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RESEARCH ARTICLE

Carbon quantity defines productivity while its quality defines community composition of bacterioplankton in subarctic ponds

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Abstract Bacterial communities in 16 oligotrophic ponds in Kilpisjärvi, subarctic Finland, were studied to test the hypothesis that dissolved organic carbon (DOC) quantity and quality differently influence bacterioplankton. The ponds were located below and above treeline at 600 m a.s.l., with 2–4 fold higher concentration of DOC below treeline. The concentration of DOC changed during the open-water season with highest values measured in mid-summer. Bacterial production, abundance, biomass were highest in mid-summer and correlated positively with the concentration of DOC. Quality indices of DOC showed that spring differed from the rest of the season. Highest specific UV-absorbance (SUVA) and humification index (HI), ratio a250/a265 and lowest fluorescence index (FI) were found during spring compared to summer and autumn, possibly indicating higher relative importance of allochthonous carbon during spring and a seasonal effect of photo-oxidation. According to Length Heterogeneity Polymerase Chain Reaction (LH-PCR) analyses, bacterial communities in spring were significantly different from those later in the season, possible due to the introduction of terrestrial bacteria associated with higher molecular weight material in spring DOC. Comparison between ponds situated above and below treeline revealed that bacteria were more abundant and productive at lower altitudes, which is probably connected to higher concentrations of DOC. The results also suggest that increased temperature and precipitation induced by global change and consequent higher allochthonous DOC runoff from the catchment could have a strong impact on biomass, productivity and community composition of micro-organisms in subarctic ponds and lakes.

Keywords Subarctic · DOC · Ponds · Bacterial biomass · Bacterial production · Bacterial community composition · Allochthonous carbon

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Introduction

Heterotrophic bacterioplankton need organic carbon as an energy source and their productivity in lakes is largely determined by the amount of allochthonous (terrestrial) DOC inputs from the catchment area (Tranvik 1988; Hessen et al. 1990, 2004; Crump et al. 2003). In small subarctic and arctic ponds, the importance of DOC inputs is also great but varies spatially. Location of the water body, soil type of the catchment, and annual variation in precipitation and runoff all have a direct impact on allochthonous carbon loads and hence on biomass and productivity of bacteria (ACIA 2005; Hobbie and Laybourn-Parry 2008). Since the amount of DOC in northern lakes is typically low (<5 mg C l^{-1}), even a small increase in DOC in runoff water may have strong and rapid impacts on lake condition (e.g. light attenuation, nutrient levels, benthic primary production) (Karlsson et al. 2009). Climate warming and increasing precipitation have significant impacts on the interaction between the lake and catchment area by increasing organic material inputs

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(ACIA 2005; McGuire et al. 2009). This carbon accumulated in soils can contribute to adding green house gases by releasing a part of the organic load from the drainage area to the atmosphere by bacterial respiration (Hanson et al. 2003; Walter et al. 2006; Laurion et al. 2010). Such changes likely occur first at the polar treeline (Hobbie and Laybourn-Parry 2008).

The most distinct decline in DOC concentration occurs when crossing the northern treeline (Vincent and Pienitz 1996; Rautio 2001), which in Scandinavia is the boundary between the mountain birch forest and treeless tundra. This boundary also represents a change in the quality of DOC (Baron et al. 1991; Rautio and Vincent 2007). This change is especially reflected in the coloured fraction of dissolved organic matter pool (CDOM), which is primarily a complex mixture of degraded terrestrial and aquatic plant material having a strong influence on the underwater light climate. Another factor influencing DOC quality in higher latitudes is photo-oxidation, which probably plays notable role in decomposing heavier carbon molecules (Lindell et al. 1995, 1996). Ponds and lakes below treeline receive higher concentrations of large molecular-sized allochthonous carbon compounds compared to those above treeline. Previous work has shown a relation between the source of organic material and its fluorescence properties, making optical spectroscopy a useful tool for the characterization of the quality of dissolved organic matter (DOM) (Kalbitz et al. 1999; Lindell et al. 1995; McKnight et al. 2001; Weishaar et al. 2003).

Tranvik (1988) estimated that <15% of allochthonous DOC is readily available for bacteria, although the large relative amount of this DOC, in comparison to autochthonous DOC, often makes it the dominant carbon source for bacteria growth. Bacteria metabolizing allochthonous carbon can provide an additional energy source for the whole food web (Jones 1992; Karlsson et al. 2002; Pace et al. 2004) and also releases bacterioplankton from dependence on phytoplankton-derived autochthonous carbon (Jansson et al. 1999).

The change in catchment vegetation characteristics is also reflected in the chemical and physical condition of the respective water bodies, such as temperature, pH and nutrient concentrations (Blom et al. 2000). This change in water quality probably has an impact on bacterioplankton dynamics. Biomass of bacteria and their biological efficiency have been reported to be dependent on inorganic nutrients (Simon et al. 1998; Granéli et al. 2004), temperature (Ochs et al. 1995; Rae and Vincent 1998), UV radiation (Laurion et al. 1997) and grazing by nanoflagellates and cladocerans (Kankaala 1988; Laybourn-Parry and Marshall 2003).

The purpose of our study was to find out how differences in the quantity and quality of DOC affect bacteria communities in subarctic ponds across the northern treeline in Finnish Lapland. This aim was achieved by studying bacterioplankton parameters (abundance, productivity, biomass, cell size and community composition) in relation to environmental variables, including detailed spectroscopic analysis of water to estimate carbon quality. Additionally, we describe the seasonal succession of bacterioplankton dynamics during the short northern summer from ice-out in May–June to freezing in September and give some insights into bacterioplankton community composition changes in northern ponds. To our knowledge, this is among the first studies to include carbon quality indices when studying shifts in natural bacterioplankton dynamics.

Methods

Field measurements, chlorophyll-*a* (chl-*a*) and nanoflagellates

We sampled 16 small, shallow ponds in the Kilpisjärvi region (69°N, 20°E) of NW Finnish Lapland along an altitudinal gradient from 485 to 950 m a.s.l. (Fig. 1; Table 1). These ponds, and especially their zooplankton community structure, have been intensively studied earlier (Rautio 1998, 2001; Mariash et al. 2011). The treeline of mountain birch (*Betula pubescens* subsp. *czerepanovii* (Orlova) Hämet-Ahti) is at around 600 m a.s.l. and eight ponds were situated below treeline and eight ponds were above or at treeline (Altitudinal). The ponds were distributed in five different areas: Malla, Tsâhkal, Jeâhkkas, Siilasvuoma and Saana (Spatial) (Fig. 1). Surface area and catchment area (mean area = 10.0 ha, range 0.6–42.0 ha) of each pond was taken using geographical information system (GIS). All 16 ponds were sampled three times in 2008: in May–June at the ice-out, in mid-July, and in September 2–3 weeks before the ponds froze (Seasonal). On each occasion, 3 separate surface water samples were collected around the pond shoreline and mixed together as a 2 L composite sample from which temperature,

Table 1 Physical characteristics from ponds below and above treeline

	<600 m	>600 m
Lake area (ha)	0.3 (0.1–0.8)	0.8 (0.1–1.4)
Drainage area (ha)	3.6 (0.6–17.2)	17.3 (0.6–41.6)
Depth (m)	1.1 (0.5–2.0)	3.0 (0.5–7.5)
Altitude (m)	527 (485–570)	825 (600–950)

Numbers outside parentheses are averages from ponds in a group. Numbers in parentheses are minimum and maximum values

conductivity and pH were measured immediately with a YSI 63 or YSI Professional probe (YSI incorporated, Yellow Springs, USA). Earlier sampling has shown that the water column in all but the two deepest ponds (Ponds 15 and 16 in Fig. 1; 7.5 and 3 m, respectively) mix constantly. A 200 ml subsample was filtered through a GFF filter and frozen until spectrophotometric and spectrofluorometric analysis of chl-*a* following the method by Nusch (1980). Bias caused by phaeopigments was removed by measuring the concentration from acidified chl-*a* samples (Yentsch and Menzel 1963) and final calculations for chl-*a* concentration were done according to Jeffrey and Walschmeyer (1997). Sample water for enumeration of heterotrophic and pigmented nanoflagellates was collected only in summer. The sample was preserved with <0.2 µm filtered formaldehyde (1% final concentration). Three 20 ml replicates were filtered through 0.6 µm, 25 mm black polycarbonate filters. Samples were stained with 4-,6-diamido-2-phenylindole (DAPI) with a final concentration of 5 µg ml^{-1}. Samples were counted for total heterotrophic (HNF) and pigmented (PNF) nanoflagellates under UV excitation with an epifluorescense microscope (Olympus BH-2) with 1,000× magnification. Green excitation was used to discriminate autotrophic and mixotrophic cells with pigments. At least 50 cells from each filter were counted.

Carbon characterization

A 200 ml aliquot was filtered for DOC (0.2 µm cellulose acetate filter) and sent to the Lapland Regional Environment Centre in Rovaniemi for immediate analysis by using standard methods (Finnish Standards Association SFS-EN 1484:1997) of the National boards of Waters in Finland. Another 200 ml subsample was filtered (0.2 µm cellulose acetate filter) to acid-washed and sample-rinsed glass bottles and stored in the dark at 4°C for further CDOM analysis to characterize the quality of carbon. CDOM absorption was measured in dual-beam mode with scanning spectrophotometer (Cary 100 UV–Vis; Varian Inc. Walnut Creek, USA) by using a 10-cm quartz acid-washed cuvette especially designed for highly transparent waters. Scanning was performed at 1 nm intervals between wavelengths 250 and 800 nm against MilliQ water. Mean value for wavelengths between 750 and 800 nm was used to minimize the noise in null correction procedure. Absorption coefficients were calculated as in Mitchell et al. (2003). Wavelengths between 300 and 650 nm were chosen to calculate nonlinear regressions for CDOM properties (Stedmon et al. 2000). A suite of quality indices was calculated to characterize organic carbon. SUVA parameter was determined from DOC normalized absorption coefficient at the

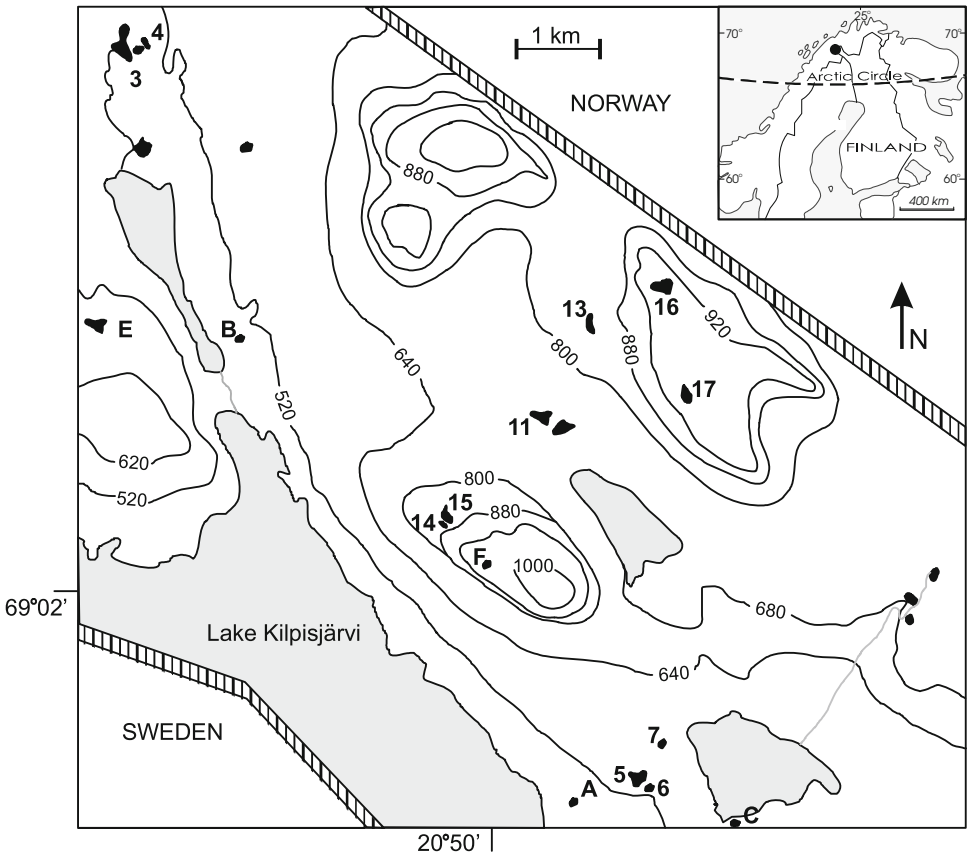

Fig. 1 Location of the 16 ponds studied in the Kilpisjärvi region in five spatial areas: Saana (ponds 11, 14–15 and *F*), Malla (ponds B and E), Siilasvuoma (ponds 3–4), Jeâhkkas (ponds 13, 16–17) and Tsâhkal (ponds A, C and 5–7). The treeline follows the 600 m contour

wavelength 254 nm (Weishaar et al. 2003). Increasing SUVA could indicate greater contribution of terrestrially derived carbon (Hood et al. 2003, 2005). Another index calculated was the ratio between absorbance at 250 and 365 nm that is known to increase with increased UV-exposure, indicating importance of photo-oxidation (Lindell et al. 1995).

In general, low molecular weight compounds, often of autochthonous origin, fluoresce intensely at the lower end and high molecular compounds of allochthonous origin at the higher end of synchronous fluorescence (SF) spectra (Stewart and Wetzel 1980). SF spectra were recorded between 200 and 700 nm with 14 nm difference between excitation and emission beams and with a slit width of 5 nm using a spectrofluorometer (Cary eclipse; Varian Inc., USA). SF spectra were corrected for blank (MilliQ), baseline and inner-filter effect (Mobed et al. 1996). Accuracy of the instrument was checked using Raman peak correction. Ratio between SF relative intensity at 470 nm over 360 nm was used as a humification index (HI), which was proposed by Kalbitz et al. (1999) to represent the degree of humification and polycondencation from sample water. Simple fluorescence emission scans from 400 to 700 nm were performed with single excitation at 370 nm to calculate Fluorescence index (FI), which is an index to analyse the origin of fulvic acids from microbial versus terrestrial sources. FI was calculated as the ratio of relative fluorescence emission intensities at 450 nm over 500 nm as proposed by McKnight et al. (2001).

Bacterial analyses

Bacterial abundance and biomass were determined from sample water preserved with <0.2 µm filtered formaldehyde (2% final concentration). Three replicates of water (2 ml each) were filtered with low pressure through a 0.2 µm, 25 mm black polycarbonate filter (Hobbie et al. 1977) 2–6 h after sampling. Bacteria were then stained with 4-,6-diamido-2-phenylindole (DAPI) with a final concentration of 5 µg ml^{-1} (Porter and Feig 1980). DAPI stained filters were stored in –20°C and in the dark. Samples were counted under UV excitation with an epifluorescense microscope (Leica Leitz DMRB) with 1,000× magnification. At least 400 cells from each filter were counted. For biomass estimation, the length and width of the cells were estimated from digital images using Cell C—program (Selinummi et al. 2005). Biovolume was converted to biomass using a coefficient of 0.308 pgC μm^{-3} (Fry 1988).

Productivity of heterotrophic bacteria was estimated using tritiated leucine incorporation (Kirchman et al. 1985) and a centrifugation method from Smith and Azam (1992). Productivity measurements started 2–6 h after sampling and were conducted in 1.5 ml Eppendorf vials with triplicate samples and duplicate controls. ^{3}H-leucine (Specific activity 73 Ci $mmol^{-1}$) was added to each vial to obtain a final leucine concentration of 20 or 30 nM, respectively. Two separate leucine concentrations were used due to different saturation points found from low and high altitude ponds. Time zero controls were terminated at this point with addition of trichloroacetic acid (TCA; 5% final concentration). Samples were incubated in the dark and cold (4°C) for 2–4 h to remove the effect of temperature. Immediately after incubation, TCA was added to stop bacterial production. Samples were then stored for 2–6 weeks at −20°C and in the dark. Leucine concentration and incubation time were estimated from saturation and incubation time curves obtained experimentally from both low, and high altitude ponds. Frozen samples were thawed at room temperature for 30 min before centrifugation. All vials were centrifuged approximately at 11,370 g (12,000 rpm) for 10 min and the supernatant was removed. This was done twice, and after the first supernatant removal, the vials were rinsed by adding 1 ml of cold TCA 5%. After the second supernatant removal, 1 ml of scintillation cocktail was added to the vials and they were vortexed. Samples were stored in the dark for at least 24 h and then radioassayed with a RackBeta scintillation counter (LKB Wallac, Model 1217-001). CPM values were converted to DPM values using the quenching curves installed in the scintillation counter. Coefficients from Simon and Azam (1989) were used to calculate leucine incorporation.

A total of 500 ml of unfiltered sample water was frozen (−20°C) and kept in the dark for community composition analysis. Bacterial DNA-extraction was done with a DNA isolation kit (PowerSoil™ DNA, MOBIO Laboratories Inc.). Frozen sample water was freeze-dried (Christ Alpha 1-4 LD Plus) to obtain at maximum 4 mg (on average 2.7 mg) of dried material. LH-PCR patterns are not significantly affected by the initial template concentration (Tiirola 2002). Dried material was diluted to bead tubes provided by the kit and 100 µl of isolated DNA (1–10 ng) was prepared following the isolation instructions. After preparing the PCR-master mix containing 10 × Biotools buffer, IRD700 labelled forward primer 27F (5′-AGAGTT TGA TCM TGG CTC AG-3) (Lane 1991) and reverse primer PRUN518r (5′-ATTACC GCG GCT GCT GG-3) (Muyzer et al. 1993), dNTPs mix, sterile water and biotools polymerase) for PCR, it was mixed in a ratio of 24 µl Master Mix to 1 µl template. Samples were then placed in a Perkin-Elmer GeneAMP 9600 and PCR was performed to amplify bacterial 16S rRNA-genes from the sample. The PCR procedure included a denaturation step of 5 min at 95°C followed by 35 amplification cycles (94°C for 30 s 52°C for 1 min and 72°C for 15 min) and the final PCR-product was stored at 4°C. Fragments were then separated

using agarose gel electrophoresis. An automated LI-COR 4200 sequencer (LI-COR BioTech, Lincoln, NE) was used to run the electrophoresis gel for 6 h or overnight using 6% Long Ranger denaturing polyacrylamide gel (FMC Bioproducts, Rocland, ME). The loading mixture (10% buffer, 33% Licor loading dye and 57% sterile water) and PCR-product were mixed in a ratio of 19 µl of loading mixture and 1 µl of PCR product and then loaded to the LI-COR sequencer. The gel was then pre-run for 30 min and the run was performed with size standards of 470, 527 and 553 base pairs. The data were analyzed using Quantity One software (Bio-Rad Laboratories, Hercules, CA) and fragments that represented over 0.5% of the summed fragment area were taken into account in the final analysis. The original method using measurements of small subunit rRNA gene PCR amplicon length heterogeneity as an illustration of community structure is based on Suzuki et al. (1998). Although this method is robust and does not allow identification of bacteria species, it still provides an efficient tool to characterize changes in community composition.

Statistical tests

Three statistical tests were run for all bacterial parameters. First, multifactorial analysis of variance was used to identify significant differences in mean values among groups (seasonal, altitudinal and spatial). Second, Pearson's correlation was used to test relationships among bacterial parameters (abundance, production, biomass and cell size) and physico-chemical properties (temperature, conductivity, pH, DOC, chl-*a*, lake area, depth and altitude) and DOM parameters (SUVA, FI, HI and a250/a365). In the analysis of bacterial productivity, temperature was not included as a variable as the productivity measurements were carried out at a constant temperature of 4°C. Finally, stepwise multiple regression analysis was also done with all data (excluding chl-*a* that was measured only for summer and autumn samples) to identify the variables best explaining variation in bacterial abundance, production, biomass and cell size (PASW Statistic 18). For the analysis, bacterial production and biomass results were transformed in logarithmic scale. In addition, independent samples *t* test was used to evaluate differences in HNF and PNF abundances between low and high altitude ponds (PASW Statistic 18).

Main fragment size classes in LH-PCR data were normalized by Bray–Curtis similarity and then analyzed (1) seasonally, (2) altitudinally and (3) spatially with permutational multivariate analysis of variance (MANOVA), similarities between groups were tested with a Pair-wise test. Statistically significant differences in MANOVA were illustrated by canonical analysis of principal coordinates (CAP). Statistic analyses for bacterial diversity were performed with Primer 6.1.12 and Permanova+ 1.0.2 (Primer-E Ltd, UK).

Results

Some physical characteristics of the ponds are shown in Table 1. In earlier studies (Rautio 1998, 2001), the ponds were divided into three groups according to their location in relation to treeline, but here the results from factor analysis based on water temperature, pH, dissolved organic carbon (DOC) and conductivity suggested separation into just two groups: low altitude ponds below treeline (<600 m a.s.l.) and high altitude ponds above treeline (>600 m a.s.l.). Results of the environmental measurements are shown in Table 2.

Highest bacterial abundance was measured in summer in both pond groups, although this was more marked in low altitude ponds. A seasonal trend was found in both low and high altitude ponds with lowest cell densities in spring and autumn and highest in summer, although cell density was about two (1.9–2.2) times higher in low altitude ponds (Fig. 2a). A similar seasonal trend was found in bacterial production in low altitude ponds with low production in spring and autumn and higher production in summer, but no such trend was evident in high altitude ponds where production remained low during the whole sampling season (Fig. 2b). Two exceptionally high values for abundance and productivity in spring could not be compared with autumn values because one pond dried out during summer and another sample was clearly contaminated with bottom sediment, therefore these two data points were removed from the statistical analyses. Bacterial production results are likely underestimates when compared to the in situ situation because the incubation temperature of 4°C was lower than the in situ water temperature during summer (ca. 14°C). On the other hand, a stable incubation temperature removes the temperature variable from potential factors affecting production rates and was done as our focus was on carbon effects. Bacterial biomass showed similar patterns to abundance and productivity. Lower values were measured in spring and autumn and clearly higher bacterial biomass was found during summer in low altitude ponds. In high altitude ponds, spring and autumn bacterial biomass were similar and only slightly higher values were recorded in summer (Fig. 2c), although the differences were not statistically significant. Cell size of bacteria showed that larger bacteria were present in low altitude ponds than in high altitude ponds (Fig. 2d).

Potential range of grazing impact on bacterioplankton was assessed by counting abundances of heterotrophic nanoflagellates (HNF) and pigmented nanoflagellates

Table 2 Seasonality of environmental characteristics of ponds below and above treeline

	Spring		Summer		Autumn	
	<600 m	>600 m	<600 m	>600 m	<600 m	>600 m
Temperature (°C)	6.0	2.0	16.5	12.0	7.4	6.4
	(1.5–11.6)	(0.6–4.5)	(15.9–17.5)	(9.7–15.7)	(5.9–8.3)	(5.0–7.8)
Conductivity (μS cm^{-1})	19.8	7.3	37.1	15.1	39.1	21.7
	(8.6–28.8)	(5.0–10.0)	(16.2–71.1)	(5.3–24.0)	(12.2–70.5)	(4.4–62.6)
pH	6.1	6.5	6.8	7.1	6.7	7.1
	(5.3–6.6)	(5.8–8.0)	(5.6–7.4)	(6.7–8.1)	(5.2–7.7)	(6.5–7.7)
Chl-*a* (μg l^{-1})	–	–	1.3	0.4	1.0	0.5
			(0.6–2.2)	(0.2–0.8)	(0.4–2.6)	(0.5–0.7)
DOC (mg l^{-1})	4.3	2.1	7.6	2.4	5.25	2.6
	(2.6–6.9)	(1.3–4.1)	(3.9–13)	(1.4–3.5)	(2.4–8.8)	(1.9–4.0)
SUVA[a]	8.3	8.6	7.1	4.7	5.4	3.4
	(4.9–11.4)	(6.5–9.9)	(4.2–12.3)	(3.4–6.4)	(3.2–9.5)	(2.5–4.6)
a250/a365[b]	4.4	4.5	4.8	5.1	5.4	5.8
	(4.2–4.7)	(4.1–5.1)	(3.0–6.8)	(4.3–5.8)	(5.3–5.6)	(4.6–6.6)
FI[c]	1.15	1.19	1.14	1.20	1.22	1.27
	(1.11–1.24)	(1.12–1.28)	(1.02–1.30)	(1.15–1.30)	(1.05–1.31)	(1.19–1.40)
HI[d]	1.10	1.14	0.90	0.94	0.86	0.87
	(0.95–1.20)	(0.95–1.39)	(0.80–1.07)	(0.79–1.15)	(0.68–1.00)	(0.83–0.95)

Numbers outside of the parentheses are seasonal averages. Numbers inside parentheses are seasonal minimum and maximum values

[a] Specific UV-absorbance (Weishaar et al. 2003)

[b] Photo-oxidation index (Lindell et al. 1995)

[c] Fluorescence index (McKnight et al. 2001)

[d] Humification index (Kalbitz et al. 1999)

(PNF) during summer. In ponds below ($n = 4$) treeline, the abundance of HNF (447 ± 149 ind ml^{-1}) was higher than in ponds above ($n = 7$) treeline (308 ± 112 ind ml^{-1}), although the difference was not statistically significant ($t = 0.99$, $df = 6.3$ and $p = 0.358$). Abundance of PNF was at the same level in both pond groups (116 ± 71 and 108 ± 26 ind ml^{-1}, respectively) and there was no statistically significant difference ($t = 0.38$, $df = 9$ and $p = 0.711$).

Changes between bacterial parameters (abundance, production, biomass and cell size) and three different factors (seasonal, altitudinal and spatial) were tested with multifactorial ANOVA to test if the found shifts in bacterial parameter patterns were statistically significant. Significant differences among means were found in almost all cases, with only exceptions in altitudinal bacterial production ($F(1.27) = 3.20$, $p = 0.085$) and bacterial cell size among seasons ($F(2.27) = 1.17$, $p = 0.327$) and space ($F(4.27) = 2.24$, $p = 0.091$) (Table 3). Seasonally, more detailed examination among all bacterial parameters with post hoc test (Tukey) revealed that summer was the most distinct season. Spatial tests confirmed the results from the altitudinal test that high altitude ponds differed from low altitude ponds.

All correlations among bacterial parameters and environmental data are provided in Table 4. Pearson's correlation analysis was used to estimate how all physico-chemical parameters were connected to bacterial parameters. The strongest significant positive correlations between bacteria and environmental parameters were found between DOC and bacterial abundance and biomass. These bacterial variables acted very similarly to each other with both having strong significant positive correlations with chl-*a* and temperature. Bacterial production was measured in stable temperature and therefore the temperature variable was removed from the analysis. Otherwise, bacterial production followed the same pattern with bacterial abundance and biomass. Lake area, depth and altitude had a significant negative correlation with bacterial abundance and biomass, but not with bacterial production. Bacterial cell size followed almost the same pattern with other bacterial variables, having a positive correlation with temperature and negative correlations with lake area and depth. Also, fluorescence index (FI) had a strong negative correlation with bacterial abundance and biomass, but characterization of dissolved organic matter (DOM) revealed that spectroscopically measured optical properties of DOM did not follow the changes in DOC concentrations

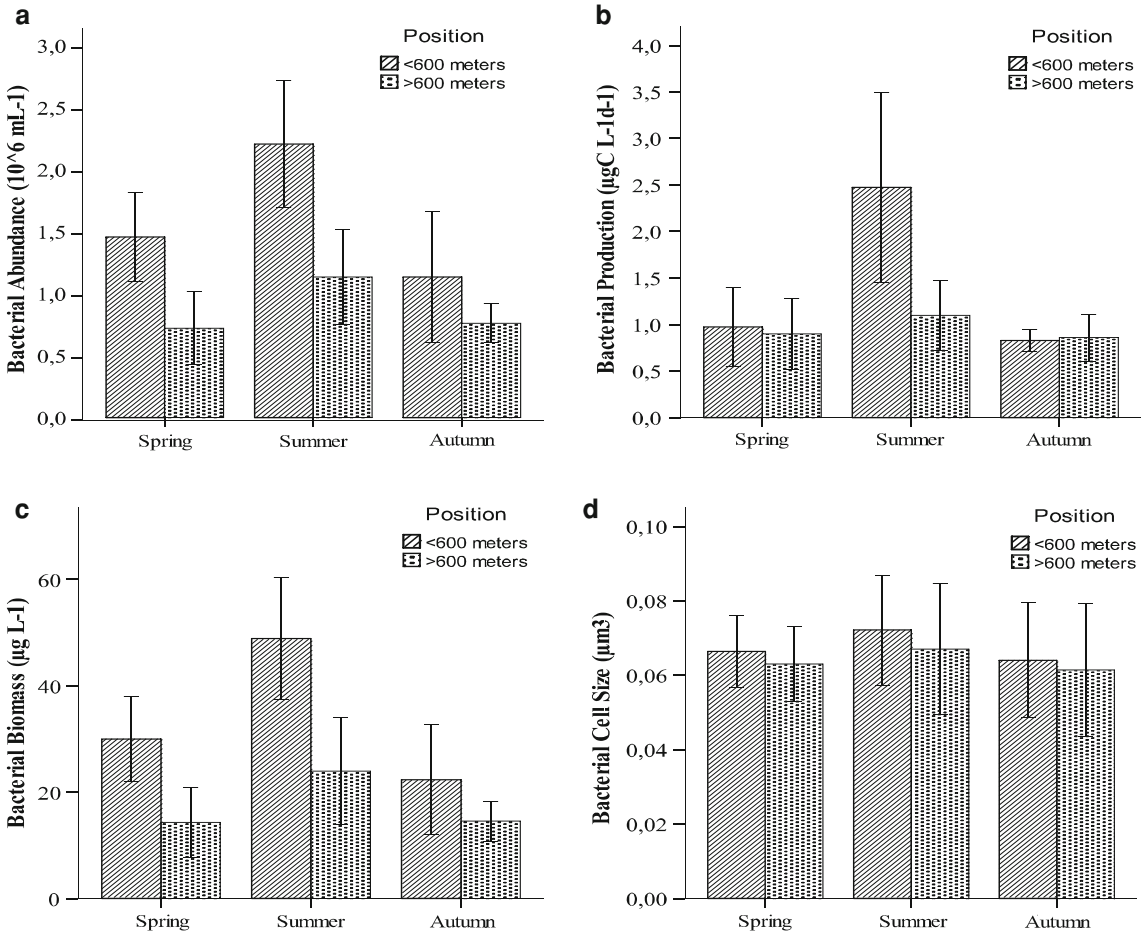

Fig. 2 Mean bacterial parameters (**a**) abundance, (**b**) production, (**c**) biomass and (**d**) cells size in the different seasons in ponds below and above treeline. *Vertical lines* represent standard error (PASW statistics 18)

(Table 2). During spring when DOC was at minimum, there was a clear peak in measured specific UV absorbance (SUVA) and FI values that were significantly negatively ($n = 37$, $r = -0.64$, $p < 0.001$) correlated. Nevertheless, there was a gradual shift in both SUVA and FI observed during the season. Similarly to Jaffe et al. (2008), significant changes in aromaticity of DOM between low and high elevation ponds were found with lower SUVA and higher FI indices, probably indicating less terrestrial associated input in higher elevation ponds. No significant correlations among bacterial variables and humidification index (HI) and photo-oxidation index (a250/a365) were found.

Stepwise multiple regression analysis was done with all data (environmental variables and DOM properties) to identify variables best explaining changes in bacterial abundance, production, biomass and cell size. DOC, temperature and depth were found to be the best explaining factors for changes in bacterial abundance and biomass with relatively good explanatory power of 54% for abundance and 58% for biomass. Bacterial production was best explained by changes in DOC, although with rather low explaining power of 24%, and temperature and lake area were found to explain variation in bacterial cell size with a power of 34% (Table 5).

All together, 42 LH-PCR profiles were run and normalized data were analyzed for possible changes in community composition. The distributions of the main fragment size classes in length heterogeneity PCR (LH-PCR) were analyzed using permutational MANOVA and canonical analysis of principal coordinates (CAP) was used to illustrate the difference. In the first run, seasonality (spring, summer and autumn, Fig. 3) and altitude (below and above the treeline) were included in MANOVA analyses. Results pointed out a significant difference among seasons ($F(2.34) = 5.10$, $p < 0.001$) but no significant difference in altitudinal ($F(1.34) = 1.63$, $p = 0.059$) group. When differences among seasons were examined more carefully with pair-wise tests, results showed a significant difference of spring versus summer ($t = 2.66$, $p < 0.001$) and spring versus autumn ($t = 2.61$, $p < 0.001$) but no difference between summer versus autumn ($t = 0.90$, $p = 0.622$). In the second run, spatial distribution (Saana, Malla, Jeâhkkas, Siilasvuoma

Table 3 Multifactorial analysis of variance for logarithmic transformed bacterial parameters (abundance, production, biomass and cell size) with three different data sets; (1) seasonally, (2) altitudinally and (3) spatially

	f	*p*
Abundance		
Seasonal	7.2	**0.003**
Altitudinal	26.3	**<0.001**
Spatial	7.9	**<0.001**
Production		
Seasonal	6.5	**0.005**
Altitudinal	3.2	0.085
Spatial	5.1	**0.003**
Biomass		
Seasonal	6.8	**0.004**
Altitudinal	25.1	**<0.001**
Spatial	7.0	**0.001**
Cell size		
Seasonal	1.2	0.326
Altitudina	4.4	**0.045**
Spatial	2.2	0.091

Significant differences are shown bold

and Tsâhkal) and seasonality (spring, summer and autumn) were included in permutational MANOVA. No significant difference was found in the spatial ($F(4.26) = 1.03$, $p = 0.427$) group and again a strong significant difference was found in the seasonal ($F(2.26) = 4.61, p < 0.001$) group. Similarly to first run, pair-wise tests showed a significant difference between spring vs. summer ($t = 2.53, p < 0.001$) and spring versus autumn ($t = 2.42$, $p < 0.001$) but no difference between summer versus autumn ($t = 1.03$, $p = 0.372$). Permutational MANOVA results clearly indicate that seasonality has the most influence in bacterioplankton community structure and spring was the most distinct season among them.

Table 5 Stepwise multiple linear regression analysis for bacterial parameters (abundance, production, biomass and cell size)

	Explaining variables	R^2	Sig.	*F*	*N*
Abundance	DOC, temperature and depth	0.544	<0.001	13.114	36
Production[a, b]	DOC	0.236	0.002	10.813	36
Biomass[a]	DOC, depth and temperature	0.583	<0.001	15.363	36
Cell size	Temperateure and lake area	0.341	0.001	8.815	36

Tested environmental variables included temperature, conductivity, pH, DOC, lake area, depth, altitude, SUVA (Specific UV-absorbance), FI (Fluorescence index), HI (Humification index) and a250/a365 (Photo-oxidation index)

[a] Bacterial production and biomass were transformed to logarithmic scale

[b] Temperature was removed from the analysis

Discussion

We studied the bacterioplankton in two environmentally distinct habitats: subarctic ponds below and above treeline. Productivity and seasonal dynamics of bacteria varied significantly between these two ecotypes and were especially related to the concentration of DOC. Highest bacterial density, biomass, productivity and cell size were recorded in mid-summer during the time of maximum DOC and temperature. Community structure of

Table 4 Pearson's correlation (*r*- and *p*-value) for bacterial parameters (abundance, production, biomass and cell size) with all ($n = 42$) the environmental data included

	Temp.	Cond.	pH	DOC	Chl-*a*[a]	Lake area	Depth	Alt.	SUVA[c]	FI[c]	HI[c]	a250/a365[c]
Abundance	**0.53 and <0.001**	0.21 and 0.173	−0.10 and 0.519	**0.67 and <0.001**	**0.65 and 0.001**	**−0.32 and 0.040**	**−0.45 and 0.003**	**−0.50 and 0.001**	0.30 and 0.068	**−0.53 and 0.001**	−0.03 and 0.859	−0.29 and 0.078
Production[b]	**x**	0.29 and 0.066	−0.14 and 0.371	**0.46 and 0.002**	**0.47 and 0.014**	−0.10 and 0.527	−0.25 and 0.114	−0.24 and 0.130	0.17 and 0.318	−0.27 and 0.104	−0.20 and 0.242	−0.19 and 0.240
Biomass[b]	**0.54 and <0.001**	0.20 and 0.198	−0.15 and 0.350	**0.67 and <0.001**	**0.64 and <0.001**	**−0.34 and 0.026**	**−0.46 and 0.002**	**−0.44 and 0.003**	0.29 and 0.082	**−0.54 and <0.001**	−0.04 and 0.793	−0.25 and 0.119
Cell size	**0.39 and 0.010**	0.11 and 0.485	−0.22 and 0.153	0.27 and 0.085	0.26 and 0.191	**−0.39 and 0.010**	**−0.40 and 0.009**	−0.05 and 0.757	0.21 and 0.214	−0.31 and 0.057	0.09 and 0.601	−0.28 and 0.087

Significant correlations are shown in bold

[a] Chl-*a* concentration values are not available from spring ($n = 27$)

[b] Production and biomass were transformed to logarithmic scale

[c] Abbreviations as in the Table 2

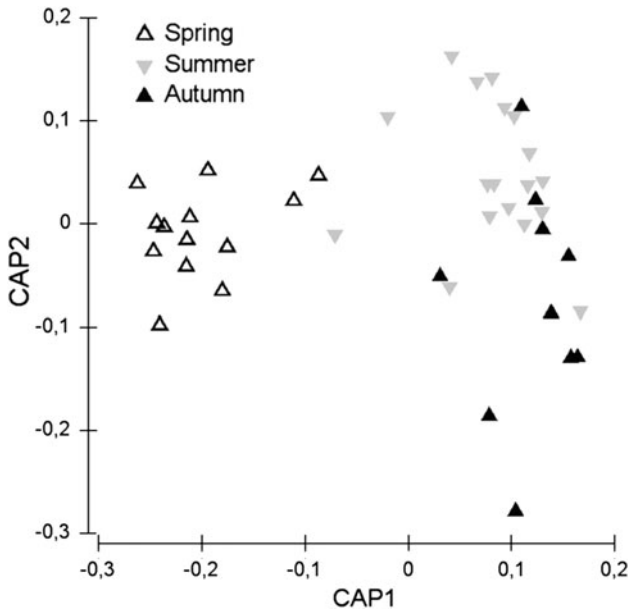

Fig. 3 Seasonal clustering of the main fragment size classes in LH-PCR analysis according to canonical analysis of principal coordinates (CAP) (Primer 6.1.12)

bacterioplankton did not follow the same pattern with the other bacterial variables but was most distinct during spring. Quality of DOC likely had an effect on bacterial community composition due to inputs of allochthonous higher-molecular weight compounds from the catchment area and associated different bacteria.

The quantity of DOC is known to be among the most important factors controlling bacterial communities in lakes in both subarctic-arctic and temperate regions (Jansson et al. 1996; Granéli et al. 2004; Vrede 2005; Sawström et al. 2007). In controlled enclosure experiments, DOC addition has had an immediate positive impact on bacteria abundance and production in an oligotrophic lake in northern Finland (Forsström et al. unpublished data) and in a High-Arctic lake in Svalbard (Hessen et al. 2004). In the present study, DOC concentration accounted for most variation in multiple regression models and was the main driver for the patterns in bacterial variables in both low and high altitude ponds, as well as for their seasonal changes. Low altitude ponds with 2–3 times higher DOC concentration had also 2–3 times higher bacteria abundance, biomass and productivity than high altitude ponds. To place our data from subarctic treeline ponds in a broader context, we compiled data from studies that have measured bacterial dynamics in different high-latitude and temperate regions (Table 6). In many of these studies, as in our study, bacteria were mostly controlled by the concentration of DOC. The values in our study fall at the lower end of this abundance/productivity range, representing values found in arctic-subarctic regions (O'Brien et al. 1997; Hobbie et al. 2000; Karlsson et al. 2001).

The seasonal dynamics of bacterial abundance, biomass and production were strongly driven by DOC concentration, and were more pronounced in low altitude ponds that showed the greatest variability in DOC. Fourteen out of 16 ponds were solid frozen until mid-May. The inoculum of bacteria in such ponds is a combination of aquatic bacteria that survive winter frozen and of terrestrial bacteria that are flushed to the pond with melting snow (Hobbie et al. 1980). Melting snow is known to have very low electrolyte content; i.e. conductivity. In Kilpisjärvi region, nutrient content in the snow is even lower than the natural amount of nutrients in the lakes and ponds (Forsström et al. 2007). Thus, nutrient- and DOC-poor melt water is probably one contributor to the low productivity of the ponds in spring while drainage water from soils with more diverse vegetation likely contributed to the increase in productivity detected in summer in lower altitude ponds. Such distinct increase in productivity was absent in high altitude ponds, most likely because their catchments were barren and unable to provide new DOC. During the growing season, the smaller water volume due to evaporation in lower altitude ponds may also have contributed to changes in bacterial activity. There are only few studies conducted on bacterial seasonality in Arctic freshwaters, all from Alaska, and in these cases bacterioplankton activity followed certain phases (Hobbie et al. 1980; Crump et al. 2003; Adams et al. 2010). In Toolik lake, productivity was at maximum in spring when also highest concentrations of DOC were measured, as a result of DOC input from rivers (Adams et al. 2010). In ponds (Barrow ponds), the seasonal cycle of DOC begins with low concentrations during the melt period and ends up with high concentrations at freeze up in September (Prentki et al. 1980). According to Hobbie et al. (1980), bacteria have their first abundance peak during spring runoff and the seasonal maximum in late summer in early August. We did not observe any peak in spring. On the contrary, bacterial activity seemed to be at its minimum during spring runoff. However, we sampled during the early phase of the runoff with most ponds still partly ice covered, while Hobbie et al. (1980) began their bacterial sampling some 10 days after the runoff, when DOC concentration had already reached its higher summer values and the bacteria were possibly benefiting from new DOC. A more detailed spring sampling would be needed to reveal the influence of runoff on DOC concentration and bacteria activity in subarctic ponds.

The spring bacterial community was also significantly different from that in other seasons, followed by a shift to a more stable bacterial community structure after the early season snow melt. One reason for the observed difference might be allochthonous bacteria introduced by melting

Table 6 Intersystem comparison among bacterial variables

Region	Site	Abundance ($\times 10^9$ l^{-1})	Biomass (μg C l^{-1})	Production (μg C l^{-1} d^{-1})	References
Arctic	Lake Toolik, Alaska	0.2–3.0	–	1.6–22.4	(Hobbie et al. 2000; O'Brien et al. 1997)
	Tundra ponds, Barrow, Alaska	2.0–6.0	–	–	(Hobbie et al. 1980)
	Ponds, high Arctic, Canada	2.1–6.5	–	0.38–0.47	(Granéli et al. 2004)
	High Arctic lake, Franz Joseph Land	0.9–1.7	–	1.2–3.9	(Panzenböck et al. 2000)
Subarctic	Lake Saanajärvi, Subarctic, Finland[a]	0.9–1.1	2.5–4.8	0.24–0.33	Forsström et al. unpublished
	Kilpisjärvi ponds, Subarctic, Finland	0.6–3.9	11.3–117.1	0.5–33.5	This study
	Subarctic lakes, Sweden	0.4–2.5	9.2–66.1	0.4–7.7	(Karlsson et al. 2001)
Temperate	Oligotrophic mesohumic, Lake Pääjärvi, Finland	1.4–3.7	–	1.8–13.6	(Tulonen 1993)
	Temperate humic Lake Mekkojärvi[a]	6.3–8.1	–	26–65	(Salonen et al. 1992)
	Eutrophic lakes, Danmark	3.1–13.6	251–684	–	(Bjørnsen 1986)

[a] Measurements were done in enclosures

snow to complement the pond bacteria that survived winter. Introduction of allochthonous bacteria is supported by our DOM characterization, where we found a clear peak of allochthonous derived carbon in our spring sampling with both measured indices (SUVA and FI). Although labile autochthonous carbon is preferentially used by aquatic bacteria, in lakes with high terrestrial inputs, it is likely that bacteria are more dependent on allochthonous DOC (Tranvik 1998; Kritzberg et al. 2004). We therefore suggest that the spring community was made of bacteria that were able to utilize allochthonous carbon as their primary energy source.

We estimated the identity of the phyla that were responsible for the community composition change using European molecular biology laboratory (EMBL) database on 16S rRNA-genes of a certain base pair (bp) length. Most of the bacterial community structure difference between spring and the two other seasons was mainly due to the absence of 16S rRNA gene lengths 522, 502 and 501 bp during spring. Both 501 and 502 bp 16rRNA gene lengths are strongly related to appearance of *Actinobacteria*. Of all reference sequences in the database, 74.1% of 501 bp and 85.7% of 502 bp long fragment sizes were assigned to *Actinobacteria*. Third, 522 bp 16rRNA gene length was assigned to *Gamma-* and *Betaproteobacteria* (41.3%), *Spirochaetes* (29.9%) and *Firmicutes* (10.4%) from all phyla identified in the database. Clone library results from the small humic lake by Taipale et al. (2009) assigned the 502 bp to A*ctinobacteria* and 522 bp to B*etaproteobacteria* supporting our assignments based on database.

A gradual decrease was measured in SUVA and an increase in FI values during the sampling season, indicating less pronounced dominance of allochthonous carbon after the spring. Seasonal differences in carbon quality indices are likely linked to decreases in less allochthonous-originated carbon due to a smaller runoff in summer. Another important factor for shifts in SUVA and FI might have been photo-oxidation where refractory DOC (high molecular weight particles) is transformed to lower molecular weight particles (Lindell et al. 1995, 1996). Increased concentrations of photodegradated DOC have shown to be beneficial to bacterioplankton growth (Lindell et al. 1995; Wetzel et al. 1995) especially in DOM rich lakes (Lindell et al. 1995). Therefore this could be one explanation for the highest bacterial densities and production observed in low altitude ponds during summer. We also measured higher HI during spring indicating that DOM entering the ponds from drainage was less usable for biota than less humified DOM during summer and autumn runoff, and therefore higher HI could be one explanation for the absence of spring production peak. Also, more stable HI values during summer and autumn could partly explain that no compositional changes in the community structure were found.

In addition to DOC in subarctic-arctic environments, temperature and predation have been observed to affect bacteria abundances (Ochs et al. 1995; Jürgens and Matz 2002). In our study, temperature was negatively dependent on altitude and the greatest temperature difference between low and high altitude ponds was 7.8°C in summer. The correlation found between bacterial variables and temperature indicates that temperature was one of the factors influencing bacterial density, biomass and cell size at high altitudes and in the beginning and end of the season. However, availability of substrates is known to overcome the negative effect of low temperature (Wiebe et al. 1992). Our data supports this assumption because both DOC and chl-*a* had stronger correlations with bacterial variables. Also in regression analysis, temperature was only a secondary explanatory variable. HNF abundances in our study were in the same range with studies by Hobbie et al. (2000) and Laybourn-Parry and Marshall (2003), with slightly but

not significantly higher abundances in ponds below treeline where, however, bacteria abundance in these ponds was higher than in above treeline suggesting that HNF predation was not severe. We assume that the top-down grazing pressure from HNF exerted only a minor control on bacterial communities (abundance, production and size) compared to bottom-up control from abiotic variables as suggested in Menge and Sutherland (1987) and Hahn and Höfle (2001).

To conclude, the quantity and quality of DOC to a large extent determines the utilization of DOC by bacterioplankton. Changes in these measures are naturally seen spatially and temporally, but they are also predicted to change in a larger global scale as a result of shifts in vegetation belts and precipitation. Arctic and subarctic lakes and ponds are especially sensitive indicators of climate change, and increased air temperatures and precipitation are likely to increase both the amount of DOC and nutrients in these systems (ACIA 2005). In the light of our results, substantial differences in the quality of carbon can be expected in association to higher runoff rates. Hence, climate change can be expected to increase the productivity of bacterioplankton and change their community structure in subarctic ponds. Whether the observed bacteria community composition shift from spring to summer resulted from selection and resource competition for different types of carbon resources or was merely a result of seasonal succession from dry (solid frozen) to liquid phase of a pond, and associated terrestrial and aquatic bacterial composition, needs more research. Understanding the drivers behind such drastic community shifts will help us make predictions for a warmer climate. Arctic limnology has developed considerably during recent years but there is still a need for better estimation of changes in bacterioplankton communities in subarctic-arctic lakes and ponds because of their crucial role in aquatic food webs as well as in green house gas release (Hanson et al. 2003; Walter et al. 2006; Laurion et al. 2010).

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