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Toni Roiha

Carbon Control of Bacterioplankton in Subarctic Lakes and Ponds

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ABSTRACT

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Subarctic water bodies vary from humic thermokarstic ponds surrounded by dense shrub to oligotrophic lakes situated in barren rocky catchments. They are subject to harsh and fluctuating environmental conditions (temperature, light, carbon and nutrients) which influence the metabolic rates and community composition of organisms living in these systems. The focus of this thesis was to describe the variability of bacteria metabolism in different types of subarctic freshwaters and to estimate the influence of dissolved organic carbon (DOC) concentration and characteristics on bacteria metabolism and bacterial community composition (BCC). The project was carried out in subarctic Finland and in Northern Quebec, and involved seasonal, spatial and experimental studies. The water bodies in Finland were generally clear and poor in DOC and nutrients and characterized by lower bacterial production (BP) than the darker and nutrient rich thermokarstic ponds in Quebec, Canada. Highest BP was measured in summer at the bottom of thermokarstic ponds, while in northern Finland ponds had the highest BP followed by lake inlets and outlets. The environmental variables that best correlated with BP were temperature, certain nutrients, DOC and the amount of humic compounds. Nutrients and DOC variables (concentration, S289, fulvic and protein compounds) also explained seasonal and spatial changes in BCC and in bacterial growth efficiency. Climate models predict higher temperatures and precipitation which should increase the amount of terrestrial carbon arriving into the lakes and alter the overall DOC composition available for bacterial metabolism. According to an experimental addition of terrestrial DOC, BP benefited from new DOC and at the same time the microbial food web moved significantly towards heterotrophy due to the increased light attenuation. Understanding how bacterial metabolism and BCC are controlled by different environmental variables can provide insights into how bacteria will manage in a changing climate.

Keywords: Bacterioplankton; dissolved organic carbon; freshwater; heterotrophy; pond; subarctic.

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original articles, which will be referred to in the text by their Roman numerals I–IV. Additional unpublished data have also been used and referred to as "Add.".

I had a significant contribution in planning, data collection and executing all studies. Planning the studies was mainly done together with MR (I, II, III, IV), IL (III), MT (I) and LF (II, additional). Data collection and sample analysis was done together with MR (I, III), LF (II, additional), IL (III), SP (IV) and MC (I). I was responsible for writing the preliminary manuscripts of I, III and IV, contributed equally with SP to the writing of IV and made a significant contribution to the writing and revision of II. All papers were finalised with the co-authors.

I Roiha T., Tiirola M., Cazzanelli M. & Rautio M. 2012 Carbon quantity defines productivity while its quality defines community composition of bacterioplankton in subarctic ponds. *Aquatic Sciences* 74: 513–525.

II Forsström L., Roiha T. & Rautio M. 2013. Responses of microbial food web to increased allochthonous DOM in an oligotrophic subarctic lake. *Aquatic Microbial Ecology* 68: 171–181.

III Roiha T., Laurion I. & Rautio M. 2015. Carbon dynamics in highly heterotrophic subarctic thaw ponds. *Biogeosciences Discussion* 12: 11707-11749.

IV Roiha T., Peura S., Cusson M. & Rautio M. 2015 Habitat and season determine the interplay between DOM pool and bacteria in subarctic freshwaters. Manuscript.

ABBREVIATIONS

BA	bacterial abundance
BB	bacterial biomass
BCC	bacterial community composition
BGE	bacterial growth efficiency
BP	bacterial production
BR	bacterial respiration
CDOM	chromophoric/colored dissolved organic matter
Chl-a	Chlorophyll-*a*
COC	colloidal organic carbon
DOC	dissolved organic carbon
DOM	dissolved organic matter
EEM	excitation-emission matrix
FI	fluorescence index
HI	humification index
HNF	heterotrophic nanoflagellate
Kd	diffuse attenuation coefficient of solar radiation
LH-PCR	length-heterogeneity-polymerase chain reaction
OTU	operational taxonomic unit (of bacteria)
PAR	photosynthetically active radiation
PARAFAC	parallel factor analysis
PNF	pigmented nanoflagellate
POC	particulate organic carbon
PP	primary production
S289	absorption spectral slope at 289 nm
S382	absorption spectral slope at 382 nm
Sr	ratio between spectral slopes at 285 nm and 375 nm
SUVA	specific ultraviolet radiation absorption
TN	total nitrogen
TP	total phosphorus

1 INTRODUCTION

1.1 Bacterioplankton in freshwater sciences

Interest in bacterioplankton research goes back to the beginning of the 19th century when the focus was on pathogens in drinking water (Welch 1935). Early studies suffered from methodological shortcomings causing severe underestimation of the role of pelagic bacterioplankton. In the 1940s the energy transfer to higher organisms through trophic steps was recognised (Lindeman 1942) and, based on this, a view of aquatic food webs was created whereby photosynthetic C from primary production was assumed solely to supply consumers and the role of bacterioplankton was merely as C consumers. It was not until the early 1980s that bacterioplankton was suggested to be an important player in the recycling of primary produced DOC back to the food web via heterotrophic flagellates (Azam *et al.* 1983, Sherr *et al.* 1988). These findings were largely due to the introduction of novel methodology (Hobbie *et al.* 1977, Porter & Feig 1980, Fuhrman & Azam 1982, Kirchman *et al.* 1985) that made estimates of BB and BP more realistic. In the 1980s it also became evident that, especially in boreal humic lakes, the amount of primary produced C was not satisfying the need of bacterioplankton production, suggesting an important secondary energy source, namely terrestrial C (Salonen *et al.* 1983, Tranvik 1988, Jones 1992).

Currently bacterioplankton is seen as an important player in two major pathways in C cycling. First, as consumers through their production bacteria recycle dead organic C into living carbon biomass (Pomeroy 1974) and secondly, via respiration bacteria produce CO_2 and release it to the biosphere (Pomeroy & Johannes 1966). Bacterioplankton may also act as a source of new DOC to aquatic ecosystems (Ogawa *et al.* 2001, Kawasaki & Benner 2006) due to DOC loss occurring during BP (Kawasaki & Benner 2006). Earlier studies suggested that this bacteria-produced DOC could be not biologically available (Stoderegger & Herndl 1998, Ogawa *et al.* 2001), but advances in fluorescence methodology have provided a detailed tool to estimate interactions between DOM and bacteria. New technology has revealed that bacterially generated DOC seems to be a significant source of the total CDOM and also that heterotrophs are a source of both,

bioavailable protein-like and refractory humic-like C (Yamashita & Tanoue 2008, Lønborg *et al.* 2009).

1.1.1 Bacterial production

Traditionally, production has been understood as the production of new organic matter in a defined time period (Boysen-Jensen 1919). In the case of microorganisms, production of new C by absorption of organic C also reflects their level of activity (Romanova & Sazhin 2011). Production of heterotrophic organisms can be studied either via direct abundance and biomass counts in a given time interval or indirectly by measuring changing rates of vital processes (DNA & protein production). These indirect measurements of incorporation of radioactive label (leucine or thymidine) are most commonly used in aquatic environments (Fuhrman & Azam 1982, Kirchman *et al.* 1985). Independent of their cell size, 63 % of bacterioplankton dry weight is formed from proteins (Simon & Azam 1989) and a relatively stable fraction of it is leucine (7.3–8.7 %) (Buesing & Marxsen 2005 and refs therein). Heterotrophic bacteria are also superior competitors for these amino-acids when compared to phytoplankton (Kirchman *et al.* 1985). Due to these factors, protein synthesis rates can be converted to estimates of organic C production. Thymidine, on the other hand, is cell size dependent and is related to the production of new DNA, and therefore it represents more the increase of the population size (thymidine) than cell growth (leucine).

Drawbacks in these incorporation methods are that both amino-acids are present in the natural environment and can also be synthesised intracellularly. This unknown fraction of incorporated unlabelled amino-acids (isotope dilution) is especially problematic with leucine and can lead to underestimation of BP because the isotope dilution conversion factor can as much as double between oligotrophic and more eutrophic environments (Simon & Azam 1989, Kirchman 1993). To minimize the effect of isotope dilution, time series and saturation curves should be established to ensure that there is significantly more external leucine available than in the natural state and to inhibit synthesis of new leucine (Buesing & Marxsen 2005 and refs therein). In turn, the thymidine method suffers from varying conversion factors that can cause significant differences in the relation between incorporation rates and C dry weight in similar environments (Scavia *et al.* 1986). Current BP methods do not either take into account viruses that are common among bacterial communities (Bratbak *et al.* 1994) indicating that communities are also likely producing viral particles (Unanue & Iriberri 1997).

1.1.2 Bacterial respiration

Respiration has been recognized as one of the key functions in the ecosystem where it represents a sink for organic matter and simultaneously produces reactants like O_2, CO_2, CH_4 and low molecular weight compounds (Williams & del Giorgio 2005). The history of respiration measurements starts with the early development of measurement of dissolved O_2 by Winkler (Winkler 1888), but it took several decades to recognize respiration as an individual process and not

merely as a correction measurement for photosynthesis. The first specific respiration studies were conducted during the late 1960s (Pomeroy & Johannes 1966, 1968) but the focus in the aquatic field was still heavily concentrated on the measurement of photosynthesis. This situation lasted nearly 30 years until del Giorgio and Peters (1993) pointed out that there was over an order of magnitude more published papers on productivity than on respiration (> 1000 vs. < 100). The topic was also acknowledged on the microbial side when del Giorgio & Cole (1998) introduced an idea of uncoupling anabolism and catabolism as a way for bacterioplankton to adapt their growth efficiency according to the changing environment. Simultaneously with an increasing interest in respiration measurements, there were also developments in the measurement methodology. BR is measured as a change in O_2 consumption or as production of CO_2 (Griffith *et al.* 1990, Biddanda *et al.* 1994, Hansell *et al.* 1995), but until the last decade the accuracy of the measurements was a problem. Recent methodology includes high resolution spectrometric sensors (Warkentin *et al.* 2007) that allow online measurements of O_2 consumption and CO_2 production making it also possible to re-evaluate assumptions regarding respiratory quotients (Berggren *et al.* 2011).

There are also methodological problems that concern BR measurements. Prefiltration is often necessary to remove zooplankton and phytoplankton from the sample. This can cause an overestimation of bacterioplankton contribution to community respiration in oligotrophic environments by removing the predation pressure from HNF (Weisse & Scheffel-Möser 1991) and nutrient competition from phytoplankton (Caron *et al.* 2000). Filtration can also cause changes in BCC because larger size fractions of bacteria can be retained by the filter and therefore favour opportunistic bacteria (Gasol & Morán 1999, Massana *et al.* 2001). It is also possible that handling procedures can cause changes in nutrient and DOC availability (Gasol & Morán 1999, Massana *et al.* 2001). As a consequence of tightly coupled predator-prey and autotrophic-heterotrophic dynamics, BR in prefiltered and long-incubated oligotrophic samples are prone to overestimations (Aranguren-Gassis *et al.* 2012).

1.1.3 Bacterial growth efficiency

Production of new C and respiration of organic to inorganic C are two main functions of heterotrophic bacterioplankton (del Giorgio & Cole 1998). These main functions can be used as an estimation of how bacterioplankton uses the obtained C. Generally it seems that heterotrophic bacteria maximize the amount rather than the efficiency of C utilized (del Giorgio & Cole 1998). BGE can be calculated as the ratio of new C produced per unit of C assimilated (del Giorgio & Cole 1998).

$$BGE = BP / (BP + BR)$$

where *BGE* is bacterial growth efficiency (%), *BP* is bacterial production and *BR* is bacterial respiration.

1.1.4 Bacterial community composition

Research on BCC has been one of the most rapidly developing fields associated with aquatic bacterioplankton (Newton *et al.* 2011). Development started during the 1990s when 16S rRNA gene sequencing was first applied to aquatic bacterial communities (Bahr *et al.* 1996). During this first generation sequencing several different methods like DGGE (Muyzer *et al.* 1993), T-RFLP (Avaniss-Aghajani *et al.* 1996) and LH-PCR (Suzuki *et al.* 1998) were applied to study the 16S rRNA gene. Results from these studies have shown that aquatic environments are inhabited by distinct bacterial communities (Zwart *et al.* 2002). During the last decade a wave of next generation sequencing has landed in aquatic bacterial research. 454 sequencing includes methods like Pyrosequencing (Margulies *et al.* 2005), Illumina (Gunderson *et al.* 2004) and Ion Torrent (Rothberg *et al.* 2011) that have been very efficient tools in studying aquatic bacterial diversity and ecosystem functioning and have provided a solution to solve very precise questions about BCC functioning. The main drawbacks of next generation sequencing are associated with handling of extremely large datasets and reducing the sequencing error within that, although software for characterizing and cleaning the data are available (Schloss *et al.* 2009, 2011).

1.2 Most important controls of bacterial metabolism

DOC (Blomqvist *et al.* 2001, Hessen *et al.* 2004), nutrients (Jansson *et al.* 1996, Granéli *et al.* 2004), nutrients and C combined (Vrede 2005, Breton *et al.* 2009, Vidal *et al.* 2011), temperature (Panzenböck *et al.* 2000, Vrede 2005), UV radiation (Sommaruga *et al.* 1997) and top-down predation (Weisse & Scheffel-Möser 1991, Hessen *et al.* 2004) have all been found to be connected to bacterial metabolic rates in freshwater ecosystems.

Aerobic bacteria use C for biomass synthesis and respiration, unlike nutrients that are only needed for biomass synthesis, making C the major limiting factor for bacterial metabolism (Kirchman 2012). Bacterioplankton are superior competitors for nutrients when compared to phytoplankton; therefore, without C eventually becoming limiting, BP would increase until the system has run out of nutrients (Bratbak & Thingstad 1985). Still nutrients are crucial to aquatic microbes for the production of new biomass. Stoichiometry of bacterioplankton (C:N:P; 50:10:1) indicates that they are extremely rich in phosphorus (Fagerbakke *et al.* 1996, Vrede *et al.* 2002). This leads to increased demand for P compared to N (Kirchman 2012). Also turnover times are faster for P, that is found in nuclei acids, lipids and nucleotides, than for N that is mainly found in proteins, making bacterioplankton more likely to be P- than N-limited (Kirchman 2012).

Low temperatures significantly decrease metabolic rates (Kirchman & Rich 1997, Pomeroy & Wiebe 2001) although diverse communities can have a high adaptation to low and fluctuating temperatures (Adams *et al.* 2010). Direct UV radiation can also inhibit bacterioplankton metabolism (Sommaruga *et al.* 1997,

Hörtnagl *et al.* 2011) but its severity to living organism is strongly regulated by attenuation by humic material. UV radiation is strongly attenuated by DOC but it also simultaneously decomposes DOC in the surface waters (Vähätalo & Wetzel 2004, Cory *et al.* 2013) therefore creating protection for living organisms. Usability of UV-decomposed C is strongly related to the original characteristics of the DOC (Tranvik & Bertilsson 2001) and in some cases it has been found to enhance (Anesio *et al.* 2005) and in others to suppress the availability of DOC (Obernosterer *et al.* 1999). Furthermore, bacterioplankton can be top-down controlled, especially in enclosure environments, by predation from HNF (Weisse & Scheffel-Möser 1991) or cladocerans (Hessen *et al.* 2004). Yet, focusing on a single limiting factor can oversimplify the functioning of the studied ecosystem because metabolic rates are more enhanced by addition of multiple limiting factors (Vrede 2005; Breton *et al.* 2009; Vidal *et al.* 2011). Furthermore, most studies have treated bacterioplankton communities as a homogeneous group and only recently has the high inherent diversity of bacterial communities been taken into consideration. High complexity indicates that different groups in bacterial communities are co-limited by multiple factors (Eiler *et al.* 2012).

In recent years, the role of DOC in controlling bacteria metabolism has received increasing attention. This interest stems from efforts for a better understanding of the global C cycle and the role of lakes in processing terrestrial C before it enters the oceans (Tranvik *et al.* 2009). Organic C in aquatic environments is found in three forms: POC, COC or DOC. Categorizing these components has been based on the methodology so that the fraction retained on a filter (0.2–1.22 μm pore size) is called POC and the filtrate includes both COC and DOC fractions. The chemical complexity of DOM leads to variation in DOM colour. Colour of the pigmented parts of DOM varies from the light yellow of fulvic acids to almost black humin (Stevenson 1982), but in exceptional conditions DOM can be degraded to almost colourless (Anderson & Stedmon 2007). Therefore DOM generally has a significant effect on the underwater light climate by attenuating blue light and UV radiation and letting through the red and yellow light of the spectrum. Especially increased attenuation of the UV part of the spectrum is due to DOM and therefore UV exposure is often linked to DOM concentrations. DOM-induced changes in light climate have a drastic influence on living organisms. Light attenuation by DOM reduces the area and volume available for benthic and pelagic primary production (Pérez-Fuentetaja *et al.* 1999, II), while on the other hand DOM provides shelter against the detrimental UV radiation (Rautio & Tartarotti 2010). DOM also has a role in pH changes. DOM of terrestrially origin mainly consists of organic acids that can cause a decrease in pH in waters with low or no bicarbonate alkalinity but on the other hand these organic acids act as buffers for further pH decrease (Lydersen 1998). DOM also plays a crucial role in detoxifying toxic and metal cations in the water body (Lydersen 1998).

O_2 consumption in water bodies is enhanced by increased DOM concentration due to increased BR rates. Photochemical degradation takes place only in the photic zone where high molecular weight C compounds are degraded to more labile forms and potentially stimulate bacterioplankton metabolism (Lindell *et al.* 1995, Stedmon & Markager 2005). Although this likely increases the

O_2 consumption, it is always coupled to O_2 production by primary production, thus preventing hypoxia in the epilimnion. Attenuation of solar energy in the epilimnetic waters, on the other hand, increases thermal stratification and increases the possibility of anoxia in the bottom waters.

Usually DOC concentrations are an order of magnitude higher than POC concentrations making DOC the most important pool of organic C in aquatic ecosystems (Wetzel 2001, Tranvik *et al.* 2009). DOC inputs are also often divided by their source. Carbon produced within the system by autotrophic organisms and macrophytes is called autochthonous C, whereas C derived from terrestrial sources (outside the system) is called allochthonous C. Terrestrial inputs are mainly soil leachates, leaf litter and debris. Autochthonous C fuels the microbial loop where high molecular weight organic C is degraded back into forms that are available to higher trophic levels (Azam *et al.* 1983) and terrestrially derived organic C further provides an excess energy source for secondary production (Tranvik 1988). Autochthonous C is generally considered to be a good and important source of C for bacterioplankton (Cole *et al.* 1988, Chen & Wangersky 1996), whereas terrestrial C is available in high quantities but its quality for organisms is considered poor (Brett *et al.* 2009, 2012). Still it has been shown that labile low molecular terrestrial C can support a significant part of secondary production (Cole *et al.* 2006, Berggren *et al.* 2010) likely due to microbial and photochemical transformations (Stedmon & Markager 2005, Laurion & Mladenov 2013). When the importance of DOC as an additional energy source for bacterioplankton was recognised the focus moved to its importance to higher trophic levels in freshwater ecosystems (Salonen & Hammar 1986). During the last decade there has been a debate about the share of terrestrial C transferred to higher trophic levels (zooplankton and fish). There are several stable isotopic ($\delta^{13}C$, $\delta^{15}N$ & $\delta^{2}H$) studies that have concluded the contribution of terrestrial C to zooplankton and fish can vary from 20 % to 70 % (Pace *et al.* 2004, Solomon *et al.* 2011, Karlsson *et al.* 2012) whereas other studies, based on C mass influxes and quality results from essential fatty acids, have concluded that their role in zooplankton diet and animal production is very small (Brett *et al.* 2009, 2012, Galloway *et al.* 2014).

All these findings are related to the complex nature of DOC. Humic substances, carbohydrates, carboxylic acids and amino acids are all components that can be found and identified by using the optical properties of CDOM. Therefore it is possible to use CDOM as a tracer of the dynamics and characteristics of the DOC components. Optical properties are analysed with a combination of spectrophotometric and spectrofluorometric measurements from which a suite of different indexes has been developed, for example to estimate the origin (McKnight *et al.* 2001), aromaticity (Kalbitz *et al.* 1999), redox-potential (Miller *et al.* 2006) and size (Retamal *et al.* 2007) of DOC. The latest advance in the fluorescent technology is applying the multivariate modelling technique, PARAFAC, to EEM (Stedmon & Bro 2008). Individual C components can be decomposed from the EEMs and their relative contributions to total fluorescence are estimated. Incorporation of this rapid and relatively inexpensive method into a range of ecological studies for understanding the biochemical role of DOM has

been suggested (Fellman *et al.* 2010), although there are still several issues with data interpretation (Ishii & Boyer 2012).

1.3 Subarctic freshwater ecosystems

There is a great diversity of aquatic ecosystems in the subarctic region among landscapes that vary from wet mineral peatlands to barren rocky catchments, and there are several features that distinguish high latitude lakes and ponds from their boreal counterparts (Vincent *et al.* 2008). The most notable difference is the length of the winter ice cover that can persist for up to 9 months (Rautio *et al.* 2011b). The long winter season is characterised by lowered temperatures and irradiance leading to lower productivity during winter, but there are still organisms that are active under the ice (Rautio *et al.* 2011a). The situation is totally different during summer when small turbid ponds especially can easily heat up to > 20 °C (Vincent *et al.* 2008), and due to primary production and terrestrial inputs there is also more C available for organisms. On top of that there is 24 h sunlight available for autochthonous producers at latitudes above the polar circle (66° N). These abiotic variables make the short summer the most important growth phase in the subarctic.

Thermokarstic permafrost thaw ponds are the most abundant water bodies in the arctic (Vincent *et al.* 2008); they are widespread over the whole circumpolar arctic and estimated to occupy 24 % of northern hemisphere land surface (Zhang *et al.* 1999, Grosse *et al.* 2013). These usually highly turbid ponds are formed in depressions caused by melting permafrost and are heavily influenced by terrestrial C. Terrestrial inputs have a major impact on the thermal and light regimes and therefore thermokarstic ponds often form stabile stratification and are O_2 depleted at the bottom (Laurion *et al.* 2010). These dark, anoxic and C-rich conditions are extremely favourable for bacterioplankton and have made the thaw ponds hot spots of greenhouse gas emissions (Walter *et al.* 2006, Laurion *et al.* 2010) and therefore possible contributors to climate warming (Schuur *et al.* 2008).

Another important type of subarctic aquatic ecosystem is clearwater lakes and ponds. These usually oligotrophic water bodies were formed in rock basins by retreating ice masses (Pienitz *et al.* 2008). Low nutrient and C content and high transparency makes these ponds and lakes especially vulnerable to increased terrestrial inputs from the catchment and in contrast to thermokarstic ponds are saturated with O_2 through the whole water column. These oligotrophic ponds and lakes are generally known for their low pelagial PP (Rautio *et al.* 2011b), but recently the focus has shifted to the importance of benthic production (Vadeboncoeur *et al.* 2008). Barren rocky pond sediments are known to have a lot of sedimented and recycled nutrients and they are not light or O_2 limited, therefore making them highly diverse and productive environments in these desert and tundra aquatic ecosystems (Rautio & Vincent 2006, Quesada *et al.* 2008).

According to climate change predictions the Subarctic and Arctic region might undergo increasing air temperatures and precipitation (Solomon *et al.* 2007).

These changes would have a large impact on their aquatic food web structures. Increased precipitation would benefit heterotrophic organisms due to improved nutrients and terrestrial C availability from the catchment (Hessen *et al.* 2004, Breton *et al.* 2009, II). Also higher light attenuation caused by terrestrial inputs would decrease the volume of photic zone and lower the overall phototrophic primary production, thereby moving subarctic water bodies towards more heterotrophic energy pathways (Pérez-Fuentetaja *et al.* 1999, II). Changes would also extend to higher trophic levels. High transparency and 24 hour radiation have created a need for zooplankton to have protection against UV radiation. Terrestrial inputs would likely increase the UV-attenuation providing shelter and making the production of UV-protecting pigments obsolete for zooplankton (Rautio *et al.* 2009). On a global scale, one of the biggest issues induced by climate change in northern environment is the accelerating mobilization of old soil organic C pools. Microbially and photochemically degraded large and old C stocks have increased the amount of CO_2 and CH_4 emitted to the atmosphere (Schuur *et al.* 2009). High greenhouse gas emissions have been measured widely over the whole northern hemisphere (Kling *et al.* 1992, Hamilton *et al.* 1994, Nakano *et al.* 2000, Walter *et al.* 2006, Desyatkin *et al.* 2009, Laurion *et al.* 2010) and CH_4 has been acknowledged to play a particularly large role in greenhouse gas emissions (Walter *et al.* 2006, Laurion *et al.* 2010) further extending the magnitude of the warming region (Laurion *et al.* 2010).

1.4 Thesis objectives

A warming climate is predicted to introduce more terrestrial organic material to subarctic aquatic ecosystems. This could make the food web structure more beneficial to heterotrophic organisms. Recently the small water bodies in the subarctic have been noted as hot spots for greenhouse gas emissions, coming mainly from bacterial metabolism. Therefore it is essential to know the factors contributing to bacterioplankton functioning in these waterbodies. This thesis aims to provide new knowledge on bacterioplankton metabolism and community composition in subarctic waters and focuses especially on:

1. Characterising the range of variability of bacterioplankton metabolism and community composition in different types of subarctic freshwaters and seasonally.
2. Estimating the role of quantity and quality of DOC for bacterioplankton functioning in natural water bodies and in experimental conditions.
3. Estimating the relative contribution of phototrophic vs. heterotrophic microbial energy pathways in different subarctic water bodies.

2 METHODS

2.1 Study area and samples

There is a high diversity of freshwater ecosystems located in high latitude subarctic regions. Data for this thesis were gathered from a series of oligotrophic ponds and lakes in the Kilpisjärvi region in north-western Finland (69º 03′ N, 20º 52′ E)) (Fig. 1) and from a series of thermokarstic ponds located in discontinuous permafrost near Kuujjuarapik in northern Quebec (55° 20′ N, 77° 30′ W). Sampling was also carried out in Seida in north-western Russia (67º 03´ N, 62º 56´ E) at a lowland tundra area and, although not yet published, some of these results are presented in the thesis and referred to as "add".

FIGURE 1 A map of the circumpolar arctic and subarctic with sampling sites (grey stars).

Kilpisjärvi is located in the most north-western corner of Finnish Lapland. A variety of ponds and lakes was chosen to represent a typical oligotrophic (low nutrient & high transparency) subarctic freshwater type. Lakes and ponds were situated along an altitudinal (473–950 m a.s.l.) gradient and were either surrounded by barren or forested catchments. The treeline of mountain birch forest (*Betula pubescens* subsp. *czerepanovii* (Orlova) Hämet-Ahti) is located at 600 m a.s.l.. Altogether 16 ponds and 3 lakes were sampled in this region. One of the lakes, Lake Saanajärvi, also served as a site for an experimental setup (II). The sites were sampled in 2008 (spring, summer and autumn) and in 2011 (winter, spring, ice-break, summer and fall).

The Kuujjuarapik region is located in discontinuous permafrost ca. 20 km east from the nearest village of Whapmagoostui-Kuujjuarapik. Five thermokarstic ponds (moderate nutrient & low transparency) were sampled along the DOC (3.9–11.9 mg l^{-1}) and colour gradient. Ponds were situated in an impermeable clay-silk bed and were surrounded by dense shrubs and sporadic tree and moss areas (Bouchard *et al.* 2011). Ponds were sampled on two separate occasions in 2009 (late winter in April and summer in August).

The Seida region is located in extensive lowland tundra and permafrost 7 km west from the small village of Seida. Altogether 8 thermokarstic ponds and 3 lakes (low nutrients & moderate transparency) with extensive moss growth on the bottom were sampled along a DOC gradient (9.5–116.8 mg l^{-1}). Ponds and lakes were sampled once in 2012 (August).

Climate, drainage-area, C input sources and climate change predictions in these areas are very different and provided a possibility to work with a large environmental gradient. Lakes and ponds in Kilpisjärvi (Finland) are typically oligotrophic with clear water and low nutrient and C concentrations (Table 1). Ponds in Kuujjuarapik (Canada) and Seida (Russia) on the other hand are typically heavily influenced by terrestrial inputs making them coloured and relatively rich in nutrients and C (Table 1). Seasonal fluctuations of bacterioplankton biomass (I, III), productivity (I, III, IV, add), respiration (IV) and community composition (I, IV) were measured. Properties of DOC were studied alongside these bacterioplankton variables. DOC concentration was measured on every sampling (I, II, III, IV, add), and the optical properties of the water were also analysed to estimate quality and source of DOC. Spectrophotometric and spectrofluorometric analyses were run to illustrate source (I, II, III, IV, add), availability (I, II, III, IV, add) and composition (IV) of CDOM. Effects of DOC were studied in the natural environment (I, III, IV, add) but also in experimentally (II). Phototrophic and heterotrophic C flows were compared in oligotrophic Saanajärvi in Kilpisjärvi (II) and in thermokarstic ponds in Kuujjuarapik (III). Chl-*a* concentration (I, II, III, IV, add), PP (II, III) and phytoplankton (II, add) or PNF (I, III) biomass were used to represent the phototrophic C energy flow. BB (I, II, III) and BP (I, II, III, IV, add), and also HNF biomass (I, II, III) were used to estimate the C flow through secondary production. Terrestrial C inputs were estimated at the same time because they are known to be beneficial to heterotrophic organisms. This hypothesis was also tested with a DOC addition experiment (II). Lastly, the role of

habitats to bacterioplankton metabolism and C quality changes was studies in Kilpisjärvi (I, IV).

2.2 Analytical methods

2.2.1 Sample collection and experimental design

During the open water season ponds were sampled either from a rubber boat or by using waders, and during winter the deepest spot was sampled through a drilled ice hole either with a Limnos or a Kemmerer water bottle sampler. Lakes were sampled from the inlet and outlet brooks using boat or waders. Generally samples were only taken from the surface waters, with the exception of KWK thermokarstic ponds where both surface and bottom were sampled. Lake Saanajärvi was used as a location for a DOC-addition experiment. Altogether 9 open ca. 75 l plastic containers were set in the south end of Lake Saanajärvi; 3 controls, 3 boreal DOC additions and 3 subarctic DOC additions. Water samples were collected 3 times (1 day, 3 day and 5 day) from the containers with a small Limnos sampler.

2.2.2 Physico-chemical properties

A multiparametric probe (YSI Inc., Yellow Springs, Ohio) was used to measure temperature, pH, conductivity and O_2 concentration in the field. Total and inorganic nutrients were analysed using standard methods (Finnish Standards Association SFS-EN 5505, 6878) of the National Board of Waters at Lammi biological station or as in Breton *et al.* (2009). DOC was analysed from filtered samples using standard methods (Finnish Standards Association SFS-EN 1484:1997) of the National Board of Waters in Finland or using a Shimadzu TOC-5000A carbon analyser calibrated with potassium biphthalate.

TABLE 1 Characteristics of study ponds and lakes situated in Kilpisjärvi, Kuujjuarapik and Seida regions. TP, TN and DOC values are presented as means of all observations ± S.D.

Region	Sites	Altitude (m a.s.l)	TP ($\mu g\ l^{-1}$)	TN ($\mu g\ l^{-1}$)	DOC ($mg\ l^{-1}$)
Kilpisjärvi	9–16	473–950	6.1±1.5	161±69	3.4±2.4
Kuujjuarapik	5	ca. 105	154±133	533±343	6.9±2.1
Seida	10	ca. 95	85±45	1431±1226	40±36

Analysis of heterotrophic components

BA was analysed with two separate methods. In I and II bacterial densities were counted from prepared slides stained with 4-,6-diamido-2-phenylindole (DAPI) using UV excitation with an epifluorescence microscope (Leica Leitz DMRB). In III bacterioplankton was stained with SYBR green I and cell abundance was

estimated using flow cytometry (FACSCalibur, Becton-Dickinson). BB estimations (I, II, III) were calculated from cell sizes converted to C using either a constant coefficient (Fry 1988) or an allometric conversion formula (Posch *et al.* 2001). Cell sizes were measured from digital images taken from DAPI-stained slides and using the Cell C program (Selinummi *et al.* 2005). ^{3}H-leucine incorporation (Kirchman *et al.* 1985) with a centrifugation method (Smith & Azam 1992) was used to estimate BP (I, II, III, IV, add). Saturation of ^{3}H-leucine incorporation was tested experimentally for concentration and time. BR was measured from prefiltered (3 µm) samples as a decrease of O_2 concentration using fibre-optic mini-sensors (Fibox 3, PreSens Precision Sensing GmbH, Regensburg, Germany) (Warkentin *et al.* 2007). BCC was analysed with two separate methods. In I the polymerase chain reaction was used to amplify bacterial 16s rRNA-genes that were analysed for length heterogeneity (LH-PCR) to illustrate differences in community composition (Suzuki *et al.* 1998). In IV bacterial 16s rRNA-genes were analysed by next generation sequencing (454 pyrosequencing; Margulies *et al.* 2005). Abundance and biomass of HNF was estimated from DAPI-stained slides using UV excitation with epifluorescence microscopy (I, II, III). Heterotrophic organisms were identified from autotrophic organism using a green excitation filter.

2.2.3 Analysis of autotrophic components

Samples were filtered onto GF/F filters from which chl-*a* was extracted into ethanol and analysed fluorometrically (I, II, III, IV, add). Photosynthesis was measured using incubations with ^{14}C solution. Screened polyethylene bags (Whirlbak) or a Rae-box were used to generate a PAR gradient (II, III) and to obtain photosynthesis–irradiance curves. Photosynthetically fixed C was normalized to chl-*a* concentration and fitted to equations depending on presence or absence of photoinhibition (Jassby & Platt 1976, Platt *et al.* 1980). The site-specific diffuse attenuation coefficient (K_dPAR) was obtained from a correlation between DOC and light (Forsström *et al.* 2015) in Kilpisjärvi region and from a correlation between DOC and total suspended solids (Watanabe *et al.* 2011) in the Kuujjuarapik region. Calculated photosynthetic parameters and K_dPAR were used for calculating depth-integrated primary production. Picoautotrophic plankton abundances were calculated either from slides under UV excitation and with a green excitation filter or by flow cytometry (FACSCalibur, Becton-Dickinson) using their own chlorophyll autofluorescence. Abundance and biomass of PNF were estimated from the same samples as HNF using UV excitation with epifluorescence microscopy (I, II, III). Autotrophic organisms were distinguished from heterotrophic organisms using a green excitation filter.

2.2.4 Analysis of carbon components

Optical and fluorescence properties of water were used to analyse the quantity and quality of DOC (I, II, III, IV, add). Three separate methods were applied for spectrophotometric data. Quantity of CDOM was estimated from the absorption

coefficient at 320 nm (a320). Pigmentation and origin of DOC was estimated from specific UV absorbance at 254 nm normalized to DOC concentration (SUVA) (Weishaar *et al.* 2003, Hood *et al.* 2005). Two individual spectral slopes and their ratios were also calculated from spectrophotometric CDOM absorption spectra. Spectral slopes in lower wavelengths (S289) are known to be related to algal derived proteins and phenols whereas spectral slopes at higher wavelengths (S382) are usually related to terrestrial fulvic and humic acids. Sr therefore represents a change in dissolved C components (Loiselle *et al.* 2009, Bracchini *et al.* 2010, Galgani *et al.* 2011). A single excitation scan was performed at 370 nm and the ratio of fluorescence emissions at 450 nm and 500 nm was used to calculate the FI that was in turn used to infer the origin (microbial or terrestrial) of fulvic acids in the sample (McKnight *et al.* 2001). A synchronous fluorescence scan was recorded from 200 nm to 700 nm with 14 nm separation between excitation and emission. The ratio between fluorescence emissions at 470 nm and 360 nm was used as an index of humification and polycondensation (HI) indicating availability of C for further usage (Kalbitz *et al.* 1999). Excitation was measured across 220–450 nm and emission across 240–600 nm with 5 and 2 nm intervals to create a 3-D EEM. EEMs were analysed with PARAFAC to identify separate fluorescent components with similar excitation-emission properties (Stedmon & Bro 2008, Fellman *et al.* 2010).

3 RESULTS AND DISCUSSION

3.1 Bacterial metabolism

BP in ponds in the Kuujjuarapik region differed significantly ($F_{2,70}$ = 122.5, p < 0.001) from ponds in the Kilpisjärvi (Tukey test; p < 0.001) and Seida (Tukey test; p < 0.001) regions with summer BP (32.5 ± 15.5 µg C $l^{-1}d^{-1}$) significantly higher than in the Kilpisjärvi (1.4 ± 1.4 µg C $l^{-1}d^{-1}$) or Seida (1.6 ± 1.5 µg C $l^{-1}d^{-1}$) regions (Fig. 2).

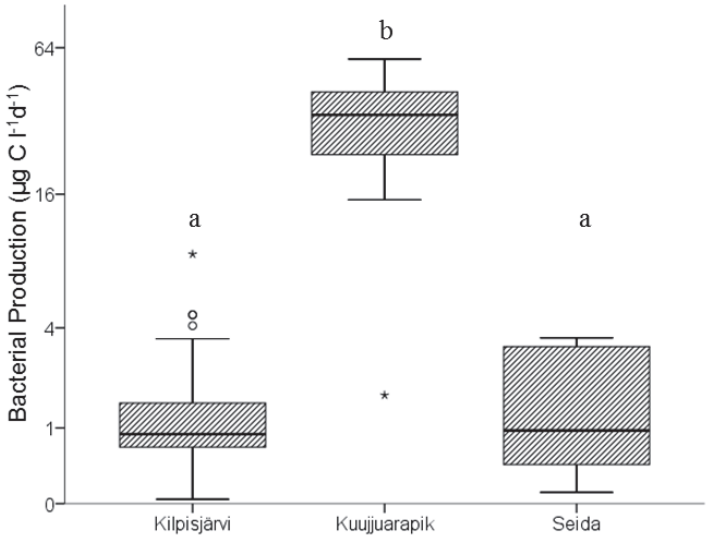

FIGURE 2 Open water BP in different studied regions. Statistically significant (p < 0.05) differences are indicated by different letters above bars.

Seasonality in BP (Fig. 3) was measured for Kilpisjärvi and Kuujjuarapik (I, III, IV). There was a significant seasonal change in BP in both Kilpisjärvi ($F_{4,50}$ = 4.0, p = 0.007) and Kuujjuarapik ponds (H = 10.0, n = 17, p = 0.002). In Kilpisjärvi waters production was found to be significantly (Tukey test; p = 0.006) higher during the summer (1.9 ± 1.3 µg C $l^{-1}d^{-1}$) than fall (0.8 ± 0.3 µg C $l^{-1}d^{-1}$). In thermokarstic

Canada there was also a clear separation between winter and summer when almost two orders of magnitude higher production was measured during summer (32.5 ± 4.5 µg C $l^{-1}d^{-1}$) than winter (0.4 ± 0.1µg C $l^{-1}d^{-1}$). BP was significantly higher in Canadian than in Russian thermokarstic ponds (H = 5.3, n = 27, p = 0.021).

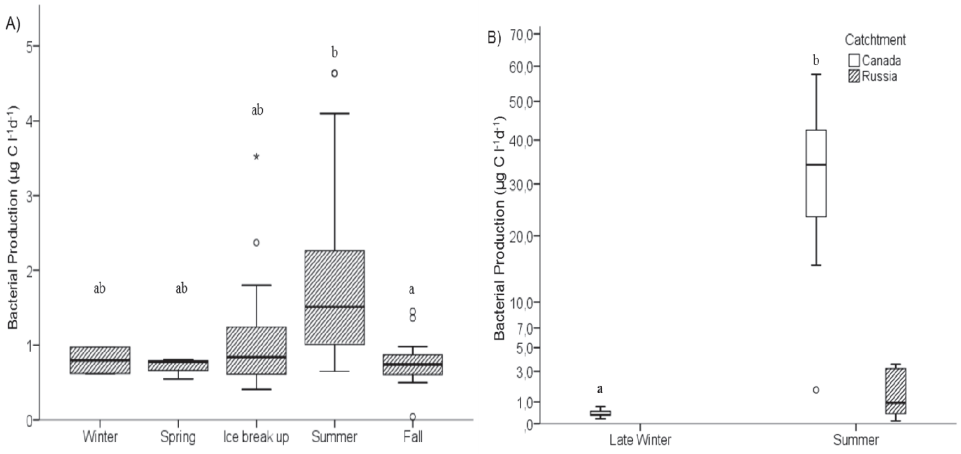

FIGURE 3 Seasonal changes of BP in A) Kilpisjärvi ponds and B) Canadian and Russian thermokarstic ponds. Statistically significant differences ($p < 0.05$) are indicated by different letters above bars.

Significant seasonal change was also observed in BR ($F_{4,36}$ = 6.1, p = 0.001) measured at Kilpisjärvi sites in 2011 (IV). Lowest BR rates were measured in summer (4.6 ± 6.8 µg C $l^{-1}d^{-1}$) and winter (5.0 ± 2.2 µg C $l^{-1}d^{-1}$), and highest values in spring (15.2 ± 5.5 µg C $l^{-1}d^{-1}$) (Fig. 4). Seasonality and uncoupling of BP and BR was clearly seen in BGE ($F_{4,36}$ = 17.6, $p < 0.0001$) that was highest during the productive summer (32.1 ± 7.6 %) period and lower during spring (4.6 ± 4.0 %) and fall (4.7 ± 3.7 %).

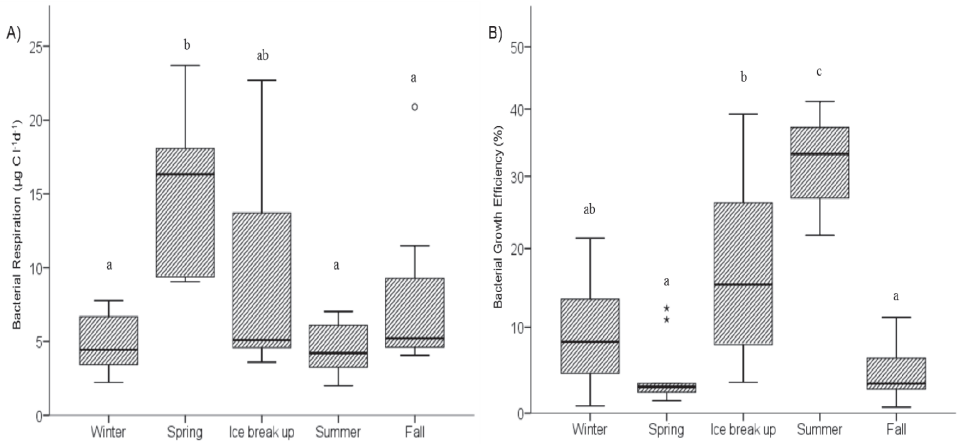

FIGURE 4 Seasonal fluctuations of A) BR and B) BGE in Kilpisjärvi water bodies. Statistically significant ($p < 0.05$) differences are indicated by different letters above bars.

In Kilpisjärvi, the aim was also to study how the landscape (I) and different habitats (IV) influence bacteria metabolism (Fig. 5). Effect of landscape was studied in ponds situated below and above the treeline (600 m a.s.l). Low altitude ponds were situated in dense mountain birch catchments whereas high altitude ponds were located in barren rocky catchments. BP was not significantly impacted by landscape change although higher average production was found in ponds in mountain birch (1.5 ± 1.1 µg C $l^{-1}d^{-1}$) than in ponds in barren rocky catchments (1.1 ± 0.9 µg C $l^{-1}d^{-1}$). Impact of habitat was studied in three different habitat zones: 1) lake inlets representing habitats influenced by allochthonous C arriving to lakes, 2) lake outlets representing C from the in-lake algal production, and 3) ponds containing C with a mixed signature of terrestrial and algal compounds. Habitat had a significant impact on bacterial production ($F_{2,82} = 4.4$, $p = 0.015$). Significantly lower BP rates (Tukey test; $p = 0.013$) were measured from the lake outlets (0.5 ± 0.4 µg C $l^{-1}d^{-1}$) whereas highest rates were found from the small ponds ($1.2. \pm 1.0$ µg C $l^{-1}d^{-1}$).

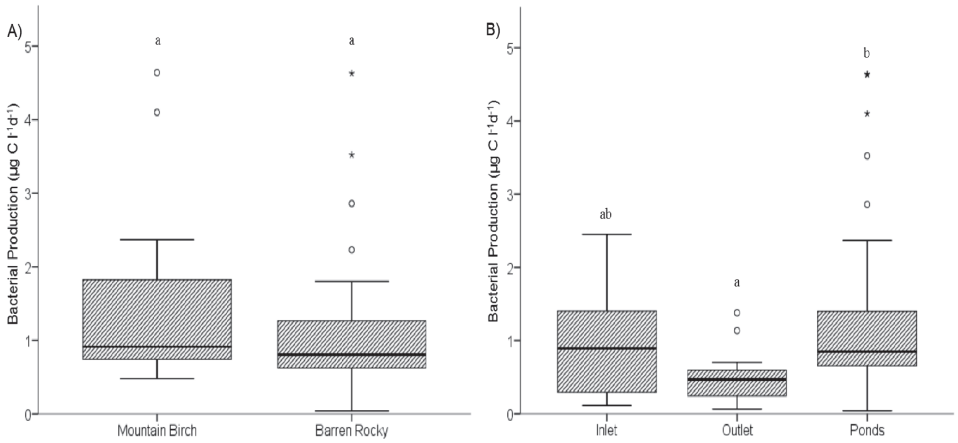

FIGURE 5 BP fluctuations according to A) landscape and B) habitat. Statistically significant differences ($p < 0.05$) are indicated by different letters above bars.

Habitat did not have as strong an impact on BR and growth efficiency as it had on bacteria production (Fig. 6). However, BGE was found significantly different in different habitats ($F_{2,27}$ = 3.5 p = 0.045) (IV). When all respiration data from Kilpisjärvi were included in the data set, the effect was not significant ($F_{2,38}$ = 1.9 p = 0.164) although higher BGEs were still measured from inlets (13.6 ± 13.6 %) and ponds (17.4 ± 14.0 %) than from outlets (8.3 ± 7.9 %).

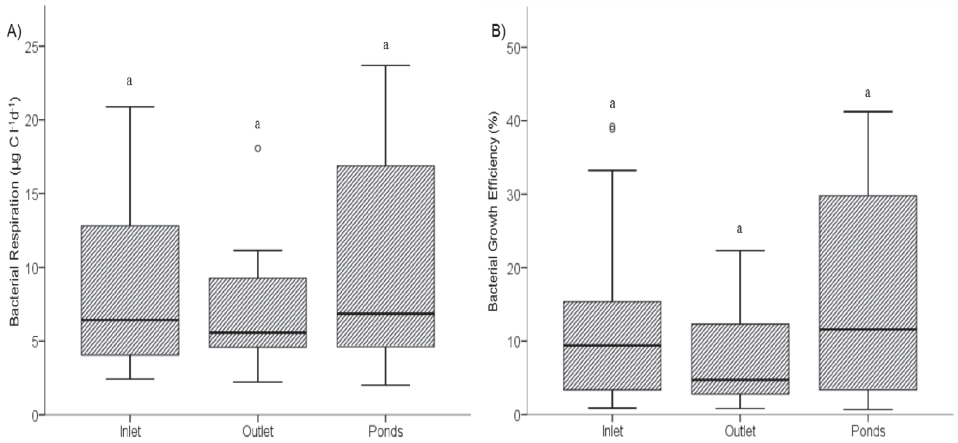

FIGURE 6 Impact of habitat on A) BR and B) BGE in Kilpisjärvi water bodies. Seasonal data are pooled in this figure. Statistically significant differences ($p < 0.05$) are indicated by different letters above bars.

3.2 Bacterial community composition

Seasonal changes in BCC in the Kilpisjärvi sites (Fig. 7) were analysed in 2008 with LH-PCR (I) and in 2011 with 454-pyrosequencing (IV). Significant differences were found among the seasons in 2008 ($F_{2,34}$ = 5.10, $p < 0.001$) but marginally significant difference between water bodies below and above the treeline ($F_{1,34}$ = 1.63, p = 0.059). Seasonal differences were mainly due to distinct bacterial communities found during the spring ice melt (spring vs. summer; t = 2.66, $p < 0.001$ and spring vs. autumn; t=2.61, $p < 0.001$). Similarly to 2008 a significant seasonal change in BCC was found in 2011 (Pseudo-$F_{2,22}$ = 3.64, $p < 0.001$) with all seasons (winter, spring, ice break up, summer, fall) being different from each other apart from the pairs winter-spring, spring-fall and summer-fall.

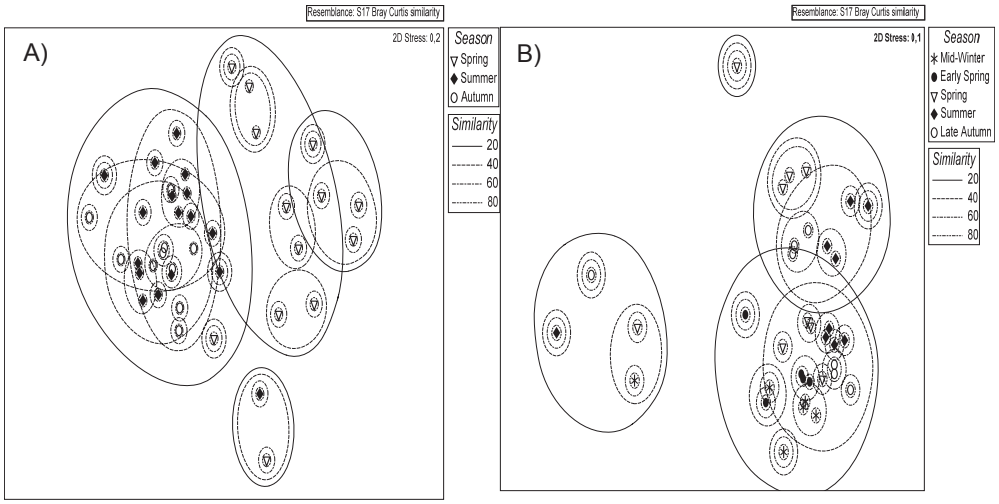

FIGURE 7 Seasonal fluctuation of BCC in Kilpisjärvi ponds, analysed with A) LH-PCR (2008) and B) pyrosequencing (2011), and illustrated with non-metric multidimensional scaling (NMDS). Similarities of sample points are illustrated with cluster analyses.

3.3 Characterization of dissolved organic carbon

Regions differed significantly from each other according to their DOC concentration (DOC; $F_{2,119}$ = 57.4, $p < 0.001$) and quality parameters (SUVA; $F_{2,118}$ = 21.3, $p < 0.001$, HI;$F_{1,106}$ = 84.5, $p < 0.001$, FI;$F_{1,89}$ = 66.9, $p < 0.001$). Lowest DOC concentrations were measured in Kilpisjärvi ponds (3.4 ± 2.4 mg l^{-1}) (Fig. 8) whereas thermokarstic ponds in Kuujjuarapik (6.9 ± 2.1 mg l^{-1}) and especially in Seida (39.6 ± 35.8 mg l^{-1}) were rich in DOC. Also quality of DOC changed according to region. DOC pigmentation (SUVA) was significantly higher in Kuujjuarapik (4.5 ± 2.6 mg C $l^{-1}m^{-1}$) and Seida (4.4 ± 1.8 mg C $l^{-1}m^{-1}$) than in

Kilpisjärvi (2.5 ± 0.8 mg C $l^{-1}m^{-1}$). Also DOC had a higher degree of humification and was of more microbial origin in Kilpisjärvi (HI; 0.89 ± 0.15, FI; 1.24 ± 0.10) than in Kuujjuarapik (HI; 0.53 ± 0.24) and in Seida (FI; 0.99 ± 0.06).

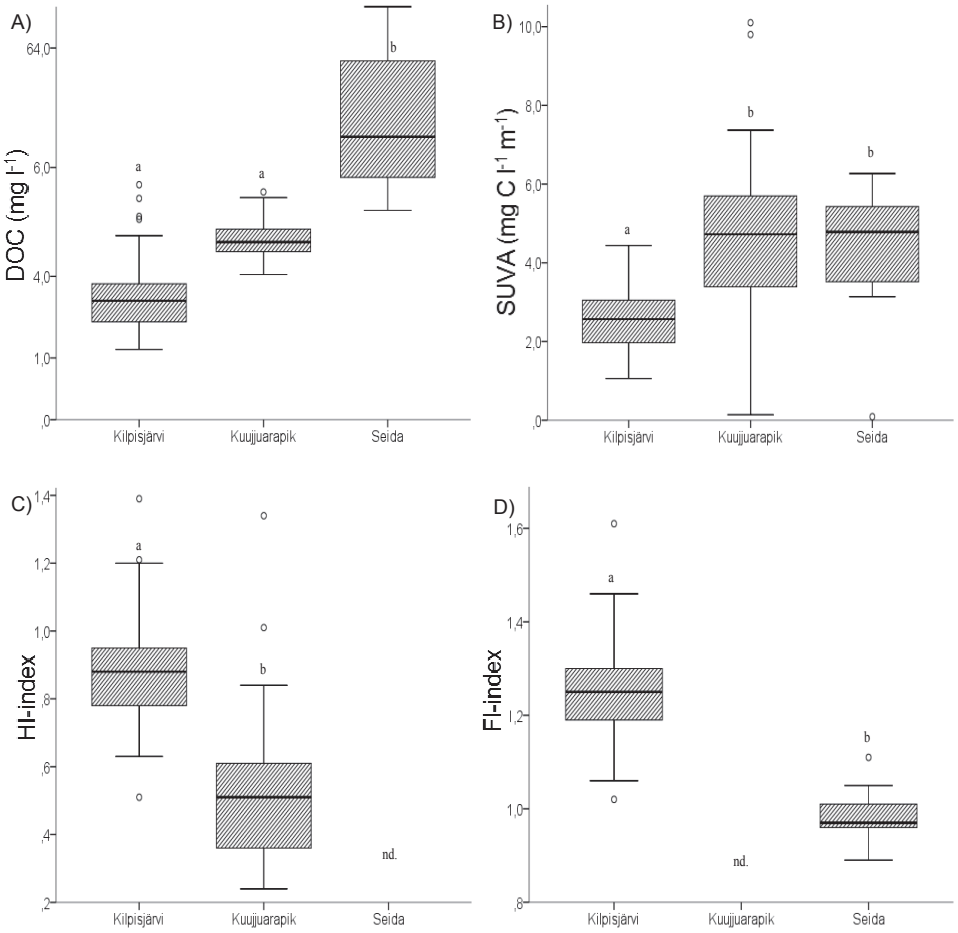

FIGURE 8 Regional fluctuations of A) DOC, B) SUVA, C) HI and D) FI. Statistically significant differences ($p < 0.05$) and cases with no data available (nd.) are indicated by different letters above bars.

There was no change in DOC concentration whereas all the DOC quality indices fluctuated significantly according to season in Kilpisjärvi ponds (SUVA: $F_{4,80} = 6.0$, $p < 0.001$; HI: $F_{4,76} = 18.0$, $p < 0.001$; FI: $F_{4,76} = 9.0$, $p < 0.001$) (Fig. 9). A significant change in SUVA takes place during the ice melt periods (ice-break) when pigmented humic terrestrial DOC enters the waterbodies. Fluorometric properties indicated that terrestrial inputs had a higher degree of humification and had more terrestrial than microbial origin during ice-break.

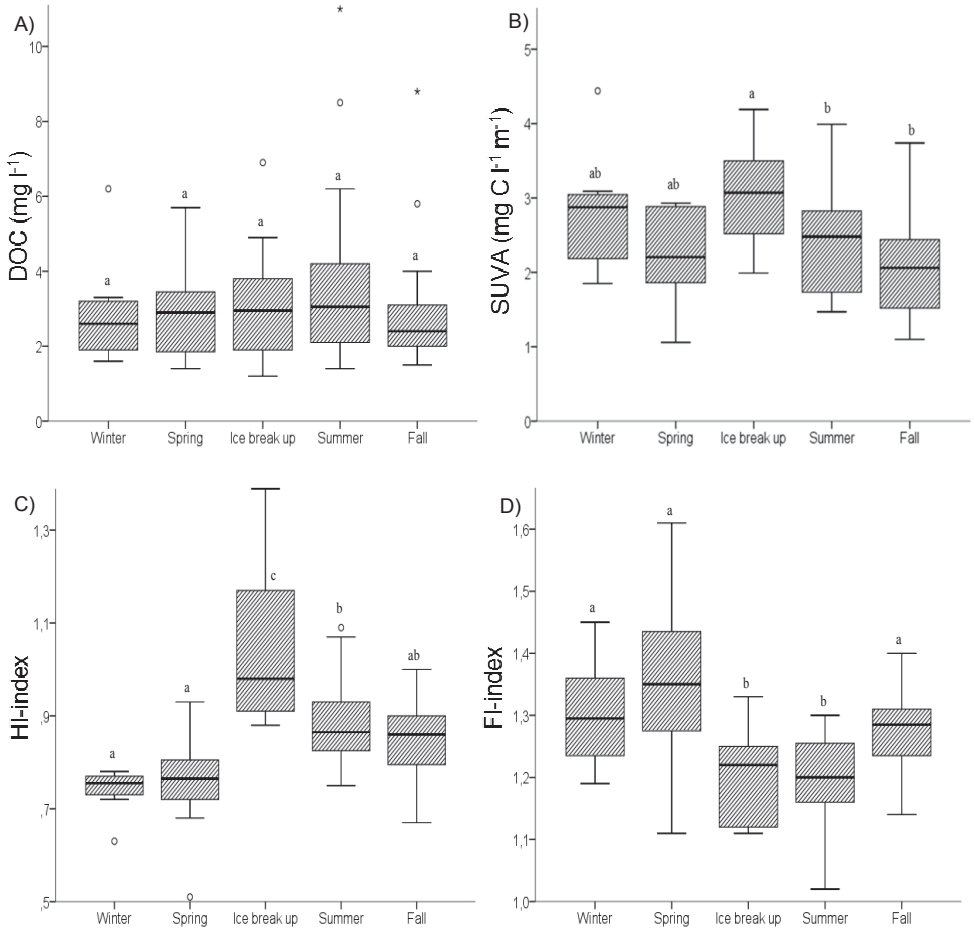

FIGURE 9 Seasonal fluctuations of A) DOC, B) SUVA, C) HI and D) FI in the Kilpisjärvi ponds. Statistically significant differences ($p < 0.05$) are indicated by different letters above bars.

Significant seasonal change in quality of DOC between winter and summer was also observed in humic Canadian thermokarstic ponds in Kuujjuarapik. During late winter the DOC was significantly less pigmented (SUVA: H=13.3, n=26, p<=0.001) and had a higher degree of humification (HI: H=11.2, n=26, p=0.001) although the amount of DOC did not change significantly between the seasons. This indicates there were terrestrial inputs from the dense shrub catchment during the open water season and also rapid degradation by microbes and UV-radiation (Fig. 10).

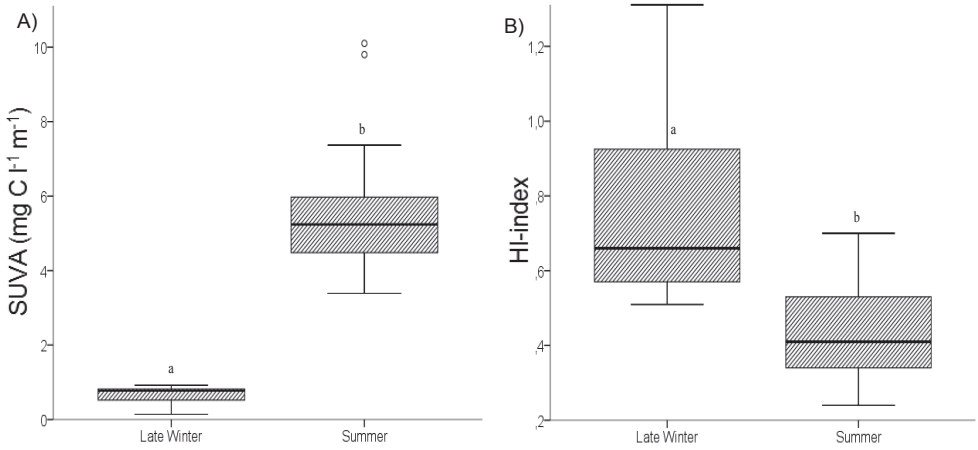

FIGURE 10 Seasonal fluctuations of A) SUVA and B) HI in the Kuujjuarapik ponds. Statistically significant differences ($p < 0.05$) are indicated by different letters above bars.

Catchment type had a significant impact on quantity (DOC: $F_{1,83} = 13.4$, $p < 0.001$) and quality of DOC (SUVA: $F_{1,83} = 4.2$, $p = 0.043$; FI: $F_{4,79} = 9.8$, $p = 0.002$) in Kilpisjärvi ponds (Fig. 11). Concentration of DOC was significantly higher in ponds with mountain birch catchments (4.1 ± 2.5 mg l^{-1}) than in ponds with barren rocky catchments (2.3 ± 0.7 mg l^{-1}). Also, according to optical indices, DOC derived from mountain birch catchments was more pigmented and had a stronger terrestrial signal than DOC from barren rocky catchments.

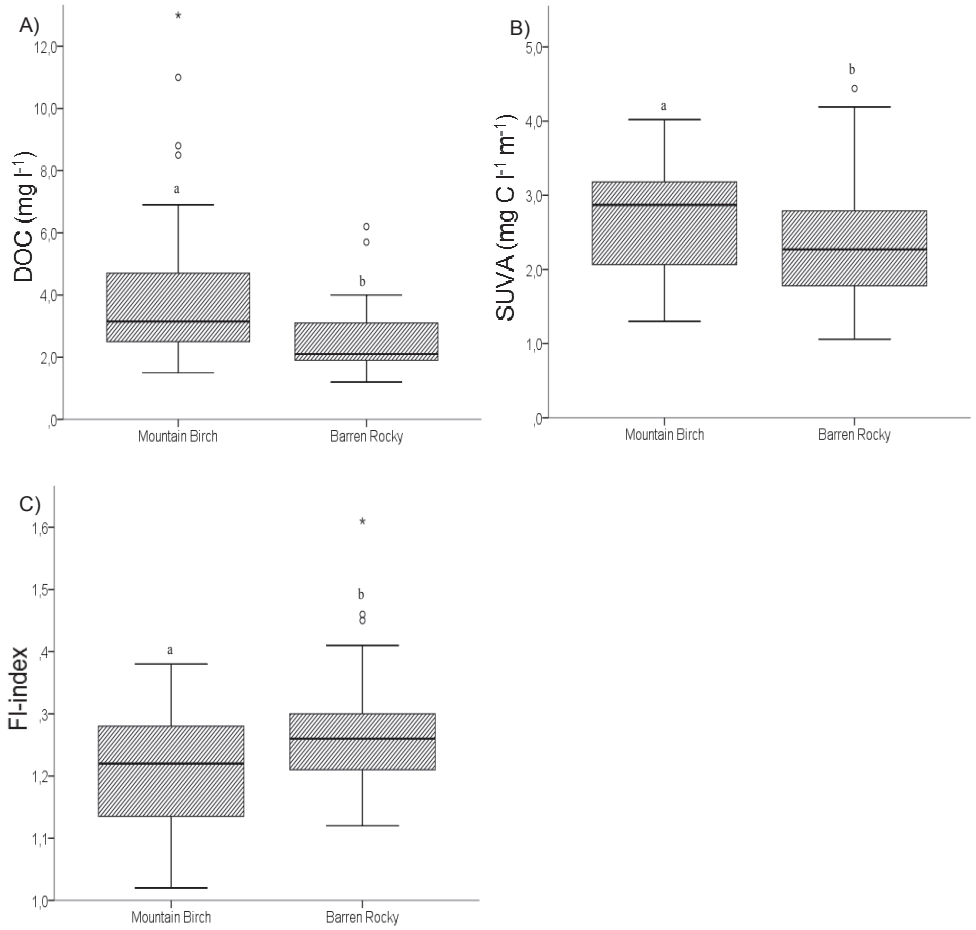

FIGURE 11 Fluctuations of A) DOC, B) SUVA and C) FI according landscape change in Kilpisjärvi. Statistically significant differences ($p < 0.05$) are indicated by different letters above bars.

Habitat had a significant influence in both concentration (DOC: $F_{2,83} = 4.3$ $p = 0.018$) and quality (HI: $F_{2,78} = 7.2$ $p = 0.001$) of DOC. More DOC with a higher content of aromatic structures was found from pond habitats than from lake inlets and outlets. Also, DOC in lake inlets seemed to be more terrestrially derived (SUVA: 2.8 ± 0.5) and the fulvic acids (FI: 1.28 ± 0.07) were more microbially produced than in outlets (SUVA: 2.4 ± 0.5, FI: 1.23 ± 0.05) or ponds (SUVA: 2.4 ± 0.9, FI: 1.23 ± 0.11), although the difference was not statistically significant (Fig. 12).

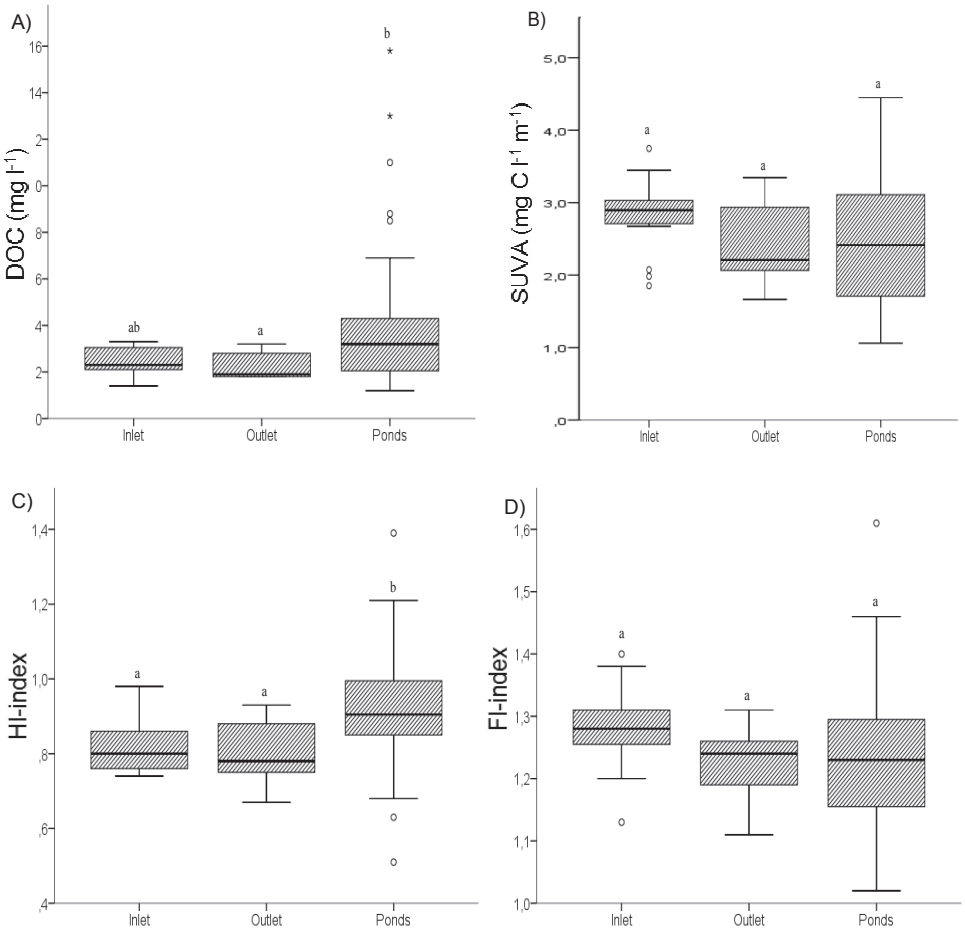

FIGURE 12 Fluctuations of A) DOC, B) SUVA, C) HI and D) FI with habitat in Kilpisjärvi ponds. Statistically significant differences ($p < 0.05$) are indicated by different letters above bars.

Kilpisjärvi sites were also analysed for individual DOC compounds in 2011 (IV). Seven individual fluorescence components were identified from 331 EEMs analysed from lakes and ponds across boreal to arctic landscapes (Fig. 13). Components were identified according to the literature (Fellman *et al.* 2010 and refs therein) and grouped to represent terrestrially (C1, C2, C3, C4 and C6) and microbially (C5) induced humic-like C and protein-like tryptophan-like C (C7).

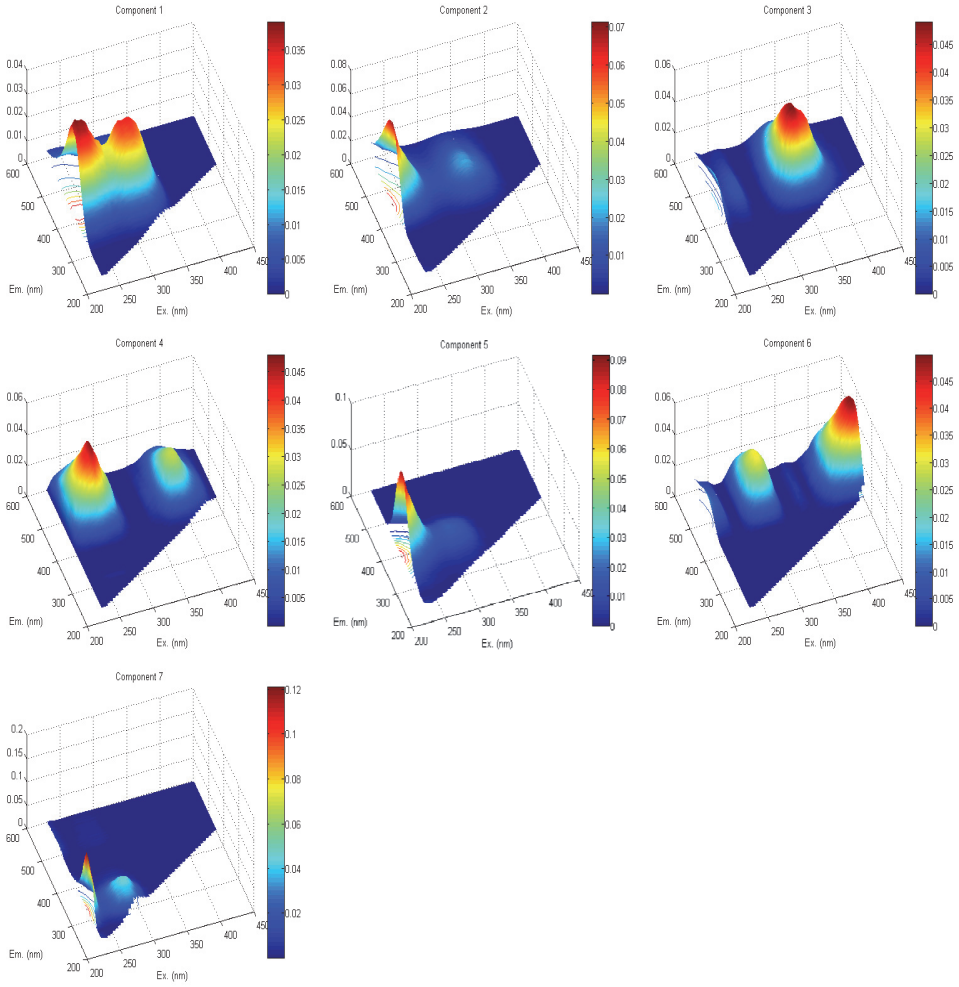

FIGURE 13 Fluorescence signatures of components C1–C7 identified from boreal and subarctic EEM scans. Components 1-4 and 6 (C1–C4 and C6) were combined to represent terrestrial humic-like components whereas component C5 was identified as fulvic microbial component and a commonly found component C7 as a protein-like (Tryptophan) component. Identification is based on Fellman et al. (2010) and refs therein.

Similarly to photo- and fluorometric indices, seasonal change in fluorescence components was identified with the proportion of protein-like clearly decreasing during the open water season whereas there was an increase especially in humic-like C of terrestrial origin, although the change was not statistically significant. From a habitat perspective, lake inlets were more influenced by terrestrial C than lake outlets and ponds (Fig. 14). Habitat had a significant influence on humic-like components C3 ($F_{2,39}$ = 4.7 p = 0.015), C4 ($F_{2,39}$ = 3.4 p = 0.044) and C6 ($F_{2,39}$ = 5.8 p = 0.006).

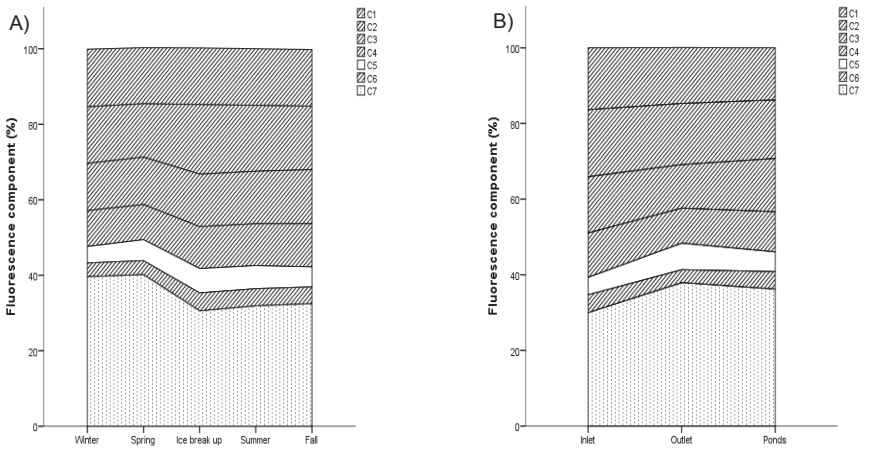

FIGURE 14 Fluctuation of fluorescence components according to A) season and B) habitat change in Kilpisjärvi.

3.4 Carbon control of bacterioplankton metabolism

In larger data sets DOC is a good predictor of bacterial metabolism (Sobek *et al.* 2003), but it does not provide any information about the source and quality of the C. Spectrophotometric and spectrofluorometric properties have been used to estimate quality and origin of DOC and have been found to explain changes in bacterial metabolism better than a simple DOC concentration measurement (McKnight *et al.* 2001, Weishaar *et al.* 2003, Guillemette & del Giorgio 2012, Laurion & Mladenov 2013).

Data from both quantity and quality of DOC (SUVA) had a positive correlation with BP. Quantity of DOC played a significant role in BP, especially in subarctic Kilpisjärvi, and in the larger Kilpisjärvi data set used in the synthesis quality of DOC (HI) was also significantly correlated with BP. In Kilpisjärvi microbially produced DOC also had a significant negative correlation with BP. One of the main differences between the clearwater Kilpisjärvi ponds and the thermokarstic ponds in Kuujjuarapik and Seida environments was the low supply of DOC in clearwater environments (DOC 3.2 ± 0.2 mg l^{-1}) and the much higher supply in the thermokarstic systems (DOC 18.9 ± 5.1 mg l^{-1}). Thermokarstic ponds in Canada were uninfluenced while the ponds in Russia were even negatively influenced by DOC, indicating that BP was regulated by factors other than DOC concentration. One other factor, terrestrial DOC, had significant correlations with BP in both Kuujjuarapik (SUVA and HI), and Seida (FI) (Table 2).

TABLE 2 Spearman's correlations between BP and carbon quantity (DOC) and quality (SUVA, HI, FI, TerC, MicbC and ProtC). Correlation results are presented for all available and spatially divided data. Correlation coefficient (R_s), p-value (p) and number of samples in analysis (n).

		All	Spatial *Kilpisjärvi*	Spatial *Seida*	Spatial *Kuujjuarapik*
DOC	Rs	0.322	0.286	-0.663	
	p	0.001	0.008	0.037	ns
	n	112	85	10	
SUVA	Rs	0.342			0.659
	p	< 0.001	ns	ns	0.004
	n	112			17
HI	Rs		0.264		-0.593
	p	ns	0.017	ns	0.012
	n		81		17
FI	Rs			-0.677	
	p	ns	ns	0.032	ns
	n			10	
TerC	Rs				
	p	ns	ns	nd	nd
	n				
MicbC	Rs	-0.367	-0.367		
	p	0.018	0.018	nd	nd
	n	41	41		
ProtC	Rs				
	p	ns	ns	nd	nd
	n				

ns = Spearman's correlation not significant
nd = data not available

In the Kilpisjärvi region the quantity of DOC had a significant positive correlation with BP during summer (Table 3). At the same time, terrestrial components had a strong positive correlation with BP while microbial and protein components were negatively correlated with BP. The carbon quality index indicating terrestrial C (SUVA) had a significant positive correlation during winter but a significant negative correlation during fall. During fall HI also had a significant positive correlation with BP. Overall BR, like BP, correlated with DOC concentration in Kilpisjärvi, but in the case of BR strong seasonal correlations between DOC and BR were found during ice-break that also seemed to be connected to terrestrial components of DOC (Table 4). Also BR was found to correlate negatively with the protein component. No significant seasonal changes were found in the relation between BGE and HI (Table 5).

Significant differences were also found between the Kilpisjärvi catchments. BP in waterbodies below the treeline was strongly influenced whereas BP in the waterbodies above the treeline was unaffected by DOC (I). In the mountain birch area C quality indexes suggested that DOC enhancing BP was mainly less recalcitrant (HI) and was likely derived from terrestrial sources (FI). This could suggest the importance of terrestrial inputs on productivity of bacteria. A similar indication of the importance of terrestrial C (subarctic DOM) in increasing BP was found in the C-addition experiment that took place in clearwater Lake Saanajärvi located above the treeline in Kilpisjärvi (II). BR and BGE were not significantly

correlated to C parameters in ponds in mountain birch areas. Concentration of DOC did not correlate with BP in ponds with barren rocky catchments, but there was a significant positive correlation found between BR and DOC. On the other hand, BGE was significantly correlated with C quality indices (HI and FI).

BP did not correlate with DOC quantity according to habitat, but there was a significant positive correlation between BR and DOC measured in ponds. In lake inlets BP correlated with less recalcitrant C (HI) that was likely of microbial origin (MicbC). A similar situation in lake inlets was found between BGE and less recalcitrant C. In ponds, terrestrial C was negatively correlated with BGE. BR did not correlate with any of the C parameters.

TABLE 3 Spearman's correlations between BP and carbon quantity (DOC) and quality (SUVA, HI, FI, TerC, MicbC and ProtC) in Kilpisjärvi region. Correlation results are presented for data divided according to season (1=winter, 2=spring, 3=ice break up, 4= summer and 5=fall), landscape (1=mountain birch and 2=barren rocky) and habitat (1=inlet, 2=outlet and 3=pond). Correlation coefficient (Rs), p-value (p) and number of samples in analysis (n).

		Seasonal					Landscape		Habitat		
		1	2	3	4	5	1	2	1	2	3
DOC	Rs				0.465		0.635				
	p	ns	ns	ns	0.022	ns	0.001	ns	ns	ns	ns
	n				24		40				
SUVA	Rs	0.714				-0.681					
	p	0.047	ns	ns	ns.	0.001	ns	ns	ns	ns	ns
	n	8				21					
HI	Rs					0.490	0.382		0.639		
	p	ns	ns	ns	ns	0.028	0.015	ns	0.010	ns	ns
	n					20	40		15		
FI	Rs						-0.341				
	p	ns	ns	ns	ns	ns	0.031	ns	ns	ns	ns
	n						40				
TerC	Rs				0.862						
	p	ns	ns	ns	0.003	ns	ns	ns	ns	ns	ns
	n				9						
MicbC	Rs				-0.697				-0.523		
	p	ns	ns	ns	0.037	ns	ns	ns	0.045	ns	ns
	n				9				15		
ProtC	Rs				-0.879						
	p	ns	ns	ns	0.002	ns	ns	ns	ns	ns	ns
	n				9						

ns = Spearman's correlation not significant

TABLE 4 Spearman's correlations between BR and carbon quantity (DOC) and quality (SUVA, HI, FI, TerC, MicbC and ProtC) in Kilpisjärvi region. Correlation results are presented for all available data (A), and data divided according to season (1=winter, 2=spring, 3=ice break up, 4= summer and 5=fall), landscape (1=mountain birch and 2=barren rocky) and habitat (1=inlet, 2=outlet and 3=pond). Correlation coefficient (R_s), p-value (p) and number of samples in analysis (n).

			Seasonal					Landscape		Habitat		
		A	1	2	3	4	5	1	2	1	2	3
DOC	Rs	0.367			0.867				0.524			0.897
	p	0.018	ns	ns	0.002	ns	ns	ns	0.010	ns	ns	0.002
	n	41			9				23			9
SUVA	Rs											
	p	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	n											
HI	Rs											
	p	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	n											
FI	Rs											
	p	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	n											
TerC	Rs				0.867							
	p	ns	ns	ns	0.002	ns	ns	ns	ns	ns	ns	ns
	n				9							
MicbC	Rs											
	p	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	n											
ProtC	Rs				-0.667							
	p	ns	ns	ns	0.050	ns	ns	ns	ns	ns	ns	ns
	n				9							

ns = Spearman's correlation not significant

TABLE 5 Spearman's correlations between BGE and carbon quantity (DOC) and quality (SUVA, HI, FI, TerC, MicbC and ProtC) in Kilpisjärvi region. Correlation results are presented for all available data (A), and data divided according to season (1=winter, 2=spring, 3=ice break up, 4= summer and 5=fall), landscape (1=mountain birch and 2=barren rocky) and habitat (1=inlet, 2=outlet and 3=pond). Correlation coefficient (R_s), p-value (p) and number of samples in analysis (n).

		Seasonal						Landscape		Habitat		
		A	1	2	3	4	5	1	2	1	2	3
DOC	Rs											
	p	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	n											
SUVA	Rs											
	p	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	n											
HI	Rs	0.347							0.447	0.541		
	p	0.028	ns	ns	ns	ns	ns	ns	0.037	0.046	ns	ns
	n	40							22	14		
FI	Rs								-0.536			-0.736
	p	ns	ns	ns	ns	ns	ns	ns	0.010	ns	ns	0.004
	n								22			13
TerC	Rs											
	p	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	n											
MicbC	Rs											
	p	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	n											
ProtC	Rs											
	p	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	n											

ns = Spearman's correlation not significant

3.5 Carbon control of bacterial community composition

Carbon also played an important role in structuring the BCC, with DOC, fulvic acids (C5) and proteins (C7), together with TP, explaining most variation of overall OTU distribution (IV). However, all C compounds correlated with some individual OTU. Similar OTUs were abundant when terrestrial humic-like components (C1-C4 and C6) were present. Very different OTUs were correlated with fulvic acids (C5) that are degraded (microbially or photochemically) humic-like compounds. BCC connection to protein-like C (C7) more resembled that of terrestrial compounds (Fig. 15). It is known that tryptophan-like C (C7) is unaffected by solar and microbial degradation (Stedmon & Markager 2005,

Laurion & Mladenov 2013) and its fluorescence signal rarely resembles pure tryptophan (Cory & McKnight 2005, Maie *et al.* 2007, Yamashita & Tanoue 2008). Therefore C7 could play a double role in the environment: as a substrate and as a degradation end-product.

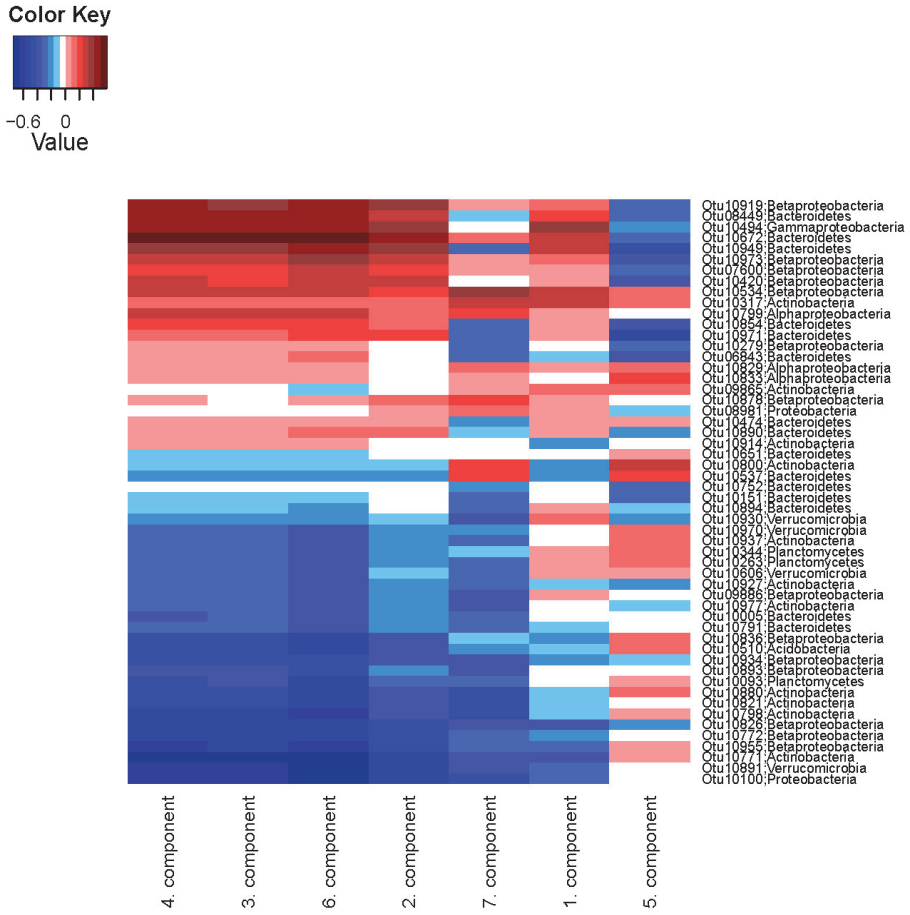

FIGURE 15 BCC Spearman correlations with carbon fluorescence components in Kilpisjärvi ponds.

3.6 Phototrophic vs. heterotrophic energy pathways

In the Kilpisjärvi region the role of different DOM additions were tested in an enclosure experiment (II). DOM additions increased the ratio between autotrophic and mixotrophic production (autotrophic:mixotrophic) in all enclosures (Fig. 16). Meanwhile the PNF:HNF abundance ratio shifted towards the heterotrophic

pathway. Although autotrophic epilimnetic production increased during the experiment, the overall production scaled to the whole water column was shifted toward heterotrophy due to increased light attenuation by the added DOM. The number of species known to be mixotrophic also increased in the enclosures.

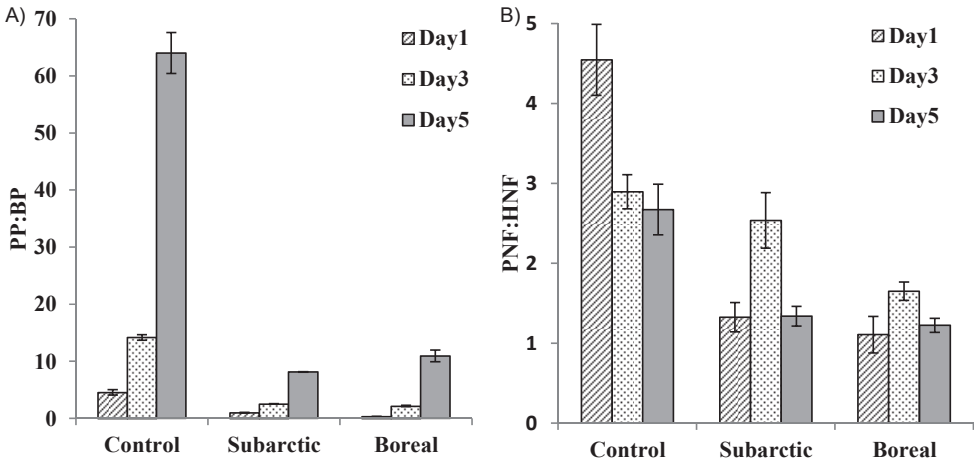

FIGURE 16 Shifts in epilimnetic autotrophic:mixotrophic versus heterotrophic processes over 5 days following DOC addition (subarctic and boreal DOC) in a subarctic Finnish lake in A) PP:BP and B) PNF:HNF abundance. Error bars represent SE.

Measured PP:BP in Kuujjuarapik thermokarstic ponds pointed to a strong heterotrophic dominance but PNF:HNF indicated to phototrophic reliance (III). During late winter, in situ PP was prevented by snow and ice cover, but when exposed to light phytoplankton C production reached the same rates as heterotrophic production, suggesting the occurrence of mixotrophic species (Fig. 17). During summer the conditions were favourable for bacterioplankton and heterotrophic production that dominated especially in the bottom waters. PNF biomass always exceeded the HNF biomass even in the bottom. Phytoplankton communities studied in the area have been dominated by flagellate Chrysophyceae species (Dupont 2009). Mixotrophic flagellates in steeply stratified ponds can likely benefit from their diurnal migration (Jones 1991) by accessing the nutrient-rich bottom waters (Jones 1991) and by using the bottom as a refuge from the zooplankton grazing (M.Sc. M. Wauthy, Université du Québec à Chicoutimi).

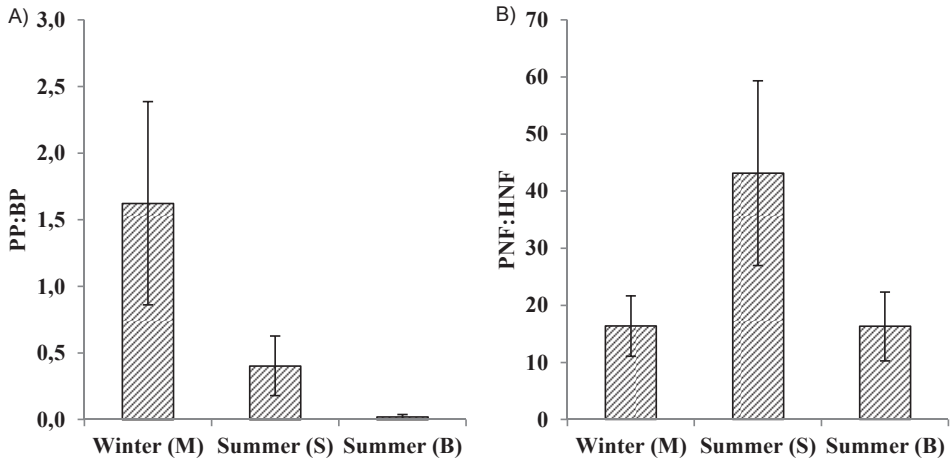

FIGURE 17 Shifts in autotrophic:mixotrophic versus heterotrophic processes in Canadian subarctic thermokarstic ponds sampled in winter at mixed water column (M) and during summer at the surface (S) and bottom (B) in A) PP:BP and B) PNF:HNF abundance ratio. Error bars represent SE.

4 CONCLUSIONS

This thesis demonstrates seasonal and spatial changes in bacterioplankton communities and C transfer in clearwater and thermokarstic subarctic aquatic environments. The role of bacterioplankton in C cycling was investigated using productivity, respiratory and community composition analysis and their changes were related to DOC quantity and quality. Analyses indicated that bacterial metabolism and community composition were strongly connected to DOC. Due to low overall DOC inputs in clearwater systems, both DOC quantity and quality were important, whereas in thermokarstic ponds with high DOC only quality was important to bacterioplankton. Seasonally both bacterial and C variables showed greatest variation between the winter ice season and the open water season with the most distinct change during ice-melt.

In subarctic clearwater systems, BP and BR were uncoupled but DOC quantity and quality still had a strong impact on both processes. In the combined data set, BP was correlated with DOC concentration and with DOC of terrestrial origin (SUVA), but it seems that the limiting factors can vary from DOC quantity (I) to TP (19 %) and to the terrestrial component of DOC (7 %) (IV). Although BR was correlated with DOC quantity, results from the multiple regression analysis indicated that variation in BR was mainly explained by TN concentration (66 %) and chl-*a* (21 %) (IV). This could indicate high respiratory and cell maintenance costs created by production of nutrient-cleaving enzymes. In thermokarstic ponds, DOC quantity was not correlated with BP, whereas the quality of DOC (terrestrial origin) in Canadian thermokarstic ponds substantially increased the microbial production. In Russian thermokarstic waterbodies C was not a limiting factor for BP. This was likely due to the different characteristics of the DOC. Although SUVA indicated that pigmentation of DOC was not significantly different between the thermokarstic sites, it is likely that DOC in Russia was much older that the Canadian counterpart and hence was not as easily accessible. Similarly two orders of magnitude lower production rates point to lower microbial degradation in Russian thermokarstic systems.

Both season and habitat were found to control BCC in subarctic Kilpisjärvi ponds. Seasonal changes were most often observed during spring (approximately

the time of ice-break). On the other hand, habitat seemed to have an impact on BCC diversity. In ponds there was a less even distribution and fewer species, whereas in larger lakes the diversity of bacteria was significantly higher. BCC was also likely influenced by temperature and the quality of substrates and nutrients. DOC quality differences were seen especially in smaller water bodies and were likely contributing to variation in BCC (I, IV). Also links between individual bacterial tribes and different DOC fractions were found (IV).

Terrestrial C is often considered a poor substrate due to its lack of essential fatty acids (Brett *et al.* 2009, 2012). Still our studies showed that terrestrial C was often strongly connected with bacterial metabolism, especially with production, probably for several reasons. The Kilpisjärvi waters were clear and had a very limited supply of DOC, causing a situation where both C quantity and quality could be limiting for organisms. In these systems, due to 24 h radiation in summer and low light attenuation in the water column, terrestrial C goes through a photochemical degradation process that is known to make higher molecular weight terrestrial humic-like C more available to organisms (Stedmon & Markager 2005, Laurion & Mladenov 2013). During summer there is also more available labile DOC produced by phytoplankton that could act as a primer for the use of more recalcitrant C (Bianchi 2011, Danger *et al.* 2013). Terrestrial C was also important in the thermokarstic systems although these were not limited by the amount of C.

Generally most oligotrophic lakes are considered heterotrophic based on their net emissions of greenhouse gases (del Giorgio *et al.* 1997), but oligotrophic relatively shallow water bodies can be considered truly autotrophic due to their high benthic production (Andersson & Brunberg 2006). Also in oligotrophic systems primary production and heterotrophic production tend to be coupled with primary production (Hobbie & Laybourn-Parry 2008).

The sensitivity of the clear water ecosystems in Kilpisjärvi to increases in C was tested with a DOC addition experiment where DOC concentration was doubled. Experiments showed that strongly phototrophic systems moved significantly towards heterotrophy. Heterotrophic microbial production increased in subarctic DOC addition treatments but a more drastic change was the decrease in photic layer depth that caused a decrease in the overall photosynthetic production. The phytoplankton community also changed towards a more mixotrophic community that could benefit from the increased DOC concentration. These changes in energy pathways indicate that the importance of secondary production can increase in cases of large environmental changes (II).

Energy pathways in thermokarstic ponds are far less studied, but due to their high DOC concentration and light attenuation they are assumed to resemble boreal humic lakes. The PP:BP ratio measured in Kuujjuarapik thermokarstic ponds (III) pointed to strong heterotrophic pathways, whereas the PNF:HNF ratio suggested phototrophic reliance. This contradiction is likely explainable by the phytoplankton community composition, which mostly consisted of mixotrophic species (Dupont 2009, Dr. Laura Forsström, University of Helsinki, pers. comm.).

In cases of large environmental changes (e.g. brownification, global change) bacterioplankton communities at the base of the food chain are the first to react.

Subarctic regions have a huge stock of organic C stored in their soils and a warming climate is expected to mobilize these stocks and to increase the delivery of terrestrial C to aquatic systems. Heterotrophs are predicted to benefit from this DOC addition meaning that more energy would cycle trough this trophic step causing significant changes to ecosystems. Therefore understanding the microbial heterotrophic processes and assessment of their sensitivity are crucial to gain a complete picture of subarctic food web interactions.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Hiilen vaikutus bakteeriplanktoniin subarktisissa järvissä ja lammissa

Subarktisilla leveyspiireillä vesimuodostumat vaihtelevat tiheän ja runsaan valuma-aluekasvillisuuden omaavista tummavetisistä palsasuolammista karujen kivikkoisten valuma-alueiden isoihin kirkasvetisiin järviin. Omat erityispiirteet pohjoisille vesistöille luovat myös huomattavan voimakkaat vuodenaikaiset ympäristöolosuhteiden (lämpötila, valo, hiili ja ravinteet) vaihtelut. Lisäksi ilmastonmuutoksen vaikutusten (lämpötila ja sadanta) on ennustettu olevan voimakkaimpia juuri pohjoisilla leveyspiireillä. Kaikilla näillä osatekijöillä on merkittävä vaikutus bakteerien metaboliaan (tuotanto ja hengitys) sekä yhteisörakenteeseen. Tämän väitöskirjan tarkoituksena on arvioida hiilen määrän ja laadun muutoksien vaikutusta subarktisien mikrobiyhteisöjen metaboliaan ja rakenteeseen vuodenaikaisesti, kokeellisesti (DOC-lisäys) sekä erilaisilla valuma-alueilla.

Niukkaravinteisella valuma-alueella mikrobituotanto ja hengitys olivat toisistaan riippumattomat ja lisäksi ne hyödynsivät eri osia liuenneesta orgaanisesta hiilestä. Mikrobien tuottaman hiilen määrä oli korkeimmillaan alku- ja keskikesällä, jolloin sulamisvesien takia lisääntynyt valuma-alueelta peräisin olevan hiilen määrä kiihdytti merkitsevästi tuotantoa. Alkukeväällä ennen jäiden lähtöä mitatut mikrobihengitysarvot olivat taasen enemmän riippuvaisia liuenneen orgaanisen hiilen sekä ravinteiden määrästä.

Subarktisille alueille ilmastonmuutosmallit ennustavat nykyistä korkeampia lämpötiloja ja lisääntyvää sadantaa, jotka lisäisivät valuma-alueelta tulevan liuenneen hiilen määrää. DOC-lisäyskokeessa mikrobituotanto kasvoi merkitsevästi, mutta erityisesti hiilen lisäys aiheutti valaistuksen vähenemistä, joka muutti koealtaita selkeästi heterotrofiseen suuntaan.

Kilpisjärven alueella bakteeriyhteisöjen muutokset olivat merkitsevästi yhteydessä vuodenaikaisuuteen sekä kasvupaikkaan. Vuodenaikaisuuden vaikutus näkyi bakteeriyhteisöjen koostumuksessa, kun taas kasvupaikan vaikutus lajirunsaudessa. Tämän lisäksi lämpötila sekä ravinteiden ja substraattien laatu vaikuttivat bakteeriyhteisöjen koostumukseen.

Ikiroudan sulamisvaihtelun muodostamissa termokarstisissa lammikoissa bakteeriplankton oli myös merkitsevästi linkittynyt hiilen laatuun, esim. kanadalaisissa termokarstisissa lammikoissa värilliset humusainekset kymmenkertaistivat mikrobien tuotannon. Termokarstiset lammikkoiden on havaittu kuormittavan ilmakehää kasvihuonekaasuilla ja myös tässä tutkimuksessa lammikoissa havaittiin suuria CO_2- ja CH_4-kaasupitoisuuksia.

Yleisesti bakteerimetabolia oli tiukasti linkittynyt liuenneeseen orgaanisen hiileen, vaikkakin vuodenaikaisuus ja valuma-alueiden erot vaikuttivat hiilen kiertoon merkittävästi: niukkaravinteisissa kohteissa sekä hiilen määrä että laatu olivat merkittävässä roolissa, kun taas termokarstisella alueella hiilen laadulla oli selkeästi vaikutus mikrobien metaboliaan.

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ORIGINAL PUBLICATIONS

I

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II

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ABSTRACT: Climate-induced changes in catchment area vegetation and runoff alter the quality and quantity of carbon that enters lakes, with implications for food webs in recipient water bodies. The effect of dissolved organic matter (DOM) on the ratio between heterotrophic and autotrophic biomass and productivity was studied in a subarctic, clear water lake in northern Finland. In a mesocosm experiment, natural DOM from a subarctic bog and a boreal lake was added to the lake water, doubling the initial dissolved organic carbon (DOC) concentration. Optical indices suggested that the subarctic DOM addition was more bioavailable, which was in line with the greater increase in bacterial biomass and production observed in this treatment. Both DOM additions increased the abundance of heterotrophic nanoflagellates (HNF) and decreased primary productivity. They also led to lower ratios of primary to bacterial production, autotrophic to mixotrophic algae and pigmented nanoflagellates (PNF) to HNF relative to the control samples, indicating a shift from a primary production-based food web towards one based on bacterial production. A comparable increase in DOM in the natural environment would lead to a considerable decrease in the euphotic layer and loss of areas available for primary production, resulting in a shift towards a heterotrophic production based food web.

KEY WORDS: Dissolved organic matter · Dissolved organic carbon · Subarctic · Microbial food web

INTRODUCTION

Permafrost thawing, soil erosion and enhanced plant growth throughout the Arctic region (ACIA 2005, Callaghan et al. 2011) all increase the transfer of organic matter from terrestrial to aquatic systems (Schuur et al. 2009). Increased availability of carbon stimulates microbial growth and respiration in lakes (Breton et al. 2009, Berggren et al. 2010a) at the expense of phytoplankton production. Transition to a net heterotrophic metabolic gas balance has been estimated to take place at dissolved organic carbon (DOC) concentrations of around 5 mg l^{-1} (Jansson et al. 2000, Prairie et al. 2002). This suggests that pelagic food webs are likely to be based on autotrophs only in very transparent, oligotrophic, low DOC lakes. Currently, high altitude and high latitude lakes which are receiving more terrestrial carbon to their waters are becoming more heterotrophic systems. Northern lakes are also becoming more DOC rich through other mechanisms, aside from climate-related changes in the catchment. The widespread increase in the concentration of DOC in lakes across the northern hemisphere is known as brownification and has been related to changes in deposition chemistry and catchment acid-sensitivity during the process of recovery from acidification (Monteith et al. 2007).

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Although the impact of DOC on lake food webs has been extensively studied, the focus has generally been on DOC quantity, and only until recently has the effect of the quality of DOC and dissolved organic matter (DOM) on lake secondary productivity begun to receive attention (Pérez & Sommaruga 2006, Berggren et al. 2010a, Guillemette & del Giorgio 2011). The quality of DOM is known to be strongly related to its source (Findlay & Sinsabaugh 2003). Autochthonous carbon (the product of *in situ* biological processes) is considered more labile than (terrestrially derived) allochthonous carbon (Søndergaard & Middelboe 1995). Differences also occur among allochthonous DOM originating from different sources; forest-derived DOM tends to be more heterogeneous, containing a proportion of young, potentially bioavailable compounds, in contrast to slow degrading and more recalcitrant bog-derived DOM (Berggren et al. 2007). Labile DOM has been shown to be more important for bacterial production than refractory DOM (Moran & Hodson 1990, Kritzberg et al. 2004). On the other hand, it seems that bacterial communities will develop according to the quality of available DOM, with some communities being more efficient in metabolizing refractory or high-molecular-weight DOM than others (Docherty et al. 2006).

The heterogeneous nature of DOM has both positive and negative effects on all trophic levels in lakes. A major negative effect of DOM is the reduction of algal growth in response to increased light attenuation (Jones 1992, Karlsson et al. 2009). Even small changes (2 mg l^{-1}) in colored DOM (CDOM) concentration are likely to cause drastic changes in light attenuation and the spectral regime (Vincent et al. 1998), leading to a decrease in the euphotic layer. However, increased DOM levels protect organisms against the harmful effects of UV radiation (Ekelund 1993, Rautio & Korhola 2002). In addition, DOM has been shown to change the availability of inorganic nutrients both directly and indirectly by stimulating bacterial growth (Jackson & Hecky 1980, Stewart & Wetzel 1980, Jones et al. 1988, Moran & Zepp 1997, Klug 2002). Bacterial production is also coupled strongly with DOM quality (Pérez & Sommaruga 2006, Berggren et al. 2010a). In addition to stimulating the microbial food web, DOM can also be an important nutritional source for zooplankton (Salonen & Hammar 1986, De Lange et al. 2003).

Increased allochthonous carbon inputs are expected to have a strong impact on the competition between organisms at the base of the subarctic food web, i.e. bacterio- and phytoplankton. These microbial organisms compete for the same resources (inorganic nutrients) but are able to utilize different carbon sources. While phytoplankton are dependent on solar radiation as an energy source to obtain inorganic carbon, bacterioplankton have the ability to use allochthonous organic carbon sources for carbon mobilization (Moran & Hodson 1990). In general, heterotrophic bacteria account for a higher proportion of planktonic biomass in oligotrophic lakes compared to meso- and eutrophic systems (Biddanda et al. 2001). In oligotrophic clear water lakes in particular, it is expected that increased allochthonous DOM will favor bacterioplankton and shift the ecosystem towards stronger heterotrophy (Kritzberg et al. 2004, Ask et al. 2009).

We tested these predictions experimentally by measuring the effect of adding allochthonous DOM on the biomass and productivity of heterotrophic and autotrophic organisms in an unproductive subarctic lake. As microbes respond to the quality of DOM (e.g. age, molecular structure and size) with highly variable rates and efficiency (Moran & Hodson 1990, Ellis et al. 1999, Berggren et al. 2009), we used DOM from 2 different sources: a boreal lake and a local subarctic bog. For logistic reasons, to avoid the so-called 'bottle effect' and to imitate natural conditions (i.e. terrestrial DOM enters the lakes in pulses following the melting season and/or heavy rainfall), we focused on the short-term effects over a few days. The experiment was conducted just above the local treeline, an ecotone where lake ecosystems are expected to show strong responses to climate change (Vinebrooke & Leavitt 2005). We also selected a lake where the initial DOC concentration was low (2 mg l^{-1}), where minor changes in DOC levels would have a strong impact on the attenuation of light, and where doubling of DOC was expected to shift the basal pelagic production and biomass dominance from algae to bacteria. We hypothesized that an increased amount of allochthonous DOM (as an energy source and through its light-absorption characteristics) would change lake productivity by causing a shift from the dominance of autotrophic to heterotrophic organisms. We further hypothesized that the 2 DOM sources would differ in quality and would therefore lead to differences in bacterial biomass and production. Finally, drawing on the results of the enclosure experiment, we considered how a similar DOC addition would change whole-lake productivity.

MATERIALS AND METHODS

A mesocosm experiment with 9 enclosures (3 controls, 3 × 2 DOM additions) was conducted in August

2008 in Lake Saanajärvi, an oligotrophic clear-water lake situated above the local treeline in northwest Finnish Lapland. The maximum depth of the lake is 24 m, the surface area is 70 ha and the catchment area, covered by sub-alpine vegetation and bare rocky surfaces, is 460 ha. Lake Saanajärvi is a dimictic lake with a short spring overturn followed by a stratification period of circa 1.5 to 2 mo, a thermocline at around 10 m, and an autumn overturn of 1.5 mo (Forsström et al. 2005). Two different DOM sources were used for the experiment: (1) DOM-rich water from a bog located 100 km southeast of the study site (68° 29' N, 22° 16' E), hereafter known as 'subarctic DOM' and (2) humic lake water from the small headwater Lake Mekkojärvi, located in southern Finland (61° 13' N, 25° 08' E), hereafter known as 'boreal DOM'. The lake has a retention time of between a few and 150 d (Arvola et al. 1992) and is surrounded by a spruce *Picea abies* and Scots pine *Pinus sylvestris* forest. Bog-water was taken from the surface of a small pond within the *Sphagnum* bog, and water from Lake Mekkojärvi was taken with a Limnos water sampler from the lake epilimnion. The 2 sources were chosen as DOM from forests and bogs are known to differ in their bioavailability (Berggren et al. 2007). As a consequence of the presence of mycorrhizal plant roots, the DOC pool in forested soils is loaded with fresh photosynthates readily available for bacterial metabolism. In contrast, the organic carbon in bog litter is generally less bioavailable compared to forest litter because it mostly consists of of slow-degrading bryophytic material. For example, *Sphagnum* species, which typically dominate the vegetation, comprise recalcitrant carbon compounds.

Open-top enclosures (diameter 400 mm, height 600 mm) were made from 0.2 mm thick low-density polyethylene (LDPE) film with high light transparency. Enclosures were placed in Lake Saanajärvi as in Galford (2000) and filled with epilimnetic water taken from Lake Saanajärvi and filtered through a <50 µm plankton net to remove zooplankton. Water taken from the bog and from Lake Mekkojärvi was filtered through 0.2 µm cellulose acetate filters to remove bacteria.

All enclosures were filled with a total volume of 42 l with a target DOC concentration for the enclosures with added DOM (+DOM) of 5 mg l^{-1} (approximately 2.5 times the DOC concentration of the lake). Initial DOC concentrations of the subarctic and boreal DOM sources were 27.8 and 20 mg l^{-1}, respectively (Table 1), so to achieve this target the added volumes were 4.7 and 6.5 l, respectively. This diluted the lake water in the +DOM treatments by 10% (subarctic enclosures) and 13% (boreal enclosures) in comparison to the control enclosures. The nutrient concentrations in the added DOM were too small, when diluted with the lake water, to have an influence on biological productivity (subarctic DOM: NO_3 < 2, NO_2 = 7, NH_4 = 7, PO_4 = 7 µg l^{-1}; boreal DOM: NO_3 < 2, NO_2 = 13, NH_4 = 4, PO_4 = 11 µg l^{-1}).

Table 1. Environmental parameters of mesocosms (enclosures) in a subarctic Finnish lake (above) and parameters related to dissolved organic matter (DOM) spectroscopy (below), for control enclosures/open water and for the DOM sources. Values for the enclosures are averages ± SD. Nutrients were analyzed only at the end of the experiment, other parameters were analyzed during every sampling. P-tot: total phosphorus; N-tot: total nitrogen; DOC: dissolved organic carbon; P^*_{max}: maximum chl *a* normalized photosynthetic capacity; E_k: light adaptation parameter, i.e. light intensity at the onset of saturation; α^*: initial slope of the *P-E* curve normalized to chl *a*; a_{CDOM}: absorption coefficient of colored DOM; a_{254}:a_{365}: absorbance ratio between 254 and 365 nm; *S*: spectral slope (of wavelength range shown); S_R: slope ratio, i.e. the ratio of $S_{275-295}$ to $S_{350-400}$; $SUVA_{254}$: specific UV absorbance at 254 nm

	Enclosures		
	Control	+DOM subarctic	+DOM boreal
Environmental parameters			
Temperature	10.5 ± 0.07	10.5 ± 0.06	10.6 ± 0.06
pH	7.5 ± 0.02	7.4 ± 0.01	7.3 ± 0.01
Conductivity (µs cm^{-1})	22.2 ± 0.09	21.7 ± 0.18	21.8 ± 0.06
P-tot (µg l^{-1})	7.3 ± 3.3	9.1 ± 2.9	7.1 ± 1.3
NO_3-N (µg l^{-1})	2.0 ± 0.00	<2	<2
N-tot (µg l^{-1})	153.3 ± 8.8	250.0 ± 40.0	246.7 ± 21.9
DOC (mg l^{-1})	2.08 ± 0.08	4.75 ± 0.17	5.56 ± 0.11
P^*_{max} (mg C mg^{-1} chl *a* h^{-1})	1.14 ± 0.46	0.51 ± 0.19	0.47 ± 0.18
E_k µmol photons (m^{-2} s^{-1})	49.9 ± 20.7	46.3 ± 18.1	50.5 ± 15.6
α^*	0.034 ± 0.01	0.011 ± 0.00	0.009 ± 0.00
	DOC sources		
	Open water	+DOM subarctic	+DOM boreal
Spectroscopy parameters			
DOC (mg l^{-1})	1.9	27.8	20.0
a_{CDOM} at 320 nm m^{-1}	4.5	138.2	140.5
a_{CDOM} at 440 nm m^{-1}	1.0	20.9	23.2
a_{254}:a_{365}	5.1	4.1	4.0
a^*_{CDOM} at 320 nm m^{-1}	2.4	5.0	7.0
$S_{300-650\ nm}$	0.0127	0.0151	0.0142
$S_{275-295\ nm}$	0.0174	0.0117	0.0124
$S_{350-400\ nm}$	0.0130	0.0171	0.0158
S_R	1.34	0.68	0.78
$SUVA_{254}$	2.9	4.5	6.4

Enclosures were sampled on Days 1 (24 h after DOM addition), 3 and 5 after the initiation of the experiment. Water temperature, pH and conductivity were all measured *in situ* using a YSI 63 handheld pH and conductivity meter. Samples for nutrients were only collected at the end of the experiment, and the analyses were carried out by the Lapland Regional Environmental Centre using the standard methods of the National Board of Waters in Finland (SFS 1990, 2004a,b). The concentration of DOC was analyzed as non-purgeable organic carbon at the Lammi Biological Station with a Shimadzu TOC-VCPH Analyzer. Measured indices of heterotrophic biomass and productivity included bacterial biomass and production, and the abundance of heterotrophic nanoflagellates (HNF). Autotrophic measures included chlorophyll *a* (chl *a*), abundance of picoautotrophs and pigmented nanoflagellates (PNF), and abundance, biomass and production of phytoplankton.

DOM absorbance was analyzed from the lake water and from both DOM sources using a Cary 300 UV-Vis spectrophotometer (Varian). For the analysis, 50 ml of sample water was filtered through pre-rinsed 0.2 µm cellulose acetate filters and stored at 4°C in the dark in acid-cleaned and pre-combusted amber glass bottles. DOM absorption was measured in dual-beam mode every 1 nm over the wavelengths 250 to 850 nm and corrected against Milli-Q water and for the absorption offset. The absorption coefficient at 320 nm (a_{CDOM} 320) was used to quantify CDOM, the absorption coefficient at 440 nm as a measure of CDOM color, and DOC-specific a_{CDOM} 320 (a^*_{CDOM} 320) as a proxy of the degree of DOM color. Spectral slopes (S) of different wavelength ranges (275 to 295, 350 to 400 and 300 to 650 nm), as well as the slope ratio (S_R), i.e. the ratio of $S_{275-295}$ to $S_{350-400}$, were calculated to obtain information on DOM quality (Helms et al. 2008). Specific UV absorbance (SUVA) at 254 nm, defined as the absorbance at 254 nm measured in inverse meters (m^{-1}) divided by the DOC concentration (mg l^{-1}) was used to quantify the variation in the source of carbon (Weishaar et al. 2003). We also calculated the absorbance ratio between 254 and 365 nm (a_{254}:a_{365}), which can be used as an additional index of DOM character (Ågren et al. 2008) and has been previously found to correlate with bacterial growth efficiency (BGE) (Berggren et al. 2007, 2009).

Bacterial biomass was calculated as a function of bacterial abundance and biovolume. The abundance was determined from black polycarbonate filters (pore size 0.2 µm, diameter 25 mm) stained with 4',6-diamidino-2-phenylindole (DAPI) (Porter & Feig 1980). Samples were counted under UV excitation with an epifluorescence microscope at 1000× magnification and a minimum of 400 cells were counted from each replicate. Bacterial biovolume estimations were carried out from digital images using the Cell C program (Selinummi et al. 2005) and biovolumes were converted to biomasses using the coefficient 308 fg C μm^{-3} (Fry 1988). Bacterial production was estimated using ^{3}H-leucine (specific activity: 73 Ci $mmol^{-1}$) incorporation (Kirchman et al. 1985). Productivity measurements started 2 to 6 h after the sampling with triplicate samples and duplicate controls. Leucine concentration (30 nM) and incubation time were estimated from Lake Saanajärvi specific saturation and incubation time curves. Samples were incubated under dark and cold (6.4°C) conditions for 3 h. The incubation temperature was approximately 4°C lower than the temperature in the enclosures and likely resulted in a slight but equal underestimation of the bacterial productivity (Adams et al. 2010) in all enclosures. After termination with trichloroacetic acid (TCA: 5% final concentration), the samples were frozen (–20°C) and stored in the dark until centrifuging and radioassaying with a RackBeta scintillation counter as in Smith & Azam (1992).

HNF and PNF were prepared for microscopy by filtering 20 to 50 ml DAPI-stained water through a 0.6 µm 25 mm black polycarbonate membrane as in Safi & Hall (1997). Slides were frozen until examination under UV excitation at 1000× magnification using a Leica Leitz DMRB epifluorescence microscope. Green excitation was used to discriminate between colorless HNF, and PNF with chloroplasts and pigments.

For chl *a* quantification, 500 ml of water from each enclosure was filtered through Whatman GF/F filters and frozen. Filters were later extracted overnight in 10 ml ethanol (90% v/v) and analyzed with a Hitachi F-4000 fluorescence spectrophotometer as in Jefferey & Humphrey (1975).

Abundances of the autotrophic community groups were assessed using 3 different sampling methods; samples were collected separately for picoautotrophs, PNF and phytoplankton abundance. There is a slight overlap in the obtained results as, for example, some autotrophs are included in both PNF and phytoplankton samples. However, there are important functional differences among the 3 categories used and we therefore present the results according to these groupings. Furthermore, as the epifluorescence technique cannot distinguish between cells that were dead at the time of sampling from those that were alive, both Utermöhl counts (Utermöhl

1958) and the epifluorescence technique are required to estimate abundance.

Water samples (50 ml) for the analysis of pico-autotrophs were first pre-filtered through 3 µm porosity membranes and then under low pressure onto 0.2 µm Anodiscs, mounted on microscope slides with immersion oil and stored at −20°C. The number of cells (minimum of 400 cells on each slide) was counted with a fluorescence microscope at 1000× magnification using a green excitation filter set to detect chlorophyll autofluorescence.

Phytoplankton abundance, biovolume and species composition were analyzed from Lugol-preserved samples using the Utermöhl technique (Utermöhl 1958). In addition to sampling the enclosures, phytoplankton was analyzed from the open water adjacent to the enclosures to assess the effect of the experimental design. The phytoplankton growth rate, r, for the most dominant species in terms of biovolume was calculated using:

$$r = \ln(N_t/N_0)/t \quad (1)$$

where N_t and N_0 are the biovolumes at the end and at the beginning of the experiment, respectively (Reynolds 2006).

Photosynthesis was analyzed *in situ* with the ^{14}C method modified from Rae & Vincent (1998) using a set of polyethylene bags (Whirlpak) with various screens to give a light transmission series of 0, 6, 25, 60 and 100% of ambient irradiance. Three replicates of 20 ml sample water spiked with ^{14}C-bicarbonate (final concentration 0.2 µCi ml^{-1}) were incubated in each bag for 2 h. After incubation, samples were filtered on GF/F filters and stored frozen. Before the laboratory analysis, 0.25 ml 0.5 N HCl was added to each filter in order to remove unbound ^{14}C. For radioactivity counting, 5 ml of a scintillation cocktail was added to each sample, and radioactivity was counted after a 24 h dark incubation with a RackBeta counter.

Photosynthetic rate to irradiance (P-E) was calculated based on a P versus E model described by Platt et al. (1980). Primary productivity (PP) values (average and standard error of 3 replicate incubations) were plotted and fitted with the following regression:

$$P = p\,(1 - e^{-\alpha I/p})\,e^{-\beta I/p} \quad (2)$$

where P (mg C m^{-2} h^{-1}) (the dependent variable, on the y-axis) is the photosynthesis rate at a given photosynthetically active radiation (PAR) intensity I (W m^{-2}) (independent variable, on the x-axis); p stands for the theoretical maximum photosynthesis rate if there were no photoinhibition, α describes the initial slope or increase of photosynthesis at low light intensities, and β attributes for the photoinhibition effect (Platt et al. 1980). E_k, the light adaptation parameter, i.e. light intensity at the onset of saturation (Talling 1957), was obtained from p and α as $E_k = p/\alpha$. Daily productivity (mg C mg^{-1} chl a m^{-2} d^{-1}) for the epilimnion of Lake Saanajärvi was integrated from the P-E curve based on the correlation between DOC and light attenuation, measured as diffuse attenuation coefficient (K_d) for PAR ($r^2 = 0.93$, calculated for a set of 18 lakes in NW Finnish Lapland) (L. Forsström unpubl. data). The DOC-correlated K_d PAR values for the control, subarctic and boreal treatments were 0.22, 0.8 and 0.7 m^{-1}, respectively. Earlier studies also showed that >85% of the between-lake variation in K_d is explained by differences in DOC concentration (Morris et al. 1995), which suggests that our light attenuation coefficients and hence water column integrated primary production values were close to true values.

Two-way ANOVA was used to test the effects of the DOC source (subarctic, boreal, control) and time (1, 3 and 5 d after the start of the experiment) on response variables that included heterotrophic and autotrophic biomass and productivity. DOC source and time were considered as fixed factors in the analysis. Normality and homogeneity of variance were checked with visual examination of residuals (Montgomery 1991). Logarithmic (base 10) transformations were applied to PP and the ratio of PP to bacterial production (BP) to achieve the ANOVA assumptions. When a factor was significant, an *a posteriori* multiple comparison test (Tukey-Kramer) was carried out to identify differences. The software JMP (SAS Institute) was used for all tests. A threshold of significance of 0.05 was adopted for all statistical tests.

RESULTS

Water chemistry and DOM quality

There were no significant differences in the measured water chemistry between the controls and treatments, with the exception of an increase in total nitrogen with both DOM additions and a slight increase in total phosphorus in the subarctic DOM enclosure (Table 1). DOC concentrations were approximately twice as high in the +DOM enclosures compared to the control enclosures.

Both DOM sources showed a high absorption of CDOM at wavelengths 320 and 440 nm, indicating high CDOM concentration and color, respectively.

They also showed signs of terrestrial dominance (high a^*_{CDOM}, high SUVA), boreal DOM more so than subarctic (Table 1). The lower S_R of subarctic DOM might indicate higher levels of degradation compared to boreal DOM. The ratio of a_{254}:a_{365}, a proxy for allochthonous organic carbon contents of low molecular weight compounds, was smaller for +DOM sources (subarctic DOM: 4.1; boreal DOM: 4.0) compared to the control (5.1).

Heterotrophic micro-organisms

Bacterial biomass varied between 11.1 and 29.1 µg ml^{-1} during the experiment and was highest and statistically different from the control in the +DOM subarctic treatment (Fig. 1A; $F_{2,16} = 6.80$, $p = 0.0073$). Biomass also increased in the +DOM boreal treatment but the increase was not statistically significant compared to the control.

BP varied between 0.36 and 1.03 µg C l^{-1} d^{-1} and was controlled by both the DOM additions and the time of the experiment (Fig. 1B; $F_{4,17} = 11.01$, $p < 0.0001$). BP was strongly stimulated by the +DOM subarctic treatment, as evidenced by 40% higher production in comparison to the control and +DOM boreal enclosures at the beginning of the experiment (Fig 1B). However, the differences levelled off afterwards and there were no differences between the treatments towards the end of the experiment. In both DOM treatments, BP was statistically lower at the end of the experiment compared to the beginning of the experiment (Fig. 1B).

The abundance of HNF varied between 0.4 and 3.9 × 10^3 cells ml^{-1}. The abundance increased significantly in all treatments during the experiment (Fig. 1C; $F_{2,17} = 53.80$, $p < 0.0001$) and was significantly higher in both +DOM treatments compared to the control (Fig. 1D; $F_{2,17} = 36.97$, $p < 0.0001$).

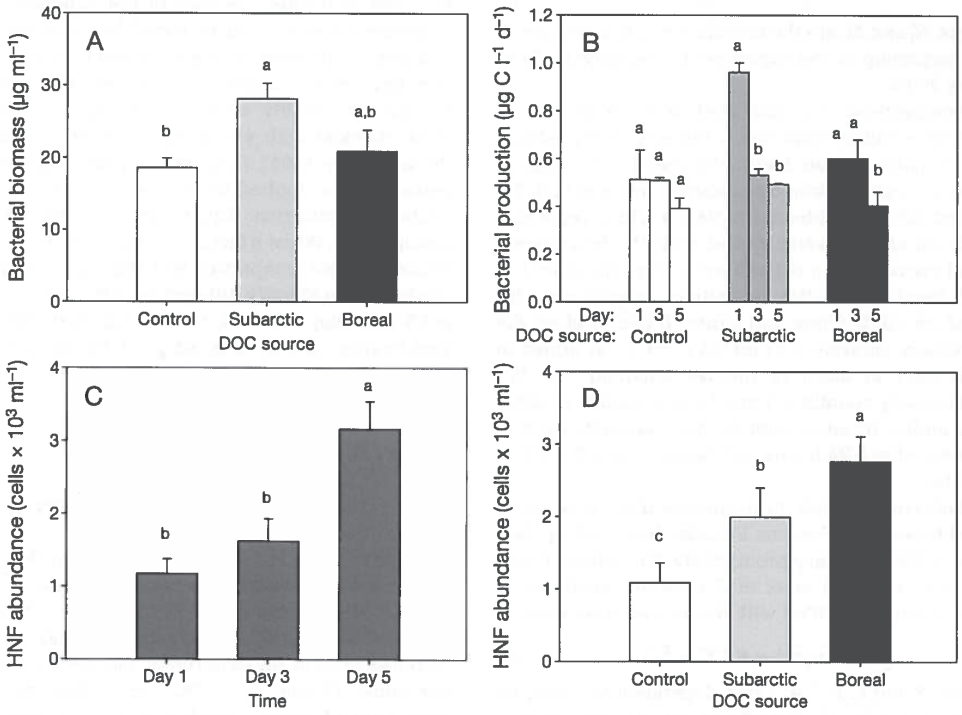

Fig. 1. Response of heterotrophic organisms over 5 d following the addition of dissolved organic matter (DOM) from a subarctic bog (Subarctic) and a boreal lake (Boreal) to enclosures in a subarctic Finnish lake. (A) Bacterial biomass; (B) bacterial productivity; (C,D) heterotrophic nanoflagellate (HNF) abundance. For each variable, only the statistically significant factors are shown (DOC source, time, or the interaction DOC source × time). Different letters above bars indicate statistically different values. In (B) the multiple comparison letters are shown within DOC source only. Error bars are SE

Autotrophic food web

Chl *a* concentration varied between 0.3 and 2.2 µg l^{-1} and increased significantly during the experiment in all enclosures (Fig. 2A; $F_{2,17} = 4.58$, $p = 0.026$), but no difference between the treatments was found.

The abundance of PNF varied between 1.0 and 6.4 × 10^3 cells ml^{-1}. Similarly to chl *a* concentration, the abundance increased significantly during the experiment in all enclosures (Fig. 2B; $F_{2,17} = 31.31$, $p < 0.0001$), but there was no difference between the treatments.

Picoautotroph abundance varied between 0.8 and 2.7 × 10^4 cells ml^{-1}. The abundance increased during the experiment in all treatments (Fig. 2C; $F_{2,17} = 10.01$, $p = 0.0013$) and was significantly higher in the +DOM boreal treatment compared to the control (Fig. 2D; $F_{2,17} = 6.29$, $p = 0.0090$).

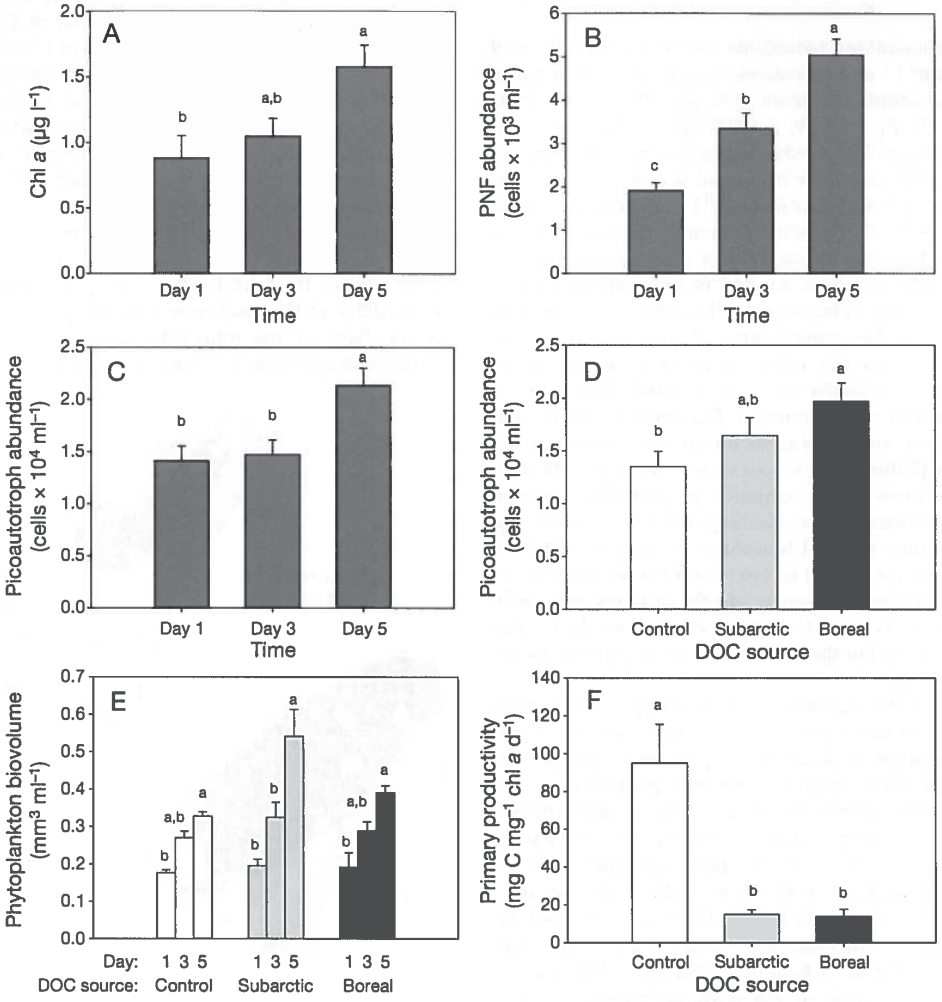

Fig. 2. Response of autotrophic organisms over 5 d following the addition of DOM to enclosures in a subarctic Finnish lake. (A) Chl *a*; (B) pigmented nanoflagellate (PNF) abundance, (C,D) picoautotroph abundance; (E) phytoplankton biovolume; (F) primary productivity. For each variable only the statistically significant factors are shown (DOC source, time, or the interaction DOC source × time). Different letters above bars indicate statistically different values. In (E) the multiple comparison letters are shown within DOC source only. Error bars are SE

Table 2. Exponential growth rate (r) (mean ± SE) of the most dominant phytoplankton species in a subarctic Finnish lake, and in control enclosures, and enclosures following the addition of dissolved organic matter (DOM) from a subarctic bog (+DOM subarctic) and a boreal lake (+DOM boreal)

Enclosures	Open water	Control	+DOC subarctic	+DOC boreal
Cyclotella sp.	−0.08	0.02 ± 0.00	0.03 ± 0.02	0.08 ± 0.04
Dinobryon crenulatum	0.11	0.30 ± 0.02	0.29 ± 0.05	0.33 ± 0.04
Uroglena sp.	0.08	0.05 ± 0.01	0.49 ± 0.09	0.26 ± 0.16
Pseudopedinella sp.	0.20	0.36 ± 0.08	0.41 ± 0.15	0.43 ± 0.06
Plagioselmis sp.	−0.47	−0.04 ± 0.09	0.11 ± 0.05	0.12 ± 0.10

Phytoplankton biovolume varied between 0.2 and 0.6 mm^3 l^{-1} and increased during the experiment in all treatments and more so in the +DOM treatments (Fig. 2E; $F_{4,17}$ = 3.19, p = 0.0398). At the end of the experiment the biovolume was significantly higher in the +DOM subarctic treatment (0.5 mm^3 l^{-1}) in comparison to the control (0.3 mm^3 l^{-1}). Higher biovolume in the +DOM subarctic treatment was mainly caused by an increase in mixotrophic chrysophyte species, especially *Uroglena* sp. and to some extent a small cryptophyte belonging to the genus *Plagioselmis* (Table 2). The growth rates of other dominant species, such as the strictly autotrophic small centric diatom, *Cyclotella* sp. and 2 small chrysophytes, *Dinobryon crenulatum* and *Pseudopedinella* sp., did not show any substantial difference between treatments (Table 2). Most species had lower growth rates in the open water compared to control enclosures, with the exception of the large colonial *Uroglena* sp.

The ratio of algal biovolume to chl *a* varied from 172 (control, Day 1) to 446 (+DOM subarctic, Day 5). It was always highest in +DOM subarctic and, with the exception of Day 3, it was lowest in control enclosures, but the differences were not statistically significant.

The DOM additions also had an impact on the light milieu in the enclosures and associated photosynthetic parameters (Table 1). E_k, the index of light saturation, varied from 16 to 88 µmol photons m^{-2} s^{-1} in the control enclosures, from 12 to 52 µmol photons m^{-2} s^{-1} in +DOM subarctic, and from 22 to 74 µmol photons m^{-2} s^{-1} in +DOM boreal. Maximum chl *a* normalized photosynthetic capacity (P^*_{max}) was on average 2-fold higher in the control than in the +DOM enclosures. The initial slope of the *P-E* curve normalized to chl *a*, α^*, which describes the initial increase of photosynthesis at low light intensities, was also highest in the control enclosures (Table 1). PP integrated to the water column respective to the epilimnion (0 to 10 m) of the lake (mg C mg^{-1} chl *a* m^{-2} d^{-1}) was significantly suppressed by the +DOM treatments (Fig. 2F; $F_{2,23}$ = 16.40, p ≤ 0.0001).

Based on previously calculated correlations between DOC and K_d PAR (r^2 = 0.93) and between DOC and K_d 320 nm (r^2 = 0.90) for the lakes in the study region (L. Forsström unpubl.), we estimated that increases in DOC comparable to this experiment would lead to a reduction in the penetration depth of 1% PAR from 21 to 6.5 m (+DOM subarctic) and 5.7 m (+DOM boreal), and a reduction in penetration of 1% 320 nm from 2 to 0.4 m (+DOM subarctic) and 0.3 m (+DOM boreal). Inside the enclosures, 88% of PAR penetrated to the bottom of the control enclosures and <60% to the bottom of the +DOM enclosures. For UV radiation, the difference was larger: while 26% of UV reached the bottom of the control enclosures, <0.1% of UV penetrated to the bottom of +DOM enclosures. In Lake Saanajärvi, the area where the euphotic layer reaches the lake bottom would decrease from 58 ha (83% of the total lake area) to approximately 24 ha (34% of the total lake area) with a DOC increase comparable to this experiment (Fig. 3).

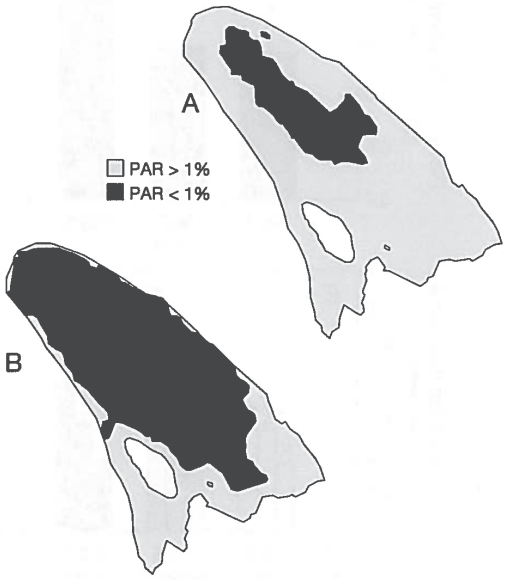

Fig. 3. Whole-lake effects of increased allochthonous DOM. Estimated euphotic area of the lake bottom with (A) current DOC concentration and (B) a DOC increase comparable to DOC additions used in the experiment. PAR: photosynthetically active radiation

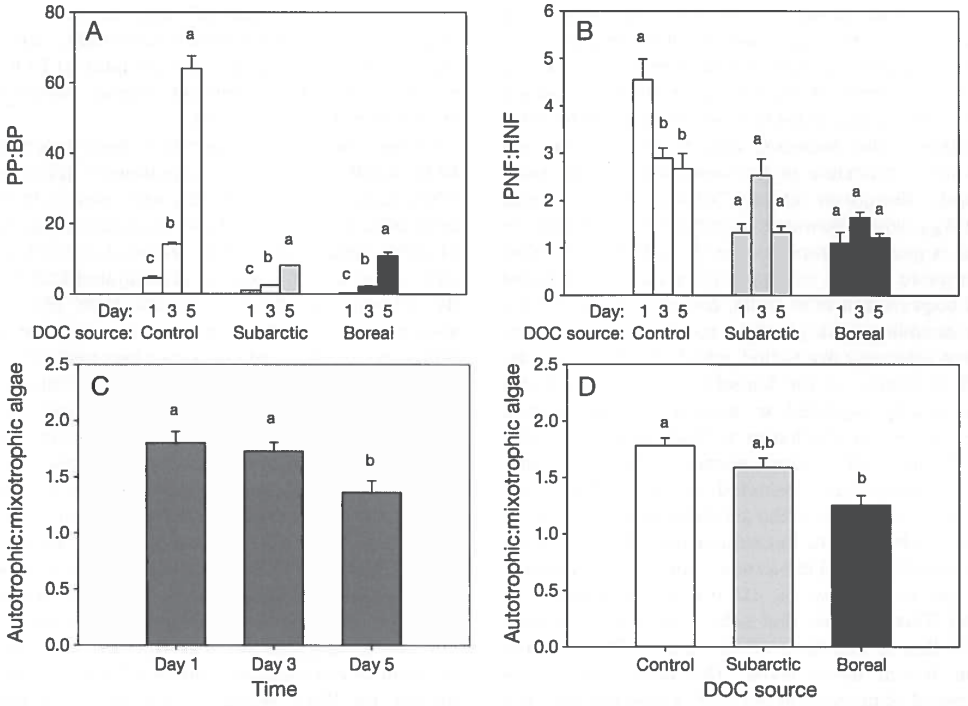

Fig. 4. Shifts in autotrophic versus heterotrophic processes over 5 d following the addition of DOM to enclosures in a subarctic Finnish lake. (A) Epilimnetic primary production:bacterial production (PP:BP); (B) pigmented nanoflagellates:heterotrophic nanoflagellates (PNF:HNF); and (C,D) autotrophic algae:mixotrophic algae. For each variable only the statistically significant factors are shown (DOC source, time, or the interaction DOC source × time). Different letters above bars indicate statistically different values. In (A) and (B) the multiple comparison letters are shown within DOC source only. Error bars are SE

Shifts between autotrophic and heterotrophic processes

The epilimnetic ratio of PP to BP, calculated based on *P-E* curves and the correlation of DOC and K_d PAR, increased significantly in all treatments during the experiment (Fig. 4A; $F_{4,17} = 35.23$, $p \leq 0.0001$). It was always highest in the control enclosures, while the 2 +DOM treatments did not differ statistically from each other.

The ratio of PNF to HNF decreased in all treatments throughout the experiment, although the decrease was statistically significant only in the control (Fig. 4B; $F_{4,17} = 9.30$, $p = 0.0004$). The control also had statistically highest PNF:HNF ratio throughout the experiment in comparison to the +DOM treatments.

The ratio of autotrophic to mixotrophic algae significantly decreased in all treatments towards the end of the experiment (Fig. 4C; $F_{2,17} = 11.45$, $p = 0.0007$). Both +DOM treatments had a smaller autotrophic to mixotrophic algae ratio, although only the +DOM boreal treatment differed significantly from the control (Fig. 4D; $F_{2,17} = 6.67$; $p = 0.0073$).

DISCUSSION

As expected, the DOM additions doubled the DOC concentration in the treatment enclosures and reached the level that is estimated to shift an aquatic ecosystem from an autotrophic to a heterotrophic based food web (Jansson et al. 2000, Prairie et al. 2002). The added DOM was poor in nutrients and did not increase the inorganic nutrient pool in the treatments in comparison to the control. Other physical and chemical parameters between the control and treatments were also similar, which allowed us to estimate the unique effect of DOM quantity and quality on the microbial food web.

The characteristics of the subarctic (bog) and boreal (lake) DOM, measured as DOM spectroscopy, differed markedly from the control DOM but less from each other. Results from DOM spectroscopy indicated the bog DOM to be more bioavailable with a higher ratio between absorbance at 254 and 365 nm — indicative of low molecular weight compounds (Berggren et al. 2010b) — and a lower $SUVA_{254}$, which however is somewhat in contrast to what is generally reported for the quality of CDOM in organic matter exported from coniferous forest and bogs (Ågren et al. 2008, Berggren et al. 2010a). Our sampling took place in the beginning of July after a relatively dry period, which could explain the 'lack of quality' in the boreal DOM, because DOM is primarily exported to aquatic systems during episodes when discharge is high and previously unsaturated soil horizons become activated. A bioassay experiment conducted in Lake Mekkojärvi estimated that 95% of the allochthonous DOM of the lake is refractory to immediate bacterial utilization (Tulonen 2004) and the annual primary production of the lake is very low, i.e. <10 g C m^{-2} (Salonen et al. 2005). This suggests that Lake Mekkojärvi contains DOM that is less bioavailable than DOM in many other boreal forest lakes. The difference in the degree of pigmentation per unit carbon between the 2 DOM sources could be due to differences in the degree of photodegradation or allochthony, but it could also indicate differences in iron concentration or the amount of non-humic allochthonous fractions, unfortunately not analyzed within this study.

Despite the apparent similarities in DOM optical variables, the heterotrophic community reacted differently to the 2 DOM additions. Subarctic DOM additions had a positive effect on bacterial biomass and productivity, while these variables in the +DOM boreal enclosures remained at the same level as in the control enclosures. HNF abundance reacted positively to both DOM additions and more so to the boreal DOM; this may partly explain the smaller bacterial biomass in the +DOM boreal enclosures, as HNF are known to be efficient bacteria grazers (Laybourn-Parry & Marshall 2003). BP showed a rapid reaction to the addition of subarctic DOM but such an increase was missing from the +DOM boreal enclosures. This suggests that boreal DOM might require a specific community to develop before it can be efficiently utilized. Using molecular microbial community analyses, Docherty et al. (2006) showed that, when exposed to a new DOM source, microbial communities will change within 72 h to correspond to the new source, regardless of the initial community structure. The fact that BP only increased at the beginning of the experiment was probably due to the combined effect of both the availability of DOC and nutrients, and the increased grazing pressure by HNF and mixotrophic algae.

Bacteria have been reported to react positively to DOC additions in many experiments (Eiler et al. 2003, Lennon & Pfaff 2005), and especially when both DOC and nutrients have been added (Granéli et al. 2004, Jansson et al. 2006, Breton et al. 2009). However, none of these studies investigated DOM quality, which, according to results from this study, should be taken into account when assessing DOM influence on bacterial and other heterotrophic communities. In an earlier study of bacterial communities in the same area, DOM quality was found to influence bacterioplankton community composition while production was more controlled by DOM quantity (Roiha et al. 2012). In a comparable study by Hessen et al. (2004), there was a decrease in bacterial biomass induced by DOC additions, despite an increase in BP. However, in their experiment bacteria were heavily grazed by zooplankton that exhibited over a 4-fold increase in biomass during the experiment. In our study, zooplankton was filtered out from the enclosures, but it is most likely that bacteria were still grazed by HNF, which increased in abundance throughout the experiment, especially in enclosures with elevated DOC.

While heterotrophic organisms showed a positive response to DOM additions, the variables representative of primary producers showed contrasting effects. Compared to the control enclosures, DOM additions led to a higher phytoplankton biovolume (+DOM subarctic) and higher numbers of picoautotrophs (+DOM boreal), but lower productivity, lower α^* and no statistical difference in chl *a* or PNF. The reasons for primary producers not showing a consistently similar response to DOM additions are probably linked to resource competition with bacteria and picoautotrophs, and a shift from autotrophy to heterotrophy among mixotrophic algal species. Due to their smaller size, bacteria and picoautotrophs are more efficient in the competition for nutrients, compared to algae (Rhee 1972, Parker et al. 1975, Smith & Kalff 1982, Callieri & Stockner 2002), which may partly explain the increase of picoautotrophs in the +DOM enclosures. Higher biovolume, evidenced in +DOM enclosures, is considered unfavorable in low nutrient concentrations (Turpin 1991). However, high biovolume can also be an indicator of the increase of (large) cells capable of mixotrophy. Indeed, the phytoplankton community of the enclosures con-

sisted of many mixotrophic species. Although mixotrophy was not specifically measured in the experiment, species that are known to be mixotrophic increased in the +DOM enclosures, especially the large colonial chrysophyte *Uroglena* sp (Table 2, Fig. 4C). Previous studies have shown that under low light intensity and/or when bacteria are added, the chlorophyll concentration per cell of mixotrophic algae reduces drastically (Sanders et al. 1990). The inconsistency in the ratio between chl *a* and phytoplankton biovolume in our study is therefore best explained by the species composition of the study lake.

Previous work found both an increase (Hessen et al. 2004, Karlsson et al. 2007) and a decrease (Blomqvist et al. 2001) in PP due to DOC additions. In our experiment, DOC additions suppressed both P^*_{max} and PP throughout the experiment. This could be another indication of a shift of mixotrophic species from auto- to heterotrophy with an excess carbon source. Previous studies showed that a threshold level of 10^6 bacteria cells ml^{-1} is required for mixotrophic algae to shift from photosynthesis to bacterivory (Sanders et al. 1990), and this threshold was exceeded in the +DOM enclosures. Another explanation for the low PP in the +DOM enclosures might be that dissolved humic material had bound iron or some other metal that algae would require for photosynthesis (Guildford et al. 1987). Because the enclosures were relatively small, the amount of PAR was high enough for photosynthesis to take place in the whole water column in both the control and the +DOM enclosures.

Measured changes in heterotrophic and autotrophic microorganisms also resulted in important shifts in the relative importance between basal autotrophic and heterotrophic food web production and biomass. Although autotrophic biomass and production remained dominant in the experiment, increasing DOC concentration was accompanied by a shift from the heavy dominance of autotrophic productivity to a more even balance of production between autotrophs and bacteria, and decreases in the ratios of PNF to HNF and of autotrophic to mixotrophic algae. Our results, therefore, are in line with earlier studies (Jansson et al. 2000, Prairie et al. 2002) that showed that pelagic systems shift towards more heterotrophic based food webs with increasing DOC concentration. In addition, the increased microbial biomass in the +DOM enclosures most likely contributed to the increased concentration of total nutrients in the DOM treatments. Changes in the N:P ratio have been shown to override some of the positive effects of carbon additions to heterotrophic microbial food webs (Karlsson et al. 2002) but such changes did not take place in our experiment because the added DOM sources did not increase the inorganic pool of the treatment enclosures.

To place our mesocosm results in a broader context, we calculated how the increase in DOM would influence the whole lake PP. The current light climate in Lake Saanajärvi allows fully developed benthic algal communities to account for a considerable proportion of primary production in the lake (L. Forsström unpubl.). An increase in DOM comparable to the additions in this experiment would mean that the area available for benthic primary production would decrease from 83 to 34 % of the total basal area of the lake. DOM would also decrease the euphotic layer for planktonic production, and reduce the depth of thermocline (Pérez-Fuentetaja et al. 1999). All these changes would lead to lower levels of PP and amplify the shift from autotrophy to heterotrophy. Previous studies have shown that phytoplankton concentration is lower at the very surface of Lake Saanajärvi, most likely due to photoinhibition (Forsström et al. 2005). An increase in DOM would protect algae from harmful UV radiation and photoinhibition, but for the total algal production of a relatively deep lake, such as Lake Saanajärvi, this has only a minor effect.

Extrapolating our results to the whole-lake level highlights one possible outcome of increasing DOM concentrations. However, further studies at different scales are needed to introduce more complexity and further assess the consequences of such changes. Mesocosm studies can never catch the complexity of whole ecosystems, and the results gained from such experiments have to be interpreted with caution (Schindler 1998, Ahn & Mitsch 2002). For example, a comparison of phytoplankton growth rates between the open water and enclosures demonstrates how growth rates were lower in natural conditions (Table 2). The difference most likely results from a combination of the lack of predation and the constant high exposure to light in the enclosures. The only exception was a large colonial chrysophyte *Uroglena* sp. which has high mobility and is better able to escape predation than other algae in the lake because of its size (diameter of the colony up to 500 µm), explaining the very similar growth rate in natural and control environments. A large-scale ecosystem experiment would mimic natural conditions better, but due to problems related to logistics, costs and replicability, a whole-lake study was not possible on this occasion. However, since our experiment focused on the short-term effects on microscopic organisms

with limited mobility, the results of our mesocosm study do represent a reasonable scenario of the outcomes of increasing DOM concentrations. A recent study comparing mesocosms of varying size up to 500 000 l concluded that mesocosms can be used to determine the limiting factors for the growth of primary producers (Spivak et al. 2011).

In summary, our results have shown that doubling of DOC from 2.5 to 5 mg l^{-1} can shift the epilimnion of an oligotrophic, clear-water lake from a system dominated by an autotrophy-based food web towards a heterotrophy-based food web. Because DOM not only promotes bacterial growth but also affects the light climate, the change towards heterotrophy is even greater if the whole water column is taken into account. Our results also show that the quality of DOM that reaches the lake is highly important. The bacterial community was mainly influenced by the more degraded and bioavailable subarctic DOM, while the picoautotrophs and the ratio of autotrophic to mixotrophic algae were more affected by the more pigmented and possibly more allochthonous boreal DOM.

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III

CARBON DYNAMICS IN HIGHLY HETEROTROPHIC SUBARCTIC THAW PONDS

by

Toni Roiha, Isabelle Laurion & Milla Rautio 2015

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IV

HABITAT AND SEASON DETERMINE THE INTERPLAY BETWEEN DOM POOL AND BACTERIA IN SUBARCTIC FRESHWATERS

by

Toni Roiha, Sari Peura, Mathieu Cusson & Milla Rautio 2015

Manuscript

Habitat and season determine the interplay between DOM pool and bacteria in subarctic freshwaters

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Running title: Relationship between habitat and bacteria in arctic lakes

Abstract

Carbon in lakes is a complex mixture of terrestrial carbon from the catchment and algal carbon from the in-lake production. Both of them serve as substrates for bacterial growth, but their composition and availability differ. Here we show how terrestrial and algal carbon compounds are linked to the bacterial metabolism and community composition (BCC) in three different habitats of subarctic freshwaters. We measured dissolved organic matter quality indices including different components of algal and terrestrial carbon together with bacterial metabolism and BCC. The samples were collected from 1) lake inlets representing habitats influenced by allochthonous carbon arriving to lakes, 2) lake outlets i.e. habitats integrating carbon from the in-lake algal production and 3) ponds that contain carbon with a mixed signature of terrestrial and algal compounds. Terrestrial drainage and associated nutrients and humic carbon compounds supported higher bacteria production but lower bacterial diversity than carbon from the algal production. There was a high variation in BCC which was best explained by the habitat-specific concentrations of nutrients, dissolved organic carbon, fulvic acids and proteins. The results also show strong variation related to pool size and seasonality, and emphasize the winter period that has previously gained little attention in aquatic studies.

INTRODUCTION

Dissolved organic matter (DOM) in surface waters is a complex mixture of humic substances, carbohydrates, carboxylic acids, amino acids and nutrients. These compounds originate from terrestrial and aquatic production, and they are a major energy source for the aquatic food webs. The main energy source for the food webs in transparent lakes (dissolved organic carbon; DOC < 5 mg L^{-1}) is assumed to be DOM produced by autotrophic phytoplankton (1, 2), but some additional energy comes from the terrestrial fraction of DOM (3, 4, 5). The availability of different fractions of DOM to bacteria differs tremendously with amino acids being readily uptaken by most bacteria, while the recalcitrant compounds in humic substances, such as lignin, can be degraded only by more specialized groups (6). The DOM quality, or proportions of different fractions of DOM may also vary depending on the type of the water body and the location within it (7, 8, 5). Further, the vegetation in the catchment has a prominent impact as the DOM from catchment with coniferous forest has been shown to support higher bacterial production than DOM from bog area (7, 9). Within the water column autochthonous amino acid-like DOM has been reported to dominate in the euphotic mixed layer whereas in the deeper layers humic-like DOM is overrepresented (10). Less is known about the horizontal and habitat-specific variations in organic carbon bioavailability.

The variation in carbon quality shapes the bacteria residing in lakes. It has been shown that bacterial community composition (BCC) and metabolism are linked to

carbon source (11, 12, 13, 14, 15, 16, 17) and to the quality of the carbon within different sources (18, 19, 20). Further, it has been shown that the composition of bacterial community plays a significant role in the rate of carbon mineralization (21), and while the bacteria are processing DOM, some compounds are produced while others get degraded (22, 23). Thus, the bacteria are influenced by the DOM milieu but also contribute to defining the quality and quantity of carbon in lakes. Another factor that needs to be taken into account especially at high latitudes is seasonality, which adds up to the changes in quantity and quality of DOM (24, 25). Seasonal changes in solar radiation, runoff, primary production and water chemistry all influence DOM properties (8). For example, DOM spectral slope distributions have been shown to differ between summer and winter (10) and under the ice DOM has been shown to have higher presence of terrestrially derived carbon (26).

DOM characteristics should also be influenced by lake morphometry, although this has received little attention. It is well known that morphometry creates differences in habitats and influences photo exposure, residence time, velocity, primary production and species composition, all of which contribute to defining DOM. For example, the size of the water body has been shown to influence the bacterial diversity (27). Similarly, the vertical location in the water column plays a critical role as photochemical processes in shallow euphotic zones make DOM more bioavailable to bacteria compared to DOM in dark (28). The efficiency of DOM transformations drops when the residence time increases, suggesting that the reactivity of organic matter is reduced as it ages (29). Thus, it can be expected

that DOM varies between different habitats of the lake and also between water bodies of different sizes, resulting in variation in microbial community compositions and microbial processes between these habitats.

Our objective was to test hypothesis that habitat-specific characteristics influence bacterial metabolism and BCC by regulating the organic matter quality. Because aquatic DOM quality (i.e. composition) reflects the dynamic interplay between DOM sources and biogeochemical reactions, we hypothesized that the DOM biogeochemistry and bioavailability have variation based on seasonality and habitat within a water body. To test this, water samples representing four different seasons were collected from nine locations in six subarctic Finnish water bodies. These included i) lake inlets representing habitats that should be influenced by allochthonous light-exposed carbon arriving to lakes, ii) lake outlets i.e. habitats that integrate carbon from in-lake algal production including euphotic and aphotic depths and iii) ponds that should contain carbon with a fast renewal time and a mixed signature of terrestrial and algal compounds. The bacterial metabolism and community composition were analyzed in relation to DOM quality (carbon compounds, spectrophotometric properties, nutrients etc.) and physical attributes of the habitats.

MATERIALS AND METHODS

Study site and sampling

We sampled three ponds, three lake inlets and three lake outlets in the Kilpisjärvi region, subarctic Finnish Lapland (69ºN, 20ºE). The sites were located between 473 and 850 m

a.s.l. in the subarctic landscape where treeline of mountain birch (*Betula pubescens* subsp. *Czerepanovii*) is at 600 m a.s.l (Table 1). All sites were sampled five times in 2011; in February (winter), in early May (spring), in mid-June just after the ice break up (ice break up), in late July (summer) and in early October (fall). Ponds were sampled in the middle of the pond and the lakes were sampled from near the inlet and outlet rivers. Samples were collected with a 2 L Limnos water sampler as integrated samples from the first meter of the water column. Water temperature was measured in the field with YSI Professional Plus (Yellow Springs, OH, USA). Total phosphorus (TP) and nitrogen (TN) concentrations were analysed from sieved (50 µm) water using standard methods (http://www.sfs.fi/). For the determination of chlorophyll a (Chl-*a*) concentrations, 1-2 L were filtered onto GF/F filters. Samples were collected in duplicate and stored at -80^{0}C until fluorometric analysis according to Nusch (30). Dissolved organic carbon (DOC) concentration was analysed from water filtered through 0.2 µm prerinsed cellulose acetate filters using Shimadzu TOC-5000A carbon analyser.

Quality measurements of carbon

A set of indicators for the quality of carbon was measured using spectrophotometric and spectrofluorometric methods. All the measurements were carried out for water that had been filtered through a 0.2 µm prerinsed cellulose acetate filter and stored in the dark at + 4°C. Scanning of absorption coefficient at 320 nm (a320), specific UV-absorbance index (SUVA) and the spectral slope (S289) was performed in a dual-beam mode with Cary 100 UV-Vis spectrophotometer (Agilent) using a 10-cm quartz cuvette. Samples were corrected against MilliQ water. Absorption coefficient at 320 nm (a_{320}) was measured as indicator of

coloured dissolved organic carbon (CDOM) concentration. Values were calculated from absorbance measurements (A_λ) at 320 using $a_\lambda = 2.303/L \times A$, where L is the length of the cuvette in meters (31). SUVA, which is an indicator of the share of terrestrially derived organic carbon (32, 33), was calculated from DOC normalized absorbance at the wavelength 254 nm with higher values indicating a higher share of terrestrial carbon compounds in the sample (34). S289, indicating the amount of carbon compounds likely related to autochthonous production (35), was calculated from the spectrophotometric measurements. For the calculation an absorption slope was calculated for the 20 nm interval between 279-299 nm. Algal derived carbon has a maximum at 289 nm, thus the higher the S289 values the bigger is the share of carbon compounds from autochthonous production (35). There are some environmental factors that could have compromised the fluorometric measurements, most important such factors being iron and pH. According to previous measurements of the lakes in the area the iron concentration is low (mean of 37 lakes 0.24 mg L^{-1}) (36) and not likely to cause a bias. Also, the pH was stable within the samples (6.5 ± 0.5) and should not interfere with the measurements. Thus, we are confident that our measurements were correct and reliably showing the true variation in carbon quality.

Composition of different humic, fulvic and protein-like carbon compounds was identified with excitation-emission matrixes (EEM) using a spectrofluorometer Cary eclipse (Agilent). They were measured across excitation (220-450 nm) and emission (240-600 nm) wavelengths with 5 and 2 nm increments, respectively. EEMs were corrected for inner filter effect (37), machine specific biases, background scattering (38) and were standardized to Raman units (R.U.) (39). Raman and Rayleigh scattering were removed

using the DOMfluor 1.7 toolbox in MATLAB 2008b (MathWorks, Natick, MA, USA) as recommended in Stedmon and Bro (40). The obtained EEMs were inserted to the parallel factor analysis (PARAFAC) model based on samples collected from > 100 lakes from boreal, subarctic and arctic lakes from Finland, Canada and Greenland (data not shown). The model was used to identify and calculate intensities of all main carbon components in the sample. Five different components (C1-C4, C6) identified from the EEMs were highly correlated with each other (correlation coefficients for all pairs > 0.87, $p < 0.0001$) and were pooled for the analyses as terrestrial humic-like compounds, while the component C5 was considered as a fulvic acid and the component C7 as protein, according to Fellman *et al*. (41) (Supplementary Fig. 1). The compounds C1-C4 and C6 are widespread terrestrial humic-like components originating e.g. from forest streams and wetlands (41, 42, 43, 44). C5 have been associated with irradiated DOM that has been microbially degraded (43). C7 resembles amino acid-like tryptophan found commonly in different freshwater environments (41).

Bacterial metabolism analyses

Bacteria production (BP) was measured using ^{3}H-leucine (specific activity 73 Ci mmol^{-1}) incorporation with a centrifugation method (45). Incubations were started within 2-6 hours after sampling using a leucine concentration of 30 nM and incubation time of 3 h according to the saturation curves in Roiha *et al*. (20). Incubations were conducted in dark in a constant temperature of 6.4±0.5 °C which deviated from the in-situ field temperatures 5.1 ± 2.1°C. TCA was added to terminate incubation (TCA; 5 % final concentration) after which the samples were stored at -20°C until centrifuging and radioassaying according to

178 Smith and Azam (45). Bacterial respiration was measured as oxygen (O_2) consumption
179 using fibre-optic O_2 mini-sensors (Fibox 3, PreSens Precision Sensing GmbH, Regensburg,
180 Germany) (46). Filtered (3 µm) water samples were incubated in top-filled 500 ml
181 Erlenmeyer vials closed with airtight silicone stopper. Samples were incubated as above
182 but in a water bath to further reduce temperature variability as this infers with O_2 sensor
183 reading. The incubations were let to stabilize for few hours before the first sensor reading.
184 Over the first five days O_2 concentrations were measured 1-2 times a day while the last
185 measurement was taken in the beginning of the next sampling trip (total incubation time 4-
186 6 weeks). BR rates were calculated from the linear slope of O_2 consumption that was
187 converted to carbon units using respiratory quotient (RQ) of 1.0. To estimate actual
188 bacteria metabolism in the sampled sites, the BP and BR values were corrected for *in-situ*
189 temperatures with Q_{10} values according to Berggren *et al*. (47). Such corrections were not
190 applied when the aim was to measure temperature-independent bacteria control. Bacterial
191 growth efficiency (BGE), i.e. bacterial production (BP) per unit of assimilated carbon was
192 calculated using equation 1.
193
194 (1) $BGE = BP/(BP + BR)$
195
196 *Bacterial community analyses*
197 Unfiltered water samples for DNA extraction were frozen within 2-4 hours of sampling.
198 300 ml subsample of the frozen water was freeze dried with an Alpha 1-4 LD plus (Christ,
199 Osterode, Germany). DNA extraction, PCR (primers 341F (5'-CCTACGGGNGGCWGCAG-3')
200 and 805R (5'-GACTACHVGGGTATCTAATCC-3'); 48) and 454-pyrosequencing were

performed as described in Peura *et al*. (49). The amplicon processing, including quality trimming and noise and chimera removal was done as outlined in Schloss *et al*. (50) using mothur (51). The sequences were assigned into operational taxonomic units (OTUs) using 97 % sequence similarity cutoff, loosely corresponding to bacterial species and OTUs were classified using taxonomic framework for freshwater bacteria introduced by Newton *et al*. (52). Two samples with likely fecal contamination were removed from the sample set. Contamination was likely caused by lowered water level during samplings. Prior to further analysis, the sequence data was resampled to smallest sample size (1153 sequences per sample) using perl script daisychopper.pl (available at http://www.genomics.ceh.ac.uk/GeneSwytch/Tools. html; 53). The sequences are available at the NCBI Sequence Read Archive under project number PRNA244724.

Statistical analysis

Differences in environmental and temperature-corrected bacterial metabolism variables between seasons and habitats were tested using a 2-way ANOVA. Season and habitat were considered as fixed factors in the analysis. Normality and homogeneity of variance were checked with visual examination of residuals (54). Square root transformations were applied to TN and Chl-a, logarithmic (base 10) transformations to a320, fulvic acids, BP and BR, and inverse (x^{-1}) transformation to S289 to achieve ANOVA assumptions. When a factor was significant, *a posteriori* multiple comparison test (Tukey-Kramer) was carried out to identify differences.

Statistical testing of the impact of season, habitat and their interaction to the bacterial community and environmental data structure was done using a Permutational Multivariate

224 analysis of variance (PERMANOVA; 55) with 999 permutations. Multiple regression
225 analyses were used to identify which environmental variables (TP, TN, Chl-a, DOC, SUVA,
226 S289, humic acids, fulvic acids and proteins) best explained the changes in bacterial
227 metabolism (BP, BR, and BGE). The absorption coefficient a320 was omitted from the
228 model due to its high Pearson correlation with DOC (r = 0.85) and humic acids (r = 0.96).
229 Best model (using forward procedure) was selected according to the lowest value of AICc
230 index. Regression equations were produced with all the dataset and separately for each
231 habitat (pond, inlet, outlet). For the statistical testing of the BCC, all OTUs with more than
232 100 sequences in the total data were retained in the analysis. Bacterial data were square
233 root transformed prior to generating a resemblance matrix of Bray-Curtis similarities.
234 Environmental data were normalised and Euclidian distances were used to generate
235 resemblance matrix. Pairwise permutation *t*-tests were performed on the factors that were
236 identified as significant in PERMANOVA to identify differences among levels. The effects of
237 season and habitat on BCC were visualized with a Principal Coordinates Analysis (PCO). A
238 similarity percentage analysis (SIMPER) was used to assess the percentage contribution of
239 each OTU to the observed dissimilarities among habitats (pond, inlet, outlet).

240 Spearman's rank correlations were used to examine relationships between the
241 resemblance matrices of BCC and environmental variables to identify the environmental
242 variables (alone or in subset) that explain best the observed patterns of BCC (BIO-ENV
243 analyses, PRIMER). For this analysis, OTU and environmental variable matrices were
244 constructed using Bray-Curtis dissimilarity (square-root transformed) and Euclidean
245 distances respectively (see 56, 57). Diversity indices and relationships between BCC and
246 carbon components were analysed with Spearman's rank correlation in R (58). Shannon

index was used to evaluate the evenness of the community, that is, how evenly the observations were distributed among OTUs (59). To measure the species richness, or the number of different OTUs in samples, we used inverse Simpson's index (60). The software JMP (JMP®, Version 10.0. SAS Institute Inc., Cary, NC, 1989-2012) was used for all univariate tests while PRIMER+PERMANOVA (version 6.1.6; 61, 55) was used for multivariate analyses. A threshold of significance of 0.05 was adopted for all statistical tests.

RESULTS

Environmental variables

Many of the environmental variables had variation based on both, the season and habitat (Table 2, Supplementary Table 1, Supplementary Fig. 2 and 3). The most drastic seasonal variation was seen in temperature which was close to zero in winter while the summer maximum was about 15°C. Total phosphorus (TP) had its maximum in the spring and in the ponds. Also total nitrogen (TN), DOC and proteins had the highest values in ponds, but the difference between ponds and other habitats was significant only in samples from under the ice (winter and spring). The indicator of algal production (S289) was always highest in the outlets but these values were significantly different only from ponds and only in winter and spring. Chlorophyll a (Chl-a), another indicator of algal carbon, was low in all samples (< 1 μg L^{-1}) and no differences between seasons or habitats were detected. Fulvic acids (indicator for microbially degraded DOC) had some habitat and seasonal variation that was expressed with ponds having the smallest amount of these compounds in winter. There were no significant differences in the indicator of the total amount of coloured DOM (CDOM; absorption coefficient a320) or in the fluorescence of humic-like compounds

(indicator of the share of terrestrial carbon in the CDOM), though those were the lowest in the outlets. Several variables in the total dataset were highly correlated with each other, with highest correlations (all $p < 0.0001$) observed between TN and TP (Pearson's correlation $r = 0.88$), DOC and humic-like substances ($r = 0.81$), DOC and TP ($r = 0.68$) and DOC and TN ($r = 0.61$).

According to PERMANOVA, there was a difference in environmental variables according to seasons (Pseudo-$F_{4,21} = 3.92$, $p < 0.001$) with all pairwise comparisons, except for winter – spring, ice breakup – summer and ice breakup – fall, suggesting different conditions ($p < 0.05$ for all). The data structure was also different between habitats (Pseudo-$F_{2,21} = 6.22$, $p < 0.001$) with the ponds being distinct from the inlets (Permutation pairwise test, $t = 2.22$, $p = 0.005$) and outlets ($t = 3.21$, $p < 0.001$) while inlets and outlets were similar.

Bacterial metabolism

Bacterial metabolism exhibited large seasonal variation (bacterial production (BP): $F_{4,29} = 8.23$, $p < 0.0001$; bacterial respiration (BR): $F_{4,27} = 3.75$, $p = 0.0150$; bacterial growth efficiency (BGR): $F_{4,27} = 18.71$, $p < 0.0001$) (Fig. 1) and there was also marked variation between the habitats for BP and BGE (Fig. 2). Highest BP values were measured for the ponds (4.5 µg C L^{-1} d^{-1} ± 3.9) and inlets (1.5 µg C L^{-1} d^{-1} ± 0.8) during the ice breakup while the maximum BP in the outlets (1.0 µg C L^{-1} d^{-1} ± 0.5) was reached in summer. In all habitats the BP was lowest in fall with values < 1 µg C L^{-1} d^{-1}. BR followed a different seasonal pattern, with the highest values measured in the ponds in the spring (20.7 µg C L^{-1} d^{-1} ± 4.4) and the lowest in the inlets in the summer (3.2 µg C L^{-1} d^{-1} ± 1.2). BGE was rather low and the maximum values, 20-39 %, were reached in the summer. There was also

293 variation between the habitats, with the ponds and inlets providing an environment that
294 allowed for higher BGE than that of the outlets (Fig. 2).
295 Multiple regression models were constructed to assess the importance of each variable that
296 was confirmed to have significant impact on the BP, BR and BGE. The models explained up
297 to 62% of the variance in BP, 87% in BR and 26% in BGE (Table 3). Overall, TN explained
298 the largest share of the bacterial metabolism (on average 45 %), but there was a lot of
299 variation between sites and processes. The highest explanatory degree was acquired for
300 the BR in ponds, where concentrations of TN and Chl-a explained 66 and 21 % of the
301 variation, respectively. When models selected algal carbon variables (i.e. S289 and Chl-a)
302 their negative coefficients showed that they were negatively linked to bacterial
303 metabolism. Models for all data and for specific habitats retained nearly the same variables,
304 however, for certain habitat – bacterial variable pairs the model could not produce any
305 significant explanatory factors. This was most likely due to the low number of observations
306 on which these data sets were based.
307
308 *Bacterial community and interactions with the environment*
309 There was a clear change in the community structure along the season (Pseudo-$F_{2,22}$ =3.64,
310 $p < 0.001$) with all season pairs except for winter-spring, spring-fall and summer-fall being
311 different from each other (Supplementary Fig. 3). Also the communities residing in the
312 habitats were different from each other (Pseudo-$F_{2,22}$ =5.76, $p < 0.001$). The pond
313 communities were more similar to the inlet (pair-wise test $t = 1.77$, $p = 0.019$) than to the
314 outlet communities ($t = 3.76$, $p < 0.001$), but also the inlet and outlet communities were
315 distinct from each other ($t = 1.61$, $p = 0.037$). The BIO-ENV analyses suggested that the

316 environmental variables that best explained the OTU distribution among habitats were TP,
317 DOC, fulvic acids and proteins (Table 4). The proteins represent the readily available,
318 amino acid-like fraction of DOM and they were the carbon compounds that alone best
319 captured most of the variability. The Spearman correlations further suggested connections
320 between certain bacterial groups and carbon fractions (Fig. 3). For example, most OTUs
321 associated with flavobacterial tribe Flavo-A3 were positively correlated with humic
322 fraction and SUVA-index. Both of these are indicators of the share of terrestrial DOC. Also
323 all OTUs associated with betaproteobacterial tribe Janb had positive correlation with SUVA.
324 The indicator for algal carbon (S289) had correlations for example to alphaproteobacterial
325 lineage LD12, betaproteobacterial LD28 and verrucomicrobial LD19. The protein fraction
326 appeared to favor only a few OTUs and all of the protein correlations were weak.
327 According to the SIMPER analysis, the difference in the BCC between habitats was caused
328 primarily by the different abundance distribution of OTUs 10973, 10878, 10854, 10771,
329 10891, 10100 and 10977. Also, the conformation of the community was distinct between
330 ponds and outlets with ponds having few very abundant OTUs, whereas the outlets were
331 harboring many small ones (Fig. 4). The ponds were more abundant especially with taxa
332 such as *Betaproteobacteria* (tribes PnecC (OTU 10973) and Lhab-A2 (OTU10878)) and
333 *Bacteroidetes* (clade bacIII-A (OTU 10854)) than inlets and outlets. Correspondingly, the
334 inlets and outlets had a higher abundance of *Actinobacteria* (tribe Myco (OTU 10771) and
335 clade acI-A (OTU 10977)), *Verrucomicrobia* (OTU 10891) and *Alphaproteobacteria* (tribe
336 LD12 (OTU 10100)). A detailed analysis of BCC revealed that the ponds and outlets had
337 rather distinct communities while the inlet community was more of a mixture of the two
338 former ones (Fig. 4). The diversity of bacterial communities was affected by habitat, but not

339 by season. According to Shannon index the communities in inlets and outlets had more
340 even communities than ponds ($\chi^2 = 13.99$, $p < 0.001$; Supplementary Table 2) and also the
341 species richness (Inverse Simpson index) was higher in inlets and outlets than in ponds (χ^2
342 $= 11.97$, $p < 0.005$).
343

DISCUSSION

Interaction between DOM quality, seasonality and habitats

The results strongly suggest that crude quantity measurements of DOC are not sufficient to demonstrate the seasonal and spatial variation in organic carbon in freshwaters, but also the quality of carbon should be taken into account. Consistent with earlier reports from the area (26, 20), the total concentration of DOC was not connected to seasonality. How ever, some of the CDOM fractions (S289, fulvic acids and proteins) did exhibit seasonal variation, which is consistent with earlier observations on fulvic acids (62, 25). We also observed seasonal variation in total phosphorus and nitrogen in ponds and in inlets, but not in outlets. The lack of variation in the outlets is in accordance with the observations of Forsström *et al*. (63) from similar environment.

Seasonal changes in carbon compounds were most pronounced in the ponds, where also the concentration was highest. Under the ice samples from ponds were especially rich with amino acids, which are often considered as an indicator of the labile fraction of DOM and can therefore be used as a predictor of DOM availability (64). This fraction has been suggested to originate from autochthonous production (65), but it can also be produced by bacterial degradation (23). Here the indicator of autochthonous production, S289, was lower in under the ice samples from ponds suggesting that the increased proportion of amino acid fraction during ice cover could originate from bacterial degradation. Thus, here the protein fraction might not predict as much the availability of the carbon, but rather the degradation rate (66).

Consistent with our hypothesis, carbon in the lake outlets was characterized by fraction, which is coming from within lake production. This observation has earlier been supported by Jonsson *et al*. (25), who suggested higher impact of phytoplankton to the carbon in lake outlets than in inlets. Also the concentration of amino acids was higher in the outlets than in the inlets. In lakes the main producers of amino acids are phytoplankton (67, 65), supporting the importance of primary production to the DOM pool in outlets. Conversely, humic substances were more typical for the ponds and inlets. The humic fraction could originate either from the terrestrial production, or from *in situ* production by microbes (68), but based on the low values of S289 it can be assumed that the contribution of fulvic and humic compounds from autochthonous production was minor (35). Thus, it seems that for these variables the volume of the pool was influencing the DOM quality with smallest and fast renewing pond waters showing the highest seasonality and terrestrial impact.

Season and habitat control the bacterial metabolism in subarctic waters

Our analyses suggest that community composition and metabolic activity of subarctic aquatic bacteria is a result of a complex interplay between the community and physical and chemical variables determining the environment. In high latitude ecosystems seasonal changes are major determinants of their physico-chemical environment (69). One of the most notable determinant is temperature, but water as a habitat levels out much of the seasonal temperature variation due to its heat absorbing capacity, which, in turn, is mainly regulated by

water volume. This could be seen in this study, with the ponds having the lowest winter and highest summer temperatures. Also the rate of temperature change followed the size of the water mass. The impact of temperature to the plankton metabolism is known to be more linear in low temperatures, where it usually decreases the metabolic rates (70, 71) and also the rate of bacterial carbon degradation (72). Thus, the low temperature combined with typically low nutrient and carbon concentrations of the harsh environment at higher latitudes usually results in slow bacterial metabolism (73). Our results are well in accordance with this notion as the BP was indeed higher in the ice breakup and summer samples than in under the ice or autumn samples, and BGE peaked in summer during the maximal temperatures.

Higher concentrations of nutrients, humic acids and proteins in ponds supported the highest BP and BGE, while the algal carbon was the greatest contributor to the secondary production in lake outlets. We did not see any link between crude DOC concentration and BP, which is controversial to some earlier studies (74, 20). In contrast, the humic fraction of DOM had a positive impact on BP. Many compounds in the humic fraction of DOC are regarded as calcitrant to bacterial degradation (6) and are reported to support less BP than the non-humic fraction of DOC (75). However, humic compounds are also highly sensitive to photodegradation (28, 43, 76), which generates products that enhance bacterial metabolism (77). The occurrence of humic-like substances was highest during the ice break up in June when also the intensity of solar radiation increased in the water column after the dark winter and was at its annual maximum. Thus, the

413 photodegradation of humic compounds was likely contributing to the increased
414 BP and more so in the shallow ponds and inlets than in the outlets. Further, the
415 potential of the humic compounds for supporting growth was likely also nutrient
416 regulated as indicated by high correlation between nitrogen and phosphorus,
417 DOC and humic compounds. Also the multiple linear regression models indicated
418 that the strongest controlling factor over bacterial metabolism was total nitrogen
419 and total phosphorus concentrations. Also previous studies have suggested
420 phosphorus alone (78, 79), or together with nitrogen (80) to be the limiting
421 factor for bacterial metabolism. In accordance with our results, the availability
422 and quality of organic carbon and the availability of inorganic P and N have been
423 suggested to be key limiting factors of BGE (7, 81).
424 Models also suggested that BP and BGE had a negative relationship to S289,
425 which is the descriptor of autochthonous primary production and BP was higher
426 in the ponds and inlets. In oligotrophic lakes autochthonous production often
427 dominates over bacterial production (5, 82) and primary production is thought to
428 support BP (83, 84). This has been suggested to lead to higher BP in outlets than
429 in inlets (85). One reason for opposite trends in our study and for the negative
430 relationship between the S289 and BP and BGE could be the seasonal effect. Most
431 studies are concentrated in open water season (e.g. 82, 85) whereas very little
432 information exists for winter season. We could see a clear seasonal impact on
433 bacterial production with highest values measured during the open water season.
434 For S289, the pattern especially in the inlets was opposite and it was exhibiting
435 the highest values in under the ice samples, possibly reflecting convective

influence from perennial benthic algae that dominate the overall algal biomass in shallow arctic waters (86, 87). Thus, in order to fully understand the interaction between autochthonous carbon and BP more efforts should be addressed to include also the winter season to sampling schemes.

Implications of carbon quality, season and habitat to bacterial community composition

The combination of molecular microbiology and chemical analyses enabled us to link certain bacterial tribes to carbon fractions across habitats. Our environmental data corroborates the experimental results that members of tribe Lhab would seem to have a preference to algal carbon over terrestrial carbon (16). Another interesting link was seen between two indicators of terrestrial carbon (humic fraction and SUVA) and OTUs associated with flavobacterial tribe Flavo-A3. Bacteria associated with this group have been previously suggested to benefit from phytoplankton exudates (88), which is opposite to what was observed here. However, in a review study 30 % of the previous occurrences of tribe Flavo-A3 were from soil habitats (52), suggesting that Flavo-A3 consists of at least two groups of bacteria with very distinct environmental preferences. Another group in the bacterial community that was associated with terrestrial carbon was tribe Janb. *Janthinobacterium*, the representative genus of tribe Janb, is described as soil bacterium (52). Thus, both Flavo-A3 and Janb could be transient members of the lake community and may originate from the catchment area. There were also groups that were associated only with algal carbon. These

459 included, for example, alphaproteobacterial tribe LD12. This tribe is a sister
460 group of highly abundant marine cluster SAR11 and has been described as typical
461 for freshwater habitats (89). The previous reports suggest that the members of
462 tribe LD12 are poor competitors and their abundance has previously been
463 reported to be negatively correlated with phytoplankton (90). How ever, it has
464 been show that generally there is a lot of variation in substrate and
465 environmental preferences within bacterial tribes (80) and even within species
466 (91, 92) and further, for LD12 specifically it has been suggested that this tribe has
467 wide variations in environmental preferences across lakes (90). Thus, it is not
468 surprising that we see variation in preferences between the members of same
469 tribe residing in different habitats.
470
471 While there were indications of certain substrate preferences for bacterial OTUs,
472 the overall composition of bacterial community was controlled by season and
473 habitat. One factor that can be assumed to influence BCC is temperature. While
474 the data for under the ice BCC of freshwater lakes is scarce, it is known that there
475 is a wide variation in bacterial adaption to extreme temperatures and certain
476 bacteria are better adapted to lower temperatures or to substantial temperature
477 changes (93). This was likely a factor in the organization of winter vs. summer
478 communities in these systems. Another factor likely contributing to differences in
479 seasonal communities was the quality and availability of substrates and
480 nutrients. It has been established that BCC will change depending on the DOC
481 source (e.g. 12, 14, 15) and quality (20). Especially in the ponds carbon quality

was very different during different seasons and likely one of the most important factors contributing to variation in BCC.

Within habitats, pond community assembly was less even and there were less species than in other habitats. This is well in accordance with previous report showing that bacterial diversity increases with lake size (27). Also the observation of difference in composition between the inlet and outlet communities is corroborated by earlier results (85). The variables best explaining differences in OTU distributions between habitats included TP, DOC, fulvic acids and proteins. As stated before, phosphorus is a typical limiting source for bacteria (78, 79), explaining the strong impact.

To conclude, our results show that pond DOM contains the best combination of carbon compounds and nutrients to support BP and stimulate BGE. Further, there are indications of distinct preferences for terrestrial vs. algal carbon among certain bacterial tribes found in subarctic waters. Our study also demonstrates how the spatial variability of DOM in subarctic waters is tightly connected to season and habitat and within those, temperature and the size of the pool are major determinants creating variation beyond what is seen within season or habitat specific studies.

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775 **Legends**

776

777 **TABLE 1** Physical characteristics of the sampled lakes and ponds.

778

779 **TABLE 2** Mean values of temperature, total phosphorus (TP), total nitrogen (TN),
780 chlorophyll-a (chl-a), dissolved organic carbon (DOC), specific UV-absorbance index
781 ($SUVA_{254}$), absorption at 320 nm (a_{320}), spectral slope at 289 nm (S289) and fluorescence
782 intensity of humic, fulvic and protein compounds of DOC in Raman units (R.U). Data are
783 shown for five seasons in 2011: winter (W), spring (S), ice break (I), summer (Su), and Fall
784 (F).

785

786 **TABLE 3** Results of different multiple linear regression models (based on lowest AICc) to
787 estimate a) bacterial production (BP), b) bacteria respiration (BR) and c) bacteria growth
788 efficiency (BGE) for all data and for the three studied habitats (pond, inlet, outlet)
789 separately. Total phosphorus (TP), humic acids (Humic), total nitrogen (TN), spectral slope
790 at 289 nm (S289) and chlorohyll-a (Chl-a) were the variables used in the regression models
791 (only significant values are listed). ns: not significant. Partial R^2 below each regression
792 coefficient, N = number of data included, total R^2 (adjusted R^2), small sample size–corrected
793 Aikaike Information Criterion Index (AICc) and root mean square errors (RMSE) are
794 shown.

795

796 **TABLE 4** Combinations of environmental variables (TP, TN, DOC, Chl-a, S289, SUVA, humic,
797 fulvic and protein), taken *k* at a time, giving the four best variables alone and the largest

798 rank correlation ρ_S between OTU and environmental variable similarity matrices; **bold**
799 indicates the best combination overall.
800
801 **FIG 1** Bacterial metabolism measured as bacterial production and respiration (μgC L^{-1} d^{-1})
802 and bacterial growth efficiency (BGE) in different seasons in subarctic Kilpisjärvi waters. W
803 = winter, S = spring, I = ice breakup, Su = summer and F = fall. The letters next to the
804 symbols indicate statistical differences between seasons. Note logarithmic scale on y-axis
805 on the left side.
806
807 **FIG 2** Average values ± SE of a) bacteria production (μgC L^{-1} d^{-1}) and b) bacteria growth
808 efficiency (BGE) between subarctic ponds, inlets and outlets. The letters above the bars
809 indicate statistical differences between sites.
810
811 **FIG 3** Heatmap visualizing the Spearman correlations between abundances of OTUs and
812 concentrations of different fractions of CDOM.
813
814 **FIG 4** Ternary plot showing the distribution of OTUs between the habitats in the dataset.
815 Axes represent the pond, inlet and outlet and the percentage of reads associated with each
816 environment. The size of the symbol indicates number of reads associated with each OTU
817 and taxonomic affiliations are indicated by colors. All OTUs with at least 20 reads are
818 included into the plot.
819
820 **Supplementary TABLE 1** Summary of ANOVAs showing the effects of Habitat (Ha), Season

(Se) and crossed factors (Ha x Se) on a) temperature, b) total phosphorus (TP), c) total nitrogen (sqrt TN), d) chlorophyll-a (sqrt chl-a), e) dissolved organic carbon (DOC), f) specific UV-absorbance index ($SUVA_{254}$), g) absorption at 320 nm ($\log a_{320}$), h) spectral slope at 289 nm (x^{-1} S289) and fluorescence intensity of i) humic, j) fulvic (log) and k) protein compounds of DOC. Significant values are shown bold.

Supplementary FIG 1 Fluorescence signatures of components C1-C7 identified from the subarctic PARAFAC model. Components 1-4 and 6 (C1-C4 and C6) were combined to represent terrestrial humic-like components whereas component C5 was identified as fulvic microbial component and a commonly found component C7 as a protein-like (Tryptophan) component. Identification is based on Fellman *et al*. 2010 and refs therein.

Supplementary FIG 2 Variation in a) dissolved organic carbon (DOC) and b) total phosphorus concentration between habitats and c) in total phosphorus between seasons. The letters above the bars indicate statistical difference between values. Error bars represent standard error. W = winter, S = spring, I = ice breakup, Su = summer, F = fall.

Supplementary FIG 3 Variation in the environmental variables between seasons and habitats. a) Temperature, b) total nitrogen (TN), c) fulvic acids, d) proteins, e) spectral slope at 289 nm (S289). The letters above the bars indicate statistical difference between values. Error bars represent standard error. W = winter, S = spring, I = ice breakup, Su = summer, F = fall.

844 **Supplementary FIG 4** Principal Coordinate Analysis (PCoA) showing a) the OTU variability
845 between seasons and b) between habitats.

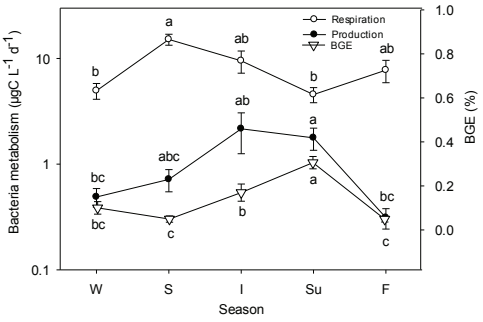


FIG 1 Bacterial metabolism measured as bacterial production and respiration (µg C L^{-1} d^{-1}) and bacterial growth efficiency (BGE) in different seasons in subarctic Kilpisjärvi waters. W=winter, S=spring, I=ice breakup, Su=summer and F=fall. The letters next to the symbols indicate statistical differences between seasons. Note logarithmic scale on the y-axis on the left side.

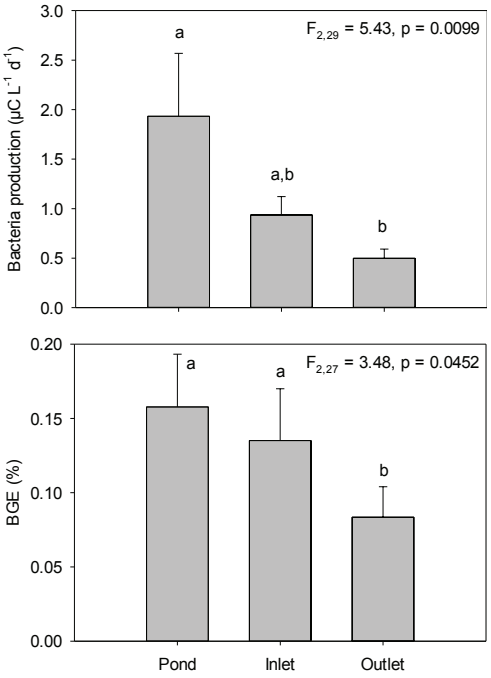


FIG 2 Average values ±SE of a)bacterial production (µg C L^{-1} d^{-1}) and b) bacterial growth efficiency (BGE) between subarctic ponds, inlets and outlets. The letters above the bars indicate statistical differences between sites.

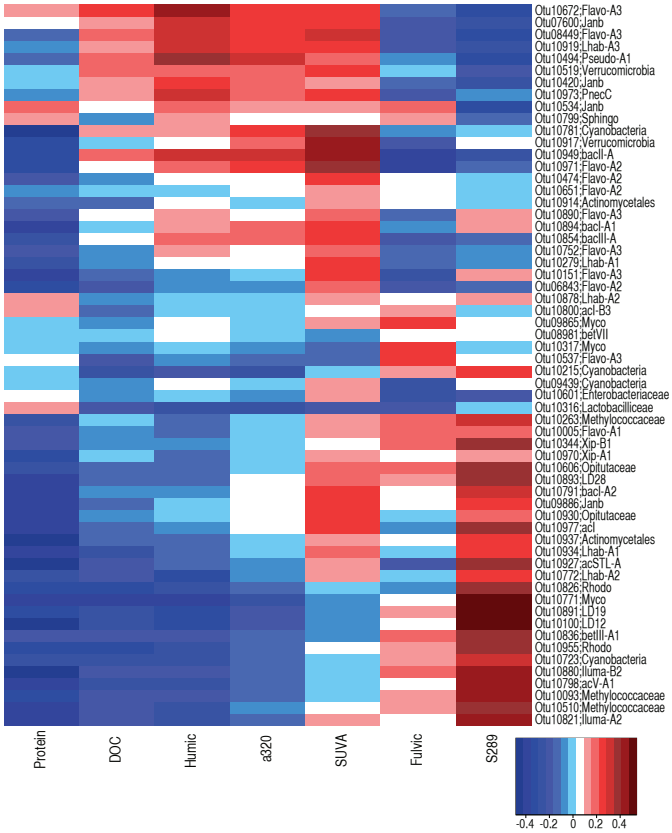


FIG 3 Heatmap visualizing the Spearman correlations between abundances of OTUs and concentrations of different fractions of CDOM.

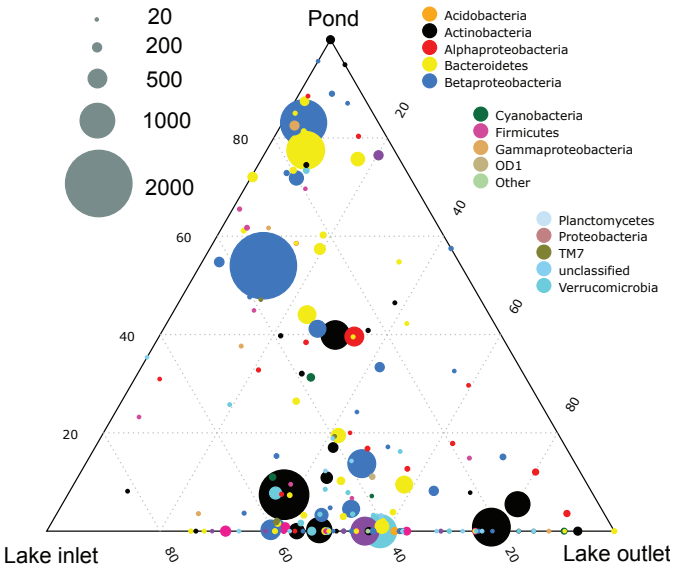
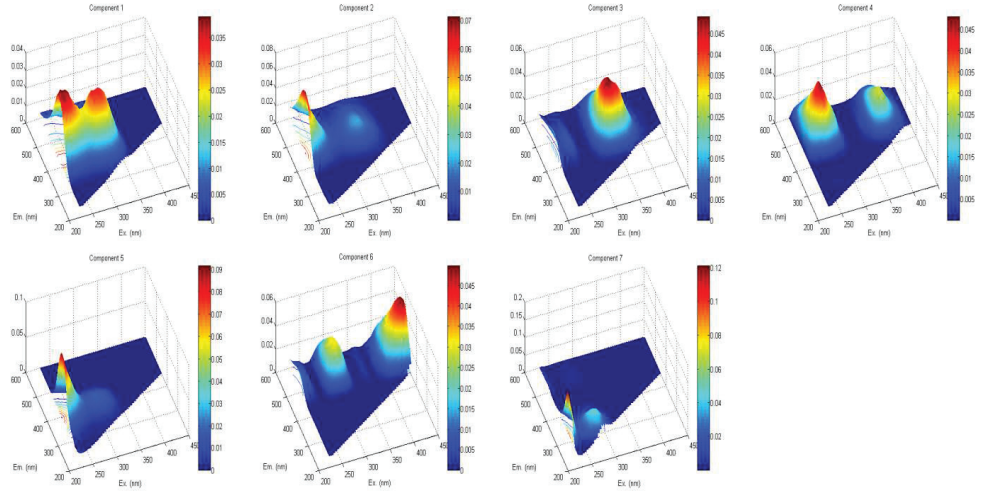
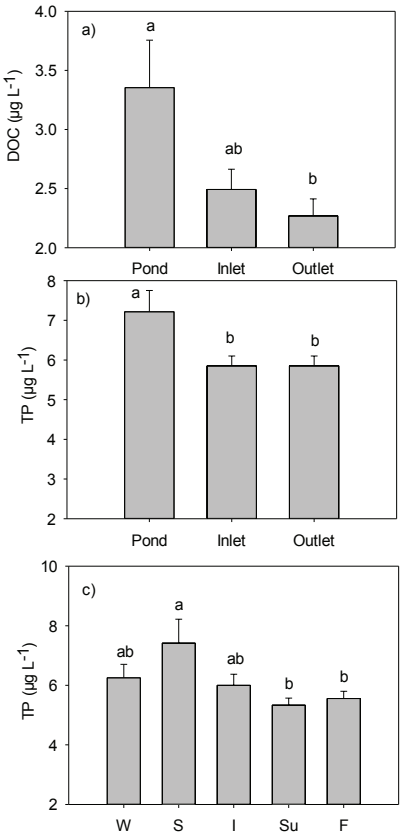


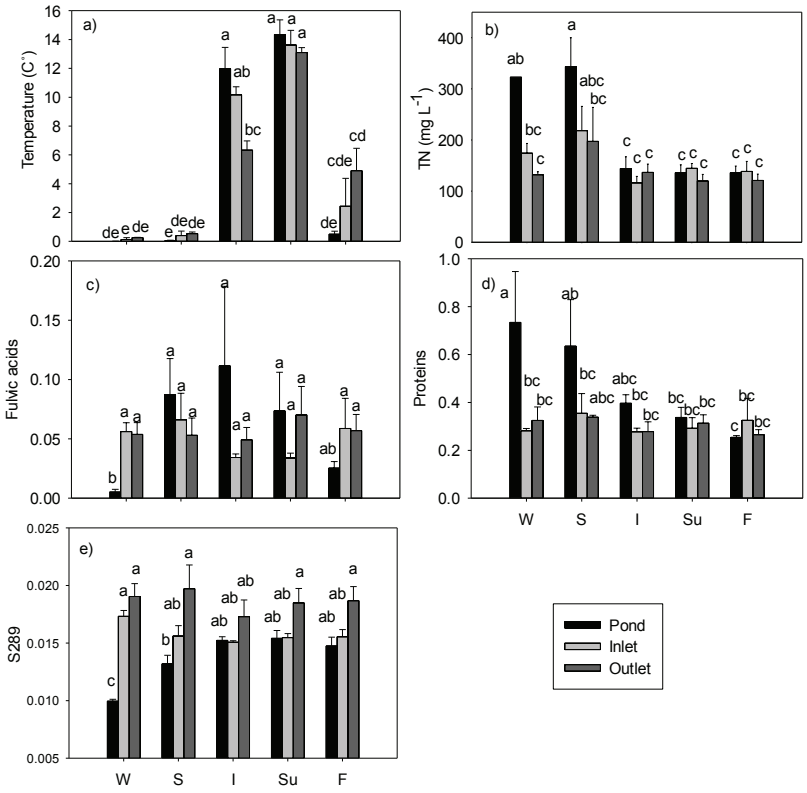
FIG 4 Ternary plot showing the distribution of OTUs between the habitats in the dataset. Axes represent ponds, inlets and outlets and the percentage of reads associated with each environment. The size of the symbol indicates number of reads associated with each OTU and taxonomic affiliations are indicated by colors. All OTUs with at least 20 reads are included into the plot.



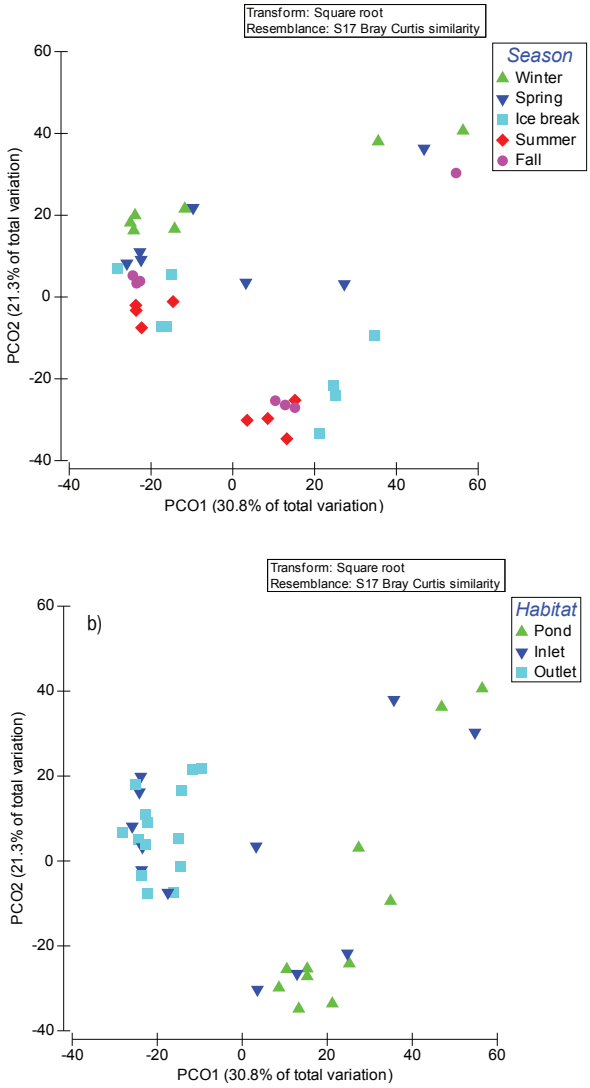
Supplementary Fig 1 Fluorescence signatures of components C1-C7 identified from the subarctic PARAFAC model. Components C1-C4 and C6 were combined to represent terrestrial humic-like component whereas component C5 was identified as fulvic microbial component and a commonly found component C7 as protein-like (tryptophan) component. Identification is based on Fellman et al. 2010 and references therein.



Supplementary Fig 2 Variation in a) dissolved organic carbon (DOC) and b) total phosphorus concentration between habitats and c) in total phosphorus between seasons. The letter above the bars indicate statistical difference between values. Error bars represent standard error. W=winter, S=spring, I=ice breakup, Su=summer, F=fall.



Supplementary Fig 3 VAriation in the environmental variables between seasons and habitats. a) Temperature, b) total nitrogen (TN), c) fulvic acids, d) proteins and e) spectral slope at 289 nm (S289). The letters above the bars indicate statistical difference between values. Error bars represent standard error. W=winter, S=spring, I=ice breakup, Su=summer, F=fall.



Supplementary Fig 4 Principal Coordinate Analysis (PCoA) showing a) the OTU variability between seasons and b) between habitats.

Table 1. Physical characteristics of the sampled lakes and ponds.

	Area (ha)	Catchment (ha)	Depth (m)	Altitude (m)
Pond 1 (Saana 15)	0.7	27	7.5	850
Pond 2 (Saana 11)	0.8	39	2.0	710
Pond 3 (Saana 12)	1.3	-	2.0	710
Lake 1 (Saanajärvi)	70	461	24.0	679
Lake 2 (Tsâhkaljärvi)	113	3396	18.0	559
Lake 3 (Kilpisjärvi)	3370	27100	57.0	473

Table 2. Mean values of temperature, total phosphorus (TP), total nitrogen (TN), chlorophyll-a (chl-a), dissolved organic carbon (DOC), specific UV-absorbance index ($SUVA_{254}$), absorption at 320 nm (a_{320}), spectral slope at 289 nm (S289) and fluorescence intensity of humic, fulvic and protein compounds of DOC in Raman units (R.U). Data are shown for five seasons in 2011: winter (W), spring (S), ice break (I), summer (Su), and Fall (F).

Site	Season	Temp (°C)	TP ($\mu g\ L^{-1}$)	TN ($\mu g\ L^{-1}$)	Chl-a ($\mu g\ L^{-1}$)	DOC ($mg\ L^{-1}$)	a_{320}	$SUVA_{254}$ ($mgC\ L^{-1}m^{-1}$)	S289	Humic (R.U.)	Fulvic (R.U.)	Protein (R.U.)
Pond	W	0.02	8.0	350	0.51	3.9	19.8	3.8	0.0099	0.7031	0.0075	0.7342
Inlet	W	0.13	6.0	174	0.11	2.8	6.6	2.6	0.0173	0.6848	0.0562	0.2815
Outlet	W	0.26	5.3	132	0.09	2.3	4.7	2.4	0.0190	0.4871	0.0538	0.3247
Pond	S	0.05	10.0	381	0.22	4.6	9.1	1.7	0.0132	0.9431	0.0872	0.6349
Inlet	S	0.40	6.6	218	0.16	2.6	6.5	2.6	0.0156	0.6465	0.0660	0.3547
Outlet	S	0.54	5.7	198	0.19	2.2	4.9	2.4	0.0197	0.4904	0.0531	0.3384
Pond	I	11.97	6.7	144	0.18	2.8	6.9	2.5	0.0152	0.7339	0.1115	0.3966
Inlet	I	10.17	5.7	116	0.29	2.5	7.7	3.2	0.0151	0.8036	0.0344	0.2771
Outlet	I	6.33	5.7	137	0.34	2.3	5.9	2.7	0.0173	0.5803	0.0491	0.2783
Pond	Su	14.35	5.7	136	0.16	2.9	7.4	2.6	0.0154	0.8631	0.0736	0.3374
Inlet	Su	13.62	5.3	145	0.20	2.6	6.7	2.7	0.0155	0.7303	0.0339	0.2924
Outlet	Su	13.10	5.0	120	0.19	2.4	5.1	2.3	0.0185	0.4702	0.0702	0.3134
Pond	F	0.50	6.0	136	0.25	2.5	6.9	2.7	0.0147	0.6996	0.0354	0.2542
Inlet	F	2.44	5.7	139	0.22	2.0	5.8	3.0	0.0155	0.6121	0.0588	0.3253
Outlet	F	4.90	5.0	121	0.54	2.2	6.2	2.4	0.0187	0.4333	0.0569	0.2652

Table 3. Results of different multiple linear regression models (based on lowest AICc) to estimate a) bacterial production (BP), b) bacteria respiration (BR) and c) bacteria growth efficiency (BGE) for all data and for the three studied habitats (pond, inlet, outlet) separately. Total phosphorus (TP), humic acids (Humic), total nitrogen (TN), spectral slope at 289 nm (S289) and chlorohyll-a (Chl-a) were the variables used in the regression models (only significant values are listed). ns: not significant. Partial R^2 below each regression coefficient, N = number of data included, total R^2 (adjusted R^2), small sample size–corrected Aikaike Information Criterion Index (AICc) and root mean square errors (RMSE) are shown.

	Intercept	TP	Humic	TN	S289	Chl-a	N	R^2 (adj. R^2)	AICc	RMSE
a) BP										
All data	-1.00	0.24	0.86	ns	ns	ns	42	0.26 (0.22)	105.65	0.816
Partial R^2		0.19	0.07							
Pond	-	ns	ns	ns	ns	ns	13	-	-	-
Partial R^2										
Inlet	4.77	ns	ns	0.007	-320.31	ns	15	0.62 (0.56)	30.12	0.495
Partial R^2				0.38	0.24					
Outlet	-	ns	ns	ns	ns	ns	14	-	-	-
Partial R^2										
b) BR										
All data	-2.45	ns	ns	0.078	ns	ns	39	0.36 (0.22)	105.65	0.816
Partial R^2				0.36						
Pond	-4.80	ns	ns	0.12	ns	-24.77	13	0.87 (0.84)	80.23	4.47
Partial R^2				0.66		0.21				
Inlet	-	ns	ns	ns	ns	ns	14	-	-	-
Partial R^2										
Outlet	-7.16	ns	ns	0.11	ns	ns	13	0.80 (0.79)	75.67	3.46
Partial R^2				0.80						
c) BGE										
All data	0.63	ns	ns	-00006	-22.10	-0.18	39	0.26 (0.20)	-52.48	0.11
Partial R^2				0.07	0.11	0.08				
Pond	-	ns	ns	ns	ns	ns	13	-	-	-
Partial R^2										
Inlet	-	ns	ns	ns	ns	ns	14	-	-	-
Partial R^2										
Outlet	-	ns	ns	ns	ns	ns	13	-	-	-
Partial R^2										

Table 4**.** Combinations of environmental variables (TP, TN, DOC, Chl-a*,* S289, SUVA, humic, fulvic and protein), taken *k* at a time, giving the four best variables alone and the largest rank correlation ρ_s between OTU and environmental variable similarity matrices; **bold** indicates the best combination overall.

k	Best variable combinations (ρ_s)			
1	Protein (0.42)	DOC (0.38)	TN (0.35)	TP (0.34)
3	TP, fulvic, protein (0.54)			
4	**TP, DOC, fulvic, protein (0.57)**	TP, humic, fulvic, protein (0.55)	TP, S289, fulvic, protein (0.54)	
5	TP, DOC, S289, fulvic, protein (0.56)	TP, DOC, humic, fulvic, protein (0.56)	TP, DOC, SUVA, fulvic, protein (0.55)	TP, S289, humic, fulvic, protein (0.57)

Supplementary table 1. Summary of ANOVAs showing the effects of Habitat (Ha), Season (Se) and crossed factors (Ha x Se) on a) temperature, b) total phosphorus (TP), c) total nitrogen (sqrt TN), d) chlorophyll-a (sqrt chl-a), e) dissolved organic carbon (DOC), f) specific UV-absorbance index ($SUVA_{254}$), g) absorption at 320 nm (log a_{320}), h) spectral slope at 289 nm (x^{-1} S289) and fluorescence intensity of i) humic, j) fulvic (log) and k) protein compounds of DOC. Significant values are shown bold.

Source of variation	df	MS	F	p-value	Source of variation	df	MS	F	p-value
a) Temperature					b) TP				
Ha	2	0.56	0.24	0.7904	Ha	2	14.15	12.88	**<0.0001**
Se	4	320.0	135.36	**<0.0001**	Se	4	6.12	5.57	**0.0019**
HaXSe	8	10.10	4.24	**0.0018**	HaXSe	8	2.10	1.91	0.0970
Residual	29				Residual	29			
C. Total	43				C. Totakl	43			
c) TN (sqrt)					d) Chl-a (sqrt)				
Ha	2	32.73	10.83	**0.0003**	Ha	2	0.027	0.87	0.4312
Se	4	38.75	12.83	**<0.0001**	Se	4	0.030	0.94	0.4530
HaXSe	8	8.83	2.92	**0.0160**	HaXSe	8	0.038	1.18	0.3430
Residual	29				Residual	29			
C. Total	43				C. Total	43			
e) DOC					f) a_{320} (log)				
Ha	2	4.97	5.10	**0.0127**	Ha	2	1.04	2.90	0.0722
Se	4	1.26	1.30	0.2950	Se	4	0.11	0.30	0.8731
HaXSe	8	0.86	0.88	0.5453	HaXSe	8	0.13	0.37	0.9277
Residual	29				Residual	27			
C. Total	43				C. Total	41			
g) SUVA					h) S289 (x^{-1})				
Ha	2	0.50	1.36	0.2736	Ha	2	1421	50.17	**<0.0001**
Se	4	0.47	1.29	0.2987	Se	4	100.9	3.56	**0.0186**
HaXSe	8	0.48	1.31	0.2801	HaXSe	8	244.1	8.62	**<0.0001**
Residual	27				Residual	27			
C. Total	41				C. Total	41			

Source of variation	df	MS	F	p-value	Source of variation	df	MS	F	p-value
i) Humic					j) Fulvic (log)				
Ha	2	0.290	2.29	0.1204	Ha	2	0.655	2.30	0.1200
Se	4	0.023	0.18	0.9464	Se	4	1.008	3.54	0.0190
HaXSe	8	0.014	0.11	0.9984	HaXSe	8	1.355	4.76	**0.0010**
Residual	27				Residual	27			
C. Total	41				C. Total	41			
k) Proteins									
Ha	2	0.120	9.87	**0.0006**					
Se	4	0.048	3.36	**0.0118**					
HaXSe	8	0.033	2.62	**0.0289**					
Residual	27								
C. Total	41								

Supplementary Table 2. Shannon and Inverse Simpson indices for different habitats.

Habitat	Shannon (± std error)	Inverse Simpson (± std error)
Pond	2.1 ± 0.8	6.2 ± 4.2
Inlet	2.9 ± 0.9	16.4 ± 10.0
Outlet	3.3. ± 0.4	18.6 ± 7.9