MICROBIAL AND PHOTOCHEMICAL CYCLING OF DISSOLVED ORGANIC MATTER IN BOREAL HEADWATER STREAMS

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Abstract

Dissolved organic matter (DOM) is a key global energy source and carbon reservoir that links terrestrial and aquatic biogeochemical cycling. Allochthonous organic matter is abundant in boreal headwater streams, and environmental changes such as variation in nutrient availability and changes to watershed landscape composition have great potential for altering the DOM source, its composition and cycling. This dissertation focuses on two of the main drivers of aquatic carbon and nutrient cycling: the photochemical and the microbial processing of DOM in boreal headwater streams; specifically (i) how the photochemical lability of DOM varies between reaches within headwater streams, among headwater streams and an associated large river reach, (ii) how stream biofilm mineralization may be regulated by watershed organic matter source and composition, increased labile carbon, nitrogen, and phosphorus availability, and (iii) whether algal carbon sources are important to supporting stream biofilms and if such sources influence the use of allochthonous DOM in nutrient-enriched streams. The results suggest changes in landscape and nutrient availability have the potential to alter the photochemical and biogeochemical cycling of DOM. DOM photolability was increased upstream relative to downstream and the river DOM. This may be due to differences in DOM source and composition, and suggests losses in photolabile DOM downstream and in the lower reaches of the watershed. The phototransformation of DOM into low molecular weight compounds and nutrients such as ammonium is likely relevant to the carbon and nutrient cycling in boreal watersheds. Results here further suggest that boreal stream biofilm mineralization of DOM is regulated by watershed DOM source and composition. Labile carbon sources, such as algal inputs, may also play an important role in regulating DOM mineralization and the processing of nutrients by these biofilms. In nutrient-impacted streams, where primary production is high relative to nutrient-poor streams, biofilms may be stimulated to incorporate algal carbon sources. Yet in the boreal streams studied here, added labile carbon rarely enhanced the mineralization of extant stream DOM suggesting autotrophic-heterotrophic interactions represent a more important priming effect relative to changing DOM source in boreal streams.

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"*A dissertation is not really about creating new knowledge, that's the end result for the world to read when the dream is over, but it's about asking yourself a question that has never been asked before and then being brave enough to go down the rabbit hole to find the answer. The rabbit hole is a scary and exciting journey with a cast of characters that are mythic… Just remember… you have to wake up at some point, Alice… there is no point of going down the rabbit hole if no one can read your story*" (Symonette, 2010).

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DOC and absorption were measured as the percent change in DOC concentration and absorption at a wavelength of 350 nm, respectively. Values are provided as the average ± standard deviation (n = 5).

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Chapter 2: Mineralization of dissolved organic matter by heterotrophic stream biofilm communities in a large boreal catchment

Figure 2.1: The delineation of the Humber River catchment both as situated in western Newfoundland, Canada (above), and shown in part in a larger scale (below) to illustrate the region and location of the study stream sites within the catchments of Crooked Feeder (CF), Big Falls Brook (BFB), Pynn's Brook (PB) and Gillams Brook (GB). A minimum of two study sites, representing upstream (triangle) and downstream (circle), were chosen for each catchment. 2-10

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treatment are provided by lower case letters (a, b, c), where only those treatments designated with different letters were found to be significantly different.

Figure 2.4: Biofilm respiration rates ($R_{Biofilm}$) provided as the ratio of treatment to control for the ambient dissolved organic matter (DOM) addition experiments along a transect of Pynn's Brook (PB-1, PB-2, and PB-3) and at two sites at Crooked Feeder (CF-1 and CF-2) in summer 2010. Substrate additions included bog DOM, pond DOM, glucose, ammonium nitrate (N) and potassium phosphate (P). Values correspond to the mean of five replicates ± one standard deviation. Results of the generalized linear model tests of the $R_{Biofilm}$ associated with each treatment are provided with asterisks as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ relative to the control (no additions). Results from the Tukey's Honestly Significant Difference tests for the absolute $R_{Biofilm}$ associated with each treatment are provided by lower case letters (a, b, c), where only those treatments designated with different letters were found to be significantly different. 2-34

Chapter 3: The impact of nutrient enrichment on dissolved organic carbon mineralization by stream biofilms

Figure 3.1: Ratio of treatment to control biofilm respiration rates ($R_{Biofilm}$) for substrate addition experiments at Leary's Brook (LB), Waterford River (WR), and Virginia River (VR). Substrate additions included glucose (C), ammonium nitrate (N), and potassium phosphate (P). Values correspond to the mean of five experimental replicates ± one standard deviation. Results of the generalized linear model tests of absolute $R_{Biofilm}$ associated with each treatment are provided with asterisks as * $p < 0.05$ relative to the control (no additions). Results from the Tukey's Honestly Significant Difference tests for the absolute $R_{Biofilm}$ associated with each treatment are provided by lower case letters (a, b, c), where only those treatments designated with different letters were found to be significantly different. 3-22

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Chapter 4: Carbon cycling and autotrophic-heterotrophic linkages within boreal stream biofilm communities

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Appendices

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Figure A2.2: Respiration rates normalized to initial dissolved organic carbon (DOC) concentration* at Crooked Feeder (CF), Pynn's Brook (PB), and Gillams Brook (GB) in autumn 2009, grouped in this figure based upon similar incubation temperature among sites (4.0 – 5.5°C). Values correspond to five replicates ± one standard deviation. A2-5

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Co-authorship statement

The research questions and experimental plans for each chapter were designed and outlined by me in collaboration with my supervisor, Dr. Susan Ziegler. The research proposal, including experimental plans, was approved by the examiners Dr. Mark Wilson, Dr. Penny Morrill, and Dr. Andrew Lang, Memorial University of Newfoundland. Samples for each experiment were collected by me, unless stated otherwise below. I conducted each experiment, unless stated otherwise below, and received support/assistance during sample collection and during the experiments and analyses as stated for each chapter individually below. Data analysis, including rate calculations and statistical analysis, and data interpretation was done by me, with my supervisor's guidance during regular meetings/discussions. Each chapter was written by me followed by reviews of multiple drafts by my supervisor, Dr. Susan Ziegler.

Chapter 1: Variation in the photochemical lability of dissolved organic matter in a large boreal watershed

The fall 2007 samples, which I used for preliminary experiments, were taken by Dr. Susan Ziegler, David Lyon, and Claire Moore-Gibbons. All other samples were collected by me with assistance from Alexandra Rouillard, Dean Strickland, Jennifer Bonnell, and Nicholas Capps. The June 2008 experiments were accomplished and samples were analyzed by Michael Hamilton as part of his honors thesis under my supervision. Michael Hamilton is included as an author on this paper because of this contribution. Rate calculations, statistical analyses, and data interpretation were done together during regular meetings of myself and my supervisor. Nicholas Capps, Aiden Dunne, Christopher Earle, Susan Hannan, Tiffany Lam, David Lyon, Claire Moore-Gibbons, and Alison Pye supported me in the laboratory in ways that range from introduction to laboratory protocols to specific instrument analyses. Dr. Geert Van Biesen, Nicole DeBond, and three anonymous reviewers provided helpful comments to this chapter/paper.

Chapter 2: Mineralization of dissolved organic matter by heterotrophic stream biofilm communities in a large boreal catchment

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Chapter 3: The impact of nutrient enrichment on dissolved organic carbon mineralization by stream biofilms

Jennifer Bonnell, Nicholas Capps, and Tiffany Lam assisted me during sample collection and substrate addition experiments at various times throughout the study. Aiden Dunne, Julia Ferguson, Susan Hannan, Tiffany Lam, Alison Pye, Dr. Geert Van Biesen, and Jamie Warren supported me during laboratory analyses and cleaning procedures. David Lyon accomplished all experiments in the Ozark streams and kindly provided the unpublished raw data set.

Chapter 4: Carbon cycling and autotrophic-heterotrophic linkages within boreal stream biofilm communities

Jennifer Bonnell, Dr. Chad and Gretchen Lane, and Wyn Rolls assisted me with sample collection during the mesocosm experiments in 2009. Aiden Dunne, Christopher Earle, Tiffany Lam, and Aaron Mcbreairty assisted me in the field in 2010 during the overnight experiments (approximately 30 hours) including sample collection. Aiden Dunne, Julia Ferguson, Susan Hannan, Tiffany Lam, Lukas Kohl, Alison Pye, and Jamie Warren assisted/supported me during the sample analyses and cleaning procedures in the laboratory. Dr. Chad Lane, Dr. Geert Van Biesen, and Natalie Szponar instructed me during the fatty acid extractions and analyses.

Thesis introduction and overview: Microbial and photochemical cycling of dissolved organic matter in boreal headwater streams

I.1 The importance of dissolved organic matter in boreal watershed biogeochemical cycling

Carbon is distributed globally among four reservoirs: the atmosphere, oceans, biosphere and lithosphere (Schlesinger, 1991). The two largest active reservoirs are soil organic matter (1.4 to 1.5 x 10^{18} g C) (Post et al., 1982; Eswaran et al., 1993; Schlesinger et al., 1995) and terrestrial plant tissue (0.2 to 0.9 x 10^{18} g C) (Bianchi, 2011). Freshwater ecosystems receive approximately 1.9 x 10^{15} g C per year from the terrestrial landscape (Cole et al., 2007). A large portion of that amount (0.4 to 0.9 x 10^{15} g C yr^{-1}) is transported as both particulate (POC) and dissolved organic carbon (DOC) to the oceans (Schlesinger and Melack, 1981; Cole et al., 2007), whereas a relatively small portion (0.2 x 10^{15} g C yr^{-1}) is buried in aquatic sediments, or returned to the atmosphere via gas exchange (at least 0.8 x 10^{15} g C yr^{-1}) (Cole et al., 2007). Twenty to sixty percent of the global soil carbon pool (90 to 500 x 10^{15} g C) is stored in high-latitude environments (tundra and taiga regions) including boreal forests and peatlands (Post et al., 1982; Gorham, 1991; Aitkenhead and McDowell, 2000; Hobbie et al., 2000; Gower et al., 2001). Boreal ecosystems cover approximately 14.5 % of the land surface (Gower et al., 2001) and consist of a large number of water bodies including wetlands (i.e. bogs, peatlands, swamps, marshes), ponds, lakes, and streams. These boreal freshwater ecosystems play an important role in the storage, transformation, and transport of terrestrial carbon and can significantly affect regional and global carbon balances.

In boreal surface waters, terrestrially derived or allochthonous dissolved organic matter (DOM; <0.45 μm) typically represents a major reservoir of carbon and nutrients,

providing an important source of energy for biogeochemical cycles in these ecosystems. Allochthonous DOM inputs into small streams, rivers, and lakes dominate processes such as microbial utilization (Tranvik, 1988) and photochemical mineralization of DOM (Bertilsson and Tranvik, 2000), as well as ecosystem metabolism enhanced by photochemical transformations of DOM (Wetzel et al., 1995; Harrison and Smith, 2009) (Fig. I.1), and contribute to the exchange of CO_2 between the aquatic environment and the atmosphere (del Giorgio et al., 1997). Understanding the cycling and fate of this key carbon reservoir may be particularly important in boreal headwater streams given their allochthonous nature.

Headwater streams are key components of watershed biogeochemical cycling and integrate the terrestrial and aquatic processing of elements such as carbon, nitrogen, and phosphorus. These low-order (1-3) streams (Vannote et al., 1980) connect upstream watersheds and tributaries with downstream rivers and lakes (Freeman et al., 2007; Wipfli et al., 2007). Headwater streams often receive the largest direct input of allochthonous DOM from wetland and forest soils (Fisher and Likens, 1973; Vannote et al., 1980), and provide important habitats for microbial, plant, and animal life (Meyer and Wallace, 2001; Meyer et al., 2007). Due to the high active microbial surface area (typically occurring as biofilms) relative to water volume (Peterson et al., 2001), headwater streams exhibit strong effects on the microbial uptake and transformation of carbon and nutrients (Peterson et al., 2001; Mulholland, 2004; Fellows et al., 2006; Alexander et al., 2007), and high CO_2 efflux (Teodoru et al., 2009). The linkage of biogeochemical and hydrological processes in headwater streams regulates the quantity, chemical composition, timing, and distances of organic matter transported to downstream ecosystems, and affects water quality, microbial metabolism, community composition, and food webs downstream (Gomi et al., 2002; Agren et al., 2007;

Alexander et al., 2007). Dissolved organic matter composition, concentration, and flux are further controlled by watershed characteristics including land cover (Hopkinson et al., 1998; Quideau et al., 2001), soil properties (Schiff et al., 1997; Quideau et al., 2001), precipitation and discharge regime (Wallis et al., 1981; Schiff et al., 1997; Schiff et al., 1998; Moore et al., 2003; Agren et al., 2007), temperature (Schiff et al., 1997; Leenheer and Croue, 2003), pH (Buffam et al., 2007; Keller et al., 2008), and the presence of microbial and photochemical degradation processes in both the soil and aquatic environments (McDowell and Likens, 1988; Brooks et al., 1999; Koehler et al., 2002; Fontaine et al., 2007).

Microorganisms play an important role in the biogeochemical cycling of DOM and have a major influence on ecosystem function in aquatic ecosystems, particularly primary production and metabolism, as well as uptake and transfer of carbon and nutrients to higher trophic levels (Kirchman, 1994; Sterner and Hessen, 1994; Wetzel, 1995; Hall and Meyer, 1998; Kaunzinger and Morin, 1998). However, not all of this material is available to the microbial community. The bioavailability of DOM varies with its source, biochemical composition, as well as carbon and nutrient concentrations (Amon and Benner, 1996; Findlay, 2003), and variations in which have major effects on aquatic food webs, trophic state, and nutrient retention or release (Findlay and Sinsabaugh, 2003).

Microbial cycling of DOM often varies between allochthonous versus autochthonous sources. Allochthonous DOM is comprised of low molecular weight (LMW) organic compounds (non-humic substrates) including carbohydrates (10 – 20 % of total DOM), amino acids (<2 % of total DOM), and carboxylic acids (3 – 11 % of total DOM) (McDowell and Likens, 1988; Volk et al., 1997; Fischer et al., 2007). These LMW substrates are labile and often removed rapidly and preferentially by microorganisms

(Kaplan and Bott, 1983). The major portion (>75 % of total DOM) of allochthonous DOM, however, is comprised of yellow to black coloured, high molecular weight (HMW) compounds including humic and fulvic acids (humic substrates) of vascular plant origin (Wallis et al., 1981; Volk et al., 1997; McKnight et al., 2001; Aitkenhead-Peterson et al., 2003; McKnight et al., 2003). Fulvic acids are rich in aliphatic and carboxyl groups, whereas humic acids exhibit a greater proportion of aromatic content, such as lignin-derived phenols (Burges et al., 1964; Wilson et al., 1987; Gaffney et al., 1996). These compounds are often considered as slow turnover substrates (Geller, 1983; Leff and Meyer, 1991; Findlay and Sinsabaugh, 1999), but they are fundamental to the energy flow and metabolism within aquatic ecosystems (Amon and Benner, 1996; Volk et al., 1997) due to their susceptibility to photochemical (Hernes and Benner, 2003) and microbial degradation processes (Tulonen et al., 1992). In contrast, organic matter derived from autochthonous sources, including macrophyte and algal production, consists mainly of rapid turnover LMW compounds (Bertilson and Jones, 2003). In boreal streams, allochthonous DOM dominates and serves as the major energy source for heterotrophic microorganisms, while autochthonous sources are usually minor due to reduced light availability and lower photosynthetic activity compared to lower DOM systems more typical of temperate regions (Tranvik, 1988, 1989; Jansson et al., 2000; Jonsson et al., 2001; Agren et al., 2008).

The composition and biogeochemical cycling of DOM in aquatic environments can further be affected by photochemical transformations of DOM (Bertilsson et al., 1999; Tranvik et al., 1999; Obernosterer and Benner, 2004). The chromophoric fraction of DOM absorbs light and its spectral and molecular properties can be altered by solar radiation (Moran et al., 2000; Osburn et al., 2001; Piccini et al., 2009). Dissolved organic matter can be photochemically transformed into LMW compounds, carbon gases such

as CO and CO_2, and nitrogen- and phosphorus-rich compounds (Francko and Heath, 1982; Wetzel et al., 1995; Bushaw et al., 1996; Moran and Zepp, 1997; Tranvik et al., 1999; Obernosterer and Benner, 2004). These photochemically altered substrates may increase the biological availability of carbon, nitrogen, and phosphorus, and may stimulate its use by microorganisms (Lindell et al., 1995; Bushaw et al., 1996; Moran and Zepp, 1997; Gao and Zepp, 1998; Tranvik et al., 1999; Bertilsson and Tranvik, 2000; Vähätalo et al., 2003; Piccini et al., 2009). This may be particularly important in boreal environments, where most of the DOM is allochthonous, characterized by a high concentration of chromophoric compounds, and thus known to be highly photoreactive (Sulzberger and Durisch-Kaiser, 2009). Investigating the variation in DOM photolability, or its relative susceptibility to be photomineralized, from headwaters to large downstream reaches of a watershed will provide information about this important feature of DOM composition and the potential variation in the role of photochemical processes in downstream ecosystems.

Headwater streams are the most vulnerable to small-scale differences (Meyer et al., 2007) and represent the first locations in watersheds that are affected by landscape changes (Peterson et al., 2001; Richardson et al., 2005; Alexander et al., 2007; Freeman et al., 2007; Arango and Tank, 2008). Describing the factors that may regulate the biogeochemical processing of elements including carbon, nitrogen, and phosphorus in headwater streams across the boreal landscape is therefore critical for determining how these watersheds will respond and adapt to environmental change.

Environmental change such as shifts in climate, hydrology, and vegetation as well as the influence of anthropogenic activities such as urbanization, agriculture, and forestry practices, have major effects on the biogeochemical cycling of carbon and nutrients in boreal ecosystems (Pastor and Post, 1988; Vitousek et al., 1997; Schindler

et al., 2004; Kortelainen et al., 2006a; Zepp et al., 2011; Olefeldt and Roulet, 2012). More specifically, shifts in organic matter composition and variability in the amount of organic matter produced and exported from terrestrial sources into aquatic ecosystems are likely to be consequences of changes in temperature and in the frequency and intensity of precipitation (Pastor and Post, 1988; Clair and Ehrman, 1996; Peterson et al., 2002; Schindler et al., 2004; Tranvik et al., 2009). Wetlands (i.e. peatlands), in particular, are extremely sensitive to environmental change. Due to the storage of significant amounts of soil carbon (Post et al., 1982; Gorham, 1991), the production and transport of organic carbon (Moore et al., 1998), and the formation and atmospheric exchange of greenhouse gases such as CO_2 and CH_4 (Bridgham et al., 1995; Moore et al., 1998), wetlands represent important components of boreal ecosystems and the global carbon cycle. Changes in climate and hydrology, increases in atmospheric CO_2 levels, as well as alterations of vegetation and soil organic matter can largely affect ecosystem production and biogeochemical cycling in these landscapes. For example, variations in the quantity and quality of accumulated plant biomass, altered carbon and nutrient regimes, and, consequently, microbial activity can affect ecosystem diversity and function (Gorham, 1991; Bridgham et al., 1995; Moore et al., 1998). Since wetlands are important suppliers of organic matter to streams (Hemond, 1990), these climate-induced changes may influence organic matter export to downstream water bodies and modify stream DOM and nutrient fluxes (Moore et al., 1998; Dinsmore et al., 2010; Olefeldt and Roulet, 2012). Further, the role of boreal watersheds as sinks or sources of CO_2 to the atmosphere will likely be modified by changes in hydrology, DOM quantity and composition (Sobek et al., 2003; Algesten et al., 2004; Kortelainen et al., 2006b; Teodoru et al., 2009).

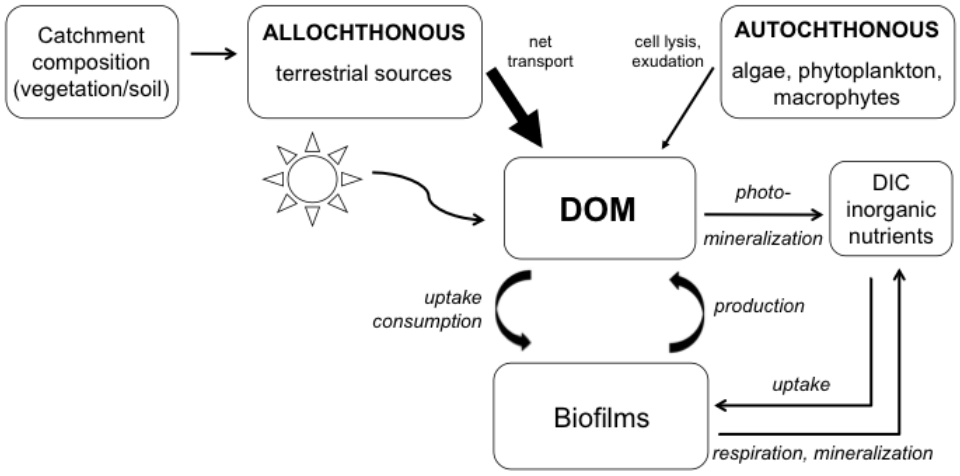

Figure I.1: Factors regulating the cycling of dissolved organic matter (DOM) in aquatic ecosystems. DOM represents a key global energy and nutrient source and carbon reservoir that integrates terrestrial and aquatic biogeochemical cycling. Allochthonous DOM is derived from soils, plant litter, and animal detritus, and enters aquatic ecosystems from the surrounding terrestrial environment mainly via hydrological processes. DOM input also originates from autochthonous sources including macrophyte and algal production. In boreal watersheds, the contribution of DOM from terrestrial sources is large compared to the amount produced within the aquatic ecosystem, and varies in its composition. Differences in the composition and role of DOM affect the photochemical lability and reactivity of DOM, as well as microbial processes including the degradation of DOM and nutrient cycling and transport.

I.2 The role of stream biofilm communities in aquatic carbon and nutrient cycling

Microorganisms play an important role in the carbon and nutrient dynamics in stream ecosystems (Fig. I.2), and influence biogeochemical processes such as organic matter decomposition and nitrogen fixation (Judd et al., 2006). Most naturally occurring microorganisms in streams appear as heterogenic biofilms attached to surfaces such as rocks and macrophytes. In addition to a variety of heterotrophic bacteria and algal species, biofilms consist of fungi, protozoa, and Archaea embedded in a thin mucilage layer. All these microorganisms excrete extracellular polymeric substances such as polysaccharides, proteins, lipids, and nucleic acids, and form a gel-like matrix enriched with dissolved nutrients (Fischer, 2003; Battin et al., 2008). The microbial community

composition in these biofilms, as well as microbial growth and activity is regulated by a variety of factors including temperature (Bertilsson et al., 1999; Tank et al., 2010), light availability (Romani and Sabater, 1999), the presence of grazers (Sabater et al., 2002), source and composition of DOM (Kaplan and Bott, 1983, 1989; Judd et al., 2006; Fellman et al., 2009), its bioavailability (Benner, 2003), and inorganic nutrients (Rier and Stevenson, 2002; Tank and Dodds, 2003). These factors and their influence on organic matter cycling can vary both seasonally (Roberts et al., 2007; Fellman et al., 2009; Hoellein et al., 2009; Hoellein et al., 2010) and spatially (Hoellein et al., 2007).

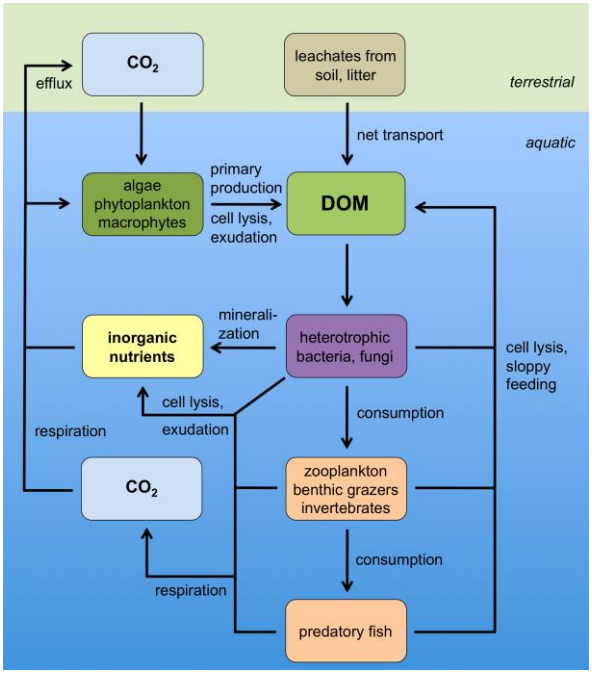

Figure I.2: Biological cycling of dissolved organic matter (DOM) in aquatic ecosystems and relationship between autotrophic and heterotrophic microorganisms and higher trophic levels. DOM, originating either from terrestrial sources such as leachates from plant litter and soils, or from autochthonous sources including photosynthesis by algae, phytoplankton, and macrophytes, are consumed and decomposed by heterotrophic bacteria and fungi, and transferred to higher trophic levels, therefore serving as the dominant carbon and energy source within aquatic ecosystems. During the consumption of algae, phytoplankton, and bacteria by zooplankton, considerable amounts of carbon and nutrients are released into the water column in a process known as “sloppy feeding”, which has a positive effect on microorganisms and small grazers. The breakdown of large particles allows soluble organic substances to dissolve out of the compact matrix which may become available as food source.

Biofilm communities in headwater streams constitute a major part of the stream ecosystem and participate largely in stream primary production, nutrient cycling, and energy flow to higher trophic levels (Peterson et al., 2001; Hoellein et al., 2010). Specifically, heterotrophic bacteria in biofilms play an important role in aquatic carbon and nutrient cycling through the uptake of organic matter from the water column, and its storage and transformation (Battin et al., 1999; Fischer, 2003; Romani et al., 2004). High molecular weight organic matter enters the biofilms by diffusion, both as allochthonous and autochthonous DOM, where it is cleaved by extracellular enzymes, enabling the uptake and utilization of the DOM molecules (Fischer, 2003). This allows bacteria in biofilms to have a significant impact on the composition and quantity of DOM in natural waters since they also retain considerable amounts of DOM (Battin et al., 1999). However, aquatic carbon and nutrient cycling is not solely connected to biofilms through the uptake of DOM by bacteria. It also occurs within the biofilms as algae exude additional internal DOM (Fischer, 2003). The utilization of algal material by microorganisms is influenced by the proximity of algae and the heterotrophic community in biofilms, as the photosynthetic activity of algae and the accumulation of algal products increases the amount of substrates available to bacteria (Cole et al., 1982; Kaplan and Bott, 1989; Romani et al., 2004). This stimulates bacterial productivity and, at the same time, most of the inorganic carbon requirements of algae can be provided by bacterial respiration (Espeland and Wetzel, 2001).

Due to their role as primary drivers of carbon and nutrient cycling in headwater streams, biofilm communities are critical to our understanding of ecosystem processes and environmental change in boreal landscapes, and must be better understood in order to elucidate carbon and nutrient dynamics. This is particularly true in boreal headwater streams, given the elevated allochthonous DOM and potential for environmental change

in these landscapes. Investigating the effects of changes in carbon and nutrient concentrations on the biogeochemical cycling of organic matter and carbon flow between the autotrophic and heterotrophic groups of headwater stream microbial biofilm communities is critical for predicting the impact of environmental change on watershed carbon and nutrient cycling including transport to downstream water bodies.

Modified carbon and nutrient concentrations, e.g. as a result of anthropogenic activities or changes in land cover due to climate change, can alter substrate sources and availability for biofilm communities and subsequently affect the biogeochemical cycling of DOM and downstream transport (Bernhardt and Likens, 2002; Ziegler and Brisco, 2004). Such changes can break the link between heterotrophic and autotrophic communities and affect processes such as respiration, as well as DOC and nutrient release and retention (Bernhardt and Likens, 2002; Scott et al., 2008; Lyon and Ziegler, 2009; Ziegler and Lyon, 2010). Specifically, elevated nutrient concentrations can increase biofilm biomass and chlorophyll content, and may affect the release of labile, autochthonous DOM (Guasch et al., 1995; Dodds et al., 2002). Increased availability of labile carbon sources, such as algal exudates, may stimulate the mineralization of more complex allochthonous stream DOM (Guenet et al., 2010) – a process analogous to "priming effects" observed in the rhizosphere, where the microbial decomposition of soil organic matter is "naturally" stimulated by photosynthetic products (Kuzyakov and Cheng, 2001; Kuzyakov et al., 2001). The causes, mechanisms, and presence of priming effects in aquatic ecosystems remain poorly understood but may be particularly important in boreal streams under nutrient enrichment conditions given their allochthonous nature. Such priming effects may contribute to CO_2 emissions from allochthonous organic matter in aquatic ecosystems (Guenet et al., 2010; Bianchi,

2011), further highlighting the importance of investigating the impact of altered carbon and nutrient regimes on the microbial cycling of organic matter in these ecosystems.

If concentrations of labile allochthonous DOM are high, thus providing an alternative carbon source for heterotrophic communities, bacterial growth and nutrient uptake may become decoupled from algal production (Findlay et al., 1991; Bernhardt and Likens, 2002). In fact, allochthonous DOM may become bioavailable for microorganisms given its susceptibility to photochemical degradation (Koehler et al., 2012). Increased loadings of allochthonous organic matter, however, can also decrease light availability for photosynthetic microorganisms and may lead to lower productivity and decreased uptake of CO_2 (Ask et al., 2012). Furthermore, increased carbon and inorganic nutrient contributions can result in enhanced microbial metabolism and the release of organic nutrients (Findlay and Sinsabaugh, 2003), which may consequently lead to stoichiometric imbalances of nutrient ratios (C:N:P) between microorganisms and their energy source (Frost et al., 2002; Cross et al., 2005; Frost et al., 2005). This influences not only microbial growth and reproduction, but also higher organisms in the food web, such as benthic grazers, that depend on microorganisms as their food source, affecting the diversity, abundance, and distribution of invertebrates and leading to altered food web structure and function (Gulis and Suberkropp, 2003; Hessen et al., 2004; Frost et al., 2005). Enhanced microbial activity in headwater streams may also influence downstream nutrient transport (Findlay and Sinsabaugh, 2003) and can alter the resilience and biological stability of the system (Wetzel, 2001). For example, higher rates of biofilm metabolism as a consequence of elevated labile carbon and nutrient concentrations can lead to greater rates of nutrient uptake, which may decrease the downstream nutrient transport (Bernhardt and Likens, 2002; Fellows et al., 2006). Conversely, increased levels of nutrients can also prevent or lower the insufficient supply

of nutrients in biofilms and may lead to reduced nutrient-specific uptake rates and increased export of nutrients to downstream ecosystems (Dodds et al., 2004).

I.3 Research focus

This dissertation is focussed on the impact of environmental change on photochemical and microbial processes, two of the main drivers of aquatic carbon and nutrient cycling in boreal watersheds. This research has resulted in four chapters entitled:

1) *Variation in the photochemical lability of dissolved organic matter in a large boreal watershed.*
2) *Mineralization of dissolved organic matter by heterotrophic stream biofilm communities in a large boreal catchment.*
3) *The impact of nutrient enrichment on dissolved organic carbon mineralization by stream biofilms.*
4) *Carbon cycling and autotrophic-heterotrophic linkages within boreal stream biofilm communities.*

The first chapter of this dissertation describes the potential for photochemical transformations in regulating the fate and cycling of DOM in boreal watersheds. In this work, I have specifically looked at whether DOM photochemical lability and the isotopic composition ($\delta^{13}C$) of photomineralized DOM varied with season, reach within headwater streams, and among headwater streams, and a large river reach in the Humber River Basin, western Newfoundland, Canada. The purpose of these investigations was to assess whether the photochemically labile fraction of DOM represents a significant proportion of DOM in terms of carbon, nitrogen, and phosphorus in a boreal watershed,

and whether photochemical transformations may represent a process responsible for losses of DOM as CO_2 in this watershed.

The research addressed in the second and third chapter of this dissertation provides information on how the mineralization of DOM by boreal stream biofilms may be regulated by watershed organic matter source and composition, and increased labile carbon, nitrogen, and phosphorus availability. Stream biofilm respiration and substrate addition experiments were conducted at contrasting sites in the Humber River Basin, chosen to provide a range in catchment composition including vegetation but in the absence of significant human influences and associated nutrient enrichment. Additionally, the same experiments were conducted in nutrient-impacted streams on the Northeast Avalon Peninsula, Newfoundland, Canada, to gain a better understanding of the impact of long-term nutrient enrichment on heterotrophic microbial mineralization of DOM in boreal headwater streams.

The research addressed in the fourth chapter contributes to our understanding of carbon cycling in boreal headwater streams by investigating the importance of autotrophic-heterotrophic linkages in nutrient-impacted urban streams on the Northeast Avalon Peninsula. I specifically addressed whether (a) algal carbon sources are important to supporting epilithic biofilms in nutrient-enriched boreal streams, and whether (b) algal or labile carbon sources may influence the use of allochthonous DOM. These are important factors to test given that terrestrial DOM sources typically dominate boreal headwater streams except under nutrient enrichment conditions when autochthonous DOM sources may have the potential to stimulate terrestrial DOM use by stream biofilm communities and thereby increase losses of this carbon reservoir to the atmosphere via increased respiration.

The research addressed in this dissertation will contribute to the understanding of the fate of terrestrial carbon in the boreal landscape and help provide information relevant to the prediction of how the cycling of carbon and nutrients may change in the future. Results from this research will form a baseline understanding relevant to key issues connected with regional land-use planning, economic development and sustainability in boreal watersheds like the Humber River Basin and implications for climate change in boreal environments around the world.

I.4 References

Agren, A., Buffam, I., Jansson, M., and Laudon, H., 2007: Importance of seasonality and small streams for the landscape regulation of dissolved organic carbon export. *Journal of Geophysical Research-Biogeosciences,* 112.

Agren, A., Berggren, M., Laudon, H., and Jansson, M., 2008: Terrestrial export of highly bioavailable carbon from small boreal catchments in spring floods. *Freshwater Biology,* 53: 964-972.

Aitkenhead, J. A. and McDowell, W. H., 2000: Soil C : N ratio as a predictor of annual riverine DOC flux at local and global scales. *Global Biogeochemical Cycles,* 14: 127-138.

Aitkenhead-Peterson, J. A., Dowell, W. H., and Neff, J. C., 2003: Sources, Production, and Regulation of Allochthonous Dissolved Organic Matter Inputs to Surface Waters. *In: Aquatic Ecosystems: Interactivity of Dissolved Organic Matter; Editors: Findlay, S. E. & Sinsabaugh, R. L.; Elsevier Science*: 25-70.

Alexander, R. B., Boyer, E. W., Smith, R. A., Schwarz, G. E., and Moore, R. B., 2007: The role of headwater streams in downstream water quality. *Journal of the American Water Resources Association,* 43: 41-59.

Algesten, G., Sobek, S., Bergstrom, A. K., Agren, A., Tranvik, L. J., and Jansson, M., 2004: Role of lakes for organic carbon cycling in the boreal zone. *Global Change Biology,* 10: 141-147.

Amon, R. M. W. and Benner, R., 1996: Bacterial utilization of different size classes of dissolved organic matter. *Limnology and Oceanography,* 41: 41-51.

Arango, C. P. and Tank, J. L., 2008: Land use influences the spatiotemporal controls on nitrification and denitrification in headwater streams. *Journal of the North American Benthological Society,* 27: 90-107.

Ask, J., Karlsson, J., and Jansson, M., 2012: Net ecosystem production in clear-water and brown-water lakes. *Global Biogeochem. Cycles,* 26: GB1017.

Battin, T. J., Butturini, A., and Sabater, F., 1999: Immobilization and metabolism of dissolved organic carbon by natural sediment biofilms in a Mediterranean and temperate stream. *Aquatic Microbial Ecology,* 19: 297-305.

Battin, T. J., Kaplan, L. A., Findlay, S., Hopkinson, C. S., Marti, E., Packman, A. I., Newbold, J. D., and Sabater, F., 2008: Biophysical controls on organic carbon fluxes in fluvial networks. *Nature Geoscience,* 1: 95-100.

Benner, R., 2003: Molecular Indicators of the Bioavailability of Dissolved Organic Matter. *In: Aquatic Ecosystems: Interactivity of Dissolved Organic Matter; Elsevier Science*: 121-137.

Bernhardt, E. S. and Likens, G. E., 2002: Dissolved organic carbon enrichment alters nitrogen dynamics in a forest stream. *Ecology,* 83: 1689-1700.

Bertilson, S. and Jones, J. B., 2003: Supply of Dissolved Organic Matter to Aquatic Ecosystems: Autochthonous Sources. *In: Aquatic Ecosystems: Interactivity of Dissolved Organic Matter; Editors: Findlay, S. E. & Sinsabaugh, R. L.; Elsevier Science*: 3-24.

Bertilsson, S., Stepanauskas, R., Cuadros-Hansson, R., Graneli, W., Wikner, J., and Tranvik, L., 1999: Photochemically induced changes in bioavailable carbon and nitrogen pools in a boreal watershed. *Aquatic Microbial Ecology,* 19: 47-56.

Bertilsson, S. and Tranvik, L. J., 2000: Photochemical transformation of dissolved organic matter in lakes. *Limnology and Oceanography,* 45: 753-762.

Bianchi, T. S., 2011: The role of terrestrially derived organic carbon in the coastal ocean: A changing paradigm and the priming effect. *Proceedings of the National Academy of Sciences of the United States of America,* 108: 19473-19481.

Bridgham, S. D., Johnston, C. A., Pastor, J., and Updegraff, K., 1995: Potential Feedbacks of Northern Wetlands on Climate-Change - an Outline of an Approach to Predict Climate-Change Impact. *Bioscience,* 45: 262-274.

Brooks, P. D., McKnight, D. M., and Bencala, K. E., 1999: The relationship between soil heterotrophic activity, soil dissolved organic carbon (DOC) leachate, and catchment-scale DOC export in headwater catchments. *Water Resources Research,* 35: 1895-1902.

Buffam, I., Laudon, H., Temnerud, J., Morth, C. M., and Bishop, K., 2007: Landscape-scale variability of acidity and dissolved organic carbon during spring flood in a boreal stream network. *Journal of Geophysical Research-Biogeosciences,* 112: G01022.

Burges, N. A., Hurst, H. M., and Walkden, B., 1964: The phenolic constituents of humic acid and their relation to the lignin of the plant cover. *Geochimica Et Cosmochimica Acta,* 28: 1547-1554.

Bushaw, K. L., Zepp, R. G., Tarr, M. A., SchulzJander, D., Bourbonniere, R. A., Hodson, R. E., Miller, W. L., Bronk, D. A., and Moran, M. A., 1996: Photochemical release of biologically available nitrogen from aquatic dissolved organic matter. *Nature,* 381: 404-407.

Clair, T. A. and Ehrman, J. M., 1996: Variations in discharge and dissolved organic carbon and nitrogen export from terrestrial basins with changes in climate: A neural network approach. *Limnology and Oceanography,* 41: 921-927.

Cole, J. J., Likens, G. E., and Strayer, D. L., 1982: Photosynthetically Produced Dissolved Organic-Carbon - an Important Carbon Source for Planktonic Bacteria. *Limnology and Oceanography,* 27: 1080-1090.

Cole, J. J., Prairie, Y. T., Caraco, N. F., McDowell, W. H., Tranvik, L. J., Striegl, R. G., Duarte, C. M., Kortelainen, P., Downing, J. A., Middelburg, J. J., and Melack, J., 2007: Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems,* 10: 171-184.

Cross, W. F., Benstead, J. P., Frost, P. C., and Thomas, S. A., 2005: Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. *Freshwater Biology,* 50: 1895-1912.

del Giorgio, P. A., Cole, J. J., and Cimbleris, A., 1997: Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature,* 385: 148-151.

Dinsmore, K. J., Billett, M. F., Skiba, U. M., Rees, R. M., Drewer, J., and Helfter, C., 2010: Role of the aquatic pathway in the carbon and greenhouse gas budgets of a peatland catchment. *Global Change Biology,* 16: 2750-2762.

Dodds, W. K., Smith, V. H., and Lohman, K., 2002: Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. *Canadian Journal of Fisheries and Aquatic Sciences,* 59: 865-874.

Dodds, W. K., Marti, E., Tank, J. L., Pontius, J., Hamilton, S. K., Grimm, N. B., Bowden, W. B., McDowell, W. H., Peterson, B. J., Valett, H. M., Webster, J. R., and Gregory, S., 2004: Carbon and nitrogen stoichiometry and nitrogen cycling rates in streams. *Oecologia,* 140: 458-467.

Espeland, E. M. and Wetzel, R. G., 2001: Effects of photosynthesis on bacterial phosphatase production in biofilms. *Microbial Ecology,* 42: 328-337.

Eswaran, H., Vandenberg, E., and Reich, P., 1993: Organic-Carbon in Soils of the World. *Soil Science Society of America Journal,* 57: 192-194.

Fellman, J. B., Hood, E., Edwards, R. T., and Jones, J. B., 2009: Uptake of Allochthonous Dissolved Organic Matter from Soil and Salmon in Coastal Temperate Rainforest Streams. *Ecosystems,* 12: 747-759.

Fellows, C. S., Valett, H. M., Dahm, C. N., Mulholland, P. J., and Thomas, S. A., 2006: Coupling nutrient uptake and energy flow in headwater streams. *Ecosystems,* 9: 788-804.

Findlay, S., Pace, M. L., Lints, D., Cole, J. J., Caraco, N. F., and Peierls, B., 1991: Weak-Coupling of Bacterial and Algal Production in a Heterotrophic Ecosystem - the Hudson River Estuary. *Limnology and Oceanography,* 36: 268-278.

Findlay, S., 2003: Bacterial Response to Variation in Dissolved Organic Matter. *In: Aquatic Ecosystems: Interactivity of Dissolved Organic Matter; Editors: Findlay, S. E. & Sinsabaugh, R. L.; Elsevier Science*: 363-379.

Findlay, S. E. G. and Sinsabaugh, R. L., 1999: Unravelling the sources and bioavailability of dissolved organic matter in lotic aquatic ecosystems. *Marine and Freshwater Research,* 50: 781-790.

Findlay, S. E. G. and Sinsabaugh, R. L., 2003: *Aquatic Ecosystems. Interactivity of Dissolved Organic Matter*: Academic Press. An imprint of Elsevier Science., 512 pp.

Fischer, H., 2003: The Role of Biofilms in the Uptake and Transformation of Dissolved Organic Matter. *In: Aquatic Ecosystems: Interactivity of Dissolved Organic Matter; Editors: Findlay, S. E. & Sinsabaugh, R. L.; Elsevier Science*: 285-313.

Fischer, H., Meyer, A., Fischer, K., and Kuzyakov, Y., 2007: Carbohydrate and amino acid composition of dissolved organic matter leached from soil. *Soil Biology & Biochemistry,* 39: 2926-2935.

Fisher, S. G. and Likens, G. E., 1973: Energy Flow in Bear Brook, New Hampshire: An Integrative Approach to Stream Ecosystem Metabolism. *Ecological Monographs,* 43: 421-439.

Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B., and Rumpel, C., 2007: Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature,* 450: 277-U210.

Francko, D. A. and Heath, R. T., 1982: UV-sensitive complex phosphorus: association with dissolved humic material and iron in a bog lake. *Journal Name: Limnol. Oceanogr.; (United States); Journal Volume: 27:3*: 564-569.

Freeman, M. C., Pringle, C. M., and Jackson, C. R., 2007: Hydrologic connectivity and the contribution of stream headwaters to ecological integrity at regional scales. *Journal of the American Water Resources Association,* 43: 5-14.

Frost, P. C., Stelzer, R. S., Lamberti, G. A., and Elser, J. J., 2002: Ecological stoichiometry of trophic interactions in the benthos: understanding the role of C : N : P ratios in lentic and lotic habitats. *Journal of the North American Benthological Society,* 21: 515-528.

Frost, P. C., Cross, W. F., and Benstead, J. P., 2005: Ecological stoichiometry in freshwater benthic ecosystems: an introduction. *Freshwater Biology,* 50: 1781-1785.

Gaffney, J. S., Marley, N. A., and Clark, S. B., 1996: Humic and fulvic acids and organic colloidal materials in the environment. *Humic and Fulvic Acids,* 651: 2-16.

Gao, H. Z. and Zepp, R. G., 1998: Factors influencing photoreactions of dissolved organic matter in a coastal river of the southeastern United States. *Environmental Science & Technology,* 32: 2940-2946.

Geller, A., 1983: Degradability of Dissolved Organic Lake Water Compounds in Cultures of Natural Bacterial Communities. *Archiv Fur Hydrobiologie,* 99: 60-79.

Gomi, T., Sidle, R. C., and Richardson, J. S., 2002: Understanding processes and downstream linkages of headwater systems. *Bioscience,* 52: 905-916.

Gorham, E., 1991: Northern Peatlands - Role in the Carbon-Cycle and Probable Responses to Climatic Warming. *Ecological Applications,* 1: 182-195.

Gower, S. T., Krankina, O., Olson, R. J., Apps, M., Linder, S., and Wang, C., 2001: Net primary production and carbon allocation patterns of boreal forest ecosystems. *Ecological Applications,* 11: 1395-1411.

Guasch, H., Marti, E., and Sabater, S., 1995: Nutrient enrichment effects on biofilm metabolism in a Mediterranean stream. *Freshwater Biology,* 33: 373-383.

Guenet, B., Danger, M., Abbadie, L., and Lacroix, G., 2010: Priming effect: bridging the gap between terrestrial and aquatic ecology. *Ecology,* 91: 2850-2861.

Gulis, V. and Suberkropp, K., 2003: Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology,* 48: 123-134.

Hall, R. O. and Meyer, J. L., 1998: The trophic significance of bacteria in a detritus-based stream food web. *Ecology,* 79: 1995-2012.

Harrison, J. W. and Smith, R. E. H., 2009: Effects of ultraviolet radiation on the productivity and composition of freshwater phytoplankton communities. *Photochemical & Photobiological Sciences,* 8: 1218-1232.

Hemond, H. F., 1990: Wetlands as the Source of Dissolved Organic-Carbon to Surface Waters. *Organic Acids in Aquatic Ecosystems,* 48: 301-313.

Hernes, P. J. and Benner, R., 2003: Photochemical and microbial degradation of dissolved lignin phenols: Implications for the fate of terrigenous dissolved organic matter in marine environments. *Journal of Geophysical Research - Oceans,* 108: 3291.

Hessen, D. O., Agren, G. I., Anderson, T. R., Elser, J. J., and De Ruiter, P. C., 2004: Carbon, sequestration in ecosystems: The role of stoichiometry. *Ecology,* 85: 1179-1192.

Hobbie, S. E., Schimel, J. P., Trumbore, S. E., and Randerson, J. R., 2000: Controls over carbon storage and turnover in high-latitude soils. *Global Change Biology,* 6: 196-210.

Hoellein, T. J., Tank, J. L., Rosi-Marshall, E. J., Entrekin, S. A., and Lamberti, G. A., 2007: Controls on spatial and temporal variation of nutrient uptake in three Michigan headwater streams. *Limnology and Oceanography,* 52: 1964-1977.

Hoellein, T. J., Tank, J. L., Rosi-Marshall, E. J., and Entrekin, S. A., 2009: Temporal variation in substratum-specific rates of N uptake and metabolism and their contribution at the stream-reach scale. *Journal of the North American Benthological Society,* 28: 305-318.

Hoellein, T. J., Tank, J. L., Kelly, J. J., and Rosi-Marshall, E. J., 2010: Seasonal variation in nutrient limitation of microbial biofilms colonizing organic and inorganic substrata in streams. *Hydrobiologia,* 649: 331-345.

Hopkinson, C. S., Buffam, I., Hobbie, J., Vallino, J., Perdue, M., Eversmeyer, B., Prahl, F., Covert, J., Hodson, R., Moran, M. A., Smith, E., Baross, J., Crump, B., Findlay, S., and Foreman, K., 1998: Terrestrial inputs of organic matter to coastal ecosystems: An intercomparison of chemical characteristics and bioavailability. *Biogeochemistry,* 43: 211-234.

Jansson, M., Bergstrom, A. K., Blomqvist, P., and Drakare, S., 2000: Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. *Ecology,* 81: 3250-3255.

Jonsson, A., Meili, M., Bergstrom, A. K., and Jansson, M., 2001: Whole-lake mineralization of allochthonous and autochthonous organic carbon in a large humic lake (Ortrasket, N. Sweden). *Limnology and Oceanography,* 46: 1691-1700.

Judd, K. E., Crump, B. C., and Kling, G. W., 2006: Variation in dissolved organic matter controls bacterial production and community composition. *Ecology,* 87: 2068-2079.

Kaplan, L. A. and Bott, T. L., 1983: Microbial Heterotrophic Utilization of Dissolved Organic Matter in a Piedmont Stream. *Freshwater Biology,* 13: 363-377.

Kaplan, L. A. and Bott, T. L., 1989: Diel Fluctuations in Bacterial-Activity on Streambed Substrata during Vernal Algal Blooms - Effects of Temperature, Water Chemistry, and Habitat. *Limnology and Oceanography,* 34: 718-733.

Kaunzinger, C. M. K. and Morin, P. J., 1998: Productivity controls food-chain properties in microbial communities. *Nature,* 395: 495-497.

Keller, W., Paterson, A. M., Somers, K. M., Dillon, P. J., Heneberry, J., and Ford, A., 2008: Relationships between dissolved organic carbon concentrations, weather, and acidification in small Boreal Shield lakes. *Canadian Journal of Fisheries and Aquatic Sciences,* 65: 786-795.

Kirchman, D. L., 1994: The Uptake of Inorganic Nutrients by Heterotrophic Bacteria. *Microbial Ecology,* 28: 255-271.

Koehler, B., von Wachenfeldt, E., Kothawala, D., and Tranvik, L. J., 2012: Reactivity continuum of dissolved organic carbon decomposition in lake water. *J. Geophys. Res.,* 117: G01024.

Koehler, S., Buffam, I., Jonsson, A., and Bishop, K., 2002: Photochemical and microbial processing of stream and soil water dissolved organic matter in a boreal forested catchment in northern Sweden. *Aquatic Sciences - Research Across Boundaries,* 64: 269-281.

Kortelainen, P., Mattsson, T., Finer, L., Ahtiainen, M., Saukkonen, S., and Sallantaus, T., 2006a: Controls on the export of C, N, P and Fe from undisturbed boreal catchments, Finland. *Aquatic Sciences,* 68: 453-468.

Kortelainen, P., Rantakari, M., Huttunen, J. T., Mattsson, T., Alm, J., Juutinen, S., Larmola, T., Silvola, J., and Martikainen, P. J., 2006b: Sediment respiration and lake trophic state are important predictors of large CO2 evasion from small boreal lakes. *Global Change Biology,* 12: 1554-1567.

Kuzyakov, Y. and Cheng, W., 2001: Photosynthesis controls of rhizosphere respiration and organic matter decomposition. *Soil Biology & Biochemistry,* 33: 1915-1925.

Kuzyakov, Y., Ehrensberger, H., and Stahr, K., 2001: Carbon partitioning and below-ground translocation by Lolium perenne. *Soil Biology & Biochemistry,* 33: 61-74.

Leenheer, J. A. and Croue, J. P., 2003: Characterizing aquatic dissolved organic matter. *Environmental Science & Technology,* 37: 18-26.

Leff, L. G. and Meyer, J. L., 1991: Biological Availability of Dissolved Organic Carbon Along the Ogeechee River. *Limnology and Oceanography,* 36: 315-323.

Lindau, C. W., Delaune, R. D., Patrick, W. H., and Lambremont, E. N., 1989: Assessment of Stable Nitrogen Isotopes in Fingerprinting Surface Water Inorganic Nitrogen Sources. *Water Air and Soil Pollution,* 48: 489-496.

Lindell, M. J., Graneli, W., and Tranvik, L. J., 1995: Enhanced Bacterial-Growth in Response to Photochemical Transformation of Dissolved Organic-Matter. *Limnology and Oceanography,* 40: 195-199.

Lyon, D. R. and Ziegler, S. E., 2009: Carbon cycling within epilithic biofilm communities across a nutrient gradient of headwater streams. *Limnology and Oceanography,* 54: 439-449.

McDowell, W. H. and Likens, G. E., 1988: Origin, Composition, and Flux of Dissolved Organic Carbon in the Hubbard Brook Valley. *Ecological Monographs,* 58: 177-195.

McKnight, D. M., Boyer, E. W., Westerhoff, P. K., Doran, P. T., Kulbe, T., and Andersen, D. T., 2001: Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography,* 46: 38-48.

McKnight, D. M., Hood, E., and Klapper, L., 2003: Trace Moieties of Dissolved Organic Material in Natural Waters. *In: Aquatic Ecosystems: Interactivity of Dissolved Organic Matter; Editors: Findlay, S. E. & Sinsabaugh, R. L.; Elsevier Science*: 71-96.

Meyer, J. L. and Wallace, J. B., 2001: Lost linkages and lotic ecology: rediscovering small streams. *Ecology: Achievement and Challenge*: 295-317.

Meyer, J. L., Strayer, D. L., Wallace, J. B., Eggert, S. L., Helfman, G. S., and Leonard, N. E., 2007: The contribution of headwater streams to biodiversity in river networks. *Journal of the American Water Resources Association,* 43: 86-103.

Moore, T. R., Roulet, N. T., and Waddington, J. M., 1998: Uncertainty in predicting the effect of climatic change on the carbon cycling of Canadian peatlands. *Climatic Change,* 40: 229-245.

Moore, T. R., Matos, L., and Roulet, N. T., 2003: Dynamics and chemistry of dissolved organic carbon in precambrian shield catchments and an impounded wetland. *Canadian Journal of Fisheries and Aquatic Sciences,* 60: 612-623.

Moran, M. A. and Zepp, R. G., 1997: Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. *Limnology and Oceanography,* 42: 1307-1316.

Moran, M. A., Sheldon, W. M., and Zepp, R. G., 2000: Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter. *Limnology and Oceanography,* 45: 1254-1264.

Mulholland, P. J., 2004: The importance of in-stream uptake for regulating stream concentrations and outputs of N and P from a forested watershed: evidence from long-term chemistry records for Walker Branch Watershed. *Biogeochemistry,* 70: 403-426.

Obernosterer, I. and Benner, R., 2004: Competition between biological and photochemical processes in the mineralization of dissolved organic carbon. *Limnology and Oceanography,* 49: 117-124.

Olefeldt, D. and Roulet, N. T., 2012: Effects of permafrost and hydrology on the composition and transport of dissolved organic carbon in a subarctic peatland complex. *J. Geophys. Res.,* 117: G01005.

Osburn, C. L., Morris, D. P., Thorn, K. A., and Moeller, R. E., 2001: Chemical and optical changes in freshwater dissolved organic matter exposed to solar radiation. *Biogeochemistry,* 54: 251-278.

Pastor, J. and Post, W. M., 1988: Response of Northern Forests to CO2-Induced Climate Change. *Nature,* 334: 55-58.

Peterson, B. J., Wollheim, W. M., Mulholland, P. J., Webster, J. R., Meyer, J. L., Tank, J. L., Marti, E., Bowden, W. B., Valett, H. M., Hershey, A. E., McDowell, W. H., Dodds, W. K., Hamilton, S. K., Gregory, S., and Morrall, D. D., 2001: Control of nitrogen export from watersheds by headwater streams. *Science,* 292: 86-90.

Peterson, B. J., Holmes, R. M., McClelland, J. W., Vorosmarty, C. J., Lammers, R. B., Shiklomanov, A. I., Shiklomanov, I. A., and Rahmstorf, S., 2002: Increasing river discharge to the Arctic Ocean. *Science,* 298: 2171-2173.

Piccini, C., Conde, D., Pernthaler, J., and Sommaruga, R., 2009: Alteration of chromophoric dissolved organic matter by solar UV radiation causes rapid changes in bacterial community composition. *Photochemical & Photobiological Sciences,* 8: 1321-1328.

Post, W. M., Emanuel, W. R., Zinke, P. J., and Stangenberger, A. G., 1982: Soil Carbon Pools and World Life Zones. *Nature,* 298: 156-159.

Quideau, S. A., Chadwick, O. A., Benesi, A., Graham, R. C., and Anderson, M. A., 2001: A direct link between forest vegetation type and soil organic matter composition. *Geoderma,* 104: 41-60.

Richardson, J. S., Naiman, R. J., Swanson, F. J., and Hibbs, D. E., 2005: Riparian communities associated with Pacific Northwest headwater streams: Assemblages, processes, and uniqueness. *Journal of the American Water Resources Association,* 41: 935-947.

Rier, S. T. and Stevenson, R. J., 2002: Effects of light, dissolved organic carbon, and inorganic nutrients on the relationship between algae and heterotrophic bacteria in stream periphyton. *Hydrobiologia,* 489: 179-184.

Roberts, B. J., Mulholland, P. J., and Hill, W. R., 2007: Multiple scales of temporal variability in ecosystem metabolism rates: Results from 2 years of continuous monitoring in a forested headwater stream. *Ecosystems,* 10: 588-606.

Romani, A. M. and Sabater, S., 1999: Effect of primary producers on the heterotrophic metabolism of a stream biofilm. *Freshwater Biology,* 41: 729-736.

Romani, A. M., Guasch, H., Munoz, I., Ruana, J., Vilalta, E., Schwartz, T., Emtiazi, F., and Sabater, S., 2004: Biofilm structure and function and possible implications for riverine DOC dynamics. *Microbial Ecology,* 47: 316-328.

Sabater, S., Guasch, H., Romani, A., and Munoz, I., 2002: The effect of biological factors on the efficiency of river biofilms in improving water quality. *Hydrobiologia,* 469: 149-156.

Schiff, S., Aravena, R., Mewhinney, E., Elgood, R., Warner, B., Dillon, P., and Trumbore, S., 1998: Precambrian shield wetlands: Hydrologic control of the sources and export of dissolved organic matter. *Climatic Change,* 40: 167-188.

Schiff, S. L., Aravena, R., Trumbore, S. E., Hinton, M. J., Elgood, R., and Dillon, P. J., 1997: Export of DOC from forested catchments on the Precambrian Shield of Central Ontario: Clues from C-13 and C-14. *Biogeochemistry,* 36: 43-65.

Schindler, D. W., Jefferson Curtis, P. J., Bayley, S. E., Parker, B. R., Beaty, K. G., and Stainton, M. P., 2004: Climate-induced changes in the dissolved organic carbon budgets of boreal lakes. *Biogeochemistry,* 36: 9-28.

Schlesinger, W. H. and Melack, J. M., 1981: Transport of organic carbon in the world's rivers. *Tellus,* 33: 172-187.

Schlesinger, W. H., 1991: Biogeochemistry - an Analysis of Global Change. *Science,* 253: 686-687.

Schlesinger, W. H., Woodwell, G. M., and Mackenzie, F. T., 1995: Soil respiration and changes in soil carbon stocks. *Oxford University Press, New York*: 159-168.

Scott, J. T., Back, J. A., Taylo, J. M., and King, R. S., 2008: Does nutrient enrichment decouple algal-bacterial production in periphyton? *Journal of the North American Benthological Society,* 27: 332-344.

Sobek, S., Algesten, G., Bergstrom, A. K., Jansson, M., and Tranvik, L. J., 2003: The catchment and climate regulation of pCO(2) in boreal lakes. *Global Change Biology,* 9: 630-641.

Sterner, R. W. and Hessen, D. O., 1994: Algal Nutrient Limitation and the Nutrition of Aquatic Herbivores. *Annual Review of Ecology and Systematics,* 25: 1-29.

Sulzberger, B. and Durisch-Kaiser, E., 2009: Chemical characterization of dissolved organic matter (DOM): A prerequisite for understanding UV-induced changes of DOM absorption properties and bioavailability. *Aquatic Sciences,* 71: 104-126.

Tank, J. L. and Dodds, W. K., 2003: Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. *Freshwater Biology,* 48: 1031-1049.

Tank, J. L., Rosi-Marshall, E. J., Griffiths, N. A., Entrekin, S. A., and Stephen, M. L., 2010: A review of allochthonous organic matter dynamics and metabolism in streams. *Journal of the North American Benthological Society,* 29: 118-146.

Teodoru, C. R., del Giorgio, P. A., Prairie, Y. T., and Camire, M., 2009: Patterns in pCO2 in boreal streams and rivers of northern Quebec, Canada. *Global Biogeochemical Cycles,* 23: GB2012.

Tranvik, L. J., 1988: Availability of Dissolved Organic Carbon for Planktonic Bacteria in Oligotrophic Lakes of Differing Humic Content. *Microbial Ecology,* 16: 311-322.

Tranvik, L. J., 1989: Bacterioplankton Growth, Grazing Mortality and Quantitative Relationship to Primary Production in a Humic and a Clearwater Lake. *Journal of Plankton Research,* 11: 985-1000.

Tranvik, L. J., Olofsson, H., and Bertilsson, S., 1999: Photochemical Effects on Bacterial Degradation of Dissolved Organic Matter in Lake Water. *Microbial Biosystems: New Frontiers. Proceedings of the 8th International Symposium on Microbial Ecology. Atlantic Canada Society for Microbial Ecology, Halifax, Canada, 1999.*

Tranvik, L. J., Downing, J. A., Cotner, J. B., Loiselle, S. A., Striegl, R. G., Ballatore, T. J., Dillon, P., Finlay, K., Fortino, K., Knoll, L. B., Kortelainen, P. L., Kutser, T., Larsen, S., Laurion, I., Leech, D. M., McCallister, S. L., McKnight, D. M., Melack, J. M., Overholt, E., Porter, J. A., Prairie, Y., Renwick, W. H., Roland, F., Sherman, B. S., Schindler, D. W., Sobek, S., Tremblay, A., Vanni, M. J., Verschoor, A. M., von Wachenfeldt, E., and Weyhenmeyer, G. A., 2009: Lakes and reservoirs as regulators of carbon cycling and climate. *Limnology and Oceanography,* 54: 2298-2314.

Tulonen, T., Salonen, K., and Arvola, L., 1992: Effects of Different Molecular-Weight Fractions of Dissolved Organic-Matter on the Growth of Bacteria, Algae and Protozoa from a Highly Humic Lake. *Hydrobiologia,* 229: 239-252.

Vähätalo, A. V., Salonen, K., Munster, U., Jarvinen, M., and Wetzel, R. G., 2003: Photochemical transformation of allochthonous organic matter provides bioavailable nutrients in a humic lake. *Archiv Fur Hydrobiologie,* 156: 287-314.

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., and Cushing, C. E., 1980: River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences,* 37: 130-137.

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H., and Tilman, G. D., 1997: Human alteration of the global nitrogen cycle: Sources and consequences. *Ecological Applications,* 7: 737-750.

Volk, C. J., Volk, C. B., and Kaplan, L. A., 1997: Chemical composition of biodegradable dissolved organic matter in streamwater. *American Society of Limnology and Oceanography,* 42: 39-44.

Wallis, P. M., Hynes, H. B. N., and Telang, S. A., 1981: The Importance of Groundwater in the Transportation of Allochthonous Dissolved Organic-Matter to the Streams Draining a Small Mountain Basin. *Hydrobiologia,* 79: 77-90.

Wetzel, R. G., 1995: Death, Detritus, and Energy-Flow in Aquatic Ecosystems. *Freshwater Biology,* 33: 83-89.

Wetzel, R. G., Hatcher, P. G., and Bianchi, T. S., 1995: Natural photolysis by ultraviolet irradiance of recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism. *Limnology and Oceanography,* 40: 1369-1380.

Wetzel, R. G., 2001: Limnology. Lake and River Ecosystems. *Elsevier Academic Press*: 1006.

Wilson, M. A., Vassallo, A. M., Perdue, E. M., and Reuter, J. H., 1987: A compositional and solid-state nuclear magnetic resonance study of humic and fulvic acid fractions of soil organic matter. *Analytical Chemistry,* 59: 551-558.

Wipfli, M. S., Richardson, J. S., and Naiman, R. J., 2007: Ecological Linkages Between Headwaters and Downstream Ecosystems: Transport of Organic Matter, Invertebrates, and Wood Down Headwater Channels. *JAWRA Journal of the American Water Resources Association,* 43: 72-85.

Zepp, R. G., Erickson, D. J., Paul, N. D., and Sulzberger, B., 2011: Effects of solar UV radiation and climate change on biogeochemical cycling: interactions and feedbacks. *Photochemical & Photobiological Sciences,* 10: 261-279.

Ziegler, S. E. and Lyon, D. R., 2010: Factors regulating epilithic biofilm carbon cycling and release with nutrient enrichment in headwater streams. *Hydrobiologia,* 657: 71-88.

Chapter 1: Variation in the photochemical lability of dissolved organic matter in a large boreal watershed

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Abstract

Boreal watersheds contain a vast quantity of terrestrially derived dissolved organic matter (DOM) originating from wetland and forest soils, yet variation in the potential for photochemical transformation of boreal aquatic DOM sources remains poorly understood. Laboratory solar radiation exposure experiments were conducted on DOM samples collected in three seasons, across nine sites, representing contrasting catchment composition and watershed position to assess variation in the photochemical lability of boreal DOM source and stable carbon isotopic signature ($\delta^{13}C$) of photomineralized DOM. Dissolved organic carbon (DOC) loss rates during laboratory exposure were lowest in summer, suggesting that DOM may have been more photo-degraded during summer. DOM from upstream portions of forested stream sites and wetland-influenced sites was more photolabile relative to downstream portions and the river DOM, suggesting potential losses in photolabile DOM downstream and in the lower reaches of the watershed. Increased a_{254}:a_{350} and spectral slope following sample exposure suggest photoproduction of low molecular weight (LMW) CDOM and/or a higher photoreactivity of high molecular weight versus LMW compounds. Photomineralization of nitrogen was regulated by organic nitrogen concentration and resulted in NH_4^+-photoproduction rates between 0.01 to 0.3 μM N hr^{-1} and ecologically significant increases in NH_4^+ for these waters. The $\delta^{13}C$ of the photomineralized DOM was positively correlated to initial DOC concentration and generally lower when intitial DOC concentrations were lower, suggesting variation in photomineralized DOM $\delta^{13}C$ may be a result of kinetic isotope fractionation. Results from this study demonstrate significant variation in the photochemical lability of boreal watershed sources of DOM. Such variation suggests landscape and environmental change has the potential to alter

the biogeochemical role photochemical transformations play in downstream portions of boreal watersheds.

1.1 Introduction

Dissolved organic matter (DOM) represents a key global energy and carbon reservoir (Hedges, 1992) that often integrates terrestrial and aquatic biogeochemical cycling (Cole et al., 2007), and is potentially sensitive to environmental change such as climate (Zepp et al., 2011) and land use (Findlay et al., 2001). Boreal watersheds contain a vast reservoir of terrestrially derived DOM originating from wetland and forest soils, which is transported through the aquatic network where it can be biogeochemically transformed via processes including photochemical transformation, particularly in downstream open waters of ponds and lakes. Terrestrially derived DOM in aquatic ecosystems consists mainly of high molecular weight (HMW) compounds, primarily in the form of fulvic and humic acids (Aitkenhead-Peterson et al., 2003) that are often considered relatively resistant to biodegradation (Findlay and Sinsabaugh, 1999). Typically measured as dissolved organic carbon (DOC), DOM concentrations in boreal streams and rivers are related to land cover, precipitation and discharge regime, acidity, soil type, and decomposition of organic matter in soil horizons (Schiff et al., 1997; Schiff et al., 1998; Moore et al., 2003; Buffam et al., 2007; Keller et al., 2008). Further, watershed location or composition can affect DOM composition and its biogeochemical processing. Headwater streams, in particular, often receive the largest direct input of terrestrially derived DOM (Fisher and Likens, 1973) and are also considered key locations for watershed biogeochemical cycling due to high active microbial surface area relative to water volume (Peterson et al., 2001). Consequently, headwater streams exhibit extensive microbial nutrient uptake (Peterson et al., 2001; Fellows et al., 2006), and high CO_2 efflux (Teodoru et al., 2009). This and the fact that headwater streams represent the first locations in watersheds that are affected by landscape changes (Peterson et al., 2001; Richardson et al., 2005; Alexander et al., 2007; Freeman et al.,

2007; Arango and Tank, 2008), signifies the importance of understanding factors regulating the chemical composition of stream DOM, including its photolability or photoreactivity, as such attributes can critically impact downstream water bodies (Koehler et al., 2002; Wipfli et al., 2007).

Variations in the discharge, storage, and transport of DOM are predicted with climate change (Clair and Ehrman, 1996; Schindler et al., 1997; Pastor et al., 2003; Freeman et al., 2004). The factors regulating DOM processing are critical for determining the result of environmental change, particularly in boreal watersheds, yet they remain poorly understood. Variations in stream DOM associated with changes in hydrology, vegetation, and soil temperature due to climate change may significantly affect carbon budgets and ecosystem processes including biogeochemical cycling and the release of CO_2 to the atmosphere at the watershed scale (Molot and Dillon, 1996; Clair et al., 2002; Algesten et al., 2004; Rantakari and Kortelainen, 2005; Sobek et al., 2005; Sobek et al., 2007). Hence, recent findings of high CO_2 efflux from headwater streams (Teodoru et al., 2009) and changing climate suggest a need to understand how DOM in boreal headwater streams varies. It is crucial to consider the factors regulating the fate and cycling of DOM as it is transported through the aquatic network, because it may be a major source of this CO_2.

Variation in the photochemical lability of DOM is one important attribute that may regulate the cycling and fate of this key carbon reservoir, particularly in the boreal environment. Due to the depletion of stratospheric ozone and the potential for increased transmission of solar ultraviolet (UV) radiation at the Earth's surface (Madronich et al., 1998), there have been tremendous strides in our understanding of the role of photochemical transformation of DOM and its implications for microbial cycling of DOM in freshwater ecosystems (Bertilsson et al., 1999; Tranvik et al., 1999; Obernosterer and

Benner, 2004). Many studies suggest an increase in biological availability of carbon, nitrogen, and phosphorus due to photomineralization and phototransformation of DOM (Bushaw et al., 1996; Moran and Zepp, 1997; Gao and Zepp, 1998; Tranvik et al., 1999), which may stimulate its use by microorganisms. However, the photoreactivity of DOM and its ecological impact varies with its source and composition (Brisco and Ziegler, 2004; Sulzberger and Durisch-Kaiser, 2009). It has been established that terrigenous (allochthonous) DOM with its high aromatic carbon content (e.g. lignin phenols) has a greater photoreactivity than internally produced (autochthonous), carbohydrate-rich DOM. Furthermore, terrestrially derived DOM bioavailability may be enhanced by photochemically-induced transformations into low molecular weight (LMW) compounds (Tranvik et al., 1999; Obernosterer and Benner, 2004; Sulzberger and Durisch-Kaiser, 2009).

Studies investigating the photochemical transformation of DOM in the boreal aquatic environment have typically focused on lakes (Tranvik et al., 1999; Bertilsson and Tranvik, 2000; Vähätalo et al., 2003) or rivers (Soumis et al., 2007). Variations in the photolability of DOM among contrasting watershed locations, e.g. headwater streams versus large lakes or main river reaches, would enable predictions regarding the delivery of photoreactive DOM to these downstream ecosystems. Such investigations, however, have been addressed to a much lesser extent. Investigating the variation in DOM photolability, or its relative susceptibility to be photomineralized or photobleached, from headwaters to large downstream reaches of a watershed will provide information about this important feature of DOM composition and the potential variation in the role of photochemical processes in downstream ecosystems. This is particularly important for boreal environments, where most of the DOM is terrestrially derived, characterized by a high concentration of colored, humic substances, and thus known to be highly

photoreactive. Furthermore, testing the effects of photochemical degradation on the chemical structure and optical properties of DOM, e.g. whether photochemical degradation proceeds to the complete photomineralization and production of CO_2 and/or CO, or whether it results in the formation of LMW compounds and potentially stimulates the microbial mineralization of DOM, will contribute to the understanding of the role of phototransformations in these watersheds.

In addition to changes in DOM chemical structure, photochemical transformations can alter the stable carbon isotopic signature ($\delta^{13}C$) of natural DOM in freshwaters (Opsahl and Zepp, 2001; Osburn et al., 2001; Vähätalo and Wetzel, 2008; Spencer et al., 2009). Investigating the $\delta^{13}C$ of the photomineralized DOM can provide information about the molecular components of the photolabile portions of DOM. It has been shown that lignin phenols, important components of terrestrially derived DOM, are highly susceptible to photodegradation (Opsahl and Benner, 1998; Benner and Kaiser, 2011), and can be used as a tracer to examine sources and the fate of terrigenous DOM in the aquatic environment (Hedges et al., 1988; Moran et al., 1991; Hedges et al., 1997; Spencer et al., 2009). Little consideration has been given to the potential fractionation associated with the photooxidation of DOM which can preferentially remove lignin, leaving a residual DOM pool enriched in carbohydrates and enriched in ^{13}C (Opsahl and Benner, 1998; Opsahl and Zepp, 2001). Assessment of the isotopic composition of photomineralized DOM from different sources is needed to decipher whether the isotopic fractionation is dependent on DOM chemical composition, and to what degree photomineralization may impact the $\delta^{13}C$ of DOM. The large terrigenous component of boreal DOM and previous studies indicating its high photoreactivity (Opsahl and Benner, 1998; Sulzberger and Durisch-Kaiser, 2009; Benner and Kaiser, 2011) suggest the stable isotopic composition may be greatly impacted by photochemical transformations.

Furthermore, changes in $\delta^{13}C$ induced by photochemical transformations may be used to determine whether the photolabile DOM is derived from similar chemical components in boreal watersheds.

The main objective of this study was to assess the variation in the photochemical lability of DOM, defined as the susceptibility of DOM to photomineralization and photobleaching, and the stable carbon isotopic signature ($\delta^{13}C$) of photomineralized DOM within a large boreal watershed. Controlled laboratory solar radiation exposure experiments were conducted on DOM samples collected in three seasons, across nine sites representing contrasting catchment composition and watershed position within the Humber River watershed, western Newfoundland, Canada. This study addressed whether DOM photochemical lability and the isotopic composition of photomineralized DOM varied with season, reaches within headwater streams and among headwater streams, and a large river reach. Further, these investigations enabled us to assess whether the photochemically labile fraction of DOM represents a significant proportion of DOM in terms of carbon, nitrogen, and phosphorus in a boreal watershed.

1.2 Methods

1.2.1 Study sites and sample collection

All samples were collected from within the Humber River watershed located in western Newfoundland, Canada, which comprises an area of 6200 km^2 (Fig. 1.1 a, b). This large boreal watershed provides sites that vary in watershed organic matter source (catchment vegetation) and headwater position (headwaters, lake) in the absence of significant human influences, allowing us to assess the variation in boreal DOM photolability among sites. Photochemical experiments were conducted on DOM samples collected from nine study sites. These sites include headwater streams chosen from a database of over 400 first to third order catchments delineated and sorted based upon their catchment vegetation composition to capture end member sites in terms of potential DOM source. Sites selected included: (1) a wetland-dominated catchment, (2) headwater streams draining primarily coniferous forest but with some variation in wetland coverage, and (3) the inlet and outlet of a large lake located in the lower reach of the Humber River itself.

Samples from the wetland-dominated catchment (23.5 km^2) were collected from a *Sphagnum* peatland bog (BOG; Fig. 1.1 c), an upstream site (Bog Feeder) immediately draining the bog (BF-1), and a downstream site (BF-2, 1 km from BF-1) situated in a forested reach of the same Bog Feeder (Fig. 1.1 c). Additionally, samples were taken from a third-order stream (Big Falls Brook; BFB) within a watershed adjacent to the bog, BF-1 and BF-2 sites (Fig. 1.1 c). Samples were collected from an upstream site (BFB-1), located in a primarily forested section with approximately 80 % forest cover, and a downstream site (BFB-2) with 20 % wetland cover located 12 km downstream from BFB-1. This downstream site represents relatively high wetland coverage for small,

accessible catchments in the Humber River watershed, where the maximum wetland coverage for first through third-order streams is 75 %, but where only 33 out of 416 stream catchments exhibit a wetland coverage greater than 25 % (mean coverage of these 33 = 36 ± 11 %).

To assess coniferous forest catchments, samples were collected from upstream (HB-1) and downstream (HB-2; 3 km from HB-1) localities of Hughes Brook (Fig. 1.1 d), which drains a watershed (113.3 km^2) dominated by coniferous forest (80 – 90 %) and less than 5 % wetland cover. Lastly, samples were taken from the inlet (DL-1) and outlet (DL-2) of Deer Lake, which is located at the downstream portion of the Humber River (Fig. 1.1 b).

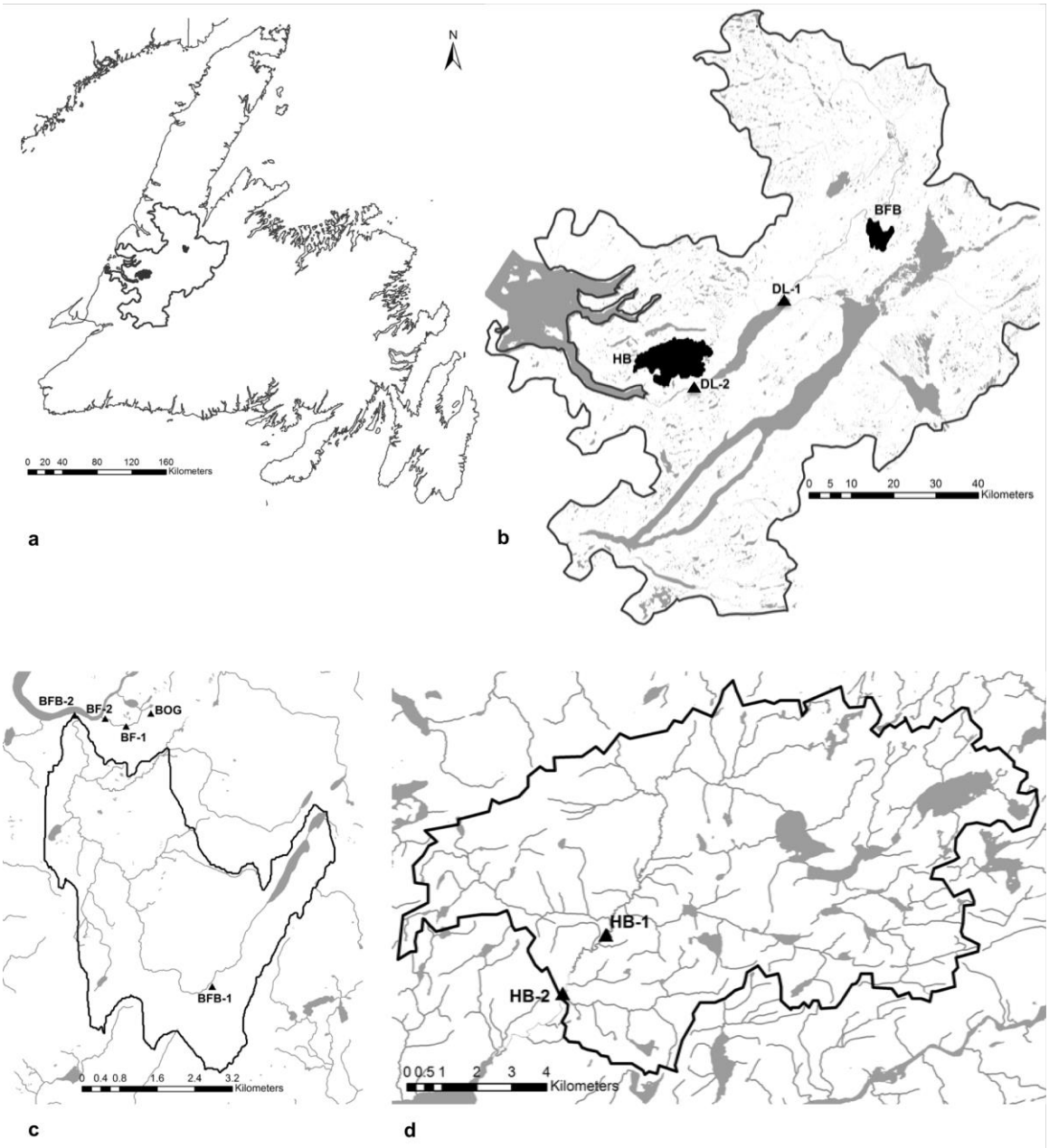

Figure 1.1: The delineation of the Humber River watershed both (a) as situated in western Newfoundland, Canada, and (b) shown in a larger scale to the right to illustrate the study sites in the watersheds of Big Falls Brook (BFB; c), Bog Feeder (BF; c), and Hughes Brook (HB; d). A minimum of two study sites, representing upstream (1) and downstream (2) or lake inlet (DL-1) and outlet (DL-2), respectively, were chosen for each catchment. This map was generated using ArcGIS.

1.2.2 Photochemical experiments

The primary goal of this study was to assess how photolability of DOM delivered from different headwater streams varied among different catchments and relative to downstream river reaches rather than to predict phototransformation rates *in situ*. Therefore, net changes following the exposure at one time point was used to estimate photochemical lability among contrasting study sites. The net change in DOM compositional characteristics was measured to assess the photochemical lability among the samples, particularly as an estimate of DOC and absorption loss, dissolved inorganic carbon (DIC) and nutrient release.

Dissolved organic matter samples were taken during three seasons (spring, summer, and fall) from each of the locations described. Samples were collected in 20 L acid-cleaned carboys and filtered using polycarbonate Nucleopore cartridge filters (0.2 µm pore size; Graver Technologies, Glasgow, DE, USA). The filtered water was distributed from a single large sample into acid-rinsed 500 mL high-density polyethylene (HDPE) bottles (Nalge Nunc International, Rochester, NY, USA). Because of the remote site locations and extent of time between exposure experiments, samples were frozen prior to transport back to the laboratory where photochemical exposure experiments were performed. This enabled us to compare samples across the watershed captured at the same time and avoid microbial degradation that would occur to differing extents given the different hold times required between experiments. Freezing water samples can, however, reduce DOC concentrations (Fellman et al., 2008) and potentially alter the initial composition of the DOM. However, we felt it most important to capture the DOM composition as close in time with collection and avoid microbial degradation. We, therefore, traded one alteration factor (microbial degradation) for another (freezing). To address this issue, we tested the effect of freezing on DOC concentrations in samples

collected at one study site within each location (Bog, DL, HB, and BFB) in summer. Furthermore, freezing and subsequent thawing of water samples has been found to greatly reduce absorption of DOM (Fellman et al., 2008); therefore we tested the effect of freezing on specific UV absorption (SUVA) in samples collected at each site along BFB, HB, and DL in fall, as well as BF in summer.

In preparation for photochemical experiments, water samples were completely defrosted while mixing on a shaker table (KS 501 digital, IKA-WERKE, Staufen, Germany). Once at room temperature, the samples were combined in a clean, pre-combusted glass flask and used to fill 300 mL quartz (5 replicates) and biological oxygen demand (BOD; 5 replicates) bottles without headspace. Quartz bottles were sealed with a gas-tight Teflon stopper, placed in a water bath within a solar simulator (Suntest XLS+, Material Testing Technology GmbH, Linsengericht, Germany) and connected to a waterchiller (NesLab, Thermo Scientific, Waltham, MA, USA) to maintain a temperature between 16 and 18˚C over the incubation period. The solar simulator was used with a high-pressure xenon lamp, with an irradiance of 765 W/m^2, and a special coated quartz UV filter generating a wavelength range of 300 to 800 nm. The spectrum of the light emitted through this filter closely matches that of natural surface solar radiation. Water samples were incubated in the solar simulator for 10 hours at 765 W/m^2. These conditions reflect a typical total radiation dose that approximates an amount that a surface water sample may experience during a several days transport in the watershed during summer. The filled BOD bottles were used to establish both initial and dark (foil covered) conditions. Dark controls were incubated in a sealed ice chest connected in line to the same water chiller system as the light-treated samples to maintain the same temperature as light samples over the same incubation period.

1.2.3 Analytical methods

Field measurements of dissolved oxygen (DO), conductivity, pH, and temperature were taken on site with an YSI 550A handheld DO instrument (YSI Incorporated, Yellow Springs, OH, USA), an ORION 122 conductivity meter (Orion Research Inc., Beverly, MA, USA), and an YSI 60 pH meter (YSI Incorporated, Yellow Springs, OH, USA), respectively. Laboratory DO measurements for the bottle incubations were conducted using an YSI-5100 DO probe (YSI Incorporated, Yellow Springs, OH, USA).

Samples for DIC and DOC analysis, as well as the stable carbon isotopic composition of these carbon pools (δ^{13}C-DIC and δ^{13}C-DOC), were collected from initial and final incubation bottles and dispensed into acid-cleaned, pre-combusted 40 mL amber glass vials and spiked with $HgCl_2$ and H_3PO_4 (HPLC grade), respectively. DIC and DOC concentrations and δ^{13}C values were determined using an AURORA 1030 TOC analyzer (O.I. Analytical, College Station, Texas, USA) coupled to a MAT252 isotope ratio mass spectrometer (Finnigan, Bremen, Germany). Values for δ^{13}C are reported in per mil units (‰) relative to the international reference standard Vienna Pee Dee Belemnite (R = 0.011237) (Sulzman, 2007). The analytical precision for δ^{13}C-DIC and δ^{13}C-DOC was 0.2 and 0.1 ‰, respectively. The carbon isotopic composition of photomineralized DOM ($\delta^{13}C_{PM}$) was calculated from changes observed in DOC concentration and δ^{13}C values using the following equation:

$$\delta^{13}C_{PM} = \frac{([DOC]_{final} \times \delta^{13}C_{final}) - ([DOC]_{initial} \times \delta^{13}C_{initial})}{[DOC]_{final} - [DOC]_{initial}} \quad \text{(eq. 1)}$$

where $[DOC]_{initial/final}$ is the DOC concentration at the beginning and end of the incubation, respectively, and $\delta^{13}C_{initial/final}$ is the initial and final stable carbon isotopic composition of the water sample in each individual bottle. This calculation was only made for samples

that exhibited significant changes in DOC concentration and $\delta^{13}C$ of DOC. For the samples analyzed in this study, the minimum detectable changes in [DOC] and $\delta^{13}C$ of DOC were 5 – 12 µM C for [DOC] = 350 – 500 µM C, 12 – 27 µM C when [DOC] = 600 – 1320 µM C, and 0.28 ‰ for the $\delta^{13}C$ of DOC.

Samples from initial and final bottles were collected for soluble reactive phosphorus (SRP) and ammonium (NH_4^+) in 15 mL Falcon tubes and analyzed with a Lachat FIA 8500 inorganic nutrient analyzer (HACH, Loveland, CO, USA) using the molybdate blue colorimetric method (detection limit 0.32 µM P) and phenol-hypochlorite method (detection limit 0.59 µM N), respectively. Samples for nitrate (NO_3^-) analysis were collected in 40 mL Falcon tubes and analyzed with a DX-100 ion chromatograph (detection limit 0.06 µM N; DIONEX, Sunnyvale, CA, USA). Samples for total dissolved nitrogen (TDN) were taken in acid-rinsed 60 mL Nalgene bottles and analyzed using a high temperature combustion total organic carbon analyzer (Shimadzu TOC-V) with a chemiluminescent NO_x detector (TNM-1; Shimadzu, Japan). Dissolved organic nitrogen (DON) was estimated by the difference of TDN and inorganic nitrogen (NH_4^+, NO_3^-). Analytical error for DON was determined from the square root of the sum of squares of the standard deviation associated with measurements of TDN, NH_4^+, and NO_3^-, and ranged from 1 to 10 % for mean concentrations ranging from 6 to 30 µM N in the samples analyzed in this study.

Samples for total dissolved iron (Fe) were taken in 120 mL brown Nalgene bottles, spiked with 1 mL nitric acid (HNO_3), and analyzed using an inductively coupled plasma optical emission spectrometer (detection limit 0.5 mg Fe L^{-1}; Perkin Elmer Optima 5300 Dual View ICP-OES, Waltham, MA, USA).

Dissolved organic matter optical characterization data were collected from initial and final samples. Absorbance measurements were performed at wavelengths spanning 200

to 800 nm using a UV/VIS scanning spectrometer with a spectral resolution of 1 nm (Lambda 25; Perkin Elmer, Waltham, MA, USA) and a 1 cm quartz cuvette, with NanoPure-UV water as a blank. The spectrophotometer was zeroed at the upper wavelength (800 nm) of the range used for each scan conducted. Absorption coefficients a_λ were calculated as:

$$a_\lambda = 2.303 \times \left(\frac{A_\lambda}{L} \right) \quad \text{(eq. 2)}$$

where A is the absorbance at wavelength λ and L is the path length (m) of the cuvette (Green and Blough, 1994). Specific UV absorption (L (mmol C)$^{-1}$ m^{-1}) was also determined for initial samples by normalizing a_{254} and a_{350} by the DOC concentration, providing a measurement of the molar absorptivity, at each of these two wavelengths, for each DOM sample (Miller, 1994). Loss in absorption due to photochemical transformation was calculated as a percent of the initial absorption at a wavelength of 350 nm lost following light exposure. Additionally, absorbance spectra were characterized using the equation:

$$a_\lambda = a_{\lambda o} e^{[-S(\lambda - \lambda_0)]} \quad \text{(eq. 3)}$$

where $a_{\lambda o}$ is the reference wavelength and S is the spectral slope. The spectral slopes for two shorter wavelength intervals 275 to 295 nm ($S_{275\text{-}295}$) and 350 to 400 nm ($S_{350\text{-}400}$) were calculated using linear regression on log-transformed data (Helms et al., 2008). Similar to results reported across a variety of sample types reported in Helms et al. (2008), we found that S using these two wavelength ranges exhibited the greatest variation in our samples. Further, broader wavelength intervals are typically not sensitive enough to identify shifts associated with the molecular weight of colored dissolved organic matter (CDOM). The ranges chosen here also provide a means to compare with

other studies since these ranges have more recently become common due to the attributes listed above (Brown, 1977; Sarpal et al., 1995; Twardowski et al., 2004; Helms et al., 2008).

To further assess photoreactivity and detect photochemically-induced changes in these experiments, photobleaching, defined as the average decrease in absorption coefficients over the 280 to 550 nm wavelength range (Δa_{avg}), was calculated for each exposure experiment using the following equation (Osburn et al., 2009):

$$\Delta a_{avg} = \frac{\sum_{\lambda=280}^{550} a(\lambda)_{initial} - a(\lambda)_{final}}{270} \quad \text{(eq. 4)}$$

where $a(\lambda)_{initial}$ and $a(\lambda)_{final}$ are the absorption coefficients for the initial and final light exposed samples with $a(\lambda)_{initial}$ having been corrected using the dark control values.

1.2.4 Statistical analyses

Significance tests (t-tests, assuming equal variances) were conducted between initial, control (dark), and final (light) samples with an α–value of 0.05 (AnalystSoft Inc., StatPlus:mac - Version 2009) to ascertain differences in spectral properties, and DIC, DOC and nutrient concentrations. Non-significant changes were considered to be below detection (BD). Further, minimum detectable differences (δ) for changes in δ^{13}C-DOC, DIC and DOC concentrations between initial and final samples were calculated based on the following equation at the 5 % significance level and 95 % confidence (Zar, 1996):

$$\delta = \sqrt{\frac{s^2}{n}}(t_{\alpha=0.05} + t_{\alpha=0.05}) \quad \text{(eq. 5)}$$

where s^2 is the pooled variance, and n is the sample size; t values are one-tailed.

Dissolved organic carbon loss rates and spectral characteristics were assessed to determine how they varied with site, in-stream location (i.e. upstream vs. downstream), and season. However, due to a lack of seasonal data, samples from the bog and Bog Feeder were not included in these analyses. Generalized linear models (GLM) were constructed to test site, in-stream location, and seasonal effects, as well as their interactions (model df = 17, data set df = 65, $\alpha = 0.05$; JMP 8.0; SAS, NC, USA). For each test conducted, chi square (χ^2) statistics from the analysis of deviance within the GLM framework were used to assess the significance of the effects tested and, where appropriate, their interactions. The assumptions of homogeneous and normal errors were checked for each GLM by plotting residuals versus fits, and by plotting residuals as probability plots (Lindsey, 1997).

Pearson correlations with an α-level of 0.05 (AnalystSoft Inc., StatPlus:mac - Version 2009) were used to test the potential role of DOM composition in regulating the photochemical lability measured as DOC loss. The initial DOC concentration, DOC loss rates, and δ^{13}C-DOC of the photomineralized DOM were all tested, where appropriate, against initial DOC, initial DOC:DON ratio, and optical measures (absorption coefficients, spectral slopes, absorption ratios a_{254}:a_{350}, and spectral slope ratios), and photobleaching measured as Δa_{254} and Δa_{350}. Likewise, the production of inorganic nitrogen as NH_4^+ and NO_3^-, and its relationship to initial DON concentration and DOC:DON ratio were tested to assess the potential role of organic nitrogen availability in regulating the photomineralization of nitrogen.

1.3 Results

1.3.1 Initial water chemistry parameters

The lowest initial pH measurement was observed at the bog site with a value of 6.0, whereas the highest pH values, between 8.2 and 8.9, were measured at the forested study sites (Table 1.1). The pH values from the frozen samples used for the light exposure experiments were elevated compared to the field measurements typically found at the study sites. Dissolved organic carbon concentrations observed in samples from wetland-influenced sites varied from 590 to 1320 µM C, and were lowest in spring and highest during the summer months (Fig. 1.2), congruent with the impact of dilution from spring flushing typical of watersheds with significant wetland coverage (Clair et al., 1996; Laudon et al., 2004; Buffam et al., 2007). Dissolved organic carbon concentrations of samples collected from forested stream sites and the lake were generally lower and ranged from 350 to 950 µM C (Fig. 1.2). Highest values were recorded in the fall likely due to increased organic matter inputs from flushing of soils during fall rain events typical of more forested catchments (Clair et al., 1996; Laudon et al., 2004). Carbon stable isotope composition (δ^{13}C-DOC) of DOM varied from -28.5 to -27.1 ‰, typical for terrestrially derived organic matter in boreal watersheds (Jonsson et al., 2001; Schumacher et al., 2006).

Total dissolved nitrogen concentrations fell within the narrow range of 12 to 18 µM N for all study sites and seasons, with BFB-1 in summer being the only exception (32 µM N). Ammonium concentrations varied from below detection (<0.6 µM N) to 3.5 µM N, and were lowest in water samples from wetland-influenced sites and highest at the downstream site of the forested stream and the lake inlet during fall. Nitrate concentrations ranged from 0.6 to 1.9 µM N for the wetland-influenced study sites and

the lake inlet, and from 3.7 to 8.0 μM N at the forested stream sites and the lake outlet. DOC:DON ratios fell within the range (40 to 65) observed in other boreal watershed studies (Bertilsson et al., 1999; Kortelainen et al., 2006), except for the forested stream sites (HB) that exhibited higher values (up to 100, Table 1.1). Elevated DOC:DON may have been a consequence of the forest logging at HB as elevated DOC export rates from forest soils have been documented in other forested watersheds (Lamontagne et al., 2000; Piirainen et al., 2002). Orthophosphate levels varied from below detection (<0.32 μM P) at the wetland-influenced sites to 3 μM P at the river and forested stream sites. Total dissolved Fe levels were below detection (<0.5 mg L^{-1}) for all sites and seasons.

DOC normalized absorption coefficients ranged from 84 to 141 L (mmol C)$^{-1}$ m^{-1} for λ_{254}, and from 21 to 41 L (mmol C)$^{-1}$ m^{-1} for λ_{350}, with the highest absorption coefficients observed at the lake inlet and outlet in fall (Table 1.1). These values result from relatively optically thick samples with a_{350} values ranging between 12 and 36 m^{-1}, similar to other brown-water studies (Minor et al., 2007). Absorption ratios a_{254}:a_{350}, often serving as indicators for the relative presence of LMW versus HMW CDOM (Helms et al., 2008), ranged from 3.1 to 4.4, varying seasonally ($p < 0.0001$, $\chi^2 = 169.6$) and among sites ($p < 0.0001$, $\chi^2 = 159.9$). Spectral slopes varied from 5.0 to 6.8 μm^{-1} for $\lambda_{275\text{-}295}$, and from 6.1 to 8.3 μm^{-1} for $\lambda_{350\text{-}400}$, and were lower compared to values reported in other studies (Helms et al., 2008), likely signifying higher concentrations of CDOM in these boreal streams relative to larger river and marine ecosystems. Spectral slope ratios (S_R) fell within the narrow range of 0.79 to 0.88 similar with other high latitude watersheds (Guéguen et al., 2011) and forested streams (Inamdar et al., 2011), except at the bog and at BF-1 where S_R were between 0.65 and 0.68. Spectral slopes and absorption

ratios were overall highest during summer, indicating the relative presence of LMW CDOM and low amounts of humic acids (Whitehead et al., 2000).

1.3.2 Freezing effect on DOC concentrations and spectral characteristics

Samples with [DOC] greater than 415 µM C and SUVA greater than 42 L (mmol C)$^{-1}$ m^{-1} can be susceptible to losses in each of these parameters upon freezing and thawing (Fellman et al., 2008). Although almost all samples in this study exhibited [DOC] > 415 µM, only one sample in our dataset exhibited both of these criteria. This one sample (HB-2 fall) was also the only site that exhibited a loss of DOC (10 %) and a very large loss in SUVA upon freezing. With the exception of the samples collected at HB-2, the summer samples tested for freezing effects on [DOC] revealed no significant differences between samples immediately analyzed (4.7 ± 0.10 to 21.2 ± 0.16 µM C) versus analyzed following freezing (4.6 ± 0.11 to 21.6 ± 0.15 µM C). Evidence for a significant freezing effect on DOC concentration was therefore not strong for samples collected as part of this study. In particular, we did not see a clear trend between [DOC] and loss upon freezing since the one sample exhibiting a loss represented a mid-range DOC concentration for this study. Freezing of DOM with relatively high [DOC] and SUVA has also been demonstrated to result in losses in SUVA as well as other spectral qualities (Spencer et al., 2007; Fellman et al., 2008). Reduction in SUVA with sample freezing was only detected in HB-2 (55 %) and BF-1 (9 %). None of the remaining study sites, where SUVA ranged from 23 ± 0.2 to 39 ± 0.3 L (mmol C)$^{-1}$ m^{-1} exhibited significant changes in spectral characteristics before and after freezing.

Results of the freezing tests suggest that DOC losses were not likely significant outside of the HB-2 study site while reduction in absorption with freezing may have been more prevalent. The lack of significant freezing effects in these samples may have been

due to the slightly lower SUVA (typically <40 L (mmol C)$^{-1}$ m^{-1}) and therefore lower relative absorption and freezing-sensitive DOM in these samples relative to those that have exhibited more susceptibility to freezing losses (Fellman et al., 2008). The tests conducted here, however, were not complete in the sense that every sample from the study was not directly tested. We must, therefore, interpret the experimental results with the understanding that some potential for alteration of DOM with freezing could have occurred. Previous investigations of such losses suggest that HMW pools of DOM are likely the most susceptible to these losses, and often these are also the most photoreactive pools. Therefore, the initial samples used here may have reduced levels of photolabile DOM relative to the ambient DOM from the study sites. This may mean that losses in DOC due to photomineralization and losses in absorption reported here might be underestimates. This is particularly so for the samples from HB-2 collected in summer. These points are taken in consideration within the interpretation of the results of this study given below.

1.3.3 Dissolved oxygen and pH changes with light exposure

Photochemical oxidation of stream, bog, and riverine DOM resulted in decreases in DO concentration, except at sites HB-1 and DL-1/2 during spring, where a change in [DO] was below detection. Losses in [DO] varied from 35 to 150 μM O_2 among study sites and seasons. Exposure of stream and riverine DOM to simulated sunlight also resulted in pH decreases, with changes ranging from 0.1 to 0.9 among study sites and seasons, indicating the formation of acidic photoproducts. Significant pH changes were not observed at DL inlet and outlet during summer.

Table 1.1: Initial water chemistry parameters including pH values, dissolved organic carbon (DOC) concentrations, dissolved organic carbon to organic nitrogen (DOC:DON) ratio, and DOC normalized absorption coefficients at 254 nm (a_{254}*) and 350 nm (a_{350}*). Values correspond to averages of 5 replicates ± standard deviation.

Site	Season	pH	DOC (μM C)	DOC:DON	a_{254}* L (mmol C)$^{-1}$ m^{-1}	a_{350}* L (mmol C)$^{-1}$ m^{-1}
Bog	Summer	6.0 ± 0.5	951 ± 38	39.6 ± 1.8	121.6 ± 5.5	34.6 ± 2.2
BF-1	Summer	6.7 ± 0.2	807 ± 21	60.7 ± 2.0	103.2 ± 4.3	34.9 ± 0.9
BF-2	Summer	7.5 ± 0.1	959 ± 56	52.9 ± 5.2	117.7 ± 4.9	37.5 ± 2.2
BFB-1	Spring	6.7 ± 0.1	786 ± 8	60.2 ± 5.2	121.1 ± 1.3	30.1 ± 0.3
BFB-1	Summer	7.3 ± 0.1	1320 ± 13	47.7 ± 0.8	84.4 ± 1.1	20.9 ± 0.4
BFB-1	Fall	7.0 ± 0.2	891 ± 24	55.3 ± 2.1	94.9 ± 2.6	22.5 ± 0.7
BFB-2	Spring	7.3 ± 0.1	587 ± 7	46.6 ± 5.4	116.8 ± 1.5	31.9 ± 0.5
BFB-2	Summer	7.9 ± 0.2	906 ± 26	61.9 ± 2.1	76.7 ± 2.2	17.7 ± 0.6
BFB-2	Fall	7.7 ± 0.2	729 ± 4	42.2 ± 1.0	95.9 ± 0.5	24.6 ± 0.2
HB-1	Spring	8.7 ± 0.1	653 ± 13	47.1 ± 2.2	117.6 ± 3.8	34.5 ± 1.0
HB-1	Summer	8.5 ± 0.3	689 ± 14	79.7 ± 7.9	96.5 ± 2.0	21.8 ± 0.5
HB-1	Fall	7.6 ± 0.2	950 ± 5	49.3 ± 1.3	123.2 ± 1.0	34.3 ± 0.2
HB-2	Spring	8.9 ± 0.2	376 ± 5	67.7 ± 2.3	104.6 ± 3.0	30.6 ± 0.7
HB-2	Summer	8.9 ± 0.2	351 ± 10	49.7 ± 4.9	105.1 ± 3.4	27.9 ± 1.0
HB-2	Fall	8.2 ± 0.1	634 ± 13	98.7 ± 5.7	120.7 ± 2.6	34.1 ± 0.7
DL-1	Spring	7.0 ± 0.2	557 ± 5	53.2 ± 2.7	128.7 ± 2.1	38.1 ± 1.3
DL-1	Summer	7.5 ± 0.1	423 ± 4	36.8 ± 2.3	113.5 ± 2.9	31.4 ± 1.7
DL-1	Fall	7.0 ± 0.1	642 ± 1	47.8 ± 4.6	139.9 ± 1.1	41.2 ± 0.5
DL-2	Spring	7.4 ± 0.1	504 ± 19	53.2 ± 13.4	114.1 ± 2.5	31.1 ± 1.0
DL-2	Summer	7.4 ± 0.1	394 ± 4	65.9 ± 5.4	117.0 ± 2.8	30.2 ± 1.3
DL-2	Fall	7.1 ± 0.2	431 ± 3	56.0 ± 1.3	141.4 ± 1.0	39.7 ± 0.5

1.3.4 Photomineralization of DOM

Photochemical oxidation of stream, bog, and riverine DOM resulted in DIC production and losses of DOC. Total carbon mineralized during the incubation, measured as an increase in DIC, varied from below detection to 65 µM C. Values were often near the detection limit for the amount of change in DIC, predominantly those samples with higher initial DIC concentrations (700 – 1200 µM C). The minimum detectable difference at these sites ranged from 14 to 57 µM C, whereas the minimum detectable difference at sites with DIC concentrations between 50 and 400 µM C ranged from 5 to 14 µM C.

Dissolved organic carbon concentrations decreased after light exposure (Fig. 1.2), whereas dark treatments did not show a significant difference from initial measurements. Values for total carbon mineralized during the incubation, measured as DOC loss, ranged from below detection to 150 µM C and were generally higher at stream sites compared to the river (site effect: $p < 0.0001$, $\chi^2 = 134.3$). Also, DOC losses were lower at the downstream location of each study site (in-stream differences among all samples: $p < 0.0001$, $\chi^2 = 99.6$; in-stream differences by site: $p < 0.0001$, $\chi^2 = 39.2$). These downstream trends were observed in the headwater streams in all seasons (no seasonal influence on upstream-downstream trends, $p = 0.29$, $\chi^2 = 2.5$), except the smallest stream directly draining the *Sphagnum* bog (BF) where an increase in DOC loss was observed downstream in the sample collected in summer (Fig. 1.3). Further, samples collected from wetland-influenced stream sites and at the upstream coniferous site exhibited the largest loss in DOC as a result of photomineralization. The trend in absolute loss was similar to the trend in relative DOC loss measured as DOC loss normalized to the initial DOC concentration. Likewise, the relative loss of DOC was generally higher at the stream sites compared to the river ($p < 0.0001$, $\chi^2 = 48.5$), with

HB-2 being an exception (Fig. 1.2). This one exception may have been a result of the loss of photolabile DOM from the initial HB-2 sample prior to the experimental irradiation, since this was the one sample that exhibited losses in DOC and SUVA with freezing. Also, DOC losses were often lower in samples collected from downstream versus upstream sites when considering each season individually (Fig. 1.2; season by site effect: $p < 0.0001$, $\chi^2 = 25.7$). The highest DOC loss of 9.6 % was observed at BF-2 in summer and corresponded to a quantitative loss of 148 $\pm$ 68 µM C. Lower DOC losses were observed in samples collected at the downstream coniferous site, as well as in samples taken from the river (DL) in summer and fall (Fig. 1.2). The lowest (measurable) DOC loss of 2.8 % at DL-2 in fall corresponded to a quantitative loss of 12 $\pm$ 4 µM C. Changes in DOC concentration at HB-2 in summer and fall, and at DL in summer were not significant.

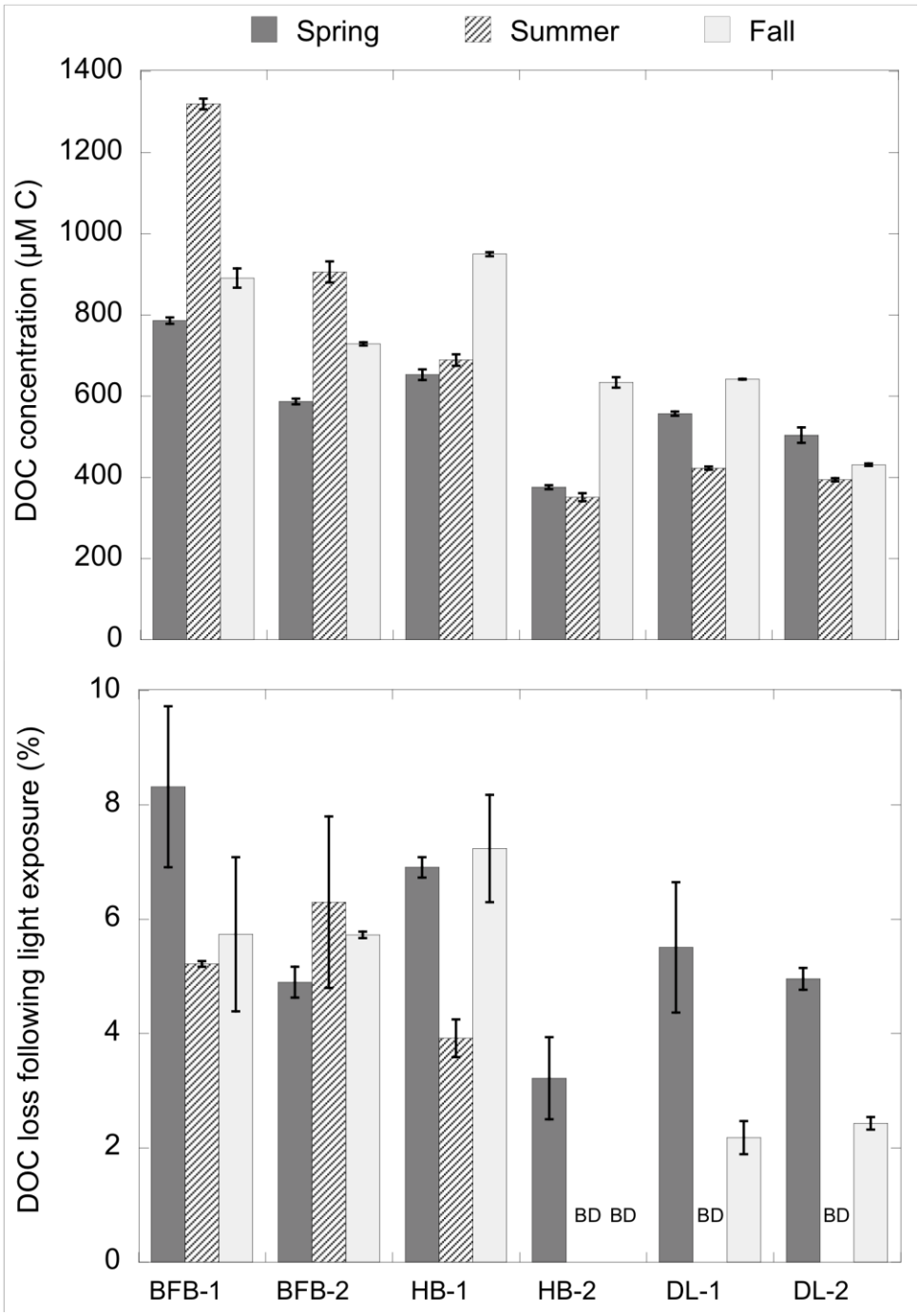

Figure 1.2: Seasonal variation in dissolved organic carbon (DOC) concentrations (top) and percent DOC loss following light exposure at six study sites within the Humber River watershed (bottom), including a wetland-dominated catchment (BFB), headwater streams draining primarily coniferous forest (HB), and the inlet and outlet of a large lake (DL) located in the lower reach of the Humber River. Values are provided as the average ± standard deviation (n = 5). BD = below detection.

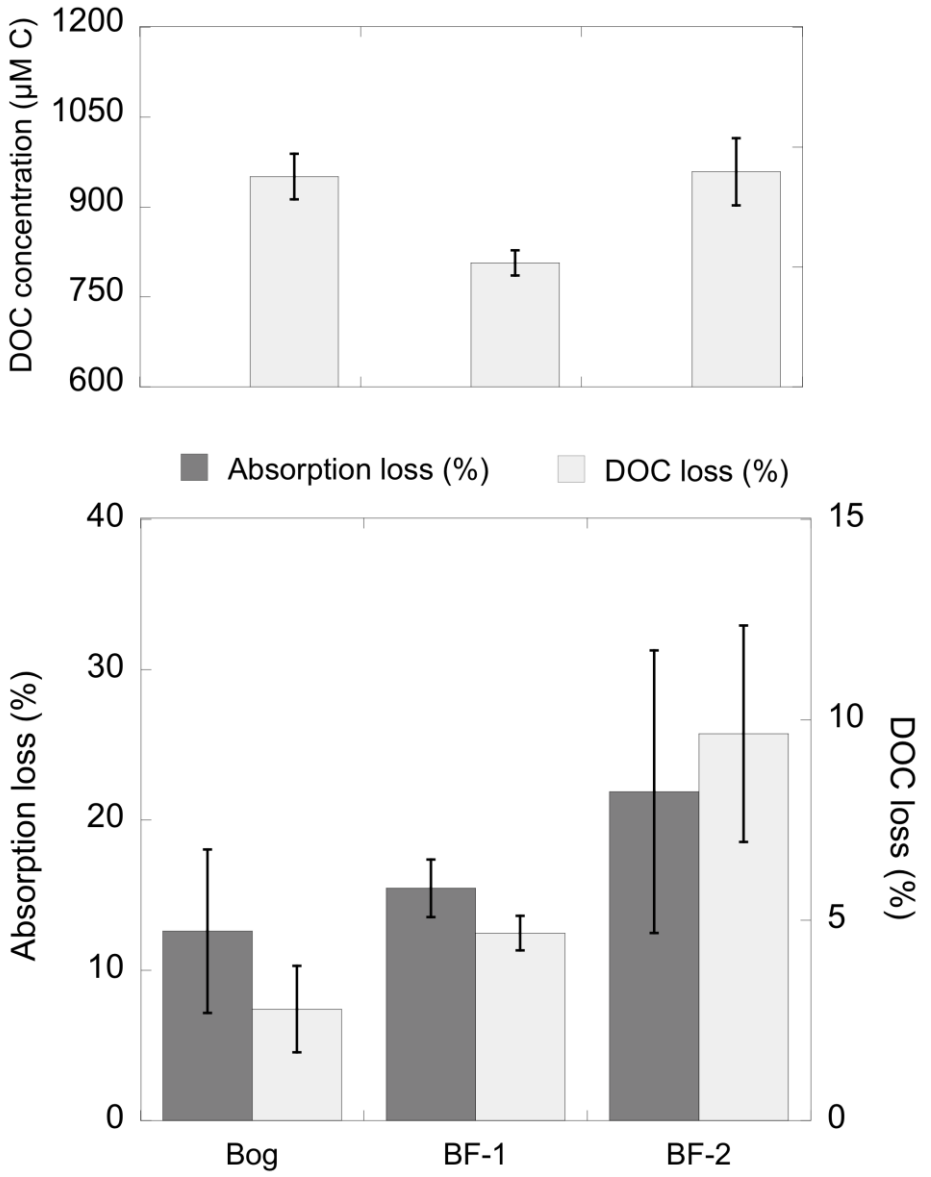

Figure 1.3: Dissolved organic carbon (DOC) concentration (top) and DOC and absorption loss following light exposure during summer (bottom). Sites represent a *Sphagnum* peatland bog, an upstream site (BF-1) immediately draining the bog, and a downstream site (BF-2) situated in a forested reach of the same stream. Losses in DOC and absorption were measured as the percent change in DOC concentration and absorption at a wavelength of 350 nm, respectively. Values are provided as the average ± standard deviation (n = 5).

1.3.5 Changes in optical properties of DOM with light exposure

Results from the photochemical experiments revealed significant changes to absorption properties (except in samples from DL-1 in summer), suggesting DOM photobleaching occurred in all samples to varying degrees (Fig. 1.4). DOC normalized absorption coefficients decreased in light-exposed samples but not in those from the dark controls, and absorption loss varied from 15 to 35 % following light exposure (Fig. 1.4). Absorption ratios a_{254}:a_{350}, spectral slope ratios (S_R), as well as spectral slopes $S_{275-295}$ and $S_{350-400}$ increased following light exposure at most sites (Fig. 1.4), indicative of greater loss of absorption at higher wavelengths. However, a significant change in $S_{350-400}$ was not detected following the exposure of the samples from both sites at BFB and at DL-1 in all seasons, and the final values for a_{254}:a_{350} and $S_{275-295}$ did not vary from the initial measurements at DL-1 collected in summer.

1.3.6 Inorganic nitrogen and phosphorus production with light exposure

Changes in SRP and NO_3^- concentrations following light exposure were below detection for all sites and seasons. Therefore, photolabile dissolved organic phosphorus (DOP) was not likely a significant fraction of the total DOP across this watershed. However, photochemical degradation of DOM resulted in the production of inorganic nitrogen in form of NH_4^+, whereas dark controls exhibited no significant difference between initial and final NH_4^+ concentrations. A minimum NH_4^+ production of 0.1 to 0.8 µM was found at a wide range of our study sites, and a high NH_4^+ production of 2.7 µM N was detected at BFB-1 in summer (Table 1.2). Minimum production values were calculated using the detection limit for the NH_4^+ analysis (0.59 µM N) when initial concentrations were below detection, resulting in a conservative estimate of NH_4^+ produced. Ammonium production was observed in samples collected during summer

and fall; however, production was not observed in any of the samples collected in spring (Table 1.2). Further, the amount of NH_4^+ produced was independent of DOC:DON ratio ($p = 0.49$), but positively correlated with initial DON concentrations ($p < 0.0001$, $R^2 = 0.47$).

Table 1.2: Initial dissolved organic nitrogen (DON) concentration and ammonium (NH_4^+) production rates over the course of the light incubation (10 hours). Data shown are average values (n = 5) ± one standard deviation. Values with asterisks represent minimum NH_4^+ production rates as initial concentrations were below detection (BD).

Site	Season	DON (μM N)	ΔNH_4^+ (μM N hr^{-1})
Bog	Summer	24.0 ± 0.4	BD
BF-1	Summer	13.3 ± 0.3	0.04* ± 0.006
BF-2	Summer	18.1 ± 1.5	0.02 ± 0.006
BFB-1	Spring	13.1 ± 1.1	BD
BFB-1	Summer	30.4 ± 0.3	0.27* ± 0.092
BFB-1	Fall	16.1 ± 0.5	0.02* ± 0.003
BFB-2	Spring	12.6 ± 1.5	BD
BFB-2	Summer	14.6 ± 0.2	0.04* ± 0.027
BFB-2	Fall	17.3 ± 0.4	BD
HB-1	Spring	13.9 ± 0.6	BD
HB-1	Summer	8.6 ± 0.8	BD
HB-1	Fall	19.3 ± 0.5	0.08 ± 0.026
HB-2	Spring	5.8 ± 0.2	BD
HB-2	Summer	7.1 ± 0.7	0.02 ± 0.002
HB-2	Fall	6.4 ± 0.3	BD
DL-1	Spring	10.5 ± 0.5	BD
DL-1	Summer	11.5 ± 0.7	BD
DL-1	Fall	13.4 ± 1.3	0.03 ± 0.010
DL-2	Spring	7.9 ± 2.0	BD
DL-2	Summer	6.0 ± 0.5	BD
DL-2	Fall	7.7 ± 0.2	0.01 ± 0.004

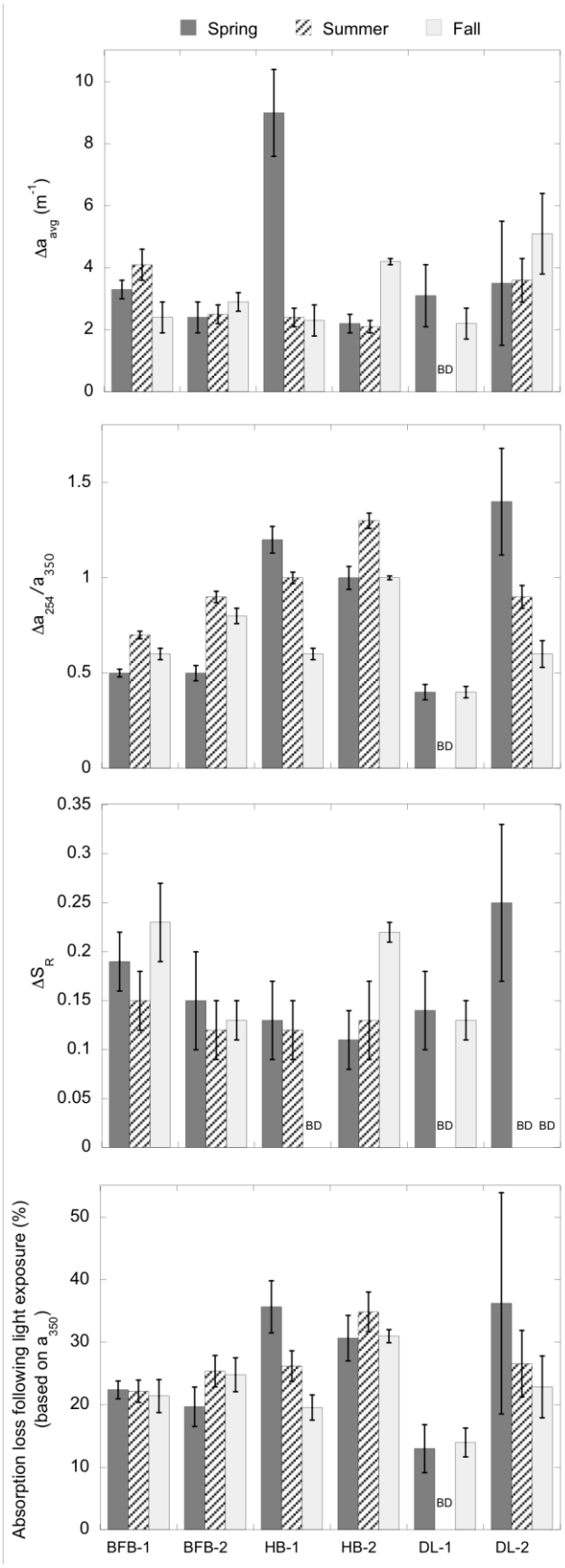

Figure 1.4: Average decrease in dissolved organic matter (DOM) absorption (Δa_{avg}), change in absorption ratio a_{254}/a_{350} ($\Delta a_{254}/a_{350}$), change in spectral slope ratio ($\Delta S_R = S_{275\text{-}295}/S_{350\text{-}400}$ final - $S_{275\text{-}295}/S_{350\text{-}400}$ initial), and relative loss in absorption at 350 nm following laboratory light exposure for the six study sites within the Humber River watershed collected during three seasons (spring, summer, fall). Sites include a wetland-dominated catchment (BFB), headwater streams draining primarily coniferous forest (HB), and the inlet and outlet of a large lake (DL) located in the lower reach of the Humber River. Numbers following the catchment abbreviation refer to the upstream (1) and downstream (2) site in each catchment. Values are provided as the average ± one standard deviation (n = 5). BD = below detection.

1.3.7 Stable carbon isotopic composition of photomineralized DOM (δ^{13}C-DOC)

Photochemical degradation of DOM induced a change in its carbon stable isotopic composition (δ^{13}C). A significant increase in δ^{13}C-DOC values was observed with light exposure of the bog, stream, and river samples, whereas dark controls did not reveal significant changes. Final δ^{13}C-DOC values were enriched by 0.2 to 1.1 ‰ relative to the starting δ^{13}C-DOC. The carbon isotopic composition of photomineralized DOM calculated from these results ranged from -30 to -41 ‰. Values were most depleted in ^{13}C relative to the initial DOC in the lake inlet sample (by 9 to 16 ‰) during spring and fall, as well as in the BFB-2 (13 ‰) and HB-2 (12 ‰) samples in spring. The δ^{13}C of photomineralized DOM was positively correlated to initial DOC concentration (Fig. 1.5) and negatively correlated to absorption coefficients a_{254} and a_{350} (Table 1.3).

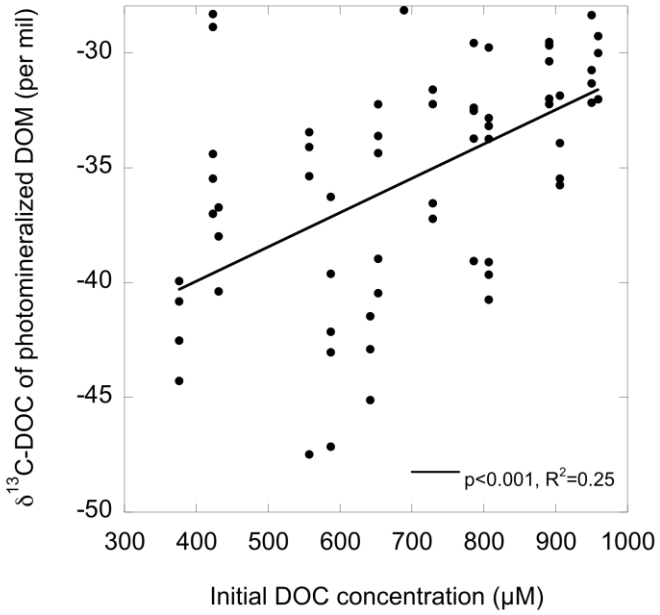

Figure 1.5: The stable carbon isotopic composition (δ^{13}C-DOC) of photomineralized dissolved organic carbon (DOC) plotted relative to initial DOC concentration for all individual replicates, study sites, and seasons.

Table 1.3: Relationships between stable carbon isotope composition ($\delta^{13}C_{PM}$; ‰) of photomineralized dissolved organic carbon (DOC), DOC loss (µM C), photobleaching (as Δa_{254} and Δa_{350}; m^{-1}), and initial DOC concentration (µM C), DOC:DON ratio, DOC normalized absorption coefficients a_{254}^* and a_{350}^* (L $(mmol\ C)^{-1}$ m^{-1}), spectral slope ratios (S_R), and a_{254}:a_{350} ratios, expressed as p- and R^2 values determined from Pearson correlations.

	Initial [DOC]		**DOC:DON**		**a_{254}^***		**a_{350}^***		**S_R**		**a_{254}:a_{350}**	
	p	R^2	p	R^2	p	R^2	p	R^2	p	R^2	p	R^2
Initial [DOC]	-	-	0.17	-	0.07	0.16	0.16	-	0.47	-	0.43	-
$\delta^{13}C_{PM}$	<0.001	0.26	0.54	-	0.04	0.28	0.05	0.25	0.71	-	0.29	-
DOC loss	<0.001	0.46	0.23	-	0.47	-	0.68	-	0.14	-	<0.001	0.16
Δa_{254}	0.006	0.41	0.85	-	-	-	-	-	-	-	-	-
Δa_{350}	0.027	0.28	0.87	-	-	-	-	-	-	-	-	-

1.4 Discussion

1.4.1 Carbon mass balance in the light exposure experiments

Mass balance for DIC production and DOC loss could not be determined in 40 % of the exposure experiments, likely for two reasons. Firstly, larger minimum detectable differences at sites with higher DIC concentrations resulted in values near the detection limit for the change in DIC. As a result, change in DIC could not be measured in the majority of the samples where mass balance for DIC production and DOC loss was not achieved. Secondly, the decrease in pH during photochemical transformation, particularly in samples with an initially lower pH (≤7), may have resulted in a gaseous loss of DIC in the final samples. This would have resulted in underestimates of DIC production. Given this lack of mass balance in some of the experiments and the greater potential error in the DIC production values, the amount of DIC produced could not be compared across all study sites. The DOC data was, therefore, used to determine the photolability of the bog, stream, and riverine DOM in this study.

1.4.2 Relationship between watershed position and DOM photolability

Photochemical transformations may be important in regulating the downstream fate of DOM to differing degrees for DOM derived from headwater streams versus the main river portions of this watershed. The results from this study suggest losses in photolabile DOM downstream in the lower reaches of the watershed and downstream within the headwater streams themselves. Photochemical lability of DOM appeared to have varied at both the smaller, in-stream headwater site, and larger, between headwater and river, scale within this watershed. Dissolved organic matter from upstream portions of the forested stream sites (HB) and the wetland-influenced sites (BFB) was more photolabile

relative to downstream portions. This consistent in-stream variation suggests that portions of the DOM pool may have undergone some photoprocessing during watershed transport and was therefore less susceptible to further photodegradation downstream. Further, the photolability of stream DOM appeared greater than DOM from the river and lake, which represent the more downstream portions of this large watershed, and is congruent with the concept of more chromophoric, photolabile DOM in stream water (Molot and Dillon, 1997).

The photomineralization of stream water DOM in this study was lower than other comparable studies of bog (16 % DOC loss; Osburn et al., 2001), forested swamp (8 %; Obernosterer and Benner, 2004), and blackwater riverine (9 – 17 % DOC loss; Smith and Benner, 2005) DOM, but not lake DOM (4 %; Obernosterer and Benner, 2004). More careful consideration, however, supports the observation that the headwater stream DOM in this watershed is more photolabile relative to downstream aquatic systems and similar to other upper watershed sources of DOM. The lower proportion of photolabile DOM in the stream water investigated here may be due, in part, to a freezing-induced reduction in the photolabile pool. Given the loss in DOC due to freezing was only detected for one sample, this was not likely the major contributing factor. Furthermore, DOC concentrations and SUVA, good predictors for freezing effects (Fellman et al., 2008), were similar between samples collected within streams where photolability was found to vary significantly. In other studies, irradiance dosages were generally greater than those used in this study. For example, the bog water was irradiated for seven days under natural sunlight at a lower latitude (Osburn et al., 2001), likely representing more than seven times the dosage used in this study. The blackwater riverine results reported in Smith and Benner (2005) were derived from single time point measurements with a dosage more than two times (64.8×10^3 kJm^{-2}) of that used in this

study, whereas relative losses of DOC due to photomineralization were not much greater than the stream DOM results from this study. The values for the swamp and lake DOM were more comparable to relative photodegradation losses reported (Obernosterer and Benner, 2004) and were estimated by extrapolating between time points to obtain losses representative of the 27.5 x 10^3 kJm^{-2} dosage used in this study. Collectively, these comparisons suggest that headwater stream DOM in this boreal watershed is typically more photoreactive compared with riverine and lake sources, and similar to some wetland-derived DOM.

1.4.3 Potential role of DOM source and composition in regulating the proportion of photolabile DOM

The variation in DOM photolability at the larger and smaller watershed scales in the Humber River watershed could potentially have been a result of differences in DOM source and composition, as previously suggested in studies of other systems (Brisco and Ziegler, 2004; Southwell et al., 2011). Specifically, losses of DOC and absorption appeared generally higher with elevated initial DOC concentrations and with a greater presence of HMW CDOM relative to LMW CDOM, estimated from a_{254}:a_{350} ratios and spectral slopes. These findings coincide with previous observations of the high photoreactivity of HMW CDOM, including aromatic compounds such as lignin-phenols, relative to LMW CDOM such as carbohydrates, which cannot be directly altered photochemically (Opsahl and Benner, 1998; Osburn et al., 2001). One exception to this observation was observed at both BFB sites in summer when DOC concentration was highest, yet photolability was low potentially due to changes in DOM chemical composition. Greater algal contributions at BFB-2 were possible given the filamentous algae and elevated nutrient concentrations observed at that time (Franke, D.,

unpublished data). Such a source could increase DOC while potentially decreasing its relative photolability. At the upstream site (BFB-1), greater input from the landscape was likely relative to groundwater in early summer, increasing DOC when discharge increased. Enhanced light availabililty in early summer, however, also likely increased autochthonous sources and/or photomineralization of DOM in the small pond just upstream of this site, reducing the relative susceptibility of the stream DOM to photomineralization. Thus, both sites (BFB-1 and BFB-2) could have been influenced by increased autochthonous sources in summer, and BFB-1 may potentially be further influenced by reduced photolabile DOM due to phototransformations upstream.

Factors such as differences in DOM inputs from peatland bog versus forested ecosystems also may have been responsible for differences in DOM chemical composition and thus photoreactivity among the sites and seasons. Contrary to in-stream differences observed at the majority of our study sites, DOM photolability increased from samples collected from the open site in the *Sphagnum* peatland bog to those from the small stream directly draining this bog (BF-1 and BF-2). Peatland bog water DOM has been previously described to be relatively non-chromophoric compared to other systems (Clair and Sayer, 1997) and thus less photoreactive, and the chemical composition of samples collected from our study sites suggests that bog DOM exhibits elevated carbohydrate contents relative to the headwater stream DOM (Bonnell, J. et al., unpublished data), perhaps due to autotrophic activity. Further, since DOM samples from the bog were collected in summer, both photochemical and active microbial decomposition due to light exposure and warmer temperatures may have reduced the aromaticity of the bog DOM (Clair et al., 1996). The increase in DOM photolability downstream from this bog was likely due to increasing forest input of terrestrial organic matter and DOM derived from deeper portions of peatland bogs in these downstream

sites. Both of these sources could potentially contribute more photoreactive compounds. For example, syringyl phenols have been found to be the most photolabile lignin phenol in DOM (Opsahl and Benner, 1998). Syringyl phenols are predominant in vascular plants including boreal coniferous trees and soils (Hedges and Mann, 1979; Ugolini et al., 1981), but are virtually absent in *Sphagnum* (Williams et al., 1998). Syringyl phenols, however, tend to exhibit increases in the relative contribution with depth in peatland soils (Williams and Yavitt, 2003).

Similarly, seasonal changes in DOM photolability documented in this study may have resulted from temporal changes in DOM chemical composition (Clair and Sayer, 1997; Lindell et al., 2000; Waiser and Robarts, 2004) caused by varying levels of photodegradation of DOM due to seasonal differences in solar radiation exposure. The amount of DOC lost during the laboratory exposure was lowest in the summer at most study sites when regarding each stream individually, suggesting that part of the DOM may have been more photo-degraded during the summer sampling period. Slightly higher initial S_R and a_{254}:a_{350} values during the summer months suggest that a relatively higher amount of LMW CDOM was present relative to other seasons. Further, the low DOC loss following light exposure in the summer samples did not correspond to lower absorption loss relative to other seasons. This may have been the result of absorption losses upon freezing that may have been less significant in the summer samples, which exhibited generally lower DOC and absorption relative to fall samples. Furthermore, freezing tests conducted here suggest changes in absorption may have been more common than DOC loss and potentially greater in fall versus summer samples.

In addition to the chemical composition of DOM, factors such as pH and iron concentrations can impact photomineralization of DOM. The presence of iron can facilitate the photodegradation of DOM to DIC (Faust and Zepp, 1993; Gao and Zepp,

1998; Sulzberger and Durisch-Kaiser, 2009) and cannot be ruled out as a potential factor regulating the photolability of DOM in the Humber River watershed. Total dissolved iron concentrations were below the elevated detection limit (<0.5 mg L^{-1}) obtained in this study. However, Fe levels have been found to range from 0.02 to 0.2 mg L^{-1} for the Humber River region (WQMA, 2000) similar to other Canadian boreal watersheds (Molot and Dillon, 2003; Wu et al., 2005; Kelton et al., 2007). In these systems, the photodegradation of DOM was strongly influenced by iron concentrations (Wu et al., 2005), even when initial [Fe] were relatively low (0.002 to 0.4 mg L^{-1}). Therefore, iron may have participated in the phototransformation of Fe-DOM carboxylates in the Humber River watershed samples studied here via ligand-to-metal charge transfer (Kelton et al., 2007), followed by the formation of polycarboxylate radicals and hydroxyl radicals, which can participate in photooxidation (Faust and Zepp, 1993; Gao and Zepp, 1998). Iron concentrations vary with catchment type and watershed DOM source (Maranger et al., 2006; Bjorkvald et al., 2008), and may explain upstream-downstream trends we observed, or differences between the study catchments. For example, higher Fe concentrations were measured at the wetland-influenced study sites (e.g. BFB, BF) relative to the forested streams and the main river in the Humber River watershed (Bonnell, J. et al., unpublished data), and may have contributed to the greater DOM photolability measured at the wetland-influenced sites relative to the forested stream sites (HB) or the main river portions.

Through possible impacts on DOM composition and photoprocessing of Fe, variation in pH can impact the photolability of DOM in natural waters (Waite and Morel, 1984; Bertilsson and Tranvik, 2000). Though potentially important in some environments, pH was not likely a major factor regulating the variation in DOM photolability observed in this study. Lowering pH significantly (i.e. by 2 or more units) has been found to cause

precipitation of more humic DOM (Aiken and Malcolm, 1987), and thereby potentially reducing the proportion of photoreactive DOM. Such large differences in pH, however, were not detected among samples or within experiments in this study. On the other hand, decreasing pH in the range of one or more units in lake or stream waters (Gennings et al., 2001; Anesio and Granéli, 2003; Vione et al., 2009) has been shown to increase photomineralization of DOM. The range in pH values we observed in our study was 6.0 to 8.7, with river samples ranging between 7.0 to 7.5 and streams 6.7 to 8.7. Differences in pH were not likely large enough to explain the differences in DOM photolability within headwater streams (greatest range 0.7) or between the headwater stream sites relative to the downstream river sites. The one exception to this was the headwater stream HB where a larger proportion of carbonate bedrock in the catchment results in elevated pH values relative to downstream portions of the watershed. The elevated pH range at the HB-1 site, however, coincided with relatively higher photomineralization of DOM relative to the downstream HB-2 and river DOM samples.

1.4.4 Potential role of HMW DOM in boreal watershed DOM photochemical transformations

Differences in DOC loss and spectral characteristics suggest that the presence of HMW CDOM plays a significant role in DOM photochemical transformations in the Humber River watershed. Although differences in DOC loss were not correlated to CDOM photobleaching ($p = 0.77$) and decreases in DOC concentration were considerably lower than the corresponding losses in absorption, similar patterns have been observed in other systems (Gao and Zepp, 1998). Either the photoreactive portion of DOM had a greater absorption than the bulk DOM pool, or the photoproducts had a lower absorption than the initial DOM pool (Gao and Zepp, 1998). Both of these

conditions allow for variations in the amount of DOC lost while observing relatively constant losses in absorption among sites and seasons and could only be distinguished through measurements of the apparent quantum yield (Johannessen and Miller, 2001) for each parameter (DOC and absorption loss), which was not accessible in these experiments.

Increases in a_{254}:a_{350} and spectral slope following simulated solar radiation, however, suggest increases in LMW photoproducts and/or a greater resistance of LMW compounds to photodegradation and a higher photoreactivity of HMW compounds, respectively (Opsahl and Benner, 1998). This shift in the relative molecular weight of CDOM that occurred during photochemical transformations has been shown in previous studies (Vodacek et al., 1997; Obernosterer and Benner, 2004; Waiser and Robarts, 2004), however several other studies reported decreases in spectral slope following light exposure (Gao and Zepp, 1998; Wu et al., 2005). The reasoning for these differences has not been well established, although differences in the spectral quality of irradiation to which CDOM was exposed (Tzortziou et al., 2007), as well as differences in source (e.g. marine versus riverine or stream DOM), chemical composition and the degradative state may be factors (Gao and Zepp, 1998). Results from our research suggest HMW CDOM is an important fraction contributing to the photolabile pool of DOM in these boreal waters.

1.4.5 Photolabile DON and DOP in boreal watersheds

Photomineralization of phosphorus was not detected throughout the sites investigated while photomineralization of nitrogen resulted in the production of NH_4^+ and was regulated by organic nitrogen availability in the Humber River watershed. The lack of DOP photomineralization was likely due to low DOP availability and lower phosphorus

photomineralization rates typical of boreal watersheds relative to nitrogen (Vähätalo et al., 2003) and in contrast to more phosphorus-rich ecosystems (Francko and Heath, 1982). The absolute NH_4^+ production rates (0.01 to 0.3 μM N hr^{-1}) in this study, however, were similar to the rates observed in natural and artificial sunlight exposure experiments on boreal DOM (peatland bog specifically) with higher DON concentrations ranging from 20 to 86 μM N (Bushaw et al., 1996; Vähätalo et al., 2003). However, NH_4^+ production rates detected in our photochemical experiments accounted for a 30 to 150 % increase in the initial NH_4^+ pool size, and as high as 450 % at BFB-1 in summer. Elevated temperatures during summer months can lead to greater reworking of DOM, resulting in DOM with a higher proteinaceous component relative to other times of the year (Ziegler and Fogel, 2003). Subsequent photochemical ammonification can make these nitrogen compounds more accessible for microbial utilization, and thus stimulate microbial degradation (Bushaw et al., 1996) and production (Vähätalo and Jarvinen, 2007). Ammonium concentrations are typically very low in these streams, particularly in boreal aquatic ecosystems, yet play an important role in microbial assimilation and nitrification processes (Peterson et al., 2001). Ammonium is usually the preferred form of DIN (Dodds et al., 1991) and therefore rapidly removed from the water column by microorganisms (Peterson et al., 2001; Pelster et al., 2008; Fellman et al., 2009). Further, the microbial community present in these streams may be able to adapt to recycling or acquiring the nitrogen compounds (e.g. NH_4^+) needed for an enhanced degradation of DOM carbon sources (Franke, D., unpublished data). Thus, the photochemical production of NH_4^+ may have important ecological implications for the carbon and nutrient cycling in the Humber River watershed, particularly in summer and fall when NH_4^+ production was measurable in samples collected.

1.4.6 Factors regulating the isotopic composition of photomineralized DOM

Photochemical transformations in this study lead to increases in the $\delta^{13}C$ of DOM similar to those reported in previous sunlight exposure studies on terrigenous and freshwater DOM (Opsahl and Zepp, 2001; Osburn et al., 2001). The lack of any relationship between $\delta^{13}C$ of the photomineralized DOM and measures such as the DOC:DON ratio and optical properties of the DOM suggests bulk DOM compositional differences was not likely the reason for the observed shift in $\delta^{13}C$. The majority of the calculated values for the carbon isotopic composition of photomineralized DOM (-30 to -35 ‰) were similar to those found in other studies and reflect the ^{13}C-depleted signature of lignin (Opsahl and Zepp, 2001). However, some of our values for the $\delta^{13}C$ of the photomineralized DOM were very low (-41 ‰), which is not typically the case for terrestrial $\delta^{13}C$ values including those of lignin, normally depleted by 4 to 7 ‰ relative to bulk vascular plant tissues (Benner et al., 1987) or lipids, normally depleted by 2 to 5 ‰ relative to bulk biomass (Hayes, 2001). It is important, however, to recognize that the DIC released as a result of photooxidation does not necessarily reflect the carbon derived from the *entire* biomolecule that was photooxidized. Typically only a portion of the photoreactive component is released as DIC during photochemical decarboxylation processes (Osburn et al., 2001; Xie et al., 2004). Although carboxylic groups on molecules may not be typically depleted relative to the molecule from which they are derived (Vogler and Hayes, 1980), kinetic isotope fractionation associated with decarboxylation and specifically photodecarboxylation can lead to isotopically deplete CO_2 that may explain the values reported in this study (Bigeleisen and Allen, 1951). Given this and the fact that lignin phenols are light relative to bulk organic matter suggests that kinetic isotope fractionation, particularly associated with decarboxylation of

photoreactive components of DOM, may in part control the $\delta^{13}C$ of DOM in boreal waters.

Relationships between $\delta^{13}C$ of the photomineralized DOM and rates of photomineralization can serve to more directly link changes in the $\delta^{13}C$ of DOM with kinetic isotope fractionation (Opsahl and Zepp, 2001; Vähätalo and Wetzel, 2008). Here the $\delta^{13}C$ of the photomineralized DOM was positively correlated to initial DOC concentration. Specifically, the $\delta^{13}C$ of the photomineralized DOM was generally lower when intitial DOC concentrations were lower. Dissolved organic carbon concentration itself was correlated with DOC photomineralization rates; however, whether DOC loss was correlated to the $\delta^{13}C$ of the photomineralized DOM could not be directly tested due to the fact that this rate was used in the calculation of the $\delta^{13}C$ value. In combination with previous studies, the results of this study suggest that photomineralization has the potential to alter the stable carbon isotope composition of DOC and DIC in boreal aquatic ecosystems. Further, such change is likely regulated more by the rates of photomineralization and less so by the composition of DOM in these watersheds.

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1.6 References

Aiken, G. R. and Malcolm, R. L., 1987: Molecular weight of aquatic fulvic acids by vapor pressure osmometry. *Geochimica Et Cosmochimica Acta,* 51: 2177-2184.

Aitkenhead-Peterson, J. A., Dowell, W. H., and Neff, J. C., 2003: Sources, Production, and Regulation of Allochthonous Dissolved Organic Matter Inputs to Surface Waters. *Aquatic Ecosystems: Interactivity of Dissolved Organic Matter; Elsevier Science*: 25-70.

Alexander, R. B., Boyer, E. W., Smith, R. A., Schwarz, G. E., and Moore, R. B., 2007: The role of headwater streams in downstream water quality. *Journal of the American Water Resources Association,* 43: 41-59.

Algesten, G., Sobek, S., Bergstrom, A. K., Agren, A., Tranvik, L. J., and Jansson, M., 2004: Role of lakes for organic carbon cycling in the boreal zone. *Global Change Biology,* 10: 141-147.

Anesio, A. M. and Granéli, W., 2003: Increased photoreactivity of DOC by acidification: Implications for the carbon cycle in humic lakes. *Journal Limnology and Oceanography,* 48: 735-744.

Arango, C. P. and Tank, J. L., 2008: Land use influences the spatiotemporal controls on nitrification and denitrification in headwater streams. *Journal of the North American Benthological Society,* 27: 90-107.

Benner, R., Fogel, M. L., Sprague, E. K., and Hodson, R. E., 1987: Depletion of 13C in lignin: implications for stable carbon isotope studies. *Nature,* 329: 708-710.

Benner, R. and Kaiser, K., 2011: Biological and photochemical transformations of amino acids and lignin phenols in riverine dissolved organic matter. *Biogeochemistry,* 102: 209-222.

Bertilsson, S., Stepanauskas, R., Cuadros-Hansson, R., Graneli, W., Wikner, J., and Tranvik, L., 1999: Photochemically induced changes in bioavailable carbon and nitrogen pools in a boreal watershed. *Aquatic Microbial Ecology,* 19: 47-56.

Bertilsson, S. and Tranvik, L. J., 2000: Photochemical transformation of dissolved organic matter in lakes. *Limnology and Oceanography,* 45: 753-762.

Bigeleisen, J. and Allen, T. L., 1951: Fractionation of the Carbon Isotopes in Decarboxylation Reactions. IV. The Relative Rates of Decomposition of 1- C12 and 1- C13 Trichloracetate Ions. *The Journal of Chemical Physics,* 19: 760-764.

Bjorkvald, L., Buffam, I., Laudon, H., and Morth, C. M., 2008: Hydrogeochemistry of Fe and Mn in small boreal streams: The role of seasonality, landscape type and scale. *Geochimica Et Cosmochimica Acta,* 72: 2789-2804.

Brisco, S. and Ziegler, S., 2004: Effects of solar radiation on the utilization of dissolved organic matter (DOM) from two headwater streams. *Aquatic Microbial Ecology,* 37: 197-208.

Brown, M., 1977: Transmission spectroscopy examinations of natural waters: C. Ultraviolet spectral characteristics of the transition from terrestrial humus to marine yellow substance. *Estuarine and Coastal Marine Science,* 5: 309-317.

Buffam, I., Laudon, H., Temnerud, J., Morth, C. M., and Bishop, K., 2007: Landscape-scale variability of acidity and dissolved organic carbon during spring flood in a boreal stream network. *Journal of Geophysical Research-Biogeosciences,* 112: G01022.

Bushaw, K. L., Zepp, R. G., Tarr, M. A., SchulzJander, D., Bourbonniere, R. A., Hodson, R. E., Miller, W. L., Bronk, D. A., and Moran, M. A., 1996: Photochemical release of biologically available nitrogen from aquatic dissolved organic matter. *Nature,* 381: 404-407.

Clair, T. A. and Ehrman, J. M., 1996: Variations in discharge and dissolved organic carbon and nitrogen export from terrestrial basins with changes in climate: A neural network approach. *Limnology and Oceanography,* 41: 921-927.

Clair, T. A., Sayer, B. G., Kramer, J. R., and Eaton, D. R., 1996: Seasonal variation in the composition of aquatic organic matter in some Nova Scotian brownwaters: A nuclear magnetic resonance approach. *Hydrobiologia,* 317: 141-150.

Clair, T. A. and Sayer, B. G., 1997: Environmental variability in the reactivity of freshwater dissolved organic carbon to UV-B. *Biogeochemistry,* 36: 89-97.

Clair, T. A., Arp, P., Moore, T. R., Dalva, M., and Meng, F. R., 2002: Gaseous carbon dioxide and methane, as well as dissolved organic carbon losses from a small temperate wetland under a changing climate. *Environmental Pollution,* 116: 143-148.

Cole, J. J., Prairie, Y. T., Caraco, N. F., McDowell, W. H., Tranvik, L. J., Striegl, R. G., Duarte, C. M., Kortelainen, P., Downing, J. A., Middelburg, J. J., and Melack, J., 2007: Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems,* 10: 171-184.

Dodds, W. K., Priscu, J. C., and Ellis, B. K., 1991: Seasonal Uptake and Regeneration of Inorganic Nitrogen and Phosphorus in a Large Oligotrophic Lake - Size-Fractionation and Antibiotic-Treatment. *Journal of Plankton Research,* 13: 1339-1358.

Faust, B. C. and Zepp, R. G., 1993: Photochemistry of Aqueous Iron(Iii) Polycarboxylate Complexes - Roles in the Chemistry of Atmospheric and Surface Waters. *Environmental Science & Technology,* 27: 2517-2522.

Fellman, J. B., D'Amore, D. V., and Hood, E., 2008: An evaluation of freezing as a preservation technique for analyzing dissolved organic C, N and P in surface water samples. *Science of the Total Environment,* 392: 305-312.

Fellman, J. B., Hood, E., Edwards, R. T., and Jones, J. B., 2009: Uptake of Allochthonous Dissolved Organic Matter from Soil and Salmon in Coastal Temperate Rainforest Streams. *Ecosystems,* 12: 747-759.

Fellows, C. S., Valett, H. M., Dahm, C. N., Mulholland, P. J., and Thomas, S. A., 2006: Coupling nutrient uptake and energy flow in headwater streams. *Ecosystems,* 9: 788-804.

Findlay, S. and Sinsabaugh, R. L., 1999: Unravelling the sources and bioavailability of dissolved organic matter in lotic aquatic ecosystems. *Marine and Freshwater Research,* 50: 781-790.

Findlay, S., Quinn, J. M., Hickey, C. W., Burrell, G., and Downes, M., 2001: Effects of land use and riparian flowpath on delivery of dissolved organic carbon to streams. *Limnology and Oceanography,* 46: 345-355.

Fisher, S. G. and Likens, G. E., 1973: Energy Flow in Bear Brook, New Hampshire - Integrative Approach to Stream Ecosystem Metabolism. *Ecological Monographs,* 43: 421-439.

Francko, D. A. and Heath, R. T., 1982: UV-sensitive complex phosphorus: association with dissolved humic material and iron in a bog lake. *Journal Name: Limnol. Oceanogr.; (United States); Journal Volume: 27:3*: 564-569.

Freeman, C., Fenner, N., Ostle, N. J., Kang, H., Dowrick, D. J., Reynolds, B., Lock, M. A., Sleep, D., Hughes, S., and Hudson, J., 2004: Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels. *Nature,* 430: 195-198.

Freeman, M. C., Pringle, C. M., and Jackson, C. R., 2007: Hydrologic connectivity and the contribution of stream headwaters to ecological integrity at regional scales. *Journal of the American Water Resources Association,* 43: 5-14.

Gao, H. Z. and Zepp, R. G., 1998: Factors influencing photoreactions of dissolved organic matter in a coastal river of the southeastern United States. *Environmental Science & Technology,* 32: 2940-2946.

Gennings, C., Molot, L., and Dillon, P., 2001: Enhanced photochemical loss of organic carbon in acidic waters. *Biogeochemistry,* 52: 339-354.

Green, S. A. and Blough, N. V., 1994: Optical-Absorption and Fluorescence Properties of Chromophoric Dissolved Organic-Matter in Natural-Waters. *Limnology and Oceanography,* 39: 1903-1916.

Guéguen, C., Granskog, M. A., McCullough, G., and Barber, D. G., 2011: Characterisation of colored dissolved organic matter in Hudson Bay and Hudson Strait using parallel factor analysis. *Journal of Marine Systems,* 88: 423-433.

Hayes, J. M., 2001: Fractionation of carbon and hydrogen isotopes in biosynthetic processes. *Stable Isotope Geochemistry,* 43: 225-277.

Hedges, J. I. and Mann, D. C., 1979: The characterization of plant tissues by their lignin oxidation products. *Geochimica Et Cosmochimica Acta,* 43: 1803-1807.

Hedges, J. I., Clark, W. A., and Cowie, G. L., 1988: Organic-Matter Sources to the Water Column and Surficial Sediments of a Marine Bay. *Limnology and Oceanography,* 33: 1116-1136.

Hedges, J. I., 1992: Global Biogeochemical Cycles - Progress and Problems. *Marine Chemistry,* 39: 67-93.

Hedges, J. I., Keil, R. G., and Benner, R., 1997: What happens to terrestrial organic matter in the ocean? *Organic Geochemistry,* 27: 195-212.

Helms, J. R., Stubbins, A., Ritchie, J. D., Minor, E. C., Kieber, D. J., and Mopper, K., 2008: Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnology and Oceanography,* 53: 955-969.

Inamdar, S., Singh, S., Dutta, S., Levia, D., Mitchell, M., Scott, D., Bais, H., and McHale, P., 2011: Fluorescence characteristics and sources of dissolved organic matter for stream water during storm events in a forested mid-Atlantic watershed. *Journal of Geophysical Research,* 116: G03043.

Johannessen, S. C. and Miller, W. L., 2001: Quantum yield for the photochemical production of dissolved inorganic carbon in seawater. *Marine Chemistry,* 76: 271-283.

Jonsson, A., Meili, M., Bergstrom, A. K., and Jansson, M., 2001: Whole-lake mineralization of allochthonous and autochthonous organic carbon in a large humic lake (Ortrasket, N. Sweden). *Limnology and Oceanography,* 46: 1691-1700.

Keller, W., Paterson, A. M., Somers, K. M., Dillon, P. J., Heneberry, J., and Ford, A., 2008: Relationships between dissolved organic carbon concentrations, weather, and acidification in small Boreal Shield lakes. *Canadian Journal of Fisheries and Aquatic Sciences,* 65: 786-795.

Kelton, N., Molot, L. A., and Dillon, P. J., 2007: Effect of ultraviolet and visible radiation on iron lability in boreal and artificial waters. *Aquatic Sciences,* 69: 86-95.

Koehler, S., Buffam, I., Jonsson, A., and Bishop, K., 2002: Photochemical and microbial processing of stream and soil water dissolved organic matter in a boreal forested catchment in northern Sweden. *Aquatic Sciences - Research Across Boundaries,* 64: 269-281.

Kortelainen, P., Mattsson, T., Finer, L., Ahtiainen, M., Saukkonen, S., and Sallantaus, T., 2006: Controls on the export of C, N, P and Fe from undisturbed boreal catchments, Finland. *Aquatic Sciences,* 68: 453-468.

Lamontagne, S., Carignan, R., D'Arcy, P., Prairie, Y. T., and Pare, D., 2000: Element export in runoff from eastern Canadian Boreal Shield drainage basins following forest harvesting and wildfires. *Canadian Journal of Fisheries and Aquatic Sciences,* 57: 118-128.

Laudon, H., Köhler, S., and Buffam, I., 2004: Seasonal TOC export from seven boreal catchments in northern Sweden. *Aquatic Sciences - Research Across Boundaries,* 66: 223-230.

Lindell, M. J., Graneli, H., and Bertilsson, S., 2000: Seasonal photoreactivity of dissolved organic matter from lakes with contrasting humic content. *Canadian Journal of Fisheries and Aquatic Sciences,* 57: 875-885.

Lindsey, J. K., 1997: Applying Generalized Linear Models. *New York; Springer (ISBN 0387982183)*: 265 pp.

Madronich, S., McKenzie, R. L., Bjorn, L. O., and Caldwell, M. M., 1998: Changes in biologically active ultraviolet radiation reaching the Earth's surface. *Journal of Photochemistry and Photobiology,* 46: 5-19.

Maranger, R., Canham, C. D., Pace, M. L., and Papaik, M. J., 2006: A spatially explicit model of iron loading to lakes. *Limnology and Oceanography,* 51: 247-256.

Miller, W. L., 1994: *Recent advances in the photochemistry of natural dissolved organic matter*: Aquatic and Surface Photochemistry; edited by G. R. Helz, R. G. Zepp, and D. G. Crosby; A. F. Lewis, New York, 111-127 pp.

Minor, E. C., Dalzell, B. L., Stubbins, A., and Mopper, K., 2007: Evaluating the photoalteration of estuarine dissolved organic matter using direct temperature-resolved mass spectrometry and UV-visible spectroscopy. *Aquatic Sciences,* 69: 440-455.

Molot, L. A. and Dillon, P. J., 1996: Storage of terrestrial carbon in boreal lake sediments and evasion to the atmosphere. *Global Biogeochemical Cycles,* 10: 483-492.

Molot, L. A. and Dillon, P. J., 1997: Colour - mass balances and colour - dissolved organic carbon relationships in lakes and streams in central Ontario. *Canadian Journal of Fisheries and Aquatic Sciences,* 54: 2789-2795.

Molot, L. A. and Dillon, P. J., 2003: Variation in iron, aluminum and dissolved organic carbon mass transfer coefficients in lakes. *Water Research,* 37: 1759-1768.

Moore, T. R., Matos, L., and Roulet, N. T., 2003: Dynamics and chemistry of dissolved organic carbon in precambrian shield catchments and an impounded wetland. *Canadian Journal of Fisheries and Aquatic Sciences,* 60: 612-623.

Moran, M. A., Wicks, R. J., and Hodson, R. E., 1991: Export of Dissolved Organic-Matter from a Mangrove Swamp Ecosystem - Evidence from Natural Fluorescence, Dissolved Lignin Phenols, and Bacterial Secondary Production. *Marine Ecology-Progress Series,* 76: 175-184.

Moran, M. A. and Zepp, R. G., 1997: Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. *Limnology and Oceanography,* 42: 1307-1316.

Obernosterer, I. and Benner, R., 2004: Competition between biological and photochemical processes in the mineralization of dissolved organic carbon. *Limnology and Oceanography,* 49: 117-124.

Opsahl, S. and Benner, R., 1998: Photochemical Reactivity of Dissolved Lignin in River and Ocean Waters. *Limnology and Oceanography,* 43: 1297-1304.

Opsahl, S. P. and Zepp, R. G., 2001: Photochemically-induced alteration of stable carbon isotope ratios (delta C-13) in terrigenous dissolved organic carbon. *Geophysical Research Letters,* 28: 2417-2420.

Osburn, C. L., Morris, D. P., Thorn, K. A., and Moeller, R. E., 2001: Chemical and optical changes in freshwater dissolved organic matter exposed to solar radiation. *Biogeochemistry,* 54: 251-278.

Osburn, C. L., O'Sullivan, D. W., and Boyd, T. J., 2009: Increases in the longwave photobleaching of chromophoric dissolved organic matter in coastal waters. *Limnology and Oceanography,* 54: 145-159.

Pastor, J., Solin, J., Bridgham, S. D., Updegraff, K., Harth, C., Weishampel, P., and Dewey, B., 2003: Global warming and the export of dissolved organic carbon from boreal peatlands. *Oikos,* 100: 380-386.

Pelster, D., Burke, J. M., Couling, K., Luke, S. H., Smith, D. W., and Prepas, E. E., 2008: Water and nutrient inputs, outputs, and storage in Canadian boreal forest wetlands: a review. *Journal of Environmental Engineering and Science,* 7: 35-50.

Peterson, B. J., Wollheim, W. M., Mulholland, P. J., Webster, J. R., Meyer, J. L., Tank, J. L., Marti, E., Bowden, W. B., Valett, H. M., Hershey, A. E., McDowell, W. H., Dodds, W. K., Hamilton, S. K., Gregory, S., and Morrall, D. D., 2001: Control of nitrogen export from watersheds by headwater streams. *Science,* 292: 86-90.

Piirainen, S., Finer, L., Mannerkoski, H., and Starr, M., 2002: Effects of forest clear-cutting on the carbon and nitrogen fluxes through podzolic soil horizons. *Plant and Soil,* 239: 301-311.

Rantakari, M. and Kortelainen, P., 2005: Interannual variation and climatic regulation of the CO2 emission from large boreal lakes. *Global Change Biology,* 11: 1368-1380.

Richardson, J. S., Naiman, R. J., Swanson, F. J., and Hibbs, D. E., 2005: Riparian communities associated with Pacific Northwest headwater streams: Assemblages, processes, and uniqueness. *Journal of the American Water Resources Association,* 41: 935-947.

Sarpal, R. S., Mopper, K., and Kieber, D. J., 1995: Absorbance properties of dissolved organic matter in antarctic sea water. *Antarctic Journal of the United States,* 30: 139-140.

Schiff, S., Aravena, R., Mewhinney, E., Elgood, R., Warner, B., Dillon, P., and Trumbore, S., 1998: Precambrian shield wetlands: Hydrologic control of the sources and export of dissolved organic matter. *Climatic Change,* 40: 167-188.

Schiff, S. L., Aravena, R., Trumbore, S. E., Hinton, M. J., Elgood, R., and Dillon, P. J., 1997: Export of DOC from forested catchments on the Precambrian Shield of Central Ontario: Clues from C-13 and C-14. *Biogeochemistry,* 36: 43-65.

Schindler, D. W., Curtis, P. J., Bayley, S. E., Parker, B. R., Beaty, K. G., and Stainton, M. P., 1997: Climate-induced changes in the dissolved organic carbon budgets of boreal lakes. *Biogeochemistry,* 36: 9-28.

Schumacher, M., Christl, I., Vogt, R. D., Barmettler, K., Jacobsen, C., and Kretzschmar, R., 2006: Chemical composition of aquatic dissolved organic matter in five boreal forest catchments sampled in spring and fall seasons. *Biogeochemistry,* 80: 263-275.

Sobek, S., Tranvik, L. J., and Cole, J. J., 2005: Temperature independence of carbon dioxide supersaturation in global lakes. *Global Biogeochemical Cycles,* 19: GB2003.

Sobek, S., Tranvik, L. J., Prairie, Y. T., Kortelainen, P., and Cole, J. J., 2007: Patterns and regulation of dissolved organic carbon: An analysis of 7,500 widely distributed lakes. *Limnology and Oceanography,* 52: 1208-1219.

Soumis, N., Lucotte, M., Larose, C., Veillette, F., and Canuel, R., 2007: Photomineralization in a boreal hydroelectric reservoir: a comparison with natural aquatic ecosystems. *Biogeochemistry,* 86: 123-135.

Southwell, M. W., Mead, R. N., Luquire, C. M., Barbera, A., Avery, G. B., Kieber, R. J., and Skrabal, S. A., 2011: Influence of organic matter source and diagenetic state on photochemical release of dissolved organic matter and nutrients from resuspendable estuarine sediments. *Marine Chemistry,* In Press, Accepted Manuscript.

Spencer, R. G. M., Bolton, L., and Baker, A., 2007: Freeze/thaw and pH effects on freshwater dissolved organic matter fluorescence and absorbance properties from a number of UK locations. *Water Research,* 41: 2941-2950.

Spencer, R. G. M., Stubbins, A., Hernes, P. J., Baker, A., Mopper, K., Aufdenkampe, A. K., Dyda, R. Y., Mwamba, V. L., Mangangu, A. M., Wabakanghanzi, J. N., and Six, J., 2009: Photochemical degradation of dissolved organic matter and dissolved lignin phenols from the Congo River. *Journal of Geophysical Research-Biogeosciences,* 114: G03010.

Sulzberger, B. and Durisch-Kaiser, E., 2009: Chemical characterization of dissolved organic matter (DOM): A prerequisite for understanding UV-induced changes of DOM absorption properties and bioavailability. *Aquatic Sciences,* 71: 104-126.

Sulzman, E. W., 2007: Stable isotope chemistry and measurement: a primer. *Stable Isotopes in Ecology and Environmental Science. Ecological Methods and Concepts.,* 2nd edition: 1-21.

Teodoru, C. R., del Giorgio, P. A., Prairie, Y. T., and Camire, M., 2009: Patterns in pCO2 in boreal streams and rivers of northern Quebec, Canada. *Global Biogeochemical Cycles,* 23: GB2012, doi:2010.1029/2008GB003404.

Tranvik, L. J., Olofsson, H., and Bertilsson, S., 1999: Photochemical Effects on Bacterial Degradation of Dissolved Organic Matter in Lake Water. *Microbial Biosystems: New Frontiers. Proceedings of the 8th International Symposium on Microbial Ecology. Atlantic Canada Society for Microbial Ecology, Halifax, Canada, 1999.*

Twardowski, M. S., Boss, E., Sullivan, J. M., and Donaghay, P. L., 2004: Modeling the spectral shape of absorption by chromophoric dissolved organic matter. *Marine Chemistry,* 89: 69-88.

Tzortziou, M., Osburn, C. L., and Neale, P. J., 2007: Photobleaching of dissolved organic material from a tidal marsh-estuarine system of the Chesapeake Bay. *Photochemistry and Photobiology,* 83: 782-792.

Ugolini, F. C., Reanier, R. E., Rau, G. H., and Hedges, J. I., 1981: Pedological, Isotopic, and Geochemical Investigations of the Soils At the Boreal Forest and Alpine Tundra Transition in Northern Alaska. *Soil Science,* 131: 359-374.

Vähätalo, A. V., Salonen, K., Munster, U., Jarvinen, M., and Wetzel, R. G., 2003: Photochemical transformation of allochthonous organic matter provides bioavailable nutrients in a humic lake. *Archiv Fur Hydrobiologie,* 156: 287-314.

Vähätalo, A. V. and Jarvinen, M., 2007: Photochemically produced bioavailable nitrogen from biologically recalcitrant dissolved organic matter stimulates production of a nitrogen-limited microbial food web in the Baltic Sea. *Limnology and Oceanography,* 52: 132-143.

Vähätalo, A. V. and Wetzel, R. G., 2008: Long-term photochemical and microbial decomposition of wetland-derived dissolved organic matter with alteration of 13C:12C mass ratio. *Limnology and Oceanography,* 53: 1387-1392.

Vione, D., Khanra, S., Man, S. C., Maddigapu, P. R., Das, R., Arsene, C., Olariu, R.-I., Maurino, V., and Minero, C., 2009: Inhibition vs. enhancement of the nitrate-induced phototransformation of organic substrates by the •OH scavengers bicarbonate and carbonate. *Water Research,* 43: 4718-4728.

Vodacek, A., Blough, N. V., DeGrandpre, M. D., Peltzer, E. T., and Nelson, R. K., 1997: Seasonal variation of CDOM and DOC in the Middle Atlantic Bight: Terrestrial inputs and photooxidation. *Limnology and Oceanography,* 42: 674-686.

Vogler, E. A. and Hayes, J. M., 1980: Carbon isotopic compositions of carboxyl groups of biosynthesized fatty acids. *Physics and Chemistry of The Earth,* 12: 697-704.

Waiser, M. J. and Robarts, R. D., 2004: Photodegradation of DOC in a shallow prairie wetland: evidence from seasonal changes in DOC optical properties and chemical characteristics. *Biogeochemistry,* 69: 263-284.

Waite, T. D. and Morel, F. M. M., 1984: Ligand exchange and fluorescence quenching studies of the fulvic acid-iron interaction: Effects of ph and light. *Analytica Chimica Acta,* 162: 263-274.

Whitehead, R. F., de Mora, S., Demers, S., Gosselin, M., Monfort, P., and Mostajir, B., 2000: Interactions of ultraviolet-B radiation, mixing, and biological activity on photobleaching of natural chromophoric dissolved organic matter: A mesocosm study. *Limnology and Oceanography,* 45: 278-291.

Williams, C. J., Yavitt, J. B., Wieder, R. K., and Cleavitt, N. L., 1998: Cupric oxide oxidation products of northern peat and peat forming plants. *Canadian Journal of Botany,* 76: 51-62.

Williams, C. J. and Yavitt, J. B., 2003: Botanical composition of peat and degree of peat decomposition in three temperate peatlands. *Ecoscience,* 10: 85-95.

Wipfli, M. S., Richardson, J. S., and Naiman, R. J., 2007: Ecological Linkages Between Headwaters and Downstream Ecosystems: Transport of Organic Matter, Invertebrates, and Wood Down Headwater Channels1. *JAWRA Journal of the American Water Resources Association,* 43: 72-85.

WQMA, 2000: Iron Contours Based on Canada-Newfoundland Water Quality Monitoring Agreement Data. *Canada-Newfoundland Water Quality Monitoring Agreement; Environment Canada & Government of Newfoundland and Labrador (Department of Environment and Conserservation - Water Resources Management Division): http://www.env.gov.nl.ca/env/waterres/quality/background/Fe.pdf*.

Wu, F. C., Mills, R. B., Cai, Y. R., Evans, R. D., and Dillon, P. J., 2005: Photodegradation-induced changes in dissolved organic matter in acidic waters. *Canadian Journal of Fisheries and Aquatic Sciences,* 62: 1019-1027.

Xie, H. X., Zafiriou, O. C., Cai, W. J., Zepp, R. G., and Wang, Y. C., 2004: Photooxidation and its effects on the carboxyl content of dissolved organic matter in two coastal rivers in the Southeastern United States. *Environmental Science & Technology,* 38: 4113-4119.

Zar, J. H., 1996: Biostatistical Analysis. *Prentice Hall, 3rd edition*: 662 p.

Zepp, R. G., Erickson, D. J., Paul, N. D., and Sulzberger, B., 2011: Effects of solar UV radiation and climate change on biogeochemical cycling: interactions and feedbacks. *Photochemical & Photobiological Sciences,* 10: 261-279.

Ziegler, S. E. and Fogel, M. L., 2003: Seasonal and diel relationships between the isotopic compositions of dissolved and particulate organic matter in freshwater ecosystems. *Biogeochemistry,* 64: 25-52.

Chapter 2: Mineralization of dissolved organic matter by heterotrophic stream biofilm communities in a large boreal catchment

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Key words

Dissolved organic matter (DOM), heterotrophic biofilm communities, DOM source and composition, boreal headwater streams, nutrient availability, priming effect.

Summary

1) Boreal headwater streams typically receive large contributions of dissolved organic matter (DOM) from wetland and forest soils, and can exhibit high CO_2 effluxes, but little is known about how stream biofilm respiration of DOM responds to carbon and nutrient substrates in boreal catchments.

2) The aim of this study was to assess (a) the response of heterotrophic biofilms to increased availability of labile carbon (C), nitrogen (N), and phosphorus (P), and (b) how this response varies across sites differing in the source of catchment organic matter.

3) Normalized biofilm respiration rates ($R_{Biofilm}$) of dissolved organic carbon (DOC) of DOM sources derived from wetland, deciduous forest, and ponds were up to 10 times greater than for coniferous forest DOM. Experimental additions of bog and pond-derived DOM stimulated $R_{Biofilm}$ in coniferous forest sites (1.5 to 2.5 times), adding further weight to the evidence for differing responses to stream DOM sources in this boreal catchment.

4) Mineralization of added glucose by the biofilms was only increased by added N and P, coinciding with a reduction in mineralization of the extant stream DOM. These findings suggest that increases in labile C, N, and P can reduce biofilm mineralization of stream dissolved organic nitrogen and phosphorus, perhaps due to the greater energy required to access the more complex DOM.

5) In 15 of 19 experiments, the addition of glucose had no effect on biofilm mineralization of stream DOC (R_{DOC}). In the presence of added N and P, however, R_{DOC} decreased by 30 to 38 % with the addition of glucose at sites with the most active biofilms in summer. When glucose was added alone, conversely, R_{DOC} was

stimulated (40 – 50 %) at these same sites in autumn, when biofilm respiration was lower and DOM more aromatic and less carbohydrate-rich. Combined, these results suggest labile C sources, such as algal exudates, may (a) compete as a source of energy and/or stimulate the incorporation rather than mineralization of the more N and P-rich stream DOM, or (b) stimulate the mineralization of stream DOM, potentially depending upon nutrient availability and the composition of stream DOM.

2.1 Introduction

Understanding the nutrient and organic matter dynamics of boreal headwater streams is essential to our ability to decipher linkages across terrestrial-aquatic boundaries and gain a predictive understanding of how this linkage may change with climate and other anthropogenic landscape changes. In particular, shifts in organic matter composition, production, and export from terrestrial sources into aquatic ecosystems are likely consequences of changes in temperature, catchment vegetation regime, and in the frequency and intensity of precipitation (Clair and Ehrman, 1996; Schindler et al., 1996; Schiff et al., 1998; Pastor et al., 2003). Such changes may already be underway in boreal or high-latitude catchments, as indicated by increasing organic carbon loadings in the rivers of these regions (Roulet and Moore, 2006).

Small headwater streams integrate the terrestrial and aquatic biogeochemical cycling of elements such as carbon (C), nitrogen (N), and phosphorus (P), and represent the first locations in catchments that are affected by landscape changes (Peterson et al., 2001; Alexander et al., 2007; Freeman et al., 2007). Low-order streams typically receive a large input of terrestrially derived or allochthonous dissolved organic matter (DOM) (Fisher and Likens, 1973), particularly in boreal regions (Aitkenhead and McDowell, 2000; Pastor et al., 2003). Further, these headwater streams exhibit a high active microbial surface area, typically as epilithic or sediment surface biofilms (Battin et al., 2003b; Hoellein et al., 2010), relative to water volume, and typically represent the most biogeochemically active components of the larger catchment (Peterson et al., 2001). Such biogeochemical processing in boreal streams is largely driven by heterotrophic biofilm microbial communities due to high DOM concentrations, limited light availability, and low rates of primary production in these ecosystems (Tranvik, 1988, 1989; Jansson et al., 2000; Jonsson et al., 2001; Agren et al., 2008). In boreal landscapes in particular,

such attributes may contribute in part to high stream CO_2 effluxes, representing a potentially significant loss of C from the landscape (Teodoru et al., 2009).

Changes in landscape composition caused by climate change or anthropogenic impacts often lead to increases in inorganic N and P levels in the aquatic environment (Schindler et al., 1996; Mattsson et al., 2005; Kreutzweiser et al., 2008). Such changes may not only increase more labile, autochthonous sources of DOM (Mallin et al., 2004), but potentially alter the fate of both autochthonous and allochthonous DOM through interactive effects on microbial activity, particularly in boreal streams. Mineralization of labile C sources by stream biofilms is typically linked to N and P availability with increased inorganic N supply often increasing the microbial demand for labile C (Reche et al., 1998; Gulis and Suberkropp, 2003; Stelzer et al., 2003). Congruent with this, increased labile C sources have been found to lead to microbial depletion of either N or P or both (Francoeur, 2001; Bernhardt and Likens, 2002; Tank and Dodds, 2003). In low nutrient streams, where organic forms of N and P may dominate, biofilms may be able to extract energy from new labile C sources when inorganic nutrients are increased and use this energy to form biomass while inhibiting the mineralization of extant DOM (Kaushal and Lewis, 2005). Similarly to results from temperate streams (Bernhardt and Likens, 2002; Ziegler and Brisco, 2004; Lane et al., 2012), heterotrophic microbial response to catchment nutrient enrichment may become limited by bioavailable C in boreal streams despite the elevated DOM typical of these systems. In fact, microbial use of extant DOM may be inhibited by increases in labile C, N, and P sources in boreal streams.

Variation in microbial growth efficiencies suggest that mineralization of extant DOM in response to new labile substrates probably also depends on nutrient availability and DOM source or composition (del Giorgio and Cole, 1998; Smith and Prairie, 2004;

Berggren et al., 2007). In boreal landscapes, the growth efficiency of bacterioplankton can vary with catchment composition and nutrient status (Berggren et al., 2007), suggesting that both DOM source or composition and nutrient availability may regulate the mineralization or fate of DOM in these landscapes. Dissolved organic nitrogen (DON) is often the major form of N in boreal catchments (Stepanauskas et al., 2000; Moore, 2009) and when inorganic nutrients are in low concentration heterotrophic biofilms may be more adept at utilizing organic forms of N and P. As such, DON, and possibly P, can play a specific role in regulating organic matter mineralization (Neff et al., 2003; Kaushal and Lewis, 2005).

Inorganic nutrient concentrations can greatly affect biomass and processes in stream biofilms in temperate streams (Guasch et al., 1995; Romani et al., 2004; Lyon and Ziegler, 2009; Hoellein et al., 2010) and may have an even more significant, and probably different, influence on boreal stream biogeochemistry, given their allochthonous nature. In temperate streams, increased C and inorganic nutrient contributions have been shown to result in enhanced microbial metabolism and the release of organic nutrients, which may lead to altered downstream nutrient transport (Findlay and Sinsabaugh, 2003) and changes in the resilience and biological stability of the ecosystem (Wetzel, 2001). For example, the availability of nutrients or production of labile algal-derived C may increase the degradation of organic matter that turns over more slowly, a process analogous to the priming effect observed in soils (Kuzyakov, 2010). To date, the causes, mechanisms and presence of the priming effect in aquatic ecosystems remain poorly understood but may in fact contribute to CO_2 emissions from allochthonous organic matter (Guenet et al., 2010; Bianchi, 2011), particularly in boreal aquatic ecosystems.

Studies conducted in boreal catchments have revealed variation in microbial respiration, growth and production in relation to location (e.g. ponds, lakes) in a catchment and/or organic matter source (e.g. algal sources, leaf leachates) (Findlay et al., 1986; Meyer et al., 1987; Jonsson et al., 2001; Berggren et al., 2009b). Such studies have typically focused on planktonic communities (Tranvik, 1988, 1989; Jansson et al., 2000; Anesio et al., 2005; Haukka et al., 2005), whereas factors regulating heterotrophic biofilm mineralization of organic matter within boreal headwater streams have been assessed to a more limited extent (Paul et al., 1991; Fischer et al., 2009). Such knowledge about stream biofilm activity, and specifically DOM mineralization, however, is critical for determining the fate of DOM and how boreal catchments will respond and adapt to environmental change and alterations to aquatic-terrestrial connectivity, including altered contributions from ponds and wetlands (Schiff et al., 1998; Davidson and Janssens, 2006; Laine et al., 2009).

This study was designed to assess (1) the response of boreal stream biofilm DOM mineralization to increased labile C, N, and P availability and (2) how this response varies across streams of contrasting catchment composition. Artificial substrates (glucose, ammonium nitrate, and potassium phosphate) were added to directly test how they influenced microbial respiration of stream DOM in short-term dark bottle incubations of biofilm-colonized tiles. Direct measurement of mineralization of the added labile C mineralized separately from the mineralization of the extant stream DOM was accomplished using ^{13}C-labeled glucose-C. Subsequent analysis of stable carbon isotopic composition (δ^{13}C) of the respired C, therefore, enabled us to test for priming effects on stream DOM mineralization. Stream water and biofilm-colonized tiles used in these experiments were collected from contrasting sites to provide a range in catchment composition (e.g. vegetation cover varying from forests to wetlands and including a pond

site), but in the absence of significant human influences and associated nutrient enrichment. Additionally, natural DOM concentrates isolated from a peatland bog and headwater pond were used in a subset of these addition experiments to more directly test these sources, which have the potential to regulate variation in biofilm respiration across the study sites.

2.2 Methods

2.2.1 Study sites

Substrate addition experiments were conducted on DOM samples collected from nine study sites in four sub-catchments located within the Humber River Basin (HRB) in western Newfoundland, Canada (Fig. 2.1). This large boreal catchment (total area: 10923 km^2) provides sites that vary in catchment organic matter source, as determined from catchment vegetation in the absence of significant human influences. The ArcGIS (ArcGIS 10; ESRI, Redlands, CA, USA) layers used to select the study sites were obtained from the LandSat 5 Thematic Mapper Satellite 2007 database provided by the Canadian Forest Service and included vegetation cover and type, soil type, geology, water bodies, and roads for the entire HRB. Within the HRB, 416 first to third order stream catchments were delineated using RiverTools (RiverTools 3.0; Rivix, LLC, Broomfield, CO, USA). Sites were selected based on accessibility and vegetation cover and included (a) two headwater streams draining wetland-dominated catchments, (b) two headwater streams draining primarily coniferous forest but with some variation in wetland coverage, and (c) one headwater stream representing a high deciduous forest coverage for the HRB (Table 2.1). The substrate addition experiments were conducted at or close to baseflow (see Appendix Table A2.1 for discharge) and consisted of two parts: (a) Labile substrate addition experiments at upstream (1) and downstream (2) sites of Big Falls Brook (BFB-1 and BFB-2) in 2008, and at Crooked Feeder (CF-1 and CF-2), Pynn's Brook (PB-1 and PB-2), and Gillams Brook (GB-1 and GB-2) in 2009, and (b) ambient DOM addition experiments at the contrasting CF and PB sites in 2010 (Fig. 2.2).

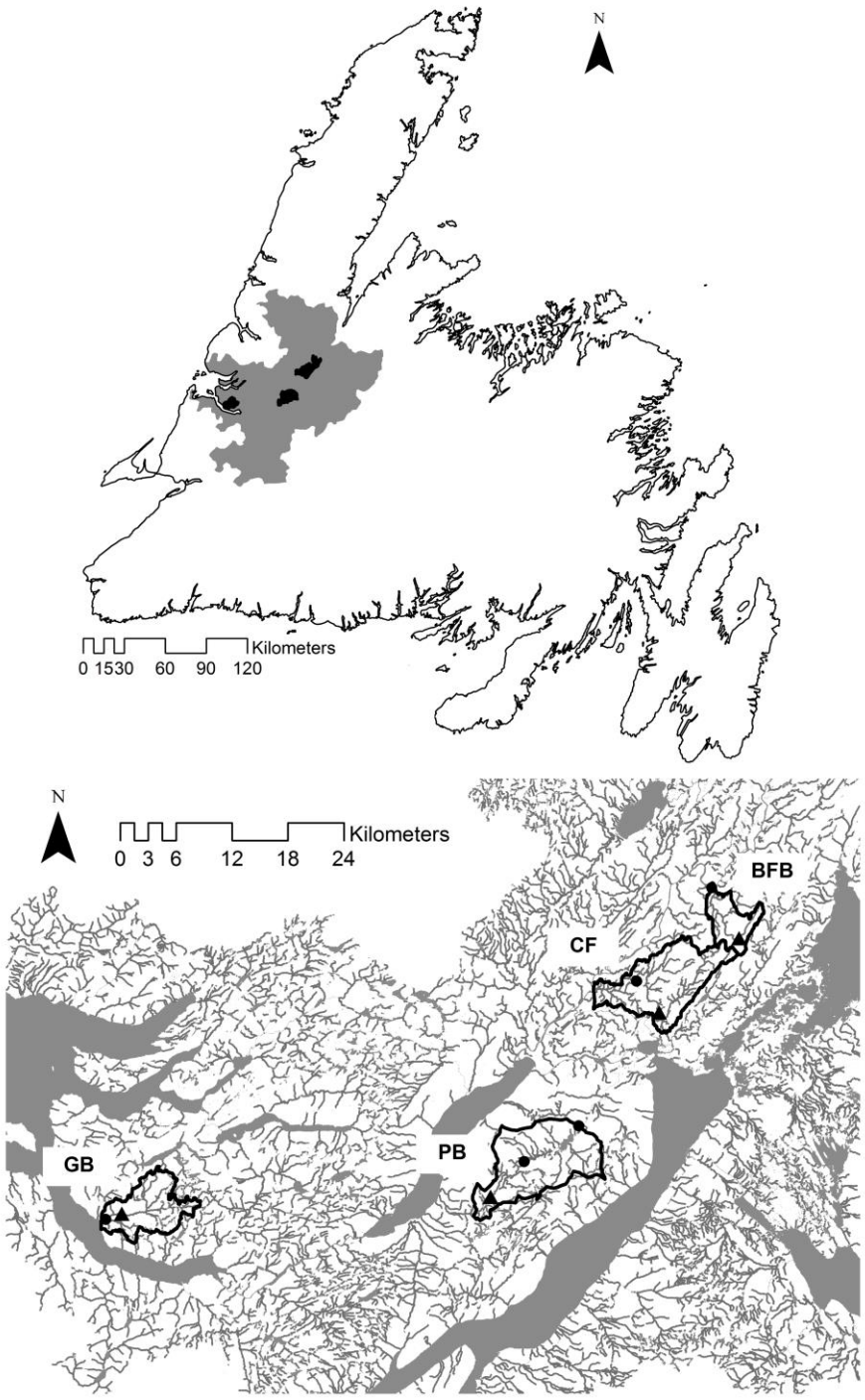


Figure 2.1: The delineation of the Humber River catchment both as situated in western Newfoundland, Canada (above), and shown in part in a larger scale (below) to illustrate the region and location of the study stream sites within the catchments of Crooked Feeder (CF), Big Falls Brook (BFB), Pynn's Brook (PB), and Gillams Brook (GB). A minimum of two study sites, representing upstream (triangle) and downstream (circle), were chosen for each catchment.

Table 2.1: Description of the study sites in the Humber River Basin (HRB), including intra-site location and abbreviation (Abbr.), estimated catchment area, distance of the intra-site location from the headwaters of each catchment, catchment vegetation cover and composition of the vegetation of each catchment given as the percentage of total cover.

Site	Intra-site location	Abbr.	Catchment area (km^2)	Distance of site location from head-waters (km)	Catchment vegetation cover	Catchment composition
Crooked Feeder	upstream downstream	CF-1 CF-2	25.9 56.9	6.9 14.9	75 % forest 25 % wetland*	Peatland bogs Black spruce (*Picea mariana*, 25 %) Balsam fir (*Abies balsamea*, 8 %) Coniferous (22 %) and deciduous shrubs (6 %)
Big Falls Brook	upstream downstream	BFB-1 BFB-2	4.6 23.5	2.3 12.3	80 % forest 20 % wetland*	Peatland bogs Black spruce (*Picea mariana*, 13 %) Balsam fir (*Abies balsamea*, 30 %) Coniferous (26 %) and deciduous shrubs (2 %)
Pynn's Brook	upstream downstream downstream	PB-1** PB-2 PB-3	6.6 28.1 83.6	2.8 10.8 17.9	80 % forest <10 % wetland	Black spruce (*Picea mariana*, 21 %) Balsam fir (*Abies balsamea*, 45 %) Coniferous (14 %) and deciduous shrubs (7 %)
Gillams Brook	upstream downstream	GB-1*** GB-2	32.3 43.5	8.2 10.4	7 % deciduous forest	White birch (*Betula papyrifera*, >95 %)

* CF and BFB represent relatively high wetland coverage for small accessible catchments in the HRB. The maximum wetland coverage for first through third-order streams in the HRB is 75 %, and only 33 out of all 416 stream catchments exhibit a wetland coverage greater than 25 % (mean coverage of these 33 = 36 ± 11 %) and all of these sites were inaccessible.

** PB-1 is draining directly from a headwater pond.

*** GB-1 was chosen to contrast with the coniferous-dominated forests typical of this watershed and is located within the largest patch of deciduous forest (1.8 km^2) almost exclusively (>95 %) comprised of white birch in this catchment. Of the catchments in the HRB that exhibit deciduous forest cover, <10 % have greater deciduous forest coverage than found in GB. Those catchments with greater deciduous forest cover were found exclusively on carbonate-rich geology, which changed the water chemistry significantly enough to render them incomparable to our coniferous forest sites.

2.2.2 Heterotrophic biofilm colonization

Heterotrophic activity, measured as dark biofilm community level respiration, was used to assess how the microbial mineralization of DOM varies among the sites studied. Pre-combusted (500°C) ceramic tiles (10 x 47 x 5 mm, ~15 cm^2 surface area), serving as solid substrate for heterotrophic biofilm colonization, were deployed at stream sites during two seasons (summer and autumn) in each of the four stream catchments (CF, BFB, PB, and GB) within the Humber River Basin. To select for the growth of heterotrophic microbial populations, tiles were incubated on racks held in open-ended, 10 cm diameter black PVC tubes that were anchored midstream at each of the stream sites and oriented in such a way to allow ambient stream flow. The tiles were fixed on the rack such that they were always at least 10 cm in from the PVC tube opening, however, complete omission of phototrophic microbial organisms cannot be guaranteed. The colonized tiles were collected within 10 to 12 weeks following their deployment. Substrate addition experiments, utilizing the heterotrophic biofilm colonized tiles at each site, were conducted in replicate (n = 5) 300 mL biological oxygen demand (BOD) bottles. Bottles were filled with stream water from a single carboy collected from each site and incubated on site for 24 hours in the dark with two colonized tiles per bottle (total biofilm surface area of ~30 cm^2). Unfiltered water was used to avoid large changes in dissolved oxygen (DO) concentrations associated with filtration. Initial experiments used to test this design were conducted with only stream water in CF, PB, and BFB, where respiration rates were found to represent <2 % of those rates measured with tiles (data not shown). The summer 2008 (BFB) and 2009 (PB, CF) experiments as well as the autumn 2008 (BFB) experiments were conducted on site. Unfortunately, the summer 2009 experiments at GB were lost due to disturbance while incubating in the stream overnight and cannot be included here. To avoid such field-related disturbance, the

samples from autumn 2009 (PB, CF, GB) and summer 2010 (PB, CF) were incubated in the field station in a chiller water bath with recirculating water of similar DO levels from a larger reservoir (NesLab, Thermo Scientific, Waltham, MA, USA) with temperature maintained within 1°C of stream water temperature at the time of collection.

Tiles from each experiment were carefully collected and stored separately in individual re-sealable plastic bags, immediately frozen, and freeze-dried. Freeze-dried tiles from each BOD bottle were sonicated separately in 100 mL NanoPure water for approximately 4 hours at room temperature. The water was filtered through a pre-combusted quartz filter (32 mm, 0.45 µM pore size, Whatman) and distributed equally into duplicate, acid-cleaned, pre-combusted 40 mL amber glass vials, spiked with H_3PO_4, and analyzed for dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) with the same NanoPure water used in the extractions as a blank. The quartz filters were freeze-dried and analyzed for C and N content and $\delta^{13}C$.

2.2.3 Labile substrate addition experiments

Substrates were added to assess how stream heterotrophic microbial respiration may be regulated by carbon and nutrient availability. The labile substrates used in these experiments were glucose (C), ammonium nitrate (N), and potassium phosphate (P), added either as single spikes (C, N, and P) or as a combination (CNP and NP; Fig. 2.2) immediately upon filling the BOD bottles with stream water. Additions were made to obtain approximate final concentrations of 83 µM C, 18 µM N, and 3 µM P (ratio 28:6:1), respectively, lower than the C:N:P-ratio typical of bacterial biomass (40:10:1) assuring adequate N and P for C substrate uptake and incorporation. The absolute concentrations used here represented N and P values approximately 5 to 10 times ambient stream concentrations for inorganic N and P and more typical of concentrations

in nutrient-impacted streams in this region (D. Franke, unpublished data). The glucose-C additions resulted in levels roughly 14 to 25 % of ambient stream DOC concentrations; a level deemed adequate enough to stimulate the use of C while remaining relative to common N and P additions. Biofilm respiration rates ($R_{Biofilm}$) were measured as microbial oxygen uptake, comparing treatments to control bottles where no substrates were added. Respiration rates are presented here as carbon consumption in units μM C hr^{-1} assuming a respiratory quotient of 1 (del Giorgio et al., 1997; del Giorgio and Cole, 1998). Additionally, biofilm respiration rates were normalized to DOC concentration (μM C hr^{-1} mM DOC^{-1}), to total biofilm biomass carbon ($C_{biomass}$) incubated (μM C hr^{-1} mM $C_{biomass}^{-1}$), and to both DOC and $C_{biomass}$ (μM C hr^{-1} mM DOC^{-1} mM $C_{biomass}^{-1}$) to assess the variation in DOC and biofilm-specific rates across the study sites investigated.

In addition to assessing the variation in labile C, N, and P limitation of heterotrophic respiration in these biofilms, the influence of labile C, with and without added inorganic N and P, on the mineralization of extant stream DOC (R_{DOC}) was determined by using ^{13}C-labeled glucose (D-Glucose-$^{13}C_6$; Sigma Aldrich, St. Louis, MO, USA). The glucose solution used in these experiments had a $\delta^{13}C$ value of 551 ‰ allowing us to distinguish it from ambient DOC. Changes in the $\delta^{13}C$ of the total inorganic carbon (TIC) were measured and used to calculate the rate of glucose-C respired ($R_{Glucose}$; μM C hr^{-1}) during the incubation using the following equation:

$$R_{Glucose} = \frac{[TIC_{final}]}{t} \times \frac{\delta^{13}C_{TIC-final} - \delta^{13}C_{TIC-initial}}{\delta^{13}C_{Glucose} - \delta^{13}C_{TIC-initial}} \quad \text{(eq. 1)}$$

where $[TIC_{final}]$ is the final TIC concentration (μM C), t is the incubation time (hrs), $\delta^{13}C_{TIC-initial/final}$ are the stable inorganic carbon isotopic values (‰) at the beginning and the end of the incubation, respectively, and $\delta^{13}C_{Glucose}$ is the $\delta^{13}C$ (551 ‰) of the glucose spike. Results were compared to respiration rates from the treatments without glucose

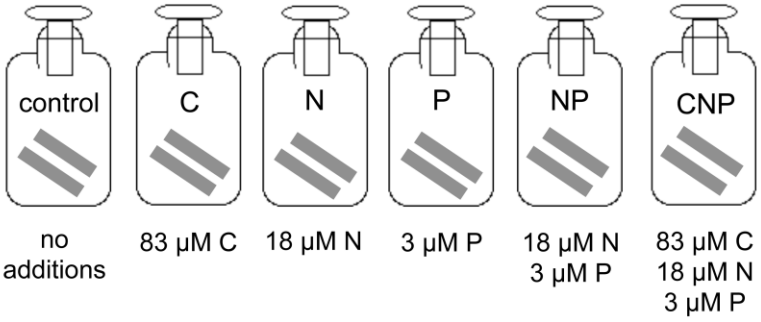
additions ($R_{control}$) to investigate whether glucose stimulated biofilm respiration. In those cases where glucose caused an increase in total respiration (R_{total}), the respiration of extant stream DOC (R_{DOC}) was calculated as the difference between R_{total} and $R_{Glucose}$ in the glucose-treated incubations. R_{DOC} was then compared with $R_{control}$ to determine if respiration of stream DOC increased as a result of the glucose treatment. More specifically, we used these data to determine whether the added glucose competed with stream DOC as a source of energy or enabled a priming effect that stimulated the mineralization of stream DOC.

2.2.4 Ambient DOM addition experiments

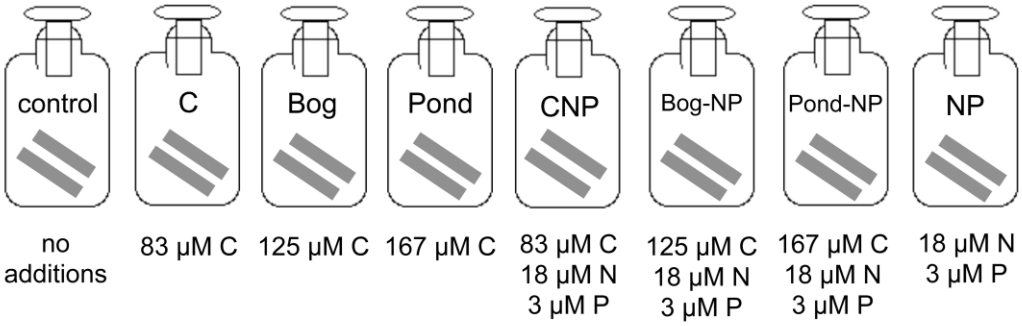
To more directly assess the difference in biofilm respiration of pond and peatland bog DOM sources in the Humber River Basin, additional experiments were conducted at sites (PB-1, PB-2, PB-3, CF-1, CF-2) that exhibited significantly different levels of biofilm respiration. Concentrates of DOM (Fig. 2.2), isolated from the headwater pond immediately upstream of PB-1 and a peatland bog located in the BFB catchment, were included in an additional set of labile substrate addition experiments as described above. Samples of these two sources were collected one day prior to the experiments from the headwater pond and the peatland bog and immediately pre-filtered through 0.2 µm polypropylene filters, and then concentrated using a RealSoft PROS/2S reverse osmosis (RO) system with two Filmtec TW30-4021 membranes (GEA Process Engineering Inc., Columbia, MD, USA). The pond and bog DOM concentrates exhibited a relative carbohydrate content of 38.5 % and 19.4 % of total C resolved and an aromatic to aliphatic (A_R:A_L) carbon ratio of 0.02 and 0.57, respectively, as determined by solid state ^{13}C Cross Polarization Magic Angle Spinning Nuclear Magnetic Resonance (^{13}C {^{1}H} CPMAS NMR). The final C concentration added with the pond and bog DOM

concentrates was 167 µM C and 125 µM C, respectively. Analysis for each individual BOD bottle from each site and treatment confirmed that inorganic nutrient concentrations in these DOM treatments were either below detection (NH_4^+, SRP) or as low as the control samples (NO_3^-; data not shown). Additionally, inorganic N and P were added to achieve the same set of treatments and concentrations as for the glucose additions described above (Fig. 2.2).

In addition to the RO retentates collected and used in the substrate addition experiments, DOM samples were collected at PB, CF, and GB at the same time water collections were made for experimental set-up by concentrating stream water using the same RO technique, and freeze-dried as described above. These samples were analyzed for the A_R:A_L ratio derived from ^{13}C {^1H} CPMAS NMR spectra and for quantifying the total hydrolysable carbohydrate content of the DOM at each site. Also, biofilm material was collected at each stream site by scrubbing rocks with a clean brush. The sample material was rinsed off with NanoPure water, collected in acid-rinsed, brown 500 mL high-density polyethylene (HDPE) bottles, shell-frozen, freeze-dried, and analyzed for $\delta^{13}C$, %C, and %N.



a) Labile substrate addition experiments at BFB in 2008 and at PB, CF, GB in 2009



b) Ambient DOM addition experiments at PB and CF in 2010

Figure 2.2: Illustration of the experimental set-up used in the (a) labile substrate addition experiments conducted at Big Falls Brook (BFB) in 2008 and at Pynn's Brook (PB), Crooked Feeder (CF), and Gillams Brook (GB) in 2009, and (b) ambient dissolved organic matter (DOM) addition experiments at Crooked Feeder and Pynn's Brook in 2010. The labile substrates used in these experiments were glucose (C), ammonium nitrate (N) and potassium phosphate (P), added as 1 mL additions to each bottle to achieve final concentrations of 83 µM C, 18 µM N and 3 µM P, respectively, assuring adequate N and P for C substrate uptake and incorporation. Concentrations used here represent N and P values roughly 5-10 times ambient stream conditions and more typical of concentrations in nutrient-impacted streams in this region. These substrates were added either as single spikes (C, N, and P) or as a combination (CNP and NP) immediately upon filling the bottles with stream water. The final carbon concentration added with the pond and bog DOM concentrates was 167 µM C and 125 µM C, respectively.

2.2.5 Field sampling

Measurements of DO, pH and temperature (T) were taken on site with an YSI 550A DO meter and an YSI 60 pH meter (YSI Incorporated, Yellow Springs, OH, USA), respectively. Dissolved oxygen measurements for the bottle incubations were conducted using an YSI-5100 DO probe with temperature compensation and stirring function (YSI Incorporated, Yellow Springs, OH, USA).

Samples for TIC, DOC, $\delta^{13}C_{TIC}$, and $\delta^{13}C_{DOC}$ analysis were collected from initial and final incubation bottles and dispensed into acid-cleaned, pre-combusted 40 mL amber glass vials and spiked with $HgCl_2$ and H_3PO_4 (HPLC grade), respectively, and stored at 4˚C until analysis within 7 days of collection. Samples for soluble reactive phosphorus (SRP), ammonium (NH_4^+), and nitrate (NO_3^-) were collected in clean 40 mL Falcon tubes and frozen until analysis. Samples for TDN were taken in acid-rinsed 60 mL HDPE Nalgene bottles and frozen until analysis. Samples for total dissolved phosphorus (TDP) analysis were collected in 100 mL amber glass bottles and preserved with sulphuric acid and stored at 4°C. Samples for absorbance measurements were taken in dark 60 mL HDPE Nalgene bottles and preserved with $HgCl_2$ and stored at 4°C until analysis. All samples for DOC, SRP, NH_4^+, NO_3^-, TDN, and TDP quantification, as well as absorbance measurements were filtered through a pre-combusted microfiber glass filter (GF/F; Whatman) upon collection.

2.2.6 Analytical methods

Total inorganic carbon and DOC concentration and their $\delta^{13}C$ values were determined at the Memorial University Stable Isotope Laboratory using an AURORA 1030 TOC analyzer (O.I. Analytical, College Station, Texas, USA) coupled to a MAT252

isotope ratio mass spectrometer (Finnigan, Bremen, Germany). Values for $\delta^{13}C$ are reported in per mil units (‰) using the international reference standard Vienna Pee Dee Belemnite (R = 0.011237) (Sulzman, 2007). The analytical precision was <0.4 ‰ for $\delta^{13}C_{TIC}$ and <0.2 ‰ for $\delta^{13}C_{DOC}$, and the maximum percent coefficient of variation was 1.1 % for TIC and 0.6 % for DOC concentrations.

Total hydrolysable carbohydrate content was determined using sulphuric acid hydrolysis (Pakulski and Benner, 1992) and the MBTH (3-methyl-2-benzothiazolinone hydrochloride) method (Johnson and Sieburth, 1977) with glucose standards (D-$C_6H_{12}O_6$; Sigma Aldrich, St. Louis, MO, USA). Glucose equivalents as glucose-C was then used to express the proportion of carbohydrate-C in the DOM samples as a percent of total dissolved organic carbon. Aromatic to aliphatic (A_R:A_L) carbon ratios were determined from spectra derived from ^{13}C {^1H} CPMAS NMR spectroscopy using a Bruker AVANCE II 600 MHZ. These analyses were conducted using frequencies of 600.33 MHz for ^1H and for ^{13}C, and a Bruker 3.2 mm MAS triple-tuned probe (H/F/C; Milton, ON, Canada). Samples were spun at 20 kHz and the temperature was maintained constant at 298 K. All experiments had a contact time of 2 ms and the Hartmann-Hahn condition was set at 62.5 kHz. 71680 scans were collected with a 2 s recycling delay. ^{13}C chemical shifts were referenced to TMS with adamantane as an external secondary reference. Based on repeated scans of the same sample and repeated fit calculations to test the reproducibility of results, it was determined that a variation of 10 % in the relative contribution of functional groups or their ratios is significantly different.

Soluble reactive P and NH_4^+ concentrations were analyzed with a Lachat FIA 8500 inorganic nutrient analyzer (HACH, Loveland, CO, USA), using the molybdate blue colorimetric method (detection limit 0.32 µM P) and phenol-hypochlorite method

(detection limit 0.59 µM N), respectively. Nitrate samples were analyzed with a DX-100 ion chromatograph (detection limit 0.057 µM N; DIONEX, Sunnyvale, CA, USA). Total dissolved N concentrations were determined using a high temperature combustion total organic carbon analyzer (Shimadzu TOC-V) with a chemiluminescent NO_x detector (TNM-1; Shimadzu, Japan). Dissolved organic N was estimated by the difference between TDN and inorganic nitrogen (NH_4^+, NO_3^-). Molar C:N ratios of the DOM were calculated by dividing DOC by DON with both in µM units. Total dissolved P was determined by Maxxam Analytics (Bedford, NS, Canada) using the antimony-phosphomolybdate calorimetric method (detection limit 0.64 µM P).

Absorbance measurements were performed at wavelengths spanning 200 to 800 nm using a UV/VIS scanning spectrometer (Lambda 25; Perkin Elmer, Waltham, MA, USA) and a 1 cm quartz cuvette, with NanoPure water as a blank. Results were used to calculate DOC normalized absorption coefficients a_{254}^* and a_{350}^* (Green and Blough, 1994), absorption ratios a_{254}:a_{350}, as well as spectral slopes (S) for the wavelength intervals 275 to 295 nm ($S_{275-295}$) and 350 to 400 nm ($S_{350-400}$) (Helms et al., 2008).

Freeze-dried biofilm and tile biomass samples were analyzed for elemental (%C, %N) and stable carbon isotope composition ($\delta^{13}C$) at the Memorial University Stable Isotope Laboratory using a Carlo Erba NA1500 Series II elemental analyzer (Milan, Italy) interfaced with a Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The analytical precision for the $\delta^{13}C$ was <0.2 ‰, and <1.8 and <3.7 % for the C and N content, respectively.

2.2.7 Statistical analyses

Significance tests (t-tests) were conducted between initial, control, and final samples ($\alpha = 0.05$; AnalystSoft Inc., StatPlus:mac - Version 2009) to ascertain differences in DO, TIC, DOC, and nutrient concentrations. Non-significant changes were considered to be below detection. To test differences in respiration rates among the individual treatments, multiple comparisons were conducted using one-way analyses of variation (ANOVA) followed by post-hoc Tukey's Honestly Significant Difference (THSD) tests (family wise error of $\alpha = 0.05$).

Water quality parameters, DOM, and biofilm (biomass) compositional characteristics as well as $R_{Biofilm}$ rates were assessed to determine how they varied with a) stream (site), b) in-stream location (intra-site; i.e. upstream vs. downstream), and c) season (temporal). Generalized linear models (GLM) were constructed to test inter-, intra-site, and temporal effects as well as their interactions (e.g. inter- by intra-site, inter- or intra-site by temporal and inter- by intra-site by temporal; JMP 8.0; SAS, NC, USA). The assumptions of homogeneous and normal error distribution were checked for each GLM by plotting residuals versus fits and by plotting residuals as probability plots (Lindsey, 1997). Respiration rates were not normally distributed and were therefore natural log-transformed (Sokal and Rohlf, 2012) and then found to meet the assumptions required in the GLMs. The data from GB-1, GB-2, and PB-3 were not included in these tests due to a lack of seasonal results from these sites. Further, due to a lack of data for carbohydrates and A_R:A_L ratios for the BFB sites, GLMs could not be constructed for these parameters. The GLMs were followed by a THSD test as a post-hoc test of all possible pair-wise effect comparisons in order to determine which level of each effect was in fact different from which others.

Pearson correlations with an α-level of 0.05 were used to test the potential role of T, DOM composition, and biofilm biomass in regulating $R_{Biofilm}$. These correlations were conducted for potential use in interpreting the GLM results described above. Respiration rates were tested against T, DOC, DON, C:N ratio, DIN, a_{254}, a_{350}, a_{254}:a_{350}, S, and biofilm biomass. Likewise, tile biofilm biomass was tested against stream T, DOC, DON, C:N, DIN, TIC, and DO to investigate whether these parameters related to tile colonization.

2.3 Results

2.3.1 Water quality characteristics

Stream water temperature did not vary significantly among the streams tested (CF, BFB, PB, GB; $p = 0.68$), however, T did vary temporally ($p < 0.01$, $\chi^2 = 14.9$), ranging between 14 and 19°C in summer and between 4 and 8°C in autumn (Table 2.2). Temperature also exhibited an intra-site by temporal effect ($p = 0.01$, $\chi^2 = 6.0$) such that the upstream sites were typically 1.5°C warmer than the downstream locations in summer. This effect was likely due to the more extreme T differences between the landscape surface and groundwater features in the landscape and the greater terrestrial influence on the upstream versus downstream sites.

Dissolved oxygen and TIC levels varied temporally ($p < 0.01$, $\chi^2 = 14.7$ and 15.7, respectively), with DO concentrations ranging from 201 to 374 µM O_2 in summer and from 273 to 527 µM O_2 in autumn, and with TIC concentrations ranging from 360 to 1180 µM C in summer and from 180 to 530 µM C in autumn. The pH values ranged between 5 and 7, being highest at the most wetland-influenced sites (CF) and lowest at the coniferous forest stream sites (PB).

Dissolved inorganic N levels exhibited an inter-site by temporal effect ($p = 0.01$, $\chi^2 = 6.5$). Highest values were recorded at the wetland-influenced sites (CF; Table 2.2). Specifically, NO_3^- concentrations ranged from 1 to 6 µM N for the wetland-influenced study sites (CF, BFB) and from 0.5 to 3 µM N at the forested stream sites (PB) and were considerably higher downstream relative to upstream in all streams. Further, NH_4^+ concentrations were below detection at CF and PB in summer and varied from 1.7 to 3.4 µM N at BFB-1 and BFB-2 in summer, respectively. In autumn, NH_4^+ levels were below detection at PB and GB and ranged between 0.7 and 3.6 µM N at BFB and CF. Total P

and SRP levels were below detection for all sites and seasons, except at CF-1 and CF-2 in autumn 2009 and summer 2010, where SRP levels were between 0.3 and 0.5 µM P.

2.3.2 Dissolved organic matter quantity and composition

Dissolved organic carbon concentrations varied among the study streams (inter-site effect; $p < 0.01$, $\chi^2 = 25.5$). These values ranged from 680 to 1200 µM C at the wetland-influenced sites (CF, BFB), with highest values recorded during the summer months, while samples collected at the forested stream sites (PB, GB) were generally lower and ranged from 320 to 610 µM C, with highest values observed in autumn (Table 2.2). The $\delta^{13}C_{DOC}$ of the DOM varied from -27.1 to -28.6 ‰.

Dissolved organic nitrogen concentrations ranged from 10 to 28 µM N, varying among streams tested (inter-site effect; $p < 0.01$, $\chi^2 = 11.7$), with highest values recorded at the wetland-influenced sites (Table 2.2). The C:N of the DOM varied from 19 to 62 (Table 2.2) and exhibited an inter-site by temporal effect ($p < 0.01$, $\chi^2 = 9.2$). The lower C:N values were observed primarily at PB in the summer months, potentially due to autochthonous inputs from the pond upstream of PB-1.

DOC normalized absorption coefficients a_{254}^* and a_{350}^* exhibited an inter-site by temporal effect ($p = 0.01$, $\chi^2 = 6.1$ and $p < 0.01$, $\chi^2 = 9.2$, respectively) and ranged from 77 to 148 L mmol C^{-1} m^{-1} and from 18 to 36 L mmol C^{-1} m^{-1}, respectively, with the highest values observed at the wetland-influenced sites (CF) and in autumn relative to summer (Appendix Table A2.1). Values measured in the summer were highest at CF, whereas values observed in autumn were also high at the forested stream sites (PB, GB) and comparable to CF. Spectral slopes varied temporally ($p = 0.02$, $\chi^2 = 5.8$) and by intra-site ($p = 0.01$, $\chi^2 = 5.9$), ranging from 6.2 to 7.9 μm^{-1} for $S_{275-295}$ and from 5.8 to 8.6 μm^{-1} for

$S_{350-400}$, with highest values generally associated with downstream sites and in autumn. Absorption ratios a_{254}:a_{350}, serving as indicators for the relative presence of low- versus high-molecular weight chromophoric dissolved organic matter (Helms et al., 2008), ranged from 3.9 to 5.2 and exhibited an inter- by intra-site effect ($p = 0.04$, $\chi^2 = 4.2$) such that values were generally higher in the forested streams and downstream relative to upstream (Appendix Table A2.1).

Carbohydrate content, relative to DOC, was highest at the wetland and pond-influenced sites (CF-1, CF-2, PB-1), particularly in summer, but was lower at CF-1 and CF-2 in autumn (Appendix Table A2.1). The A_R:A_L of DOM was also highest at the wetland (CF-1 and CF-2) and pond-influenced sites (PB-1). A_R:A_L values varied between upstream and downstream sites and were considerably lower at PB-1 in autumn relative to the summer, whereas at CF these ratios were higher in autumn than in the summer (Appendix Table A2.1).

Table 2.2: Water chemistry parameters for temperature (T), dissolved oxygen (DO, given as concentration and saturated oxygen), dissolved inorganic nitrogen (DIN), dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) concentrations, and molar C:N ratios. The T and discharge values were obtained from single *in situ* measurements while the remaining parameters are reported as the mean ± one standard deviation (n = 5).

Site	Season	T	DO	DIN	DOC	DON	C:N
		°C	µM O_2 / %	µM N	µM C	µM N	
CF-1	Summer 2009	18.5	362 / 129	1.1	960	25	39
CF-1	Summer 2010	18.2	201 / 55	4.4	604	18	33
CF-1	Autumn 2009	5.5	468 / 117	4.5	683	21	46
CF-2	Summer 2009	16.6	374 / 123	1.1	1022	28	37
CF-2	Summer 2010	18.0	234 / 57	5.9	590	14	43
CF-2	Autumn 2009	5.5	495 / 117	3.4	695	26	40
BFB-1	Summer 2008	15.9	297 / 97	5.3	1194	28	43
BFB-1	Autumn 2008	8.3	308 / 85	4.0	930	15	62
BFB-2	Summer 2008	14.4	338 / 105	2.5	675	22	31
BFB-2	Autumn 2008	8.3	273 / 77	1.8	720	13	54
PB-1	Summer 2009	19.5	306 / 107	0.5	320	16	19
PB-1	Summer 2010	18.2	211 / 55	1.9	325	14	23
PB-1	Autumn 2009	4.0	461 / 106	2.3	407	10	40
PB-2	Summer 2009	17.9	310 / 104	2.5	363	15	24
PB-2	Summer 2010	18.0	234 / 56	2.1	436	13	35
PB-2	Autumn 2009	4.0	527 / 115	1.9	609	17	36
PB-3	Summer 2010	18.0	222 / 55	1.1	585	17	34
GB-1	Autumn 2009	5.5	502 / 129	2.9	555	12	47
GB-2	Autumn 2009	5.5	517 / 129	3.7	419	18	23

2.3.3 Experimental tile biofilm biomass

In the 2009 experiments, tile biofilm biomass, measured as total C content, varied temporally ($p = 0.03$, $\chi^2 = 4.7$) and by inter- ($p = 0.01$, $\chi^2 = 6.5$) and intra-site ($p = 0.04$, $\chi^2 = 3.9$). Tile colonization was highest at the wetland-influenced streams, particularly at the downstream site of CF in autumn. Elevated tile colonization in autumn relative to summer was also observed at the remaining wetland-influenced stream sites (BFB-1 and BFB-2), except at CF-1 where tile colonization was higher during the summer (Appendix Table A2.2). Such intra-site and temporal differences, however, were not evident at the forested stream sites. Tile biomass was significantly lower in summer 2010 than observed during the previous summer. In the 2009 experiments, tile N content exhibited an inter-site by temporal interaction ($p = 0.02$, $\chi^2 = 5.6$) such that N biofilm content was highest at the forested stream sites in summer and at the wetland-influenced streams sites in autumn (Appendix Table A2.2). Tile biomass was positively, though weakly, correlated to stream DON ($p = 0.04$, $R^2 = 0.21$) but not correlated ($p > 0.14$) to any other water chemistry parameters assessed in this study.

2.3.4 Tile biofilm respiration within the Humber River Basin

Tile biofilm respiration ($R_{Biofilm}$) rates, measured as changes in DO and presented here as C consumption, exhibited inter- by intra-site ($p = 0.0004$, $\chi^2 = 12.5$), inter-site by temporal ($p < 0.0001$, $\chi^2 = 45.1$) and intra-site by temporal ($p = 0.0013$, $\chi^2 = 10.3$) interactions. Additionally, $R_{Biofilm}$ rates were controlled by temperature within summer ($p < 0.0001$, $\chi^2 = 18.8$) and autumn ($p = 0.0094$, $\chi^2 = 6.7$).

In summer 2008 and 2009, $R_{Biofilm}$ rates were highest at the wetland-dominated sites (CF-1 and CF-2; both 10 µM C hr^{-1}) and at the pond-influenced site (PB-1; 6 µM C

hr^{-1}), coinciding with a higher water temperature (Table 2.2), whereas $R_{Biofilm}$ rates at the upstream (1.2 µM C hr^{-1}) and downstream site (0.3 µM C hr^{-1}) of BFB and at the downstream site of PB (0.7 µM C hr^{-1}) were relatively low (Table 2.3). However, $R_{Biofilm}$ rates were significantly lower when repeating these experiments at CF and PB in the following summer (Table 2.3), likely due to the lower biofilm colonization compared to the previous year. As observed during the previous summer, $R_{Biofilm}$ rates were again highest at the pond (PB-1) and wetland-influenced sites in 2010 (e.g. CF-1). Autumn 2008 and 2009 $R_{Biofilm}$ rates were significantly lower than in the summer 2008 and 2009 experiments (Table 2.3), likely due to lower water temperatures. Contrary to the summer, $R_{Biofilm}$ rates were highest at BFB-2 and GB-1 (4 and 6 µM C hr^{-1}, respectively) and lowest at PB-2 (0.4 µM C hr^{-1}) and CF-1 (0.5 µM C hr^{-1}) in autumn (Table 2.3). Overall, the $R_{Biofilm}$ rates normalized to biomass as well as $R_{Biofilm}$ normalized to DOC concentration followed the trend in the absolute respiration rates (Table 2.3).

Considering all data from summer and autumn, $R_{Biofilm}$ rates were positively correlated to the initial DOC concentration, though DOC concentration only explained a minor fraction of the variation in $R_{Biofilm}$ ($p = 0.03$, $R^2 = 0.10$). The a_{350}^* ($p = 0.03$, $R^2 = 0.09$) and $S_{350-400}$ ($p = 0.03$, $R^2 = 0.09$) were also positively but weakly correlated with $R_{Biofilm}$ and likely due to the correlation between DOC concentration and a_{350}^* ($p = 0.05$, $R^2 = 0.07$) and $S_{350-400}$ ($p = 0.03$, $R^2 = 0.10$). The a_{254}:a_{350} and $S_{275-295}$ were also correlated with DOC (both $p < 0.01$, $R^2 = 0.27$ and $R^2 = 0.24$, respectively). These measures were almost significantly correlated to $R_{Biofilm}$ ($p = 0.09$ and 0.07, respectively) reflecting, again, a potentially weak correlation with $R_{Biofilm}$. Further, $R_{Biofilm}$ rates were not correlated ($p > 0.14$) to any other biofilm or water chemistry parameters assessed in this study.

Table 2.3: Biofilm respiration rates in absolute terms ($R_{Biofilm}$), normalized to dissolved organic carbon concentration ($R_{Biofilm}$/DOC), and normalized to both biomass and DOC concentration ($R_{Biofilm}$/DOC & Biomass). Respiration rates represent changes in dissolved oxygen and are presented here as carbon consumption, assuming a respiratory quotient of 1. Values correspond to averages of 5 experimental replicates ± one standard deviation.

Site	Season	$R_{Biofilm}$	$R_{Biofilm}$/DOC	$R_{Biofilm}$/DOC & Biomass
		μM C hr^{-1}	μM C hr^{-1} mM DOC^{-1}	μM C hr^{-1} mM DOC^{-1} mM $C_{Biomass}^{-1}$
CF-1	Summer 2009	10.1 ± 0.3	10.6 ± 0.4	118.1 ± 4.7
CF-1	Summer 2010	0.7 ± 0.1	1.1 ± 0.1	19.3 ± 1.2
CF-1	Autumn 2009	0.5 ± 0.1	0.7 ± 0.2	8.7 ± 2.3
CF-2	Summer 2009	9.6 ± 0.1	9.4 ± 0.2	122.4 ± 4.1
CF-2	Summer 2010	0.3 ± 0.1	0.5 ± 0.1	7.7 ± 2.1
CF-2	Autumn 2009	1.8 ± 0.6	2.6 ± 0.9	10.8 ± 3.7
BFB-1	Summer 2008	1.2 ± 0.1	1.0 ± 0.1	16.7 ± 2.2
BFB-1	Autumn 2008	3.9 ± 0.8	4.2 ± 0.9	35.2 ± 7.6
BFB-2	Summer 2008	0.3 ± 0.1	0.4 ± 0.1	7.0 ± 1.2
BFB-2	Autumn 2008	2.3 ± 0.4	3.2 ± 0.5	43.0 ± 7.6
PB-1	Summer 2009	6.0 ± 0.3	18.7 ± 2.5	240.1 ± 32.7
PB-1	Summer 2010	0.8 ± 0.2	2.5 ± 0.6	40.1 ± 9.9
PB-1	Autumn 2009	1.8 ± 0.1	4.3 ± 0.3	68.4 ± 5.2
PB-2	Summer 2009	0.7 ± 0.1	2.0 ± 0.3	25.4 ± 3.4
PB-2	Summer 2010	0.2 ± 0.1	0.4 ± 0.1	5.9 ± 1.5
PB-2	Autumn 2009	0.4 ± 0.1	0.6 ± 0.1	11.9 ± 2.8
PB-3	Summer 2010	0.5 ± 0.1	0.9 ± 0.1	10.8 ± 0.8
GB-1	Autumn 2009	5.7 ± 0.1	10.3 ± 0.5	149.5 ± 7.7
GB-2	Autumn 2009	1.9 ± 0.1	3.8 ± 0.3	60.3 ± 5.0

2.3.5 Response of biofilm respiration to labile C, N, P additions

Increased $R_{Biofilm}$, relative to the control respiration, occurred with inorganic N and P added in conjunction with C (CNP treatment) at the upstream sites of both BFB and CF in summer 2008 and 2009, respectively ($p < 0.01$; Fig. 2.3). Adding C, N, P, and NP alone did not affect $R_{Biofilm}$ at these sites. In contrast to the wetland-influenced sites, no significant differences were observed between control and treatment measurements at the upstream pond-influenced site (PB-1; Fig. 2.3). At the downstream sites of CF and BFB, increased $R_{Biofilm}$ rates were measured when adding solely glucose-C (C treatment; $p = 0.01$; Fig. 2.3) additionally to the CNP treatment effect ($p < 0.01$). At the downstream site of PB (PB-2), however, increased $R_{Biofilm}$ rates were measured with the addition of CNP only ($p = 0.02$), whereas adding C, N, P, and NP alone did not show a treatment effect at this site (Fig. 2.3). Respiration rates for the CNP treatments were significantly different from the remaining treatments (C, N, P, NP), except in BFB and at PB-2 where CNP was not significantly different from the glucose treatment (BFB-1: $p = 0.28$, BFB-2: $p = 0.81$, PB-2: $p = 0.12$). Aside from the CNP treatment, only the glucose treatment at CF-2 was significantly different from any other treatment in addition to the control.

As seen in the 2008 and 2009 summer experiments, no treatment effect was observed within the addition experiments conducted in summer 2010 when adding N and P without an added source of C (Fig. 2.4). $R_{Biofilm}$ was co-limited by labile C, N, and P at CF-1 ($p < 0.01$), CF-2 ($p < 0.01$), as well as PB-2 ($p = 0.02$) and PB-3 ($p < 0.01$). Glucose stimulated $R_{Biofilm}$ at CF-1 ($p < 0.01$), CF-2 ($p < 0.01$) and PB-3 ($p = 0.03$; Fig. 2.4). Again, congruent with the results from the previous year experiments, no treatment effect was noted when adding C, N, and P, in any combination, at the upstream site directly draining the headwater pond (PB-1; Fig. 2.4).

The respiration measured as part of the nutrient addition experiments conducted in autumn 2008 and 2009 exhibited a C, N, and P co-limitation (CNP treatment) only at PB-1 ($p = 0.01$) and PB-2 ($p = 0.02$), and increased $R_{Biofilm}$ rates were measured following the addition of glucose-C only at PB-2 (C treatment; $p = 0.03$). The $R_{Biofilm}$ rates associated with the CNP treatments at both PB sites and the C treatment at PB-2 did not differ from any other treatments other than the control, likely due to the higher relative variation caused by lower $R_{Biofilm}$ rates at these sites. In contrast to summer, the addition of C, N, and P did not elicit a significant effect in any combination at either the upstream or downstream sites of CF, BFB, and GB in autumn (data not shown).

In some cases, the pond and bog DOM additions elicited an increase in $R_{Biofilm}$ relative to the control (Fig. 2.4) though significantly reduced relative to the glucose treatment and often not significantly different from the NP treatments. At CF-1, $R_{Biofilm}$ increased with the addition of DOM concentrates from the pond ($p = 0.02$) and bog ($p = 0.03$) and with the addition of N and P in conjunction with these C sources ($p = 0.02$; Fig. 2.4). Respiration rates were similar for the pond +NP and bog +NP treatments, but were only about one third of the rates observed in the glucose +NP treatment.

Respiration rates at the downstream sites of CF (CF-2; pond: $p = 0.15$; bog: $p = 0.12$) and PB (PB-2; pond: $p = 0.44$; bog: $p = 0.29$) did not vary significantly relative to the treatments without the bog and pond DOM additions (Fig. 2.4). The addition of the pond and bog DOM in combination with N and P, however, elicited an increase in $R_{Biofilm}$ relative to the control at these sites (CF-2: $p < 0.01$ for pond/bog +NP; PB-2: $p = 0.04$ for pond +NP and $p = 0.03$ for bog +NP) as well as at PB-3 ($p = 0.03$ for pond +NP and $p = 0.01$ for bog +NP; Fig. 2.4). The pond DOM also stimulated $R_{Biofilm}$ at PB-3 ($p = 0.01$). The increase in $R_{Biofilm}$ rates in the glucose +NP treatment relative to the control was approximately twice as high relative to the bog/pond +NP treatments at PB-2 and PB-3,

and roughly four times higher at CF-2. The glucose +NP treatments exhibited significant differences in $R_{Biofilm}$ relative to all other treatments ($p < 0.01$ in all instances), except at PB-2 where the $R_{Biofilm}$ rates within the CNP treatments were not significantly different from the bog ($p = 0.06$), bog +NP ($p = 0.32$), and pond +NP treatments ($p = 0.26$), and at PB-3 where the glucose +NP treatments were not significantly different from the bog +NP ($p = 0.07$) and pond +NP treatments ($p = 0.77$). The $R_{Biofilm}$ rates associated with the glucose treatment were significantly different from the other treatments in addition to the control ($p < 0.01$ for all treatments) only at the CF-2 site. In all other instances where a C treatment effect was observed (CF-1, PB-3), the $R_{Biofilm}$ rates of the C treatments were significantly different from the CNP treatments, but did not differ from the remaining treatments (bog, pond, bog +NP, pond +NP, NP).

The addition of glucose ($p = 0.26$), pond ($p = 0.28$) and bog DOM ($p = 0.37$), either added alone or combined with inorganic N and P ($p = 0.27$ for glucose +NP, $p= 0.40$ for pond +NP, and $p = 0.30$ for bog +NP), did not elicit a treatment effect at PB-1.

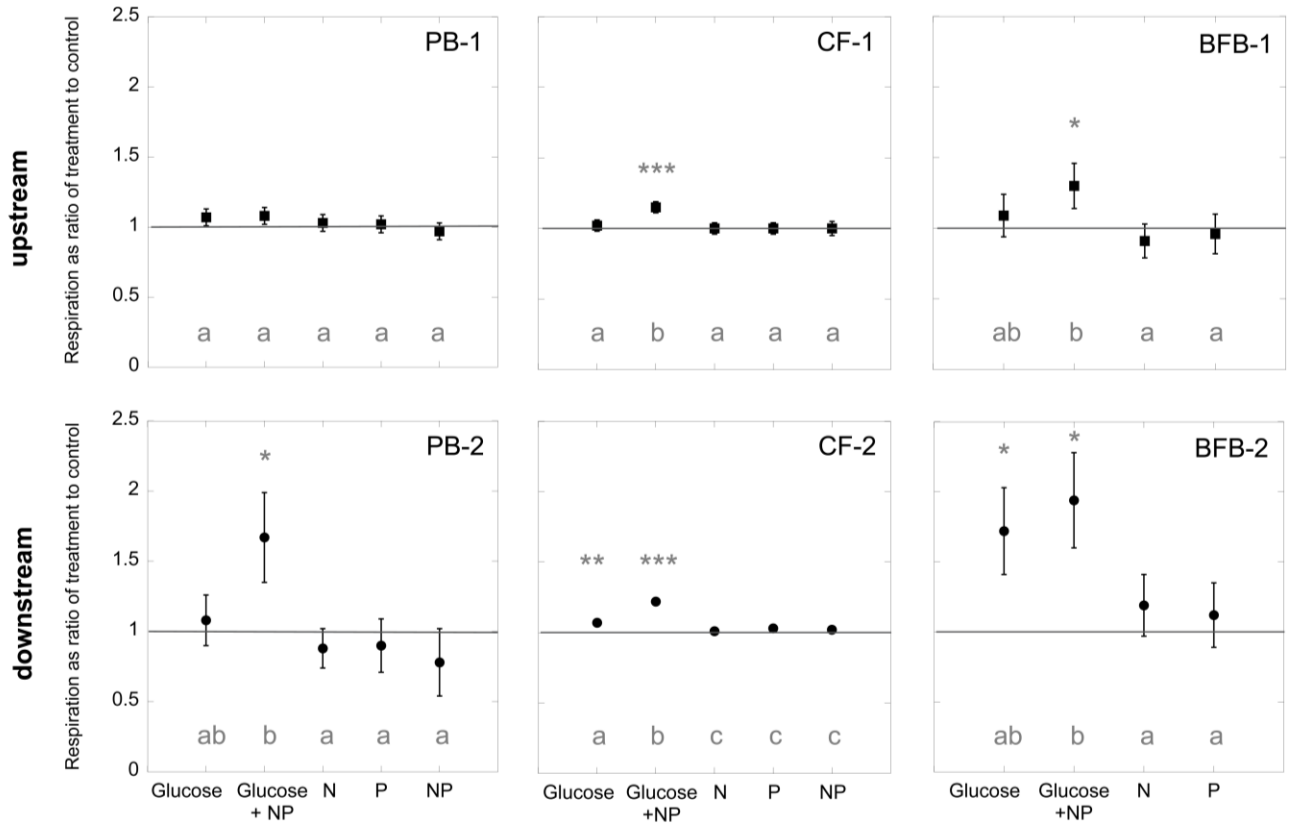


Figure 2.3: Biofilm respiration rates ($R_{Biofilm}$) provided as the ratio of treatment to control for labile substrate addition experiments at Pynn's Brook (PB) and Crooked Feeder (CF) in summer 2009, and at Big Falls Brook (BFB) in summer 2008. Substrate additions included glucose, ammonium nitrate (N), and potassium phosphate (P). Values correspond to the mean of five replicates ± one standard deviation. Results of the generalized linear model tests of the absolute $R_{Biofilm}$ associated with each treatment are provided with asterisks as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ relative to the control (no additions). Results from the Tukey's Honestly Significant Difference tests for the absolute $R_{Biofilm}$ associated with each treatment are provided by lower case letters (a, b, c), where only those treatments designated with different letters were found to be significantly different.

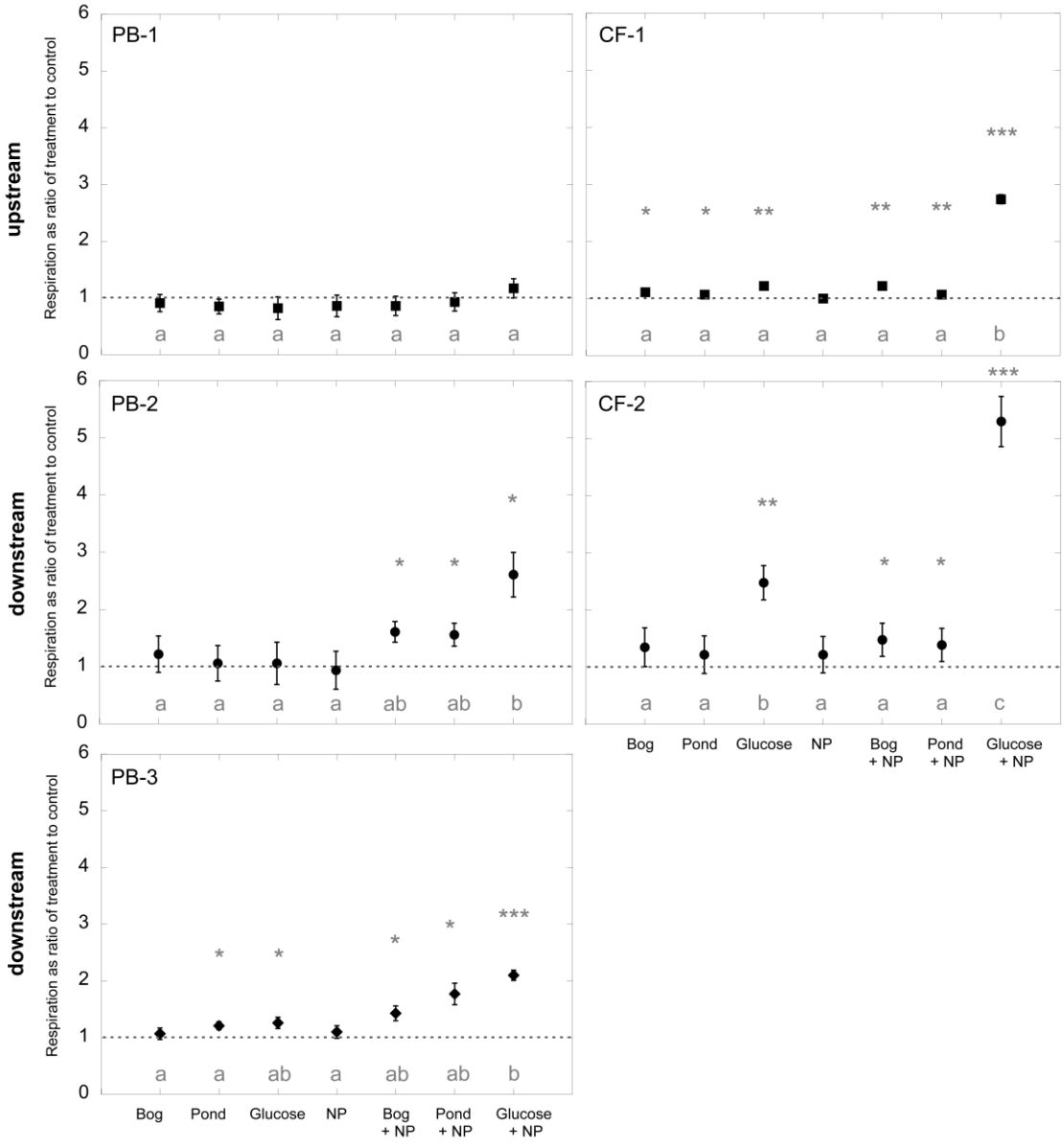


Figure 2.4: Biofilm respiration rates ($R_{Biofilm}$) provided as the ratio of treatment to control for the ambient dissolved organic matter (DOM) addition experiments along a transect of Pynn's Brook (PB-1, PB-2, and PB-3) and at two sites at Crooked Feeder (CF-1 and CF-2) in summer 2010. Substrate additions included bog DOM, pond DOM, glucose, ammonium nitrate (N), and potassium phosphate (P). Values correspond to the mean of five replicates ± one standard deviation. Results of the generalized linear model tests of the $R_{Biofilm}$ associated with each treatment are provided with asterisks as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ relative to the control (no additions). Results from the Tukey's Honestly Significant Difference tests for the absolute $R_{Biofilm}$ associated with each treatment are provided by lower case letters (a, b, c), where only those treatments designated with different letters were found to be significantly different.

2.3.6 Effect of glucose amendment on biofilm respiration of stream DOC

The added glucose associated with the substrate addition experiments either (1) increased $R_{Biofilm}$ at the expense of the mineralization of ambient stream DOC (R_{DOC}) or (2) enhanced biofilm R_{DOC}. From the nine instances across all summer experiments conducted where added glucose increased total respiration (CF-1, CF-2, BFB-1, BFB-2, PB-2, PB-3), only the CNP treatment from CF-1 and CF-2 in summer 2009 exhibited a significant difference between R_{DOC} and the associated control respiration ($R_{control}$; Table 2.4; CF-1: $p = 0.01$, CF-2: $p < 0.01$), signifying an effect of the added glucose on R_{DOC}. However, R_{DOC} was not different from $R_{Glucose}$ (Table 2.4; CF-1: $p = 0.39$, CF-2: $p = 0.07$), but considerably lower than the respective control measurements ($R_{control}$; Table 2.4; CF-1: $p = 0.01$, CF-2: $p < 0.01$). These results indicate preferential microbial mineralization of labile C in the form of glucose at CF-1 and CF-2 when N and P were available, rather than a stimulation of the mineralization of stream DOC.

In autumn 2009, the addition of glucose-C appeared to have stimulated microbial respiration of stream DOC at CF-1 and CF-2, whereas the addition of CNP or any other treatments did not exhibit differences between R_{DOC} and $R_{control}$. For the glucose-only treatment the rate of glucose-C respired was low at both CF-1 and CF-2, while R_{DOC} was not significantly different (CF-1: $p = 0.41$, CF-2: $p = 0.11$) from R_{total} (Table 2.4). The R_{total} and R_{DOC} for the glucose only treatments at these two sites, however, were significantly higher than the control treatments (below detection, $p < 0.01$), indicating a stimulation of R_{DOC} equivalent to an increase of >40 % and >50 % in the respiration of extant stream DOC at CF-1 and CF-2, respectively.

Table 2.4: Effect of glucose amendment on biofilm respiration of stream dissolved organic carbon (DOC) at Crooked Feeder upstream (CF-1) and downstream (CF-2) in summer and autumn. In summer, only the glucose-carbon (C) + nitrogen (N) and phosphorus (P) treatment exhibited a significant difference between the respiration of ambient stream DOC (R_{DOC}) and the associated control respiration ($R_{control}$), whereas in autumn the addition of glucose-C appeared to have stimulated microbial respiration of stream DOC. The amount of total carbon respired is expressed as R_{total}, and the amount of glucose-C respired as $R_{Glucose}$. Values correspond to changes in total inorganic carbon and refer to 5 experimental replicates ± one standard deviation. BD: Below detection.

Site/Season	**Treatment**	**$R_{control}$** μM C hr^{-1}	**R_{total}** μM C hr^{-1}	**$R_{Glucose}$** μM C hr^{-1}	**R_{DOC}** μM C hr^{-1}
CF-1 Summer	Glucose-C +NP	1.04 ± 0.31	1.41 ± 0.22	0.69 ± 0.08	0.72 ± 0.24
CF-2 Summer	Glucose-C +NP	1.93 ± 0.27	2.23 ± 0.21	1.03 ± 0.04	1.20 ± 0.22
CF-1 Autumn	Glucose-C	BD	0.42 ± 0.01	0.01	0.41 ± 0.01
CF-2 Autumn	Glucose-C	BD	0.60 ± 0.08	0.01	0.53 ± 0.09

2.4 Discussion

Biofilm respiration and its response to labile C, N, and P varied spatially and temporally in the Humber River Basin, suggesting boreal headwater stream responses to environmental change vary and depend upon DOM source and composition. Biofilms in streams located adjacent to ponds and within wetland-dominated catchments exhibited higher rates of $R_{Biofilm}$ relative to biofilms from forested catchments, contrasting with the lack of such variation in bacterioplankton respiration in other boreal catchments (Berggren et al., 2007). Intra-stream differences in respiration and responses to substrate additions observed in this study, however, suggest a combination of landscape DOM source and potential in-stream processing may influence the mineralization of DOM by stream biofilms. The use of the natural DOM additions in our experiments provided more direct evidence of higher lability of bog and pond sources of DOM compared with forested stream DOM in these catchments. The stream $R_{Biofilm}$ heterogeneity and the varied response of extant stream DOC mineralization (R_{DOC}) to labile C addition suggest they were regulated by DOM composition and the availability of N and P. While adding glucose typically had no effect on R_{DOC} across the study sites, differential effects at the most active sites suggest labile substrates, analogous with algal inputs, can have opposing effects on the fate of stream DOM. Labile substrate additions both competed as a source of energy and/or stimulated the incorporation rather than mineralization of the more N and P-rich extant stream DOM and caused a priming effect and stimulated the mineralization of extant DOM when $R_{Biofilm}$ was lower and DOM more aromatic. The lack of response to added inorganic N and P indicates that priming effects on DOM mineralization in these streams was primarily generated by labile C inputs to the most developed biofilms.

2.4.1 Catchment and sub-catchment scale variation in biofilm respiration

Spatial and temporal variation in $R_{Biofilm}$ and DOM composition were captured in these experiments and suggest landscape inputs and in-stream processing can regulate losses of stream DOM via $R_{Biofilm}$. Biofilm respiration weakly correlated with DOC concentration and biofilm biomass weakly correlated with DON concentration such that highest biomass and $R_{Biofilm}$ were associated with the wetland-influenced sites. These sites also exhibited the highest DOC and DON concentrations and aromaticity. DON and DOC concentrations, however, explained a small proportion of the variation in biomass and $R_{Biofilm}$, respectively, indicating other factors such as temperature and DOM composition likely regulated biofilm activity and potential for losses of DOM in this landscape.

Grouping $R_{Biofilm}$ results by temperature suggests catchment composition and associated DOM and its chemistry likely played a role in $R_{Biofilm}$ variation across these sites. Elevated biofilm biomass and $R_{Biofilm}$ at the wetland and pond-influenced sites relative to the forested stream sites, particularly in summer, was congruent with variation in DON concentration and DOM carbohydrate content among these sites. These data emphasize how organic matter contributions from wetlands can be an important energy source for aquatic food webs (Wetzel, 1992; Mulholland, 1997; Fellman et al., 2008) and potentially more so than forest inputs (Fellman et al., 2008). In boreal catchments, however, this energy may simply be lost via respiration given our observations of $R_{Biofilm}$ coupled with lower stream water bacterial growth efficiencies documented in other mire versus forest-dominated boreal catchments (Berggren et al., 2007).

Landscape and temporal variation in microbial activity, as observed in other boreal catchments likely fueled by discrete DOM inputs (Naiman et al., 1987; Fisk et al., 2003; Berggren et al., 2009a), suggest greater potential for mineralization of DOM from

deciduous forest and pond-influenced sites in these catchments. The forested streams exhibited seasonal variation in $R_{Biofilm}$, likely fueled by changes in DOM concentration and composition observed and associated with the increased discharge and defoliation during autumn relative to summer as noted in other boreal catchments (Laudon et al., 2004). Increased input of fresh deciduous leaf litter leachate, often considered an important stream DOM source in temperate systems (McDowell and Fisher, 1976; Kaplan and Bott, 1983; Meyer et al., 1998), likely supported the elevated $R_{Biofilm}$ at the most deciduous-dominated site. The high respiration rates at the pond-influenced site, however, coincided with greater DOM carbohydrate content and higher biofilm $\delta^{13}C$ in summer (Appendix Tables A2.1 and A2.2) indicative of autochthonous DOM from the headwater pond located directly upstream and its incorporation at this site (France, 1995; Karlsson et al., 2003; Kritzberg et al., 2004). Autochthonous C sources are typically less abundant in humic-rich systems likely due to rapid consumption. Decreased DOM bioavailability coupled with the changes in DOM composition reflective of increased allochthonous inputs, such as increased C:N and aromaticity and decreased carbohydrate content, reflect this rapid loss of autochthonous C downstream of PB-1. The lack of response to the addition of C, N, and/or P observed at pond-influenced PB-1 contrasts with these downstream sites where additions (pond DOM, glucose) stimulated $R_{Biofilm}$. In combination, these results indicate that elevated microbial activity in the pond influenced PB-1 site was likely fueled by sources of labile C (i.e. carbohydrates) (Olapade and Leff, 2005; Wilcox et al., 2005).

2.4.2 The role of inorganic N and P availability in regulating boreal stream biofilm respiration

Increases in N and P levels caused by changes in landscape composition due to climate change or anthropogenic impacts (Schindler et al., 1996; Mattsson et al., 2005; Kreutzweiser et al., 2008) may not only increase more labile, autochthonous sources of DOM (Mallin et al., 2004) but alter the fate of both autochthonous and allochthonous DOM in these boreal streams. Results here suggest the mineralization, and therefore fate, of more complex stream DOM in response to increased N and P differs markedly from more labile substrates analogous to autochthonous sources such as exudates. Even at sites where dissolved organic C and biofilm biomass was elevated, the addition of N and P did not elicit an increase in DOM mineralization – only the mineralization of the labile glucose congruent with other studies where increased inorganic N supply led to an increased microbial demand for labile C (Berggren et al., 2007; Lutz et al., 2011). Mineralization of labile C sources by stream biofilms is typically linked to N and P availability (Reche et al., 1998; Gulis and Suberkropp, 2003; Stelzer et al., 2003) with the addition of labile C and stimulation of microbial activity leading to depletion of either N or P or both (Francoeur, 2001; Bernhardt and Likens, 2002; Tank and Dodds, 2003).

The preferential use of the labile glucose, in the presence of added N and P, over the extant stream DOM may have been due to a reduced need for N and P from the more complex DOM, which likely requires greater energy to access (e.g. enzyme production). The biofilms may be able to extract energy from the added glucose and produce DON (or DOP) from DIN (or DIP) when inorganic nutrients are increased while simultaneously inhibiting the mineralization of DOM (Kaushal and Lewis, 2005). In this study, the added glucose at the most wetland-influenced sites in summer reduced extant stream DOM mineralization but increased the overall C mineralization supported by the

added glucose when N and P were simultaneously added. The few instances where C limitation was observed with the addition of solely glucose or DOM concentrates, for example at CF and BFB, coincided with higher stream water DIN concentrations relative to other seasons, or other sites during the same season, or both. This further suggests increased inorganic N supply likely aids in increased microbial mineralization of more labile C substrates preferentially over more complex DOM.

In these boreal catchments, where DON is the major form of N as noted in other similar systems (Stepanauskas et al., 2000; Moore, 2009), inorganic nutrient concentrations were low and may result in heterotrophic biofilms more adept at utilizing organic N and P. Although previous studies have shown that some stream microorganisms take up DIN preferentially (Kaushal and Lewis, 2005) when DIN availability is low, DON can play a specific role in regulating organic matter mineralization (Neff et al., 2003; Kaushal and Lewis, 2005). The correlation between biofilm biomass development and stream DON concentration observed here signifies the potential role of DON in regulating heterotrophic biofilms and their activity in these streams. This may have been a factor contributing to the lack of response to the inorganic N and P additions in these biofilms.

Contrasting with the lack of response of $R_{Biofilm}$ to the added inorganic N and P in this study, a number of temperate, boreal, and arctic lake studies typically found bacterioplankton growth and/or community respiration was limited by inorganic N and/or P availability (Hessen et al., 1994; Jansson et al., 1996; Vrede et al., 1999; Carlsson and Caron, 2001; Graneli et al., 2004; Smith and Prairie, 2004). In these other studies, however, the extant stream nutrient concentrations were typically at least 10-fold higher (e.g. 50 – 100 µM N) than those in this study with the actual additions also proportionally higher than those used here (e.g. N: 5 – 10x higher). This suggests that microbial

respiration can be stimulated by N or P due to transient exposure to higher levels of inorganic N and P, but typically when previous exposure to N and P is high unlike in these study streams. To address this further, similar studies need to be conducted in boreal streams having a history of nutrient enrichment. In such systems, where biofilm exposure and resulting adaption have taken place, the more complex interactions among different functional groups (e.g. autotrophs and heterotrophs) not investigated here can be accounted for in an assessment of stream DOM fate.

2.2.3 Little evidence for a priming effect on biofilm mineralization of boreal stream DOM

Increased access to labile C sources, such as algal exudates made available through primary production, can at times stimulate the mineralization of more complex allochthonous stream DOM (Guenet et al., 2010). Using ^{13}C as a tracer for the added glucose in these experiments, we found that the mineralization of boreal stream DOM was typically not influenced by the glucose additions, with or without NP. In fact, stream DOM mineralization was reduced with the addition of glucose at the most wetland-influenced sites in summer. However, of all 19 experiments conducted, we observed that adding solely glucose at the two most wetland-influenced sites in autumn stimulated the mineralization of ambient stream DOM indicating this type of priming effect is rare in the stream biofilms of this region. The fact that the priming effect only occurred in the absence of added N and P suggests that the mineralization of stream DOM can be an important mechanism used by biofilms to obtain N and P. This further suggests the mineralization of DOM in response to new labile substrates greatly depends upon nutrient availability likely due to changes in microbial growth efficiencies with nutrient availability (del Giorgio and Cole, 1998; Smith and Prairie, 2004; Berggren et al., 2007).

Analogous to results from temperate streams (Bernhardt and Likens, 2002; Ziegler and Brisco, 2004; Lane et al., 2012), these findings suggest that heterotrophic microbial response to catchment nutrient enrichment may become limited by bioavailable C in boreal streams despite the elevated DOM typical of these systems. Furthermore, results here suggest increased labile DOM sources that may come with nutrient enrichment may not simply lead to increased mineralization of more complex stream DOM sources. Interactive effects of DOM composition and biofilm development or structure need to be considered.

Changes in DOM composition indicative of a decrease in lability in autumn relative to summer are congruent with the stimulation of its mineralization with the added glucose in the wetland-dominated sites in autumn. Lower DOM concentrations were observed at the wetland sites in autumn relative to summer, and DOM present in autumn exhibited a greater aromaticity based upon greater A_R:A_L ratios and a_{254} (McKnight et al., 1994; McKnight and Aiken, 1998; Weishaar et al., 2003) and a lower carbohydrate content, suggesting a lower bioavailability compared to summer. In summer, rather lower C:N and greater carbohydrate content suggests greater DOM lability that likely contributed to the lack of a priming effect from the added glucose. Greater biofilm biomass, particularly at the downstream site of the most wetland-influenced stream in autumn, signifies greater biofilm development and likely a more complex composition, for example a larger presence of fungi (Lane et al., 2012). More complex biofilms can incorporate a wider array of substrate types and exhibit greater metabolic capabilities (Battin et al., 2003a; Artigas et al., 2011; Lane et al., 2012), potentially enabling processes responsible for priming effects as noted in other systems and observed here in the wetland-dominated sites. These DOM and biofilm characteristics may have enabled the stimulation of the uptake of relatively slow turnover organic matter observed in autumn at these more

wetland-dominated sites. Further study is required to tie DOM composition to the overall impact of priming effects on boreal stream biofilm activity and DOM mineralization.

In this study of boreal stream DOM mineralization by heterotrophic biofilms, we found evidence for differential effects of added N and P on more complex versus simple, labile organic substrates. These effects suggest that increased nutrient enrichment may increase losses of more labile versus more complex substrates in these streams. This differential loss, however, may be altered by priming effects fueled by increased labile organic substrates with nutrient enrichment. The limited evidence for such priming effects in this study suggests the need for similar studies in long-term nutrient-impacted systems, where biofilm communities have developed in response to elevated concentrations of inorganic N and P and where C limitation may be more prevalent.

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2.6 References

Agren, A., Berggren, M., Laudon, H., and Jansson, M., 2008: Terrestrial export of highly bioavailable carbon from small boreal catchments in spring floods. *Freshwater Biology,* 53: 964-972.

Aitkenhead, J. A. and McDowell, W. H., 2000: Soil C : N ratio as a predictor of annual riverine DOC flux at local and global scales. *Global Biogeochemical Cycles,* 14: 127-138.

Alexander, R. B., Boyer, E. W., Smith, R. A., Schwarz, G. E., and Moore, R. B., 2007: The role of headwater streams in downstream water quality. *Journal of the American Water Resources Association,* 43: 41-59.

Anesio, A. M., Graneli, W., Aiken, G. R., Kieber, D. J., and Mopper, K., 2005: Effect of humic substance photodegradation on bacterial growth and respiration in lake water. *Applied and Environmental Microbiology,* 71: 6267-6275.

Artigas, J., Gaudes, A., Munoz, I., Romani, A. M., and Sabater, S., 2011: Fungal and Bacterial Colonization of Submerged Leaf Litter in a Mediterranean Stream. *International Review of Hydrobiology,* 96: 221-234.

Battin, T. J., Kaplan, L. A., Newbold, J. D., Cheng, X. H., and Hansen, C., 2003a: Effects of current velocity on the nascent architecture of stream microbial biofilms. *Applied and Environmental Microbiology,* 69: 5443-5452.

Battin, T. J., Kaplan, L. A., Newbold, J. D., and Hansen, C. M. E., 2003b: Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature,* 426: 439-442.

Berggren, M., Laudon, H., and Jansson, M., 2007: Landscape regulation of bacterial growth efficiency in boreal freshwaters. *Global Biogeochemical Cycles,* 21: 1-9.

Berggren, M., Laudon, H., and Jansson, M., 2009a: Hydrological Control of Organic Carbon Support for Bacterial Growth in Boreal Headwater Streams. *Microbial Ecology,* 57: 170-178.

Berggren, M., Laudon, H., and Jansson, M., 2009b: Aging of allochthonous organic carbon regulates bacterial production in unproductive boreal lakes. *Limnology and Oceanography,* 54: 1333-1342.

Bernhardt, E. S. and Likens, G. E., 2002: Dissolved organic carbon enrichment alters nitrogen dynamics in a forest stream. *Ecology,* 83: 1689-1700.

Bianchi, T. S., 2011: The role of terrestrially derived organic carbon in the coastal ocean: A changing paradigm and the priming effect. *Proceedings of the National Academy of Sciences of the United States of America,* 108: 19473-19481.

Carlsson, P. and Caron, D. A., 2001: Seasonal variation of phosphorus limitation of bacterial growth in a small lake. *Limnology and Oceanography,* 46: 108-120.

Clair, T. A. and Ehrman, J. M., 1996: Variations in discharge and dissolved organic carbon and nitrogen export from terrestrial basins with changes in climate: A neural network approach. *Limnology and Oceanography,* 41: 921-927.

Davidson, E. A. and Janssens, I. A., 2006: Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature,* 440: 165-173.

del Giorgio, P. A., Cole, J. J., and Cimbleris, A., 1997: Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature,* 385: 148-151.

del Giorgio, P. A. and Cole, J. J., 1998: Bacterial growth efficiency in natural aquatic systems. *Annual Review of Ecology and Systematics,* 29: 503-541.

Fellman, J. B., D'Amore, D. V., Hood, E., and Boone, R. D., 2008: Fluorescence characteristics and biodegradability of dissolved organic matter in forest and wetland soils from coastal temperate watersheds in southeast Alaska. *Biogeochemistry,* 88: 169-184.

Findlay, S. E. G., Meyer, J. L., and Risley, R., 1986: Benthic Bacterial Biomass and Production in 2 Blackwater Rivers. *Canadian Journal of Fisheries and Aquatic Sciences,* 43: 1271-1276.

Findlay, S. E. G. and Sinsabaugh, R. L., 2003: *Aquatic Ecosystems. Interactivity of Dissolved Organic Matter*: Academic Press. An imprint of Elsevier Science., 512 pp.

Fischer, H., Bergfur, J., Goedkoop, W., and Tranvik, L., 2009: Microbial leaf degraders in boreal streams: bringing together stochastic and deterministic regulators of community composition. *Freshwater Biology,* 54: 2276-2289.

Fisher, S. G. and Likens, G. E., 1973: Energy Flow in Bear Brook, New Hampshire: An Integrative Approach to Stream Ecosystem Metabolism. *Ecological Monographs,* 43: 421-439.

Fisk, M. C., Ruether, K. F., and Yavitt, J. B., 2003: Microbial activity and functional composition among northern peatland ecosystems. *Soil Biology & Biochemistry,* 35: 591-602.

France, R. L., 1995: Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. *Marine Ecology Progress Series,* 124: 307-312.

Francoeur, S. N., 2001: Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. *Journal of the North American Benthological Society,* 20: 358-368.

Freeman, M. C., Pringle, C. M., and Jackson, C. R., 2007: Hydrologic connectivity and the contribution of stream headwaters to ecological integrity at regional scales. *Journal of the American Water Resources Association,* 43: 5-14.

Graneli, W., Bertilsson, S., and Philibert, A., 2004: Phosphorus limitation of bacterial growth in high Arctic lakes and ponds. *Aquatic Sciences,* 66: 430-439.

Green, S. A. and Blough, N. V., 1994: Optical Absorption and Fluorescence Properties of Chromophoric Dissolved Organic Matter in Natural Waters. *Limnology and Oceanography,* 39: 1903-1916.

Guasch, H., Marti, E., and Sabater, S., 1995: Nutrient enrichment effects on biofilm metabolism in a Mediterranean stream. *Freshwater Biology,* 33: 373-383.

Guenet, B., Danger, M., Abbadie, L., and Lacroix, G., 2010: Priming effect: bridging the gap between terrestrial and aquatic ecology. *Ecology,* 91: 2850-2861.

Gulis, V. and Suberkropp, K., 2003: Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology,* 48: 123-134.

Haukka, K., Heikkinen, E., Kairesalo, T., Karjalainen, H., and Sivonen, K., 2005: Effect of humic material on the bacterioplankton community composition in boreal lakes and mesocosms. *Environmental Microbiology,* 7: 620-630.

Helms, J. R., Stubbins, A., Ritchie, J. D., Minor, E. C., Kieber, D. J., and Mopper, K., 2008: Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnology and Oceanography,* 53: 955-969.

Hessen, D. O., Nygaard, K., Salonen, K., and Vahatalo, A., 1994: The Effect of Substrate Stoichiometry on Microbial Activity and Carbon Degradation in Humic Lakes. *Environment International,* 20: 67-76.

Hoellein, T. J., Tank, J. L., Kelly, J. J., and Rosi-Marshall, E. J., 2010: Seasonal variation in nutrient limitation of microbial biofilms colonizing organic and inorganic substrata in streams. *Hydrobiologia,* 649: 331-345.

Jansson, M., Blomqvist, P., Jonsson, A., and Bergstrom, A. K., 1996: Nutrient limitation of bacterioplankton, autotrophic and mixotrophic phytoplankton, and heterotrophic nanoflagellates in Lake Ortrasket. *Limnology and Oceanography,* 41: 1552-1559.

Jansson, M., Bergstrom, A. K., Blomqvist, P., and Drakare, S., 2000: Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. *Ecology,* 81: 3250-3255.

Johnson, K. M. and Sieburth, J. M., 1977: Dissolved carbohydrates in seawater. I, A precise spectrophotometric analysis for monosaccharides. *Marine Chemistry,* 5: 1-13.

Jonsson, A., Meili, M., Bergstrom, A. K., and Jansson, M., 2001: Whole-lake mineralization of allochthonous and autochthonous organic carbon in a large humic lake (Ortrasket, N. Sweden). *Limnology and Oceanography,* 46: 1691-1700.

Kaplan, L. A. and Bott, T. L., 1983: Microbial Heterotrophic Utilization of Dissolved Organic Matter in a Piedmont Stream. *Freshwater Biology,* 13: 363-377.

Karlsson, J., Jonsson, A., Meili, M., and Jansson, M., 2003: Control of zooplankton dependence on allochthonous organic carbon in humic and clear-water lakes in northern Sweden. *Limnology and Oceanography,* 48: 269-276.

Kaushal, S. S. and Lewis, W. M., 2005: Fate and transport of organic nitrogen in minimally disturbed montane streams of Colorado, USA. *Biogeochemistry,* 74: 303-321.

Kreutzweiser, D. P., Hazlett, P. W., and Gunn, J. M., 2008: Logging impacts on the biogeochemistry of boreal forest soils and nutrient export to aquatic systems: A review. *Environmental Reviews,* 16: 157-179.

Kritzberg, E. S., Cole, J. J., Pace, M. L., Graneli, W., and Bade, D. L., 2004: Autochthonous versus allochthonous carbon sources of bacteria: Results from whole-lake C-13 addition experiments. *Limnology and Oceanography,* 49: 588-596.

Kuzyakov, Y., 2010: Priming effects: Interactions between living and dead organic matter. *Soil Biology & Biochemistry,* 42: 1363-1371.

Laine, J., Minkkinen, K., and Trettin, C., 2009: Direct Human Impacts on the Peatland Carbon Sink. *In: Carbon Cycling in Northern Peatlands, Geophysical Monograph Series 184, American Geophysical Union 10.1029/2008GM000808*: 71-78.

Lane, C. S., Lyon, D. R., and Ziegler, S. E., 2012: Cycling of two carbon substrates of contrasting lability by heterotrophic biofilms across a nutrient gradient of headwater streams. *Aquatic Sciences. Doi: 10.1007/s00027-012-0269-0*.

Laudon, H., Köhler, S., and Buffam, I., 2004: Seasonal TOC export from seven boreal catchments in northern Sweden. *Aquatic Sciences - Research Across Boundaries,* 66: 223-230.

Lindsey, J. K., 1997: Applying Generalized Linear Models. *New York; Springer (ISBN 0387982183)*: 265 pp.

Lutz, B. D., Bernhardt, E. S., Roberts, B. J., and Mulholland, P. J., 2011: Examining the coupling of carbon and nitrogen cycles in Appalachian streams: the role of dissolved organic nitrogen. *Ecology,* 92: 720-732.

Lyon, D. R. and Ziegler, S. E., 2009: Carbon cycling within epilithic biofilm communities across a nutrient gradient of headwater streams. *Limnology and Oceanography,* 54: 439-449.

Mallin, M. A., McIver, M. R., Ensign, S. H., and Cahoon, L. B., 2004: Photosynthetic and heterotrophic impacts of nutrient loading to blackwater streams. *Ecological Applications,* 14: 823-838.

Mattsson, T., Kortelainen, P., and Raike, A., 2005: Export of DOM from boreal catchments: impacts of land use cover and climate. *Biogeochemistry,* 76: 373-394.

McDowell, W. H. and Fisher, S. G., 1976: Autumnal Processing of Dissolved Organic-Matter in a Small Woodland Stream Ecosystem. *Ecology,* 57: 561-569.

McKnight, D. M., Andrews, E. D., Spulding, S. A., and Aiken, G. R., 1994: Aquatic Fulvic Acids in Algal-Rich Antarctic Ponds. *Limnology and Oceanography,* 39: 1972-1979.

McKnight, D. M. and Aiken, G. R., 1998: Sources and age of aquatic humus. *In: Hessen and Tranvik (eds.) Aquatic Humic Substances, Ecological Studies,* 133: 9-39.

Meyer, J. L., Edwards, R. T., and Risley, R., 1987: Bacterial Growth on Dissolved Organic Carbon from a Blackwater River. *Microbial Ecology,* 13: 13-29.

Meyer, J. L., Wallace, J. B., and Eggert, S. L., 1998: Leaf litter as a source of dissolved organic carbon in streams. *Ecosystems,* 1: 240-249.

Moore, T. R., 2009: Dissolved Organic Carbon Production and Transport in Canadian Peatlands. *In: Carbon Cycling in Northern Peatlands, Geophysical Monograph Series 184, American Geophysical Union 10.1029/2008GM000816*: 229-236.

Mulholland, P. J., 1997: Dissolved organic matter concentration and flux in streams. *Journal of the North American Benthological Society,* 16: 131-141.

Naiman, R. J., Melillo, J. M., Lock, M. A., Ford, T. E., and Reice, S. R., 1987: Longitudinal Patterns of Ecosystem Processes and Community Structure in a Sub-Arctic River Continuum. *Ecology,* 68: 1139-1156.

Neff, J. C., Chapin, F. S., and Vitousek, P. M., 2003: Breaks in the cycle: dissolved organic nitrogen in terrestrial ecosystems. *Frontiers in Ecology and the Environment,* 1: 205-211.

Olapade, O. A. and Leff, L. G., 2005: Seasonal response of stream biofilm communities to dissolved organic matter and nutrient enrichments. *Applied and Environmental Microbiology,* 71: 2278-2287.

Pakulski, J. D. and Benner, R., 1992: An Improved Method for the Hydrolysis and MBTH Analysis of Dissolved and Particulate Carbohydrates in Seawater. *Marine Chemistry,* 40: 143-160.

Pastor, J., Solin, J., Bridgham, S. D., Updegraff, K., Harth, C., Weishampel, P., and Dewey, B., 2003: Global warming and the export of dissolved organic carbon from boreal peatlands. *Oikos,* 100: 380-386.

Paul, B. J., Duthie, H. C., and Taylor, W. D., 1991: Nutrient Cycling by Biofilms in Running Waters of Differing Nutrient Status. *Journal of the North American Benthological Society,* 10: 31-41.

Peterson, B. J., Wollheim, W. M., Mulholland, P. J., Webster, J. R., Meyer, J. L., Tank, J. L., Marti, E., Bowden, W. B., Valett, H. M., Hershey, A. E., McDowell, W. H., Dodds, W. K., Hamilton, S. K., Gregory, S., and Morrall, D. D., 2001: Control of nitrogen export from watersheds by headwater streams. *Science,* 292: 86-90.

Reche, I., Pace, M. L., and Cole, J. J., 1998: Interactions of photobleaching and inorganic nutrients in determining bacterial growth on colored dissolved organic carbon. *Microbial Ecology,* 36: 270-280.

Romani, A. M., Giorgi, A., Acuna, V., and Sabater, S., 2004: The influence of substratum type and nutrient supply on biofilm organic matter utilization in streams. *Limnology and Oceanography,* 49: 1713-1721.

Roulet, N. and Moore, T. R., 2006: Environmental chemistry - Browning the waters. *Nature,* 444: 283-284.

Schiff, S., Aravena, R., Mewhinney, E., Elgood, R., Warner, B., Dillon, P., and Trumbore, S., 1998: Precambrian shield wetlands: Hydrologic control of the sources and export of dissolved organic matter. *Climatic Change,* 40: 167-188.

Schindler, D. W., Bayley, S. E., Parker, B. R., Beaty, K. G., Cruikshank, D. R., Fee, E. J., Schindler, E. U., and Stainton, M. P., 1996: The effects of climatic warming on the properties of boreal lakes and streams at the Experimental Lakes Area, northwestern Ontario. *Limnology and Oceanography,* 41: 1004-1017.

Smith, E. M. and Prairie, Y. T., 2004: Bacterial metabolism and growth efficiency in lakes: The importance of phosphorus availability. *Limnology and Oceanography,* 49: 137-147.

Sokal, R. R. and Rohlf, F. J., 2012: Biometry: the principles and practice of statistics in biological research. *4th edition. W. H. Freeman and Co.: New York.*: 937 pp.

Stelzer, R. S., Heffernan, J., and Likens, G. E., 2003: The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. *Freshwater Biology,* 48: 1925-1937.

Stepanauskas, R., Laudon, H., and Jorgensen, N. O. G., 2000: High DON bioavailability in boreal streams during a spring flood. *Limnology and Oceanography,* 45: 1298-1307.

Sulzman, E. W., 2007: Stable isotope chemistry and measurement: a primer. *In: Stable Isotopes in Ecology and Environmental Science. Ecological Methods and Concepts.,* 2nd edition: 1-21.

Tank, J. L. and Dodds, W. K., 2003: Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. *Freshwater Biology,* 48: 1031-1049.

Teodoru, C. R., del Giorgio, P. A., Prairie, Y. T., and Camire, M., 2009: Patterns in pCO2 in boreal streams and rivers of northern Quebec, Canada. *Global Biogeochemical Cycles,* 23: GB2012.

Tranvik, L. J., 1988: Availability of Dissolved Organic Carbon for Planktonic Bacteria in Oligotrophic Lakes of Differing Humic Content. *Microbial Ecology,* 16: 311-322.

Tranvik, L. J., 1989: Bacterioplankton Growth, Grazing Mortality and Quantitative Relationship to Primary Production in a Humic and a Clearwater Lake. *Journal of Plankton Research,* 11: 985-1000.

Vrede, K., Vrede, T., Isaksson, A., and Karlsson, A., 1999: Effects of nutrients (phosphorous, nitrogen, and carbon) and zooplankton on bacterioplankton and phytoplankton - a seasonal study. *Limnology and Oceanography,* 44: 1616-1624.

Weishaar, J. L., Aiken, G. R., Bergamaschi, B. A., Fram, M. S., Fujii, R., and Mopper, K., 2003: Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental Science & Technology,* 37: 4702-4708.

Wetzel, R. G., 1992: Gradient-Dominated Ecosystems - Sources and Regulatory Functions of Dissolved Organic Matter in Freshwater Ecosystems. *Hydrobiologia,* 229: 181-198.

Wetzel, R. G., 2001: Limnology. Lake and River Ecosystems. *Elsevier Academic Press*: 1006.

Wilcox, H. S., Wallace, J. B., Meyer, J. L., and Benstead, J. P., 2005: Effects of labile carbon addition on a headwater stream food web. *Limnology and Oceanography,* 50: 1300-1312.

Ziegler, S. E. and Brisco, S. L., 2004: Relationships between the isotopic composition of dissolved organic carbon and its bioavailability in contrasting Ozark streams. *Hydrobiologia,* 513: 153-169.

Chapter 3: The impact of nutrient enrichment on dissolved organic carbon mineralization by headwater stream biofilm communities

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Dissolved organic carbon (DOC), nutrient enrichment, boreal and temperate headwater streams, priming effect.

Abstract

This study aimed to investigate how stream heterotrophic biofilm mineralization of dissolved organic carbon (DOC) was impacted by nutrient enrichment and how this impact may vary between boreal and temperate streams and with variation in DOC attributed to hydrological regime. Increased biofilm respiration ($R_{Biofilm}$) rates were associated with lower DOC concentrations at the nutrient-rich boreal sites. This suggests algal inputs and greater mineralization and perhaps incorporation of these labile carbon (C) sources by the more active microbial communities play a significant role in these stream reaches relative to streams with lower nutrient concentrations. This labile C mineralization, however, may not effectively stimulate extant DOC mineralization analogous to a priming effect, at least not through water column labile inputs. Further, $R_{Biofilm}$ was significantly higher in the temperate streams compared to the boreal sites, however, despite the differences in $R_{Biofilm}$ rates between the two regions, the biofilms responded similarly to the substrate additions. In particular, $R_{Biofilm}$ generally increased when adding labile C, regardless of region. At the sites with lower nutrient concentrations we found a C, nitrogen (N), and phosphorus (P) co-limitation and observed a lack of response in $R_{Biofilm}$ when adding C, N, P separately. The sites already elevated in N and P, however, exhibited an increase in $R_{Biofilm}$ with the added C and CNP, and a lack of response when adding solely N and/or P. These findings suggest that nutrient enrichment may increase the capacity of biofilms to use and mineralize labile C substrates in headwater streams. The potential importance of nutrients in regulating microbial activity was further highlighted during a stormflow experiment, when changes in hydrology led to lower $R_{Biofilm}$ presumably due to the reduction of N and P concentrations.

3.1 Introduction

Headwater streams are typically dominated by terrestrial allochthonous dissolved organic matter sources, and were often thought to simply transport that organic matter (Wipfli et al., 2007). However, headwater streams are now recognized as key components of watershed biogeochemical cycling. Due to the high active microbial surface area relative to water volume, headwater streams are characterized by high microbial activity including processes such as transformation of organic matter and CO_2 flux (Peterson et al., 2001; Alexander et al., 2007). In boreal streams, such processing is largely driven by heterotrophic biofilm microbial communities due to high dissolved organic carbon (DOC) concentrations (Aitkenhead and McDowell, 2000; Pastor et al., 2003), limited light availability, and low rates of primary production in these ecosystems (Tranvik, 1988, 1989; Jansson et al., 2000; Jonsson et al., 2001; Agren et al., 2008). In lower DOC systems more typical of temperate regions, however, algal-produced or autochthonous substrates can also play a vital role (Carr et al., 2005). Nutrient levels often regulate algal biomass (Tank and Dodds, 2003), and consequently affect the production of labile autochthonous substrates and transfer through the aquatic food web, and influence DOC pools and carbon cycling in headwater streams.

The biogeochemical cycling of DOC is potentially sensitive to anthropogenic alteration, particularly changes in watershed hydrology, vegetation, and associated changes in DOC and nutrient concentrations (Findlay et al., 2001; Findlay, 2005; Arango and Tank, 2008). Such changes may alter the fate of both autochthonous and allochthonous dissolved organic matter through interactive effects on microbial activity and aquatic nutrient transport (Bernhardt and Likens, 2002; Stelzer et al., 2003; Olapade and Leff, 2005). Increased loadings of bioavailable DOC into nutrient-rich waters, for example, can occur as a result of increased algal biomass and primary production (Meon

and Kirchman, 2001; Dodds et al., 2002). This often results in enhanced microbial metabolism, including high respiration and subsequent increases in the demand for nitrogen and/or phosphorus (Francoeur, 2001; Bernhardt and Likens, 2002; Tank and Dodds, 2003), leading to a reduced downstream nutrient transport. A greater availability of labile DOC sources can also increase the degradation of more slow turnover organic matter (Franke et al., 2013) and may contribute to CO_2 emissions from terrestrially derived DOC (Guenet et al., 2010; Bianchi, 2011), particularly in boreal aquatic ecosystems. Further, enhanced microbial metabolism results in the release of organic nutrients, and leads to stoichiometric imbalances of nutrient ratios (C:N:P) between microorganisms and their energy source (Frost et al., 2002; Frost et al., 2005). This influences not only microbial growth and reproduction, but also higher organisms in the food web, such as benthic grazers, that depend on microorganisms as their food source, leading to altered food web structure and function (Dodds et al., 2004; Frost et al., 2005). Microbial activity can also lead to a transformation of DOC into more slow turnover compounds, resulting in a lower bioavailability for downstream microbial communities (Sun et al., 1997; Hopkinson et al., 1998). Likewise, labile nitrogen compounds can be consumed rapidly by microorganisms and converted into more slow turnover substrates, affecting nitrogen uptake rates further downstream (Dodds et al., 2004).

Dissolved organic carbon and nutrient concentrations are often correlated with discharge (McDowell and Likens, 1988; McDiffett et al., 1989; Hinton et al., 1997). Changes in carbon and nutrient levels can, therefore, also occur during stormflow events, typically resulting in increased DOC and dissolved organic nitrogen (DON) concentrations, and contributions of more bioavailable, less degraded terrestrial organic matter (e.g. carbohydrates), often followed by greater microbial activity relative to

baseflow (Wondzell and Swanson, 1996; Gremm and Kaplan, 1998; Stepanauskas et al., 2000; Buffam et al., 2001; Sanderman et al., 2009). However, some studies conducted in watersheds with high DOC concentrations also showed a decrease in carbohydrate content during stormflow, followed by lower microbial activity (Meyer et al., 1988). Such factors affecting the amount, composition, and bioavailability of DOC and nutrients can be important for controlling the degradation of organic matter, ecosystem productivity, and, thus, biogeochemical cycling of carbon and nutrients.

Investigating the relationship between microbial DOC cycling under changing DOC and nutrient conditions will increase our understanding of carbon and nutrient cycling in headwater streams and the potential impacts of environmental change on these dynamics. Stream biofilm respiration represents one important fate of DOC and, therefore, a useful measure for determining the potential influence of nutrients on the net mineralization of more complex stream DOC versus labile substrates such as glucose or autochthonous C sources. To elucidate the impact of long-term nutrient enrichment on the heterotrophic microbial mineralization of DOC, substrate addition experiments were conducted in three historically nutrient-impacted boreal headwater streams in eastern Newfoundland, Canada. These boreal stream results were compared to similar results from four temperate streams representing a nutrient gradient typical of temperate streams in North America (Lyon and Ziegler, 2009) to assess large scale regional variation in these effects. Results from these experiments were collectively assessed to investigate how nutrient enrichment and DOC character influences stream biofilm respiration and the factors limiting stream biofilm respiration.

3.2 Methods

3.2.1 Study sites

To gain a better understanding of the impact of long-term nutrient enrichment on heterotrophic microbial mineralization of DOC in boreal headwater streams, we conducted manipulative substrate addition experiments, previously accomplished in a relatively pristine boreal watershed (Franke et al., 2013), within more urban nutrient-enriched stream sites. Three boreal streams, including Leary's Brook (LB), Waterford River (WR), and Virginia River (VR) on the Northeast Avalon Peninsula in Newfoundland, Canada, were chosen. The upstream sites of LB and VR are influenced by forest (e.g. balsam fir (*Abies balsamea*); forest floor: broom moss (*Dicranum scoparium*) and feathermoss (*Ptilium*)) and wetland (bogs and fens) cover (WQMA, 2012) and represent relatively unimpacted sites. The stream flow continues through the city of St. John's, where main roads, industrial parks and agriculture have a major impact on the downstream water quality (Table 3.1). Waterford River originates at Bremigans Pond, a remote area west of the city. However, the Trans-Canada-Highway and other main roads may influence the upstream site of WR. The downstream location of WR is influenced by the drainage of waste water as well as inputs of septic sludge, waste oil, and chemicals from a waste disposal site located nearby (WQMA, 2012). Sampling and experiments were conducted at upstream and downstream sites in summer 2009 during baseflow in all three streams, and additionally during stormflow at VR-1 and VR-2 to capture base- versus stormflow conditions in order to provide further contrast in stream water and dissolved organic matter chemistry.

The sites upstream and downstream of nutrient impacts on the Northeast Avalon Peninsula in Newfoundland were compared to two pairs of temperate streams

(Northwest Arkansas region of the Ozark Plateau, USA), where each pair represented sites with similar catchment characteristics but contrasting nutrient enrichment. Four headwater streams were investigated, including Cecil Creek (CC), Mill Creek (MC), Spavinaw Creek (SC), and Columbia Hollow (CH). These streams are influenced by forest cover, pasture, and low-density urban development (Lyon and Ziegler, 2009). Cecil Creek and Mill Creek are tributaries of the Buffalo National River. Cecil Creek is relatively pristine, whereas Mill Creek receives high nutrient loadings from agricultural land use (Mott et al., 2000). Spavinaw Creek and Columbia Hollow are part of the Lake Eucha–Spavinaw Basin, an important drinking water supply in Northeast Oklahoma. These streams are highly impacted by agriculture; mainly pasture with a high density of poultry houses (Haggard et al., 2001; Haggard et al., 2005). All research sites were located in forested areas, except CH, which was located in pasture with only a few small trees in its riparian zone. Sampling and experiments were conducted in fall 2006 at baseflow conditions.

3.2.2 Experimental approach

Substrate addition experiments were conducted at each of the six stream sites on the Northeast Avalon Peninsula in summer 2009, identical to those outlined in Franke et al., 2013 (Chapter 2). Additionally, similar experiments were performed at the four stream sites in the Ozarks in fall 2006. All experiments were carried out in replicate 300 mL biological oxygen demand (BOD) bottles, filled with stream water from each site, and with two heterotrophic biofilm colonized tiles (ceramic, 10 x 47 x 5 mm, total biofilm surface area of ~30 cm^2) added per bottle. Due to the high bacterial cell abundance in CH and SC ($2.1 \times 10^6 \pm 8.8 \times 10^4$ cells mL^{-1} and $8.9 \times 10^4 \pm 5.4 \times 10^3$ cell mL^{-1}, respectively), the stream water used in the Ozark experiments was filtered sequentially

through 10, 1, 0.2, and 0.1 µm cartridge filters. Bacterial cell numbers decreased after filtration ($1.1 \times 10^6 \pm 1.7 \times 10^4$ cells mL^{-1} at CH and $3.4 \times 10^4 \pm 1.5 \times 10^3$ cell mL^{-1} at SC). Bacterial cell numbers were very low at MC and CC (only one cell for every 25 – 50 grids).

The labile substrates used in these experiments were glucose (C), ammonium nitrate (N), and potassium phosphate (P), added as 1 mL additions to each bottle to achieve final concentrations of 83 µM C, 18 µM N, and 3 µM P in the boreal streams. The glucose-C additions resulted in levels roughly 12 to 35 % of ambient stream DOC concentrations to assure stimulation of the use of C while remaining relative to adequate N:P ratios typical of concentrations found at the nutrient-impacted stream sites in this region. The substrates used in the Ozark streams were added to achieve final concentrations of 60 µM C at CC, MC, SC, and 120 µM C at CH, as well as 15 µM N and 1.5 µM P at CC and MC, and 60 µM N and 6 µM P at SC and CH, respectively, providing adequate labile C and the appropriate ratio of N:P across these streams, which range in over an order of magnitude in terms of inorganic nutrient concentrations (Table 3.1). In the case of CC and MC, where nutrients and DOC are fairly low, the addition of DOC was likely in excess of labile C in any of these streams (>50 % total DOC pool). The higher addition of glucose at CH was based on the elevated autotrophic activity in this stream, which likely entails a greater input of labile C substrates (Haggard et al., 2005; Lyon and Ziegler, 2009) in addition to the elevated DOC at this site. Substrates were added either as single spikes (C, N, and P), or as a combination (CN, CP, CNP, NP). The bottles from the experiments conducted in the boreal streams were incubated for 24 hours in a dark water bath (NesLab, Thermo Scientific, Waltham, MA, USA) in the laboratory at a constant water temperature of 15.5°C. The bottles from the experiments conducted in the Ozarks were incubated simultaneously at one site in the dark at a

temperature of 22 ± 1°C. To assess treatment effects on heterotrophic microbial activity, respiration rates, as microbial oxygen uptake, were measured using an YSI-5100 dissolved oxygen (DO) probe (YSI Incorporated, Yellow Springs, OH, USA). Respiration rates are presented here as carbon consumption in units μM C hr^{-1}, assuming a respiratory quotient of 1 (del Giorgio et al., 1997; del Giorgio and Cole, 1998). Additionally, biofilm respiration ($R_{Biofilm}$) rates were normalized to DOC concentration (μM C hr^{-1} mM DOC^{-1}), and to DOC concentration and biofilm biomass (μM C hr^{-1} mM DOC^{-1} mM $C_{biomass}^{-1}$) to assess the variation in DOC and biofilm-specific rates across the study sites investigated. Biofilm biomass data were not collected during the experiments in the Ozarks, so $R_{Biofilm}$ were only normalized to DOC concentration (μM C hr^{-1} mM DOC^{-1}). In the boreal streams, however, tiles from each experiment were collected individually in clean plastic bags, immediately frozen and freeze-dried (Labconco, Kansas City, MO, USA), and used for biomass analysis as outlined in Franke et al., 2013. Additionally, epilithic stream biofilm material was collected at each stream site, shell-frozen, and freeze-dried. Further, DOC and nutrient uptake were measured as changes in dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP). Respiration rates, as well as DOC and nutrient uptake rates were compared with control treatments where no substrates have been added.

In addition to the assessment of variation in labile C, N, and P limitation of heterotrophic biofilm respiration, the influence of labile C on the mineralization of extant stream DOC (R_{DOC}) was determined by using ^{13}C-labeled glucose (551 ‰, D-Glucose-$^{13}C_6$; Sigma Aldrich, St. Louis, MO, USA), identical to those experiments described in detail in Franke et al., 2013. To address whether labile C stimulated the respiration of ambient stream DOC analogous to a priming effect, we determined the net amount of glucose that was respired during each experiment where glucose was added. The

difference between the total respiration (R_{total}) and glucose C respired ($R_{Glucose}$) in the glucose treated incubations was then compared with the respiration from the treatments without glucose additions ($R_{control}$; Franke et al., 2013).

3.2.3 Analytical methods

Tile and stream biofilm material collected during the experiments was analyzed for stable carbon and nitrogen isotopic composition ($\delta^{13}C$, $\delta^{15}N$) and biomass (%C, %N) with a Carlo Erba NA1500 Series II elemental analyzer (Milan, Italy) interfaced with a MAT252 isotope ratio mass spectrometer (Finnigan, Bremen, Germany). Epilithic stream biofilm chlorophyll content was determined by extracting lyophilized biofilm samples in 90 % acetone followed by centrifugation and absorbance measurements at wavelengths of 430, 630, 647, 664, 750 nm using a UV/VIS Spectrometer (Lambda 25; Perkin Elmer, Waltham, MA, USA) and acetone as a blank. Previous comparisons between freshly frozen and freeze-dried biofilms yield no significant difference in chlorophyll content (D. Lyon, unpublished data).

Analyses for TIC and DOC concentrations, as well as $\delta^{13}C_{TIC}$ and $\delta^{13}C_{DOC}$, were conducted within 10 days of sampling using an AURORA 1030 TOC analyzer (O.I. Analytical, College Station, Texas, USA) coupled to a MAT252 isotope ratio mass spectrometer (Finnigan, Bremen, Germany). Total dissolved phosphorus (TDP), SRP and ammonium (NH_4^+) concentrations were measured with a Lachat FIA 8500 inorganic nutrient analyzer (detection limits: 0.64 µM P, 0.32 µM P, 0.59 µM N, respectively; HACH, Loveland, CO, USA). Nitrate (NO_3^-) samples were analyzed with a DX-100 ion chromatograph (detection limit: 0.057 µM N; DIONEX, Sunnyvale, CA, USA). Samples for total dissolved nitrogen (TDN) were analyzed using a high temperature combustion total organic carbon analyzer (Shimadzu TOC-V) with a chemiluminescent NO_x detector

(TNM-1; Shimadzu, Japan). Dissolved organic nitrogen was estimated from the difference between TDN and total inorganic nitrogen (NH_4^+, NO_3^-).

Spectral measurements were performed at wavelengths spanning 200 to 800 nm using a UV/VIS scanning spectrometer (Lambda 25; Perkin Elmer, Waltham, MA, USA) and a 1 cm quartz cuvette, with NanoPure-UV water as a blank.

3.2.4 Statistical analyses

Significance tests were conducted between initial, control, and final samples with an α–value of 0.05 (AnalystSoft Inc., StatPlus:mac - Version 2009) to ascertain differences in DO, TIC, DOC, and nutrient concentrations. Non-significant changes were considered to be below detection. To determine whether $R_{Biofilm}$ rates measured in the boreal streams may have varied with site (inter-site effect) and in-stream (intra-site effect) location, generalized linear models (GLM) were constructed to test these effects, as well as their interactions (JMP 8.0; SAS, NC, USA). To test differences in $R_{Biofilm}$ rates among the individual treatments (C, CNP, CN, CP, N, P, NP), multiple comparisons were conducted using one-way analyses of variation (ANOVA) followed by post-hoc Tukey's Honest Significant Difference tests (family wise error of α = 0.05). Furthermore, Pearson correlations with an α-level of 0.05 were used to test the potential role of DOC and nutrient concentrations and biofilm biomass in regulating $R_{Biofilm}$ rates. Respiration rates were tested against DOC, DON, DIN, and – in case of the boreal streams – biofilm C and N content.

3.3 Results

3.3.1 Water chemistry – Boreal versus temperate streams

In the boreal streams, ambient stream water temperature was between 15 and 16°C at all study sites, with all the boreal experiments conducted at 15.5°C. The Ozark streams water temperatures varied between 18 and 25°C, with all the experiments conducted at 22 ± 1°C. Dissolved oxygen concentrations ranged from 310 to 400 μM O_2 in both boreal and Ozark streams, and were higher downstream relative to upstream in the boreal streams (Table 3.1).

Dissolved organic carbon concentrations varied from 420 to 690 μM C at the upstream sites of the boreal streams, and from 200 to 250 μM C at the downstream sites LB-2 and VR-2, whereas WR-2 exhibited DOC concentrations of up to 500 μM C (Table 3.1). The higher DOC concentrations at the upstream sites at VR and LB were likely due to large organic matter inputs from wetlands or ponds located upstream of the study sites. DOC concentrations were significantly lower (70 – 115 μM C) in the Ozark streams, except at CH (430 μM C; Table 3.1). DOC normalized absorption coefficients a_{254}, often used as indicators for DOM aromaticity (Weishaar et al., 2003), varied from 119 to 145 L mmol C^{-1} m^{-1} at the upstream sites, and from 65 to 89 L mmol C^{-1} m^{-1} at the boreal downstream sites and at the temperate stream sites. DOC normalized absorption coefficients a_{350} ranged from 33 to 40 L mmol C^{-1} m^{-1} at the upstream sites, and from 3 to 18 L mmol C^{-1} m^{-1} at the boreal downstream sites and at the temperate stream sites. This suggests aromatic content of the DOC was relatively higher upstream compared to the boreal downstream and temperate stream sites. Absorption ratios a_{254}:a_{350} were increased at the boreal downstream and temperate stream sites compared to the boreal

upstream sites, indicating a higher relative presence of low molecular weight DOC downstream in the boreal streams and in the temperate streams.

Dissolved organic nitrogen concentrations varied from 11 to 18 μM N at the upstream sites, and from 12 to 50 μM N at the downstream sites of the boreal streams (Table 3.1). Dissolved inorganic nitrogen levels ranged from 1 to 3 μM N upstream, and from 10 to 19 μM N downstream (Table 3.1). SRP levels were below detection at all boreal stream sites (<0.3 μM P). Total P concentrations were below detection (<0.6 μM P) at all upstream sites, and ranged from 1 to 3 μM P at the downstream sites (Table 3.1). A significant reduction in N and P concentrations was observed at VR-2 during stormflow relative to baseflow (Table 3.1). Both DIN and SRP concentrations were considerably higher at the study sites in the Ozarks compared to the values measured in the boreal streams. DIN concentrations at CH, for example, were as high as 1376 μM N, which accounted for the total N content at this site (Table 3.1). SRP levels varied from 0.1 μM P at CC and MC, to 1.1 and 3.1 μM P at SC and CH, respectively (Table 3.1).

3.3.2 Geochemical characterization of epilithic stream biofilm communities on site

The boreal stream biofilm communities found on site exhibited C:N ratios between 5.0 and 6.7 – contrasting to the higher biofilm C:N of 7.3 to 19.5 measured in the Ozark streams (Lyon and Ziegler, 2009) – $\delta^{13}C$ values from -31.2 to -28.2 ‰, and $\delta^{15}N$ values between 4.8 and 6.7 ‰, typical of freshwater autotrophs (Fry, 1991; Hamilton and Lewis, 1992; Kendall et al., 2001). Biofilm C content ranged from 47 to 55 wt.%, and N content varied from 9 to 11 wt.%. Both C and N content were higher downstream compared to upstream.

Chlorophyll a content varied between 59 and 75 % (0.02 – 2.51 μg chl mg C^{-1}) of the total chlorophyll content, and was higher downstream relative to upstream in the

boreal streams. Chlorophyll b ranged from 12 to 25 % (0.01 – 0.41 µg chl mg C^{-1}), and chlorophyll c varied from 6 to 17 % (0.01 – 0.45 µg chl mg C^{-1}), both being higher upstream than downstream. In contrast, total biofilm chlorophyll content in the Ozark streams ranged from 2.8 µg chl mg C^{-1} in CC to 19.9 µg chl mg C^{-1} in CH, with chl a varying from 75 to 79 %, chl b from 11 to 16 %, and chl c from 6 to 12 % of the total chlorophyll content (Lyon and Ziegler, 2009).

3.3.3 Geochemical characterization of the tile biofilm communities

Heterotrophic biofilm biomass colonized on the tiles collected in the boreal streams and measured as C and N content was generally higher at the downstream sites relative to upstream (Table 3.2). Biofilm C content varied from 2.7 to 6.6 µmol C per tile among most of the study sites, except at the downstream site of LB-2, where biofilm C content was significantly higher (18.5 µmol C per tile) compared to all other sites (Table 3.2). Likewise, biofilm N content ranged from 0.2 to 0.9 µmol N per tile among the study sites, whereas biofilm N content at LB-2 was 3.4 µmol N. Also, tile biofilm biomass was slightly reduced at the downstream site of VR when collected for the stormflow experiments, whereas tile biomass was more than two times lower at the upstream site during stormflow relative to baseflow. Reflecting the tile biofilm C and N content, the tile biofilm C:N ratios were higher upstream (12 – 14) compared to downstream (5 – 10). This may suggest the incorporation of more allochthonous DOC sources upstream and autochthonous sources and/or perhaps greater availability of N and P downstream. Tile biomass was not determined for the Ozark stream tiles.

Table 3.1: Initial water chemistry parameters at Leary's Brook (LB), Waterford River (WR), and Virginia River (VR), with 1 representing a relatively unimpacted upstream site, and 2 representing a nutrient-enriched downstream site of each stream, as well as Cecil Creek (CC), Mill Creek (MC), Spavinaw Creek (SC), and Columbia Hollow (CH). Measurements were taken for total inorganic carbon (TIC), dissolved oxygen (DO), dissolved organic carbon (DOC), dissolved organic (DON) and inorganic nitrogen (DIN), total dissolved (TDP) and soluble reactive phosphorus (SRP) concentrations. Values correspond to averages of five replicates derived from initial bottles used in the experiments. BD: Below detection.

Site	**Event**	**TIC**	**DO**	**DOC**	**DON**	**DIN**	**TDP**	**SRP**
		µM C	µM O_2	µM C	µM N	µM N	µM P	µM P
				Boreal streams				
LB-1	Baseflow	108	330	686	18.1	0.9	BD	BD
LB-2	Baseflow	213	331	246	12.7	19.1	0.9	BD
WR-1	Baseflow	232	313	423	11.5	3.1	BD	BD
WR-2	Baseflow	300	359	497	45.7	18.0	2.8	BD
VR-1	Baseflow	178	344	423	12.7	0.7	BD	BD
VR-2	Baseflow	443	385	211	48.5	19.1	0.9	BD
VR-1	Stormflow	181	325	558	11.8	0.6	BD	BD
VR-2	Stormflow	370	354	206	27.2	9.7	BD	BD
				Ozark streams				
CC	Baseflow	-	375	113	6	BD	-	0.1
MC	Baseflow	-	399	104	14	59	-	0.1
SC	Baseflow	-	335	70	38	206	-	1.1
CH	Baseflow	-	356	428	BD	1376	-	3.1

Table 3.2: Seasonal description of the biofilm communities on the tiles (10 x 47 x 5 mm), particularly stable carbon isotope composition ($\delta^{13}C$), total carbon (C) and nitrogen (N) content, and biofilm C:N ratios. Prior to analysis, freeze-dried tiles were sonicated in 100 mL NanoPure water for approximately 4 hours at room temperature. The water was filtered through a pre-combusted quartz filter (0.45 µM pore size) and distributed equally into duplicate, acid-cleaned, pre-combusted 40 mL amber glass vials, and spiked with H_3PO_4. Values correspond to averages of five replicates derived from initial bottles used in the experiments ± one standard deviation.

Site	Event	$\delta^{13}C$	C biomass	N biomass	Biofilm C:N
		‰	µmol C per tile	µmol N per tile	
LB-1	Baseflow	-28.7 ± 0.6	6.7 ± 0.14	0.5 ± 0.012	14 ± 0.1
LB-2	Baseflow	-26.7 ± 0.3	18.5 ± 0.34	3.5 ± 0.016	5 ± 0.4
WR-1	Baseflow	-25.3 ± 0.8	4.2 ± 0.06	0.3 ± 0.006	13 ± 0.5
WR-2	Baseflow	-25.0 ± 0.8	5.9 ± 0.21	0.6 ± 0.004	10 ± 0.1
VR-1	Baseflow	-29.4 ± 0.6	6.5 ± 0.09	0.4 ± 0.005	15 ± 0.1
VR-2	Baseflow	-32.0 ± 0.5	4.5 ± 0.14	0.9 ± 0.005	5 ± 0.1
VR-1	Stormflow	-26.4 ± 0.5	2.7 ± 0.02	0.2 ± 0.003	12 ± 0.1
VR-2	Stormflow	-26.1 ± 0.4	4.1 ± 0.04	0.7 ± 0.004	5 ± 0.5

3.3.4 Heterotrophic biofilm respiration and DOC mineralization under changing DOC and nutrient conditions

In the boreal streams, heterotrophic $R_{Biofilm}$ rates, measured as changes in DO and reported as C consumption units, exhibited an inter- ($p = 0.01$, $\chi^2 = 6.7$) and intra-site effect ($p < 0.01$, $\chi^2 = 20.0$), and were low at the upstream sites of VR and LB (below detection and 0.2 ± 0.1 μM C hr^{-1}, respectively) and greater at the downstream sites (0.9 ± 0.1 μM C hr^{-1} and 1.7 ± 0.2 μM C hr^{-1}, respectively). Respiration rates ($R_{Biofilm}$) at the upstream and downstream sites of WR did not differ significantly from each other (0.7 ± 0.1 μM C hr^{-1} at WR-1, 0.8 ± 0.2 μM C hr^{-1} at WR-2). Heterotrophic $R_{Biofilm}$ rates measured in the Ozark streams were considerably higher than the ones observed in the boreal streams and varied from 1.8 to 2.2 μM C hr^{-1} at CC, MC, and SC, and from 9.6 to 11.1 μM C hr^{-1} at CH. The $R_{Biofilm}$ rates measured in the boreal streams were negatively correlated with stream DOC concentration ($p < 0.01$, $R^2 = 0.43$, $\chi^2 = 11.3$). Further, the $R_{Biofilm}$ rates measured in the boreal streams were positively but weakly correlated with initial DON ($p = 0.01$, $R^2 = 0.03$, $\chi^2 = 6.9$) and DIN ($p = 0.01$, $R^2 = 0.12$, $\chi^2 = 6.3$) concentrations, suggesting that nutrients play an important role controlling the biofilm microbial activity and use of DOC in these streams. Furthermore, $R_{Biofilm}$ rates measured in the boreal streams were correlated with biofilm biomass (C content: $p = 0.03$, $R^2 = 0.57$; N content: $p < 0.01$, $R^2 = 0.73$). The $R_{Biofilm}$ rates measured in the Ozark streams were positively correlated with DOC ($p < 0.01$, $R^2 = 0.95$), DIN ($p < 0.01$, $R^2 = 0.93$), and SRP concentration ($p < 0.01$, $R^2 = 0.75$).

The $R_{Biofilm}$ rates normalized to the initial DOC concentration varied from below detection at VR-1 and 0.3 μM C hr^{-1} mM DOC^{-1} at LB-1 to 4.0 μM C hr^{-1} mM DOC^{-1} and 6.7 μM C hr^{-1} mM DOC^{-1} at VR-2 and LB-2, respectively. DOC normalized $R_{Biofilm}$ rates at

both sites of WR ranged between 1.5 and 1.7 μM C hr^{-1} mM DOC^{-1}. DOC normalized $R_{Biofilm}$ rates measured in the Ozark streams were, again, considerably higher than the ones observed in the boreal streams and fell within the small range between 18.2 and 24.2 μM C hr^{-1} mM DOC^{-1}.

Biofilm respiration rates normalized to DOC concentration and biomass varied from 2.2 μM C hr^{-1} mM DOC^{-1} mM $C_{biomass}^{-1}$ at LB-1 to 49.7 μM C hr^{-1} mM DOC^{-1} mM $C_{biomass}^{-1}$ at VR-2, whereupon rates at LB-2, WR-1 and WR-2 did not vary much from each other (12.9 – 19.6 μM C hr^{-1} mM DOC^{-1} mM $C_{biomass}^{-1}$). The reduction of N and P concentrations (Table 3.1) and lower $R_{Biofilm}$ rates during stormflow indicate a decreased DOC bioavailability at VR-2 during stormflow (18 μM C hr^{-1} mM DOC^{-1} mM $C_{biomass}^{-1}$) relative to baseflow (50 μM C hr^{-1} mM DOC^{-1} mM $C_{biomass}^{-1}$). At VR-1, however, increased DOC concentrations during the stormflow event coincided with an increase in the normalized $R_{Biofilm}$ rates (7 μM C hr^{-1} mM DOC^{-1} mM $C_{biomass}^{-1}$) relative to baseflow (below detection).

3.3.5 Carbon and nutrient limitation of biofilm DOC mineralization

Substrate addition experiments conducted in the urban boreal streams revealed a microbial C, N, and P co-limitation based on $R_{Biofilm}$ at the upstream sites of VR and LB ($p < 0.01$ relative to control). The upstream site of WR, however, exhibited a labile C ($p = 0.01$) and P limitation ($p = 0.02$; Fig. 3.1). At LB-1, the $R_{Biofilm}$ rates associated with the CNP treatment were not significantly different from the $R_{Biofilm}$ rates of all other treatments (C/P/NP: $p = 0.42$, N: $p = 0.21$). At WR-1, $R_{Biofilm}$ rates associated with the P treatment were not significantly different from the $R_{Biofilm}$ rates of all other treatments (C: $p = 0.11$, CNP: $p = 0.18$, N: $p = 0.99$, NP: $p = 0.85$) were not significant. Further, a labile C limitation was observed at all nutrient-impacted downstream sites ($p < 0.01$), indicating

that heterotrophic $R_{Biofilm}$ may be limited by labile C when inorganic nutrients are more available. Congruent with this, the addition of inorganic N and P additionally to the ambient nutrient levels already present did not show an effect at VR-2, LB-2, and WR-2 (Fig. 3.1). A C and CNP treatment effect was observed at LB-2 ($p < 0.01$ relative to control and all other treatments). However, C and CNP effects were not significantly different ($p = 0.62$) from each other. At WR-2, a significant difference was observed between the CNP and N ($p = 0.03$), P ($p = 0.04$), and NP ($p = 0.05$), but not between C and CNP ($p = 0.88$) treatments. Also, the C treatment effect was not significantly different from the N ($p = 0.24$), P ($p = 0.31$), or NP ($p = 0.34$) treatment effects. The changing nutrient levels and potential variation in DOC bioavailability at VR-2 during stormflow led to an increased C and CNP limitation (approximately three times higher) relative to baseflow, and also caused the N and P limitation (Fig. 3.2), which was not observed during baseflow.

Similarly to the results from the nutrient-enriched boreal downstream sites, substrate addition experiments conducted in the Ozark streams revealed a strong labile C limitation at three of the four sites (MC, SC, CH; Fig. 3.3). $R_{Biofilm}$ rates increased at MC when adding C in conjunction with N (CN treatment), P (CP treatment), and both (CNP treatment), indicating a C and P co-limitation (Fig. 3.3). Significant differences were observed among C, CN, CP, CNP and all other treatments at MC ($p < 0.001$) and CH ($p < 0.001$; Fig. 3.3). At SC, a significant difference was observed between CN, CNP and N, P, and NP ($p = 0.01 – 0.04$), however, not between C and CP and all other treatments (Fig. 3.3). Further, at the lowest nutrient site CC, increased $R_{Biofilm}$ rates were observed with the addition of CN and CNP, suggesting a strong C and N co-limitation and, to a smaller extent, a C, N, and P co-limitation (Fig. 3.3). A significant difference was observed between CN as well as CNP and all other treatments ($p < 0.001$ for all

treatments; Fig. 3.3). No treatment effect was observed at any of the study sites when adding N and P without sources of labile C (N, P, NP; Fig. 3.3).

Dissolved organic carbon uptake rates, measured in the control and treatment bottles, were below detection at both sites of VR and WR. Net DOC uptake rates, determined in the control bottles, were 1.7 ± 0.1 and 0.3 ± 0.02 μM C hr^{-1} at LB-1 and LB-2, respectively. Increased DOC uptake rates (2.1 – 2.7 μM C hr^{-1}) were observed with the addition of C and CNP at LB-1 and LB-2 (Fig. 3.4), as well as with the addition of P at LB-1, suggesting that the biofilm community may be limited by C and P. Nutrient uptake rates were below detection at all the boreal study sites. Further, net DOC uptake rates, determined in the control bottles, were below detection at CC, MC, and SC, and were 0.4 ± 0.2 μM C hr^{-1} at CH (Fig. 3.4). The DOC, DIN, and SRP uptake rates measured in the Ozark streams reflected the C and nutrient limitation pattern observed in the $R_{Biofilm}$. Increased net uptake rates were observed following the addition of C (alone and in combination with N and/or P) at CH and SC (Fig. 3.4), however, uptake rates were significantly lower at SC (BD – 4.1 μM C hr^{-1}, 0.2 – 0.7 μM N hr^{-1}, 0.03 – 0.05 μM P hr^{-1}) compared to CH (BD – 9.2 μM C hr^{-1}, 6.9 – 8.8 μM N hr^{-1}, 0.1 μM P hr^{-1}). Biofilms at MC were co-limited by C and P, in part congruent with the $R_{Biofilm}$ results. Increased DOC uptake rates (relative to the control) were observed at MC when adding C in conjunction with P (CP treatment; Fig. 3.4), and DIN and SRP uptake was only measurable in the CP and CNP treatment (0.3 – 0.4 μM N hr^{-1}, 0.03 μM P hr^{-1}). At CC, increased DOC uptake rates (relative to the control) were observed when adding C in conjunction with N (CN and CNP treatments), and, to a lesser extent, with P (CP and CNP treatments; Fig. 3.4). The N and P uptake rates, however, were only measureable in the CNP treatment (0.3 μM N hr^{-1}, 0.03 μM P hr^{-1}). These results suggest that the

biofilm community present at CC may be co-limited by C and N, and, though of smaller importance, by P.

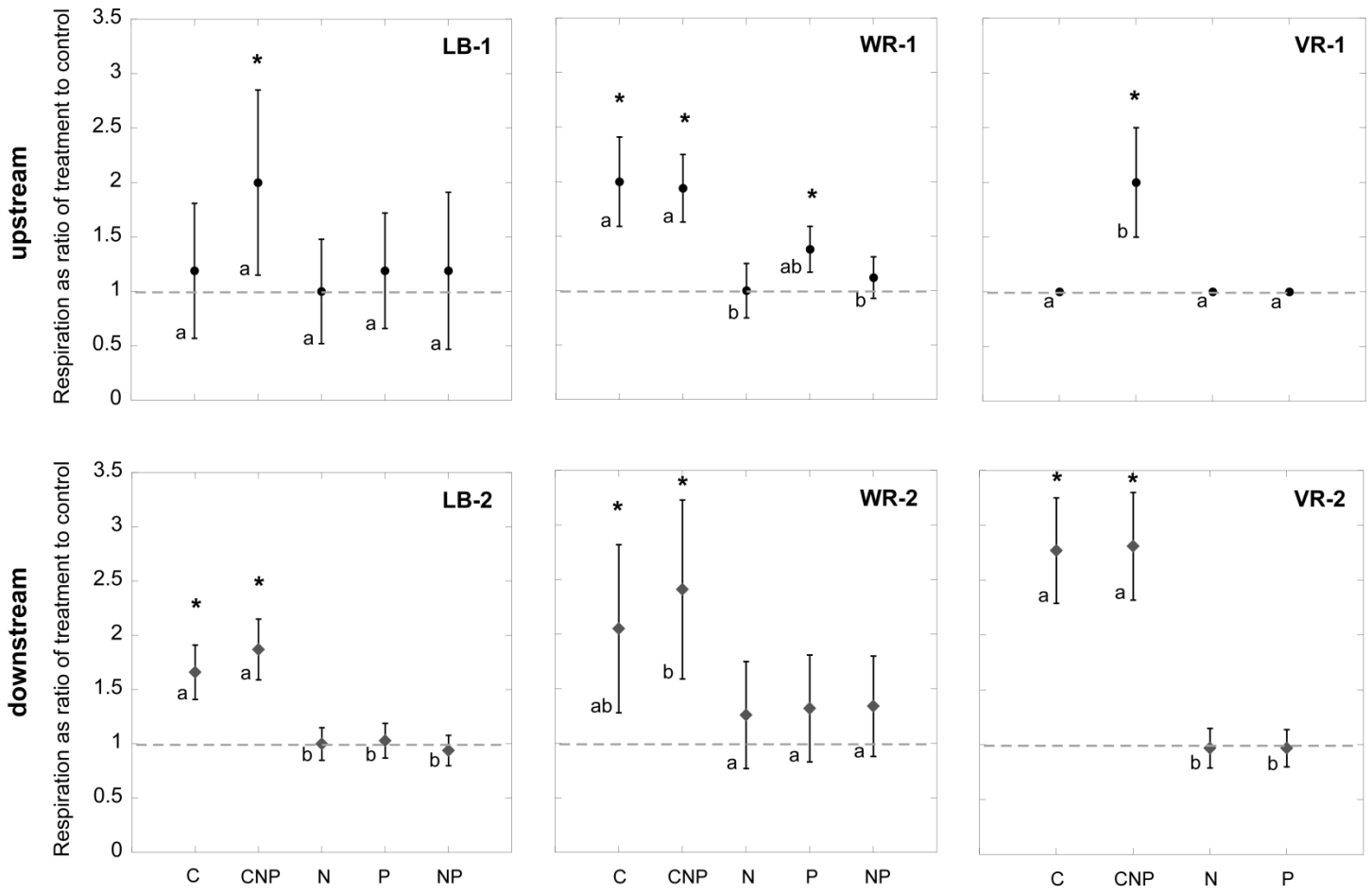

Figure 3.1: Ratio of treatment to control biofilm respiration rates ($R_{Biofilm}$) for substrate addition experiments at Leary's Brook (LB), Waterford River (WR), and Virginia River (VR). Substrate additions included glucose (C), ammonium nitrate (N), and potassium phosphate (P). Values correspond to the mean of five experimental replicates ± one standard deviation. Results of the generalized linear model tests of absolute $R_{Biofilm}$ associated with each treatment are provided with asterisks as * $p < 0.05$ relative to the control (no additions). Results from the Tukey's Honestly Significant Difference tests for the absolute $R_{Biofilm}$ associated with each treatment are provided by lower case letters (a, b), where only those treatments designated with different letters were found to be significantly different.

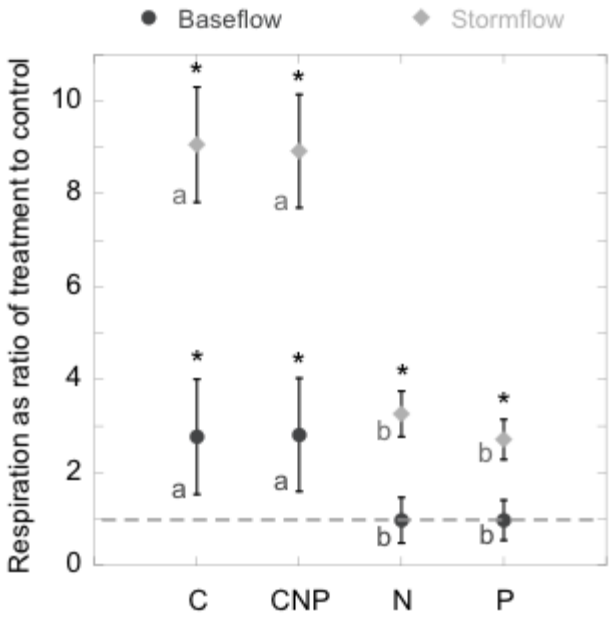

Figure 3.2: Ratio of treatment to control biofilm respiration rates ($R_{Biofilm}$) for substrate addition experiments at Virginia River downstream (VR-2) during baseflow and stormflow. Substrate additions included glucose (C), ammonium nitrate (N), and potassium phosphate (P). Values correspond to the mean of five replicates ± one standard deviation. Results of the generalized linear model tests of absolute $R_{Biofilm}$ associated with each treatment are provided with asterisks as * $p < 0.05$ relative to the control (no additions). Results from the Tukey's Honestly Significant Difference tests for the absolute $R_{Biofilm}$ associated with each treatment are provided by lower case letters (a, b), where only those treatments designated with different letters were found to be significantly different.

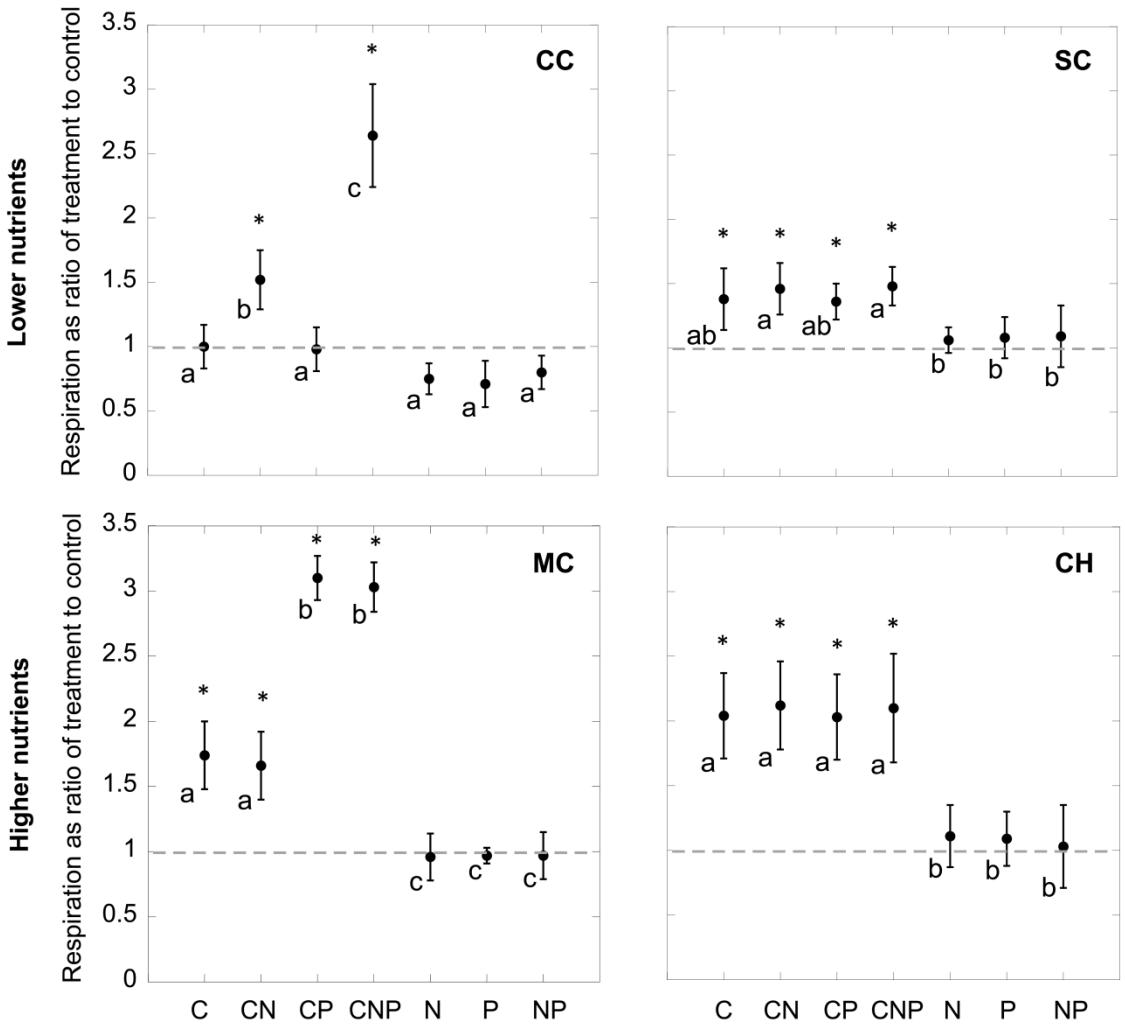

Figure 3.3: Ratio of treatment to control biofilm respiration rates ($R_{Biofilm}$) for substrate addition experiments paired by watersheds and along a nutrient gradient in the Ozarks – Cecil Creek (CC), Mill Creek (MC), Spavinaw Creek (SC), and Columbia Hollow (CH). Substrate additions included glucose (C), ammonium nitrate (N), and potassium phosphate (P). Values correspond to the mean of five replicates ± one standard deviation. Results of the generalized linear model tests of absolute $R_{Biofilm}$ associated with each treatment are provided with asterisks as * $p < 0.05$ relative to the control (no additions). Results from the Tukey's Honestly Significant Difference tests for the absolute $R_{Biofilm}$ associated with each treatment are provided by lower case letters (a, b, c), where only those treatments designated with different letters were found to be significantly different.

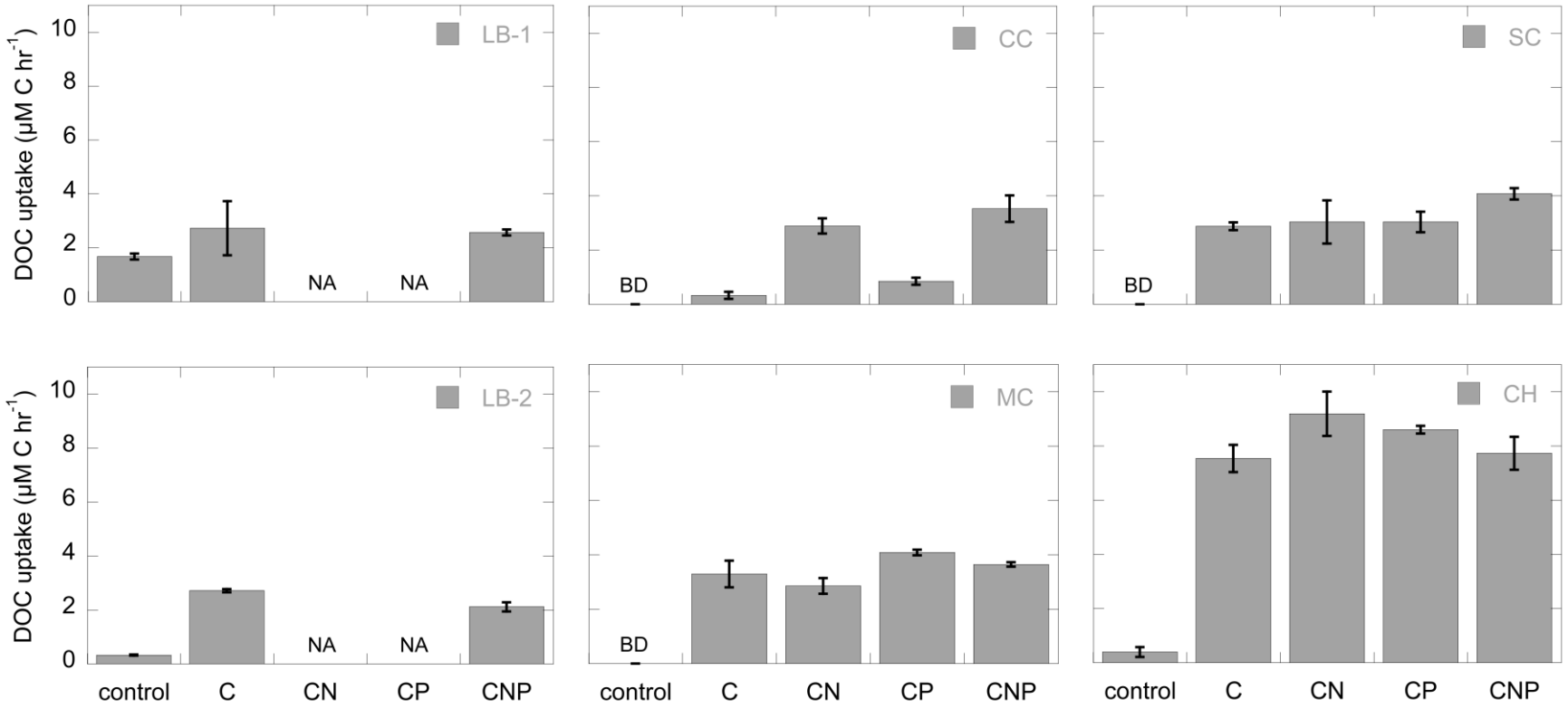

Figure 3.4: Dissolved organic carbon (DOC) uptake rates during substrate addition experiments at Leary's Brook upstream (LB-1) and downstream (LB-2), Cecil Creek (CC), Mill Creek (MC), Spavinaw Creek (SC), and Columbia Hollow (CH), measured as control (no additions) and in the carbon (C), nitrogen (N), and phosphorus (P) treatments. All other boreal sites were not included in this figure since DOC uptake was below detection. BD: Below detection. NA: Not applicable.

3.3.6 Effect of glucose amendment on biofilm respiration of extant boreal stream DOC

The influence of labile C, with and without added inorganic N and P, on the mineralization of extant boreal stream DOC (R_{DOC}) was investigated by determining changes in the $\delta^{13}C$ of the TIC and comparing the rate of glucose C respired ($R_{Glucose}$) during the incubation to the respiration rates from the treatments without glucose additions ($R_{control}$). The respiration of extant stream DOC (R_{DOC}) was calculated as the difference between total respiration (R_{total}) and $R_{Glucose}$ in the glucose treated incubations. R_{DOC} was then compared with $R_{control}$ to determine whether the added glucose competed with stream DOC as a source of energy or enabled a priming effect that stimulated the mineralization of stream DOC. Although $R_{control}$ was measureable across all sites using DO and across four of six study sites using TIC measurements, R_{DOC} was below detection across all sites within all treatments that included glucose, except at LB-1 in the C treatment (though not significantly different from $R_{control}$), signifying low R rates for extant DOC (Table 3.3). At the upstream sites, most R rates, based upon changes in TIC, were below detection with the only measureable rates for LB-1 across all treatments and CNP for VR-1. The addition of glucose, with or without added N and P, did not affect biofilm respiration at LB-1 and WR-1 (Table 3.3). At VR-1, however, the addition of glucose stimulated R_{total} in the CNP treatment, but not R_{DOC}, indicating that the increase in R was solely fueled by the added glucose (Table 3.3). In particular, $R_{Glucose}$ was not significantly different from R_{total} and R_{DOC} was below detection in the CNP treatment, whereas R_{total} was below detection in the solely C treatment and NP only treatment at VR-1 (Table 3.3). At the downstream sites, R rates based upon TIC production were all detectable, but neither C or CNP treatments lead to increased R_{DOC} (Table 3.3), rather R

was fueled, again, primarily by the added glucose-C in both the C and CNP treatments, and labile glucose was used preferentially relative to the ambient DOC.

Table 3.3: Respiration rates (μM C hr^{-1}) from the treatments without glucose additions ($R_{control}$), as well as total respiration (R_{total}) and glucose carbon respired ($R_{Glucose}$) in the glucose treated incubations at Leary's Brook (LB), Waterford River (WR), and Virginia River (VR). Values correspond to averages of five replicates ± one standard deviation. BD: Below detection.

Site		C treatment			CNP treatment		
	$R_{control}$	R_{total}	$R_{Glucose}$	R_{DOC}	R_{total}	$R_{Glucose}$	R_{DOC}
LB-1	0.13 ± 0.03	0.17 ± 0.08	0.03 ± 0.01	0.13 ± 0.08	0.19 ± 0.05	0.13 ± 0.01	BD
WR-1	BD	BD	BD	BD	BD	BD	BD
VR-1	BD	BD	BD	BD	0.34 ± 0.01	0.37 ± 0.02	BD
LB-2	0.37 ± 0.07	0.78 ± 0.01	0.66 ± 0.09	BD	0.59 ± 0.02	0.61 ± 0.05	BD
WR-2	0.15 ± 0.03	0.22 ± 0.03	0.32 ± 0.03	BD	0.30 ± 0.10	0.35 ± 0.05	BD
VR-2	0.26 ± 0.05	1.15 ± 0.01	1.07 ± 0.09	BD	0.99 ± 0.24	1.09 ± 0.16	BD

3.4 Discussion

Elucidating the potential influence of nutrients on the net mineralization of more complex stream DOC versus labile substrates such as glucose or autochthonous C sources provides some predictive understanding of C and nutrient cycling in headwater streams. Such knowledge is useful when trying to understand how headwater streams will respond and adapt to altered nutrient regimes due to human activities and/or climate change. Thus, the aim of this study was to investigate how variation in nutrient enrichment and DOC character influences the stoichiometric (CNP) limitations of heterotrophic biofilm respiration in both boreal and temperate regions. The intention was to determine whether differences in patterns of labile C, N, and/or P limitation with nutrient enrichment are common across these regions.

Biofilm respiration measured in both the boreal and temperate streams studied here was correlated with DOC and nutrient concentrations but in ways suggested of different controls. Greater $R_{Biofilm}$ rates were measured with lower DOC and higher nutrient concentrations in the boreal streams, suggesting higher nutrient concentrations may lead to increased autochthonous C inputs, which may stimulate $R_{Biofilm}$. Further, $R_{Biofilm}$ was significantly higher in the temperate streams compared to the boreal sites, however, despite the differences in $R_{Biofilm}$ rates between the two regions, the biofilms responded similarly to the substrate additions, i.e. $R_{Biofilm}$ generally increased when adding labile C, regardless of region. The CNP co-limitation and C limitation patterns generally observed at the nutrient-poor sites and the NP-rich sites, respectively, suggest that nutrient enrichment may increase the capacity of biofilms to use and mineralize labile C substrates in headwater streams. This labile C mineralization, however, may not effectively stimulate extant DOC mineralization, at least not through water column labile inputs. Combined, these findings suggest algal inputs and the greater incorporation of

these sources by the more active microbial communities may play a significant role in the nutrient-rich stream reaches relative to streams with lower nutrient concentrations.

3.4.1 Evidence for the impact of biofilm activity on stream DOC and its dependence upon N and P availability

The correlations and patterns observed for $R_{Biofilm}$ measured in these boreal and temperate streams suggest stream biofilms can have an important role in regulating DOC concentration and that this role is influenced by nutrient availability. The $R_{Biofilm}$ rates measured in the boreal streams were higher at the downstream sites relative to upstream, congruent with lower DOC and higher nutrient concentrations downstream. This coincided with the lower aromatic content of the DOC and a higher relative presence of low molecular weight chromophoric DOC (measured as increased a_{254}:a_{350}) downstream compared to the upstream sites of these streams. Furthermore, the higher tile biofilm C:N ratios upstream compared to downstream suggest the incorporation of more allochthonous DOC sources upstream and autochthonous sources and/or perhaps greater availability of N and P downstream. Further, the $R_{Biofilm}$ rates determined in the boreal streams were considerably lower compared to the temperate streams, again, coinciding with generally lower DOC concentrations and higher nutrient levels in combination with the likely higher biomass and temperature in the temperate streams compared to the boreal streams. The lower DOC concentrations could be associated with the lower organic matter content in the soils adjacent to the temperate study sites in contrast to the high organic matter content in soils typical of boreal stream watersheds (Schlesinger, 1984). The negative correlation between $R_{Biofilm}$ and DOC concentration observed within the boreal streams and when comparing boreal versus temperate streams may suggest co-correlation effects such as greater nutrient concentrations

leading to increased autochthonous inputs and, thus, stimulated $R_{Biofilm}$. The $R_{Biofilm}$ rates measured within the temperate streams, however, were positively correlated with DOC – in line with other studies (e.g. Bernhardt and Likens, 2002; Franke et al., 2013) – and, again, increased with higher nutrient levels. Long-term nutrient enrichment at the downstream boreal sites as well as in the temperate streams may have led to higher biofilm colonization (biomass) and, thus, a greater proportion of DOC mineralized relative to the unimpacted sites. Combined, these findings suggest that microbial respiration can be stimulated by transient exposure to N and/or P, and/or by increased availability of autochthonous C sources potentially due to higher nutrient levels, and highlight the importance of nutrients and likely labile autochthonous C sources in regulating $R_{Biofilm}$ in these stream reaches.

The potential importance of nutrients and labile C sources in regulating microbial activity was confirmed during the stormflow experiment conducted at Virginia River, when changes in hydrology led to lower $R_{Biofilm}$ presumably due to the reduction of N and P concentrations. Thus, we found a N and P limitation during stormflow, which was not observed during baseflow. The contrast in the $R_{Biofilm}$ response to storm- relative to baseflow further suggests a significant role of autochthonous sources of energy in the boreal downstream sites. In particular, the response to the added C and CNP was approximately three times greater at the downstream site of Virginia River during stormflow compared to baseflow, indicating a decreased DOC bioavailability during stormflow perhaps due to the changing proportion of allochthonous versus autochthonous DOC (Leff and Meyer, 1991; Wiegner et al., 2009). Algae may be extremely sensitive to changes in hydrology (Wyatt et al., 2012); thus the stormflow event at Virginia River may have affected algal productivity and, hence, the availability of important labile energy sources for heterotrophic metabolism.

3.4.2 Stream biofilms limited by labile C in nutrient-rich conditions

Nutrient enrichment may increase the capacity of biofilms to use and mineralize labile C substrates in headwater streams, and the processing of nutrients may become limited by labile C. Despite the differences in $R_{Biofilm}$ rates between the boreal and temperate regions, the biofilms present in both boreal and temperate streams studied here responded similarly to the substrate additions. In particular, $R_{Biofilm}$ generally increased when adding labile C within the more nutrient-impacted sites, regardless of region. We found a CNP co-limitation when adding C, N, P simultaneously and observed a lack of response in $R_{Biofilm}$ when adding C, N, P separately at the nutrient-poor upstream sites of the urban boreal streams and at the sites with lower nutrient concentrations in the Ozarks (CC and MC), similarly to the results observed in the substrate addition experiments conducted in relatively pristine streams in the Humber River watershed (Franke et al., 2013). The biofilms in these nutrient-poor streams may not be adapted to high N and P concentrations, and/or may be limited by other nutrients not investigated in this study (Franke et al., 2013). At the upstream site of Waterford River, however, we found a C limitation, coinciding with the higher DIN concentration at this site relative to the upstream sites of Virginia River and Leary's Brook, and the smaller differences in biofilm C:N ratio compared to the nutrient-rich downstream sites. The nutrient-enriched downstream sites of the boreal streams and the nutrient-rich temperate stream sites (SC and CH), where N and P were already available, exhibited an increase in $R_{Biofilm}$ with the added C and CNP. This coincided with the lack of response when adding N and P (without C) additionally to the N and P that were already elevated in the stream water at those sites. The lack of response to the added N and P in both boreal and temperate study streams may be due to a stimulated incorporation of

labile DOC into biomass by the heterotrophic biofilms rather than a stimulation of $R_{Biofilm}$, which cannot be detected by measuring solely $R_{Biofilm}$ (Franke et al., 2013).

Dissolved organic carbon uptake rates measured in this study further highlight the potential importance of labile C sources for supporting these heterotrophic biofilms, particularly at the nutrient-enriched sites. DOC uptake rates were only measurable at the sites that exhibited the greatest biofilm biomass (LB-1 and LB-2) relative to the other boreal study sites, and at the most nutrient-rich site in the temperate streams (CH), which likely exhibited elevated autotrophic activity and a greater input of labile C substrates (Haggard et al., 2005; Lyon and Ziegler, 2009). In the boreal stream with the highest tile biomass (LB), rates of DOC uptake exceeded those of the temperate streams despite the likely lower biomass and lower temperature in the boreal stream relative to the temperate streams. In all temperate streams, DOC uptake (except the low rate at CH) was only observed when labile C was provided, signifying labile C limitation of biofilm activities in line with the substrate addition effects on $R_{Biofilm}$. Further, DOC uptake was stimulated by the added labile C at the boreal sites but not in presence of NP, suggesting that biofilm activity overall ($R_{Biofilm}$ and DOC use) was primarily limited by labile C. In the temperate streams, however, there was evidence for the need for N and/or P in addition to the labile C to stimulate uptake of DOC at CC and CH. Furthermore, even in the nutrient-impacted boreal study sites net nutrient uptake could not be measured in contrast to the temperate streams, where net uptake rates reflected the C and nutrient limitation pattern observed in the $R_{Biofilm}$, i.e. increased net uptake rates were typically observed following the addition of C (alone and in combination with N and/or P).

3.4.3 Lack of evidence for a priming effect on biofilm mineralization of boreal stream DOC

Boreal headwater streams are typically dominated by terrestrial DOC sources, while autochthonous sources are usually minor due to reduced light availability, and therefore, lower photosynthetic activity compared to lower DOC systems more typical of temperate regions (Tranvik, 1988, 1989; Jansson et al., 2000; Jonsson et al., 2001; Agren et al., 2008). Under nutrient enrichment conditions, however, labile C sources, such as algal exudates made available through primary production, may have the potential to stimulate terrestrial DOC use by stream biofilm communities, and may alter differential losses of more labile versus more complex substrates in these streams (Franke et al., 2013). Potential increases in the sources of labile autochthonous substrates and alterations of DOC pools may therefore change carbon cycling with nutrient enrichment in boreal headwater streams. The ^{13}C-glucose additions used in this study enabled us to more directly test the role of increased labile DOC on the uptake of extant stream DOC; however, they did not turn out to be congruent with the observations in these streams where lower DOC coincided with greater nutrients and potential autochthonous sources of DOC. Labile C (as glucose) stimulated $R_{Biofilm}$ at all downstream sites studied in the boreal streams, however, a stimulation of stream DOC mineralization analogous to a priming effect was not observed, regardless whether glucose was added as a single spike or as a combination with NP. The biofilms responded to the labile additions by using it as a source of energy over the extant stream DOC – in line with the findings for DOC uptake described for Leary's Brook above. This suggests stream water additions likely regulated labile C uptake but did not necessarily stimulate extant DOC uptake. The inconsistency of the findings from the ^{13}C-glucose additions versus stream water chemistry, where lower DOC was found in sites

with higher nutrients, points to possible biofilm autotrophic-heterotrophic coupling that might fuel DOC uptake rather than direct changes in stream water organic matter pools. In order to further investigate potential effects of labile or autochthonous C sources on the fate of stream DOC potentially relevant in elevated N and P environments, it would be helpful to study autotrophic-heterotrophic linkages by following the influence of newly fixed or autochthonous C production on stream DOC mineralization. Even though $R_{Biofilm}$ is an important measure to understanding C cycling as it provides one important fate for DOC, it does not necessarily provide information about whether added labile compounds may further increase the incorporation of nutrient-rich dissolved organic matter, which was not determined here.

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4.6 References

Agren, A., Berggren, M., Laudon, H., and Jansson, M., 2008: Terrestrial export of highly bioavailable carbon from small boreal catchments in spring floods. *Freshwater Biology,* 53: 964-972.

Aitkenhead, J. A. and McDowell, W. H., 2000: Soil C : N ratio as a predictor of annual riverine DOC flux at local and global scales. *Global Biogeochemical Cycles,* 14: 127-138.

Alexander, R. B., Boyer, E. W., Smith, R. A., Schwarz, G. E., and Moore, R. B., 2007: The role of headwater streams in downstream water quality. *Journal of the American Water Resources Association,* 43: 41-59.

Arango, C. P. and Tank, J. L., 2008: Land use influences the spatiotemporal controls on nitrification and denitrification in headwater streams. *Journal of the North American Benthological Society,* 27: 90-107.

Bernhardt, E. S. and Likens, G. E., 2002: Dissolved organic carbon enrichment alters nitrogen dynamics in a forest stream. *Ecology,* 83: 1689-1700.

Bianchi, T. S., 2011: The role of terrestrially derived organic carbon in the coastal ocean: A changing paradigm and the priming effect. *Proceedings of the National Academy of Sciences of the United States of America,* 108: 19473-19481.

Buffam, I., Galloway, J. N., Blum, L. K., and McGlathery, K. J., 2001: A stormflow/baseflow comparison of dissolved organic matter concentrations and bioavailability in an Appalachian stream. *Biogeochemistry,* 53: 269-306.

Carr, G. M., Morin, A., and Chambers, P. A., 2005: Bacteria and algae in stream periphyton along a nutrient gradient. *Freshwater Biology,* 50: 1337-1350.

del Giorgio, P. A., Cole, J. J., and Cimbleris, A., 1997: Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature,* 385: 148-151.

del Giorgio, P. A. and Cole, J. J., 1998: Bacterial growth efficiency in natural aquatic systems. *Annual Review of Ecology and Systematics,* 29: 503-541.

Dodds, W. K., Smith, V. H., and Lohman, K., 2002: Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. *Canadian Journal of Fisheries and Aquatic Sciences,* 59: 865-874.

Dodds, W. K., Marti, E., Tank, J. L., Pontius, J., Hamilton, S. K., Grimm, N. B., Bowden, W. B., McDowell, W. H., Peterson, B. J., Valett, H. M., Webster, J. R., and Gregory, S., 2004: Carbon and nitrogen stoichiometry and nitrogen cycling rates in streams. *Oecologia,* 140: 458-467.

Findlay, S., Quinn, J. M., Hickey, C. W., Burrell, G., and Downes, M., 2001: Effects of land use and riparian flowpath on delivery of dissolved organic carbon to streams. *Limnology and Oceanography,* 46: 345-355.

Findlay, S. E. G., 2005: Increased carbon transport in the Hudson River: unexpected consequence of nitrogen deposition? *Frontiers in Ecology and the Environment,* 3: 133-137.

Francoeur, S. N., 2001: Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. *Journal of the North American Benthological Society,* 20: 358-368.

Franke, D., Bonnell, E. J., and Ziegler, S. E., 2013: Mineralization of dissolved organic matter by heterotrophic stream biofilm communities in a large boreal catchment. *Accepted for publication in Freshwater Biology*.

Frost, P. C., Stelzer, R. S., Lamberti, G. A., and Elser, J. J., 2002: Ecological stoichiometry of trophic interactions in the benthos: understanding the role of C : N : P ratios in lentic and lotic habitats. *Journal of the North American Benthological Society,* 21: 515-528.

Frost, P. C., Cross, W. F., and Benstead, J. P., 2005: Ecological stoichiometry in freshwater benthic ecosystems: an introduction. *Freshwater Biology,* 50: 1781-1785.

Fry, B., 1991: Stable Isotope Diagrams of Freshwater Food Webs. *Ecology,* 72: 2293-2297.

Gremm, T. J. and Kaplan, L. A., 1998: Dissolved carbohydrate concentration, composition, and bioavailability to microbial heterotrophs in stream water. *Acta Hydrochimica Et Hydrobiologica,* 26: 167-171.

Guenet, B., Danger, M., Abbadie, L., and Lacroix, G., 2010: Priming effect: bridging the gap between terrestrial and aquatic ecology. *Ecology,* 91: 2850-2861.

Haggard, B. E., Storm, D. E., and Stanley, E. H., 2001: Effect of a point source input on stream nutrient retention. *Journal of the American Water Resources Association,* 37: 1291-1299.

Haggard, B. E., Stanley, E. H., and Storm, D. E., 2005: Nutrient retention in a point-source-enriched stream. *Journal of the North American Benthological Society,* 24: 29-47.

Hamilton, S. K. and Lewis, W. M., 1992: Stable Carbon and Nitrogen Isotopes in Algae and Detritus from the Orinoco River Floodplain, Venezuela. *Geochimica Et Cosmochimica Acta,* 56: 4237-4246.

Hinton, M. J., Schiff, S. L., and English, M. C., 1997: The significance of storms for the concentration and export of dissolved organic carbon from two Precambrian Shield catchments. *Biogeochemistry,* 36: 67-88.

Hopkinson, C. S., Buffam, I., Hobbie, J., Vallino, J., Perdue, M., Eversmeyer, B., Prahl, F., Covert, J., Hodson, R., Moran, M. A., Smith, E., Baross, J., Crump, B., Findlay, S., and Foreman, K., 1998: Terrestrial inputs of organic matter to coastal ecosystems: An intercomparison of chemical characteristics and bioavailability. *Biogeochemistry,* 43: 211-234.

Jansson, M., Bergstrom, A. K., Blomqvist, P., and Drakare, S., 2000: Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. *Ecology,* 81: 3250-3255.

Jonsson, A., Meili, M., Bergstrom, A. K., and Jansson, M., 2001: Whole-lake mineralization of allochthonous and autochthonous organic carbon in a large humic lake (Ortrasket, N. Sweden). *Limnology and Oceanography,* 46: 1691-1700.

Kendall, C., Silva, S. R., and Kelly, V. J., 2001: Carbon and nitrogen isotopic compositions of particulate organic matter in four large river systems across the United States. *Hydrological Processes,* 15: 1301-1346.

Leff, L. G. and Meyer, J. L., 1991: Biological Availability of Dissolved Organic Carbon Along the Ogeechee River. *Limnology and Oceanography,* 36: 315-323.

Lyon, D. R. and Ziegler, S. E., 2009: Carbon cycling within epilithic biofilm communities across a nutrient gradient of headwater streams. *Limnology and Oceanography,* 54: 439-449.

McDiffett, W., Beidler, A., Dominick, T., and McCrea, K., 1989: Nutrient concentration-stream discharge relationships during storm events in a first-order stream. *Hydrobiologia,* 179: 97-102.

McDowell, W. H. and Likens, G. E., 1988: Origin, Composition, and Flux of Dissolved Organic Carbon in the Hubbard Brook Valley. *Ecological Monographs,* 58: 177-195.

Meon, B. and Kirchman, D. L., 2001: Dynamics and molecular composition of dissolved organic material during experimental phytoplankton blooms. *Marine Chemistry,* 75: 185-199.

Meyer, J. L., Mcdowell, W. H., Bott, T. L., Elwood, J. W., Ishizaki, C., Melack, J. M., Peckarsky, B. L., Peterson, B. J., and Rublee, P. A., 1988: Elemental Dynamics in Streams. *Journal of the North American Benthological Society,* 7: 410-432.

Mott, D. N., Hudson, M. R., and Aley, T., 2000: Nutrient loads traced to interbasin groundwater transport at Buffalo National River, Arkansas. *Inside Earth,* 3: 6-11.

Olapade, O. A. and Leff, L. G., 2005: Seasonal response of stream biofilm communities to dissolved organic matter and nutrient enrichments. *Applied and Environmental Microbiology,* 71: 2278-2287.

Pastor, J., Solin, J., Bridgham, S. D., Updegraff, K., Harth, C., Weishampel, P., and Dewey, B., 2003: Global warming and the export of dissolved organic carbon from boreal peatlands. *Oikos,* 100: 380-386.

Peterson, B. J., Wollheim, W. M., Mulholland, P. J., Webster, J. R., Meyer, J. L., Tank, J. L., Marti, E., Bowden, W. B., Valett, H. M., Hershey, A. E., McDowell, W. H., Dodds, W. K., Hamilton, S. K., Gregory, S., and Morrall, D. D., 2001: Control of nitrogen export from watersheds by headwater streams. *Science,* 292: 86-90.

Sanderman, J., Lohse, K. A., Baldock, J. A., and Amundson, R., 2009: Linking soils and streams: Sources and chemistry of dissolved organic matter in a small coastal watershed. *Water Resources Research,* 45: W03418.

Schlesinger, W. H., 1984: Soil Organic Matter: a Source of Atmospheric CO2. *The Role of Terrestrial Vegetation in the Global Carbon Cycle: Measurement by Remote Sensing, John Wiley & Sons Ltd*: 111-127.

Stelzer, R. S., Heffernan, J., and Likens, G. E., 2003: The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. *Freshwater Biology,* 48: 1925-1937.

Stepanauskas, R., Laudon, H., and Jorgensen, N. O. G., 2000: High DON bioavailability in boreal streams during a spring flood. *Limnology and Oceanography,* 45: 1298-1307.

Sun, L., Perdue, E. M., Meyer, J. L., and Weis, J., 1997: Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. *Limnology and Oceanography,* 42: 714-721.

Tank, J. L. and Dodds, W. K., 2003: Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. *Freshwater Biology,* 48: 1031-1049.

Tranvik, L. J., 1988: Availability of Dissolved Organic Carbon for Planktonic Bacteria in Oligotrophic Lakes of Differing Humic Content. *Microbial Ecology,* 16: 311-322.

Tranvik, L. J., 1989: Bacterioplankton Growth, Grazing Mortality and Quantitative Relationship to Primary Production in a Humic and a Clearwater Lake. *Journal of Plankton Research,* 11: 985-1000.

Weishaar, J. L., Aiken, G. R., Bergamaschi, B. A., Fram, M. S., Fujii, R., and Mopper, K., 2003: Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental Science & Technology,* 37: 4702-4708.

Wiegner, T. N., Tubal, R. L., and MacKenzie, R. A., 2009: Bioavailability and export of dissolved organic matter from a tropical river during base- and stormflow conditions. *Limnology and Oceanography,* 54: 1233-1242.

Wipfli, M. S., Richardson, J. S., and Naiman, R. J., 2007: Ecological Linkages Between Headwaters and Downstream Ecosystems: Transport of Organic Matter, Invertebrates, and Wood Down Headwater Channels. *JAWRA Journal of the American Water Resources Association,* 43: 72-85.

Wondzell, S. M. and Swanson, F. J., 1996: Seasonal and storm dynamics of the hyporheic zone of a 4th-order mountain stream .2. Nitrogen cycling. *Journal of the North American Benthological Society,* 15: 20-34.

WQMA, 2012: Water Quality Monitoring Agreement. *Environment and Conservation, Government of Newfoundland and Labrador, Canada: http://www.env.gov.nl.ca/env/waterres/quality/background/wqma_sites.html*.

Wyatt, K., Turetsky, M., Rober, A., Giroldo, D., Kane, E., and Stevenson, R. J., 2012: Contributions of algae to GPP and DOC production in an Alaskan fen: effects of historical water table manipulations on ecosystem responses to a natural flood. *Oecologia,* 169: 821-832.

Chapter 4: Carbon cycling and autotrophic-heterotrophic linkages within boreal stream biofilm communities

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Dissolved organic carbon (DOC), autochthonous carbon, stable carbon isotopes, phospholipid fatty acids (PLFA), nutrient enrichment, priming effect.

Abstract

The extent of autotrophic-heterotrophic linkages was investigated in nutrient-impacted boreal streams, specifically whether (1) autotrophic carbon sources are important to supporting epilithic biofilms in nutrient-enriched boreal streams, and (2) autotrophic or labile carbon sources (e.g. glucose) influence the mineralization of extant stream organic matter. Primary production, community respiration, nutrient uptake, and carbon cycling within biofilms were monitored at three study sites within two nutrient-impacted boreal streams during fall 2009 and summer 2010, using *in situ* mesocosms. Phospholipid fatty acid (PLFA) biomarkers and a ^{13}C-labeling approach were employed to trace autochthonous or newly fixed carbon (C_{NF}) generated during the experiments. Dissolved organic carbon (DOC) released from the biofilms was not directly linked to C_{NF} but was associated with photoautotrophic activity and nutrients. Surprisingly, net DOC release into the stream water only represented between 16 and 21 % of net primary production (NPP) at the most nutrient-rich sites, with C_{NF} released as DOC representing only 3 to 10 % of NPP in the light incubations. At the sites with lower nutrient concentrations, net DOC release into the stream water represented between 82 and 117 % of NPP, but with C_{NF} released as DOC representing only 1 % of NPP in the light incubations. The proportion of C_{NF} derived from NPP as well as the uptake of C_{NF} into eukaryotic and heterotrophic biofilm PLFA were higher at the most nutrient-rich site relative to the sites with lower nutrient concentrations. Also, the ratios of C_{NF} into heterotrophic bacterial relative to eukaryotic PLFA observed in this study were similar to those found in temperate streams where DOC concentrations were lower and nutrient levels significantly higher. Priming effects associated with glucose additions were not observed in these urban boreal stream biofilms. However, light-mediated activities such as algal production were found to increase the mineralization of organic matter overall in

these biofilms. In combination, these results suggest that tight autotrophic-heterotrophic linkages may lead to priming effects in boreal stream biofilms primarily supported through autotrophic organic matter sources not easily observed using external substrate additions.

4.1 Introduction

The microbial community growing as epilithic biofilm on submerged rocks plays an important role in the biogeochemical cycling of carbon and nutrients in headwater streams (Hoellein et al., 2010). Aquatic biofilms consist of a mixture of heterotrophic (bacteria and fungi) and autotrophic (cyanobacteria and algae) microorganisms embedded in a polysaccharide matrix (Lock et al., 1984). These biofilm communities have a major influence on ecosystem function in headwater streams, particularly stream primary production and metabolism, as well as uptake and transfer of carbon and nutrients to higher trophic levels (Ford and Lock, 1987; Mayer and Likens, 1987; Winterbourn, 1990; Mulholland et al., 1991; Dodds et al., 2000).

Microbial community composition and activity often vary with the quantity and availability of carbon and nutrients, which commonly correspond to dissolved organic carbon (DOC) sources and composition (Leff and Meyer, 1991; Findlay et al., 2003; Judd et al., 2006). Terrestrially derived or allochthonous dissolved organic matter is typically the major form of organic carbon in aquatic ecosystems and the main energy and nutrient (nitrogen and phosphorus) source for heterotrophic microorganisms (Fisher and Likens, 1973). The quantity and composition of these sources vary with catchment vegetation and soil properties (Schiff et al., 1997; Quideau et al., 2001), hydrology (Schiff et al., 1998), acidity (Buffam et al., 2007), and the presence of photochemical and microbial degradation processes (McDowell and Likens, 1988; Koehler et al., 2002). Heterotrophic microbial activity can also be dependent on autochthonous sources of DOC, comprised largely of labile algal exudates, which may stimulate the degradation and use of DOC by heterotrophic bacteria (Cole, 1982; Haack and McFeters, 1982; Kaplan and Bott, 1989; Romani and Sabater, 1999). The extent to which heterotrophic biofilm communities take up allochthonous DOC or use primarily autochthonous DOC,

depends on the growth conditions of biofilms. For example, light availability (Romani et al., 2004) and nutrient levels (Tank and Dodds, 2003) often regulate algal biomass, and consequently affect the uptake of CO_2 and transfer of autochthonous carbon through the aquatic food web.

Environmental changes, specifically carbon and nutrient enrichment, can alter substrate sources and availability for microorganisms and subsequently affect the biogeochemical cycling of DOC (Bernhardt and Likens, 2002; Ziegler and Brisco, 2004). Changes in carbon and nutrient concentrations may be followed by an alteration in biofilm community composition, activity levels, and interspecies interactions (Guasch et al., 1995; Gulis and Suberkropp, 2003; Haukka et al., 2006). Such changes can break the link between heterotrophic and autotrophic communities and affect processes such as respiration, as well as nutrient release and retention (Bernhardt and Likens, 2002; Scott et al., 2008; Lyon and Ziegler, 2009).

In boreal streams, allochthonous DOC dominates and serves as the major energy source for heterotrophic microorganisms, while autochthonous sources are usually minor due to reduced light availability, and therefore, lower photosynthetic activity compared to lower DOC systems more typical of temperate regions (Tranvik, 1988, 1989; Jansson et al., 2000; Jonsson et al., 2001; Agren et al., 2008). Given this allochthonous dominance and the fact that DOC may be susceptible to greater rates of loss through microbial mineralization under nutrient enrichment conditions, priming effects may be particularly important in boreal streams under nutrient enrichment conditions. With elevated nutrient availability, autochthonous DOC sources may potentially stimulate allochthonous DOC use by stream biofilm communities and thereby increase losses of this carbon reservoir to the atmosphere via increased respiration. Labile carbon sources have the potential to stimulate the mineralization of allochthonous DOC by some boreal stream biofilm

communities (Franke et al., 2013a) and such a mechanism may contribute to CO_2 emissions from aquatic ecosystems via increased respiration (Guenet et al., 2010; Bianchi, 2011). Enhanced microbial uptake and depletion of bioavailable DOC with nutrient enrichment may also result in more slow turnover, less bioavailable components in the residual DOC pool (Ziegler and Brisco, 2004). Potential increases in the sources of labile autochthonous substrates and alterations of DOC pools suggest the potential for changes in carbon cycling with nutrient enrichment in boreal headwater streams.

The research addressed here contributes to our understanding of carbon cycling in boreal headwater streams by investigating the importance of autotrophic-heterotrophic linkages in nutrient-impacted streams. Specifically we asked whether (1) autotrophic carbon sources are important to supporting epilithic biofilms in nutrient-enriched boreal streams, and (2) autotrophic or labile carbon sources influence the mineralization of extant stream DOC. Primary production, community respiration, nutrient uptake, and carbon cycling within biofilms were monitored at three study sites within two nutrient-impacted boreal streams during fall 2009 and summer 2010, using *in situ* mesocosms. A ^{13}C-labeling approach was used to trace autochthonous carbon generated during the experiments. Biofilm samples were collected for both bulk stable carbon isotopes ($\delta^{13}C$) and the $\delta^{13}C$ of phospholipid fatty acid (PLFA) biomarkers. These $\delta^{13}C$ signatures enabled the calculation of the flow of autotrophic carbon through the heterotrophic bacterial communities in these biofilms (Boschker and Middelburg, 2002; Kritzberg et al., 2004; Van den Meersche et al., 2004; Pace et al., 2007), and were used to quantify the proportion of newly fixed carbon assimilated by heterotrophic bacteria relative to net primary production. Furthermore, a comparison of the data from the light and dark enclosure experiments was used to test whether autotrophic DOC can stimulate the

heterotrophic mineralization or use of organic matter or induce a priming effect in these streams.

4.2 Methods

4.2.1 Study sites

Three study sites in two urbanized boreal streams were chosen on the Northeast Avalon Peninsula, Newfoundland, Canada: Virginia River upstream (VR-1) and downstream (VR-2), and Rennie's River downstream (RR-2). The upstream sites of these streams are influenced by forest (e.g. Balsam Fir (*Abies balsamea*); forest floor: Broom Moss (*Dicranum scoparium*) and Feathermoss (*Ptilium crista-castrensis*)) and wetland (bogs and fens) cover (WQMA, 2012), and represent relatively unimpacted sites. Rennie's River's headwaters include a number of ponds, which are all located in Pippy Park in St. John's. The headwaters to Virginia River are marshes located in the district of Airport Heights with the downstream reaches running through a highly urbanized and residential area, where paved roads and industrial parks have a major impact on the downstream water quality. Both, Rennie's River and Virginia River drain into Quidi Vidi Lake. Experiments were conducted at VR-1 and VR-2 in early fall in 2009, and at RR-2 and VR-2 in summer 2010.

4.2.2 Tests prior to experiments

Prior to the incubation experiments, mesocosm chambers were tested for gas and water tightness. The chambers, containing a 10 µM PO_4^{3-} solution, were placed in a large container filled with de-ionized water. Initial samples were collected from both the mesocosm chambers and the large container, and again following a 24-hour incubation at room temperature. Differences in phosphate concentration in the chambers and the large container were below detection. Further, the chambers were incubated in the large container again but with slightly aerated NanoPure water inside the chamber and oxygen

(O_2) saturated lake water in the large container to assess the significance of O_2 exchange across the chamber silicone seal. Dissolved oxygen (DO) was measured in the chamber at the beginning and the end of the incubation. Results exhibited a 1.1 % decrease in chamber DO after 24 hours. Additionally, an hourly DO profile was obtained at VR-1 and VR-2 in fall 2009 (see appendix A4) and compared to the profile-integrated estimate using the endpoints (dawn-dusk) measurement. The results from these tests showed that the dawn-dusk measurement is adequate to estimate a daily net rate of DO production, but can underestimate the average hourly rate.

4.2.3 Light and dark mesocosm experiments using a ^{13}C-bicarbonate labeling approach

To capture a full day of sunlight, experiments were set up before sunrise and continued until sunset. Stream water substrate, representative for the study site benthos, was collected by scrubbing randomly selected rocks (n = 6) with a clean brush. The biofilm material was rinsed off with NanoPure water, collected in acid-rinsed, brown 500 mL high-density polyethylene bottles, and returned to the laboratory, immediately freeze-dried (Labconco, Kansas City, MO, USA), and analyzed (see below) for the initial stable carbon (C) and nitrogen (N) isotopic composition (δ^{13}C, δ^{15}N), chlorophyll content, biomass (%C, %N), and PLFA.

Meanwhile, triplicate clear (light) and opaque (dark) 12 L polycarbonate enclosures, equipped with peristaltic pumps to maintain stream water circulation, were filled with stream water substrate (6 – 7 rocks each) randomly collected from the stream at each study site. Clear containers were used to measure net phototrophic activity, whereas opaque containers were used to measure net heterotrophic activity only. Eight liters of unfiltered stream water, collected and spiked with ^{13}C-labeled bicarbonate (2000

‰) 12 hours prior to the experiments to allow for the distribution of the ^{13}C-labeled bicarbonate among the inorganic carbon species in the water, were added to the mesocosms and circulated for approximately 15 minutes before initial mesocosm samples were taken. Final samples were collected at the end (after approximately 8 hours) of the incubation, just after sunset.

Mesocosm community respiration was measured as the change in DO and total inorganic carbon (TIC) in the dark chambers. Likewise, net primary production (NPP) was measured as the change in DO and TIC in the light chambers. Net uptake and release rates of DOC and nutrients (nitrogen and phosphorus) were determined as the difference between initial and final concentrations. The $\delta^{13}C_{TIC}$ and $\delta^{13}C_{DOC}$ as well as the biofilm PLFA were measured to determine the quantity and fate of newly fixed C (C_{NF}) in these biofilms (biofilm primary production). Changes in DO were measured on site, using a YSI 550A handheld DO instrument (YSI Incorporated, Yellow Springs, OH, USA). Samples for TIC and DOC analysis, as well as $\delta^{13}C_{TIC}$ and $\delta^{13}C_{DOC}$, were collected in acid-cleaned, pre-combusted 40 mL amber glass vials and spiked with $HgCl_2$ and H_3PO_4 (HPLC grade), respectively. Samples for soluble reactive phosphorus (SRP), ammonium (NH_4^+), nitrate (NO_3^-), and total dissolved nitrogen (TDN) were collected in acid-rinsed 60 mL Nalgene bottles. Samples for total dissolved phosphorus (TDP) analysis were collected in 100 mL amber glass bottles and preserved with sulphuric acid. All samples for DOC, SRP, NH_4^+, NO_3^-, TDN, and TDP quantification were filtered using a 0.45 µm Whatman microfiber glass filter.

The fraction of biofilm C derived from TIC that was fixed by autotrophs (newly fixed carbon, C_{NF}) during the incubation period ($F_{Biofilm}$) was calculated similarly to Lyon and Ziegler (2009) as:

$$F_{Biofilm} = \frac{\delta^{13}C_{Biofilm\text{-}final} - \delta^{13}C_{Biofilm\text{-}initial}}{\delta^{13}C_{TIC-initial} - \delta^{13}C_{Biofilm\text{-}initial}} \quad \text{(eq. 1)}$$

where $\delta^{13}C_{Biofilm\text{-}initial/final}$ is the bulk biofilm $\delta^{13}C$ (‰) collected at the beginning (initial) and the end (final) of the incubation, respectively; $\delta^{13}C_{TIC\text{-}initial}$ is the $\delta^{13}C$ of the ^{13}C-labeled TIC (‰) from the beginning of the incubation. The rate of C_{NF} incorporation into the biofilm (C-specific biofilm primary production, $PP_{Biofilm}$, mmol C mol^{-1} $C_{Biofilm}^{-1}$ hr^{-1}) was then determined as:

$$PP_{Biofilm} = \frac{F_{Biofilm} \times C_{Biofilm} \times 10^3}{C_{Biofilm} \times t} \quad \text{(eq. 2)}$$

where $C_{Biofilm}$ (mol) is the biofilm C content within each container and t is the incubation period (hrs). Equation 2 was multiplied by 10^3 to convert the units mol C mol^{-1} $C_{Biofilm}^{-1}$ hr^{-1} to mmol C mol^{-1} $C_{Biofilm}^{-1}$ hr^{-1}.

Primary production as net DOC released (PP_{DOC}, µmol C mol $C_{Biofilm}^{-1}$ hr^{-1}) was calculated based on the fraction of DOC derived from TIC that was fixed during the incubation (F_{DOC}), and the change in the $\delta^{13}C_{DOC}$ in the mesocosms over the incubation period (Lyon and Ziegler, 2009):

$$F_{DOC} = \frac{\delta^{13}C_{DOC-final} - \delta^{13}C_{DOC-initial}}{\delta^{13}C_{TIC-initial} - \delta^{13}C_{DOC-initial}} \quad \text{(eq. 3)}$$

$$PP_{DOC} = \frac{[DOC]_{final} \times F_{DOC} \times V}{C_{Biofilm} \times t} \quad \text{(eq. 4)}$$

where $[DOC]_{initial/final}$ represents the DOC concentration at the beginning (initial) and the end (final) of the incubation (µM C), respectively, and V is the volume (L) of stream water in each enclosure. The relationship between PP_{DOC} and total net DOC release (R_{DOC}, %) was used to express the proportion of DOC derived from autochthonous C, and was calculated similar to Lyon and Ziegler (2009) as:

$$R_{DOC} = \frac{[DOC]_{final} \times F_{DOC}}{[DOC]_{final} - [DOC]_{initial}} \times 100 \quad \text{(eq. 5)}$$

Equation 5 was multiplied by 100 to convert the units to a percentage.

Biofilm PLFA were used as biomarkers to quantify the proportion of C_{NF} assimilated by heterotrophic bacteria relative to NPP as well as biofilm autotrophs based upon their PLFA. PLFA concentration and $\delta^{13}C_{PLFA}$ were measured in freeze-dried biofilm samples collected at RR-2 in summer and VR-2 in fall. Polyunsaturated PLFA (18:2ω6, 18:3ω6, 18:3ω3, 20:5ω3, 22:5ω6) were used to represent algal PLFA (Napolitano, 1999; Boschker et al., 2005); terminally branched fatty acids (i15:0, a15:0, i16:0) were used to represent heterotrophic bacterial PLFA (Parker et al., 1967; Napolitano, 1999). The following equations were used to measure the rate of C_{NF} into individual biofilm PLFA and specific groups of PLFA, similarly to Lyon and Ziegler (2009). The fraction of PLFA (F_{PLFA}) carbon derived from C_{NF} was determined as:

$$F_{PLFA} = \frac{\delta^{13}C_{PLFA-final} - \delta^{13}C_{PLFA-initial}}{\delta^{13}C_{TIC-initial} - \delta^{13}C_{PLFA-initial}} \quad \text{(eq. 6)}$$

The F_{PLFA} was used to calculate the rates of C_{NF} incorporation into PLFA normalized to both biofilm C and individual PLFA C as:

$$\text{Biofilm C-normalized } C_{NF} \text{ incorporation rate} = \frac{F_{PLFA} \times C_{PLFA} \times 10^3}{C_{Biofilm} \times t} \quad \text{(eq. 7)}$$

$$\text{Concentration-normalized } C_{NF} \text{ incorporation rate} = \frac{F_{PLFA} \times C_{PLFA} \times 10^3}{C_{PLFA} \times t} \quad \text{(eq. 8)}$$

where $\delta^{13}C_{PLFA\text{-}initial/final}$ is the $\delta^{13}C$ of PLFA in biofilm samples (‰) collected from each container at the beginning (initial) and the end (final) of the incubation, respectively; $C_{Biofilm}$ is the total biofilm carbon (mol $C_{Biofilm}$) within each mesocosm, C_{PLFA} is the amount

of C present for individual PLFA (μmol C_{PLFA}) in the final biofilm samples, and t is the incubation time (hrs). Units of the $C_{Biofilm}$-normalized and concentration-normalized C_{NF} rates are given as nmol PLFA-C_{NF} mol $C_{Biofilm}^{-1}$ hr^{-1} and nmol PLFA-C_{NF} μmol PLFA-C_{total}^{-1} hr^{-1}, respectively.

4.2.4 Mesocosm experiments with ^{13}C-Glucose

A second part to this mesocosm experiment was conducted at RR-2 only and included another set of light and dark incubations to track stream DOC mineralization in relation to C_{NF} inputs and the added glucose. Samples were taken from the mesocosms at the beginning near sunrise and at dusk similarly as described above. The final sampling, however, was conducted the next morning to determine whether differences in DOC mineralization overnight occurred between chambers that previously received light (i.e. C_{NF} inputs) versus those held in the dark for the entire 24 hours. This approach also included a set of dark incubations that received ^{13}C-labeled glucose (42 μM C; 551 ‰) to determine if respiration of extant stream organic matter increased as a result of an added labile substrate. The glucose was added at dusk after approximately 10 hours of incubation. The mineralization of glucose-C ($R_{Glucose}$; μM C hr^{-1}) and stream organic carbon ($R_{Stream\text{-}OC}$; μM C hr^{-1}) were determined and compared to that measured in the dark control that did not receive any ^{13}C-glucose:

$$R_{Glucose} = \frac{[TIC_{final}]}{t} \times \frac{\delta^{13}C_{TIC-final} - \delta^{13}C_{TIC-initial}}{\delta^{13}C_{Glucose} - \delta^{13}C_{TIC-initial}} \quad \text{(eq. 9)}$$

$$R_{Stream-OC} = \frac{([TIC]_{final} - [TIC]_{initial})}{t} - R_{Glucose} \quad \text{(eq. 10)}$$

where $[TIC]_{initial/final}$ is the initial and final TIC concentration (μM C), respectively, t is the incubation time (hrs), $\delta^{13}C_{TIC\text{-}initial/final}$ are the stable inorganic carbon isotope values (‰) of

the TIC pool at the beginning (initial) and the end (final) of the incubation, respectively, and $\delta^{13}C_{Glucose}$ is the $\delta^{13}C$ (551 ‰) of the glucose added.

4.2.5 Analytical methods

Concentrations of TIC, DOC, their $\delta^{13}C$ values, as well as TDN, NH_4^+, NO_3^-, dissolved organic nitrogen (DON), TDP, and SRP concentrations were determined using the same instruments, methods and detection limits described in detail in Franke et al. (2013a). The analytical precision ranged between 0.2 and 0.4 ‰ for $\delta^{13}C_{TIC}$, and between 0.1 and 0.2 ‰ for $\delta^{13}C_{DOC}$. The maximum percent coefficient of variation (CV) was 1.1 % for TIC and 4.5 % for DOC analysis.

Freeze-dried biofilm samples were analyzed for C and N content (%) as well as $\delta^{13}C$ and $\delta^{15}N$ using a Carlo Erba NA1500 Series II elemental analyzer (Milan, Italy) interfaced with a Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The analytical precision for $\delta^{13}C$ and $\delta^{15}N$ ranged between 0.01 and 0.2 ‰, and the analytical error for the C and N content was between 0.2 and 3.7 %. Biofilm chlorophyll content was determined using the spectrophotometric method (Jeffrey and Humphrey, 1975). Biofilm samples were extracted in 90 % acetone followed by centrifugation and absorbance measurements at wavelengths of 430, 630, 647, 664, 750 nm using a UV/VIS Spectrometer (Lambda 25; Perkin Elmer, Waltham, MA, USA) and 90 % acetone as a blank.

Phospholipid fatty acid extractions were conducted on freeze-dried biofilm samples by isolating phospholipids from the total organic or lipid extractions of the lyophilized biofilm sample using solid phase extraction with silicic acid (White et al., 1979; Vestal and White, 1989; White and Ringelberg, 1998). In order to perform gas chromatography

(GC) analyses, fatty acid methyl esters (FAMEs) were derivatized from phospholipids using single-step saponification and methylation procedure (Findlay, 2004). To determine the change in $\delta^{13}C$ imparted by the added methyl carbon, a small aliquot of the methanol used during methylation was analyzed for its $\delta^{13}C$ composition using an AURORA 1030 TOC analyzer (O.I. Analytical, College Station, Texas, USA) coupled to a MAT252 isotope ratio mass spectrometer (Finnigan, Bremen, Germany). FAME identification and quantification was conducted using an Agilent 6890 GC (capillary column: BPX-70, 50 m, 0.22 mm internal diameter, 0.25 µm stationary phase thickness; 1 mL, splitless injection; SGE Analytical Science, Austin, TX, USA) equipped with a flame ionization detector (FID) and interfaced with an Agilent 5973 inert mass selective detector (Agilent Technologies, Mississauga, ON, Canada), respectively. The $\delta^{13}C$ of the FAMEs were determined with an Agilent 6890 GC interfaced to a MAT252 isotope ratio mass spectrometer with a GC-C-III combustion interface (ThermoFinnigan, Bremen, Germany). Phospholipid recovery was determined by adding a phospholipid standard (1,2-diheptadecanoyl-sn-glycero-3-phosphocholine; Avanti Polar Lipids, Alabaster, AL, USA) to biofilm subsamples (>74 % recovery). Additionally, blank extractions were conducted under the same conditions to verify the lack of any contamination during sample handling and procedure.

4.2.6 Statistics

Significance tests (t-tests) were conducted between initial and final mesocosm samples with an α–value of 0.05 (AnalystSoft Inc., StatPlus:mac - Version 2009) to ascertain differences in DO, TIC, DOC, and nutrient (e.g. TDN, NH_4^+, SRP) concentrations, and PLFA content, as well as $\delta^{13}C_{DOC}$, $\delta^{13}C_{TIC}$, and $\delta^{13}C_{PLFA}$. Non-significant changes were considered to be below detection. Significance tests were also

performed on biofilm C-normalized and concentration-normalized C_{NF} incorporation rates/ratios in the light incubations to ascertain differences among prokaryotic and eukaryotic microbial groups, and between sites.

Pearson correlations with an α-level of 0.05 (AnalystSoft Inc., StatPlus:mac - Version 2009) were used to test the potential role of water quality parameters in regulating the development of the stream biofilms. For instance, biofilm C:N ratios were tested against stream DOC, DON, DOC:DON, DIN, SRP, and TDP to investigate whether these parameters influenced biofilm growth and stoichiometry. Furthermore, chlorophyll a, b, and c content of the stream biofilms was tested against biofilm C and N content, C:N, $\delta^{13}C$, and $\delta^{15}N$ to determine how carbon and nutrient availability within the biofilms influenced the development and potential autotrophy of the biofilm communities. Also, NPP and PP_{DOC} in the light incubations were tested against chlorophyll content to assess the relationship between the measures of primary production and these measures of biofilm autotrophic potential. Further, $PP_{Biofilm}$ was tested for a correlation with the flux of C_{NF} into heterotrophic, eukaryotic and total PLFA.

4.3 Results

4.3.1 Water quality characteristics and dissolved organic matter composition

The two study streams, Rennie's River and Virginia River, varied in their water quality characteristics and dissolved organic matter composition (Table 4.1). The upstream site of VR exhibited low nutrient levels and relatively high DOC concentrations (685 µM C; Table 4.1). Soluble reactive phosphorus levels were 0.3 µM P, whereas TDP concentration was below detection (<0.64 µM P). Dissolved inorganic nitrogen concentration was 2.8 µM N and DON was 10.9 µM N (Table 4.1). Both study sites located downstream at VR and RR are characterized by higher nutrient levels and relatively lower DOC concentrations (280 – 290 µM C at VR-2 and 400 µM C at RR-2, respectively; Table 4.1). Total P levels were 0.9 µM P and SRP levels were 0.6 µM P. Inorganic N concentrations were approximately twice as high at VR-2 relative to RR-2, ranging from 19.1 to 20.5 µM N at VR-2, whereas DIN levels were 10.1 µM N at RR-2 (Table 4.1). Dissolved organic nitrogen concentrations were between 48.5 and 50.9 µM N at VR-2 and 17.1 µM N at RR-2 (Table 4.1). Further, TIC concentrations varied from 120 µM C at VR-1 and 380 µM C at RR-2 to 650 µM C at VR-2 (Table 4.1). Dissolved oxygen concentrations ranged from 281 µM O_2 at RR to 341 µM O_2 at VR (Table 4.1). Experiments were conducted at 20.8°C at RR, and at 11.2 and 14.9°C at VR in fall 2009 and summer 2010, respectively.

4.3.2 Epilithic stream biofilm characteristics and composition

Epilithic stream biofilm C:N ratios were approximately 25 at the nutrient-poor site (VR-1) and varied between 8 and 12 at the nutrient-rich stream sites (VR-2, RR-2; Table 4.2), suggesting a more N-rich community at the downstream VR-2 and RR-2 sites

relative to the upstream VR-1 site. Stream site epilithic biofilm C:N ratio was positively correlated to stream DOC:DON ($p = 0.03$), DOC ($p = 0.03$), and TDP ($p = 0.02$). However, biofilm C:N ratio was not correlated to DON ($p = 0.23$), DIN ($p = 0.10$), and SRP ($p = 0.16$).

Epilithic stream biofilm N content varied from 0.8 to 1.7 Wt. %, with highest values measured at VR-2 (Table 4.2). The $\delta^{15}N$ of the stream biofilms was 2 ‰ at VR-1, and between 5 and 7 ‰ at VR-2 and RR-2 (Table 4.2). Even though the elevated $\delta^{15}N$ at VR-2 and RR-2 may be important indicators for nutrient (e.g. inorganic N) enrichment (Lindau et al., 1989; Wayland and Hobson, 2001; Savage, 2005), $\delta^{15}N$ values were not correlated to stream DIN ($p = 0.30$) and DON ($p = 0.48$) in this study.

Stream biofilm C content varied from 11 to 17 Wt. %, with highest values recorded at VR-1 (Table 4.2). The $\delta^{13}C$ of the stream biofilms were -28 ‰ at VR-1 and RR-2, and from -19 to -23 ‰ at VR-2 (Table 4.2). The differences in the $\delta^{13}C$ among the biofilms likely indicate greater algal-derived C within the epilithic biofilms at VR-2 relative to VR-1 and RR-2 (Fry, 1991; Karlsson et al., 2003). At VR-1 and RR-2, these labile sources might not be as readily available, and the microorganisms present at this site may use a greater proportion of allochthonous C sources (Rounick and Winterbourn, 1986; France and Schlaepfer, 2000; Jonsson et al., 2001; Pace et al., 2007).

Chlorophyll a, indicating the presence of photoautotrophic microorganisms including cyanobacteria, red and green algae (Sigee, 2006), exhibited the highest proportion of the total chlorophyll (66 to 87 %) and ranged in concentration from 0.7 to 23.9 µg chl a per mg biofilm C (Table 4.2). As expected, lower values were recorded at the relatively shaded and nutrient-poor stream site (VR-1), whereas high values were measured at the open, nutrient-rich sites (e.g. VR-2). Chlorophyll b, present in green algae only (Rowan, 1989; Bianchi and Canuel, 2011) and chlorophyll c, typically present

in diatoms, dinoflagellates, and brown algae (Sigee, 2006), exhibited a relatively low proportion of the total chlorophyll (5 to 17 %), varying from 0.1 to 3.6 µg chl mg $C_{Biofilm}^{-1}$ (Table 4.2). Chlorophyll content did not correlate with the biofilm C content (chl a: p = 0.60, chl b: p = 0.77, chl c: p = 0.59), N content (chl a: p = 0.08, chl b: p = 0.06, chl c: p = 0.18), biofilm C:N (chl a: p = 0.25, chl b: p = 0.30, chl c: p = 0.33), $\delta^{13}C$ (chl a: p = 0.44, chl b: p = 0.29, chl c: p = 0.61), or $\delta^{15}N$ (chl a: p = 0.38, chl b: p = 0.50, chl c: p = 0.42).

Total PLFA abundance was 0.51 and 0.34 µg PLFA mg biofilm^{-1} in the samples collected at VR-2 in fall 2009 and at RR-2 in summer 2010, respectively. The PLFA 16:0 (0.09 – 0.22 µg PLFA mg biofilm^{-1}), 16:1ω7 (0.07 – 0.11 µg PLFA mg biofilm^{-1}), 18:1ω9c and 18:1ω9t (0.03 – 0.09 µg PLFA mg biofilm^{-1}) as well as 18:2ω6 (0.01 – 0.02 µg PLFA mg biofilm^{-1}) were the most abundant among the biofilm samples collected at VR-2 and RR-2. Other PLFA present in smaller quantities (<0.02 µg PLFA mg biofilm^{-1}) included 14:0, i15:0, a15:0, 15:0, i16:0, 17:0, 18:0, 18:3ω6, 18:3ω3, 20:4ω6, 22:0, 20:5ω3, and 22:5ω6. The biofilm C-normalized C content of each individual PLFA present in the biofilms and total C content per sample are listed in Table 4.3. The proportion of heterotrophic bacterial and eukaryotic fatty acids was 3 – 9 % and 8 – 17 % of the total biofilm PLFA, respectively (Table 4.3). The heterotrophic bacterial to eukaryotic PLFA ratios ranged from 0.15 to 0.62 (Table 4.3), and were not significantly different among the study sites (p = 0.07) or light and dark treatments (RR: p = 0.22, VR: p = 0.18). The PLFA listed in Table 4.3 were detected consistently across the samples collected at VR-2 and RR-2, however, only the PLFA 14:0, i15:0, a15:0, 16:0, 16:1ω7, 18:0, 18:2ω6, 18:3ω3, 18:1ω9c, 18:1ω9t, 20:5ω3 were analyzed for $\delta^{13}C_{PLFA}$. The initial $\delta^{13}C_{PLFA\text{-}individual}$ varied from -40 to -30 ‰. The variation in isotopic composition among dark and light treatments is specified in appendix A4.

Table 4.1: Initial water chemistry parameters at a nutrient-poor (VR-1) and a nutrient-rich stream site (VR-2) in Virginia River in fall 2009, and at a nutrient-rich site in Virginia River (VR-2) and Rennie's River (RR-2) in summer 2010. Measurements were taken for temperature (T; °C), total inorganic carbon (TIC; μM C), dissolved oxygen (DO; μM O_2), dissolved organic carbon (DOC; μM C), dissolved organic (DON; μM N) and inorganic nitrogen (DIN; μM N), soluble reactive phosphorus (SRP; μM P) and total dissolved phosphorus (TDP; μM P) concentrations. Values correspond to averages of 3 analytical replicates.

Site	Season	T	TIC	DO	DOC	DON	DIN	SRP	TDP
VR-1	Fall 2009	11.2	117	341	685	10.9	2.8	0.3	BD
VR-2	Fall 2009	11.2	418	327	286	50.9	20.5	0.6	0.9
VR-2	Summer 2010	14.9	647	335	288	48.5	19.1	0.6	0.9
RR-2	Summer 2010	20.8	382	281	406	17.7	10.1	0.4	0.9

Table 4.2: Seasonal description of the stream biofilm communities at Virginia River (VR) and Rennie's River (RR), particularly stable carbon ($\delta^{13}C$; ‰) and nitrogen ($\delta^{15}N$; ‰) isotope composition, carbon (C; Wt. %) and nitrogen (N; Wt. %) content, biofilm C:N ratios, and chlorophyll (Chl; μg Chl mg^{-1} $C_{Biofilm}$) a, b, and c content. Values correspond to averages of 3 analytical replicates ± one standard deviation.

Site	Season	$\delta^{13}C$	C	$\delta^{15}N$	N	Biofilm C:N	Chl a	Chl b	Chl c
VR-1	Fall 2009	-28.2 ± 0.2	17.2 ± 0.6	1.7 ± 0.1	0.8 ± 0.2	24.5 ± 0.8	0.7 ± 0.05	0.1 ± 0.01	0.1 ± 0.01
VR-2	Fall 2009	-22.7 ± 0.5	12.5 ± 6.8	6.9 ± 0.1	1.7 ± 0.3	8.3 ± 3.1	23.9 ± 0.09	3.6 ± 0.08	2.5 ± 0.15
VR-2	Summer 2010	-18.6 ± 0.3	14.8 ± 0.1	5.2 ± 0.1	1.5 ± 0.1	11.2 ± 0.1	9.7 ± 0.93	2.0 ± 0.21	0.6 ± 0.03
RR-2	Summer 2010	-28.0 ± 0.2	10.8 ± 0.1	7.0 ± 0.1	1.1 ± 0.1	11.7 ± 0.1	4.4 ± 0.44	0.2 ± 0.03	0.4 ± 0.07

Table 4.3: Biofilm carbon-normalized total carbon content and carbon content of individual fatty acids (μmol C_{PLFA} mol $C_{Biofilm}^{-1}$) present in initial, light and dark biofilm samples collected at Rennie's River in summer 2010 and at Virginia River in fall 2009, as well as proportion of heterotrophic and eukaryotic fatty acids relative to the total fatty acid content (Wt. %). Values were derived from single fatty acid extractions.

		Rennie's River (RR-2)							**Virginia River (VR-2)**						
Microbial Groups	**Fatty Acids**	**Initial**	**Light**			**Dark**			**Initial**	**Light**			**Dark**		
	Total	**3.42**	**3.71**	**3.23**	**2.97**	**3.12**	**1.36**	**2.94**	**4.57**	**4.65**	**6.89**	**5.42**	**4.81**	**4.18**	**6.55**
Heterotrophic bacteria (HB)	i15:0	0.06	0.05	0.09	0.09	0.06	-	0.09	0.05	0.11	0.22	0.11	0.08	0.05	0.15
	a15:0	-	-	0.05	0.05	0.04	-	0.07	0.03	0.19	0.29	0.18	0.14	0.11	0.24
	i16:0	0.05	0.04	0.05	0.06	0.06	0.08	0.03	0.05	0.05	0.07	0.05	0.04	0.04	0.06
	Wt. %	*3.1*	*2.6*	*6.0*	*6.8*	*5.1*	*5.5*	*6.6*	*3.0*	*7.7*	*8.5*	*6.5*	*5.4*	*4.7*	*6.9*
Eukaryotes (EUK), e.g. green algae, diatoms, fungi	18:2ω6	0.20	0.26	0.23	0.15	0.14	0.09	0.15	0.06	0.12	0.22	0.18	0.17	0.14	0.27
	18:3ω6	-	0.10	0.07	0.09	0.08	0.05	0.07	0.19	0.04	0.05	0.04	0.05	0.04	0.06
	18:3ω3	0.11	0.11	0.08	0.08	0.08	0.05	0.05	0.04	0.22	0.31	0.24	0.24	0.29	0.34
	20:5ω3	0.13	0.11	0.10	0.09	0.10	0.05	0.08	-	0.26	0.29	0.32	0.17	0.25	0.20
	22:5ω6	0.05	0.06	0.03	0.04	0.05	-	0.06	0.09	0.05	0.07	0.05	0.05	0.05	0.06
	Wt. %	*14.0*	*17.4*	*15.5*	*15.1*	*14.7*	*18.3*	*14.5*	*8.1*	*14.8*	*13.6*	*15.3*	*14.1*	*18.4*	*14.0*
General	14:0	0.11	0.09	0.17	0.09	0.07	-	0.10	0.06	0.24	0.38	0.21	0.09	0.06	0.28
	15:0	-	-	-	-	-	-	-	-	0.07	0.09	0.06	0.06	0.07	0.08
	16:0	0.93	1.22	0.81	0.77	0.89	0.32	0.74	1.97	1.57	2.26	1.81	1.59	1.48	2.02
	16:1ω7	0.78	0.63	0.72	0.68	0.78	0.23	0.65	0.82	1.09	1.77	1.39	1.33	0.96	1.86
	17:0	0.22	0.13	0.13	-	0.04	-	0.19	-	0.06	0.08	0.07	0.04	0.04	0.05
	18:0	0.18	0.20	0.12	0.12	0.15	0.08	0.11	0.13	0.12	0.16	0.15	0.14	0.11	0.14
	18:1ω9t	0.17	0.23	0.14	0.16	0.16	0.12	0.14	0.23	0.14	0.15	0.18	0.14	0.12	0.17
	18:1ω9c	0.45	0.48	0.45	0.50	0.40	0.29	0.38	0.85	0.32	0.48	0.38	0.48	0.37	0.59
	Wt. %	*82.9*	*80.0*	*78.6*	*78.1*	*80.2*	*76.1*	*78.9*	*88.9*	*77.5*	*78.0*	*78.2*	*80.5*	*76.8*	*79.2*
HB:EUK ratio		*0.22*	*0.15*	*0.38*	*0.45*	*0.35*	*0.30*	*0.46*	*0.36*	*0.52*	*0.62*	*0.42*	*0.38*	*0.26*	*0.49*

4.3.3 Net primary production and community respiration

Net primary production (NPP), measured as net TIC uptake and net release of DO, occurred in the light incubations, while dark incubations exhibited net TIC production and net decreases in DO. Net oxygen production was 7.2 ± 2.3 μmol O_2 mol $C_{Biofilm}^{-1}$ hr^{-1} and net TIC uptake was 23.2 ± 5.3 μmol C mol $C_{Biofilm}^{-1}$ hr^{-1} in the light enclosures at VR-1 (Fig. 4.1a). At VR-2, net oxygen production was 54.3 ± 10.0 μmol O_2 mol $C_{Biofilm}^{-1}$ hr^{-1} and net TIC uptake was 82.2 ± 9.0 μmol C mol $C_{Biofilm}^{-1}$ hr^{-1} in the light mesocosms in fall (Fig. 4.1a). In summer, 51.8 ± 4.8 μmol O_2 mol $C_{Biofilm}^{-1}$ hr^{-1} were produced and net TIC uptake was 122.8 ± 9.2 μmol C mol $C_{Biofilm}^{-1}$ hr^{-1} in the light mesocosms (Fig. 4.1a). At RR-2, oxygen production was 45.2 ± 3.3 μmol O_2 mol $C_{Biofilm}^{-1}$ hr^{-1} and net TIC uptake was 93.2 ± 9.2 μmol C mol $C_{Biofilm}^{-1}$ hr^{-1} in the light mesocosms (Fig. 4.1a). A correlation between NPP (as C uptake) and biofilm chlorophyll a ($p = 0.12$), b ($p = 0.10$), and c ($p = 0.25$) content was not observed.

Net oxygen consumption was 15.4 ± 5.5 μmol O_2 mol $C_{Biofilm}^{-1}$ hr^{-1} and net TIC production was 25.3 ± 3.8 μmol C mol $C_{Biofilm}^{-1}$ hr^{-1} in the dark chambers at VR-1 (Fig. 4.1b). At VR-2, net oxygen consumption was 24.7 ± 5.1 μmol O_2 mol $C_{Biofilm}^{-1}$ hr^{-1} and net TIC production was 39.7 ± 5.9 μmol C mol $C_{Biofilm}^{-1}$ hr^{-1} in the dark enclosures in fall (Fig. 4.1b). In summer, net oxygen consumption was 14.3 ± 0.2 μmol O_2 mol $C_{Biofilm}^{-1}$ hr^{-1} and 27.7 ± 11.5 μmol C mol $C_{Biofilm}^{-1}$ hr^{-1} were produced in the dark chambers (Fig. 4.1b). At RR-2, net oxygen consumption was 28.9 ± 0.4 μmol O_2 mol $C_{Biofilm}^{-1}$ hr^{-1} and 51.9 ± 8.6 μmol C mol $C_{Biofilm}^{-1}$ hr^{-1} were produced in the dark enclosures (Fig. 4.1b).

The DO and TIC data presented here revealed that the respiratory quotients (RQ = $+\Delta CO_2/-\Delta O_2$) and photosynthetic quotients (PQ = $+\Delta O_2/-\Delta CO_2$) ranged from 1.4 to 1.9 and from 0.3 to 0.7, respectively, and cannot be assumed to be equal to 0.8 – 1.5 in this

study as it is often reported for freshwater and marine systems (Bell and Kuparinen, 1984; del Giorgio et al., 1997; Robinson et al., 1999; Goto et al., 2008). However, wide ranges in PQ and RQ, especially seasonally, have been reported in other studies, for example in the presence of anaerobic degradation processes (Glud, 2008). Here, we present C uptake and release rates when describing NPP processes as using the direct C rates data is likely more accurate when estimating C production and mineralization (Wetzel and Likens, 2000; Glud, 2008). The DO measurements were used to verify the C data and show a similar trend in each mesocosm (for details see appendix A4).

Gross primary production (GPP) was estimated from the sum of NPP and community respiration (as changes in TIC), assuming that community respiration measured in the dark was equivalent to the respiration that occurred in the light chambers. Lowest rates were measured at VR-1 (46.9 – 49.3 μmol C mol $C_{Biofilm}^{-1}$ hr^{-1}) and highest rates were observed at VR-2 and RR-2 (108.3 – 193.7 μmol C mol $C_{Biofilm}^{-1}$ hr^{-1}; Fig. 4.1c). Photosynthesis to respiration (P:R) ratios varied from 1.8 to 2.4 at VR-1, 3.0 to 4.0 at VR-2 in fall 2009, 4.1 to 7.2 at VR-2 in summer 2010, and from 2.8 to 3.3 at RR-2, indicating biofilms were net autotrophic at all of these sites (Odum, 1956).

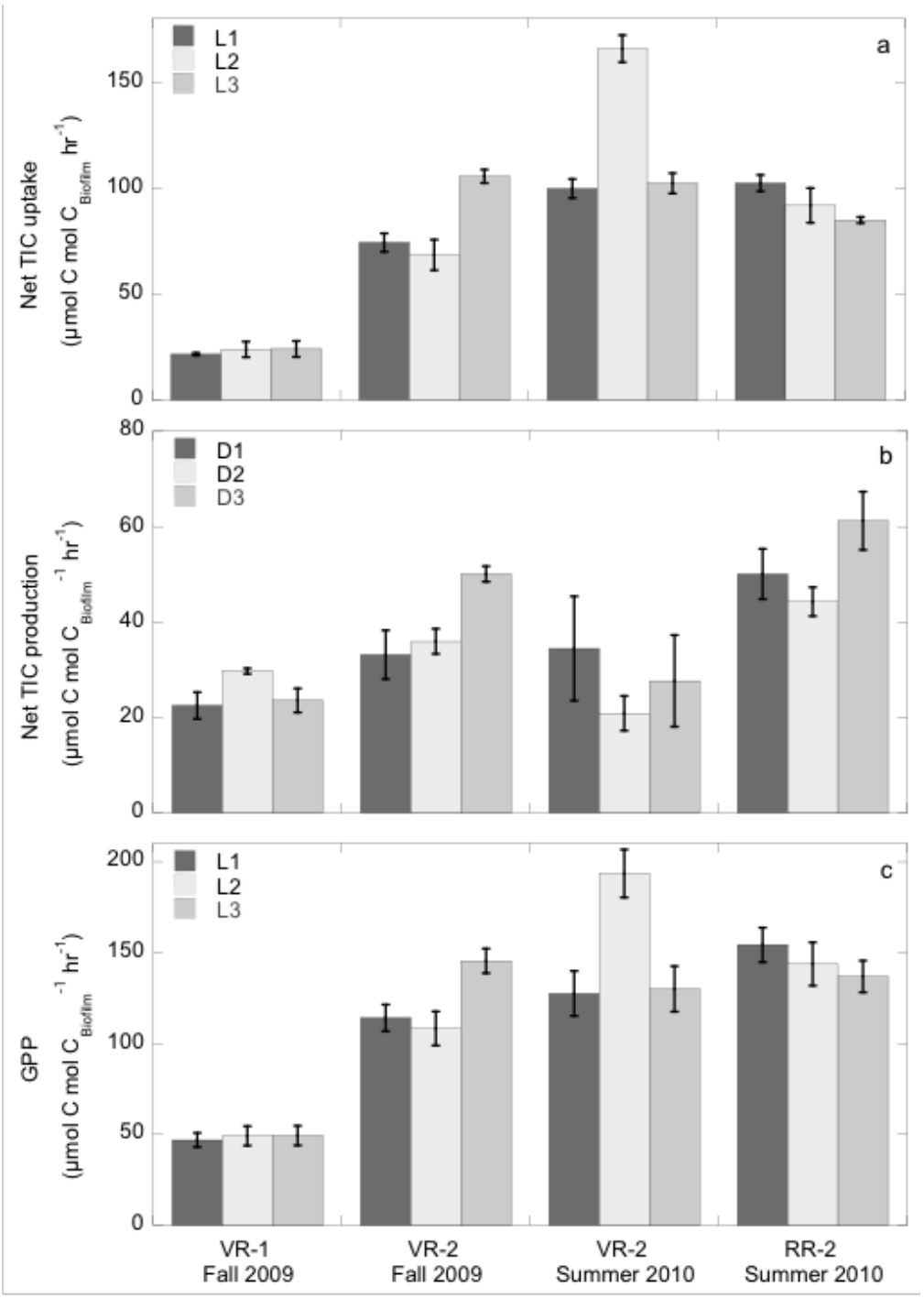

Figure 4.1: Biofilm-normalized net inorganic carbon (TIC) uptake (net primary production; a) in the light mesocosms (L1, L2, L3) and production (community respiration; b) in the dark mesocosms (D1, D2, D3), and gross primary production (GPP; c) estimated from the sum of net primary production of each light mesocosm and average community respiration at Virginia River upstream (VR-1), Virginia River downstream (VR-2), and Rennie's River downstream (RR-2). Values correspond to 3 analytical replicates per mesocosm ± one standard deviation.

4.3.4 DOC release and uptake

Total net DOC release during the light incubation was lower than NPP at the VR sites and surprisingly higher than NPP at RR-2. The net DOC released varied from below detection (in chambers L1 and L3; Table 4.4a) to 19.2 ± 11.2 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1} (in chamber L2; Table 4a) in the light mesocosms at VR-1. The large standard deviation in L2 represents the variation in the individual chamber analytical replicates (n = 3); however, changes between initial and final measurements for this chamber were significant (p = 0.02). At VR-2, the average net DOC released was 17.9 ± 2.3 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1} and 20.2 ± 6.5 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1} in summer and fall, respectively. At RR-2, net DOC release in the light incubations was 109.4 ± 19.5 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1}. The increase in $\delta^{13}C_{DOC}$ in the light enclosures was 3.5 ‰ at VR-2 and 6.3 ‰ at RR-2 in summer, and below detection at VR-1 and 2.9 ‰ at VR-2 in the fall. Primary production as net DOC released (PP_{DOC}), measured based on the fraction of DOC derived from TIC that was fixed during the incubation, and the change in $\delta^{13}C_{DOC}$ over the incubation period, was between 0.8 and 2.2 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1} at VR-2 and between 1.4 and 1.9 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1} at RR-2. PP_{DOC} was below detection at VR-1. These PP_{DOC} values represent only 1 % of biofilm NPP at RR-2, less than 1 % at VR-1, and 3 to 10 % at VR-2 (Fig. 4.2). A correlation between PP_{DOC} released and biofilm chlorophyll a (p = 0.16), b (p = 0.29), and c (p = 0.19) content was not observed. Furthermore, net DOC uptake in the dark incubations varied from below detection to 11.3 ± 7.1 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1} at VR-1 and VR-2, and was below detection in the dark mesocosms at RR-2.

Carbon-specific biofilm PP in the light enclosures of RR-2 and VR-2, measured based on the fraction of biofilm C derived from TIC that was fixed during the incubation and the change in $\delta^{13}C_{Biofilm}$ over the incubation period, was similar at the two sites and

varied from 0.8 to 1.5 mmol C mol $C_{Biofilm}^{-1}$ hr^{-1} (Fig. 4.3). Carbon-specific biofilm PP was below detection at VR-1.

Table 4.4a: Total net dissolved organic carbon (DOC) release to stream water normalized to biofilm biomass (µmol C mol $C_{Biofilm}^{-1}$ hr^{-1}) in the light incubations (L1, L2, L3) at Virginia River upstream (VR-1), Virginia River downstream (VR-2), and Rennie's River downstream (RR-2). Values correspond to 5 replicates ± one standard deviation. NS: Not significant.

Site	Season	DOC release		
		L1	L2	L3
VR-1	Fall 2009	NS	19.2 ± 11.2	NS
VR-2	Fall 2009	23.9 ± 4.3	18.0 ± 4.2	18.7 ± 2.7
VR-2	Summer 2010	11.9 ± 1.2	NS	24.0 ± 2.0
RR-2	Summer 2010	135.5 ± 15.9	89.0 ± 5.5	103.8 ± 9.8

Table 4.4b: Total net dissolved organic carbon (DOC) uptake from stream water normalized to biofilm biomass (µmol C mol $C_{Biofilm}^{-1}$ hr^{-1}) in the dark incubations (D1, D2, D3) at Virginia River upstream (VR-1), Virginia River downstream (VR-2), and Rennie's River downstream (RR-2). Values correspond to 5 replicates ± one standard deviation. NS: Not significant.

Site	Season	DOC uptake		
		D1	D2	D3
VR-1	Fall 2009	NS	10.8 ± 0.6	NS
VR-2	Fall 2009	NS	10.3 ± 3.7	12.2 ± 6.1
VR-2	Summer 2010	2.4 ± 1.0	3.9 ± 1.7	7.6 ± 2.1
RR-2	Summer 2010	NS	NS	NS

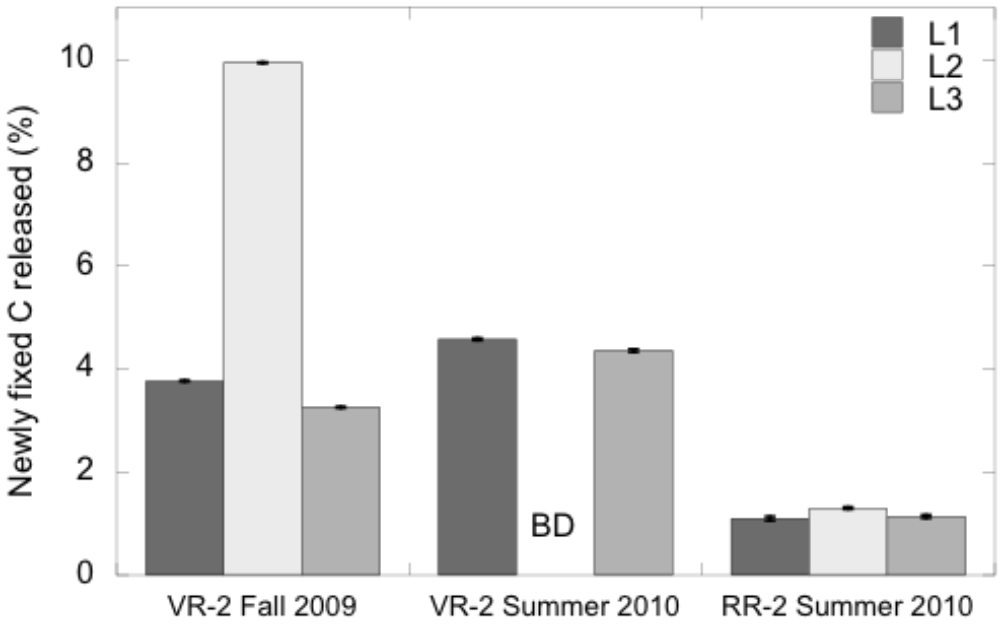

Figure 4.2: Newly fixed carbon released as dissolved organic carbon during the course of light incubation at Virginia River (VR-2) and Rennie's River (RR-2) given as a percentage of net primary production. Values correspond to mesocosm averages (L1, L2, L3) ± one standard deviation. BD: Below detection.

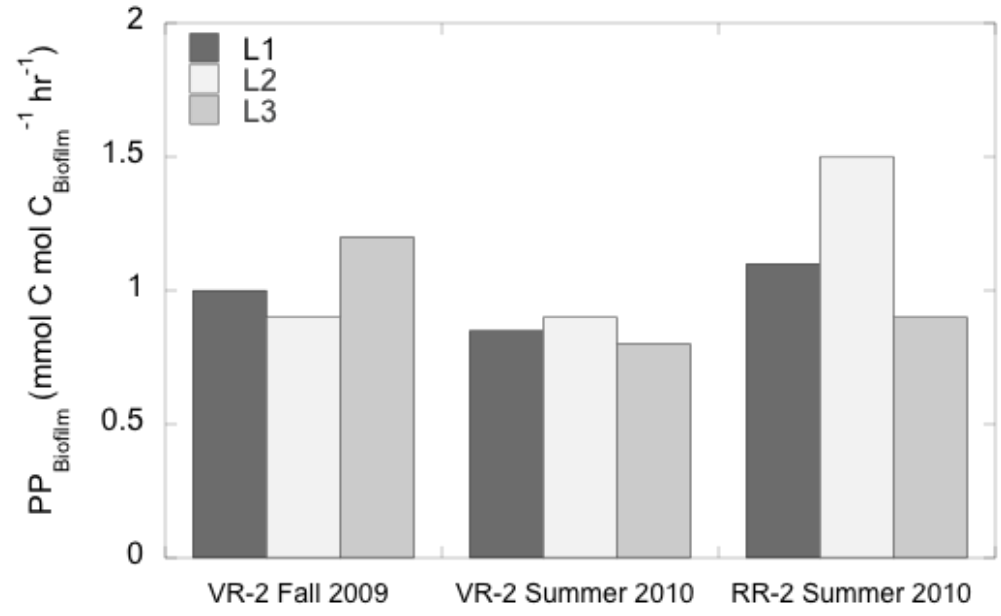

Figure 4.3: Carbon-specific biofilm primary production ($PP_{Biofilm}$) in the light mesocosms (L1, L2, L3) at Virginia River (VR-2) and Rennie's River (RR-2).

4.3.5 Newly fixed C incorporation into biofilm PLFA

Total biofilm C-normalized C_{NF} incorporation rates into PLFA in the light incubations varied from 9.71 to 14.39 nmol PLFA-C_{NF} mol $C_{Biofilm}^{-1}$ hr^{-1} at RR-2, whereupon biofilm C-normalized C_{NF} incorporation rates specifically determined for heterotrophic and eukaryotic PLFA ranged from 0.10 to 0.30 nmol PLFA-C_{NF} mol $C_{Biofilm}^{-1}$ hr^{-1} and from 0.79 to 1.39 nmol PLFA-C_{NF} mol $C_{Biofilm}^{-1}$ hr^{-1}, respectively (Table 4.5). At VR-2, total biofilm C-normalized C_{NF} incorporation rates ranged from 14.07 to 22.06 nmol PLFA-C_{NF} mol $C_{Biofilm}^{-1}$ hr^{-1} with biofilm C-normalized C_{NF} incorporation rates specifically determined for heterotrophic and eukaryotic PLFA having varied from 0.46 to 1.00 nmol PLFA-C_{NF} mol $C_{Biofilm}^{-1}$ hr^{-1} and from 1.55 to 2.36 nmol PLFA-C_{NF} mol $C_{Biofilm}^{-1}$ hr^{-1}, respectively (Table 4.5). Total biofilm C-normalized C_{NF} incorporation rates were not significantly different between RR-2 and VR-2 ($p = 0.07$), however, biofilm C-normalized C_{NF} incorporation rates specifically determined for heterotrophic ($p = 0.03$) and eukaryotic PLFA ($p = 0.02$) were significantly different among the two study sites. A significant correlation was observed between biofilm-specific PP and the rate of C_{NF} into eukaryotic PLFA ($p = 0.04$), but not into heterotrophic bacterial ($p = 0.06$) and total ($p = 0.14$) PLFA. The ratios of C_{NF} incorporation into heterotrophic bacterial relative to eukaryotic PLFA ranged from 0.07 to 0.29 at RR-2, and from 0.28 to 0.42 at VR-2 (Table 4.5), and were not significantly different between the two sites ($p = 0.08$). Biofilm C-normalized C_{NF} incorporation rates were below detection in the dark incubations at both RR-2 and VR-2.

The C_{NF} incorporation into biofilm PLFA in the light mesocosms, expressed as the total PLFA-C_{NF} relative to total PLFA-C, varied from 30.09 to 40.81 nmol PLFA-C_{NF} µmol PLFA-C_{total}^{-1} hr^{-1} at RR-2, whereupon C_{NF} incorporation into heterotrophic bacterial and eukaryotic PLFA ranged from 1.80 to 3.92 nmol PLFA-C_{NF} µmol PLFA-C_{total}^{-1} hr^{-1} (5 – 10

% of total C_{NF} in the PLFA attributed to heterotrophic bacterial PLFA; Table 4.5), and from 7.23 to 8.36 nmol PLFA-C_{NF} µmol PLFA-C_{total}^{-1} hr^{-1} (19 – 25 % of total C_{NF} in the PLFA attributed to eukaryote PLFA; Table 4.5), respectively. PLFA concentration-normalized rates of C_{NF} incorporation into biofilm PLFA in the light incubations at VR-2 were slightly lower (but not significant: $p = 0.14$) compared to RR-2, and ranged from 28.97 to 31.83 nmol PLFA-C_{NF} µmol PLFA-C_{total}^{-1} hr^{-1}. PLFA concentration-normalized rates of C_{NF} incorporation specifically into heterotrophic bacterial and eukaryotic PLFA, however, was higher (both $p = 0.02$) at VR-2 compared to RR-2 and varied from 7.23 to 8.36 nmol PLFA-C_{NF} µmol PLFA-C_{total}^{-1} hr^{-1} (10 – 12 % of total C_{NF} in the PLFA attributed to heterotrophic bacterial PLFA; Table 4.5), and from 8.35 to 8.68 nmol PLFA-C_{NF} µmol PLFA-C_{total}^{-1} hr^{-1} (27 – 29 % of total C_{NF} in the PLFA attributed to eukaryote PLFA; Table 4.5), respectively. The ratios of C_{NF} incorporation into heterotrophic bacterial relative to eukaryotic PLFA ranged from 0.22 to 0.48 (Table 4.5) and were not significantly different between the two sites ($p = 0.19$). PLFA concentration-normalized rates of C_{NF} incorporation into biofilm PLFA were below detection, as expected, in the dark mesocosms at both RR-2 and VR-2.

Table 4.5: Uptake rate of newly fixed carbon into biofilm phospholipid fatty acids (PLFA) provided as the biofilm carbon-normalized rate (C_{NF}, nmol PLFA-C_{NF} mol $C_{Biofilm}^{-1}$ hr^{-1}) and percent of total C_{NF} incorporated into the biofilm PLFA allocated to specific PLFA or PLFA groups (C_{NF} as % PLFA-C_{NF}, %) in the light incubations at Rennie's River and Virginia River downstream. Values were derived from single fatty acid extractions and three analysis replicates (L1, L2, L3).

		Rennie's River (RR-2)						**Virginia River (VR-2)**					
Microbial groups	**Fatty acids**	**L1**		**L2**		**L3**		**L1**		**L2**		**L3**	
		C_{NF}	**C_{NF} as % PLFA-C**	**C_{NF}**	**C_{NF} as % PLFA-C**	**C_{NF}**	**C_{NF} as % PLFA-C**	**C_{NF}**	**C_{NF} as % PLFA-C**	**C_{NF}**	**C_{NF} as % PLFA-C**	**C_{NF}**	**C_{NF} as % PLFA-C**
	Total	**14.4**		**14.2**		**9.7**		**14.1**		**22.6**		**17.6**	
Heterotrophic bacteria (HB)	i15:0	0.1	5.4	0.2	6.4	0.2	5.9	0.2	5.5	0.4	6.2	0.2	6.1
	a15:0	-	-	0.1	3.2	0.0	1.2	0.3	5.0	0.6	6.1	0.3	5.9
	Total HB	*0.1*	*5.4*	*0.3*	*9.6*	*0.2*	*7.1*	*0.5*	*10.5*	*1.0*	*12.2*	*0.6*	*12.0*
Eukaryotes (EUK), e.g. green algae, diatoms, fungi	18:2ω6	0.8	9.0	0.5	5.7	0.4	8.9	0.4	10.2	0.7	10.0	0.5	9.7
	18:3ω3	0.4	11.3	0.3	9.0	0.2	9.6	1.0	15.4	1.3	13.4	1.1	15.3
	20:5ω3	0.2	4.9	0.2	5.2	0.2	5.5	0.2	3.2	0.3	3.6	0.3	3.3
	Total EUK	*1.4*	*25.2*	*1.0*	*19.9*	*0.8*	*24.0*	*1.6*	*28.8*	*2.4*	*27.0*	*2.0*	*28.2*
General (G)	14:0	0.3	8.7	0.6	9.0	0.3	12.1	1.1	15.6	1.6	13.7	0.8	12.6
	16:0	5.7	14.1	4.5	13.6	2.9	12.3	5.6	12.3	7.8	10.9	6.5	11.6
	16:1ω7	3.6	17.1	4.6	15.4	3.3	15.8	4.2	13.4	7.8	13.7	6.1	14.3
	18:0	0.6	8.6	0.6	12.4	0.4	10.6	0.2	6.0	0.4	7.2	0.3	6.2
	18:1ω9c	0.6	7.3	0.5	8.4	0.4	7.9	0.2	5.4	0.3	6.6	0.3	6.5
	18:1ω9t	2.2	13.6	2.1	11.8	1.5	10.1	0.7	8.0	1.3	8.7	1.0	8.6
	Total G	*12.9*	*69.4*	*12.9*	*70.5*	*8.7*	*68.9*	*12.1*	*60.6*	*19.2*	*60.8*	*15.0*	*59.7*
HB:EUK ratio		0.07	0.22	0.29	0.48	0.24	0.30	0.30	0.36	0.42	0.45	0.28	0.43

4.3.6 Net phosphorus and nitrogen uptake and production

Net P uptake or production rates were below detection in all instances, except at VR-1, where a net production of 0.01 μM P hr^{-1} as SRP was observed in the light enclosures. Net TDN production was 0.2 μM N hr^{-1} in the light incubations at VR-1, whereas 1.3 μM N hr^{-1} were consumed in the light enclosures at VR-2. Thereof, 0.09 μM N hr^{-1} were produced as NH_4^+ in the light incubations at VR-1 and 0.1 μM N hr^{-1} were consumed as NH_4^+ in the light enclosures at VR-2. Changes in TDN or NH_4^+ were not detectable in the light incubations at RR-2. Furthermore, changes in TDN were not observed in the dark enclosures at VR-2, whereas TDN production was 0.1 and 0.3 μM N hr^{-1} in the dark incubations at VR-1 and RR-2, respectively. Changes in NH_4^+ were not detectable in the dark enclosures.

4.3.7 The influence of labile C availability on the respiration of ambient stream DOC

The mesocosm experiments at RR-2 included a second set of light (n = 3) and dark (n = 6) incubations that were used to assess the influence of C_{NF} and added glucose on the mineralization of stream DOC. All mesocosms were set-up and filled with stream water within 30 minutes of sunrise, however, this second set was not sampled until approximately one hour following sunrise. The initial DOC concentration in the light and dark mesocosms that were spiked with ^{13}C-bicabonate and sampled first ranged from 405 to 410 μM C (Fig. 4.4). The second set of mesocosms, sampled over an hour later, exhibited elevated DOC concentrations (475 – 490 μM C; Fig. 4.4) relative to the earlier set, likely a result of the later sampling time having occurred following sunrise. Congruent with this, the initial TIC concentration in this second set of mesocoms was lower (275 – 295 μM C) than the initial measurements from the first set of mesocosms

(382 – 387 µM C). Here, estimates of the differences between initial and final samples are reported for each treatment individually.

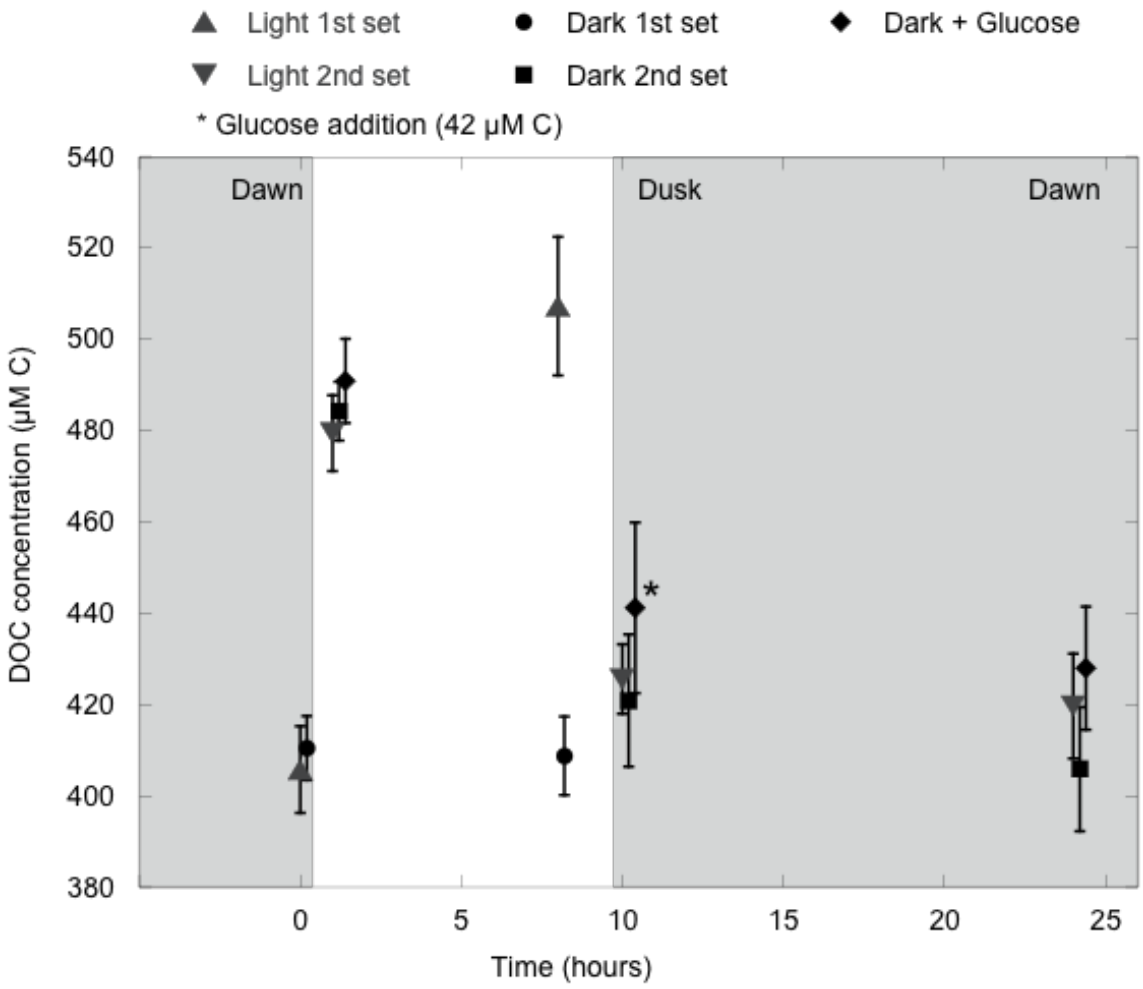

Figure 4.4: Changes in dissolved organic carbon (DOC) concentration in the light (n = 6) and dark chambers (n = 9) incubated in Rennie's River over a 24-hour incubation period. Values represent mesocosm averages (n = 3 each) ± one standard deviation.

Note: The initial DOC concentration (t = 0) and the measurement taken after 8 hours were derived from the mesocosms that were spiked with ^{13}C-bicarbonate (first set), whereas the measurements taken after one hour, 10 hours, and 24 hours were derived from the second set of light mesocosms, assuming that the same processes occurred in all 6 light mesocosms. Symbols are aligned in the order of sampling.

* Three of the six dark incubations received ^{13}C-labelled glucose (42 µM C; 551 ‰) at dusk as a labile substrate to determine if respiration of stream DOC increased as a result of the glucose treatment. The remaining three dark incubations served as a control.

While net DOC uptake was not measurable in the ^{13}C-bicarbonate labeled dark mesocosms of the first set (Table 4.4b), it was measureable and varied from 53 to 69 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1} in the second set of dark mesocosms incubated from sunrise till dusk (approximately 10 hours). This second set of dark incubations proceeded through the night until sunrise, and exhibited a total net DOC uptake between 37 and 85 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1} during the whole incubation (approximately 24 hours). Hence, only 5 to 12 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1} were used during the incubation at night. The net TIC production, or respiration, also decreased during the night. While the average TIC production that occurred from sunrise till sunset was 24 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1} in these dark enclosures (Table 4.4a), the average net TIC production from sunrise until the next morning (24 hours) was only 14 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1} during the course of night incubation (dusk to dawn; Table 4.6b). The drop in water temperature (to approximately 12°C) during the night was likely a major factor reducing microbial activity in the overnight incubations. As expected, net DOC release in the light incubations was negligible (0.1 and 0.8 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1} measured in L1 and L3, respectively) during the incubation at night. The mesocosm chamber L2 exhibited a net DOC uptake of 11 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1} during the incubation at night. Average net TIC production was 32 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1} in these enclosures from dusk until sunrise (Table 4.6b).

Three of the six dark incubations received ^{13}C-labeled glucose (42 µM C; 551 ‰) at dusk as a labile substrate to determine if respiration of extant stream DOC increases as a result of added labile C. The remaining three dark incubations served as a control. In all three instances where glucose was added, microbial respiration did not increase relative to the incubations without glucose. Also, the amount of C respired (16.0 ± 5.8 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1}) was significantly lower in the incubations where glucose was added relative to the enclosures that likely received labile C from algal sources

throughout the light incubation (32 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1}). The proportion of glucose that was respired ($R_{Glucose}$) in the glucose treatment mesocosms was relatively small (3 – 4 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1}), whereas the average respiration of stream organic carbon ($R_{Stream\text{-}OC}$) was 12.8 ± 5.1 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1} (from dusk to dawn). Furthermore, $R_{Stream\text{-}OC}$ in the glucose treatment mesocosms did not differ significantly from the incubations without glucose (control, 14.2 ± 8.0 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1}; $p = 0.30$) or R_{total} (16.0 ± 5.8 µmol C mol $C_{Biofilm}^{-1}$ hr^{-1}; $p = 0.29$) when glucose was added. These results suggest that the added glucose did not stimulate the microbial use of extant stream organic matter at RR.

Table 4.6a: Respiration rates presented as total inorganic carbon (TIC, µmol C mol $C_{Biofilm}^{-1}$ hr^{-1}) production in the mesocosms, as well as primary production presented as TIC uptake in the light mesocosms from dawn to dusk. Each set of mesocosms included three light and three dark chambers. Values correspond to mesocosm averages (n = 3) ± one standard deviation.

1st mesocosm set	2nd mesocosm set
Dark (TIC production)	
51.9 ± 8.6	23.6 ± 5.8
Light (TIC uptake)	
93.2 ± 9.2	87.6 ± 1.0

Table 4.6b: Respiration rates presented as total inorganic carbon (TIC, µmol C mol $C_{Biofilm}^{-1}$ hr^{-1}) production in the mesocosms from dusk to dawn. Values were derived from the second mesocosm set and included three light and three dark chambers. Additionally, three dark chambers were spiked with glucose at dusk. Values correspond to mesocosm averages (n = 3) ± one standard deviation.

Dark control	*Dark+Glucose*	*Light*
14.2 ± 8.0	16.0 ± 5.8	32.2 ± 3.2

4.4 Discussion

Headwater streams are “hot spots” of biogeochemical transformations in the watershed, yet boreal catchments are responsible for the transport of significant quantities of organic matter (Post et al., 1982; Gorham, 1991; Molot and Dillon, 1996; Aitkenhead and McDowell, 2000; Pastor et al., 2003). Questions abound regarding the role of stream biofilms in the biogeochemical cycling of this organic matter, including its mineralization to CO_2, and the response of that mineralization to environmental change. Environmental changes, specifically nutrient enrichment, are followed by an alteration in biofilm community composition, activity levels, and interspecies interactions. External DOC and nutrient sources can break the link between heterotrophic and autotrophic communities and affect processes such as respiration as well as nutrient release and retention (Bernhardt and Likens, 2002; Scott et al., 2008; Lyon and Ziegler, 2009). The aim of this study was to investigate the importance of autotrophic-heterotrophic linkages in nutrient-impacted boreal streams, specifically whether (1) autotrophic carbon sources are important to supporting epilithic biofilms in nutrient-enriched boreal streams, and (2) whether autotrophic or labile carbon sources influence the use of allochthonous DOC.

Dissolved organic carbon released from biofilms was not clearly linked to C_{NF} but was stimulated by autotrophic activity and nutrients in the boreal urban streams studied here. A greater proportion of organic C was derived from C_{NF} during primary production at the most nutrient-rich site compared to the sites with lower nutrient concentrations. Net DOC release into the stream water was, however, lower than NPP. Further, the uptake of C_{NF} into eukaryotic and heterotrophic biofilm PLFA was higher at the most nutrient-rich site relative to the sites with lower nutrient concentrations. These findings suggest that higher nutrient levels in these streams can result in increased autotrophic activity and a higher capacity of the biofilms to incorporate autochthonous C.

Priming effects associated with glucose additions were not observed in these urban boreal stream biofilms. However, priming effects on the mineralization of stream DOC may occur with increases in autotrophic C inputs – sources that cannot be easily mimicked through C additions but rather through following the influence of newly fixed or autochthonous C production on stream DOC mineralization. Results here suggest that light-mediated activities, whether through autotrophic activity itself or the C released by this activity, were important to supporting greater organic matter mineralization by these biofilms.

4.4.1 Nutrient and carbon dynamics in nutrient-rich boreal stream biofilms

Gross primary production, NPP, net DOC release, PP_{DOC}, and $PP_{Biofilm}$ measured in the light mesocosms in this study were higher at the nutrient-rich sites (VR-2 and RR-2) relative to the nutrient-poor site (VR-1), coinciding with higher stream DIN, DON, SRP, and TP concentrations, as well as lower DOC concentrations at VR-2 and RR-2 relative to VR-1. This also coincided with biofilm chlorophyll content, C and N content, $\delta^{13}C$ and $\delta^{15}N$, as well as P:R ratios indicative of more autotrophic biofilms. Nitrogen uptake rates (as TDN and NH_4^+) were only detectable in the light mesocosms at VR-2, the most nutrient-rich site in this study, indicating that stream water N plays a significant role in supporting primary production at this site and biofilm N demand likely increases with increasing algal production. The lower N uptake and release rates at VR-1 and RR-2 suggest that these biofilms were likely fueled by N produced and cycled within the biofilm. The fact that TDN uptake could not be measured in the dark incubations indicates that TDN uptake may be dominated by photoautotrophic microorganisms (Hall and Tank, 2003; Lyon and Ziegler, 2009). Heterotrophs may, however, not be able to use the N substrates without a labile C source, which may be available in the light

incubations due to autotrophic production (Lyon and Ziegler, 2009; Lane et al., 2012; Franke et al., 2013a).

Net DOC release in the light mesocosm incubations did not exhibit a clear relationship with NPP and the minor fraction of C_{NF} in the DOC released highlights the potential importance of detrital sources of DOC from these biofilms. This was evident through the higher net DOC release compared to NPP at RR-2, indicative of excess DOC released by the biofilms in the light incubations at this site. This also suggests that a major proportion of the released DOC at the sites with lower nutrient concentrations was derived from older C stored within the biofilm rather than fixed C during the incubation, which is, again, suggestive of a more detrital component in these biofilms. These findings coincided with the lower PLFA concentrations of the boreal stream biofilm communities studied here relative to temperate systems (Lyon and Ziegler, 2009). Aggregates of such detrital material, often described as freely floating flocs or “snow”, provide important surface areas for microbial colonization and may contain significant amounts of DOC and nutrients (Fischer, 2003; Sinsabaugh and Foreman, 2003).

The minor fraction of C_{NF} in the DOC released further suggests that the DOC produced was not necessarily released into the stream water at these sites but rather stored and cycled within the biofilm. The findings here suggest a tight autotrophic-heterotrophic linkage in these biofilms, and reveal that higher nutrient levels can result in a greater capacity to incorporate autochthonous C. At VR-2, the most nutrient-rich site with the highest algal abundance, a greater proportion of DOC was derived from C_{NF} during primary production. Furthermore, the uptake of C_{NF} into eukaryotic and heterotrophic biofilm PLFAs was higher at the most nutrient-rich site (VR-2) relative to the site with lower nutrient concentrations (RR-2). The ratios of C_{NF} incorporation into heterotrophic bacterial relative to eukaryotic PLFA were, however, not significantly

different between the two study sites, and were similar to the ratios found in temperate streams (Lyon and Ziegler, 2009) that exhibited lower DOC concentrations and much higher nutrient levels compared to the boreal streams studied here. The absolute rates of autotrophy in these boreal streams were, however, significantly lower than observed in highly nutrient-impacted temperate streams (Lyon and Ziegler, 2009), further indicating the potential importance of nutrients. More specifically, Lyon and Ziegler (2009) measured a greater proportion of C_{NF} released as DOC in temperate streams (5 – 30 % compared to 1 – 10 % in our study). The PP_{DOC} rates and $PP_{Biofilm}$ rates determined in this study were also lower (4 – 140 and 3 – 6 times, respectively) compared to the rates measured in the nutrient-rich temperate streams (Lyon and Ziegler, 2009). These findings coincided with the lower PLFA concentrations of the boreal stream biofilm communities relative to the temperate stream biofilms (Lyon and Ziegler, 2009). Additional to the importance of nutrients, climatic conditions, i.e. temperature, may affect the biofilm composition by regulating the decomposition of organic matter by heterotrophs (Schiff et al., 1998; Davidson and Janssens, 2006; Bragazza et al., 2009; Chapin et al., 2011) and likely more so than algal production. This was evident through the relatively lower distribution of heterotrophic PLFA compared to algal PLFA in the boreal stream biofilms studied here relative to temperate streams (Lyon and Ziegler, 2009), whereas the proportion of algal PLFA was relatively similar when comparing these two systems.

The rates of C_{NF} incorporated into the biofilm exceed the estimates of GPP significantly by roughly an order of magnitude. This, again, suggests that C_{NF} is primarily retained in the biofilm while the DOC released and C respired by the biofilm is not significantly supported by the C_{NF} but likely largely supported by allochthonous C sources. This is supported by the fact that PP_{DOC} was a low percentage of NPP. The

DOC releases discussed here were light-mediated since they were not measured in the dark mesocosms, where uptake or no change at all in DOC was detected. Although DOC released did not reflect newly fixed autotrophic C sources, results from this study suggest that autotrophic C sources may play a significant role supporting microbial activity in the biofilms, which could influence DOC cycling including its release.

4.4.2 The influence of labile carbon availability on the respiration of ambient stream organic carbon

Allochthonous DOC sources typically dominate boreal headwater streams. Under nutrient enrichment conditions, however, autochthonous DOC sources may have the potential to stimulate allochthonous DOC use by stream biofilm communities. Labile DOC additions did not stimulate the microbial mineralization of ambient organic carbon in these boreal streams; however, observations here do suggest autotrophic activity stimulates biofilm mineralization. Total respiration was significantly lower in the incubations where glucose was added relative to the enclosures that had received labile C from algal sources from the previous light incubation prior to dusk. Photosynthetically-produced labile C sources may have provided energy and C supporting the increased mineralization by the biofilms in the previously exposed to the light relative to the dark glucose or control incubations. However, the increased respiration could have been attributed to any of the biofilm components, including respiring autotrophs, since total average respiration (as dusk-dawn measurement) was almost twice as high in the mesocosms that were held in the light throughout the day relative to the control mesocosms incubated in the dark and the mesocosms that were spiked with glucose. Therefore, we cannot determine if increases in allochthonous stream C mineralization occurred with autotrophic activity. Results here, however, are important in distinguishing

a significant difference between priming via substrate addition and the potential for priming via more direct autotrophic inputs – a more likely scenario given the autotrophic-heterotrophic linkages demonstrated in this and other studies.

Labile substrates, analogous with algal inputs, can have opposing effects on the fate of stream DOC potentially depending upon nutrient availability and/or biofilm activity (Franke et al., 2013a; Franke et al., 2013b). The mineralization rates measured in the enclosures that had received labile C from algal sources from the previous light incubation prior to dusk may in part be influenced by the availability of inorganic N and P in these streams as well as the activity of the biofilms. In particular, labile C such as algal products may limit the biofilms present at the relatively nutrient-enriched RR-2 site where N and P were available. The biofilms may be able to extract energy from labile C when N and P are provided. This was also observed in the substrate addition experiments (N and P in combination with ^{13}C-glucose) conducted in the urban streams on the Northeast Avalon Peninsula (Franke et al., 2013b), which are adjacent and similar to RR-2, as well as in selected pristine streams in the Humber River watershed (Franke et al., 2013a). Also, the substrate additions in most cases likely stimulated the incorporation rather than mineralization of the more N- and P-rich extant stream organic matter (Franke et al., 2013a). This may have also occurred in the dark incubations here that received glucose. Hence, lower mineralization rates were measured compared to the incubations that were held in the light throughout the day and which may have contained labile C sources and likely more active biofilms including respiring autotrophs. All together, these effects suggest that increased nutrient enrichment may increase losses of more labile versus more complex substrates in these streams. The differential loss of more complex versus simple substrates, however, may be altered by priming effects fueled by increased labile sources with nutrient enrichment.

The results from this study show that photosynthetically-produced labile C sources may be of significance in these urban boreal streams as they likely allow the mineralization of organic matter by microbial communities, whether in form of newly fixed, older, or allochthonous organic matter. Autotrophic-heterotrophic microbial interactions and the influence of potential priming effects need to be further investigated in a large variety of aquatic ecosystems contrasting in watershed organic matter source and composition as well as nutrient availability in order to further contribute to the understanding of microbial carbon and nutrient cycling in the aquatic environment and the link between aquatic and terrestrial carbon cycles, as well as the responses of ecosystems to anthropogenic perturbations.

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4.6 References

Agren, A., Berggren, M., Laudon, H., and Jansson, M., 2008: Terrestrial export of highly bioavailable carbon from small boreal catchments in spring floods. *Freshwater Biology,* 53: 964-972.

Aitkenhead, J. A. and McDowell, W. H., 2000: Soil C : N ratio as a predictor of annual riverine DOC flux at local and global scales. *Global Biogeochemical Cycles,* 14: 127-138.

Bell, R. T. and Kuparinen, J., 1984: Assessing Phytoplankton and Bacterioplankton Production during Early Spring in Lake Erken, Sweden. *Applied and Environmental Microbiology,* 48: 1221-1230.

Bernhardt, E. S. and Likens, G. E., 2002: Dissolved organic carbon enrichment alters nitrogen dynamics in a forest stream. *Ecology,* 83: 1689-1700.

Bianchi, T. S., 2011: The role of terrestrially derived organic carbon in the coastal ocean: A changing paradigm and the priming effect. *Proceedings of the National Academy of Sciences of the United States of America,* 108: 19473-19481.

Bianchi, T. S. and Canuel, E. A., 2011: Chemical Biomarkers in Aquatic Ecosystems. *Princeton University Press (Feb 28 2011)*: 392 pages.

Boschker, H. T. S. and Middelburg, J. J., 2002: Stable isotopes and biomarkers in microbial ecology. *Fems Microbiology Ecology,* 40: 85-95.

Boschker, H. T. S., Kromkamp, J. C., and Middelburg, J. J., 2005: Biomarker and carbon isotopic constraints on bacterial and algal community structure and functioning in a turbid, tidal estuary. *Limnology and Oceanography,* 50: 70-80.

Bragazza, L., Buttler, A., Siegenthaler, A., and Mitchell, E. A. D., 2009: Plant Litter Decomposition and Nutrient Release in Peatlands. *In: Carbon Cycling in Northern Peatlands, Geophysical Monograph Series 184, American Geophysical Union 10.1029/2008GM000808*: 99-110.

Buffam, I., Laudon, H., Temnerud, J., Morth, C. M., and Bishop, K., 2007: Landscape-scale variability of acidity and dissolved organic carbon during spring flood in a boreal stream network. *Journal of Geophysical Research-Biogeosciences,* 112: G01022.

Chapin, F. S., Matson, P. A., and Vitousek, P. M., 2011: Decomposition and Ecosystem Carbon Budgets. Principles of Terrestrial Ecosystem Ecology: Springer New York, 183-228.

Cole, J. J., 1982: Interactions between Bacteria and Algae in Aquatic Ecosystems. *Annual Review of Ecology and Systematics,* 13: 291-314.

Davidson, E. A. and Janssens, I. A., 2006: Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature,* 440: 165-173.

del Giorgio, P. A., Cole, J. J., and Cimbleris, A., 1997: Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature,* 385: 148-151.

Dodds, W. K., Evans-White, M. A., Gerlanc, N. M., Gray, L., Gudder, D. A., Kemp, M. J., López, A. L., Stagliano, D., Strauss, E. A., Tank, J. L., Whiles, M. R., and Wollheim, W. M., 2000: Quantification of the Nitrogen Cycle in a Prairie Stream. *Ecosystems,* 3: 574-589.

Findlay, R. H., 2004: Determination of microbial community structure using phospholipid fatty acid profiles. *In: Kowalchuk G. A., De Bruijn F. J., Head I. M., Akkermans A. D. L., Van Elsas J. D. (eds) - Molecular Microbial Ecology Manual. Kluwer Academic*: 983–1004.

Findlay, S. E. G., Sinsabaugh, R. L., Sobczak, W. V., and Hoostal, M., 2003: Metabolic and structural response of hyporheic microbial communities to variations in supply of dissolved organic matter. *Limnology and Oceanography,* 48: 1608-1617.

Fischer, H., 2003: The Role of Biofilms in the Uptake and Transformation of Dissolved Organic Matter. *In: Aquatic Ecosystems: Interactivity of Dissolved Organic Matter; Editors: Findlay, S. E. & Sinsabaugh, R. L.; Elsevier Science*: 285-313.

Fisher, S. G. and Likens, G. E., 1973: Energy Flow in Bear Brook, New Hampshire: An Integrative Approach to Stream Ecosystem Metabolism. *Ecological Monographs,* 43: 421-439.

Ford, T. E. and Lock, M. A., 1987: Epilithic Metabolism of Dissolved Organic Carbon in Boreal Forest Rivers. *Fems Microbiology Ecology,* 45: 89-97.

France, R. L. and Schlaepfer, M. A., 2000: C-13 and N-15 depletion in components of a foodweb from an ephemeral boreal wetland compared to boreal lakes: putative evidence for microbial processes. *Hydrobiologia,* 439: 1-6.

Franke, D., Bonnell, E. J., and Ziegler, S. E., 2013a: Mineralization of dissolved organic matter by heterotrophic stream biofilm communities in a large boreal catchment. *Accepted for publication in Freshwater Biology*.

Franke, D., Lyon, D. R., and Ziegler, S. E., 2013b: The impact of nutrient enrichment on dissolved organic carbon mineralization by headwater stream biofilm communities.

Fry, B., 1991: Stable Isotope Diagrams of Freshwater Food Webs. *Ecology,* 72: 2293-2297.

Glud, R. N., 2008: Oxygen dynamics of marine sediments. *Marine Biology Research,* 4: 243-289.

Gorham, E., 1991: Northern Peatlands - Role in the Carbon-Cycle and Probable Responses to Climatic Warming. *Ecological Applications,* 1: 182-195.

Goto, N., Miyazaki, H., Nakamura, N., Terai, H., Ishida, N., and Mitamura, O., 2008: Relationships between electron transport rates determined by pulse amplitude modulated (PAM) chlorophyll fluorescence and photosynthetic rates by traditional and common methods in natural freshwater phytoplankton. *Fundamental and Applied Limnology,* 172: 121-134.

Guasch, H., Marti, E., and Sabater, S., 1995: Nutrient enrichment effects on biofilm metabolism in a Mediterranean stream. *Freshwater Biology,* 33: 373-383.

Guenet, B., Danger, M., Abbadie, L., and Lacroix, G., 2010: Priming effect: bridging the gap between terrestrial and aquatic ecology. *Ecology,* 91: 2850-2861.

Gulis, V. and Suberkropp, K., 2003: Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology,* 48: 123-134.

Haack, T. K. and McFeters, G. A., 1982: Nutritional Relationships among Microorganisms in an Epilithic Biofilm Community. *Microbial Ecology,* 8: 115-126.

Hall, R. O. and Tank, J. L., 2003: Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. *Limnology and Oceanography,* 48: 1120-1128.

Haukka, K., Kolmonen, E., Hyder, R., Hietala, J., Vakkilainen, K., Kairesalo, T., Haari, H., and Sivonen, K., 2006: Effect of nutrient loading on bacterioplankton community composition in lake mesocosms. *Microbial Ecology,* 51: 137-146.

Hoellein, T. J., Tank, J. L., Kelly, J. J., and Rosi-Marshall, E. J., 2010: Seasonal variation in nutrient limitation of microbial biofilms colonizing organic and inorganic substrata in streams. *Hydrobiologia,* 649: 331-345.

Jansson, M., Bergstrom, A. K., Blomqvist, P., and Drakare, S., 2000: Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. *Ecology,* 81: 3250-3255.

Jeffrey, S. W. and Humphrey, G. F., 1975: New Spectrophotometric Equations for Determining Chlorophylls a, B, C1 and C2 in Higher Plants, Algae and Natural Phytoplankton. *Biochemie Und Physiologie Der Pflanzen,* 167: 191-194.

Jonsson, A., Meili, M., Bergstrom, A. K., and Jansson, M., 2001: Whole-lake mineralization of allochthonous and autochthonous organic carbon in a large humic lake (Ortrasket, N. Sweden). *Limnology and Oceanography,* 46: 1691-1700.

Judd, K. E., Crump, B. C., and Kling, G. W., 2006: Variation in dissolved organic matter controls bacterial production and community composition. *Ecology,* 87: 2068-2079.

Kaplan, L. A. and Bott, T. L., 1989: Diel Fluctuations in Bacterial-Activity on Streambed Substrata during Vernal Algal Blooms - Effects of Temperature, Water Chemistry, and Habitat. *Limnology and Oceanography,* 34: 718-733.

Karlsson, J., Jonsson, A., Meili, M., and Jansson, M., 2003: Control of zooplankton dependence on allochthonous organic carbon in humic and clear-water lakes in northern Sweden. *Limnology and Oceanography,* 48: 269-276.

Kritzberg, E. S., Cole, J. J., Pace, M. L., Graneli, W., and Bade, D. L., 2004: Autochthonous versus allochthonous carbon sources of bacteria: Results from whole-lake C-13 addition experiments. *Limnology and Oceanography,* 49: 588-596.

Leff, L. G. and Meyer, J. L., 1991: Biological Availability of Dissolved Organic Carbon Along the Ogeechee River. *Limnology and Oceanography,* 36: 315-323.

Lindau, C. W., Delaune, R. D., Patrick, W. H., and Lambremont, E. N., 1989: Assessment of Stable Nitrogen Isotopes in Fingerprinting Surface Water Inorganic Nitrogen Sources. *Water Air and Soil Pollution,* 48: 489-496.

Lock, M. A., Wallace, R. R., Costerton, J. W., Ventullo, R. M., and Charlton, S. E., 1984: River Epilithon - toward a Structural-Functional Model. *Oikos,* 42: 10-22.

Lyon, D. R. and Ziegler, S. E., 2009: Carbon cycling within epilithic biofilm communities across a nutrient gradient of headwater streams. *Limnology and Oceanography,* 54: 439-449.

Mayer, M. S. and Likens, G. E., 1987: The Importance of Algae in a Shaded Headwater Stream as Food for an Abundant Caddisfly (Trichoptera). *Journal of the North American Benthological Society,* 6: 262-269.

Molot, L. A. and Dillon, P. J., 1996: Storage of terrestrial carbon in boreal lake sediments and evasion to the atmosphere. *Global Biogeochemical Cycles,* 10: 483-492.

Mulholland, P. J., Steinman, A. D., Palumbo, A. V., Elwood, J. W., and Kirschtel, D. B., 1991: Role of Nutrient Cycling and Herbivory in Regulating Periphyton Communities in Laboratory Streams. *Ecology,* 72: 966-982.

Napolitano, G. E., 1999: Fatty acids as trophic and chemical markers in freshwater ecosystems. *In M. T. Arts and B. C. Wainman [eds.], Lipids in freshwater ecosystems. Springer*: 21-44.

Odum, H. T., 1956: Primary Production in Flowing Waters. *Limnology and Oceanography,* 1: 102-117.

Pace, M. L., Carpenter, S. R., Cole, J. J., Coloso, J. J., Kitchell, J. F., Hodgson, J. R., Middelburg, J. J., Preston, N. D., Solomon, C. T., and Weidel, B. C., 2007: Does terrestrial organic carbon subsidize the planktonic food web in a clear-water lake? *Limnology and Oceanography,* 52: 2177-2189.

Parker, P. L., Van Baalen, C., and Larry Maurer, L., 1967: Fatty Acids in Eleven Species of Blue-Green Algae: Geochemical Significance. *Science,* 155: 707-708.

Pastor, J., Solin, J., Bridgham, S. D., Updegraff, K., Harth, C., Weishampel, P., and Dewey, B., 2003: Global warming and the export of dissolved organic carbon from boreal peatlands. *Oikos,* 100: 380-386.

Post, W. M., Emanuel, W. R., Zinke, P. J., and Stangenberger, A. G., 1982: Soil Carbon Pools and World Life Zones. *Nature,* 298: 156-159.

Quideau, S. A., Chadwick, O. A., Benesi, A., Graham, R. C., and Anderson, M. A., 2001: A direct link between forest vegetation type and soil organic matter composition. *Geoderma,* 104: 41-60.

Robinson, C., Archer, S. D., and Williams, P. J. L., 1999: Microbial dynamics in coastal waters of East Antarctica: plankton production and respiration. *Marine Ecology-Progress Series,* 180: 23-36.

Romani, A. M. and Sabater, S., 1999: Effect of primary producers on the heterotrophic metabolism of a stream biofilm. *Freshwater Biology,* 41: 729-736.

Romani, A. M., Guasch, H., Munoz, I., Ruana, J., Vilalta, E., Schwartz, T., Emtiazi, F., and Sabater, S., 2004: Biofilm structure and function and possible implications for riverine DOC dynamics. *Microbial Ecology,* 47: 316-328.

Rounick, J. S. and Winterbourn, M. J., 1986: Stable Carbon Isotopes and Carbon Flow in Ecosystems. *Bioscience,* 36: 171-177.

Rowan, K. S., 1989: Photosynthetic pigments of algae. *Cambridge University Press (July 28 1989), Cambridge, UK*: 350 pages.

Savage, C., 2005: Tracing the influence of sewage nitrogen in a coastal ecosystem using stable nitrogen isotopes. *Ambio,* 34: 145-150.

Schiff, S., Aravena, R., Mewhinney, E., Elgood, R., Warner, B., Dillon, P., and Trumbore, S., 1998: Precambrian shield wetlands: Hydrologic control of the sources and export of dissolved organic matter. *Climatic Change,* 40: 167-188.

Schiff, S. L., Aravena, R., Trumbore, S. E., Hinton, M. J., Elgood, R., and Dillon, P. J., 1997: Export of DOC from forested catchments on the Precambrian Shield of Central Ontario: Clues from C-13 and C-14. *Biogeochemistry,* 36: 43-65.

Scott, J. T., Back, J. A., Taylo, J. M., and King, R. S., 2008: Does nutrient enrichment decouple algal-bacterial production in periphyton? *Journal of the North American Benthological Society,* 27: 332-344.

Sigee, D., 2006: Freshwater Microbiology. *John Wiley & Sons Inc., Hoboken, NJ, USA*: 524 p.

Sinsabaugh, R. L. and Foreman, C. M., 2003: Integrating Dissolved Organic Matter Metabolism and Microbial Diversity: An Overview of Conceptual Models. *In: Aquatic Ecosystems: Interactivity of Dissolved Organic Matter; Editors: Findlay, S. E. & Sinsabaugh, R. L.; Elsevier Science*: 425-454.

Tank, J. L. and Dodds, W. K., 2003: Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. *Freshwater Biology,* 48: 1031-1049.

Tranvik, L. J., 1988: Availability of Dissolved Organic Carbon for Planktonic Bacteria in Oligotrophic Lakes of Differing Humic Content. *Microbial Ecology,* 16: 311-322.

Tranvik, L. J., 1989: Bacterioplankton Growth, Grazing Mortality and Quantitative Relationship to Primary Production in a Humic and a Clearwater Lake. *Journal of Plankton Research,* 11: 985-1000.

Van den Meersche, K., Middelburg, J. J., Soetaert, K., van Rijswijk, P., Boschker, H. T. S., and Heip, C. H. R., 2004: Carbon-nitrogen coupling and algal-bacterial interactions during an experimental bloom: Modeling a C-13 tracer experiment. *Limnology and Oceanography,* 49: 862-878.

Vestal, J. R. and White, D. C., 1989: Lipid Analysis in Microbial Ecology - Quantitative Approaches to the Study of Microbial Communities. *Bioscience,* 39: 535-541.

Wayland, M. and Hobson, K. A., 2001: Stable carbon, nitrogen, and sulfur isotope ratios in riparian food webs on rivers receiving sewage and pulp-mill effluents. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* 79: 5-15.

Wetzel, R. G. and Likens, G. E., 2000: Limnological Analysis. *Springer; 3rd ed. edition*: 444 pp.

White, D. C., Davis, W. M., Nickels, J. S., King, J. D., and Bobbie, R. J., 1979: Determination of the Sedimentary Microbial Biomass by Extractable Lipid Phosphate. *Oecologia,* 40: 51-62.

White, D. C. and Ringelberg, D. B., 1998: Signature lipid biomarker analysis. *In: R. S. Burlage, R. Atlas, D. Stahl, G. Geesey and G. Sayler [eds.], Techniques in Microbial Ecology. Oxford University Press*: 255-272.

Winterbourn, M. J., 1990: Interactions among Nutrients, Algae and Invertebrates in a New-Zealand Mountain Stream. *Freshwater Biology,* 23: 463-474.

WQMA, 2012: Water Quality Monitoring Agreement. *Environment and Conservation, Government of Newfoundland and Labrador, Canada: http://www.env.gov.nl.ca/env/waterres/quality/background/wqma_sites.html*.

Summary and general conclusions

Recent recognition of the role of inland waters in the global carbon (C) cycle has fueled interest in understanding factors regulating watershed C cycling and its linkages to the terrestrial environment (Tranvik, 1992; Cole and Caraco, 2001; Cole et al., 2007; Guenet et al., 2010). Understanding the nutrient and organic matter dynamics of boreal headwater streams is essential to our ability to decipher biogeochemical linkages across such terrestrial-aquatic boundaries. This dissertation addressed questions regarding the role of photochemical processes and stream biofilms in the biogeochemical cycling of dissolved organic matter (DOM) in boreal headwater streams. This included the mineralization of DOM to CO_2 and the response of that mineralization to environmental change, such as nutrient enrichment and variation in watershed landcover and its connectivity with the aquatic landscape.

The mineralization or fate of DOM is likely regulated by DOM source or composition and nutrient availability in boreal landscapes (Berggren et al., 2007). Due to the prevalence of allochthonous organic matter in boreal headwater streams, environmental changes have great potential for altering DOM source and, by extension, its composition and cycling. From studies conducted in more temperate streams it is known that increases in labile C and nutrient availability can alter substrate sources for microorganisms. Since autotrophs and heterotrophs may rely less on regenerated nutrients and photosynthetically-derived C, respectively, such changes in C and nutrient availability can break the link between autotrophic and heterotrophic communities and affect processes such as respiration, nutrient release and retention (Bernhardt and Likens, 2002; Scott et al., 2008; Lyon and Ziegler, 2009). In low nutrient boreal streams, where organic forms of nitrogen (N) and phosphorus (P) may be the primary reservoirs of nutrients (Stepanauskas et al., 2000; Moore, 2009), biofilms may be able to extract energy from new labile C sources when inorganic nutrients are increased and use this energy to form biomass while inhibiting the mineralization of

extant DOM (Kaushal and Lewis, 2005). Furthermore, photomineralization and phototransformation of organic matter may regulate the cycling and fate of this key C reservoir due to the potential increase in the biological availability of C, N, and P (Bushaw et al., 1996; Moran and Zepp, 1997; Gao and Zepp, 1998; Tranvik et al., 1999), which may stimulate its use by microorganisms. This is particularly important for boreal environments, where most of the DOM is allochthonous, characterized by a high concentration of colored, humic substances, and thus expected to be highly photoreactive.

Due to their important role in the storage, transformation, and transport of terrestrial C and their influence on regional and global C balances, boreal headwater stream biogeochemical processes must be better understood in order to elucidate C and nutrient dynamics, particularly with regard to environmental change. However, such studies accomplished in boreal watersheds are limited. Most work investigating the photochemical transformation of DOM in the boreal aquatic environment has been conducted in ponds, lakes and reservoirs (Tranvik et al., 1999; Bertilsson and Tranvik, 2000; Vähätalo et al., 2003) due to the static nature and light availability in these ecosystems. Headwater streams may not represent hot spots for photochemical transformations in the aquatic landscape; however, they are locations that are most intimately associated with the landscape, and therefore often subjected to the elevated inputs of allochthonous DOM. Understanding whether headwater stream DOM varies in its potential to be phototransformed is critical as it represents an important source of DOM to these other downstream water bodies that receive more light (Franke et al., 2012). Further, most studies investigating the microbial cycling of DOM in boreal watersheds have focused on planktonic communities (Tranvik, 1988, 1989; Jansson et al., 2000; Anesio et al., 2005; Haukka et al., 2005). Even though the biogeochemical processing in boreal streams is largely driven by heterotrophic biofilm microbial communities due to high DOM concentrations, limited light availability and low rates of primary production in these ecosystems (Tranvik, 1988, 1989; Jansson et al., 2000; Jonsson et al., 2001; Agren et al., 2008), factors regulating heterotrophic biofilm

mineralization of organic matter within boreal headwater streams have been assessed to a more limited extent (Paul et al., 1991; Fischer et al., 2009; Franke et al., 2013a; Franke et al., 2013b) and remain poorly understood. Likewise, autotrophic-heterotrophic microbial linkages have been assessed to a very limited extent (Scott et al., 2008; Lyon and Ziegler, 2009; Risse-Buhl et al., 2012). No such studies have been conducted in boreal headwater streams with the exception of the investigations in this dissertation (Franke and Ziegler, 2013). However, given the allochthonous dominance of DOM in boreal headwater streams, autochthonous DOM sources may potentially stimulate allochthonous DOM use by stream biofilm communities, particularly under nutrient enrichment conditions, highlighting the potential for changes in C cycling with nutrient enrichment in boreal headwater streams.

Within the context of nutrient enrichment and variation in watershed landcover and its connectivity with the aquatic landscape, the main objectives of this dissertation were to:

(1) Assess the variation in the photochemical lability of DOM, defined as the susceptibility of DOM to photomineralization and photobleaching within a large boreal watershed, specifically whether (a) DOM photochemical lability varied with season, reaches within headwater streams and among headwater streams, and a large river reach, and whether (b) the photochemically labile fraction of DOM represents a significant proportion of DOM in terms of C, N, and P in a boreal watershed.

(2) Assess how heterotrophic biofilm mineralization of stream DOM (a) varies within a large boreal watershed and (b) is influenced by labile forms of C, N, and P.

(3) Investigate how nutrient enrichment and DOM character influences stream biofilm respiration and the factors limiting stream biofilm respiration.

(4) Investigate the importance of autotrophic-heterotrophic linkages in nutrient-impacted boreal streams, specifically whether (a) autotrophic C sources are important to supporting epilithic biofilms in nutrient-enriched boreal streams, and (b) whether autotrophic or labile C sources influence the use of allochthonous DOM.

Investigations included laboratory solar radiation exposure experiments, identification of DOM sources using stable isotopes, bioassay experiments to investigate heterotrophic biofilm mineralization of DOM, and *in situ* mesocosm experiments to track C and nutrient cycling through autotrophic and heterotrophic components of biofilm communities using stable isotopic labeling approaches. Results suggest boreal headwater streams in this study are poorly buffered against nutrient pollution given the lack of labile C sources with which to process N and P. Specifically, the following main conclusions can be summarized from this dissertation:

Variation in the photochemical lability of DOM suggests landscape and environmental change has the potential to alter the biogeochemical role photochemical transformations play in downstream portions of boreal watersheds. In particular, DOM from upstream portions of forested stream sites and wetland-influenced sites may be more photolabile relative to downstream portions and riverine DOM, suggesting potential losses in photolabile DOM downstream and in the lower reaches of the watershed (Franke et al., 2012). Further, photomineralization and phototransformation of DOM can result in the production of low molecular weight chromophoric dissolved organic matter and nutrients such as ammonium (Franke et al., 2012), which may potentially be important for the microbial processing of DOM in boreal watersheds.

Results from the substrate addition experiments in this thesis suggest catchment DOM source may play a significant role in regulating biofilm respiration rates in boreal headwater streams. Boreal stream biofilm respiration varied significantly with rates in pond-influenced sites and wetland-dominated catchments up to ten times those found for sites in coniferous forest catchments (Franke et al., 2013a). Direct additions of pond and peatland-derived DOM confirmed these site differences. Confirming similar differences in DOM reactivity typically assessed using planktonic freshwater microbial communities (Tranvik, 1988; Jansson et al., 2000), these results provide insight more relevant to microbial communities active in headwater streams. Further, results here suggest the mineralization, and therefore

fate, of more complex stream DOM in response to increased N and P likely differs markedly from more labile substrates analogous to autochthonous sources such as exudates. Manipulative N and P additions did not elicit an increase in DOM mineralization. Only the mineralization of the added glucose by the biofilms was increased with added N and P, signifying potential increases in N and P availability can increase microbial demand for labile C (Lutz et al., 2011; Franke et al., 2013a). Such labile C sources may stimulate the mineralization of stream DOM potentially depending upon the composition of stream DOM (Franke et al., 2013a). However, increased mineralization rates when labile C, N, and P were added in combination often coincided with a reduction in the mineralization of the extant stream DOM, suggesting the additions likely compete with stream DOM as a substrate for microbial mineralization and/or stimulate the incorporation rather than mineralization of the stream DOM (Franke et al., 2013a). These results provide a first look at potential effects of labile or autochthonous C sources on the fate of stream DOM potentially relevant in elevated N and P environments.

Findings from the same experiments conducted in historically nutrient-impacted boreal streams further highlight the potential importance of labile C sources for DOM mineralization by boreal stream biofilm communities. Results here suggest nutrient enrichment may increase the capacity of biofilms to use and mineralize labile C substrates and the processing of nutrients may become limited by labile C (Franke et al., 2013b). This labile C mineralization, however, may not effectively stimulate extant DOM mineralization, at least not through water column labile inputs. In fact, priming effects associated with labile C, N, and P additions were – again – not typically observed in these stream biofilms. Light-mediated activities, however, whether through autotrophic activity itself or the C released by this activity, may be important to supporting greater DOM mineralization by boreal stream biofilm communities, whether in form of newly fixed, older, or allochthonous organic matter (Franke and Ziegler, 2013). Biofilms are likely stimulated to incorporate autochthonous C

where primary production is likely highest, such as nutrient-enriched streams. This has implications for both C and nutrient cycling in boreal streams.

In order to further contribute to the understanding of microbial C and nutrient cycling in the boreal aquatic environment and the link between aquatic and terrestrial C cycles, as well as the responses of ecosystems to climatic or anthropogenic perturbations on the fate of boreal stream DOM, it would be helpful to investigate:

(1) The influence of photochemical transformations of DOM on DOM fate and nutrient cycling in boreal watersheds. This could be accomplished by combining photochemical exposure experiments with substrate addition experiments, i.e. investigating the mineralization of photochemical transformation products by boreal stream biofilm communities and the influence of these products on the mineralization of extant stream DOM and nutrient cycling. This study showed evidence for changes in the photolability of DOM from headwaters to larger reaches suggesting a loss of photolabile DOM. Such processes could control the fate of DOM, both through photomineralization and microbial mineralization, as well as nutrient cycling, and should be further investigated.

(2) Potential priming effects on the mineralization of stream DOM via more direct autotrophic inputs instead of glucose additions. This could be accomplished by following the influence of newly fixed or autochthonous C production on stream DOM mineralization. Results here suggest that light-mediated activities were important to supporting greater organic matter mineralization by these biofilms, however, such sources cannot be easily mimicked through labile C (i.e. glucose) additions and need more attention.

These proposed investigations should be conducted in a variety of aquatic ecosystems contrasting in watershed organic matter source and composition, nutrient levels, as well as stream site location to account for potential changes in landscape composition and nutrient availability, as well as effects on the downstream processing of DOM.

References

Agren, A., Berggren, M., Laudon, H., and Jansson, M., 2008: Terrestrial export of highly bioavailable carbon from small boreal catchments in spring floods. *Freshwater Biology,* 53: 964-972.

Anesio, A. M., Graneli, W., Aiken, G. R., Kieber, D. J., and Mopper, K., 2005: Effect of humic substance photodegradation on bacterial growth and respiration in lake water. *Applied and Environmental Microbiology,* 71: 6267-6275.

Bernhardt, E. S. and Likens, G. E., 2002: Dissolved organic carbon enrichment alters nitrogen dynamics in a forest stream. *Ecology,* 83: 1689-1700.

Bertilsson, S. and Tranvik, L. J., 2000: Photochemical transformation of dissolved organic matter in lakes. *Limnology and Oceanography,* 45: 753-762.

Bushaw, K. L., Zepp, R. G., Tarr, M. A., SchulzJander, D., Bourbonniere, R. A., Hodson, R. E., Miller, W. L., Bronk, D. A., and Moran, M. A., 1996: Photochemical release of biologically available nitrogen from aquatic dissolved organic matter. *Nature,* 381: 404-407.

Cole, J. J. and Caraco, N. F., 2001: Carbon in catchments: connecting terrestrial carbon losses with aquatic metabolism. *Marine and Freshwater Research,* 52: 101-110.

Cole, J. J., Prairie, Y. T., Caraco, N. F., McDowell, W. H., Tranvik, L. J., Striegl, R. G., Duarte, C. M., Kortelainen, P., Downing, J. A., Middelburg, J. J., and Melack, J., 2007: Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems,* 10: 171-184.

Fischer, H., Bergfur, J., Goedkoop, W., and Tranvik, L., 2009: Microbial leaf degraders in boreal streams: bringing together stochastic and deterministic regulators of community composition. *Freshwater Biology,* 54: 2276-2289.

Franke, D., Hamilton, M. W., and Ziegler, S. E., 2012: Variation in the photochemical lability of dissolved organic matter in a large boreal watershed. *Aquatic Sciences - Research Across Boundaries,* 74: 751-768.

Franke, D., Bonnell, E. J., and Ziegler, S. E., 2013a: Mineralization of dissolved organic matter by heterotrophic stream biofilm communities in a large boreal catchment. *Accepted for publication in Freshwater Biology*.

Franke, D., Lyon, D. R., and Ziegler, S. E., 2013b: The impact of nutrient enrichment on dissolved organic carbon mineralization by headwater stream biofilm communities.

Franke, D. and Ziegler, S. E., 2013: Carbon cycling and autotrophic-heterotrophic linkages within boreal stream biofilm communities.

Gao, H. Z. and Zepp, R. G., 1998: Factors influencing photoreactions of dissolved organic matter in a coastal river of the southeastern United States. *Environmental Science & Technology,* 32: 2940-2946.

Guenet, B., Danger, M., Abbadie, L., and Lacroix, G., 2010: Priming effect: bridging the gap between terrestrial and aquatic ecology. *Ecology,* 91: 2850-2861.

Haukka, K., Heikkinen, E., Kairesalo, T., Karjalainen, H., and Sivonen, K., 2005: Effect of humic material on the bacterioplankton community composition in boreal lakes and mesocosms. *Environmental Microbiology,* 7: 620-630.

Jansson, M., Bergstrom, A. K., Blomqvist, P., and Drakare, S., 2000: Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. *Ecology,* 81: 3250-3255.

Jonsson, A., Meili, M., Bergstrom, A. K., and Jansson, M., 2001: Whole-lake mineralization of allochthonous and autochthonous organic carbon in a large humic lake (Ortrasket, N. Sweden). *Limnology and Oceanography,* 46: 1691-1700.

Kaushal, S. S. and Lewis, W. M., 2005: Fate and transport of organic nitrogen in minimally disturbed montane streams of Colorado, USA. *Biogeochemistry,* 74: 303-321.

Lutz, B. D., Bernhardt, E. S., Roberts, B. J., and Mulholland, P. J., 2011: Examining the coupling of carbon and nitrogen cycles in Appalachian streams: the role of dissolved organic nitrogen. *Ecology,* 92: 720-732.

Lyon, D. R. and Ziegler, S. E., 2009: Carbon cycling within epilithic biofilm communities across a nutrient gradient of headwater streams. *Limnology and Oceanography,* 54: 439-449.

Moore, T. R., 2009: Dissolved Organic Carbon Production and Transport in Canadian Peatlands. *In: Carbon Cycling in Northern Peatlands, Geophysical Monograph Series 184, American Geophysical Union 10.1029/2008GM000816*: 229-236.

Moran, M. A. and Zepp, R. G., 1997: Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. *Limnology and Oceanography,* 42: 1307-1316.

Neff, J. C., Chapin, F. S., and Vitousek, P. M., 2003: Breaks in the cycle: dissolved organic nitrogen in terrestrial ecosystems. *Frontiers in Ecology and the Environment,* 1: 205-211.

Paul, B. J., Duthie, H. C., and Taylor, W. D., 1991: Nutrient Cycling by Biofilms in Running Waters of Differing Nutrient Status. *Journal of the North American Benthological Society,* 10: 31-41.

Risse-Buhl, U., Trefzger, N., Seifert, A. G., Schonborn, W., Gleixner, G., and Kusel, K., 2012: Tracking the autochthonous carbon transfer in stream biofilm food webs. *Fems Microbiology Ecology,* 79: 118-131.

Scott, J. T., Back, J. A., Taylo, J. M., and King, R. S., 2008: Does nutrient enrichment decouple algal-bacterial production in periphyton? *Journal of the North American Benthological Society,* 27: 332-344.

Stepanauskas, R., Laudon, H., and Jorgensen, N. O. G., 2000: High DON bioavailability in boreal streams during a spring flood. *Limnology and Oceanography,* 45: 1298-1307.

Tranvik, L. J., 1988: Availability of Dissolved Organic Carbon for Planktonic Bacteria in Oligotrophic Lakes of Differing Humic Content. *Microbial Ecology,* 16: 311-322.

Tranvik, L. J., 1989: Bacterioplankton Growth, Grazing Mortality and Quantitative Relationship to Primary Production in a Humic and a Clearwater Lake. *Journal of Plankton Research,* 11: 985-1000.

Tranvik, L. J., 1992: Allochthonous Dissolved Organic Matter as an Energy Source for Pelagic Bacteria and the Concept of the Microbial Loop. *Hydrobiologia,* 229: 107-114.

Tranvik, L. J., Olofsson, H., and Bertilsson, S., 1999: Photochemical Effects on Bacterial Degradation of Dissolved Organic Matter in Lake Water. *Microbial Biosystems: New Frontiers. Proceedings of the 8th International Symposium on Microbial Ecology. Atlantic Canada Society for Microbial Ecology, Halifax, Canada, 1999.*

Vähätalo, A. V., Salonen, K., Munster, U., Jarvinen, M., and Wetzel, R. G., 2003: Photochemical transformation of allochthonous organic matter provides bioavailable nutrients in a humic lake. *Archiv Fur Hydrobiologie,* 156: 287-314.

A1: Appendix for Chapter 1

Variation in the photochemical lability of dissolved organic matter in a large boreal watershed

A1.1 Dissolved organic carbon and absorption loss following light exposure

Table A1.1: Percent dissolved organic carbon (DOC) loss following light exposure at six study sites within the Humber River Basin, including a *Sphagnum* peatland bog, an upstream site (BF-1) immediately draining the bog, and a downstream site (BF-2) situated in a forested reach of the same stream, a wetland-dominated catchment (BFB), headwater streams draining primarily coniferous forest (HB), and the inlet and outlet of a large lake (DL) located in the lower reach of the Humber River. Losses in DOC and absorption were measured as the percent change in DOC concentration. Values are provided as averages (n=5) ± one standard deviation. NS: Changes were not significant.

Site	Spring	Summer	Fall
Bog	-	2.78 ± 1.08	-
BF-1	-	4.68 ± 0.43	-
BF-2	-	9.65 ± 2.70	-
BFB-1	8.32 ± 1.41	5.22 ± 0.05	5.74 ± 1.35
BFB-2	4.90 ± 0.27	6.30 ± 1.50	5.73 ± 0.06
HB-1	6.91 ± 0.18	3.92 ± 0.33	7.24 ± 0.94
HB-2	3.22 ± 0.72	NS	NS
DL-1	5.51 ± 1.14	NS	2.18 ± 0.29
DL-2	4.96 ± 0.19	NS	2.43 ± 0.11

Table A1.2: Dissolved organic carbon (DOC) loss (μM C) following light exposure at the peatland bog, Bog Feeder (BF), Big Falls Brook (BFB), Hughes Brook (HB), and the inlet and outlet of Deer lake (DL) located in the lower reach of the Humber River. Values are provided as averages (n=5) ± one standard deviation. NS: Changes were not significant.

Site	Spring	Summer	Fall
Bog	-	44.57 ± 26.87	-
BF-1	-	37.72 ± 26.14	-
BF-2	-	148.33 ± 107.02	-
BFB-1	65.38 ± 25.21	68.83 ± 29.61	51.16 ± 24.43
BFB-2	28.76 ± 7.99	57.12 ± 27.22	41.76 ± 5.58
HB-1	45.13 ± 19.14	27.05 ± 17.97	68.76 ± 18.67
HB-2	12.11 ± 4.73	NS	NS
DL-1	30.68 ± 14.81	NS	14.01 ± 4.02
DL-2	20.95 ± 9.58	NS	10.47 ± 3.88

Table A1.3: Percent absorption loss following light exposure at six study sites within the Humber River Basin, including a *Sphagnum* peatland bog, an upstream site (BF-1) immediately draining the bog, and a downstream site (BF-2) situated in a forested reach of the same stream, a wetland-dominated catchment (BFB), headwater streams draining primarily coniferous forest (HB), and the inlet and outlet of a large lake (DL) located in the lower reach of the Humber River. Losses in absorption were measured as the percent change in absorption at a wavelength of 350 nm. Values are provided as averages (n=5) ± one standard deviation. NS: Not significant.

Site	Spring	Summer	Fall
Bog	-	12.61 ± 5.44	-
BF-1	-	15.46 ± 1.92	-
BF-2	-	21.89 ± 9.40	-
BFB-1	22.39 ± 1.43	22.17 ± 1.78	21.40 ± 2.63
BFB-2	19.69 ± 3.15	25.38 ± 2.51	24.81 ± 2.71
HB-1	35.67 ± 4.17	26.21 ± 2.43	19.58 ± 2.01
HB-2	30.67 ± 3.64	34.86 ± 3.16	30.97 ± 1.05
DL-1	13.00 ± 3.84	NS	13.97 ± 2.29
DL-2	36.22 ± 17.70	26.58 ± 5.31	22.88 ± 4.95

Table A1.4: Average decrease in dissolved organic matter (DOM) absorption (Δa_{avg}) in comparison to absorption losses at a wavelength of 254nm (Δa_{254}) and 350nm (Δa_{350}); changes in absorbance ratios a_{254}:a_{350} (Δa_{254}:a_{350} = a_{254}:a_{350} final - a_{254}:a_{350} initial) and changes in spectral slope ratios (ΔS_R=$S_{275-295}$:$S_{350-400}$ final - $S_{275-295}$:$S_{350-400}$ initial). Averages and standard deviations are shown for 5 replicates. BD = below detection.

Site	Season	Δa_{avg} (m^{-1})	Δa_{254} (m^{-1})	Δa_{350} (m^{-1})	Δa_{254}:a_{350}	ΔS_R
Bog	Summer	2.9 ± 1.6	7.1 ± 3.2	4.1 ± 1.7	0.3 ± 0.06	BD
BF-1	Summer	2.3 ± 0.5	BD	4.4 ± 0.5	0.6 ± 0.03	0.14 ± 0.04
BF-2	Summer	5.3 ± 2.5	BD	7.9 ± 3.4	0.7 ± 0.16	0.11 ± 0.03
BFB-1	Spring	3.3 ± 0.3	11.6 ± 0.9	5.3 ± 0.3	0.5 ± 0.02	0.19 ± 0.03
BFB-1	Summer	4.1 ± 0.5	10.4 ± 1.1	6.7 ± 0.5	0.7 ± 0.02	0.15 ± 0.03
BFB-1	Fall	2.4 ± 0.5	8.3 ± 1.1	4.3 ± 0.5	0.6 ± 0.03	0.23 ± 0.04
BFB-2	Spring	2.4 ± 0.5	5.8 ± 0.6	3.7 ± 0.6	0.5 ± 0.04	0.15 ± 0.05
BFB-2	Summer	2.5 ± 0.3	7.4 ± 0.6	4.1 ± 0.4	0.9 ± 0.03	0.12 ± 0.03
BFB-2	Fall	2.9 ± 0.3	7.2 ± 0.9	4.5 ± 0.5	0.8 ± 0.04	0.13 ± 0.02
HB-1	Spring	9.0 ± 1.4	10.6 ± 2.1	8.0 ± 0.9	1.2 ± 0.07	0.13 ± 0.04
HB-1	Summer	2.4 ± 0.3	6.6 ± 0.7	3.9 ± 0.4	1.0 ± 0.03	0.12 ± 0.03
HB-1	Fall	2.3 ± 0.5	8.4 ± 2.1	6.4 ± 0.7	0.6 ± 0.03	BD
HB-2	Spring	2.2 ± 0.3	4.2 ± 1.2	3.6 ± 0.4	1.0 ± 0.06	0.11 ± 0.03
HB-2	Summer	2.1 ± 0.2	5.4 ± 1.0	3.4 ± 0.3	1.3 ± 0.04	0.13 ± 0.04
HB-2	Fall	4.2 ± 0.1	9.0 ± 0.4	6.7 ± 0.0	1.0 ± 0.01	0.22 ± 0.01
DL-1	Spring	3.1 ± 1.0	2.8 ± 1.3	2.8 ± 0.8	0.4 ± 0.04	0.14 ± 0.04
DL-1	Summer	BD	BD	BD	BD	BD
DL-1	Fall	2.2 ± 0.5	3.7 ± 1.9	3.7 ± 0.6	0.4 ± 0.03	0.13 ± 0.02
DL-2	Spring	3.5 ± 2.0	5.6 ± 2.4	4.7 ± 2.3	1.4 ± 0.28	0.25 ± 0.16
DL-2	Summer	3.6 ± 0.7	4.3 ± 1.1	3.1 ± 0.6	0.9 ± 0.06	BD
DL-2	Fall	5.1 ± 1.3	6.7 ± 2.0	3.9 ± 0.8	0.6 ± 0.07	BD

A1.2 Changes in $\delta^{13}C_{DOC}$ following light exposure

Table A1.5: Seasonal initial stable carbon isotope composition ($\delta^{13}C_{DOC\text{-}initial}$) of the dissolved organic matter (DOM) in samples collected at Bog Feeder (BF-1, BF-2), Big Falls Brook (BFB-1, BFB-2), Hughes Brook (HB-1, HB-2), and Deer Lake (DL-1, DL-2), and $\delta^{13}C_{DOC}$ of the photomineralized DOM ($\delta^{13}C_{photomin}$) following light exposure in spring, summer, and fall samples. Values correspond to averages (n=5) ± one standard deviation. NS: Changes were not significant.

Site	Seasonal $\delta^{13}C_{DOC\text{-}initial}$	$\delta^{13}C_{photomin}$ Spring	$\delta^{13}C_{photomin}$ Summer	$\delta^{13}C_{photomin}$ Fall
BF-1	-28.0 ± 0.1	-	-35.6 ± 4.2	-
BF-2	-27.9 ± 0.2	-	-30.3 ± 1.2	-
BFB-1	-27.8 ± 0.1	-33.4 ± 3.5	-30.4 ± 2.3	-30.8 ± 1.3
BFB-2	-28.2 ± 0.3	-41.6 ± 4.0	-33.0 ± 3.2	-34.4 ± 2.9
HB-1	-27.7 ± 0.4	-35.9 ± 3.6	-37.6 ± 7.3	-30.3 ± 1.6
HB-2	-27.7 ± 0.8	-39.0 ± 6.7	NS	NS
DL-1	-27.5 ± 0.1	-40.5 ± 8.7	NS	-43.2 ± 1.8
DL-2	-27.4 ± 0.3	-32.8 ± 4.0	NS	-40.5 ± 6.6

A1.3 Minimum detectable differences for changes in DIC and DOC concentrations and $\delta^{13}C$

Table A1.6: Limit of detection or minimum detectable differences determined for changes in dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) concentrations as well as DOC isotopes ($\delta^{13}C_{DOC}$). Values were calculated between initial and final samples. The method employed is provided in the "Statistics" section in chapter 1.

Site	Season	DIC	DOC	$\delta^{13}C_{DOC}$
BFB-1	Spring	7.07	20.97	0.20
	Summer	8.04	18.75	0.19
	Fall	7.79	20.45	0.12
BFB-2	Spring	8.29	6.65	0.23
	Summer	14.10	22.64	0.28
	Fall	5.16	7.08	0.37
HB-1	Spring	27.81	17.79	0.19
	Summer	16.13	14.94	0.25
	Fall	17.24	27.38	0.17
HB-2	Spring	53.59	5.31	0.27
	Summer	56.64	11.13	0.40
	Fall	52.82	39.25	0.24
DL-1	Spring	5.22	12.32	0.19
	Summer	8.19	5.23	0.15
	Fall	16.31	6.75	0.21
DL-2	Spring	7.09	7.97	0.27
	Summer	13.14	3.87	0.12
	Fall	5.46	4.47	0.11

A1.4 Freezing effect on dissolved organic carbon concentrations

Table A1.7: Dissolved organic carbon (DOC) concentrations (mg C L^{-1}) before and after freezing to test the effect of freezing on DOC concentrations in samples collected at one study site within each location (Bog, Deer Lake (DL), Hughes Brook (HB), and Big Falls Brook (BFB)) in summer 2010. Values correspond to averages of 3 replicates ± standard deviation. NS: Not significant.

	Bog	**DL**	**HB**	**BFB**
Before freezing	21.18 ± 0.16	4.65 ± 0.10	7.82 ± 0.63	10.48 ± 0.12
After freezing	21.59 ± 0.15	4.58 ± 0.11	6.89 ± 0.52	10.42 ± 0.11
Effect	NS	NS	10.2 %	NS

Table A1.8: Comparison of absorption coefficients normalized to dissolved organic carbon concentration (L (mmol C)$^{-1}$ m^{-1}) to test the effect of freezing on specific UV absorption (SUVA) in samples collected at each site along BFB, HB, and DL in fall, as well as BF in summer. Values correspond to averages of 3 replicates ± standard deviation. NS: Not significant.

Site	**Season**	**Field measurements**	**Frozen samples**	**Effect**
HB-1	Fall	34.3 ± 0.2	34.3 ± 0.2	NS
HB-2	Fall	75.8 ± 0.8	34.1 ± 0.7	55.0%
DL-1	Fall	39.2 ± 0.3	41.2 ± 0.5	NS
DL-2	Fall	38.8 ± 0.3	39.7 ± 0.5	NS
BFB-1	Fall	23.1 ± 0.2	22.5 ± 0.7	NS
BF-1	Summer	38.3 ± 0.4	34.9 ± 0.9	8.9%
BF-2	Summer	37.6 ± 0.3	37.5 ± 2.2	NS

A2: Appendix for Chapter 2

Mineralization of dissolved organic matter by heterotrophic stream biofilm communities in a large boreal catchment

A2.1 Water quality parameters and dissolved organic matter composition

Table A2.1: Dissolved organic carbon normalized absorption coefficients a_{254}^* and a_{350}^*, absorption ratios a_{254}:a_{350}, spectral slopes (S) for the wavelength ranges 275 to 295 nm and 350 to 400 nm, carbohydrate content (CHO) in glucose carbon equivalents as percent of total stream dissolved organic carbon, aromatic to aliphatic carbon ratios (A_R:A_L) as determined via CP-MAS ^{13}C-NMR, total inorganic carbon (TIC) concentration and discharge**. Values correspond to averages of five replicates derived from initial bottles used in the experiments.

Site	Season	a_{254}^*	a_{350}^*	a_{254}:a_{350}	$S_{275\text{-}295}$	$S_{350\text{-}400}$	CHO	A_R:A_L	TIC	Discharge
		L mmol C^{-1} m^{-1}			μm^{-1}	μm^{-1}	%		µM C	m^3 s^{-1}
CF-1	Summer 2009	136	35	3.9	6.2	6.7	14.9	0.25	360	0.36
CF-1	Summer 2010	-	-	-	-	-	-	-	781	-
CF-1	Autumn 2009	148	36	4.1	6.4	7.6	7.7	0.27	300	0.26
CF-2	Summer 2009	124	25	5.0	7.7	8.6	28.5	0.21	380	1.06
CF-2	Summer 2010	-	-	-	-	-	-	-	881	0.07
CF-2	Autumn 2009	132	30	4.4	6.6	7.9	5.7	0.32	354	0.64
BFB-1	Summer 2008	84	21	4.0	6.3	7.7	-	-	388	-
BFB-1	Autumn 2008	95	23	4.2	6.6	8.2	-	-	233	-
BFB-2	Summer 2008	77	18	4.3	6.8	7.9	-	-	814	-
BFB-2	Autumn 2008	96	25	3.9	6.2	7.8	-	-	390	-
PB-1	Summer 2009	102	22	4.6	7.3	6.9	19.3	0.31	829	0.06
PB-1	Summer 2010	81	18	4.5	7.2	5.4	-	0.22	1179	0.20
PB-1	Autumn 2009	136	32	4.3	6.7	6.9	19.0	0.18	530	0.16
PB-2	Summer 2009	91	19	4.8	7.3	7.3	8.0	0.18	712	0.17
PB-2	Summer 2010	99	23	4.3	6.9	5.9	-	0.41	676	0.20
PB-2	Autumn 2009	135	26	5.2	7.9	8.2	8.5	0.23	303	1.12
PB-3	Summer 2010	81	18	4.5	6.7	5.8	-	0.31	454	-
GB-1	Autumn 2009	136	29	4.7	7.4	7.7	10.2	0.11	184	0.30
GB-2	Autumn 2009	138	32	4.3	6.7	7.9	10.2	0.42	221	0.45

** Stream discharge measurements were conducted using a Swoffer flow meter (Model 2100; Swoffer Instruments Inc., Seattle, WA, USA) at each study site. Total discharge was calculated by measuring flow velocity at 60 % depth and cross-sectional area at regular intervals across each stream site. The number of intervals (9 – 25) varied with the width of each stream and flow conditions. Additionally, water level was monitored at PB, CF and GB using *in situ* Onset HOBO Water Level Loggers (Bourne, MA, USA).

A2.2 Tile biofilm characteristics

Table A2.2: Description of the tile (10 x 47 x 5 mm) biofilm communities including stable carbon isotope composition ($\delta^{13}C$; ‰), total carbon (C; µM C per tile) and nitrogen (N; µM N per tile) content and biofilm C:N ratios. Values correspond to averages of five replicates derived from initial bottles used in the experiments ± one standard deviation.

Site	Season	$\delta^{13}C$	C biomass	N biomass	Biofilm C:N
CF-1	Summer 2009	-30.8 ± 0.4	45 ± 0.1	5 ± 0.12	8 ± 0.2
CF-1	Summer 2010	-28.8 ± 0.3	28 ± 1.1	3 ± 0.02	9 ± 0.4
CF-1	Autumn 2009	-29.9 ± 0.4	41 ± 1.1	4 ± 0.02	11 ± 0.3
CF-2	Summer 2009	-28.7 ± 0.3	38 ± 0.9	3 ± 0.02	12 ± 0.2
CF-2	Summer 2010	-29.8 ± 0.3	29 ± 0.3	4 ± 0.04	7 ± 0.1
CF-2	Autumn 2009	-29.4 ± 0.3	119 ± 1.2	12 ± 0.11	10 ± 0.2
BFB-1	Summer 2008	-28.7 ± 0.9	31 ± 1.1	4 ± 0.02	8 ± 0.4
BFB-1	Autumn 2008	-28.3 ± 0.9	59 ± 0.6	5 ± 0.06	11 ± 0.3
BFB-2	Summer 2008	-27.9 ± 0.5	30 ± 1.3	3 ± 0.02	9 ± 0.3
BFB-2	Autumn 2008	-27.1 ± 0.6	37 ± 2.1	3 ± 0.06	13 ± 0.1
PB-1	Summer 2009	-25.8 ± 0.7	39 ± 0.8	6 ± 0.05	6 ± 0.4
PB-1	Summer 2010	-28.5 ± 0.3	31 ± 0.6	5 ± 0.05	6 ± 0.3
PB-1	Autumn 2009	-29.7 ± 0.7	32 ± 0.2	3 ± 0.03	12 ± 0.1
PB-2	Summer 2009	-29.0 ± 0.1	39 ± 0.1	6 ± 0.02	6 ± 0.3
PB-2	Summer 2010	-28.4 ± 0.1	30 ± 0.2	4 ± 0.05	7 ± 0.2
PB-2	Autumn 2009	-28.9 ± 0.6	26 ± 1.2	2 ± 0.02	11 ± 0.1
PB-3	Summer 2010	-28.5 ± 0.1	42 ± 0.1	7 ± 0.05	6 ± 0.1
GB-1	Autumn 2009	-31.0 ± 0.6	34 ± 0.7	3 ± 0.04	10 ± 0.1
GB-2	Autumn 2009	-29.4 ± 0.6	32 ± 0.2	5 ± 0.06	7 ± 0.1

A2.3 Stream site epilithic biofilm characteristics

Table A2.3: Seasonal description of the stream biofilm communities on site, particularly stable carbon ($\delta^{13}C$; ‰) and nitrogen ($\delta^{15}N$; ‰) isotope composition, carbon (C; Wt. %) and nitrogen (N; Wt. %) content, biofilm C:N ratios, and chlorophyll (Chl; µg chl mg^{-1} C^{-1}) a, b, and c content. Values correspond to averages of 3 replicates. Biofilm samples were not available for CF-1 due a lack of accessible epilithic substrata at the study site.

Site	Season	$\delta^{13}C$	C	$\delta^{15}N$	N	Biofilm C:N	Chl a	Chl b	Chl c
CF-2	Summer 2009	-26.8	46.8	6.8	11.7	4.7	0.67	0.15	0.16
CF-2	Summer 2010	-26.8	7.1	3.4	0.7	11.8	1.26	0.35	0.17
CF-2	Autumn 2009	-27.4	2.6	5.7	0.2	13.0	12.66	3.47	3.87
BFB-1	Summer 2008	-27.0	6.1	5.4	0.6	11.3	6.38	1.66	1.78
BFB-1	Autumn 2008	-28.1	5.7	2.6	0.5	12.3	0.13	0.02	0.03
BFB-2	Summer 2008	-27.9	11.5	6.7	0.3	12.8	6.21	1.69	1.85
BFB-2	Autumn 2008	-27.0	10.9	5.7	0.3	12.9	0.11	0.02	0.05
PB-1	Summer 2009	-25.6	48.7	7.8	12.3	4.6	0.55	0.14	0.07
PB-1	Summer 2010	-32.3	22.4	2.1	2.2	11.7	2.90	0.92	0.17
PB-1	Autumn 2009	-30.7	17.6	1.7	1.7	11.8	0.91	0.34	0.21
PB-2	Summer 2009	-27.1	45.3	6.4	11.0	4.8	0.61	0.04	0.02
PB-2	Summer 2010	-25.0	4.0	3.3	0.3	13.9	2.39	0.86	0.07
PB-2	Autumn 2009	-28.1	4.8	2.0	0.5	12.1	2.78	1.04	0.58
PB-3	Summer 2010	-24.1	1.7	4.1	0.2	13.8	1.14	0.08	0.02
GB-1	Autumn 2009	-27.6	49.2	5.5	10.8	5.3	0.41	0.15	0.12
GB-2	Autumn 2009	-31.7	42.5	7.8	8.5	5.9	1.35	0.29	0.20

Site epilithic biofilm chlorophyll a represented between 62.7 and 85.0 % of the total chlorophyll and ranged in concentration from 0.1 to 12.7 µg chl a per mg biofilm C, while chlorophyll b concentration varied from 0.02 to 3.5 µg chl per mg C (6.2 to 25.9 %), and chlorophyll c varied from 0.02 to 3.9 µg chl per mg C (1.9 to 18.7 %). Chlorophyll content did not correlate to the epilithic biofilm carbon content (chl a: $p = 0.10$, chl b: $p = 0.08$, chl c: $p = 0.16$), nitrogen content (chl a: $p = 0.13$, chl b: $p = 0.10$, chl c: $p = 0.20$), biofilm C:N (chl a: $p = 0.14$, chl b: $p = 0.12$, chl c: $p = 0.24$), $\delta^{13}C$ (chl a: $p = 0.96$, chl b: $p = 0.85$, chl c: $p = 0.97$), or $\delta^{15}N$ (chl a: $p = 0.72$, chl b: $p = 0.93$, chl c: $p = 0.49$).

The C:N varied between the tile biofilms (6.1 – 13.0) and the epilithic stream biofilms (4.7 – 13.9). Tile biofilm C:N ratios were positively correlated to stream C:N ($p < 0.01$, $R^2 = 0.46$), but not correlated to stream dissolved organic carbon (DOC; $p = 0.09$), dissolved organic nitrogen (DON; $p = 0.87$), and dissolved inorganic nitrogen (DIN; $p = 0.92$). Stream site epilithic biofilm C:N was not correlated to stream C:N ($p = 0.97$), DOC ($p = 0.55$), DON ($p = 0.39$), and DIN (0.48).

A2.4 DOC mineralization within the Humber River Basin

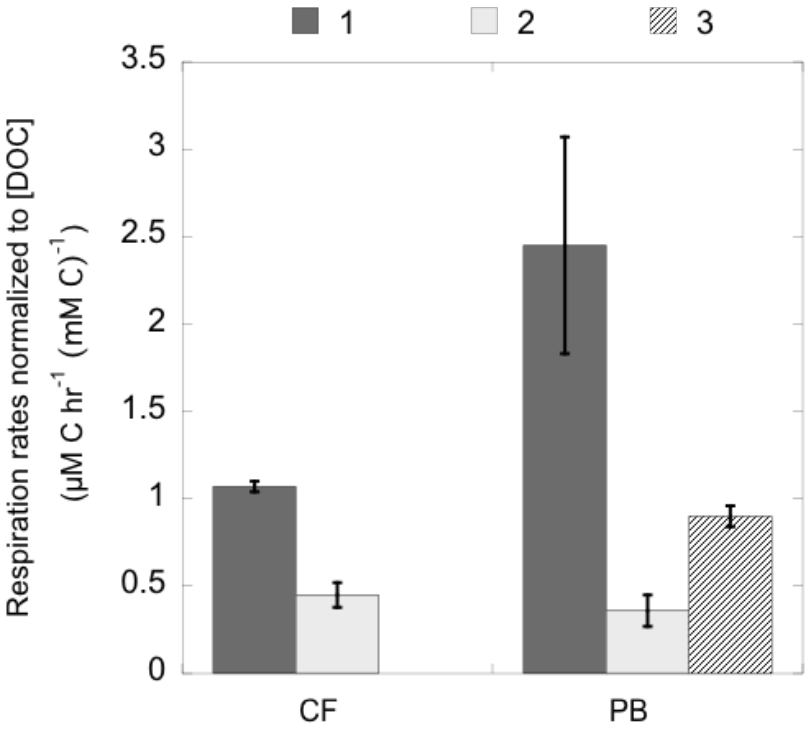

Figure A2.1: Respiration rates normalized to initial dissolved organic carbon (DOC) concentration* at Crooked Feeder (CF-1 and CF-2) and Pynn's Brook (PB-1, PB-2, and PB-3) in summer 2010, grouped in this figure based upon similar incubation temperature among sites (18.0 – 18.2°C). Values correspond to five replicates ± one standard deviation.

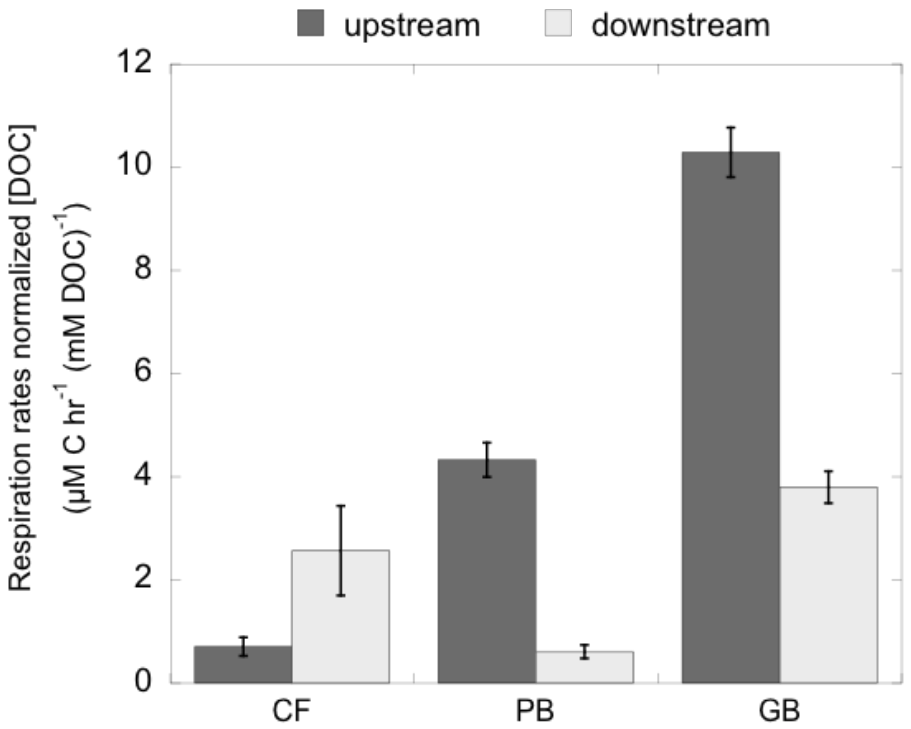

Figure A2.2: Respiration rates normalized to initial dissolved organic carbon (DOC) concentration* at Crooked Feeder (CF), Pynn's Brook (PB), and Gillams Brook (GB) in autumn 2009, grouped in this figure based upon similar incubation temperature among sites (4.0 – 5.5°C). Values correspond to five replicates ± one standard deviation.

*Biofilm respiration was regulated by DOC concentration ($p = 0.03$, $R^2 = 0.10$), however, the absolute biofilm respiration rates showed the same pattern as rates of respiration normalized to DOC concentration indicating that composition of the DOM rather than its concentration was likely more important. Also, congruent with the lack of a significant (tile) biomass effect on absolute respiration rates (C content: $p = 0.79$; N content: $p = 0.93$), biofilm respiration rates normalized to biomass followed the trend in the absolute respiration rates. The fact that respiration was not regulated by biomass suggests that perhaps a large portion of what was measured as attached biomass was not necessarily related to active biomass, which is not surprising given that biofilms form extrapolymeric substances that would appear as carbon biomass but may not correspond to increases in respiration.

A2.5 Tukey Honestly Significance Tests

Tukey's Honestly Significant Difference tests (family wise error of $\alpha = 0.05$; AnalystSoft Inc., StatPlus:mac - Version 2009) were conducted as post-hoc tests of all possible pair-wise effect comparisons in order to determine which level of each effect (tested with Generalized Linear Models, see chapter 2) was in fact different from which others.

Table A2.4a: Interactions between CNP treatments and remaining substrate addition treatments, reported as p-values derived from Tukey's Honest Significant Difference (THSD) tests. THSD tests were conducted when a CNP treatment effect was observed (based on t-tests). The data table is organized based on season and description in text; p-values > 0.05 are considered as non-significant.

Site	Season	C	N	P	NP	Bog	Bog-NP	Pond	Pond-NP
CF-1	Summer 2009	0.003	0.011	0.003	0.003	-	-	-	-
CF-2	Summer 2009	< 0.001	< 0.001	< 0.001	< 0.001	-	-	-	-
BFB-1	Summer 2009	0.276	0.012	0.036	-	-	-	-	-
BFB-2	Summer 2009	0.807	0.022	0.013	-	-	-	-	-
PB-2	Summer 2009	0.122	0.019	0.024	0.008	-	-	-	-
CF-1	Summer 2010	< 0.001	-	-	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
CF-2	Summer 2010	< 0.001	-	-	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
PB-2	Summer 2010	0.026	-	-	0.014	0.061	0.324	0.026	0.264
PB-3	Summer 2010	0.011	-	-	0.002	0.001	0.066	0.007	0.772
PB-1	Autumn 2009	0.405	0.405	0.446	0.366	-	-	-	-
PB-2	Autumn 2009	1.000	1.000	0.992	1.000	-	-	-	-

Table A2.4b: Interactions between C-addition treatments and remaining substrate addition treatments, reported as p-values derived from Tukey's Honest Significant Difference (THSD) tests. THSD tests were conducted when a C treatment effect was observed (based on t-tests). The data table is organized based on season and description in text; p-values > 0.05 are considered as non-significant.

Site	Season	CNP	N	P	NP	Bog	Bog-NP	Pond	Pond-NP
CF-2	Summer 2009	< 0.001	0.014	0.043	0.030	-	-	-	-
BFB-2	Summer 2009	0.807	0.127	0.077	-	-	-	-	-
CF-1	Summer 2010	< 0.001	-	-	0.710	0.988	1.000	0.946	0.946
CF-2	Summer 2010	< 0.001	-	-	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
PB-3	Summer 2010	0.011	-	-	0.993	0.980	0.993	1.000	0.282
PB-2	Autumn 2009	1.000	1.000	0.996	1.000	-	-	-	-

Table A2.4c: Interactions between bog-NP treatments and remaining substrate addition treatments, reported as p-values derived from Tukey's Honest Significant Difference (THSD) tests. THSD tests were conducted when a bog-NP treatment effect was observed (based on t-tests); p-values > 0.05 are considered as non-significant.

Site	Season	C	CNP	NP	Bog	Pond	Pond-NP
CF-1	Summer 2010	1.000	< 0.001	0.710	0.988	0.946	0.946
CF-2	Summer 2010	< 0.001	< 0.001	0.855	0.996	0.855	1.000
PB-2	Summer 2010	0.894	0.324	0.776	0.983	0.894	1.000
PB-3	Summer 2010	0.993	0.066	0.772	0.682	0.968	0.728

Table A2.4d: Interactions between pond-NP treatments and remaining substrate addition treatments, reported as p-values derived from Tukey's Honest Significant Difference (THSD) tests. THSD tests were conducted when a pond-NP treatment effect was observed (based on t-tests); p-values > 0.05 are considered as non-significant.

Site	Season	C	CNP	NP	Bog	Pond	Bog-NP
CF-1	Summer 2010	0.946	< 0.001	0.999	1.000	1.000	0.946
CF-2	Summer 2010	< 0.001	< 0.001	1.000	1.000	1.000	1.000
PB-2	Summer 2010	0.936	0.264	0.841	0.993	0.936	1.000
PB-3	Summer 2010	0.282	0.772	0.066	0.047	0.190	0.728

Table A2.4e: Interactions between bog-DOM addition treatments and remaining substrate addition treatments, reported as p-values derived from Turkey's Honest Significant Difference (THSD) tests. THSD tests were conducted when a bog-DOM treatment effect was observed (based on t-tests); p-values > 0.05 are considered as non-significant.

Site	Season	C	CNP	NP	Bog-NP	Pond	Pond-NP
CF-1	Summer 2010	0.988	< 0.001	0.988	0.988	1.000	1.000

Table A2.4f: Interactions between pond-DOM addition treatments and remaining substrate addition treatments, reported as p-values derived from Tukey's Honest Significant Difference (THSD) tests. THSD tests were conducted when a pond-DOM treatment effect was observed (based on t-tests); p-values > 0.05 are considered as non-significant.

Site	Season	C	CNP	NP	Bog	Bog-NP	Pond-NP
CF-1	Summer 2010	0.946	< 0.001	0.999	1.000	0.946	1.000
PB-3	Summer 2010	1.000	0.007	0.999	0.968	0.190	1.000

A3: Appendix for Chapter 3

The impact of nutrient enrichment on dissolved organic carbon mineralization by headwater stream biofilm communities

A3.1 DOC mineralization in urbanized boreal streams

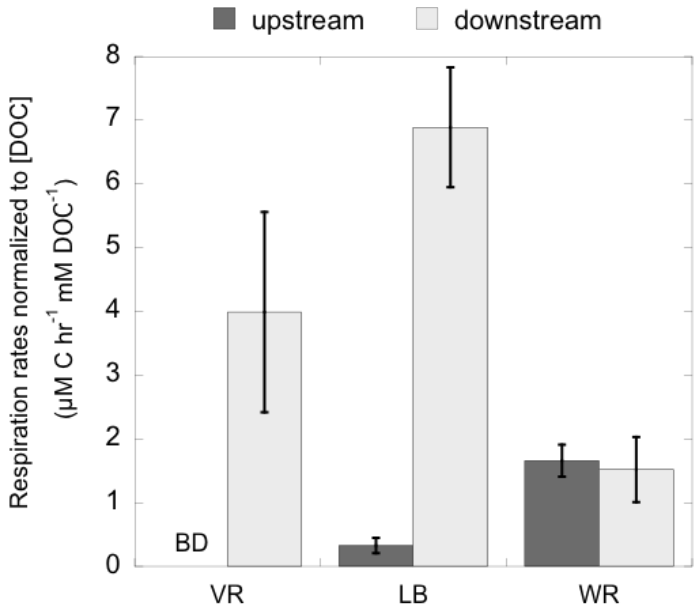

Figure A3.1: Boreal stream biofilm respiration rates normalized to dissolved organic carbon (DOC) concentration at unimpacted upstream and nutrient-rich downstream sites of Virginia River (VR), Leary's Brook (LB), and Waterford River (WR) during baseflow conditions. Values correspond to averages of five replicates ± one standard deviation.

A3.2 DOC mineralization in temperate streams

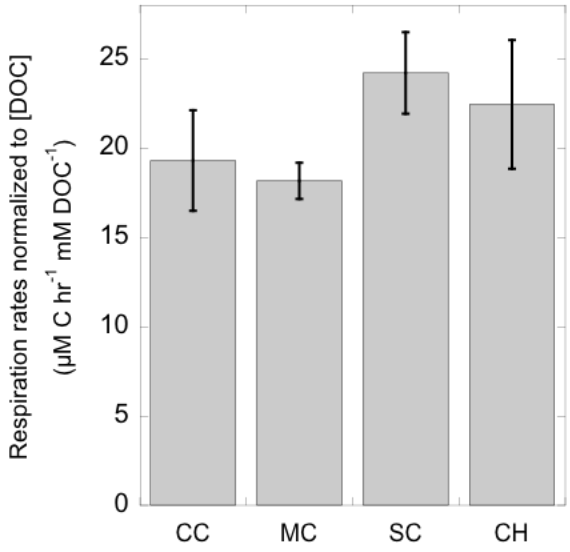

Figure A3.2: Biofilm respiration rates normalized to dissolved organic carbon (DOC) concentration along a nutrient gradient in the Ozark streams – Cecil Creek (CC), Mill Creek (MC), Spavinaw Creek (SC), and Columbia Hollow (CH). Values correspond to averages of three replicates ± one standard deviation.

A4: Appendix for Chapter 4

Carbon cycling and autotrophic-heterotrophic linkages within boreal stream biofilm communities

A4.1 Mesocosm experiments – Experimental set-up

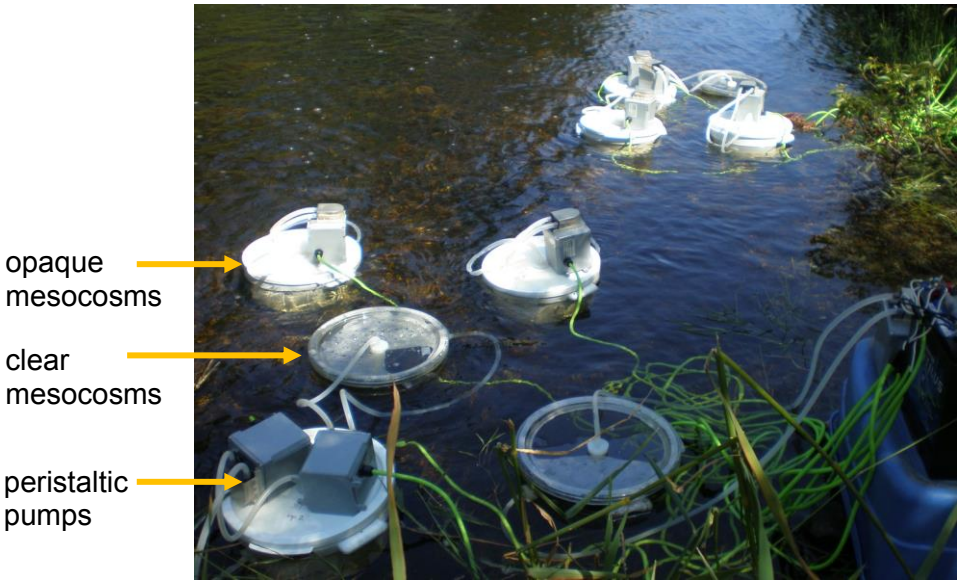

Figure A4.1: Experimental set-up of the mesocosm experiments described in detail in the method section in chapter 4. Clear (light) and opaque (dark) 12 L polycarbonate enclosures, equipped with peristaltic pumps to maintain stream water circulation, were filled with stream water substrate (6 – 7 rocks each) randomly collected from the stream at each study site. Eight liters of unfiltered stream water, initially spiked with ^{13}C-labeled bicarbonate as a tracer for autochthonous carbon generated during the experiments, were added to the mesocosms.

A4.2 Hourly oxygen profile in light and dark mesocosms

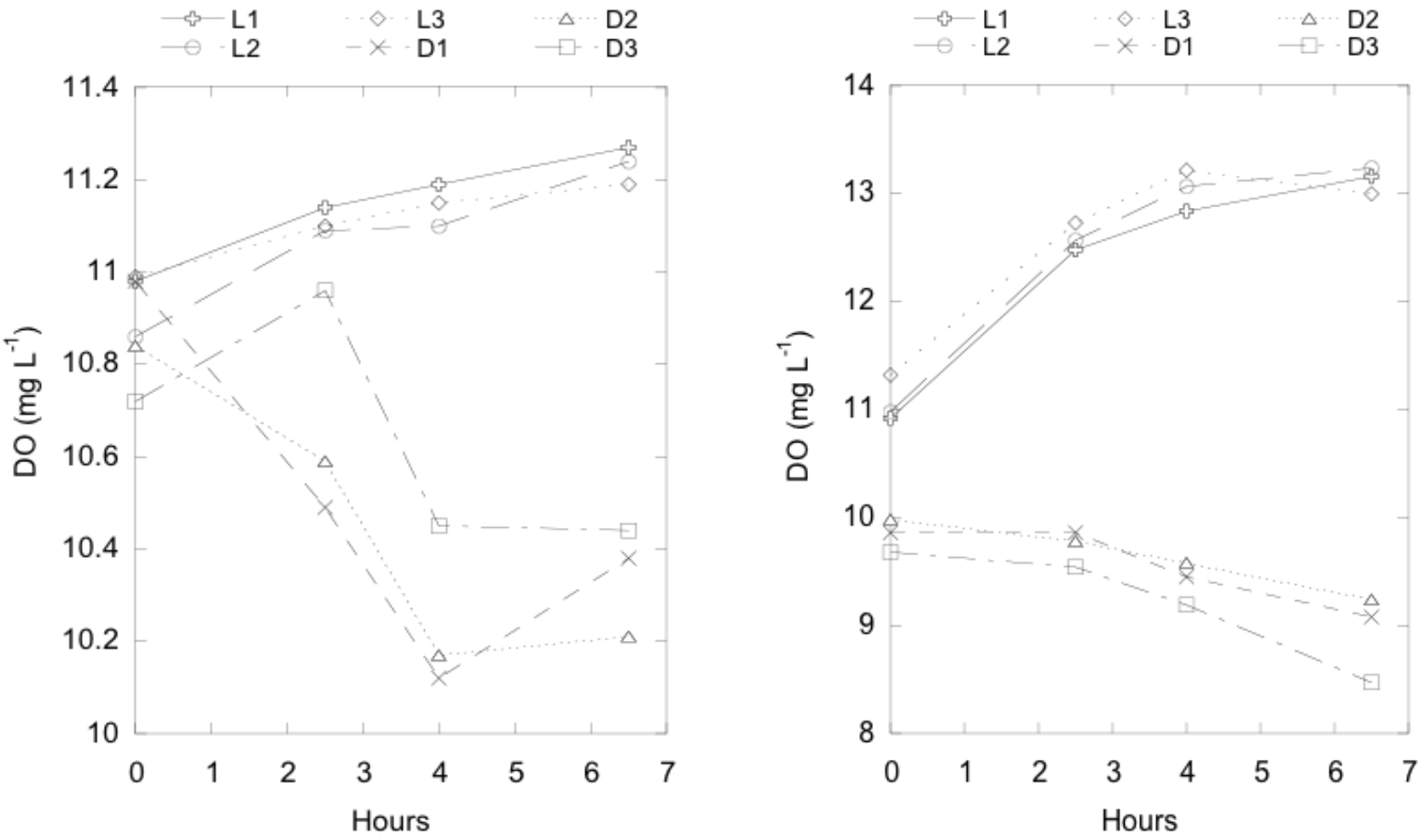

Figure A4.2: Hourly dissolved oxygen (DO) in light (L1, L2, L3) and dark (D1, D2, D3) mesocosms at Virginia River upstream (left) and downstream (right) in fall 2009.

A4.3 Change in dissolved oxygen in light and dark mesocosms

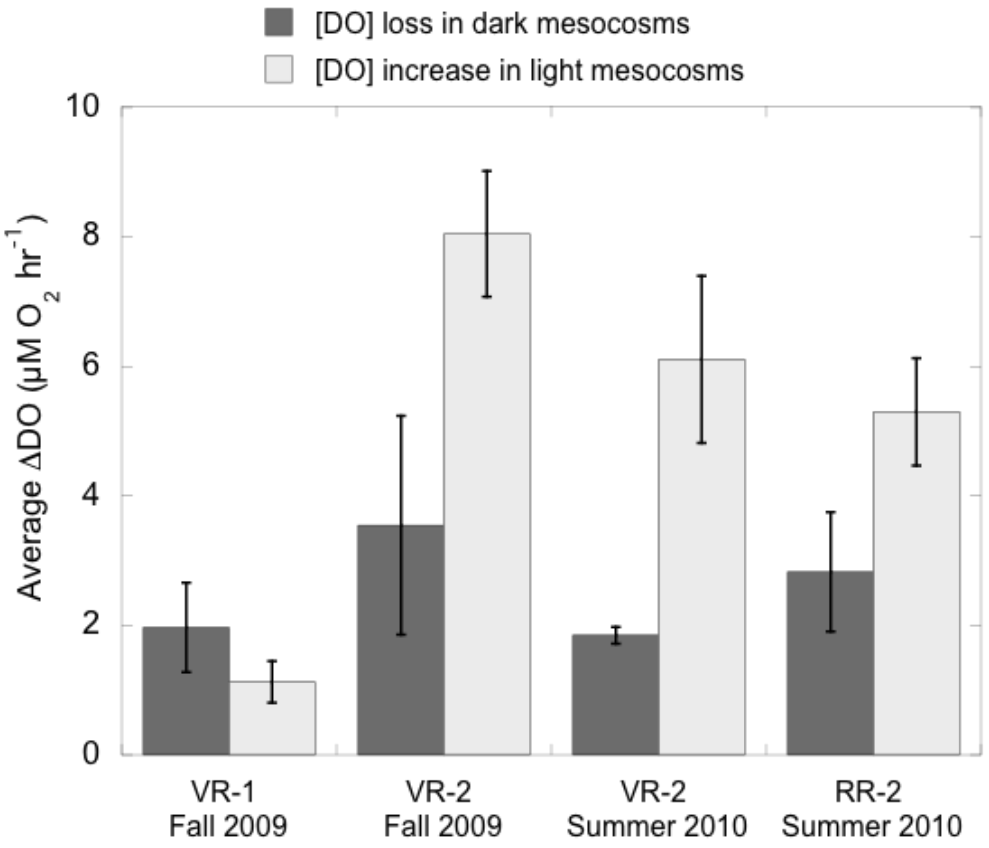

Figure A4.3: Average change in dissolved oxygen (DO) in light and dark mesocosms at Virginia River upstream (VR-1) and downstream (VR-2), and at Rennie's River (RR-2). Values correspond to mesocosm averages (n = 3) ± one standard deviation.

A4.4 Primary production as net DOC released

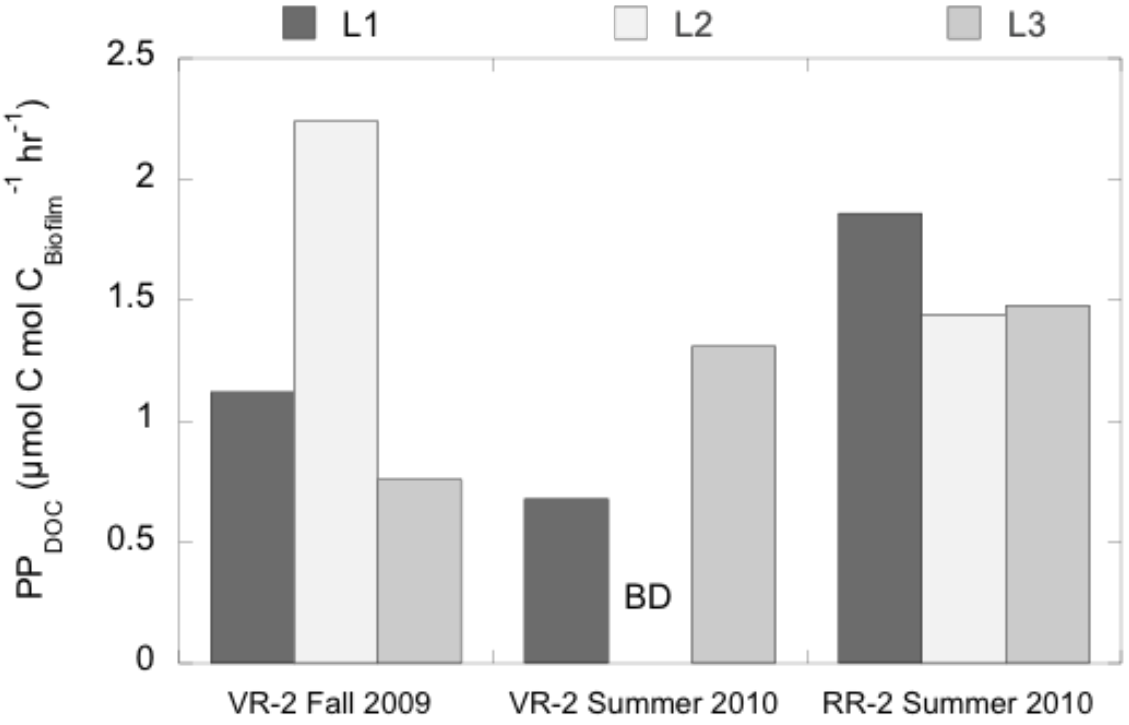

Figure A4.4: Primary production as net dissolved organic carbon (DOC) released (PP_{DOC}) in the light mesocosms (L1, L2, L3) at Rennie's River and Virginia River downstream. BD: Below detection.

A4.5 Change in $\delta^{13}C_{PLFA}$

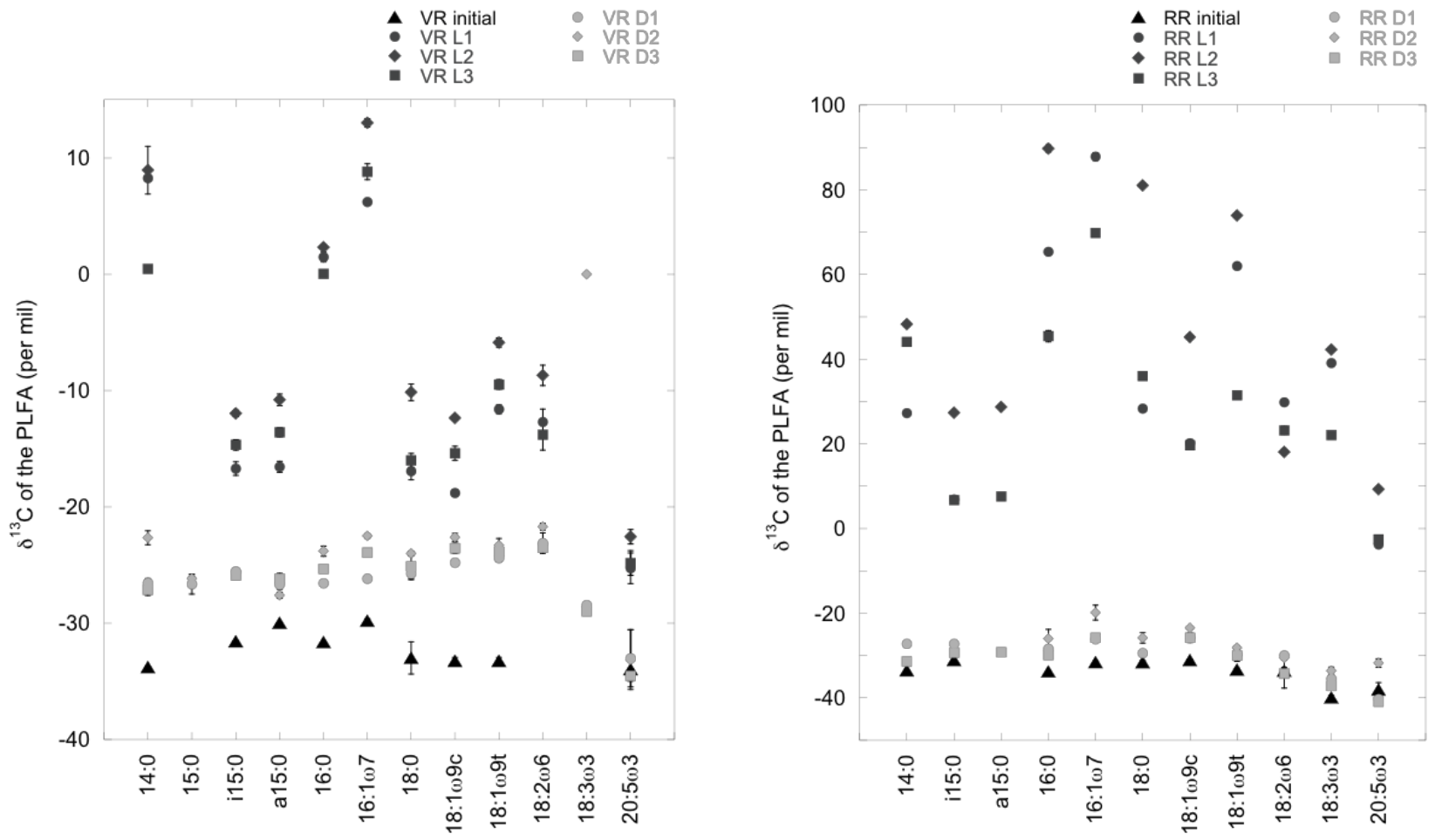

Figure A4.5: Stable carbon isotope composition ($\delta^{13}C$) of the phospholipid fatty acids (PLFA) in initial, light, and dark samples at Virginia River (VR, left) and Rennie's River (RR, right). Values correspond to analytical averages (n = 3) ± one standard deviation.