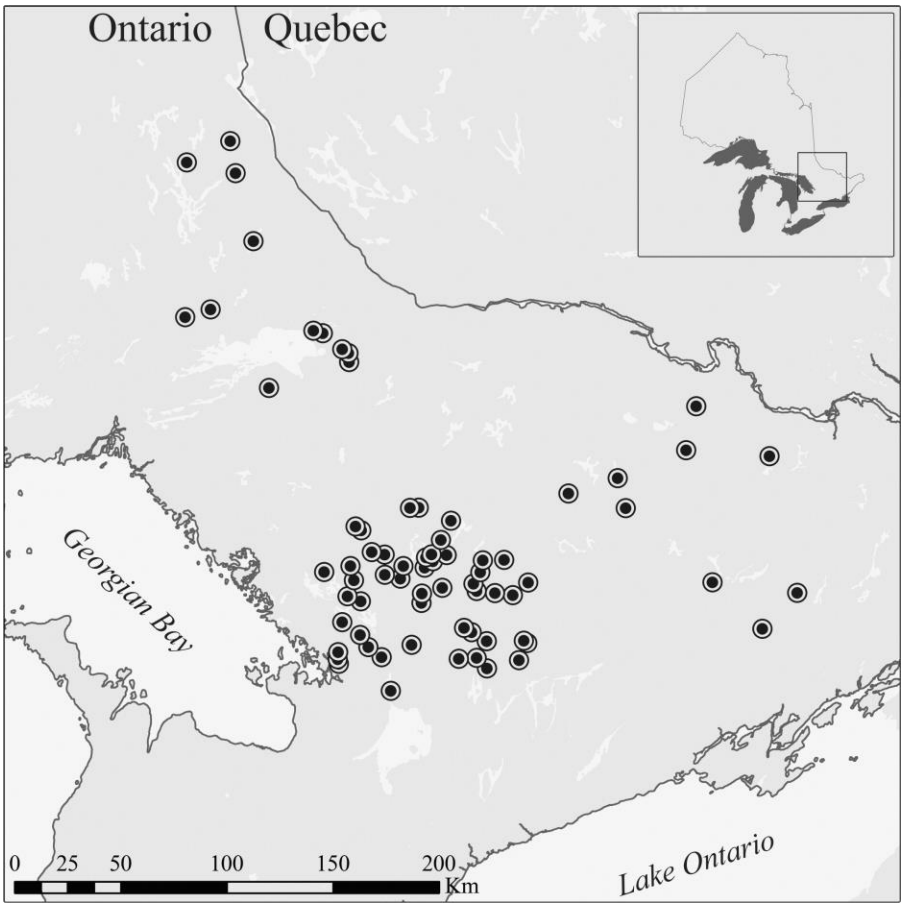

Figure 3.1. Map of study sites in the Ontario temperate forest. Lakes were selected to represent gradients of DOM quantity and quality.

3.2.2 Chemical characteristics

Lakes were sampled within a period of one month during the phytoplankton peak growing season (end of August to beginning of October) in 2015 and 2016. Temperature and dissolved oxygen were recorded in one-meter intervals using a YSI EXO-2 multiparameter sonde (WSI Incorporated, Yellow Springs, Ohio, USA). Thermocline depth in each lake was identified as the depth of highest temperature change. In stratified lakes, dissolved oxygen (DO) concentrations (mg L^{-1}) were calculated as the average of all measurements recorded in the hypolimnion recorded with the YSI multiparameter sonde. In non-stratified lakes, DO concentrations were calculated as the average of all the records taken within the water column. Standard protocols for Secchi depth were used to estimate the depth of the euphotic zone.

Composite epilimnion water samples were collected by mixing samples taken at three depths in the epilimnion using a Van Dorn sampler. Sampling depths were determined as the 25^{th}, 50^{th}, and 75^{th} percentiles of the total epilimnion depth (from surface to thermocline). Composite samples collected for the analysis of DOM quantity, DOM quality, and phytoplankton and cyanobacteria biomass were collected in dark 1 L Nalgene bottles (air-tight). Composite samples that were collected for the analysis of nutrients were filtered through an 80 μm mesh on-site and collected in polyethylene terephthalate bottles (for DOC and color), borosilicate tubes (N and P), and polypropylene bottles (Fe). All samples were kept refrigerated at 4 °C until processed within 24 h from sampling, except subsamples collected for Fe, which were preserved with HNO_3 (0.20 mL per 85 mL of sample).

Composite epilimnion water samples were analyzed for DOM quantity (DOC), DOM quality (Specific UV Absorbance; SUVA), DOM source (Fluorescence Index; FI), total nitrogen (TN), total phosphorus (TP), and dissolved iron (Fe).

Dissolved organic matter concentration, measured as DOC concentration (mg L^{-1}) from water samples filtered through a GF/F glass fiber filter (Pall Life Sciences, Mississauga, ON, Canada), was determined by colorimetry in a Technicon AutoAnalyzer II (Seal Analytics, Mequon, Wisconsin, USA). DOM quality was determined as Specific UV Absorbance (SUVA; L mg C^{-1} m^{-1}), a measurement of absorbance at 254 nm and indicator of aromaticity (Weishaar et al., 2003). Absorbance measurements on 0.45 μm filtered water samples were performed on a Cary 300 Bio UV-Visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) in a 1 cm path quartz cuvette. Absorbance measurements were corrected for DOC concentrations to calculate the SUVA index. DOM source was assessed by FI, an indicator of DOM precursor material (McKnight et al., 2001; Cory & McKnight, 2005). FI was calculated as the ratio of the intensity of the emissions at 470 to 520 nm at excitation 370 nm, measured in a Cary Eclipse spectrofluorometer (Agilent Technologies, Santa Clara, CA, USA) with a 75 Hz Zenon lamp as the excitation source. All samples with absorbance higher than 0.2 m^{-1} at 254 nm were diluted to avoid interferences during the reading and dilution factors were applied to the calculation of SUVA and FI. Water color was measured as the absorbance at 410 nm

in a Shimadzu UVmini-1240 spectrophotometer (Shimadzu, Kyoto, Japan). Absorbance measurements were then converted into true color units (TCU) using a calibration regression built with Hazen's Cobalt-Platinate Standards (1 mg Pt L^{-1} = 1 TCU).

Total nitrogen concentration (μg L^{-1}) was measured by oxidative pyrolysis and chemiluminescence in a Shimadzu TOC-VCPH with TNM-1 and ASI-V autosampler (Shimadzu, Kyoto, Japan). TP was measured by colorimetry in a Skalar San++ Continuous Flow Analyzer (Skalar Analytical, Breda, The Netherlands) after converting all P to orthophosphate in sulfuric acid-persulfate medium at 121 °C in an autoclave for 30 min. Fe was measured as total dissolved Fe (TDFe) through Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at pH < 2 in a Perkin Elmer SCIEX ELAN ICP-MS (Perkin Elmer, Waltham, Massachusetts, US.A). Minimum detection limits for DOC, TN, TP, and Fe were 4, 100, 0.32, and 0.83 μg L^{-1}, respectively.

3.2.3 Phytoplankton and cyanobacteria biomass

Chlorophyll-*a* (chl-*a*) and phycocyanin (PC) concentrations were used as proxies of phytoplankton and cyanobacteria biomass, respectively. Chl-*a* and PC are often used as proxies (Steele, 1962; Gregor et al., 2007; Brient et al., 2008), even though the correlation between pigment concentration and biomass can be influenced by environmental factors and community composition (Kasprzak et al., 2008; Boyer et al., 2009). For each lake, two samples of 500 mL epilimnion water were filtered through 0.7 μm GF/F glass fiber filters (GE Healthcare Life sciences) with the filters subsequently preserved at -80 °C until assessment for pigment content. Chl-*a* was quantified following methods described in Jeffrey & Humphrey (1975), whereas PC was quantified following methods described in Lawrenz et al. (2011). Chl-*a* was extracted in 90% (*v/v*) acetone over 24h at -20 °C and measured via fluorometry on a Trilogy fluorometer (Turner Designs, Sunnyvale, Ca, USA). PC samples were suspended in 1 mL of phosphate buffer solution (0.1 M) adjusted to pH 6. Samples were then disrupted to ensure cell lysis, freeze-thawed (3x) in liquid nitrogen followed by sonication (5 sec pulses in 2 min cycles). Following sonication, 3 mL of phosphate buffer was added and samples were incubated for 24h at 4 °C. Prior to measuring absorbance in Cary 3000 spectrophotometer

(Agilent Technologies, Santa Clara, CA, USA) in a 1 cm path-length quartz cuvette, the extracts were centrifuged (6000 g for 5 min) and the supernatant was filtered through 0.22 µm glass fiber syringe filters (Acrodisc Supor Membrane, Pall Life Sciences, Port Washington, NY, USA).

3.2.4 Statistical analyses

Brownification-driven changes in the trophic status of lakes were assessed through a linear model between DOC (indicator of brownification) and TP (indicator of eutrophication) was assessed using a linear regression in R (Venables & Ripley, 2002). Boundaries between trophic status were identified as TP thresholds (oligotrophic-mesotrophic threshold = 10µg TP L^{-1}; mesotrophic-eutrophic threshold = 35 µg TP L^{-1}, Dodds et al., 1998).

Lakes were classified based on their mixing regime (i.e., presence or absence of a thermocline) and DOM source using the following FI thresholds: allochthonous = FI < 1.3; mixed = 1.3 < FI < 1.45; autochthonous = FI > 1.45 based on Gabor et al. (2014). Differences in chl-*a* and PC between lake classes were assessed through two-way ANOVA in SigmaPlot 12.0 (Systat Software, San Jose, CA). In addition, differences in chl-*a* and PC between stratified lakes with anoxic (DO < 2 mg L-1) and oxic hypolimnion were determined through one-way ANOVA, followed by post-hoc Holm-Sidak paired test.

Correlations among environmental variables (depth, hypolimnion DO, DOC, SUVA, FI, water colour, TN, TP, and Fe) in non-stratified and stratified lakes were assessed through principal component analysis (PCA) using the package 'FactoMineR' (Lê & Husson, 2008) in R. Variable loadings were scaled from -1 to +1, and chl-*a* and PC were input in the PCA as supplementary variables (i.e., they were not considered for the determination of the principal components, but their coordinates were calculated afterwards).

Models to predict chl-*a* and PC from lake characteristics, DOM quantity and quality, and nutrient concentrations (depth, hypolimnion DO, DOC, SUVA, FI, water

color, TN, TP, and Fe) were performed through random forest analyses using the 'party' package (Hothorn et al., 2006) in R. Random forest analysis is a recursive partitioning method that consists of building multiple regression (or classification) trees and is suited to small *n* (observations) and large *p* (predicting variables) studies (Strobl et al., 2009). To ensure robust results, 1000 iterations were performed and five variables were considered for each node. Predicting variables were ranked according to their importance.

Results from the random forest models were compared to results from partial least squares regression (PLSR) models using the package 'caret' (Kuhn, 2008) in R. PLSR is a multiple regression method that identifies the most important predictors and is a suitable method for studies with few observations and many related variables (Carrascal et al., 2009). Similar to PCA, PLSR reduces the number input variables to a lower number of 'latent factors' (or components) that are then used to predict the response variable. Accuracy of the PLSR models was ensured by using repeated k-fold cross validation.

3.3 Results

Classification of lakes based on their mixing regime (i.e., presence/absence of thermocline) resulted in two classes: non-stratified lakes (n = 14) and stratified lakes (n = 57; Table 1). Non-stratified lakes were generally shallower (average depth = 5.63 m), darker (average water color = 62.61 TCU), and presented higher DOC concentration (average DOC = 7.79 mg L^{-1}) than stratified lakes (average depth = 20.75 m; average water color = 25.39 TCU; average DOC = 4.75 mg L^{-1}). Nutrient concentrations were larger in non-stratified (541.00, 22.17, and 582.86 µg L^{-1} for TN, TP, and Fe, respectively) than in stratified lakes (351.91, 10.58, 92.98 µg L^{-1} for TN, TP, and Fe, respectively), however, they presented large variability among systems in both classes. Both, chl-*a* and PC concentrations were higher and presented lower standard deviation in non-stratified (6.92 ± 0.70 and 27.54 ± 2.04 µg L^{-1}, for chl-*a* and PC) than stratified lakes (3.16 ± 1.74, and 9.77 ± 4.79 µg L^{-1}, for chl-*a* and PC, respectively).

Table 3.1 Descriptive statistics for lake characteristics (depth and hypolimnion DO), DOM quantity (DOC), source (FI), and quality (SUVA), water colour, major nutrients (TN, TP, Fe), and pigment concentrations (chl-*a*,PC) for non-stratified and stratified lakes.

	Non-stratified (n=14)			Stratified (n=57)		
	Average ± SD	Min	Max	Average ± SD	Min	Max
Depth (m)	5.63 ± 2.27	2.30	10.00	20.75 ± 7.78	7.50	65.00
HypoDO (mg L^{-1})	-	-	-	4.75 ± 3.16	0.02	11.3
DOC (mg L^{-1})	7.79 ± 2.22	4.40	12.40	4.96 ± 1.50	2.00	9.00
FI	1.35 ± 0.16	1.18	1.80	1.33 ± 0.16	1.08	1.98
SUVA (L mgC^{-1} m^{-1})	3.20 ± 1.20	1.23	5.00	2.79 ± 0.78	1.14	5.22
Colour (TCU)	62.61 ± 54.33	10.30	201.00	25.59 ± 18.58	8.35	88.60
TN (µg L^{-1})	541.00 ± 123.56	371.00	790.00	351.91 ± 89.04	200.00	601.00
TP (µg L^{-1})	22.17 ± 11.85	10.70	48.50	10.58 ± 5.94	4.30	32.20
Fe (µg L^{-1})	582.86 ± 1019.84	10.00	3750.00	92.98 ± 176.49	0.00	1180.00
Chl-*a* (µg L^{-1})	6.92 ± 0.70	0.37	1.26	3.16 ± 1.74	-0.01	1.27
PC (µg L^{-1})	27.54 ± 2.04	0.96	1.91	9.77 ± 4.79	-0.94	1.87

Dissolved organic carbon was significantly correlated with TP ($r^2 = 0.36$, $p < 0.01$; Figure 3.2). All lakes classified as oligotrophic (TP < 10 µg L^{-1}) were stratified lakes and the only two eutrophic lakes (TP > 35 µg L^{-1}) in our dataset were non-stratified.

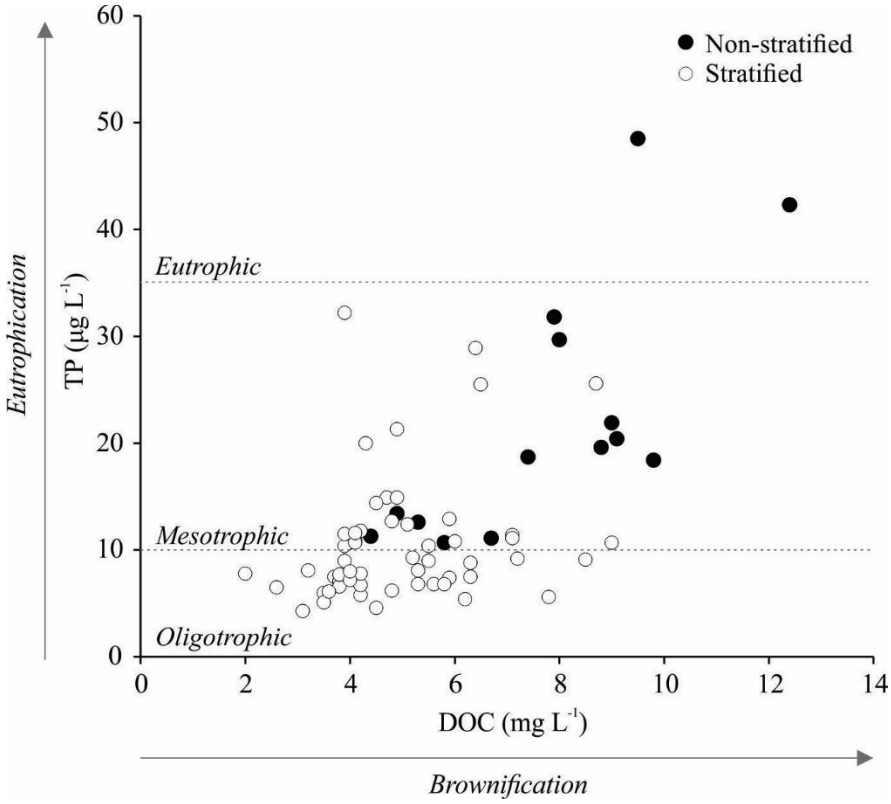

Figure 3.2 Correlation between DOC and TP concentrations in the study sites, classified according to their mixing regime. Trophic states determined as per Dodds et al. (1998).

Further classification of lakes based on their DOM source (allochthonous, mixed, and autochthonous DOM, based on FI), resulted into six classes (Figure 3.3a). In non-stratified lakes, chl-*a* was significantly larger in lakes with DOM from allochthonous (log(chl-*a*) = 0.90 µg L^{-1}) and mixed sources (1.08 µg L^{-1}) than those with autochthonous DOM (0.51 µg L^{-1}). No significant differences in chl-*a* concentration were identified among stratified lakes with different DOM sources. However, non-stratified lakes with DOM from allochthonous and mixed sources presented higher chl-*a* than stratified lakes with the same DOM sources (0.52 and 0.54 µg L^{-1}, for stratified lakes with allochthonous and mixed DOM sources, respectively). No significant differences in PC concentrations were identified between lakes with different stratification regimes or DOM sources; however, lowest PC values were typically observed in stratified lakes. In stratified lakes, chl-*a* concentration was significantly larger in lakes with an anoxic hypolimnion (0.70 µg L^{-1}), compared to lakes with an oxic hypolimnion (0.43 µg L^{-1}), but no significant differences were detected in PC concentrations (Figure 3.3b).

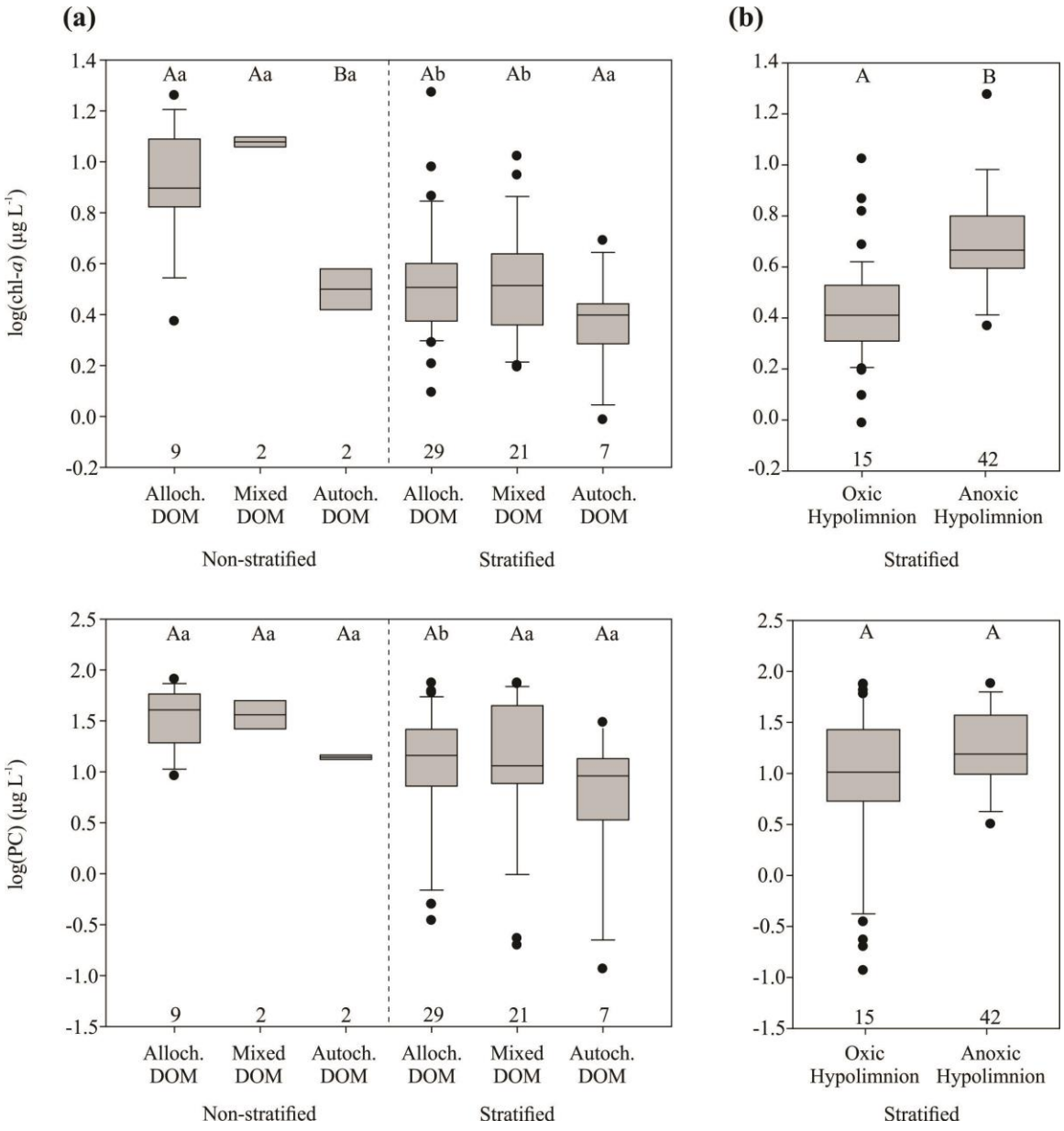

Figure 3.3(a) Effects of mixing regime and DOM source classes on chl-a (top) and PC (bottom). (b) Effects of hypolimnion anoxia on chl-*a* and PC in stratified lakes. Differences among depth and DOM source classes were tested using two-way ANOVA analyses followed by Holm-Sidak post-hoc test. Uppercase letters represent differences within stratification state; lowercase letters represent differences within DOM sources. Numbers represent number of samples (n) in each group.

In non-stratified lakes, the first two PCA components explained 82.38% of the total variance (Figure 3.4a). The loadings of the input variables indicated that the first component increased with increasing water color ($r^2 = 0.97$, $p < 0.01$), DOC ($r^2 = 0.90$, p

< 0.01), TP (r^2 = 0.90, p < 0.01), TN (r^2 = 0.86, p < 0.01), Fe (r^2 = 0.86, p < 0.01), and SUVA (r^2 = 0.85, p < 0.01), and with decreasing depth (r^2 = 0.61, p < 0.01) and FI (lower FI associated with allochthonous DOM; r^2 = 0.61, p < 0.01). The second component was significantly correlated to FI (r^2 = 0.72, p < 0.01). Chl-*a* and PC were associated with increases in the first component (r^2 = 0.67 and 0.58, respectively, p < 0.01).

In stratified lakes, the first two PCA components explained 62.11% of the total variance (Figure 3.4b). Similar to non-stratified lakes, the first component was driven by water color (r^2 = 0.84, p < 0.01), Fe (r^2 = 0.79, p < 0.01), DOC (r^2 = 0.69, p < 0.01), TP (r^2 = 0.60, p < 0.01), TN (r^2 = 0.57, p < 0.01), and SUVA (r^2 = 0.55, p < 0.01), and negatively correlated to hypolimnion DO (r^2 = 0.68, p < 0.01) and depth (r^2 = 0.67, p < 0.01). The second component was correlated directly to FI (r^2 = 0.65, p < 0.01), TN (r^2 = 0.54, p < 0.01) and TP (r^2 = 0.49 p < 0.01), and negatively to SUVA (r^2 = 0.73, p < 0.01), water color (r^2 = 0.43, p < 0.01), and hypolimnion DO (r^2 = 0.33, p < 0.01). In stratified lakes, chl-*a* and PC were associated with increases in the first component (r^2 = 0.53, 0.27, respectively, p < 0.01).

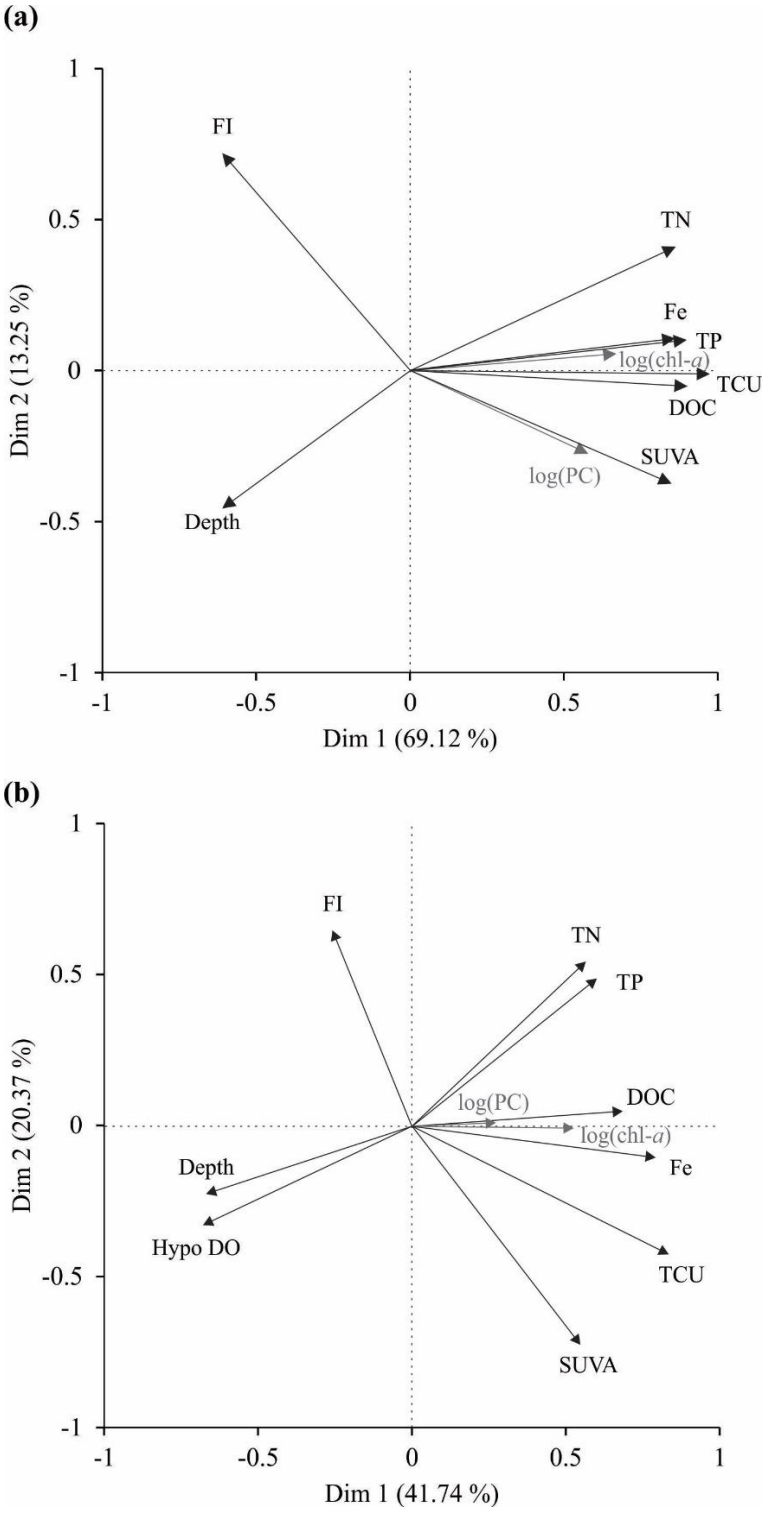

Figure 3.4 Results from principal component analysis on lake, DOM, and nutrient characteristics in non-stratified (n=14; top) and stratified (n=57; bottom) lakes. Chl-*a* and PC are included as supplementary variables (i.e., they were not included in the determination of the principal components, instead their coordinates were calculated afterwards).

Results from the random forest analyses using all continuous predicting variables (depth, hypolimnion DO, DOC, SUVA, FI, water color, TN, TP, and Fe), identified depth, water color, and DOC as the three variables with highest relative importance in terms of explaining the variation of chl-a among systems (Figure 3.5a). Comparing observed chl-a concentrations with modelled values resulted in a significant correlation ($r^2 = 0.63$; $p < 0.01$). The top three most important variables predicting PC concentration according to the random forest model were depth, water color, and TN (Figure 3.5b). There was a statistically significant correlation between observed and modelled PC values ($r^2 = 0.34$, $p < 0.01$).

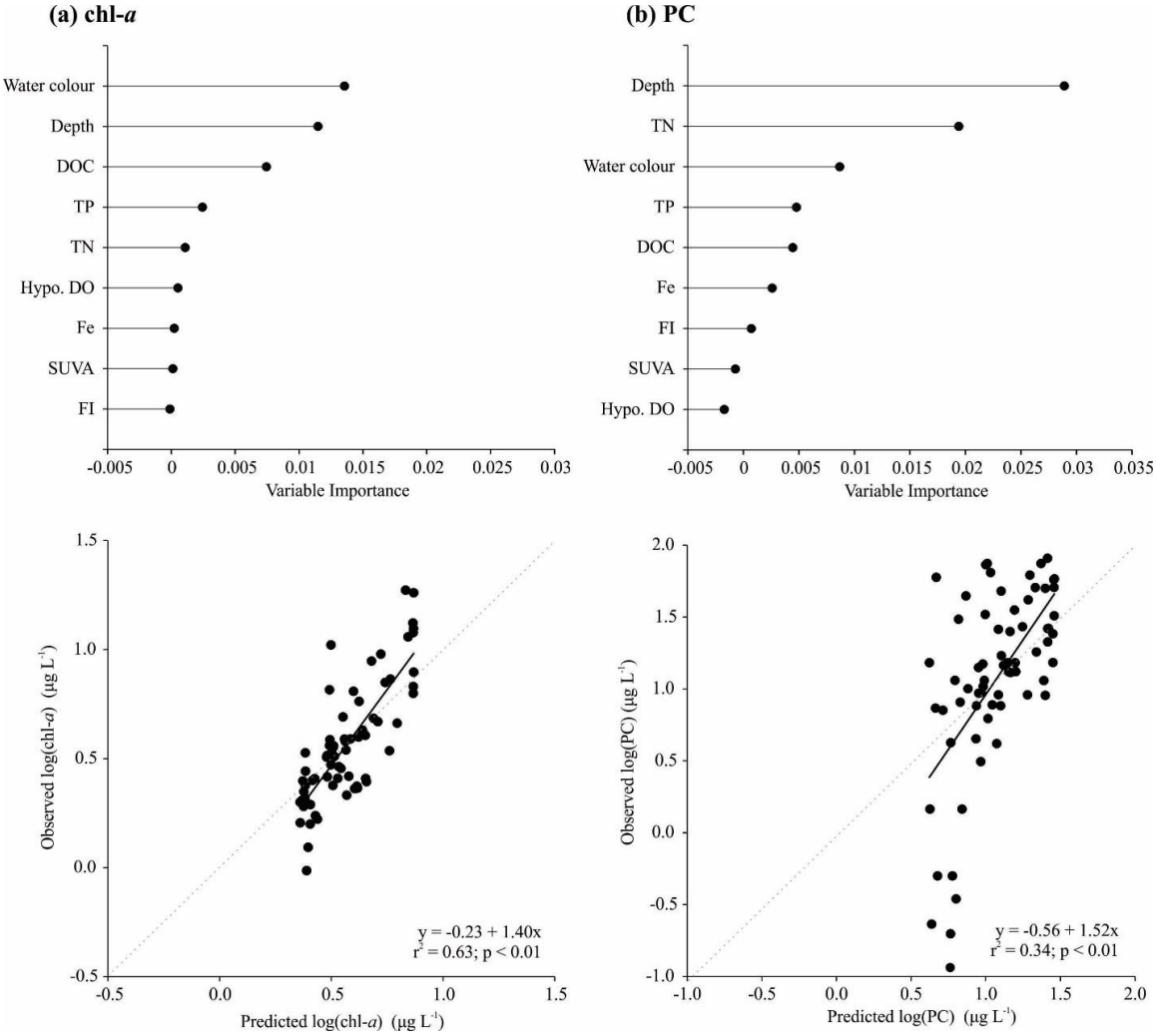

Figure 3.5 Results from random forest analysis to predict chl-*a* (left) and PC (right). Top panels represent variable importance, bottom panels represent correlations between pigment concentration values (µg L^{-1}) predicted from the models (mod; x-axis) and observed values.

The results of the random forest models generally coincided with results from the PLSR models (Table 3.2). The PLSR model for chl-*a* explained 51.42 % of the variance in the data in the first two components, and identified depth, water colour, and DOC as the most important predictors (r^2 = 0.50). The PLSR model predicting PC explained 51.34 % of the variance in one component (r^2 = 0.17), and identified depth, water colour, DOC, and TN as the most important predictors.

Table 3.2 Comparison of results from the random forest and PLSR models predicting chl-*a* and PC. Accuracy of random forest models tested as the correlation between observed and modeled values.

	Random Forest	PLSR
Chl-*a*		
Accuracy	$r^2 = 0.63$ $p < 0.01$	# components = 2 % variance = 51.42 RMSE = 0.21 $r^2 = 0.50$
Variable importance (ranked)	1. TCU 2. Depth 3. DOC 4. TP 5. TN 6. Fe 7. Hypolimnion DO 8. SUVA	1. Depth 2. DOC 3. TCU 4. TP 5. TN 6. Fe 7. SUVA 8. Hypolimnion DO
PC		
Accuracy	$r^2 = 0.34$ $p < 0.01$	# components = 1 % variance = 51.34 RMSE = 0.61 $r^2 = 0.17$
Variable importance (ranked)	1. Depth 2. TN 3. TCU 4. TP 5. DOC	1. DOC 2. Depth 3. TN 4. TCU 5. TP 6. SUVA 7. Fe 8. FI

3.4 Discussion

Nutrients can enter lakes in their inorganic forms through weathering of soils and lithology (e.g., P, Fe; Dillon & Kirchner, 1975), atmospheric deposition (e.g., N, Sulphur; Dupont et al., 2005), or as part of organic compounds (e.g., N, P, Fe; Findlay, 2003). Once in lakes, nutrients may be deposited on the sediments, where they can be stored, or re-mobilized through changes in redox conditions (e.g., P, Fe, sulphur, manganese; Wetzel, 2001), a process known as internal loading (Søndergaard et al., 2003). Our results indicate that brownification (using DOC as a proxy) is associated with eutrophication (using TP as a proxy) of temperate lakes (Figure 3.2), emphasizing the role of DOM as a nutrient vector in temperate lakes (Jones, 1998). This finding suggests

that lakes in areas prone to the mobilization of soil organic compounds are also at risk of experiencing eutrophication. However, lake characteristics, like depth and mixing regime, can modulate total lake nutrient pools, productivity, and therefore their sensitivity to brownification.

Non-stratified lakes were generally shallower, darker, more nutrient-rich, and more productive than deeper stratified lakes. Among non-stratified lakes, those with DOM from allochthonous and mixed sources presented higher chl-a concentrations. In these lakes, DOM acts as a nutrient vector, supplying macro- (TN, TP) and micro-nutrients (Fe) to lake primary producers, and also by reducing light availability. The increasing nutrient concentrations, water color, and DOC result in increasing phytoplankton biomass (chl-a). Previous research suggests that the brownification of shallow lakes reduces light penetration, limiting benthic primary production, and prompting the shift from benthic to pelagic production (Brothers et al., 2014; Rivera-Vasconcelos et al., 2018). In addition to limiting benthic production, allochthonous nutrient supplies can favor phytoplankton growth, as DOM maintains in solution nutrients that otherwise would precipitate under oxic conditions (e.g., P, Fe; Jones, 1998). However, allochthonous DOM is generally more refractory than autochthonous DOM (McKnight et al., 2003; Xiao et al., 2018). This shift towards more refractory DOM is represented by the increase in SUVA with a shift from autochthonous (high FI) to allochthonous (low FI) DOM. Refractory DOM is associated with decreased nutrient availability, as nutrients are more strongly bound to DOM complexes (Maranger & Pullin, 2003). Therefore, even though brownification may result in larger nutrient pools, the bioavailable nutrient pool may not significantly increase (Findlay, 2003). Despite this, shifts towards more refractory DOM (i.e., higher SUVA) are associated with increases in PC, suggesting that cyanobacteria have competitive advantages as allochtonous DOM increases in non-stratified lakes.

In brown waters, some cyanobacteria are able to access nutrients from DOM complexes or by adapting to darker conditions (Ekvall et al., 2013; Urrutia-Cordero et al., 2017). Mixotrophic cyanobacteria (e.g., *Cyanothece sp.*) can uptake macronutrients (N and P) bound to DOM (Maranger & Pullin, 2003). Certain cyanobacteria (e.g.,

Dolichospermum) may be able to obtain Fe from DOM complexes by producing Fe-scavenging compounds (i.e., siderophores) that confer them an advantage under Fe-limiting conditions (Trick & Kerry, 1992; Sorichetti et al., 2014, 2016). Furthermore, accessory pigments (phycobiliproteins), present in all cyanobacteria, allowing them to grow in light-limiting environments (Zevenboom et al., 1981). Brownification may not only benefit cyanobacteria growth, but also promote toxin production (Ekvall et al., 2013), as microcystin (a common cyanotoxin) has been suggested to function as a siderophore (Saito et al., 2008; Li, 2011). Furthermore, reduced light availability can abate photosynthesis, reducing cellular C:N ratios, and inducing the storage of N in N-rich compounds like microcystin (van de Waal, 2009).

In stratified lakes, the shift from autochthonous to allochthonous DOM does not significantly influence chl-*a* or PC. Instead, hypolimnion anoxia may be a driver of phytoplankton biomass (but not cyanobacteria biomass), as decreases in hypolimnion DO in stratified lakes were associated with increasing macronutrients (TN and TP). Reduced forms of P (PO^{4-}) and Fe (Fe^{2+}) are soluble in anoxic/hypoxic conditions (Curtis, 1993; Molot & Dillon, 2003). Internal nutrient loading driven by hypolimnion anoxia/hypoxia has been described as a major driver of phytoplankton and cyanobacteria growth by relieving P- and Fe-limitation (Søndergaard et al., 2003; Molot et al., 2010; Nürnberg et al., 2013; Molot et al., 2014; Orihel et al., 2017). Internal loading of TP and Fe benefits picocyanobacteria species living in the thermocline (Gervais et al., 1997; Drakare et al., 2003) and cyanobacteria species that use buoyancy to descend to the nutrient-rich, hypolimnetic waters (Carey et al., 2012). Results from the PCA analysis indicated that, in stratified lakes, DOM quantity (DOC) and quality (SUVA) are associated with changes in water color. In addition, DOC was directly correlated with Fe, and inversely correlated with hypolimnion DO. Therefore, brownification will result in decreased light, higher Fe, and hypolimnion anoxia, which will in turn result in the resuspension of sediment P.

Results from the random forest models identified depth and water color as major predictors of both chl-*a* and PC. This finding suggests that physical, rather than chemical, characteristics control primary production (Figure 3.5). However, the correlation between water color and phytoplankton pigment concentrations could reflect increased cellular

pigment content per unit phytoplankton biomass rather than increased phytoplankton biomass, as primary producers favor pigment production to compensate for decreased light availability (Felip & Catalan, 2000). Nonetheless, our results support the idea that phytoplankton communities successfully adapt to the browning conditions in the observed DOC, SUVA, and water color ranges. In addition, phytoplankton may benefit from a decrease in harmful UV light penetration associated with increased water color (Williamson et al., 2016).

The importance of TN and TP as predictors of both chl-*a* and PC was higher than Fe. This finding suggests that macronutrient availability (rather than micronutrient availability) limited phytoplankton and cyanobacteria growth in the study lakes. FI (indicator of DOM source), SUVA (indicator of DOM quality), and hypolimnion DO (indicator of hypolimnion redox conditions) were often identified as the least important predictors of chl-*a* and PC. While not being identified as important predictors, they provide valuable insight on the mechanisms, internal (hypolimnion DO) or external (SUVA, FI), that drive primary production in lakes.

Brownification-driven changes in light and nutrient availability can increase primary production in the observed ranges (DOC = 2 – 12 mg L^{-1}). Shallow lakes will be more sensitive to changes in DOM loads, as DOM quantity and quality directly control nutrient concentrations and light penetration. In deeper stratified lakes, brownification will result in increases in water color, Fe concentration, and hypolimnion anoxia, which in turn will induce the resuspension of sediment P. Changes in light and macronutrients driven by moderate inputs of allochthonous DOM will promote the growth of phytoplankton and specifically cyanobacteria in temperate lakes.

3.5 Conclusions

Global changes, including increasing temperatures, shifts in hydrologic connectivity, and decreases in atmospheric acid deposition, are causing the brownification of northern lakes. Shifts in DOM quantity and quality cause alterations in the physical and chemical characteristics of lakes and could induce a shift from oligo- to

eutrophic conditions. As current brownification trends are expected to continue, shallow lakes are likely to experience greater cyanobacteria growth and be at risk of developing potentially harmful cyanobacteria blooms, altering ecosystem function, and putting aquatic ecosystem services at risk. This risk will continue until a threshold in browning is exceeded, and the benefits of more nutrients are offset by the costs of less light, reducing lake production.

3.6 References

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Chapter 4

4 Brownification, primary production, and the transfer of essential fatty acids in temperate oligotrophic lakes

4.1 Introduction

The current trend of climate-change-driven increases in allochthonous dissolved organic matter (DOM) loads supplied to northern lakes (Kritzberg & Erkström, 2012; Creed et al., 2018), a process known as brownification, is altering the biomass and nutritional quality of primary producers (Urrutia-Cordero et al., 2016) available to primary consumers (Sterner et al., 1992, Karlsson et al., 2009; Kissman et al., 2017). Allochthonous DOM is composed of darker, more refractory, compounds (i.e., aromatic and with a high molecular weight) than autochthonous DOM and thereby alters the physico-chemical environments of freshwater lakes (McKnight et al., 2003). For example, allochthonous DOM reduces light penetration (Karlsson et al., 2009) that results in increased heat retention and a shallower, warmer, and more stable epilimnion (Houser, 2006; Porcal et al., 2009). Allochthonous DOM can function as an energy (C) and nutrient (nitrogen (N), phosphorus (P), iron (Fe)) source for primary producers (Ged & Boyer, 2013); however, the bioavailability of the increased nutrient pool declines with shifts to more refractory DOM (Findlay, 2003; Sorichetti et al. 2016; Soares et al., 2017). Low to moderate increases in allochthonous DOM supplies in clear oligotrophic lakes can enhance primary production by supplying additional energy and nutrients to phytoplankton (Seekell et al., 2015; Tanentzap et al., 2014), but high contents of allochthonous DOM in brown oligotrophic lakes have been associated with low primary production due to light limitation (Ask et al., 2009).

Brownification induces changes in phytoplankton communities (Finstad et al., 2014; Solomon et al., 2015). Specific phytoplankton groups, including cyanobacteria, have developed adaptations to brownification-driven changes in light and nutrient conditions that allow them to outcompete eukaryotic algae (Jones, 1998; Urrutia-Cordero et al., 2016). For example, cyanobacteria have phycobiliproteins, accessory pigments that allow them to photosynthesize under lower-light conditions (Oliver & Ganf, 2000). Some cyanobacteria (e.g., *Aphanizomenon sp.*) are able to regulate their buoyancy and thus

their position in the water column especially with respect to being positioned in the metalimnion and hypolimnion where nutrients accumulate (Carey et al., 2012). Some cyanobacteria (e.g., *Dolichospermum sp.*) are able to synthesize organic ligands (i.e., siderophores) that scavenge Fe from DOM and transport it into the cell (Trick & Kerry, 1992, Sorichetti et al., 2014). Finally, some cyanobacteria (e.g., *Cyanothece sp.*) resort to mixotrophy to access C and other nutrients (including P and Fe) from DOM (Jones, 1998; Deininger et al., 2017) when light and nutrients are limiting. In addition to cyanobacteria, mixotrophic flagellates, including cryptophytes, dinoflagellates, and raphydophytes, are typically found in highly-colored lakes, as they can also adjust their position in the water column thereby benefitting from spatially discrete organic nutrient pools (Arvola, 1984).

The cascading effects of brownification translated up the food web have been debated (Brett et al., 2017). The debate has focused on the essential fatty acid (EFA) composition of basal resources (Kelly et al., 2014; Galloway et al., 2014). EFAs are polyunsaturated fatty acids (PUFAs; i.e., fatty acids with multiple double bonds in their C chain) that are involved in maintaining cell structure, metabolism, growth and reproduction. EFAs generally cannot be synthesized *de novo* by consumers in sufficient quantities to meet their needs (Brett & Mülller-Navarra, 1997; Vance & Vance, 2008); however, recent studies have observed the presence of specific desaturases that support the synthesis of omega-3 (n-3) unsaturated fatty acids from saturated ones in several lineages of invertebrates (Kabeya et al., 2018). Allochthonous DOM is enriched in long-chain saturated fatty acids (LC-SFA), fatty acids without double bonds and with 20 or more atoms in their chains, but its PUFA content is restricted to mostly omega-6 (n-6) PUFAs (Hixson et al., 2015; Taipale et al., 2014). In contrast, phytoplankton synthesize higher concentrations of both n-3 and n-6 PUFAs, although there are exceptions among taxa (Strandberg et al., 2015a). Having an obligate requirement for both n-3 and n-6 PUFAs, consumers must rely on autochthonous resources to meet their n-3 requirements (Hixson et al., 2015). Among n-3 PUFAs, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) are primarily synthesized by phytoplankton and are essential for consumer's growth, reproductive success and neural development (Ravet et al., 2003; Brett et al., 2006).

Essential fatty acid content and composition in phytoplankton vary. Some phytoplankton (e.g., chrysophytes, cryptophytes, diatoms, dinoflagellates, and raphydophytes) are considered high quality resources due to their ability to synthesize significant amounts of EPA, DHA, and other n-3 PUFAs. Other phytoplankton (e.g., chlorophytes and cyanobacteria) are considered low quality resources due to the low content of EPA and DHA (Brett & Müller-Navarra, 1997; Brett et al., 2000; Strandberg et al., 2015a); however, chlorophytes, in particular, can contain appreciable quantities of short chain n-3 PUFAs (Napolitano, 1999), α-linoleic acid (ALA; 18:3n-3) and stearidonic acid (SDA; 18:4n-3) that are precursors of EPA and DHA. While differences in PUFA content among phytoplankton taxa are greater than differences within each taxa (Galloway & Winder 2015; Taipale et al., 2016), environmental conditions (e.g., light or nutrient availability) can influence PUFA content in phytoplankton (Guschina & Harwood, 2009; Fuschino et al., 2011). In fact, shifts in phytoplankton community composition driven by major changes in environmental conditions (e.g., brownification and eutrophication) may lead to changes in the production and subsequent transfer of PUFAs in lake pelagic food webs (Strandberg et al., 2015b; Taipale et al., 2016, 2018).

Both autochthonous and allochthonous C is transferred from the base of the food web to zooplankton and, from there, to higher trophic levels (fish), by bacteria that assimilate decomposing phytoplankton and extracellular substances from living phytoplankton (i.e., the microbial loop; Tranvik, 1992), or by zooplankton that graze phytoplankton and particulate organic matter (Cole et al., 2011). Heterotrophic bacteria are typically poor-quality resources that contain little or no PUFAs; rather, they contain odd-chain and branched-chain SFAs and mono-unsaturated fatty acids (Ratledge & Wilkinson, 1988; Hiltunen et al., 2015). However, heterotrophic flagellates can trophically-upgrade fatty acids with each trophic transfer through the microbial loop where they are then preferentially retained by consumers (Bec et al., 2006; Desvilletes & Bec, 2009). Consequently, the microbial loop can improve the quality of the C transfer pathway despite the loss of energy due to the upgrade of PUFAs. The transfer of PUFAs to higher trophic levels is further dependent on the feeding modes, life histories, reproduction cycles and PUFA requirements of primary consumers (Persson & Vrede, 2006; Perhar et al., 2013). For example, cladocerans are generalist filter-feeders

(although they exhibit some selectivity, particularly regarding particle size, DeMott, 1982), with greater EPA content (Persson & Vrede, 2006; Hiltunen et al., 2014, 2016), and faster growth and reproduction rates relative to copepods. Copepods are more selective, with greater DHA content than cladocerans (Persson & Vrede, 2006; Hiltunen et al., 2014, 2016). However, there is also evidence that zooplankton actively retro-convert dietary DHA back to EPA (in the case of cladocerans; Fink & Windisch, 2018) and EPA to DHA (in the case of copepods; Sargent et al., 1995), although this process is considered to be energetically expensive.

Brownification-driven changes in phytoplankton production and community composition could reduce the production and transfer of PUFAs in lakes (Hessen et al., 1990). Brownification may influence the PUFA pool size and transfer by: (1) supplying additional terrestrial resources (rich in LC-SFA); (2) favoring heterotrophic bacteria growth (resulting in enriched odd-chain and branched-chain SFA); (3) enhancing the microbial loop and subsequently the fatty acid transfer through this pathway; and (4) provoking shifts phytoplankton composition, from higher-quality autotrophic eukaryotes (resulting in enriched n-3 PUFAs, EPA and DHA) to lower-quality cyanobacteria (Figure 4.1). Here, we hypothesized that brownification of lakes enhances primary production but selects for cyanobacteria, and as primary production and associated fatty acid pools increase, cyanobacteria will shift the fatty acid pool to lower PUFA (especially n-3) in phytoplankton, which in turn will lower PUFA in consumers. We tested these hypotheses in a comparative, space-for-time substitution study (Carpenter, 1991), representing the effects of the process of brownification on content and composition of DOM in lakes in the temperate biome.

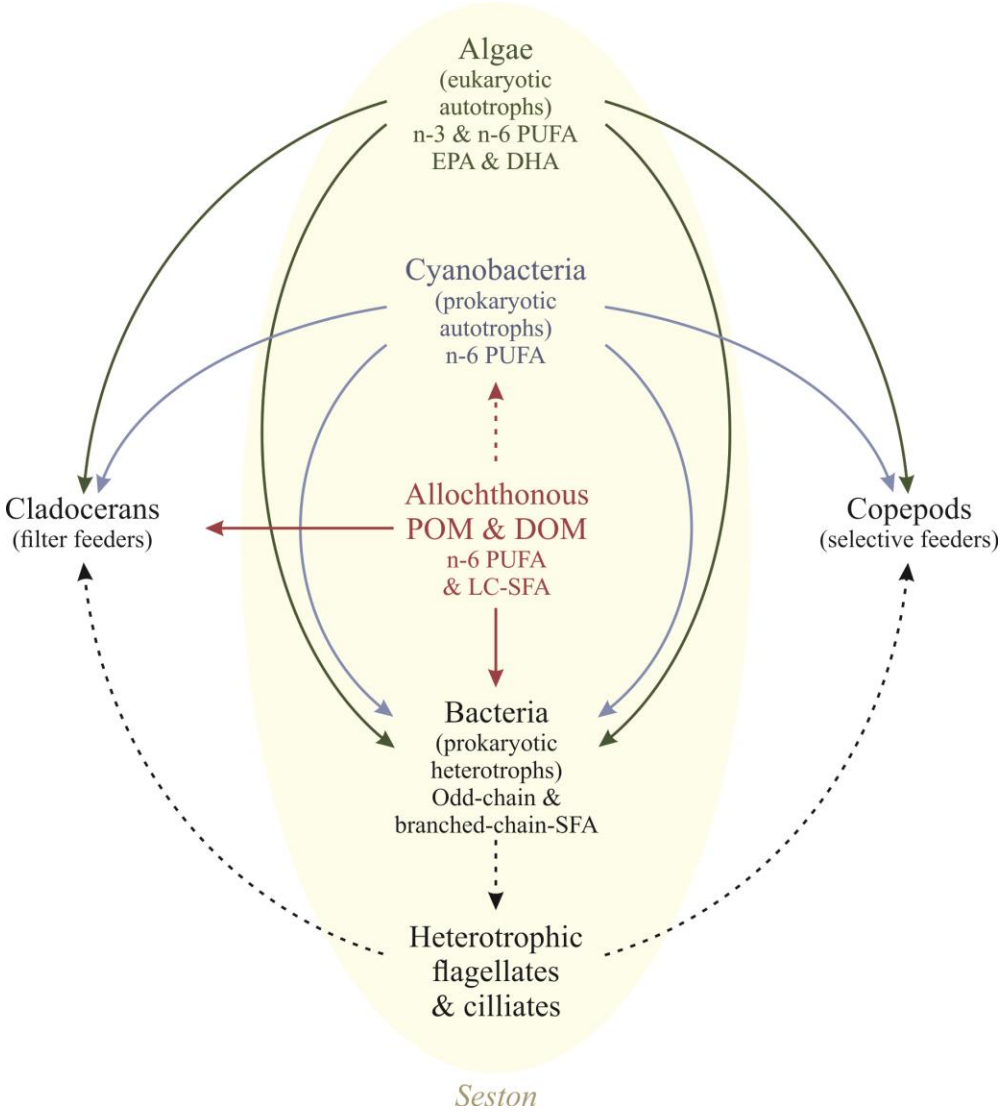

Figure 4.1. Diagram of a pelagic food web identifying main diet sources for consumers and their fatty acid indicators. Dashed arrows represent microbial loop.

4.2 Methods

4.2.1 Study lakes

Lakes were situated in the Ontario temperate biome, a region where the average annual temperature is 5.2°C and the average annual precipitation is 832 mm (weatheroffice.gc.ca). Lakes were situated on the Precambrian Shied, composed of metasedimentary rocks and soils dominated by acidic podzols (Chapman & Putnam, 1973). Contributed catchments to lakes were generated in ArcMap 10.2 (ESRI, 2014);

lake polygons (Ontario Hydrographic Network; waterbodies) were used as pour points on a 20 m digital elevation model (Ontario Digital Elevation Model, version 2.0.0) that was hydrologically conditions using depression filling algorithm (Tarboton et al., 1991). Contributing catchments were covered by forests and wetlands. The forest was dominated by sugar maple (*Acer saccharum* Marsh), yellow birch (*Betula lutea* F. Michx), and beech (*Fagus grandifolia* Ehrh), but with some white pine (*Pinus strobus* L.), balsam fir (*Abies balsamea* L.) Mill) and eastern hemlock (*Tsuga canadensis* L. Carr) (Perera et al., 2011). The wetlands ranged from 0.5 to 50% of the catchment area, based on the Ontario Wetland Inventory (revised 2013; Ontario Ministry of Natural Resources). As wetlands are an important source of organic C (Creed et al., 2008, Creed et al., 2013), lakes were selected to represent the range of proportion of wetlands.

4.2.2 Lake samples

A total of 30 lakes were selected to represent a range in proportion of wetlands (Figure 4.2). Each lake was sampled during the peak growing season for phytoplankton (August-September) in 2016 at its deepest point. Temperature measurements were taken in 1 m intervals down the water column using a YSI EXO-2 multiparameter sonde (YSI Incorporated, Yellow Springs, Ohio, USA). Thermocline depth was estimated as the depth of greatest change in temperature in the water column. Composite epilimnion water samples were collected by combining three water samples collected at equal intervals, from surface to thermocline depth, using a Van Dorn sampler. The three depths at which water was collected were equally spaced, and the shallowest and deepest sample were at the same distance from the surface and the thermocline, respectively.

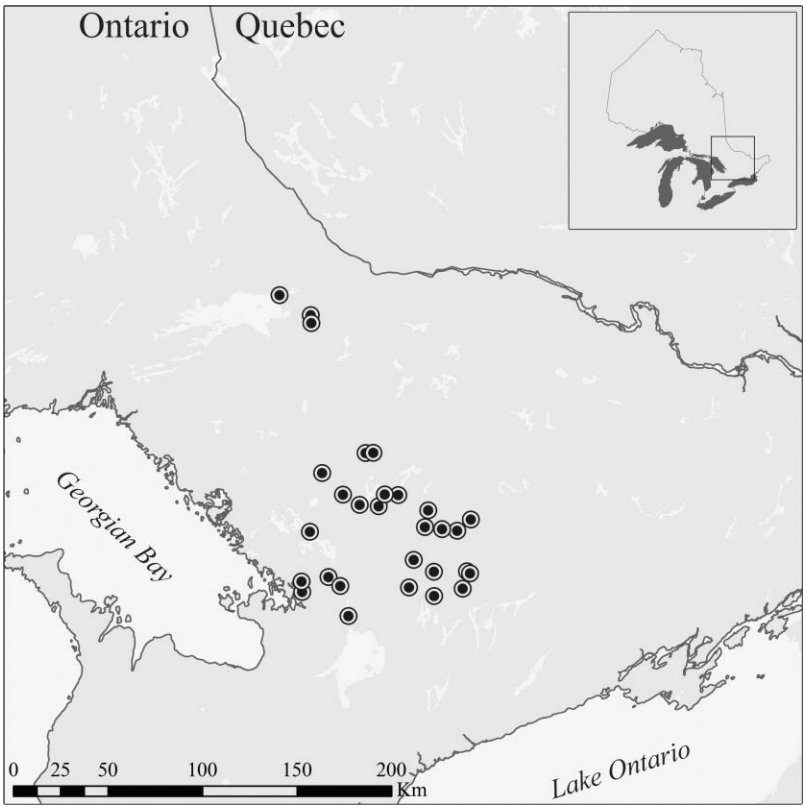

Figure 4.2. Study lakes in central Ontario.

Lake trophic status was assigned based on total P (TP; μg L^{-1}). Oligotrophic lakes had a TP concentration < 10 μg L^{-1} and eutrophic lakes had a TP > 35 μg L^{-1} (OECD, 1982; Dodds et al., 1998). A 50 mL subsample from the composite epilimnion sample was collected in a borosilicate tube and used to measure TP. All subsamples were kept on ice in coolers during transport and then at 4 °C prior to analysis in the laboratory. P in the water sample was converted to orthophosphate in sulphuric acid-persulfate medium in an autoclave at 121 °C for 30 min and then measured by colorimetry in a Skalar San++ Continuous Flow Analyzer (Skalar Analytical, Breda, The Netherlands).

Lake browning indicators included dissolved organic carbon (DOC; mg L^{-1}) as a proxy for DOM quantity, and Specific UV Absorbance (SUVA; L mg C^{-1} m^{-1}), the ratio of absorbance at 254 nm to DOC concentration, as a proxy for DOM quality (Weishaar et al., 2003). Subsamples for DOM quantity and quality were collected from the composite epilimnion samples. A composite epilimnion sample (1.5 L) for water chemistry analysis was filtered through a nylon filter (Nitex) of 80 μm porosity. A 500 mL subsample was

collected in a polyethylene terephthalate bottle for analysis of DOC. A 500 mL subsample was collected in a dark Nalgene™ bottle for determination of SUVA. DOC was measured from pre-ashed GF/F-filtered water samples by colorimetry using a Technicon AutoAnalyzer II (Seal Analytics, Mequon, Wisconsin, USA). Absorbance measurements were performed on a Cary 300 Bio UV-Visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) in a 1 cm path quartz cuvette.

Chlorophyll-*a* (chl-*a*, μg L^{-1}) was measured as a proxy for phytoplankton biomass and phycocyanin (PC, μg L^{-1}) was measured as a proxy for cyanobacteria biomass. Two 500 mL subsamples of the composite epilimnion sample were sampled, one for chl-*a* and the other for PC. The 500 mL subsamples were filtered through pre-ashed GF/F glass-fiber filters (GE Healthcare Life Sciences) within 24 h after sample collection. Filters were preserved at -80 °C until pigment extraction and quantification. Chl-*a* was extracted by submerging the filters in 90% acetone solution at -20 °C for 24 h. Chl-*a* determination was performed by fluorometry on a Trilogy fluorometer (Turner Designs, Sunnyvale, CA, USA) and quantified as per Jeffrey & Humphrey (1975). PC was extracted by placing filters in tubes with 1 mL of phosphate buffer solution (0.1 M, pH 6) and then freeze-thawing then 3× using liquid nitrogen. To ensure cell lysis, filters were further sonicated (5 sec pulses in 2 min cycles) and 3 mL of the phosphate buffer was added prior to PC extraction at 4 °C for 24 h. The supernatant was separated from the filter by centrifugation (6000 g for 5 min), filtered through 0.22 μm glass fiber syringe filters (Acrodisc Supor Membrane, Pall Life Sciences, Port Washington, NY, USA), and PC was determined by absorbance in a Cary 3000 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) in a 1 cm path-length quartz cuvette.

Fatty acid content was measured in seston and zooplankton. Seston and zooplankton samples were collected for fatty acid analysis by towing 60 μm (seston) and 156 μm (zooplankton) mesh-size plankton nets from thermocline to the lake surface in each of the 30 study lakes. Nets were towed until enough biomass was collected. Seston samples were subsequently filtered, on-site, through an 80 μm mesh-size filter on site to remove zooplankton and other suspended particles. Seston samples were filtered through 0.45 μm nitrocellulose filters (Sigma Aldrich, USA) then backwashed with 2 mL of lake water. The filtered seston samples were stored in Falcon tubes® at -80 °C. Zooplankton

samples were collected in Whirl-Pak™ bags and kept frozen on dry-ice during transport and in an ultra-low temperature freezer (set to -80 °C) prior to laboratory analysis. Zooplankton samples were thawed and then sorted into cladocerans and copepods while ensuring that no phytoplankton remained on them. Seston, cladocerans and copepod samples were freeze-dried prior to fatty acid extraction and quantification. Lipids were extracted from the freeze-dried samples with chloroform-methanol (2:1 by volume); methanolic sulfuric acid was added as a catalyst and samples were heated at 90 °C for 90 min to transmethylate the fatty acids. Fatty acids were then extracted to n-hexane, concentrated, and analyzed through gas chromatography on a Shimadzu GC-210 plus with flame ionization detector using a SP-2560 column (Supelco Inc.). Helium was used as carrier gas (average flow of 20 cm sec^{-1}) and a splitless injection technique was used. The temperature was maintained at 50 °C for 1 min, then increased to 180 °C at the rate of 15°C min^{-1}, then increased to 240 °C at a rate of 2 °C min^{-1}, where it was maintained for 23 min. The standard fatty acids methyl ester mix GLC68F (Nu-Chek Prep.) was used for peak identification and quantification. Fatty acid concentrations were converted from dry weight to C ratios (μg FA mg C^{-1}). Seston and zooplankton C content was measured from freeze-dried samples through mass spectrometry by dry combustion using an EA (Costech Analytical Technologies, Valencia, CA, USA). Fatty acid retention by consumers was calculated as the ratio between consumer fatty acid content (in μg FA mg C^{-1}) to seston fatty acid content (μg FA mg C^{-1}).

4.2.3 Statistical analyses

Significance of correlations of DOC vs. chl-*a* and PC were performed using linear and logarithmic regression models in Sigma Plot 12.0 (Systat Software, San Jose, CA). Significant differences in fatty acid composition between seston, cladocerans and copepods (as μg FA mg C^{-1}) were assessed through one-way ANOVA in Sigma Plot 12.0. Significance of correlations of brownification indicators (DOC, SUVA), lake trophic status (TP), chl-*a* and PC vs. fatty acid composition (μg FA mg C^{-1}) of seston, cladocerans and copepods) and fatty acid retention by consumers (i.e., the ratio of consumer fatty acid content to seston fatty acid content) were performed using linear regression models. All statistical analyses were performed using SigmaPlot 12.0 at a

significance level $p < 0.05$. To ensure that changes in specific fatty acid fractions were independent from each other, correlations between predictor variables and fatty acid composition were also run on the percentage of each fatty acid fraction over corresponding total fatty acid content in seston, cladocerans or copepods.

4.3 Results

There was a range in environmental conditions (DOC, SUVA, TP) among the study lakes. DOC ranged from 2.60 to 9.80 mg L^{-1}, and SUVA ranged from 1.22 to 5.00 L mg C^{-1} m^{-1}. There was a weak correlation between DOC and SUVA ($r^2 = 0.13$; $p = 0.06$). TP in the lakes ranged from 6.00 to 48.50 μg L^{-1} (average TP = 12.85 ± 8.92 μg L^{-1}); 14 lakes were oligotrophic (TP between 0 and 10 μg L^{-1}), 15 lakes were mesotrophic (TP between 10 and 35 μg L^{-1}), and one lake (Brandy Lake) was eutrophic (48.50 μg L^{-1}) (Table 4.1).

Table 4.1. Brownification drivers (DOC and SUVA), trophic status (TP) and responses (chl-*a* and PC) for all study sites.

ID	DOC (mg L^{-1})	SUVA (L mg C^{-1} m^{-1})	TP (µg L^{-1})	Chl-*a* (µg L^{-1})	PC (µg L^{-1})
BAS	5.80	2.76	10.70	2.36	9.13
BEL	2.60	1.98	6.50	2.00	15.23
BRA	9.50	5.00	48.50	7.89	50.90
COU	5.30	1.23	12.60	3.47	15.23
CRY	4.90	2.40	21.30	1.67	6.23
DAV	5.30	2.33	8.10	1.73	8.09
DEP	9.10	2.78	20.40	12.52	26.45
DEV	4.20	2.69	11.80	3.87	1.46
FOU	5.60	1.58	6.80	0.97	0.12
FOX	7.10	4.01	11.10	7.33	21.28
HEA	4.40	1.69	11.30	3.80	14.63
KAS	4.20	2.61	7.80	1.91	4.23
KOS	3.80	3.01	6.60	2.38	7.13
LON	5.20	2.78	9.30	3.57	7.76
LOM	5.90	2.05	7.40	2.86	9.13
MAC	7.40	2.32	18.70	11.45	50.15
MAP	3.70	2.91	7.50	1.24	0.50
MAR	5.10	3.39	12.40	2.15	13.02
MEN	7.10	3.93	11.40	3.44	26.36
MIN	9.80	2.85	18.40	11.98	24.22
MOR	5.50	2.91	9.00	2.91	9.50
OXB	4.00	2.75	7.20	1.58	11.50
PAI	3.90	2.52	10.40	3.62	0.50
RAV	3.50	2.59	6.00	3.37	7.36
RIL	4.10	4.83	10.90	4.04	25.12
SPA	4.90	2.32	13.40	2.63	13.23
TEA	6.30	2.82	7.50	3.89	15.23
TWE	3.20	2.39	8.10	2.77	0.23
WAS	7.90	3.34	31.80	6.31	32.38

Environmental conditions (DOC, SUVA, TP) had an effect on phytoplankton (and cyanobacteria) biomass (i.e., seston). DOC was correlated to both chl-*a* ($r^2 = 0.53$) and PC ($r^2 = 0.28$) (Figure 4.3). SUVA was correlated with PC only ($r^2 = 012$). TP was correlated to DOC ($r^2 = 0.45$) and SUVA ($r^2 = 0.19$). In addition, TP was positively correlated to chl-*a* ($r^2 = 0.27$) and PC ($r^2 = 0.16$).

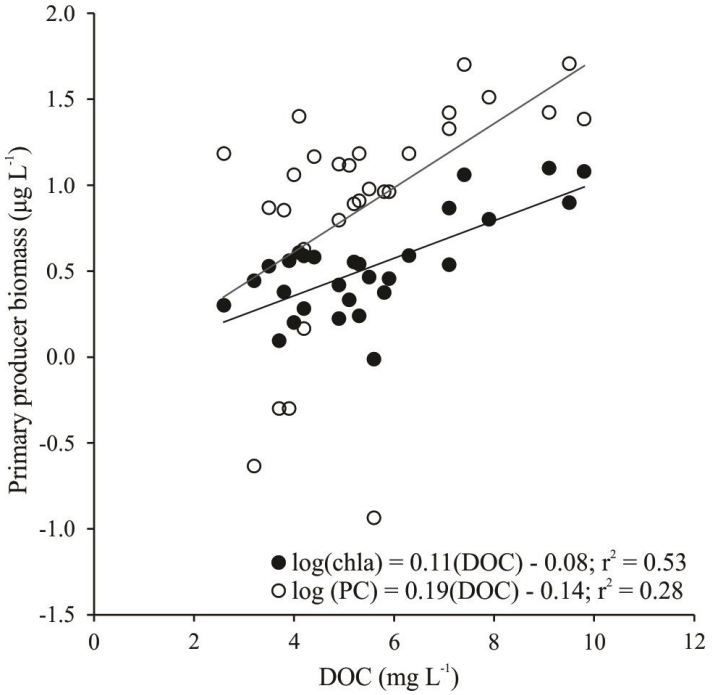

Figure 4.3. Correlation between DOM concentration (DOC) and phytoplankton (chl-a) and cyanobacteria (PC) biomass. Lines represent significant correlations ($p < 0.05$).

We examined the effect of environmental conditions (DOC, SUVA, TP) on the content of each fatty acid fraction (n-3 PUFA, n-6PUFA, EPA, DHA, LC-SFA, odd-chain and branched-chain SFA) in seston and consumers (Table 4.2). DOC was not correlated to the content of any of the assessed fatty acids in seston. However, SUVA was negatively correlated with n-6 PUFA ($r^2 = 0.12$), DHA ($r^2 = 0.12$), odd-chain and branched-chain SFA ($r^2 = 0.16$), and long-chain SFA ($r^2 = 0.12$) in seston (Table 4.1). Neither DOC nor SUVA were correlated with any of the assessed PUFA content (n-3, n-6, EPA, or DHA) in consumers. TP was negatively correlated with DHA ($r^2 = 0.19$) in seston.

Table 4.2. Slopes of significant ($p < 0.05$) correlations between (z-scored standardized) brownification drivers and fatty acid composition (in µg FA mg C^{-1}) in seston, cladocerans, and copepods, and fatty acid retention in cladocerans and copepods.

	DOC		SUVA		TP		Chl-*a*		PC		Seston FA	
	slope	r^2	slope	r^2	slope	r^2	slope	r^2	slope	r^2	slope	r^2
Seston												
n-3							-1.33	0.13	-1.3	0.12		
n-6			-1.54	0.12					-1.58	0.17		
EPA							-1.63	0.33	-1.46	0.25		
DHA			-0.96	0.12	-1.05	0.19	-1.22	0.29	-1.33	0.33		
Odd/branched			-1.78	0.16								
LC-SFA			-2.69	0.33			-1.57	0.16	-1.46	0.13		
Cladocerans												
n-3												
n-6											-4.42	0.40
EPA												
DHA					1.67	0.14					-2.74	0.23
Odd/branched							2.33	0.16	2.95	0.22		
LC-SFA												
Copepods												
n-3												
n-6												
EPA												
DHA												
Odd/branched	3.41	0.15			3.07	0.12	4.59	0.29	4.20	0.26		
LC-SFA												
Cladocerans Retention												
n-3												
n-6												
EPA							1.03	0.15				
DHA			1.03	0.21	1.04	0.25	0.9	0.22	0.89	0.25		
Odd/branched			1.60	0.15					1.15	0.18		
LC-SFA			2.08	0.30			1.43	0.19	1.26	0.17		
Copepods Retention												
n-3												
n-6												
EPA							1.33	0.22				
DHA					0.86	0.27	1.99	0.19	0.77	0.13		
Odd/branched			1.37	0.18			1.07	0.13	1.77	0.16		
LC-SFA			1.8	0.21			1.27	0.16				

The fatty acid content was significantly smaller (but more variable) in seston than in cladocerans and copepods, except for LC-SFA which was in low concentration in all three groups (average log (LC-SFA) = 0.26 µg mg C^{-1} in seston, 0.38 µg mg C^{-1} in cladocerans and 0.47 µg mg C^{-1} in copepods) (Figure 4.4). Furthermore, DHA content was lower in cladocerans (average log (DHA) = 1.58 µg mg C^{-1}) than copepods (1.72 µg mg C^{-1}), but there were no significant differences in the other fatty acids among zooplankton groups.

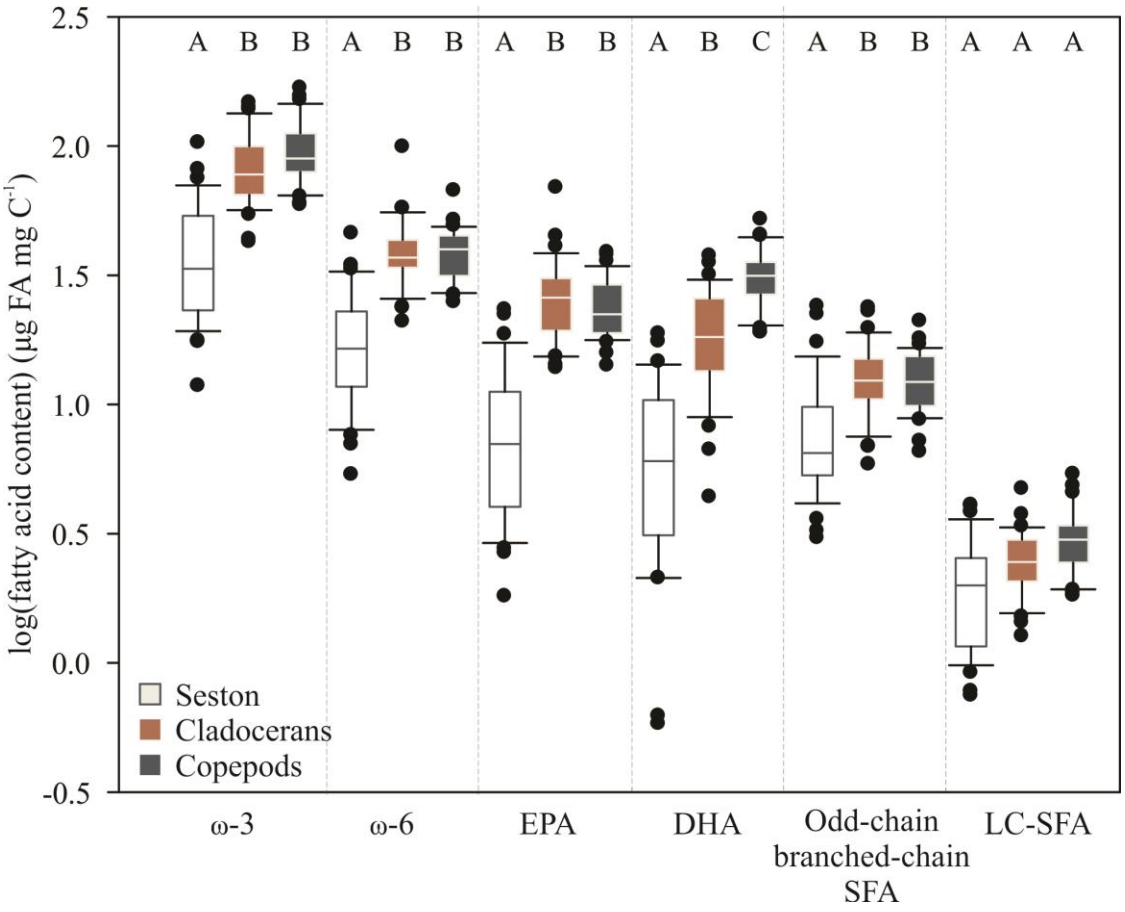

Figure 4.4. Fatty acid concentration in seston, copepods, and cladocerans. Letters represent significant differences between organism fatty acid content calculated from one-way ANOVA and post-hoc Holm-Sidak test.

We examined the effect of ecological conditions (chl-*a*, PC) on the content of each fatty acid fraction (n-3 PUFA, n-6PUFA, EPA, DHA, LC-SFA, odd-chain and branched-chain SFA) in seston (Figure 4.5) and consumers (Table 4.2). Phytoplankton biomass (chl-*a*) was negatively correlated with n-3 PUFA (r^2 = 0.13), EPA (r^2 = 0.33)

and DHA (r^2 = 0.29) in seston; and cyanobacteria biomass (PC) was negatively correlated with n-3 PUFA (r^2 = 0.12), n-6 PUFA (r^2 = 0.17), EPA (r^2 = 0.25) and DHA (r^2 = 0.33) in seston. Both chl-*a* and PC were negatively correlated with LC-SFA (r^2 = 0.16 for chl-*a* and 0.13 for PC) in seston, but positively correlated with LC-SFA in cladocerans (r^2 = 0.16 for chl-*a* and r^2 = 0.22 for PC) and copepods (r^2 = 0.29 for chl-*a* and r^2 = 0.26 for PC). DHA content in seston correlated negatively with DHA content in cladocerans (r^2 = 0.23), and n-6 PUFA in seston correlated negatively with n-6 PUFA in cladocerans (r^2 = 0.40). None of the fatty acid fractions in seston correlated with fatty acid content in copepods. When the percentage of each fatty acid fraction over total fatty acid content was assessed (rather than the concentration), all predictors (DOC, SUVA, TP, chl-*a*, and PC) were significantly correlated with the percentage of odd-chain and branched-chain SFA over total fatty acid content (rather than their concentration) in copepods but only chl-*a* and PC were correlated with the percentage of odd-chain and branched-chain SFA in cladocerans (Table 4.3).

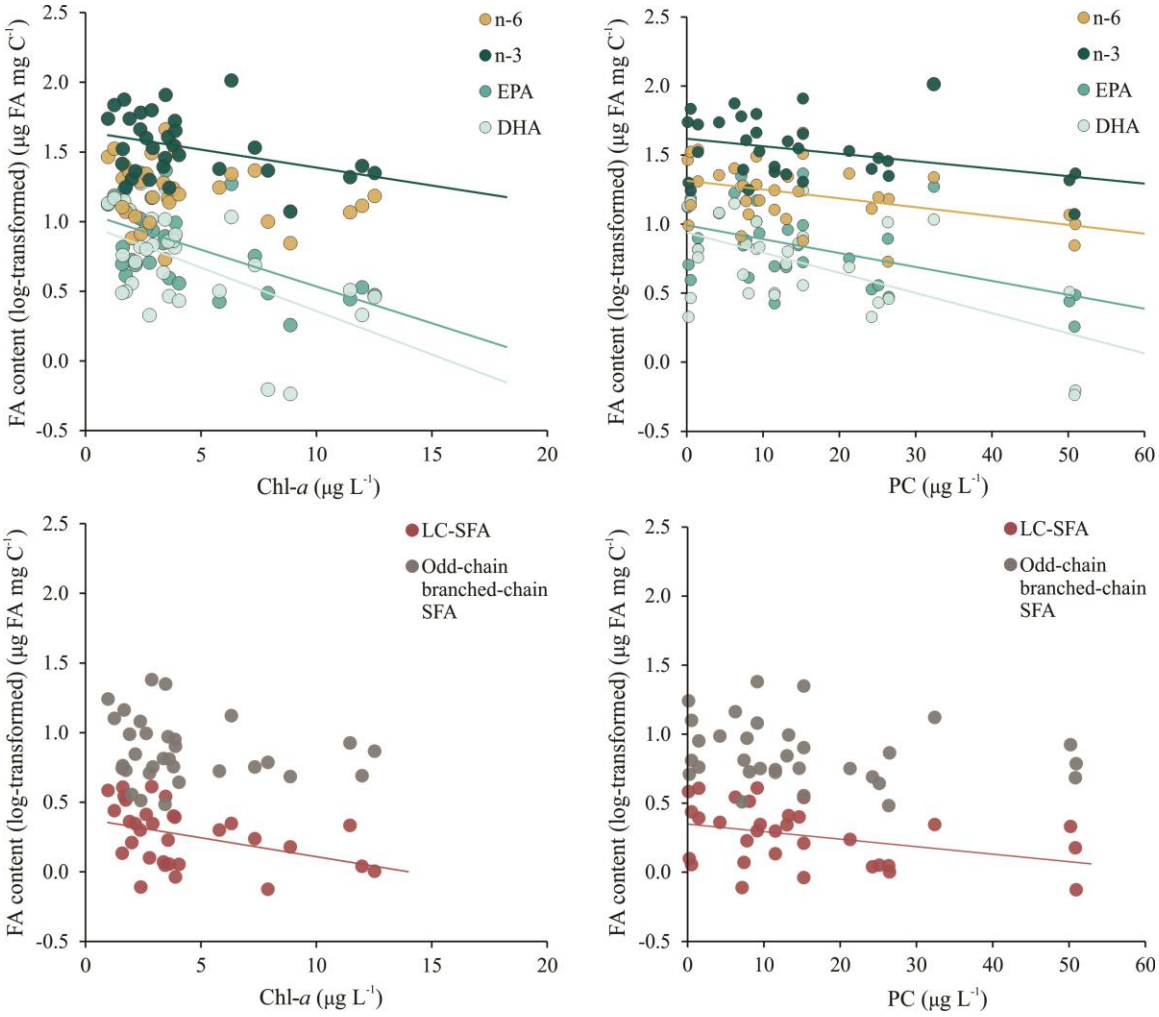

Figure 4.5. Correlation between seston PUFA and chl-a (left column) and PC (right column). Lines represent significant correlations ($p < 0.05$); r^2 values and slopes can be found in Table 4.1

Table 4.3. Significant ($p < 0.05$) correlations between (z-score transformed) indicators of brownification (DOC, SUVA), trophic status (TP), and responses of brownification (chl-a, PC), and seston and consumers fatty acid content measured as the percentage of each fatty acid fraction over the total fatty acid content.

	DOC		SUVA		TP		Chl-*a*		PC		Seston FA	
	slope	r^2	slope	r^2	slope	r^2	slope	r^2	slope	r^2	slope	r^2
Seston												
n-3												
n-6												
EPA							-0.50	0.17				
DHA							-0.49	0.17				
Odd/branched												
LC-SAFA												
Cladocerans												
n-3												
n-6												
EPA	-1.23	0.18	-0.99	0.14			-1.09	0.14			1.25	0.3
DHA					1.02	0.14						
Odd/branched							0.62	0.27	0.64	0.33	0.48	0.17
LC-SAFA											0.09	0.18
Copepods												
n-3												
n-6												
EPA											0.95	0.34
DHA												
Odd/branched	0.63	0.4	0.41	0.17	0.55	0.3	0.67	0.44	0.75	0.51		
LC-SAFA											0.13	0.21

We examined the environmental (DOC, SUVA, TP) and ecological (chl-*a*, PC) controls on the fatty acid retention in consumers (measured as the ratio of consumer to seston fatty acid content) (Table 4.2). DOC did not correlate to any of the fatty acid retention in consumers, however, SUVA was positively correlated to odd chain and branched-chain SFA and LC-SFA retention in both cladocerans ($r^2 = 0.15$ for odd-chain SFA and $r^2 = 0.30$ for LC-SFA) and copepods ($r^2 = 0.18$ for odd-chain and $r^2 = 0.21$ for branched-chain SFA and LC-SFA). SUVA was also positively correlated to DHA retention in cladocerans ($r^2 = 0.21$). Chl-*a* was correlated with retention of PUFA (EPA and DHA) in both cladocerans and copepods, and it was correlated with retention of LC-SFA in cladocerans ($r^2 = 0.19$) and retention of odd-chain and branched-chain SFA ($r^2 = 0.13$) and LC-SFA in copepods ($r^2 = 0.16$). PC was correlated with retention of DHA in both cladocerans ($r^2 = 0.25$) and copepods ($r^2 = 0.13$), with retention of odd-chain and

branched-chain SFA and LC-SFA in cladocerans (r^2 = 0.18 and 0.17, respectively), and retention of odd-chain and branched-chain SFA in copepods (r^2 = 0.16).

The transfer of PUFA to consumers through the microbial loop was further assessed through correlations between seston PUFA content (EPA and DHA) and the ratio of PUFA to bacterial fatty acids (odd-chain and branched-chain SFA) in consumers (Figure 4.6). Correlations existed between seston EPA and the ratio of EPA to bacterial fatty acids in cladocerans (r^2 = 0.22) and copepods (r^2 = 0.31). In addition, a correlation existed between DHA content in seston and the ratio of DHA to bacterial fatty acids ratio in copepods (r^2 = 0.20), but not in cladocerans.

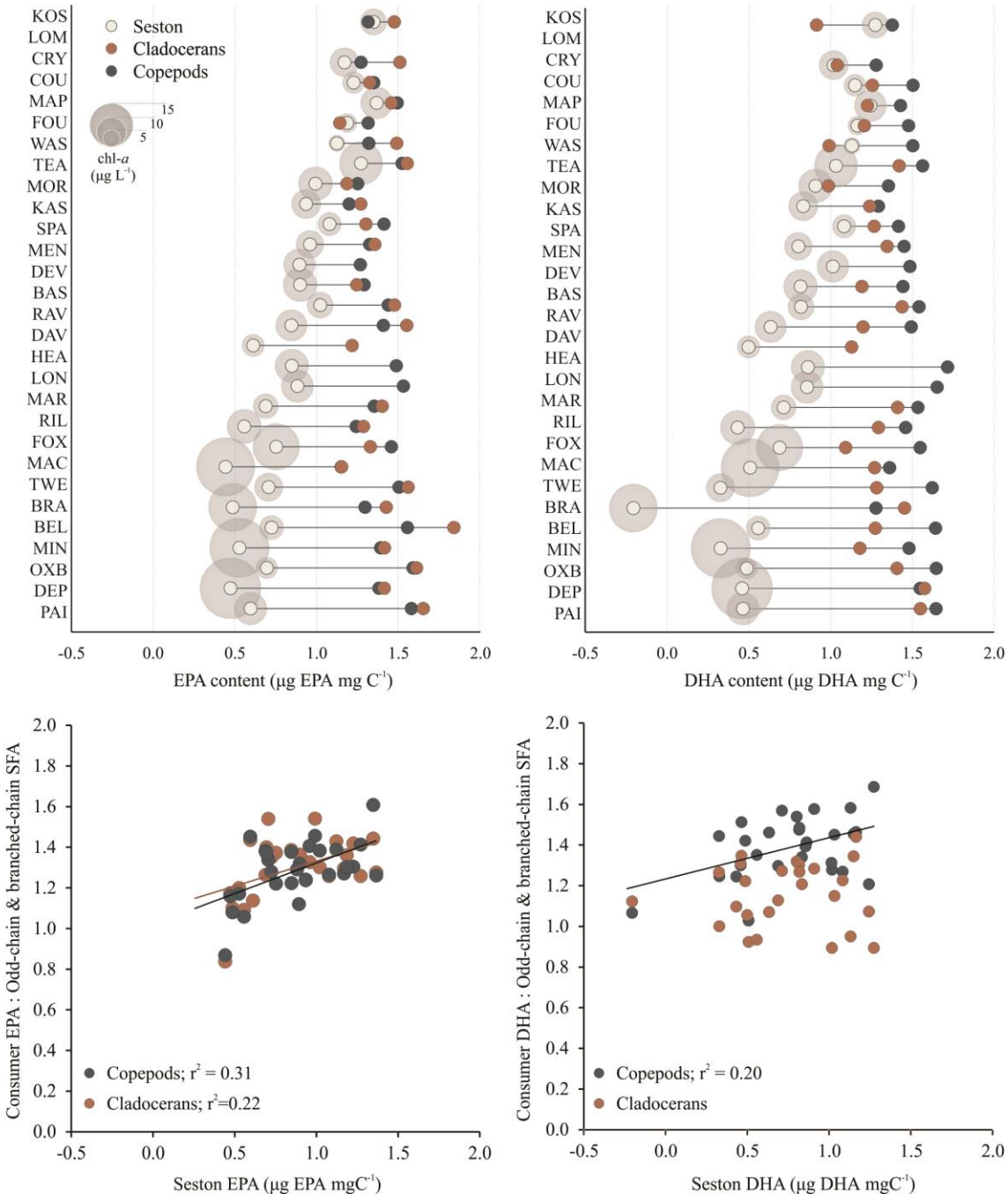

Figure 4.6. Seston, cladocerans, and copepod EPA (top left) and DHA (top right) content in all the study lakes. Seston circle sizes represent phytoplankton biomass (chl-*a*). Bottom panels represent consumers' EPA:odd-chain and branched-chain SFA (bottom left) and DHA:odd-chain and branched-chain SFA (bottom right) ratios as a function of seston EPA and DHA content. Lines represent significant correlations ($p<0.05$).

4.4 Discussion

Brownification alters the pathways and efficiency of energy and nutrient transfers in lake food webs (Karlsson et al., 2009; Finstad et al., 2014). This alteration may occur directly, by increasing the availability of low-quality terrestrial resources (Karlsson et al., 2009), changing the phytoplankton community composition (Jones, 1998), or promoting the microbial loop because of additional C resources (Tranvik, 1992). Alternatively, this alteration may occur indirectly, by changing lake environmental conditions (Solomon et al., 2015) that in turn alter the production of PUFA by phytoplankton (Guschina and Hardwood, 2009). We hypothesized that brownification directly affects the phytoplankton community, increasing biomass and favoring cyanobacteria, consequently resulting in a reduction in the availability and transfer of PUFA to primary consumers.

We found that brownification led to increased DOM and shifts towards more refractory DOM (higher SUVA, associated with darker water color (McKnight et al., 2003)) in the lakes. The additional DOM inputs led to an increase in phytoplankton biomass (i.e., chl-*a*) and a shift in the phytoplankton community to a greater prevalence of cyanobacteria (i.e., PC). We also found that brownification-driven changes in the phytoplankton community were associated with a decline in PUFA in seston; specifically, increases in PC were correlated to decreases in seston n-3 PUFA (including EPA and DHA) and seston n-6 PUFA. Shifts towards more *refractory* DOM (i.e., larger SUVA), rather than more DOM (i.e., larger DOC), drove the decline in seston DHA and n-6 PUFA. Cyanobacteria have competitive advantages in lakes with more refractory DOM. Cyanobacteria have accessory pigments that allow them to photosynthesize under lower light conditions (Oliver & Ganf, 2000), some species can scavenge Fe from DOM-Fe complexes (Sorichetti et al., 2014, 2016), and some species can shift from autotrophy to mixotrophy to consume DOM (Poerschmann et al. 2004; Wilken et al., 2018) in these nutrient-poor lake waters. Irrespective of the strategy, the increase in the cyanobacteria could drive declines in the content of PUFA of seston (Müller-Navarra et al., 2004).

However, changes in environmental conditions driven by brownification may have further impacted PUFA production. For example, increases in temperature and CO_2 concentrations (Mayorga et al., 2005; Porcal et al., 2009) can *decrease* PUFA production in phytoplankton, as unsaturation of fatty acids is typically highest at low temperature

and CO_2 concentrations (Thompson, 1996, Fuschino et al. 2011). Furthermore, changes in nutrient conditions can *decrease* PUFA pools. Nutrient limitation can lead to slower growth and reduced cellular division resulting in greater cellular fatty acid stores (Thompson, 1996; Guschina & Harwood, 2009). Therefore, brownification and associated increased nutrient loads (Jones, 1998) can *decrease* in PUFA pools by favoring faster and larger growth and therefore reducing the cellular fatty acid storage. Alternatively, decreases in the penetration of UV radiation (Williamson et al., 1996) can *increase* PUFA pools by reducing PUFA oxidative damage (Harwood, 1998).

Despite the observed difference in PUFA content in phytoplankton (lower in cyanobacteria), we found no difference in PUFA content in zooplankton. Compared to seston, consumers had a larger content of PUFA (and odd-chain and branched-chain SFA, but not LC-SFA), reflecting the preferential retention of these fatty acids. Increases in PUFA content from seston to herbivores, and from herbivores to carnivores, have been previously observed, as these compounds are preferentially retained to maintain somatic growth and reproduction (Persson & Vrede, 2006). Greater classification (to the species level) within zooplankton taxa could help unravel differences between feeding types (Hessen & Leu, 2006; Guschina & Harwood, 2009). However, differences in EPA and DHA were observed even at the class level, with cladocerans (filter-feeders) having a larger average EPA content and copepods (selective-feeders) having a larger DHA content. The DHA content in copepods makes them the major DHA transfer pathway to top predators (Strandberg et al., 2015b). Importantly, there was no decrease in zooplankton PUFA content associated with brownification. Instead, the PUFA content in zooplankton was relatively homogenous among lakes irrespective of DOM properties. The PUFA content of consumers is defined, in part, by phylogenetic origin and life history (Persson & Vrede, 2006), making them quasi-homeostatic (i.e., their nutritional characteristics are, to an extent, independent of their diet) to fatty acid variability in seston (Brett et al., 2009). The quasi-homeostatic PUFA content in consumers is also reflected by the lack of correlation between environmental (DOC, SUVA, TP) and ecological (chl-*a*, PC) controls vs. consumer PUFA (except for the negative correlations in n-6 PUFA and DHA content between seston and cladocerans). Both, the enrichment and the consumers' quasi-homeostatic PUFA content suggest that zooplankton have

strategies (e.g., greater consumption of –PUFA-poor resources or reliance on the microbial loop) to compensate for the decline in seston quality.

We found an association between chl-*a* and PC and bacterial fatty acids (odd-chain and branched-chain SFA) in cladocerans and copepods, indicating greater reliance on fatty acid transfer through the microbial loop with increasing phytoplankton and cyanobacteria biomass (Hiltunen et al., 2015). These findings are further supported by the percentage of odd-chain and branched-chain SFA (rather than concentration), especially in copepods, confirming the brownification-driven increase in the uptake of bacterially-derived fatty acids. In the microbial loop, autochthonous and allochthonous C is assimilated by bacteria and transferred to zooplankton via two additional trophic transfers (bacteria-ciliates-heterotrophic flagellates-zooplankton; Berglund et al., 2007). Since each trophic transfer results in PUFA enrichment, heterotrophic flagellates can have significantly higher PUFA content than the original resource; a phenomenon known as 'trophic upgrading' (Bec et al., 2006, 2010). Under PUFA-poor phytoplankton conditions, heterotrophic flagellates might be a greater quality resource for zooplankton (Desvilletes & Bec, 2009). In our study lakes, the ratios of EPA and DHA to bacterial fatty acids (odd-chain and branched-chain SFA) in copepods decline with increasing EPA and DHA seston content. This correlation is observed only when considering the ratio of EPA (and not DHA) to bacterial fatty acids in cladocerans. These correlations further suggest that, when brownification drive declines in seston quality, consumers supplement their diet with PUFA transferred through the microbial loop to meet their requirements. The decline in seston PUFA quality and consumers' reliance on supplementary PUFA sources result in greater differences between seston and consumer PUFA and greater PUFA retention by consumers.

4.5 Conclusions

This study did a space-for-time substitution for brownification. The degree of browning in our lakes was relatively low, as DOM concentrations ranged from 2 to 10 mg DOC L^{-1}, and changes in DOM composition from SUVA of 1 L mg C^{-1} m^{-1} (labile DOM) to 5 L mg C^{-1} m^{-1} (refractory DOM). This degree of browning resulted in declines in seston PUFA (despite the increase in phytoplankton biomass, due to the increasing

prevalence of PUFA-poor cyanobacteria), but not in consumers' PUFA content. Furthermore, under PUFA-poor seston conditions, consumers did not experience major changes in their PUFA composition, nor did they present an increasing reliance on terrestrial fatty acids (LC-SFA). These results suggest that brownification could increase the transfer of PUFAs through the microbial loop, allowing primary consumers to adapt to the lower quality of phytoplankton (i.e., consumers find alternative pathway for PUFA). In contrast, in other studies, where the degree of browning in the lakes was higher (i.e., DOM concentrations that are more typical in boreal lakes (> 15 mg L^{-1}); Taipale et al., 2015, 2017), resulted in decreases in zooplankton PUFA content. Even though phytoplankton communities are typically dominated by PUFA-rich raphydophytes in these darker systems (Taipale et al., 2016), primary production is low (Kelly et al., 2014), and raphydophytes are too large to be directly consumed by zooplankton, therefore limiting the transfer of PUFA to consumers (i.e., consumers have a lower biomass) and eventually to top predators.

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Chapter 5

5 Conclusions

5.1 Research Findings

The planet has entered an era of major global atmospheric changes. Alterations in terrestrial-aquatic linkages are consequences of these global changes, including increased temperatures (Kirtman et al., 2013), intensification of hydrologic cycle (Huntington, 2006), and atmospheric pollution (Evans et al., 2012; SanClements et al., 2012). One such alteration is the browning of northern lakes (Monteith et al., 2007; Creed et al., 2018). However, the susceptibility of a lake to experience brownification and the consequences for food webs are largely unknown. This thesis aimed to provide scientific knowledge to improve predictability of which lakes are susceptible to brownification and its consequences on food webs.

The first finding is that DOM loads to lakes are driven by atmospheric and hydrologic changes that in turn lead to the destabilization of soil C (Kritzberg & Erkstrom, 2012; Williamson et al., 2015). Hydrologic connectivity (i.e., the water-mediated transfer of matter across the landscape; Bracken et al., 2013; Turnbull et al., 2008) promotes aquatic C export. Aquatic C export peaks during the spring snowmelt and fall storm hydrologic connectivity periods. In addition to droughts, extreme events are projected to increase, with wet areas becoming wetter, and dry areas becoming drier (Trenberth, 2011). While declines in hydrologic connectivity reduce the transfer of C from terrestrial to aquatic systems as dissolved organic matter (DOM), extreme precipitation events concentrated in the hydrologic connectivity seasons may promote its export. Temperature acts as an enhancer that promotes atmospheric export during the dry periods (Kirschbaum, 1995; Webster et al., 2008). Therefore, a large proportion of the annual atmospheric C export occurs during the hydrologic disconnectivity summer period. Ecotones, the transition zones between wetlands and uplands, are major C stores (Webster et al., 2008) and hotspots of atmospheric C export as CO_2 (Creed et al., 2013). Decreases in hydrologic connectivity contribute to greater C export to the atmosphere from the ecotone and the wetland, where microbial respiration is often suppressed by

oxygen limitation (Lohse et al., 2009). Thus, prolonged and more intense droughts will favour the export of soil C to the atmosphere, especially in lowland-dominated catchments where there are deeper deposits of C. With climate predictions suggesting warmer temperatures and longer droughts, the increase in soil respiration could potentially prompt the shift of forest soil from C sink to C source (Bellamy et al., 2005; Schulze & Freibauer, 2005). In addition, the greater export of stored C will further contribute to climate warming, resulting in a positive feedback that will increase the risk of greater destabilization of C stores (Davidson & Janssens, 2006). Since the rate of atmospheric change is greater in northern systems (Smith et al., 2015), northern lakes are more susceptible to experience brownification (Creed et al., 2018).

The second finding from this thesis is that moderate increases in allochthonous DOM inputs alter the physical and chemical environment of lakes and enhance lake primary production. Brownification is not only associated to changes in DOM quantity, but also shifts in DOM quality towards refractory (i.e., more aromatic, higher molecular weight) compounds (McKnight et al., 2003). Shifts in DOM quality are caused by the more refractory compounds in allochthonous (externally derived) DOM compared to the more labile (i.e., more aliphatic, lower molecular weight) autochthonous (in-lake produced) DOM (Bertilsson & Jones, 2003). Allochthonous DOM inputs increase water colour and supply nutrients to aquatic systems, subsequently reducing light penetration and inducing the shift from oligotrophic to eutrophic conditions (Jones, 1992; Findlay, 2003), inducing the shift from oligotrophic to eutrophic conditions. Shallow non-stratified lakes are more susceptible to experience brownification. In these shallow systems, allochthonous DOM inputs increase lake nutrient concentrations and decrease light availability, prompting the shift from benthic to pelagic production (Brothers et al., 2014). In addition, alterations in nutrient availability favour cyanobacteria that can access the nutrients in DOM complexes (i.e., mixotrophy, siderophore production). In contrast, in deeper stratified lakes, allochthonous DOM creates darker conditions, acts as a supply of micronutrients (iron), and promotes hypolimnion anoxia. Changes in the hypolimnion redox conditions mobilize sediment nutrient, specially phosphorus, soluble in anoxic conditions. Changes in light availability, and the increase in nutrient concentration by direct supply from allochthonous DOM and indirectly through internal loading (Molot &

Dillon, 2003) favour primary producers. An increase in nutrients in the hypolimnion water benefits cyanobacteria living in the thermocline (Gervais et al., 1997; Drakare et al., 2003), or those that are able to regulate their buoyancy (Carey et al., 2012).

Both, phytoplankton and cyanobacteria (using chl-*a* and PC concentrations as biomass proxies) responded to lake physical characteristics, being highest in shallower and darker lakes. After physical conditions, macronutrients (nitrogen and phosphorus) were major indicators of phytoplankton and cyanobacteria growth, suggesting that these systems are limited by macronutrient (and not micronutrient) availability. Phytoplankton, and cyanobacteria specifically, adapted to the changes in light driven by the moderate allochthonous DOM inputs (2-12 mg dissolved organic C L^{-1}). However, larger inputs of allochthonous DOM could induce light-limiting conditions, constraining phytoplankton growth, and reducing overall ecosystem productivity (Karlsson et al., 2009).

The third finding is that the brownification-driven changes in phytoplankton alter the pathways and efficiency of C transfer. DOM and phytoplankton constitute the basal resources for pelagic food webs (Ask et al., 2009; Brett et al., 2017). Brownification can change the quantity and quality of basal resources (Karlsson et al., 2009). Differences in quality refer to the content of essential fatty acids (EFA) in the basal resources. Eukaryotic autotrophs are generally considered better-quality resources than prokaryotic autotrophs (i.e., cyanobacteria) and allochthonous DOM (Kelly et al., 2014; Galloway & Winder, 2015). Eukaryotic autotrophs (except for green algae) contain high concentrations of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), which are lacking or in low concentrations in cyanobacteria or terrestrial plants (Brett & Muller-Navarra, 1997; Brett et al., 2000). The brownification-driven promotion of cyanobacteria in clear oligotrophic lakes is associated with a decline in the EPA and DHA content of basal resources. However, the decline in quality does not have significant consequences in the EPA and DHA content in primary consumers. Under poor-quality resource circumstances, zooplankton rely on PUFA transferred from heterotrophic bacteria to heterotrophic flagellates through the microbial loop. The degradation of autochthonous and allochthonous resources by heterotrophic bacteria results in PUFA enrichment, as PUFA are preferentially retained in each trophic transfer

(Persson & Vrede, 2006, Bec et al., 2006). Thus, under poor-quality resource conditions, heterotrophic flagellates may be a better resource for zooplankton (Desvilettes & Bec, 2009). However, reliance on microbial loop may not be enough to sustain secondary production under greater DOM loads (Strandberg et al., 2015; Taipale et al., 2016).

If current atmospheric and hydrologic trends persist, allochthonous DOM inputs into temperate lakes will continue to rise (Solomon et al., 2015). While the moderate increases in DOM (2 – 10 mg dissolved organic C L^{-1}) promote primary production and cyanobacteria growth, reducing the production (but not the transfer) of EFAs, larger allochthonous DOM inputs could suppress lake primary production due to light limitation (Ask et al., 2009). This will result in further declines in resource quantity and quality (Strandberg et al., 2015; Taipale et al., 2016) due to smaller primary production, shifts towards greater reliance on allochthonous resources (Karlsson et al., 2009), and consequently reduction in overall ecosystem production (Finstad et al., 2014).

5.2 Significance

This study contributes to improve our understanding on the multiple factors and complex interactions at play in brownification. The three manuscripts sequentially identify landscape sources of C, track its transfer from terrestrial to aquatic ecosystems, and then its transfer from primary producers to consumers. Key findings of this thesis include: (1) identification of the drivers of soil C destabilization and export to the atmospheric and aquatic fates; (2) the consequences of shifts in lake DOM quantity and quality on primary production and cyanobacteria dominance; and (3) the implications of changes in basal resource quantity and quality for aquatic consumers. These findings motivate future research that investigates terrestrial and aquatic linkages and the consequences of catchment processes for aquatic ecosystem function (Figure 5.1).

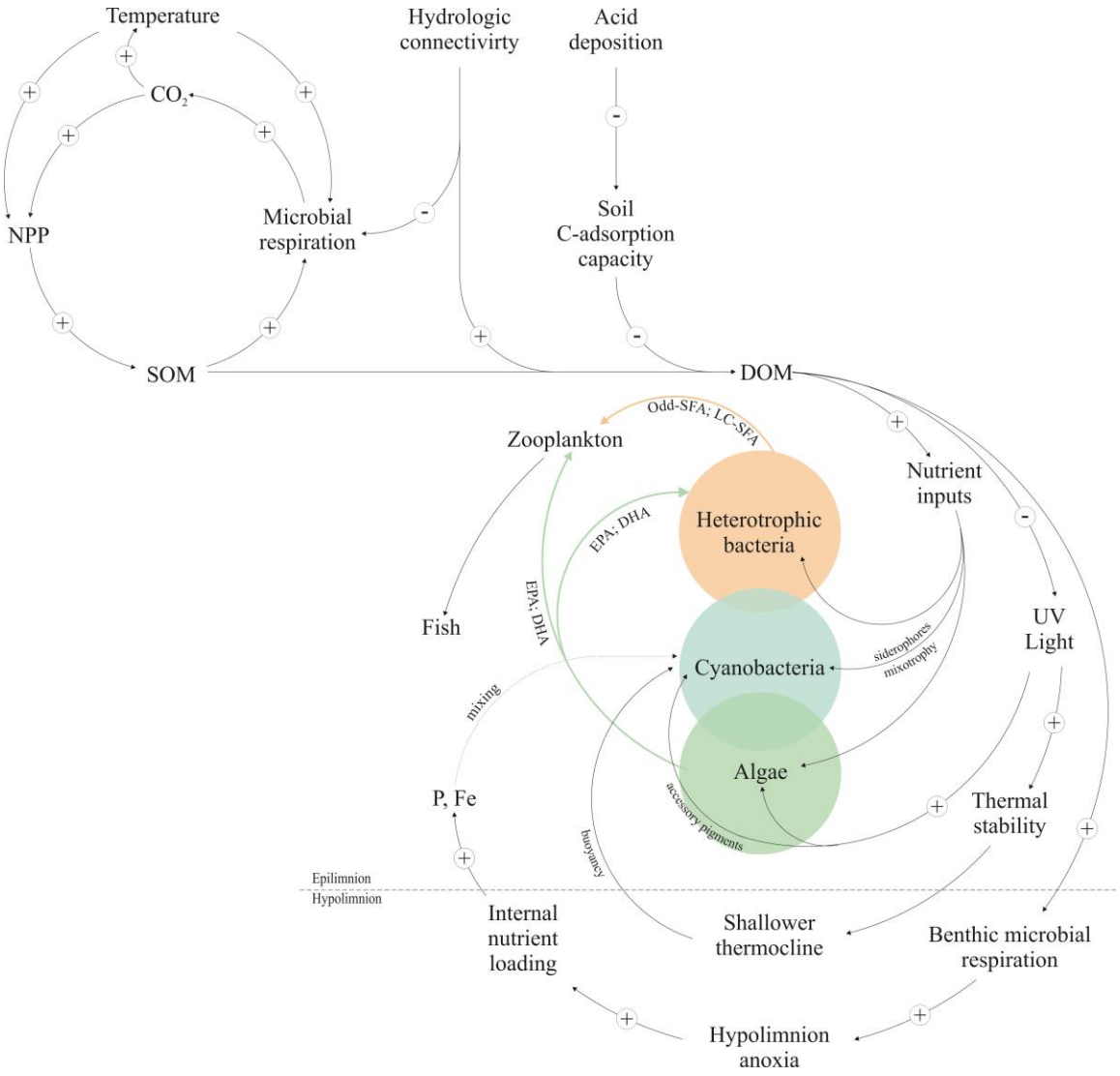

Figure 5.1. Causes and consequences of soil carbon mobilization (top left) and lake brownification (bottom) in northern forested landscape

In addition, this study establishes the baseline to understand how brownification-driven changes in aquatic ecosystem functions can result in losses of aquatic ecosystem services that pose risks to human health. Brownification-driven increases in cyanobacteria can restrict the capacity of lakes to provide cultural and recreational services (Tuvendal & Elmqvist, 2011). In addition, brownification-linked changes colour, odour, taste and toxin content of water may lead to its restriction as a drinking water source (Weyhenmeyer et al., 2014). Finally, declines in the production and transfer of

essential fatty acids and potentially reducing lake secondary production could pose a health risk for communities that rely in these aquatic resources.

5.3 Recommendations for Future Research

The drivers and consequences of brownification are diverse and often result of interacting factors and synergistic effects. Future studies should consider all C fractions (including inorganic C and particulate organic C), precursors of DOC, and the transformations they undergo during hydrologic connectivity and disconnectivity periods. These studies should be performed in comparable catchments in different biomes (e.g., tropical vs. temperate vs. boreal); especially since boreal systems store large amounts of C, susceptible to destabilization after permafrost melt. These studies should also focus on the characterization of the composition, molecular structure, reactivity, and degradability of DOM compounds. Elemental and molecular composition analyses based on chromatography and spectrometry provide detailed information of DOM composition (Creed et al., 2015). Besides these, optical measurements (based on absorbance and fluorescent spectroscopy, like the ones used in this study), provide inexpensive and reliable information on DOM source and characteristics (Cory & McKnight, 2005). DOM characterization should be combined with its ecological role on physical and chemical conditions of lakes and its effects on aquatic organisms. Large spatial scale studies are necessary to cover the diversity of DOM composition and catchment and lake characteristics. Complementary laboratory and mesocosm experiments would help explore the effect of single or synergistic drivers (e.g., temperature, light, nutrient access) on phytoplankton communities and cyanobacteria dominance. Cyanobacteria are a diverse group of organisms with different adaptations to brownification.

A limitation of this study was the focus on indirect measurements of phytoplankton and cyanobacteria biomass (i.e., chlorophyll-*a* and phycocyanin concentrations) to assess the implications of brownification on lake primary production. Future studies should expand the focus on cellular pigment concentrations - that may vary as a function of environmental conditions and community composition (e.g., phytoplankton may increase pigment production in order to maintain photosynthetic performance when light availability is low; Kasprzak et al., 2008; Boyer et al., 2009) – to

other indicators of phytoplankton biomass (e.g., cell counts or biovolume in bulk phytoplankton samples and species or genera specific) should be considered to improve our understanding on shifts in phytoplankton community composition.

A potential of this study was to examine the consequences of brownification for the nutritional quality of aquatic food webs through the analysis of the availability and transfer of essential fatty acids. Future studies should include stable isotope analysis to identify the main dietary sources (allochthonous vs. autochthonous; Solomon et al., 2015; Karlsson et al., 2009) for consumers, and the C transfer pathways within aquatic systems. In addition to essential fatty acids, the quality of aquatic food webs can be assessed through other nutritional compounds (e.g., essential amino acids; Taipale et al., 2017), or pollutants (e.g., mercury; French et al., 2014). Future trophic studies should incorporate these analysis to better identify C sources and pathways and, when including top predators (i.e., piscivorous fish) they provide a better understanding of the role of bottom-up controls on overall ecosystem productivity.

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Appendices

Appendix A: Soil carbon characteristics in c38

Table A1. Average organic carbon pool (g C m^{-2}) in soils collected from each of the topographic positions. FFL = freshly fallen litter; LFH = forest floor layer composed of > 1 year old litter, fibric and hemic layers; and A horizon in uplands to a maximum depth of 5 cm or the top 10 cm of peat in wetlands. Data include average values from triplicate samples.

Transect	Position	Point	FFL	LFH	Ah	Ae	Total
T15	Wetland	IW	135.50	3216.80	4441.14		7793.50
		OW	88.39	3413.14	4442.20		7943.73
	Ecotone	TS	165.94	978.48	5491.84	3078.52	9714.78
		FS	164.36	902.54	2734.92	2470.47	6272.28
	Upland	BS	134.83	792.18	1578.32	1565.47	4070.80
		CR	141.68	1267.10	2552.18	3070.18	7031.12
		SH	119.43	791.71	1707.36	3720.28	6338.79
T35	Wetland	IW	89.38	3237.61	4042.65		7369.64
		OW	127.24	2805.07	4402.57		7334.87
	Ecotone	TS	158.28	1492.36	6471.74	1136.43	9258.82
		FS	185.85	1737.39	5844.32	2295.86	10063.42
	Upland	BS	112.03	1843.56	3868.59	1891.94	7716.11
		CR	124.21	1084.42	3257.07	2284.59	6750.29
		SH	108.24	2200.36	3289.66	1768.13	7366.39

Table A2. Average SOC sorption capacity (mol m^{-2}) in soils collected from each of the topographic positions. FFL = freshly fallen litter; LFH = forest floor layer composed of > 1 year old litter, fibric and hemic layers; and A horizon in uplands to a maximum depth of 5 cm or the top 10 cm of peat in wetlands. Data include average values from triplicate samples.

Transect	Position	Point	FFL	LFH	Ah	Ae
T15	Wetland	IW		1.30	2.59	
		OW		1.50	5.80	
	Ecotone	TS		0.09	12.35	18.15
		FS		0.17	5.72	17.24
	Upland	BS		0.08	3.50	5.57
		CR		0.13	4.57	10.25
		SH		0.14	3.01	14.93
T35	Wetland	IW		0.31	0.72	
		OW		0.27	0.63	
	Ecotone	TS		0.07	30.13	3.41
		FS		0.05	7.128	6.92
	Upland	BS		0.08	1.21	4.61
		CR		0.12	6.20	6.13
		SH		0.24	4.68	3.92

Appendix B: Carbon Budget in c38

Although the present study focuses on the export of SOC from c38 to the aquatic and terrestrial fates, the forest ecosystem in TLW, where c38 is located, has been extensively studied for over 30 years. We rely on previous work in done in c38 in order to give a broader view of C cycling in the catchment. The annual net C fixation (Net Primary Production; NPP) in the TLW was estimated at 5580 kg C ha^{-1} y^{-1} by Morrison et al. (1993) by measuring C content in all three components (foliage, fruit, branches, stem bark, stem wood, and roots) and extrapolated through logarithmic regressions to calculate above- and below-ground biomass per hectare. Soil C inputs were derived from the abovementioned values, assuming a 100% leaf turnover rate and 48% C content in leaf phytomass (Morrison, 1990); and a 50% root turnover rate for belowground biomass.

SOC pools were measured along hillslope transects in the FFL, LFH, Ah, and Ae horizons across different topographic features as indicated in the Methods section (see results in Table A1). SOC pools were found to be relatively stable at long term, with no significant differences in SOC content between 1981 and 1996 (Morrison and Foster, 2001). In addition to SOC content, soil C sorption capacity was estimated by measuring Fe and Al oxyhydroxide concentrations in the same positions and horizons (Table S1). These compounds can bind to soil DOC, increasing C residence time in soil and limiting microbes' accessibility to it (Kalbitz et al., 2000). While the present study does not include soil DOC quantity and quality in different positions due to discontinuity in the data, a previous study identified its spatial variability in the catchment and its role in microbial respiration. Creed et al. (2013) identified the role of topography at regulating the concentration and sorption of DOC to soil and defined the ecotone as CO_2 hotspot, as this landscape position acts as a trap of DOC.

Despite not including DOC in our analysis, the model used to estimate atmospheric C export as a function of soil temperature and moisture, SOC concentration, and soil sorption capacity, developed for the same study area and time period has been proven to accurately predict CO2 efflux (adj. $r^2 = 0.72$, $p < 0.05$). This model was applied using daily values of soil temperature and moisture and constant SOC and sorption capacity to calculate daily atmospheric C export. During the winter period, soil

temperature and moisture were considered constant, however empirical measurements from early spring reported low soil CO2 efflux values.

The modelled atmospheric C export was considered to represent both soil autotrophic and heterotrophic respiration. The ratio of soil autotrophic to heterotrophic respiration was considered to be 50:50 (Hanson et al., 2000) and kept constant throughout the seasons in order to estimate a catchment C balance (Figure A1).

Aquatic C export was measured at the catchment outlet (see Methods section) and missing data was interpolated using regression models with discharge as the explanatory variable.

In the C balance (Figure A1), Gross Primary Production (GPP) has been calculated as the sum of NPP, aboveground autotrophic respiration, and soil autotrophic respiration. Therefore, the Net Ecosystem Exchange (NEE) can be calculated as NPP – Soil Heterotrophic Respiration, which ranges between 3719 and 4581 kg C ha^{-1} y^{-1} (or 66.6 % to 82.1 % of the annual NPP) in 2007 and 2008 respectively. However, if accounting only SOC inputs (assumed constant at 3085 kg C ha^{-1} y^{-1}) and outputs (annual soil heterotrophic respiration), SOC export exceeds the inputs between 120.5 % and 148.5 % in 2007 and 2008 respectively (see section 2.4).

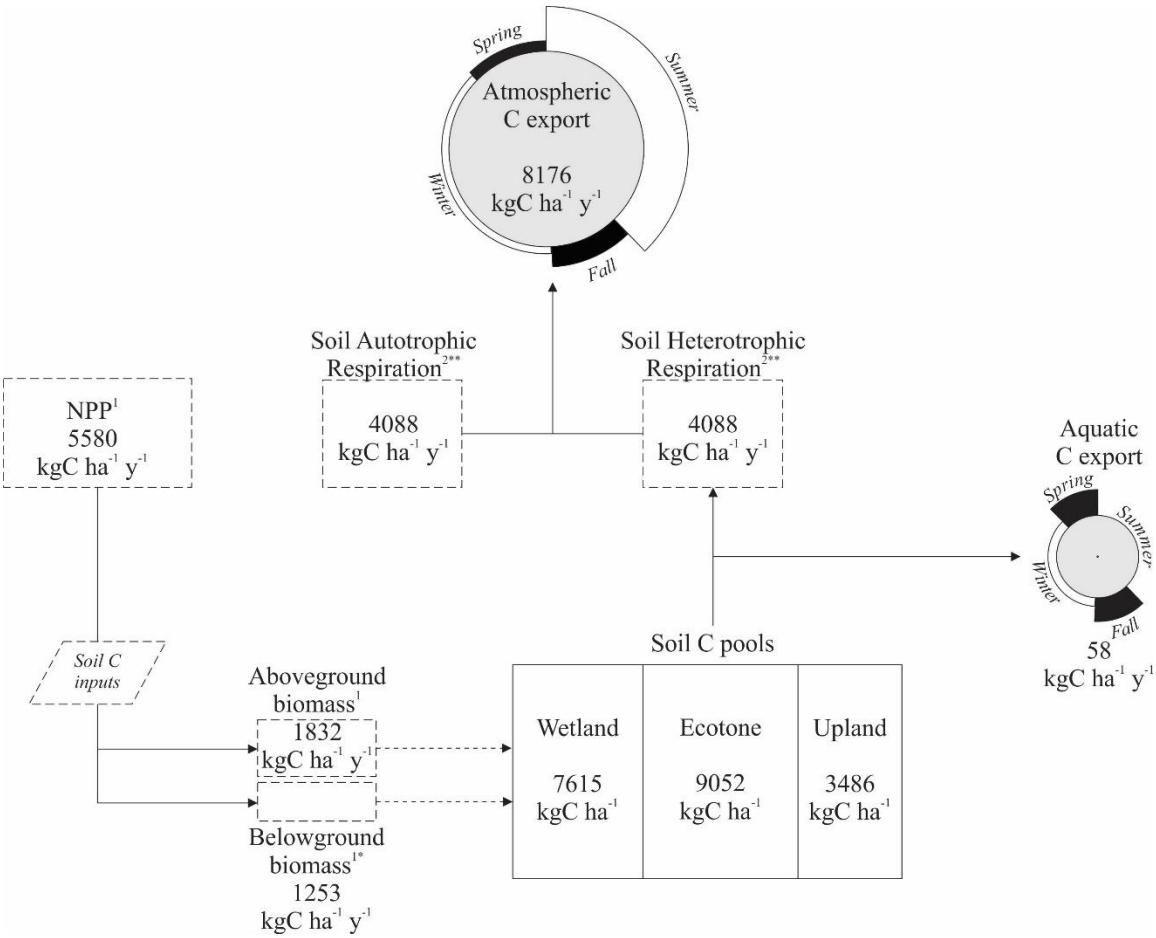

Figure A1. C cycling in c38. Boxes represent C pools and circles represent catchment C export; their sizes are proportional to the C pool/flux. Dashed boxes represent values taken from literature (1Morrison et al., 1993, 2Hanson et al., 2000) or inferred from literature and results from the study *Fine root turnover rate estimated to be 50% as per Majdi et al., 2005; **calculated from the reported atmospheric C export and the ratio of autotrophic to heterotrophic respiration estimated to be 50:50 as per Hanson et al., 2000). C export is divided into two hydrologic disconnectivity (summer and winter; white) and two connectivity (fall storms and spring snowmelt; black) periods. The proportion of each season within the circle represents its duration (averaged for the five years in the 2006-2010 period) and its thickness represents the median daily C export (in Table 2.1).

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Publications:

Senar O.E., Creed, I.F., Strandberg, U., Arts, M.T. (submitted). Brownification-driven changes in polyunsaturated fatty acid production and transfer in temperate oligotrophic lakes. *Freshwater Biology.*

Senar, O.E., Creed, I.F. (in preparation). Catchment-fed cyanobacteria blooms in temperate lakes.

Lamothe, K.A., Dong, H., **Senar, O.E.**, Teichert, S., Creed, I.F., Kreutzweiser, D.P., Schmiegelow, F.K.A., Venier, L. (2018). Demand for non-provisioning ecosystem services as a driver of change in the Canadian boreal zone. *Environmental Reviews*.

Senar, O. E., Webster, K. L., & Creed, I. F. (2018). Catchment-scale shifts in the magnitude and partitioning of carbon export in response to changing hydrologic connectivity in a northern hardwood forest. *Journal of Geophysical Research: Biogeosciences*, 123(8): 2337-2352.

Creed, I. F., Bergström, A. K., Trick, C. G., et al. (2018). Global change-driven effects on dissolved organic matter composition: Implications for food webs of northern lakes. Global change biology, 24(8), 3692-3714.

Conferences & Workshops:

Senar OE*, Creed IF. 2018. Is brownification of lakes triggering cyanobacteria blooms in northern lakes?. Interdisciplinary Freshwater Harmful Algal Bloom Workshop. April 15-17, Toronto, ON.

Senar OE*, Creed IF. 2017. Catchment-fed cyanobacterial blooms in brownified temperate lakes. American Geophysical Association Fall Meeting, December 11-15, New Orleans, LA.

Senar OE*, Creed IF, Webster K. Climate driven changes in hydrological connectivity influences the magnitude and fate of carbon export in temperate forests. American Water Resources Association Spring Specialty Conference, April 30-May 3, Snowbird, UT. **Invited.**

Senar OE*, Freeman E*, Creed IF, Trick CG. 2017. Brownification of surface waters promotes cyanobacteria in oligotrophic lakes. Canadian Conference for Fisheries Research - Society of Canadian Limnologists Meeting. January 5-8, Montreal, QC.

Senar OE*, Creed IF, Kidd K, Trick C. 2016. Dissolved organic matter promotes cyanobacterial dominance in oligotrophic lakes. International Association of Great Lakes Research Meeting, June 6-10, Guelph, ON.

Senar OE*, Creed IF. 2015. Dissolved organic matter promotes cyanobacterial dominance in oligotrophic lakes. North American Lake Management Society. 35th International Symposium. November 17-20, Saratoga Springs, NY.