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Bacterial community succession in an Arctic lake–stream system (Brattegg Valley, SW Spitsbergen)

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The factors affecting prokaryotic and virus structure dynamics and bacterial community composition (BCC) in aquatic habitats along a ca. 1500 m of the Brattegg Valley lake–stream system (SW Spitsbergen) composed of three postglacial lakes created by Brattegg Glacier meltwater were examined. A high number of small-volume prokaryotic cells were found in the recently-formed, deep, upper, subglacial lake. Bacterial taxonomic diversity assessed by PCR-DGGE indicated that the lakes' BCC differed; with its richness decreasing down the system. Fluorescence *in situ* hybridisation (FISH) revealed a BCC increase; mainly in α-Proteobacteria, Cytophaga/Flavobacterium and Actinobacteria. We found increasing β-Proteobacteria and Actinobacteria numbers and a decrease in α-Proteobacteria down the system. Our results indicate that glacial meltwater sustains an active bacterial community which is then stimulated or partly eliminated by the proglacial lakes' higher temperature and nutrient input. We conclude that the prokaryotic succession along this lake–stream system occurs through changes in the structure of dominant bacterial communities, their replacement and elimination.

Introduction

Rapid glacier melting during the last decades in different areas of the globe have focused scientific attention on various aspects of global warming and its consequences for the polar regions (Jania and Hagen 1996, Oerlemans 1998 Bernstein *et al*. 2007, Vincent *et al*. 2009).

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River–lake systems

In favourable topography, accelerated glacier runoff results in the formation of new, previously nonexistent lakes from which the fluvio-lacustrine hydrological systems originate (Milner *et al*. 2008). The glacier-supplied lakes accumulate large amounts of water which transports

nutrients and microorganisms to newly-formed proglacial lakes, affecting the microbial composition of those lakes (Mindl *et al*. 2007). The newly-formed habitat colonization can also occur by transfer of organisms from other habitats containing developed microbiota. Major roles are played by surface runoff (Crump *et al*. 2012), airborne transport (Shinn *et al*. 2003, Harding *et al*. 2011), animal migrations (Mindl *et al*. 2007, Van Geest *et al*. 2007) and sea aerosols (Bigg and Leck 2001).

The rivers flowing through the lakes form a fluvial–lacustrine system with combined lentic (lake) and lotic (river) landscape "patches", together with the transitory zones between them. This system provides transport and exchange of matter and microorganisms. The lakes are specific places in this system where particle selection and transported matter sedimentation occur (Milner *et al*. 2008). These enable selection and exchange of planktonic organisms with different reproduction strategies.

Glaciers as a source of nutrients and microorganisms

Currently, glaciers are seen not only as an abiotic cryosphere component but also as environments (ecosystems) inhabited by microbial life (Hodson *et al*. 2008) harbouring viruses, autotrophic and heterotrophic bacteria, protists and algae (Skidmore *et al*. 2000, Christner *et al*. 2003, Foong *et al*. 2010, Stibal *et al*. 2012, Cameron *et al*. 2012, Hell *et al*. 2013, Bellas *et al*. 2013, Mieczan *et al* 2013). Microorganisms in glaciers were found in many habitats: on glacial surfaces (Yoshimura *et al*. 1997, Zeng *et al*. 2013, Mieczan *et al*. 2013), in ice cores (Skidmore *et al*. 2000, Christner *et al*. 2003, Segawa *et al*. 2010), glacial streams (Elster *et al*. 1997, Battin *et al*. 2001, Wilhelm *et al*. 2013), drainage systems of glaciers (Řehák *et al*. 1990), subglacial sediments (Foght *et al*. 2004, Bhatial *et al*. 2006) and glacier beds (Sharp *et al*. 1999). Abundant and active microbial communities are found in glacier's cryoconite holes (Mueller *et al*. 2001, Hodson *et al*. 2008, Bellas *et al*. 2013). Glaciers are also places of accumulation of organic matter and nutrients (Hodson *et al*. 2004, Stibal *et al*. 2008, Hood *et al*. 2009). Melting glaciers mobilise ice-locked organic matter with implications for downstream carbon cycling (Singer *et al*. 2012) and heterotrophic activity (Milner *et al*. 2009). For proglacial microbial communities, besides meltwater an additional source of nutrients is glacial flour generated by glacial erosion (Hodson *et al*. 2004, 2005). In the summer, meltwater with fine-grained particles generated by glacial erosion and abrasion of solid material is introduced to nearby situated proglacial lakes (Priscu *et al*. 2002).

Bacterial community composition shift

One of the basic problems in microbial ecology of the polar regions is to understand the processes that underpin the composition of communities (Pavoine and Bonsall 2011, Barberán *et al*. 2014). Most studies of bacterial community composition have focused only on single environment types, such as lakes or streams. Comparisons across environments are less common (Dolan 2005, Crump *et al*. 2007) and interactions among organisms populating these environments are rarely investigated (Crump *et al*. 2004, Adams *et al*. 2014). The data currently available suggest that bacterial community composition in freshwater is controlled by dispersal (biogeography) or environmental factors (habitat quality), or both, and that these controls operate across a spatial range from local to regional. Variations in bacterioplankton community composition, assessed by PCR-based community fingerprinting techniques, often correlate with physical, chemical and biological factors (Muylaert *et al*. 2002, Yannarell and Triplett 2004). Bacteria were identified as members of globally-distributed freshwater phylogenetic clusters within the α- and β-Proteobacteria, the Cytophaga–Flavobacteria–Bacteroides group and the Actinobacteria (Crump *et al*. 2003). Data on microbial succession in arctic fluvial-lacustrine systems are rare (Mindl *et al*. 2007, Milner *et al*. 2009). The studies are mainly focused on the changes in bacterial community composition on a geographical scale (e.g. Crump *et al*. 2007). While glaciers in recent years have become increasingly recognized as a habitat for

diverse and active microbial communities, the microbial ecology of supraglacial lakes, against the background of the observed climate changes, remain poorly understood. It is particularly interesting that deep mountain lakes are emerging at the foot of glaciers, and these rapidly-formed hydrologically-connected systems now provide a unique opportunity to study initial formation, colonization and development of microbial succession in a fluvio-lacustrine system along the chronosequences.

Arctic Brattegg Valley lake–stream system

The Brattegg Valley lake–stream system is an example of a multi-related system, with variable hydrology, trophy and lake systems. The aim of this study was to assess the spatial shifts in prokaryotes abundance and diversity along a longitudinal transect down from the three proglacial lakes. We also evaluated the effects of bacterial growth limiting factors, such as temperature and nutrient content along the valley transect. Our particular interest was centred on assessing the changes in the prokaryotic plankton of this ecologically diverse ecosystem and on the continuum of the supraglacial meltwater stream and pelagic communities in adjacent lakes along this transect.

We hypothesized that in a connected series of lakes, the lake communities changed with distance from the upstream lake and with changes in environmental conditions, considerably affecting the structure of the lakes' microbiocenosis and their species richness.

Material and methods

Study area

The Brattegg river valley is located in Wedel Jarlsberg Land, near the SW coast of Spitsbergen. In summer, the valley is usually devoid of snow cover. Permanent snow cover is generally formed in the second half of September and lasts until the first decade of June (Przybylak and Araźny 2006). The area comprises a part of the coast belonging to the catchment of the Brattegg river. This catchment, with an area of 8.2 km^2, represents a typical partly-glaciered arctic lake-river system composed of three lakes (upper, middle and Lake Myrktjørna located below the first two) of different sizes (9.1, 1.3 and 13.6 ha, respectively) and depths (max. 40.3, 6.7 and 6.9 m, respectively), connected by the Brattegg river (Fig. 1). Only a small part of the catchment (0.37 km^2), is permanently ice-covered. Three of the lakes differ, not only in the morphometric parameters, but also in the time of formation. The Brattegg river originates in the upper (young, deep and cold) cirque lake recharged from glacial ablation which is situated at an altitude of ~234 m a.s.l. Farther down, having crossed the frontal moraine, it falls into the middle small lake situated at 139 m a.s.l. and then flows over dertitial massif rocks to the large (and oldest) Lake Myrktjørna (72 m a.s.l.). The first bathymetric measurements of these lakes were made in 2011 by D. Górniak and H. Marszałek. From Lake Myrktjørna, the river flows through the belt of the older moraine at 95 m a.s.l. into the valley to join the outflow from the Werenskiold glacier and flows into Nottingham Bay of the Greenland Sea. The length of the river, including the lakes, is only 4.05 km. The orographic borders of the catchment area are formed by the summits of Angellfjellet (590.6 m), Brattegg (645.0 m), Jahnfjellet (643.0 m) and Gullichsenfjellet (583.0 m). The slope of the river is substantial, with an average of 6% (Marszałek and Wąsik 2013). Basic lake environmental parameters are given in Table 1.

Sample collection

Water samples were collected at 9 stations along the Brattegg Valley lake–stream system during the summers of 2010 and 2011 (in the first half of August) (Fig. 1). Each year, samples were taken twice at an interval of three weeks. Since the obtained values of microbiological parameters in both seasons were similar, average values are presented here. Samples from lake inlets and outlets were taken from a depth of ≤ 0.25–0.5 m and approximately 1.5 m from the shores. Samples of surface water (≤ 0.5 m) from above the deep-

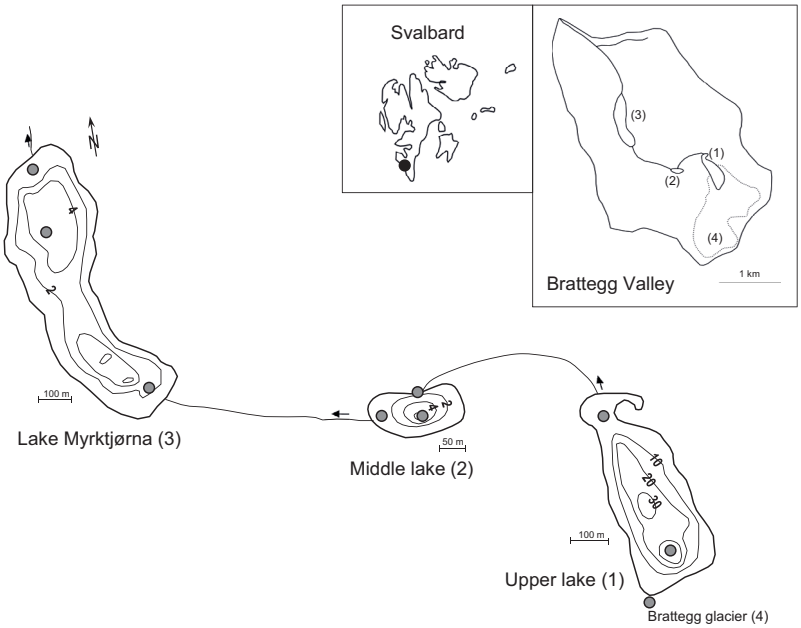

Fig. 1. Brattegg Valley lake–stream system and locations of sampling sites (gray dots).

est points of the lakes were collected with a 5-l Niskin bottle cast from a small pontoon. All samples were transferred to sterile acid-washed polyethylene 2-l containers previously rinsed with distilled water, and immediately transported to the nearby field laboratory at the Wrocław University "Baranowski" summer research station. The samples were processed within two hours after return to the field laboratory. Filtration for microscopic examination, as well as chlorophyll *a*, dissolved organic carbon (DOC) concentration and total suspended solids (TSS) were all analysed in the field laboratory and cells for molecular examination were deposited on polycarbonate membranes by vacuum filtration.

Environmental conditions

The surface water temperature, conductivity, dissolved oxygen (DO) and pH were measured *in situ* at the sampling sites using the WTW GmbH (Germany) equipment. Chlorophyll *a* (Chl *a*), dissolved organic carbon (DOC), total nitrogen (N_{tot}), N-NH_4, N-NO_3, P-PO_4, and total suspended solids (TSS) contents were determined. Alkalinity was measured in the laboratory by colorimetric titration with HCl. The chemical composition of water, including major ions and trace elements, was determined using an atomic adsorption spectrometry (AAS) method with graphite tray (Perkin-Elmer GmbH, Germany) in triplicates.

Chlorophyll *a* concentration

Samples for chlorophyll-*a* estimation (500 ml) were filtered onto Whatman GF/F filters (∅ 25 mm) and stored frozen at –20 °C. Chlorophyll *a* (corrected for phaeo-pigments) was then extracted with 96% ethanol and measured by the Shimadzu UV/VIS 1200 spectrophotometer according to Nusch (1980) using an excitation wavelength of 435 nm and an emission wavelength of 675 nm. Standards (Spinach; C-5753) were obtained from Sigma (Germany).

Dissolved organic carbon (DOC) concentration

Forceps and plastics were soaked in 10% Decon 90 (Decon Laboratories) detergent for > 2 days and thoroughly rinsed in Milli-Q water. Samples were vacuum-filtered through a ∅ 25 mm, 0.2 μm pore-size polycarbonate membrane and

Table 1. Sampling locations, and environmental and microbiological parameters characterizing the Brattegg Valley lake–stream system. DO = dissolved oxygen; DOsat = dissolved oxygen saturation; TSS = total suspended solids; TCN = total cell number; PB = prokaryote biomass; ACV = average cell volume; Cocci, curved, rods = cell morphotypes; VLP = virus-lake particles. nd = no. data

Variable	Upper lake			Middle lake			Myrktjørna		
	Inlet	Surface	Outlet	Inlet	Surface	Outlet	Inlet	Surface	Outlet
Coordinates	77°03´04.0´´N 15°16´11.0´´E	77°03´16.7´´N 15°15´20.3´´E	77°03´16.6´´N 15°15´22.4´´E	77°03´09.3´´N 15°15´33.8´´E	77°03´09.3´´N 15°14´33.8´´E	77°03´08.9´´N 15°14´11.2´´E	77°03´20.7´´N 15°14´36.2´´E	77°03´20.7´´N 15°12´36.2´´E	77°03´45.2´´N 15°12´11.3´´E
Area (ha)		9.05			1.28			13.63	
Altitude (m a.s.l.)	256	234	232	141	139	138	74	72	71
Max depth (m)		40.3			6.7			6.9	
Temperature (°C)	0.9	1.3	1.6	2.7	3.9	2.9	4.5	7.3	7.3
Conductivity (μS cm^{-1})	22.9	25.8	26.6	28.0	30.0	32.0	34.7	36.1	38.0
DO (mg l^{-1})	1.90	2,22	3.22	3.16	2.80	2.64	4.22	3.27	5.05
DOsat (%)	18.2	18.5	24.3	22.4	21.7	19.8	33.3	29.2	40.0
TSS (mg l^{-1})	99.7	22.5	nd	nd	8.1	nd	nd	8	nd
pH	6.88	6.95	7.19	7.18	7.0	7.14	7.14	7.24	7.07
TCN ($\times 10^6$ cells ml^{-1})	0.85	0.97	0.89	0.54	0.50	0.52	0.56	1.05	1.59
PB (μg C l^{-1})	17.86	16.39	17.39	12.78	13.78	12.66	12.47	30.28	30.59
ACV (μm^3)	0.06	0.05	0.08	0.07	0.08	0.07	0.06	0.1	0.11
Rods (% in TCN)	88.6	80.65	84.78	81.8	85.77	82.73	80.78	71.63	75.65
Cocci (% in TCN)	10.2	16.74	9.82	10.14	9.92	7.65	9.66	12.71	9.59
Curved (% in TCN)	2.43	2.62	5.41	8.07	4.32	9.63	9.57	15.67	14.77
VLP ($\times 10^6$ ml^{-1})	nd	1.71	1.53	1.07	0.62	0.66	0.68	2.59	2.61
VLP/TCN ratio	nd	1.76	1.72	1.98	1.24	1.32	1.21	2.47	1.64

the filtrate was collected directly into Falcon tubes and frozen at –20 °C for measurement. Analyses were performed with a Shimadzu TOC 5000 total organic carbon analyser calibrated with a prepared potassium hydrogen phthalate solution (0 to 5.0 mg C l^{-1}) in Milli-Q water, each of calibrating solution was acidified and sparged. A coefficient of variance < 2% of the concentration was obtained in 3 to 5 replicate analyses of each sample (Dunalska 2009). Samples for DOC (non-purgeable organic carbon, NPOC) determination were HCl acidified at pH 2 and blown with oxygen for 10 min to purge CO_2. The DOC and CO_2 concentrations were determined with a detection limit and accuracy of 650 mg C l^{-1} and coefficient of variation ≤ 1%.

Total suspended solids

Total suspended solid (TSS) concentration was measured by gravimetric analysis according to Strickland and Parsons (1972). Known volumes of water were filtered through preweighed Whatman GF/C glass fibre filters in the laboratory and the filters were then dried and re-weighed using standard laboratory practices. The suspended load concentration was then calculated from weight differences.

Total cell number (TCN), average cell volume (ACV), prokaryotic biomass (PB) and cell morphotype diversity

Total cell numbers (TCNs) were calculated using the direct epifluorescent filter technique (DEFT). Sub-samples (2 ml) were fixed with buffered formalin to a final concentration of 2%. TCNs were determined by epifluorescence microscopy using 4´,6-diamidino-2-phenylindole (DAPI) on black Nuclepore polycarbonate 0.2-μm pore-size membrane filters (Porter and Feig 1980), under an Olympus BX41 microscope with a 100 W Hg lamp and 100/1.30 CFI 60 oil immersion objective, with a digital DS-Fi 1C camera and a filter block of wavelengths EX 360–380, DM 400, BA 420. Between 300 and 400 DAPI-stained cells in at least 20 digital images of each filter were counted and measured. The field images were analysed with Nikon NIS-Elements F 3.0 and MultiScan ver. 14.02 (computer scanning systems). Prokaryote number, cell volume and total bacterial biomass were evaluated through image analysis (Sieracki *et al.* 1985, Świątecki 1997). The biomass was calculated by converting DAPI-stained cell volume to carbon units using the biomass conversion factor 170 fg C μm^3 (Norland 1993). To determine the cell morphotype diversity, frequencies of bacterial morphological forms (cocci, rods and cylindrical curved) in the water samples were evaluated (Nübel *et al.* 1999). Counts of the three independently-prepared replicate samples were averaged for the representative concentrations.

Photosynthetic picoplankton (FPP) enumeration

Buffered formalin-fixed samples without staining, were pelleted by low pressure (5–10 kPa) vacuum filtration on a Nucleopore filter membrane with a porosity of 0.2 μm. The subsample volume was 5 ml. After filtration, the membrane was dried in the dark and mounted on slides using Citifluor (Agar scientific, UK) and was then frozen at –20 °C until microscopic analysis. Photosynthetic cells were counted using fluorescence microscopy with blue EX 450–490 nm and 510–560 nm green light (Putland and Rivkin 1999). Microscopic analysis was performed according to the methodology described above.

Virus-lake particles numbers (VLP)

Half millilitre subsamples were preserved in buffered formalin (final concentration 2%) and stained with SYBR Gold (Invitrogen, Life Technologies, UK) for 15 min in the dark at room temperature. We used a concentrated 2.5×10^{-3} ml^{-1} (1:2500) commercial stock solution in DMSO diluted in MQ water deprived of viruses by filtration (Chen *et al.* 2001). Samples were filtered through 0.02 μm pore-size membranes Anodisc (Whatman), air dried in room temperature in the dark, and mounted on glass slides. Green fluorescent particles in 20 randomly-selected fields were counted (max. 20 000 per sample).

Fluorescence *in situ* hybridisation (FISH)

Water samples were fixed for a maximum of 24 hours at 4 °C in a final concentration of 4% paraformaldehyde, and for Actinobacteria in 1:1 EtOH/PFA. Resultant 20 ml subsamples were then vacuum-filtered onto 0.2-μm pore-size (∅ 47 mm) white polycarbonate membrane filters (Millipore GTTP) mounted in a bottle-top filter holder (Nalgene), rinsed with particle-free sterile MiliQ water, air-dried and frozen at –20 °C for analysis (Pernthaler *et al*. 2001).

The Bacteria and Archaea target oligonucleotide probes used in this study (*see* Table 2) were commercially-labelled with fluoresceinisothiocyanate at the 5´ end (Oligo, Poland). The filters, with deposited cells, were cut into sections and one section of each filter was tested in parallel with the NON 338-Cy3 (a probe complementary to EUB 338 probe) to control non-specific binding (Wallner *et al*. 1993). The hybridization buffer contained 0.9 M NaCl, 20 mM Tris/HCl, pH 7.3 and 0.01% SDS (Pernthaler *et al*. 2001). The concentration of formamide in the hybridization buffer varied from 0%–35% (v/v), depending on the oligonucleotide probe used (Table 2). For each sample, 20 μl of hybridization buffer was mixed with 5 pmol of the oligonucleotide probe and applied to the filter sections. Samples were hybridized during 3.5 h in a dark humid chamber at 46 °C and sections were then rinsed for a few seconds with sterile MilliQ water, air-dried in the dark and mounted on glass slides with 4:1 Citifluor:Vecta Shield (Citifluor UK, Vector Laboratories, Inc., CA, USA) containing 1 μg ml^{-1} DAPI. An epifluorescence microscope was used to study the bacteria in hybridized sections processed for FISH. Total cell numbers were calculated by DEFT after DAPI staining, following the FISH procedure, and the results for each probe were averaged for each station. The FISH counts were compared with previous counts of DAPI-stained cells and calculated as percentages of the total cell number (TCN).

Extraction of total DNA and PCR amplification of the partial 16S rRNA gene

The 500 ml water samples were vacuum-filtered through a 0.2-μm pore-size (∅ 47 mm) white polycarbonate membrane filter (Millipore GTTP) mounted on a sterile bottle top filter holder (Nalgene). Membranes were stored at –20 °C until analysed. Total DNA was extracted using an

Table 2. FISH (fluorescence *in situ* hybridization) oligonucleotide probes used in this study.

Probe	Sequence (5´–3´)	Specificity	rRNA target site (*E. coli* numbering)	FA[a] (%)	References
EUB338 I–III	GCTGCCTCCCGTAGGAGT	Bacteria	16S (338–355)	35	Daims *et al*. 1999
ARCH915	GTGCTCCCCCGCCAATTCCT	Archaea	16S (915–934)	0	Stahl and Amann 1991
ALF968	GGTAAGGTTCTGCGCGTT	α-Proteobacteria	16S (968–985)	35	Neef 1997
BET42a	GCCTTCCCACTTCGTTT GCCTTCCCACATCGTTT[b]	β-Proteobacteria	23S (1027–1043)	35	Manz *et al*. 1992
GAM42a	GCCTTCCCACATCGTTT GCCTTCCCACTTCGTTT[b]	γ-Proteobacteria	23S (1027–1043)	35	Manz *et al*. 1992
HGC69a	TATAGTTACCACCGCCGT	Actinobacteria	23S (1901–1981)	20	Roller *et al*. 1994
CF319a	TGGTCCGTGTCTCAGTAC	Cytophaga–Flavobacterium	16S (319–336)	35	Manz *et al*. 1996
NON338	ACTCCTACGGGAGGCAGC	Non nspecific	16S (338–355)	35	Wallner *et al*. 1993

[a] Formamide concentration in the hybridization buffer. [b] Unlabeled competitor.

UltraClean™ Water DNA Isolation Kit (MoBio, Carlsbad, CA) in accordance with the manufacturer's protocol. DNA quality and yield were measured with a NanoVue™ spectrophotometer (GE Healthcare Life Science, Germany). The extracted DNA was stored at –20 °C until further use. DNA quality and quantity were checked by electrophoresis in 1% (w/v) horizontal agarose gel run in 1% TBE (tris-borate-ethylenediaminete-triacetate, pH 8.3) buffer and stained with 0.5 μg ml^{-1} ethidium bromide. A 1 kb ladder was used as a reference for molecular size marker.

Denaturing gradient gel-electrophoresis (DGGE)

To confirm bacterial taxonomic diversity in the three lakes, a DGGE analysis of samples was performed. Dominant bacterial phylotypes were distinguished by DGGE analysis, and electrophoresis was performed with a D-Code Universal Mutation Detection System (BioRad Laboratories, USA). Each PCR reaction contained 35.2 μl of PCR-grade water, 5 μl of 10× buffer (Sigma Aldrich Co.), 6.0 μl of 25 mM $MgCl_2$ (Sigma Aldrich Co.), 1.2 μl of 20 mg ml^{-1} BSA (Sigma Aldrich Co.), 0.4 μl of 25 mM dNTP (Sigma Aldrich Co.), 0.5 μl of 20 μM in each primer, 0.2 μl of 5 U μl^{-1} Taq DNA polymerase (Sigma Aldrich Co.) and 1.0 μl of DNA template (approximately 1–2 ng μl^{-1}), with a total volume of 50 μl. The primers used were 341f with GC clamp (5´-GC-CC TAC GGG AGG CAG CAG-3´) complementary to position 341 to 357 and 907r (CCG TCA ATT CMT TTG AGT TT) complementary to positions 926 to 907 (*Escherichia coli* numbering) (Muyzer *et al*. 1993, 1998). The samples were loaded in 6% acrylamide gels with a denaturing gradient of 35%–70% (where 100% denaturant is 7 M urea and 40% formamide). The gels were run at 60 V for 17 h at 60 °C. The electrophoretic products were stained by gently agitating the gel for 30 min in 100 ml of 1× TAE containing 5 μl 1:10 000 dilution of SYBR Gold nucleic acid stain (Invitrogen, Life Technologies, UK) in DMSO and then rinsed with Milli-Q water for 30 min. DGGE banding patterns were visualized with UV trans-illumination and photographed using the Gel Doc 2000 gel documentation system (BioRad Laboratories, USA). Gel bands were identified using a GelCompar software to create the presence–absence matrix as described by Crump and Hobbie (2005). Each band represents a bacterial Operational Taxonomic Unit (OTU). To analyse bacterial phylotypes, the presence or absence of band in each line of a DGGE gel was converted into binary matrix for statistical analysis, and a distance matrix of anti-similarity was calculated from this binary matrix.

Statistical analyses

Direct cell counts and measurement results are reported as means and standard deviations of all replicates.

To analyse cell morphotype diversity describing the frequency of morphological forms in each size class, based on cell shapes (cocci, rods, curved forms) in five classes of volumes (< 0.05, 0.05–0.1, 0.1–0.5, 0.5–1.0, > 1.0 μm^3), a modified Shannon-Wiener index was used (Gurienowich 1995, Nübel *et al*. 1999). A modified Shannon-Wiener index was calculated for cell morphotypes determined by fluorescent microscopy. Spearman's rank correlation was used to evaluate interdependences between studied microbial traits and environmental factors. A multiple stepwise regression analysis was conducted for five main microbiological parameters (dependent variables): total cell number (TCN), prokaryote biomass (PB), average cell volume (ACV) and morphotype diversity (rods and curved cell percentages of TCN). The analysis identified the explaining variable for a model whose correlation with the explained variable was the strongest and determined a model with significant parameters. Subsequent steps involved the selection of successive explaining variables whose values were most strongly correlated with the rest from these steps. The extended model was characterised by the significance of all parameters. The use of a regression model allowed for a function to be created which described how the expected value of an explained variable depended on the explaining variables.

Canonical correspondence analysis (CCA) was conducted to determine species–environment relationships. In the analysis, microbiological data (presence or absence of a band in each line of a DGGE gel) for each sample were analyzed in relation to all environmental parameters measured. The similarity between the DGGE band patterns was assessed by the Dice coefficient, and clustering analysis was performed using the unweighted pair-group method and arithmetic averages (UPGMA) for dendrogram construction. Species richness was determined by the number of bands resolved by PCR-DGGE in one sample line.

All data were analysed statistically using Statistica version 10 (StatSoft Inc.) and Canoco ver. 4.5 for Windows (ter Braak and Šmilauer 2002) for the correspondence analysis.

Results

Physico-chemical properties

Several environmental parameters in the Brattegg lake–stream system varied considerably (Table 1). The water temperature measured in the upper lake's initial and greatest subglacial inflow differed from that in the outflow from Lake Myrktjørna by more than 6 °C. In addition to temperature, the amount of total suspended solids (TSS) (8 and 190 mg l^{-1}, respectively) and oxygenation (18.2% and 40%, respectively) of the system's surface water also differed. pH of all surface-water samples was neutral, only glacier meltwater had a slightly lower pH. The water had low conductivity (22.9–34.7 μS cm^{-1}), with the maximum recorded in Lake Myrktjørna. TSS was the highest (99.7 mg l^{-1}) in the water flowing into the upper lake. Because of its location and great depth, the upper lake traps glacial sediments, which results in low mean TSS (8 mg l^{-1}) of the two lakes downstream. Although the studied lakes were poor in nutrients, the upper lake's surface water was richer in phosphates (0.06 mg l^{-1}) than the surface water of Lake Myrktjørna's (0.04 mg l^{-1}), with the minimum found in the middle lake (0.02 mg l^{-1}). Lake Myrktjørna had the highest DOC (max. 0.780 mg l^{-1}) and chlorophyll-*a* concentrations (max. 0.8 μg l^{-1}). This lake also had the highest surface-water N-NO_3 concentration (0.07 mg l^{-1}). Intermediate values of DOC, P-PO_4, N-NH_4 and N-NO_3 were found in the upper lake, and the lowest in the middle lake. Both the upper- and middle-lake chlorophyll-*a* concentrations were extremely low (0.1 μg l^{-1}), eight times lower than those in Lake Myrktjørna (0.780 μg l^{-1}) (Tables 2 and 3). Canonical correspondence analysis indicated that in the upper lake the strongest environmental factor was TSS, but in the middle lake N-NO_3 content. The lowest lake was under the influence of pH, P-PO_4 and the content of chlorophyll *a* (Fig. 2).

Microbial abundance, biomass, biovolume, morphotype diversity virus-like particle numbers.

Prokaryotic cell abundance was the highest in Lake Myrktjørna, intermediate in the upper lake and the smallest in the middle lake (Tables 1 and 3). The surface prokaryotic total cell number (TCN) means (± SD) were; 0.97 (± 0.26) × 10^6 cells ml^{-1} in the upper lake, 0.50 (± 0.23) × 10^6 cells ml^{-1} in the middle and 1.05 (± 0.41) × 10^6 cells ml^{-1} in Lake Myrktjørna. The prokaryotic biomass means (± SD) were: 16.39 (± 6.62) μg C l^{-1} in the upper lake, 13.78 (± 0.5) μg C l^{-1} in the middle and 30.28 (± 1.38) μg C l^{-1} in Lake Myrktjørna. The highest prokaryotic biomass recorded was 30.59 (± 1.45) μg C l^{-1} in the Lake Myrktjørna outflow. The prokaryotic average cell volume (ACV) was the lowest in the cold upper lake (0.05 ± 0.01 μm^3), and the highest in Lake Myrktjørna (0.1 ± 0.02 μm^3). Hence, cell biovolume increased two-fold down the lake–stream system.

Prokaryotic cell morphotype diversity (percentage of forms in TCN) changed down the water system, with rods dominating in the upper lake 88.6% (± 1.32%) decreasing downstream to 71.6% (± 2.38%) in the Lake Myrktjørna outlet. Surface-water curved cells increased from 2.4% in the upper lake to 15.7% in Lake Myrktjørna (Table 1). The FPP numbers in upper and middle lakes were similar [0.62 (± 0.07) × 10^4 ml^{-1} and 0.65 (± 0.03) × 10^4 ml^{-1}, respectively], in the upper lake they varied in a wider range, and in

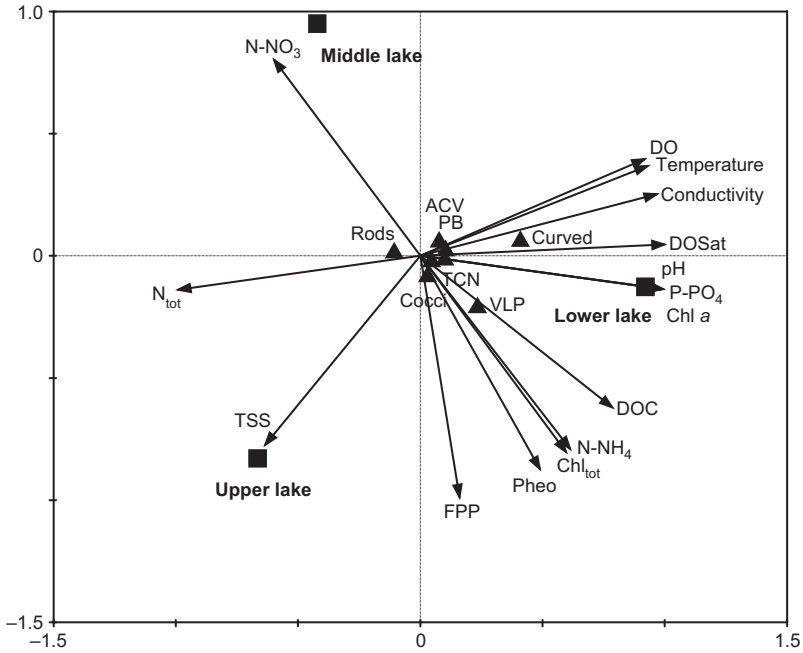

Fig. 2. Triplots (CCA) along the two main axes of variation for the prokaryotic structure parameters (triangles), significant environmental variables ($p < 0.05$) and sampled lakes (squares). TCN = total cell number; PB = prokaryote biomass; cocci, curved, rods are cell morphotypes; VLP = viruse lake particles; DOC = dissolved organic carbon; DO = dissolved oxygen, DOsat = dissolved oxygen saturation; Chl *a* = Chlorophyll *a*; Chl_{tot} = total chlorophyll, Pheo = pheophytin; FPP = photosynthetic picoplankton; TSS = total suspended solids; N_{tot} = total nitrogen.

Table 3. Structure of prokaryoticcells, viruses and limnological properties of the three study lakes; surface water in summer 2011. TCN = total cell number; PB = prokaryote biomass; ACV = average cell volume; FPP = photosynthetic picoplankton; VLP = virus-lake particles, DOC = dissolved organic carbon; N_{tot} = total nitrogen; Chl *a* = chlorophyll *a*; Cocci, curved, rods = cell morphotypes; Pheo = Pheophytin.

Variable	Upper lake	Middle lake	Lake Myrktjørna
TCN ($\times 10^6$ cells ml^{-1})	0.84*	0.92	1.19
PB (μg C l^{-1})	17.11	25.73	31.59
ACV (μm^3)	0.06	0.10	0.11
Rods (% in TCN)	84.85	76.40	64.94
Cocci (% in TCN)	13.31	13.48	18.18
Curved (% in TCN)	1.84	10.11	16.88
FPP ($\times 10^5$ cells ml^{-1})	0.62	0.65	0.52
VLP (10^6 ml^{-1})	1.22	1.12	2.68
VLP/TCN ratio	1.94	1.05	2.32
Chl *a* (μg l^{-1})	0.1	0.1	0.8
Pheo/Chl *a* ratio	72	25	7.5
DOC (mg l^{-1})	0.468	0.403	0.671
N_{tot} (mg l^{-1})	0.6	0.7	0.175
$P\text{-}PO_4$ (mg l^{-1})	0.06	0.02	0.04
$N\text{-}NH_4$ (mg l^{-1})	0.04	0.02	0.04
$N\text{-}NO_3$ (mg l^{-1})	0.06	0.02	0.07
pH	6.9	7.0	7.2

* Mean values.

Lake Myrktjørna they were the lowest [0.52 (± 0.05) $\times 10^4$ ml^{-1}].

The VLP number was intermediate in the upper lake [1.71 (± 0.25) $\times 10^6$ ml^{-1}] and the lowest [0.62 (± 0.11) $\times 10^6$ ml^{-1}] in the middle lake. VLP reached its maximum in Lake Myrktjørna [2.59 (± 0.89) $\times 10^6$ ml^{-1}], i.e. it was more than four times the greater than in the middle lake. The VLP/TCN ratio was the highest in Lake Myrktjørna (2.5) and 1.2 in the middle lake. The chlorophyll-*a*/pheophytin ratio varied from 72 in the upper lake to 8.5 in Lake Myrktjørna (Tables 1 and 3).

Dependencies

Modified Shannon-Wiener's index calculated for cell morphotypes ranged from 0.69 in the middle lake to 0.93 in Lake Myrktjørna and was correlated Spearman's with TCN, ACV and the curved cell presence ($r = 0.71$, $r = 0.68$ and $r = 0.76$, respectively). Strong positive Spearman's correlations between TCN ($r = 0.90$), biomass ($r = 0.73$) and viral abundance were found, the number of viruses was also positively correlated with DOC ($r = 0.83$) and Chl *a* ($r = 0.77$) concentrations ($p < 0.05$, $n = 36$).

Spearman's rank correlation revealed dependences between prokaryotic abundance (TCN) and lake area, dissolved organic carbon (DOC), chlorophyll *a* and P-PO_4 concentrations, as well as number of phages (VLP). Positive correlations between prokaryotic biomass (PB) and lake area, dissolved oxygen saturation (DOsat), DOC, Chl *a*, and VLP number were also found. Correlations also existed between prokaryotic cell biovolume (ACV) and temperature, conductivity, dissolved oxygen (DO), and chlorophyll *a* concentration. Percentage of curved cells in TCN correlated with temperature, conductivity, DO, DOsat, and Chl *a* concentration. Negative correlations were found between TCN and N-NO_3, PB and N-NO_3, ACV and the elevation of the lakes a.s.l. and total suspended solids (TSS), percentage of curved cells in TCN and the elevation of the lakes a.s.l. and TSS (Table 4).

CCA (Fig. 2) revealed the strong influence of almost all environmental factors on microbiocenoses in Lake Myrktjørna.

Multiple stepwise regression analysis revealed factors affecting the main microbiological parameters. The TCN was mainly affected by the DOC and the BB by DOsat, Chl *a* and DOC. Cells biovolume was determined by temperature, Chl *a* and TSS. The percentage of curved cells in TCN affect mainly temperature and the content of TN (Table 5).

Bacterial community composition

The DGGE banding pattern revealed 28 dominant operational taxonomic units (OTUs) in the Brattegg Valley lake–stream system. The OTU numbers were 18 in the upper and middle lakes and 14 in Lake Myrktjørna. The meltwater flowing into the upper lake contained 8 dominant OTUs, and there were the following fluctuations: 19 in the upper lake outflow and 20 in the middle lake inflow, 17 in the middle lake outflow and 19 in the Lake Myrktjørna inflow and 12 in the Lake Myrktjørna outflow. Our results showed a general decrease in dominant bacterial OTUs down the Brattegg Valley lake–stream system. In addition, OTU UPGMA cluster analysis revealed that dominant bacteria populations were quite similar in the upper and middle lakes, and rather different in Lake Myrktjørna (Fig. 3). Nine joint OTUs were present in all three lakes, with six being common for the upper and middle lakes, and only one common for the middle lake and Lake Myrktjørna. There were no joint OTUs for the upper lake and Lake Myrktjørna. We also found three unique OTUs in the upper lake, two in the middle and four in Lake Myrktjørna (Fig. 4).

CCA revealed the effects of environmental factors on dominant bacterial OTUs. While in the upper and middle lake communities were affected by TSS and N_{tot}, in Lake Myrktjørna they were affected by Chl *a*, conductivity and DO (Fig. 5).

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The taxonomic structure of prokaryotic microorganisms along the system was quite diverse. Bacteria were the dominant group, with a maximum of 90.4% of the total prokaryotes in the middle lake and a minimum of 73.6% in the upper lake. Archaea averaged 4.3% in the middle lake and 7.9% in Lake Myrktjørna (Table 6). The dominant group of bacterioplankton were

Table 4. Spearman's rank correlations between total cell number (TCN), prokaryotes biomass (PB), average cell volume (ACV), percentage of curved cells in TCN and the environmental variables: dissolved oxygen (DO), dissolved oxygen saturation (DOSat), total suspended solids (TSS), dissolved organic carbon (DOC), Chlorophyll *a* (Chl *a*), and virus-like particles (VLP). Significant results at the $p < 0.05$ are indicated with an asterisk (*), $n = 36$.

Variable	TCN	PB	ACV	Percentage of curved
Altitude	–0.22	–0.17	–0.63*	–0.83*
Area	0.66*	0.72*	0.11	0.26
Temperature	0.16	0.19	0.77*	0.83*
Conductivity	0.18	0.19	0.60*	0.85*
DO	0.17	0.24	0.57*	0.76*
DOsat	0.32	0.47*	0.44	0.64*
TSS	–0.10	0.10	–0.76*	–0.83*
DOC	0.89*	0.81*	0.51	0.42
Chl *a*	0.78*	0.79*	0.83*	0.77*
P-PO_4	0.77*	0.69	0.03	0.13
N-NO_3	–0.97*	–0.92*	–0.25	–0.50
VLP	0.90*	0.73*	0.41	0.41

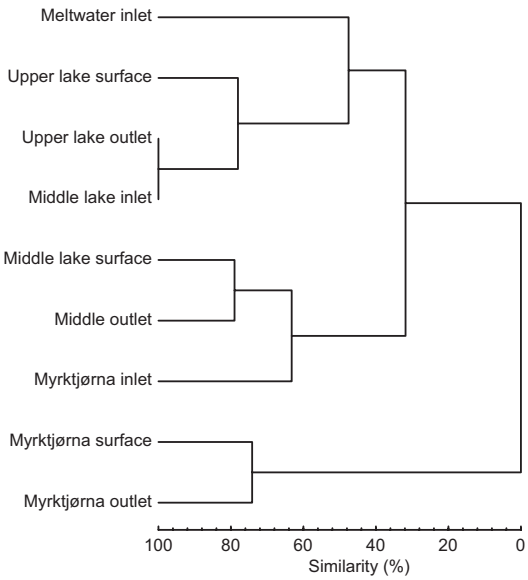

Fig. 3. Results of the cluster analysis based on DGGE band position for the stations located along the transect. Banding patterns were subjected to GELCOMPAR cluster analysis (UPGMA; Dice correlation coefficient).

β-Proteobacteria (31.9% in Lake Myrktjørna), although the Actinobacteria cluster made up a considerable portion of procaryotes. γ-Proteobacteria were very few (3.6%–3.8%) and none were found in the upper lake. α-Proteobacteria ranged between 19.5% in the upper lake and 11.1% in Lake Myrktjørna. While γ-Proteobacteria were not widely present, α-Proteobacteria, β-Proteobacteria and Actinobacteria were characteristic for the Brattegg Valley water system. The abundances of Actinobacteria and Cytophaga–Flavobacterium increased down the system while α-Proteobacteria numbers decreased.

Discussion

Environmental conditions

The upper lake in the Brattegg Valley lake–stream system, receiving water directly from the Brattegg Glacier head, was a trap for glacial sediments due to its location and considerable depth, which significantly reduced deposition of solids in the reservoirs below. This large, deep, cold lake was relatively rich in nutrients and microorganisms. As described by Hodson *et al*. (2004), glacial sediments could support bacterial growth due to the high phosphorus content of up to 1 mg g^{-1}. Nevertheless, bioavailability of this phosphorus is highly correlated with rates of sediment delivery, which is composed of unweathered primary mineral phases (mainly apatite) (Hodson *et al*. 2004).

Microbial activity in polar freshwater ecosystems is limited mainly by low temperatures and availability of organic and inorganic nutrients (Pomeroy and Wiebe 2001, Adams *et al*. 2010). Although a temperature increase from the start to the end of this system was recorded, the water temperature in the lowest lake did not exceed 10 °C. As observed in other arctic lakes, prokaryote metabolic activity was limited by temperature (Adams *et al*. 2010). The water temperature increase causes more intensive growth of phy-

Table 5. Factors determined by the multiple regression that best explain variability in the five main microbiological parameters (dependent variables): total cell number (TCN), prokaryotes biomass (PB), average cell volume (ACV) and morphotype diversity (percentages of rods and curved cells in TCN), in the Brattegg Valley lake–stream system (n = 36). DOC = dissolved organic carbon, DOsat = dissolved oxygen saturation, Chl *a* = chlorophyll *a*, TSS = total suspended solids, Temp. = temperature, N_{tot} = total nitrogen.

Parameters	Factors								r^2	p
	DOsat	Temp.	Chl *a*	DOC	TSS	N-NO_3	Altitude (m a.s.l.)	N_{tot}		
TCN				81%					r^2 = 0.97	< 0.003
PB	72%		14%	11%					r^2 = 0.53	< 0.04
ACV		41%	23.7%		9%		5%		r^2 = 0.91	< 0.02
Percentage of rods		41%				7.6%			r^2 = 0.41	< 0.05
Percentage of curved		87%						8%	r^2 = 0.87	< 0.002

toplankton and, thus, more dissolved oxygen in the water. Anoxia is a common feature in these turbid environments, both under the ice in winter and in the hypolimnia in summer. All three lakes usually remain ice-covered for approximately six and a half months. Numerous studies have elucidated the strong relationship between bacterial-specific growth rate and temperature (Ram *et al*. 2005, Vrede 2005). Shiah and Duclow (1994) reported that temperature limitation is more important than substrate limitation for specific estuarine bacterial growth rates in cold seasons. It is possible that the relatively high abundance of prokaryotes in the upper lake was caused by the glacier supplying both nutrients and prokaryotic cells (Stibal *et al*. 2012, Bradley *et al*. 2014).

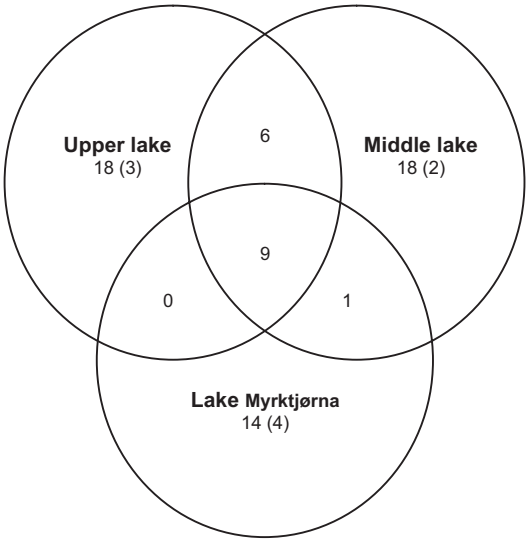

Fig. 4. Number of operational taxonomic units (OTUs) revealed by DGGE related to lakes. Black numbers: all (unique) OTUs in lakes water, bold numbers: OTUs shared with neighboring lake.

Nutrients

The link between bacterial biomass and chlorophyll-*a* content in Lake Myrktjørna also indicates an increase in the amount of easily assimilable

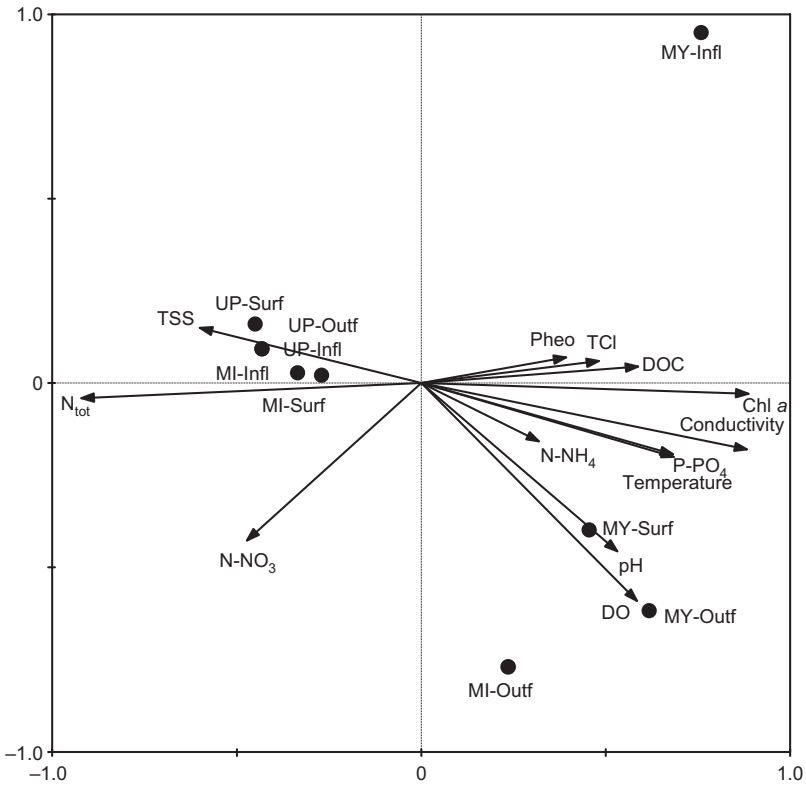

Fig. 5. Bacterial communities along Brattegg lake–stream system DGGE banding patterns and environmental factors. Canonical Correspondence analysis (CCA), UP = upper lake, MI = middle lake, MY = Lake Myrktjørna (Outl = Outlet, Surf = Surface, Inl = Inlet), DOC = dissolved organic carbon, DO = dissolved oxygen, DOsat = dissolved oxygen saturation, Chl *a* = chlorophyll *a*, Pheo = pheophytin, TSS = total suspended solids, N_{tot} = total nitrogen.

organic matter and the strong impact of phytoplankton on the prokaryote communities. The chlorophyll-*a*-to-pheophytin ratio is regarded as an indicator of phytoplankton physiological status (Cota *et al*. 1994, Janukowicz 2006). Considering this assumption, Lake Myrktjørna phytoplankton had the highest activity across the system and produced autochthonous organic matter available for heterotrophic bacteria. To some extent, this may explain the increase in activity and changes in the structure of the bacterioplankton in the lake. Dead microbes from the glacier meltwater, such as bacteria or microalgae, may also be a source of organic matter for live bacteria. Several authors reported the important role of glaciers as sources of organic matter; particularly DOC (Stibal *et al*. 2006, Stibal *et al*. 2008, Anesio *et al*. 2009), and others have described glaciers as places inhabited by diverse communities of microorganisms which colonize new habitats on melted glacier forelands (Edwards *et al*. 2013).

Prokaryotic community shift

One of our most important findings was the clearly noticeable change in the prokaryote abundance and biomass along the studied lake–stream system. Clear changes were also found in the cell biovolume, the value in warmer Lake Myrktjørna being twice that in the upper lake (0.11 μm^3 and 0.05 μm^3, respectively). Although in the entire Brattegg system, summer water temperatures do not exceed 10 °C, the 6.4 °C increase from 0.9 °C in the upper lake to 7.3 °C in Lake Myrktjørna resulted in prokaryote cell biomass growth. This eight-fold increase in the Lake Myrktjørna water temperature almost doubled the biomass content. Here, even temperatures under 10 °C stimulated prokaryotic community, and both cell size and microbiocenotic morphological structure provided indicators of prokaryotic cell structural changes throughout the Brattegg system.

The increase in plankton curved-cell percentage throughout the system indicates changes in substrate availability, particularly organic carbon (Dunalska *et al*. 2004). The availability of the organic matter (OM) is related to its age. The older, highly modified refractory OM originating from the glacier or soil is barely more metabolized by heterotrophic microorganisms than younger, labile, freshly-produced OM (Hood *et al*. 2009). The curved cells are associated with a capacity to digest refractory DOC (RDOC) (Dunalska *et al*. 2004), which is unavailable for the majority of aquatic bacteria. RDOC originates in the catchment area, where soil bacteria exert specialized utilization (McLaughlin and Kaplan 2013). Similar observations were made by Roiha *et al*. (2012) and Crump *et al*. (2012), who reported that catchment runoff has a strong effect on biomass, productivity and community composition of microorganisms in sub-Arctic ponds and lakes.

Changes in the prokaryotic community cell size and morphological structure in the river and stream system of the Brattegg Valley may be caused by changes in the amount and quality of available organic matter. The effect of the drainage basin on the waters of the lowest lake in the system and making the microbiocenosis community grow by supplying more cells from the soil may also be an important factor. As described by Crump *et al*. (2003, 2007), the drainage basin could supply the lake with dead cells, but also nutrients, that stimulate autoch-

Table 6. Bacterial structure (phylotypes) in the surface water of the three studied Brattegg Valley lakes. Values are percentages (mean ± SD, n = 36) of hybridized cells in the total DAPI counts.

Lake	Bacteria	Proteobacteria			Cytophaga–Flavobacterium	Actinobacteria	Archaea
		α	β	γ			
Upper	73.6 ± 2.7	19.5 ± 3.5	19.0 ± 1.7	0	11.5 ± 1.6	18.2 ± 3.0	6.9 ± 1.2
Middle	90.4 ± 1.1	10.1 ± 0.9	18.7 ± 0.2	3.6 ± 0.1	16.4 ± 1.4	27.4 ± 3.3	4.3 ± 1.0
Myrktjørna	87.1 ± 1.6	11.1 ± 1.9	31.9 ± 4.4	3.8 ± 0.8	19.0 ± 2.2	29.3 ± 3.8	7.9 ± 0.8

thonous bacterioplankton growth. Obviously, the transport of microorganisms from the glacier is also taking place. Due to low water retention, the short distance from the upper lake, the small size and a considerable inclination of the slope, the middle-lake inlet practically contains the upper lake water. For these reasons there were small changes in the bacterioplankton structure in the middle lake.

The Brattegg lake–stream system VLP number was correlated with prokaryote abundance and DOC, thus highlighting biocenotic links. The results received by Säwström *et al*. (2007) suggest that virioplankton may be maintained in polar environments even though host density is low and often increases slowly. The metabolic state of the host cell is a critical factor in the phage life cycle (Weinbauer 2004, Ram *et al*. 2005, Säwström *et al*. 2008). In this regard, the high VLP/TCN ratio in Lake Myrktjørna may indicate higher activity of the community of prokaryotes in this lake.

Bacterial community composition shift

The bacterial community composition in the Brattegg lake–stream water system changed along the system. Specific environmental conditions, primarily physical (temperature, UV) and chemical factors (organic and mineral compounds) favour organisms originally adapted to other conditions (Kaštovská *et al*. 2005). Our findings suggest that bacterial succession in a high Arctic lake–stream system involves simplification of community composition. This confirms the results obtained by Sigler *et al*. (2002) in glacial forefield soils where the number of dominant bacterial types decreased with succession. Although little published information is available on prokaryotic-plankton structural changes in arctic lake–stream systems (Crump *et al*. 2007, Ylla *et al*. 2013), the small number of OTUs detected in the glacial water suggests that these microbiocenoses vary greatly. However, the relatively high bacteria diversity in the upper lake, decreasing down the system, indicates elimination of bacteria poorly-adapted to environmental change. The strongest selection of community of glacial origin takes place in the middle lake. Moreover, greater bacteria diversity in the streams than in the lakes in the Arctic was previously described by Crump *et al*. (2012), Portillo *et al*. (2012) and Adams *et al*. (2014).

In the Brattegg Valley, greater bacteria diversity in the stream than in the lakes results from biologically-active microbial mats in the watercourses. The presence of microbial mats, phyto- and zooplankton (D. Górniak and H. Marszałek unpubl. data) and fish (D. Górniak and H. Marszałek pers. obs.) in Lake Myrktjørna affects food-network development by increasing organic matter quantity and quality in these waters. This undoubtedly affects the structure and function of the lake's microbiocenosis, as evidenced by the changes in the number and composition of dominant bacterial phylotypes.

Examination of the bacterioplankton structure indicated its re-development in the lake system. The increased percentage of Actinobacteria and β-Proteobacteria throughout the system suggests a clear link with the quality of organic matter and nutrient sources. This was accompanied by a simultaneous decrease in α-Proteobacteria. The increased amounts of Actinobacteria and Cytophaga–Flavobacterium throughout the system points to the soil being their primary source as Actinobacteria are considered typical colonizers of Arctic soil environments (Yergeau *et al*. 2010, Schütte *et al*. 2010). Recent bacterioplankton structure studies have also revealed their widespread occurrence in freshwater (Allgaier and Grossart 2006), where some members of this phylum can utilise humic substances. Cytophaga–Flavobacterium have also been reported in many habitat in the biosphere, including rocks and sea-ice in Antarctica, and sediments of lakes in addition to β-Proteobacteria, one of the most common heterotrophic bacteria in freshwater (Kirchman 2002, Sommaruga and Casamayor 2009).

Summary

The microorganism growth in the studied arctic river-lacustrian system was determined by changing environmental conditions. Changes in nutrient content and water physicochemical conditions throughout the system caused strong

selection pressure on microorganisms moving from the upper lake to the lower-lying reservoirs. Here, the upper lake was subject to the strong influence of ablation water, with a reduction in most measured water chemistry and microbial parameters in the middle lake, while Lake Myrktjørna is a typical example of ultra-oligotrophic arctic lakes with a well-developed food web and ecological sustainment. However, this does not mean that microbiocenosis reaching Lake Myrktjørna from the upper parts of the system did not affect microbial diversity, since this is achieved by explicit selection. It was also confirmed by the different dominant bacterial taxa in Lake Myrktjørna as compared with those in the upper and middle lakes. The abundance of prokaryotes and their higher taxonomical biodiversity in the upper lake as compared with that in the lower-lying reservoirs was noteworthy. It appears that the limited transport of organic matter and nutrients down the Brattegg Valley system had no direct impact on changes in prokaryote structure, because these lakes are regulated by changing abiotic conditions such as temperature, suspended solids, oxygen saturation which affect their colonization and the development of prokaryotic community. We conclude that succession in the Brattegg Valley lake–stream system comprised changes in bacterial structure, together with a decrease in species richness. The ecological succession of planktonic prokaryotes in the arctic fluvio-lacustrine system consisted of two steps: (1) selection of glacial derived microorganisms by changing environmental conditions and (2) post-adaptational colonization.

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