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Springtime dynamics, productivity and activity of prokaryotes in two Arctic fjords

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Abstract In the Kongsfjorden–Krossfjorden system (Spitsbergen), increasing temperatures enhance glacier melting and concomitant intrusion of freshwater. These altered conditions affect the timing, intensity, and composition of the phytoplankton spring bloom in Kongsfjorden; yet, the effects on prokaryotes (bacteria and archaea) are not well understood. The aim of this study was to examine springtime prokaryote communities in both fjords as a function of hydrographic and phytoplankton variability. Prokaryote community composition was studied in two consecutive years by molecular fingerprinting of the 16S rRNA gene. In addition, we measured bacterial abundance, productivity (³H-Leucine uptake), and single-cell activity

using catalyzed reporter deposition fluorescence in situ hybridization combined with microautoradiography. Differences in bacterial and archaeal communities were found between Kongsfjorden and Krossfjorden. Furthermore, an increase in productivity, abundance, and proportion of active bacterial cells was observed during the course of spring. Bacteroidetes were the most abundant bacterial group among the assessed taxa in both Kongsfjorden and Krossfjorden. Multivariate analysis of the microbial community fingerprints revealed a strong temporal shaping of both the bacterial and archaeal communities in addition to a spatial separation between the two fjords. A significant part of the observed bacterial variation could be explained by cyanobacterial biomass, as deduced from pigment analysis, and by phosphate concentration. Archaea were mainly controlled by abiotic factors. We speculate that the bacterial response to hydrographic changes and glacier meltwater is mediated through shifts in phytoplankton abundance and composition, whereas archaea are directly influenced by abiotic environmental variables.

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Introduction

Global warming changes the environmental conditions that shape the polar marine ecosystem, thereby potentially changing its dynamics, productivity, and composition (Hop et al. 2002; Vincent et al. 2011). Warming of polar regions reduces sea-ice cover which may alter all three marine microbial domains (eukaryota, bacteria, archaea), such as found for the Beaufort Sea (Canadian Arctic, Comeau et al.

2011). Furthermore, it enhances the input of (sediment-rich) freshwater from melting glaciers and inland snow fields (Olsen et al. 2011). This may result in increased stratification with a top layer of turbid freshwater that enhances light attenuation and affects phytoplankton composition and activity. Several studies reported a shift from larger algal species (mainly diatoms >20 µm) to small-sized nano- and picophytoplankton (<2 µm) species in response to increased freshwater input (Daufresne et al. 2009; Li et al. 2009; Coupel et al. 2012; Piquet et al. 2014) or reduced sea-ice cover (Comeau et al. 2011). This apparent phytoplankton downsizing can have significant effects on higher trophic levels in polar marine ecosystems (Duarte et al. 2012).

An alteration in the composition and productivity of phytoplankton communities is also expected to affect prokaryotic production, composition, and diversity. To address these latter effects, we studied the prokaryotic communities in Kongsfjorden and Krossfjorden, two adjacent glacial fjords located on the west coast of Spitsbergen (79°N). This Arctic fjord system is strongly influenced by the West Spitsbergen Current (WSC) transporting relatively warm (3–7 °C) and salty (35.1–35.3) Atlantic waters (AW) into the common mouth (Svendsen et al. 2002; Cottier et al. 2005). However, the inner sections of the fjords receive input from cold, freshwater derived from melting glaciers and snow fields during spring and summer. Maximum meltwater runoff in summer leads to a salinity-driven stratification of the water column with a concomitant sediment discharge and increased turbidity, which appears more pronounced in Kongsfjorden than in Krossfjorden (Keck et al. 1999; Svendsen et al. 2002; Hegseth and Tverberg 2013; Piquet et al. 2014). During AW influenced years, *Phaeocystis pouchetii* dominates the spring blooms in Kongsfjorden, while in other years diatoms dominate (*Chaetoceros* sp., *Thalassiosira* sp., and *Fragilariopsis* sp.) the spring blooms (Leu et al. 2006; Hodal et al. 2012; Hegseth and Tverberg 2013). Small-sized phytoplankton species belonging to the nano- and picosize range typically dominate during post-bloom low-nutrient conditions (Piquet et al. 2014).

Summertime Kongsfjorden–Krossfjorden microbial community is dominated by Alphaproteobacteria, including the SAR11 and RCA cluster (Piquet et al. 2010). Furthermore, members of the Gammaproteobacteria and Cytophaga–Flavobacter–Bacteroides (CFB) group formed a large proportion of the identified bacteria (Arnosti 2008; Zeng et al. 2009; Piquet et al. 2010). Gammaproteobacteria were frequently retrieved during cultivation from sediment samples of Kongsfjorden (Srinivas et al. 2009). Many of the microorganisms identified in the Kongsfjorden–Krossfjorden system were found in marine microbial diversity studies carried out elsewhere in the Arctic (Ravenschlag

et al. 1999; Bano and Hollibaugh 2002; Brinkmeyer et al. 2004; Groudieva et al. 2004; Garneau et al. 2005; Malmstrom et al. 2007; Arnosti 2008; Kellogg and Deming 2009; Collins et al. 2010). Identification of the typical freshwater associated Betaproteobacteria in both fjords has been linked to glacial meltwater input (Glöckner et al. 1999; Piquet et al. 2010). Betaproteobacteria were abundant in summer meltwater pools on Arctic pack ice (Brinkmeyer et al. 2003, 2004). Generally, betaproteobacterial abundance decreases with increasing salinity (Garneau et al. 2005). Consequently, it was suggested that marine microbial communities in the Kongsfjorden–Krossfjorden system are shaped by both water mass origin (Atlantic, Arctic) and meltwater input (Zeng et al. 2009; Piquet et al. 2010). Limited information is available, however, on how phytoplankton spring bloom dynamics govern bacterial diversity, composition, and activity. In this study, we present springtime dynamics of prokaryotic communities (bacteria and archaea) of Kongsfjorden and Krossfjorden by means of 16S rRNA gene fingerprinting, bacterial abundance, production, and the activity of five specific bacterial groups using catalyzed reporter deposition fluorescence in situ hybridization combined with microautoradiography (Micro-CARD-FISH; MCF).

Materials and methods

Sampling and sample handling

Samples were obtained twice a week, from May 22, 2007 to June 25, 2007 (mid- to late spring), and from April 9, 2008 to May, 12 2008 (early to mid-spring) in Kongsfjorden (78°57'54"N, 11°51'24"E) and Krossfjorden (79°10'00"N, 11°46'00"E) with Kings Bay RV *Teisten* (Table 1). Samples were obtained from the surface and at 20 m depth with an occasional sampling at other depths. The five sampling sites represent a transect from ocean- to the glacier-influenced part of the fjords: Site “O” is located at the intersection of both fjords with the ocean; sites “M” and “KM” are located in the middle of Kongsfjorden and Krossfjorden, respectively; and sites “G” and “KG” are located near the glaciers of Kongsfjorden and Krossfjorden, respectively (Fig. 1). For molecular analyses, 1.5–2 L of seawater was successively filtered through 2- and 0.2-µm-pore-size polycarbonate filters with a 47 mm diameter (Merck Millipore, Massachusetts, USA). Samples were flash-frozen in liquid nitrogen and stored at –80 °C until analysis in the home laboratory (University of Groningen) and the NIOZ-Yerseke Marine Microbiology Laboratory. Pigment composition and phytoplankton biomass (chlorophyll *a*) was determined using high-performance liquid chromatography (HPLC) in combination with CHEMTAX

Table 1 Average salinity, temperature (°C), and nutrient data (μM): NO_x (nitrate + nitrite), PO₄, Silica, and N:P ratio in early and late spring

Station	Salinity	Temperature	NO _x	PO ₄	Silica	N:P ratio
<i>Early spring</i>						
O	34.93 (±0.05)	1.35 (±0.38)	8.25 (±2.48)	0.63 (±0.15)	3.54 (±1.14)	13.10
M	34.92 (±0.05)	1.14 (±0.26)	7.10 (±2.34)	0.56 (±0.13)	3.47 (±1.06)	12.69
G	34.76 (±0.10)	0.05 (±0.57)	7.33 (±1.57)	0.57 (±0.15)	3.38 (±0.85)	12.82
KM	34.89 (±0.03)	0.10 (±0.40)	8.71 (±2.06)	0.62 (±0.11)	3.76 (±0.85)	14.11
KG	34.36 (±0.93)	−0.30 (0.64)	9.17 (±1.76)	0.64 (±0.11)	4.06 (±0.44)	14.22
<i>Late spring</i>						
O	34.56 (±0.21)	2.91 (±1.38)	0.62 (±0.68)	0.09 (±0.06)	0.86 (±1.12)	6.98
M	34.41 (±0.34)	2.74 (±1.49)	1.08 (±2.19)	0.12 (±0.07)	0.61 (±0.56)	9.11
G	33.99 (±1.01)	3.07 (±1.60)	1.23 (±0.74)	0.19 (±0.07)	0.82 (±0.57)	6.61
KM	34.49 (±0.29)	2.89 (±1.26)	0.36 (±0.36)	0.09 (±0.05)	0.46 (±0.28)	3.94
KG	34.36 (±0.65)	3.14 (±0.86)	0.80 (±1.10)	0.14 (±0.06)	0.56 (±0.48)	5.69

The average and standard deviation were calculated for surface and 20-m-depth samples; see Piquet et al. (2014) for more details. Stations are ocean (O), Kongsfjorden middle (M) and glacier (G), Krossfjorden middle (KM), and glacier (KG). Early spring data from the period April 9, 2008 to May 12, 2008, late spring data: May 22, 2007 to June 26, 2007

analysis as previously described (Piquet et al. 2014). In short, HPLC-CHEMTAX data revealed that diatoms and *Phaeocystis* sp. were replaced by small nano- and picophytoplankton during late spring, coinciding with low nutrient availability. The innermost stations showed higher relative abundances of nano- and picophytoplankton throughout, notably of cyanophytes and cryptophytes. Further detailed information on sample collection and processing can be found in Piquet et al. (2014).

Prokaryotic abundance

Prokaryotic abundance was determined on a DAPI (4',6-diamidino-2-phenylindole) stained subsection of each Micro-CARD-FISH (MCF) filter (see below). Cells were counted under a Zeiss Axioplan 2 (Carl Zeiss MicroImaging, GmbH, München, Germany) microscope equipped with a 100-W Hg lamp and DAPI filter. Approximately 1000 DAPI-positive cells were counted per sample with a minimum of 10 microscopy fields evenly distributed over the filter.

Prokaryotic production

Prokaryotic production of the free-living community was only determined for the early to mid-spring samples of 2008 by the leucine incorporation assay using the microcentrifugation method (Smith and Azam 1992). Samples were stored onboard in the dark at in situ temperature and processed upon arrival in the laboratory. Approximately 15 mL of seawater was pre-filtered through a 3-μm polycarbonate filter to exclude sediment and potential prokaryotic grazers (Kirchman and Ducklow 1993).

Samples were transferred to four 2-mL screw cap microfuge tubes (1.7 mL each). Three tubes per sample were spiked with ³H-leucine (80 Ci mmol^{−1}, final concentration 20 nM) and incubated in the dark at 4 °C for 60 min. The fourth tube served as a negative control, to which trichloroacetic acid (TCA, final concentration 5 %) was added 10 min prior to spiking with ³H-Leucine. The incubation was terminated by addition of 100 % TCA to a final concentration of 5 %. Subsequently, the fixed samples were centrifuged at 10,000 g, the supernatant siphoned off, washed with 5 % TCA and 80 % EtOH, air-dried, 2 mL of scintillation cocktail (Emulsion Scintillation Plus scintillation liquid, PerkinElmer, Inc. Waltham, MA, USA) was added, and the disintegrations per min (DPM) were counted in a Tri-carb scintillation analyzer (Packard 1600CA, Downers Grove, IL, USA). The DPMs of the negative control were subtracted from the average of the triplicate samples. Leucine incorporation rates were calculated and subsequently converted to carbon production applying a conversion factor of 3594 g C mol Leu^{−1} (Simon and Azam 1989).

Prokaryotic community composition and group-specific activity

Community composition and activity were determined using MCF (Lee et al. 1999; Pernthaler et al. 2002a). Samples (10 mL) were pre-filtered through a 3-μm polycarbonate filter to exclude sediment and larger organisms and transferred to 15-mL Greiner tubes. Cells were spiked with [³H]-leucine (155 Ci mmol^{−1}, final concentration 20 nM), incubated at 4 °C for 4 h, fixed with paraformaldehyde (2 % final concentration) stored

Fig. 1 Map of the Kongsfjorden and Krossfjorden bathymetry and sample sites. The five sampling stations ocean (O), Kongsfjorden middle and glacier (M and G), Krossfjorden middle, and glacier stations (KM and KG) are shown

overnight at 4 °C, and finally filtered onto a 25-mm, 0.2- μ m GTTP filter (Merck Millipore, Massachusetts, USA), supported by a 25-mm nitrocellulose filter. GTTP filters were air-dried, loosely packed in aluminum foil, and held at -20 °C until further processing in the home laboratory. A negative control was included, to which paraformaldehyde (2 % final concentration) was added before spiking and incubating with [3 H]-leucine.

The CARD-FISH procedure was carried out according to Pernthaler et al. (2002a, b). Briefly, cells were

permeabilized with lysozyme (Sigma-Aldrich, Germany), and the filters were cut into six pieces. One piece was used for the determination of prokaryotic abundance as described above, and the other five were used for hybridization with specific oligonucleotide probes (Table 2) at a final concentration of $2.5 \text{ ng } \mu\text{L}^{-1}$ for 12 h at 37 °C. Filters were washed, and signal amplification was conducted with tyramide-Alexa488 (Molecular probes Europe BV) for 30 min at 37 °C. After hybridizing the filter sections, microautoradiography was carried out according to

Table 2 Probes used for Micro-CARD-FISH analysis of the bacterial community and selected bacterial groups

Target group	Probe	References
Bacteria	Eub338 I–III + non338	1, 2, 3
Betaproteobacteria	Bet42a + nonGam42a	4
Bacteroidetes	CF319	5
<i>Roseobacter</i>	Ros537	6
SAR86	SAR86	7, 8

(1) Daims et al. (1999), (2) Amann et al. (1990), (3) Amann et al. (1995), (4) Manz et al. (1992), (5) Manz et al. (1996), (6) Eilers et al. (2001), (7) Eilers et al. (2000), (8) Morris et al. (2002)

Alonso-Sáez et al. (2008), whereby the filter sections were embedded in the autoradiography emulsion (KODAK NTB-2; Sigma-Aldrich, Germany) with the cells facing the emulsion at 4 °C for 48 h. Slides were developed and fixed according to the manufacturer's recommendations (KODAK Dektol developer and fixer; Sigma-Aldrich, Germany). Slides were dried and finally stained with DAPI (Life Technologies Ltd., Paisley, UK). Quantification was done with a “Zeiss Axioplan 2” microscope equipped with a 100-W Hg lamp and the appropriate filters for the detection of Alexa488 and DAPI fluorescence. A minimum of 400 DAPI-positive cells and at least 10 microscopic fields were analyzed for hybridized (selected bacterial groups) and [³H]-leucine-positive (active) cells. The average abundance of bacteria (Eubacteria probe + cells) and specific bacterial groups was normalized to total DAPI-stained cells and to total bacteria, respectively.

Fingerprinting of the prokaryotic communities by denaturing gradient gel electrophoresis (DGGE)

Total DNA was extracted from the 2- to 0.2-μm-size fraction using the MoBio DNA extraction kit as in Piquet et al. (2010). Polymerase chain reaction (PCR) was performed to amplify 16S rRNA gene fragments from the bacterial and archaeal fraction of the communities. The primers used are listed in Table 3. A nested PCR was

performed to amplify archaeal 16S rRNA gene fragments. The initial step consisted of the amplification of the full-length archaeal 16S rRNA gene, followed by the specific DGGE-PCR. For the first reaction, a 25 μL PCR mixture was used consisting of 1X HotStar PCR Buffer (Qiagen Inc.), 2.5 mM of MgCl₂, 200 μM of dNTP's (Roche Applied Science, USA), 0.1 mg/mL bovine serum albumin (Fermentas, USA), 200 nM of the primers A2F and U1492R (see Table 3), and 0.5 Units of HotStar Taq DNA polymerase (Qiagen Inc.). The PCR consisted of an initial denaturation at 95 °C for 15 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 30 s and elongation at 72 °C for 2 min, followed by a final elongation step at 72 °C for 7 min. Amplicons were purified using the E.Z.N.A. Cycle-Pure Kit (Omega Bio-Tek, USA), eluted in 30 μL of elution buffer, and 1 μL was used as template for the second PCR. The archaeal DGGE-PCR was performed with 1x Illustra PCR Buffer (GE Healthcare, UK), 200 μM of dNTPs (Roche Applied Science, USA), 2.5 % v/v of dimethyl sulfoxide (Sigma-Aldrich, Germany), 200 nM of the primers PARCH519R-GC and SAF-341F (Table 3), and 1 Unit of Illustra Taq DNA polymerase (GE Healthcare, UK). The PCR consisted of an initial denaturation at 95 °C for 3 min and 10 touchdown cycles in which the annealing temperature decreased by 0.5 °C per cycle (denaturation at 95 °C for 1 min, at 60–55 °C for 1 min, and elongation at 72 °C for 2 min). This was followed by 25 cycles of denaturation at 95 °C for 1 min, annealing at 56 °C for 1 min, and elongation at 72 °C for 2 min, followed by a final elongation step at 72 °C for 30 min. For bacteria, we ran a direct DGGE-PCR. The 25 μL bacterial DGGE-PCR consisted of 1x Illustra Buffer (GE Healthcare, UK), 200 μM dNTPs (Roche Applied Science, USA), 0.1 mg/mL bovine serum albumin (Fermentas, USA), 2.5 % v/v of dimethyl sulfoxide (Sigma-Aldrich, Germany), 200 nM of the primers Bac-F968-GC and Bac-R1401c, and 1 Unit of Illustra Taq DNA polymerase (GE Healthcare, UK). The PCR was performed as described for the second step of the archaeal reaction. PCR was carried out using a

Table 3 List of primers used in this study to assess the bacterial and archaeal community composition by DGGE fingerprinting

Primer name	Sequence, 5'–3'	References
A2F	TTCCGGTTGATCCYGCCGGA	1
U1492R	GGTTACCTTGTTACGACTT	1
PARCH519R-GC	CGCCCCCGCGCGCGGGCGGGGCGGGGCGGGGGACGGGGGGTTACCGCGGCKGCTG	2
SAF-341F	CTAYGGGGCGCAGCAGG	3
Bac-F968-GC	CGCCCCCGCGCGCGGGCGGGGCGGGGCGGGGCGGGGGGAACGCGAAGAACCTTAC	4
Bac-R1401c	CGGTGTGTACAAGRCCCGGGAACG	3

(1) van der Wielen et al. (2005), (2) Ovreas et al. (1997), (3) Nicol et al. (2003), (4) Heuer et al. (1997)

thermal cycler (Thermal Cycler 2720, Applied Biosystems, Foster City, CA, USA).

DGGE-PCR mixtures were purified using E.Z.N.A. CyclePure columns (Omega Bio-Tek, USA). After purification, the DNA concentration in each sample was determined spectrophotometrically and adjusted to a final concentration of 200 ng of DNA in 28 μL , supplemented with 2 μL of loading buffer (Piquet et al. 2008) and loaded on the DGGE gel. The DGGE was run on the Ingeny phorU[®] system (Ingeny International, Goes, the Netherlands). Denaturing polyacrylamide gels (8 % w/v acrylamide) were made according to the manufacturer's instructions. The gradients used were from 45 to 60 % v/v for bacterial DGGE amplicons and 40–55 % v/v for archaeal amplicons. Samples and reference samples were subjected to electrophoresis at 100 V for 18 h in a $0.5 \times \text{TAE}$ buffer at 60 °C. Reference samples were made by combining samples that covered the full gel gradient and re-amplifying these to generate larger quantities. After electrophoresis, DGGE gels were silver-stained using an automated gel stainer (Hoefer Processor Plus, Amersham Biosciences).

DGGE fingerprints were analyzed using the BioNumerics software package (Applied Maths NV, Belgium). Bands were automatically assigned and checked for false bands generated by silver stain spots in the gel. For microbial diversity analysis, we designated distinguishable bands in the gel as an operational taxonomic unit (OTU) using the band-matching tool of BioNumerics. Bands occurring at the same height in different samples were considered to belong to the same OTU, whereas their intensities were used as a semiquantitative estimate of their relative abundance in the original sample. OTU position and intensity data were exported to the open-source statistical software package PAST (Hammer et al. 2001) and used to calculate the Shannon diversity index of each sample.

Data processing

Statistical analyses were carried out in STATISTICA 8.0[®] (StatSoft, Inc., Tulsa, USA). Multifactorial ANOVAs were carried out for environmental variables, biotic variables, chlorophyll *a* (chl-*a*), prokaryotic abundance, and bulk heterotrophic production. Environmental variables, biotic variables, and chl-*a* concentration were extracted from Piquet et al. (2014). Ordination analysis of DGGE and environmental data sets was performed using the CA package (Greenacre 2007) and the Vegan package in R (Oksanen et al. 2014). Canonical correlation analysis (CCA) was run on the relative abundance of OTUs, as identified from 16S rRNA gene DGGE profiles, and environmental variables (abiotic and biotic). Separate CCAs were run on 130 bacterial and 134 archaeal DGGE profiles.

Environmental variables used in the CCA were temperature, salinity, depth, nutrient concentrations (NO_x, PO₄, Si), N:P ratio, time (years), and location. We also included the biotic variable chl-*a*, and relative pigment abundances of the major phytoplankton groups, as previously calculated using HPLC pigment fingerprinting followed by CHEMTAX (Piquet et al. 2014). The significance of each variable was determined using the environmental (envfit) function of Vegan.

Results

Prokaryotic abundance, bacterial composition, and single-cell activity

The average prokaryotic abundance in Kongsfjorden increased from $2.8 \pm 0.9 \times 10^5$ cells mL^{-1} on April 14, 2008 to $6.0 \pm 1.4 \times 10^5$ cells mL^{-1} on May 12, 2008 (data not shown). Late spring (2007 campaign) average prokaryotic abundance in Kongsfjorden was 9.8×10^5 cells mL^{-1} . A similar trend was found for Krossfjorden where the average prokaryotic abundance increased from $2.1 \pm 0.5 \times 10^5$ cells mL^{-1} on the April 16, 2008 to $3.9 \pm 1 \times 10^5$ cells mL^{-1} on the May 6, 2008 and an average of $12 \pm 0.4 \times 10^5$ cells mL^{-1} in late spring samples.

Over the same period, the contribution of bacteria to DAPI-stained cells increased from 75 % in early spring, to 87 % in mid- and late spring. In early spring, the majority of cells in both Kongsfjorden (Fig. 2) and Krossfjorden (Fig. 3) at all stations and both depths consisted of non-active bacteria. Cells that hybridized to the Bacteroidetes, *Roseobacter*, SAR86, and betaproteobacterial probes were also largely inactive and together comprised no more than 37 % of the total number of DAPI-stained cells. The only exception occurred in early spring surface samples of Krossfjorden where Bacteroidetes at Stations O and M, and *Roseobacter* at Station G added up to 57 % of the total number of DAPI-stained cells. In mid-spring (Kongsfjorden) and late spring (Kongsfjorden and Krossfjorden), cells hybridizing to the Bacteroidetes probe showed a high relative abundance at all stations, reaching up to 67 % in late spring surface samples of Station M and KG, of which 97 % were active (Figs. 2, 3). With an average relative abundance of 8 %, the *Roseobacter* group maintained generally low relative abundances at all stations and depths. Yet, the proportion of active cells within this group strongly increased in mid- and late spring to up to 100 %. The proportion of SAR86 and Betaproteobacteria to DAPI-stained cells remained very low in all samples with only very few active cells. For these groups, no general patterns were detectable over the sampling period, depths, or location.

Fig. 2 Percentage of CARD-FISH groups (*Bacteroidetes*, *Roseobacter*, *SAR86*, Betaproteobacteria, bacteria) relative to the total number of DAPI-stained cells in Kongsfjorden during the spring bloom period. Data are depicted for surface and 20 m depth, at the ocean (O), middle (M), and glacier (G) stations and for early, mid-, and late spring. Cells taking up ^3H -Leucine (Leu-active) are depicted in gray and Leu-inactive cells in black

The ratio of active versus non-active cells at all stations and depths combined increased for all groups from early to mid- to late spring (Fig. 4). In early spring, the active bacterial community exhibited a disproportionally low activity compared to its abundance (below the 1:1 trend line). In mid-spring, the relative abundance of active cells in the specific bacterial groups increased proportional to their abundance, with the exception of *Bacteroidetes* exhibiting a relatively low number of active cells. In late

spring, however, the number of active *Bacteroidetes* cells increased also (Fig. 4).

Prokaryotic heterotrophic production

Prokaryotic heterotrophic production as determined by leucine incorporation and measured from early to mid-spring of 2008 followed a similar trend as the contribution

Fig. 3 Percentage of CARD-FISH groups (*Bacteroidetes*, *Roseobacter*, *SAR86*, *Betaproteobacteria*, and *Bacteria*) relative to the total number of DAPI-stained cells in Kongsfjorden during the spring bloom period. Data are depicted for surface and 20 m depth, at the ocean (O), middle (KM), and glacier (KG) stations and for early and late spring. Cells taking up ^3H -Leucine (Leu-active) are depicted in gray and Leu-inactive cells in black

of active cells to the total DAPI-stained cells. In early spring, all Kongsfjorden and Krossfjorden stations revealed low prokaryotic production rates (<11.5 and $16.2 \text{ ng C L}^{-1} \text{ h}^{-1}$ for Kongsfjorden and Krossfjorden, respectively) (Fig. 5). Heterotrophic prokaryotic production increased thereafter reaching at the mid-bloom period in Kongsfjorden $206.2 \pm 78.5 \text{ ng C L}^{-1} \text{ h}^{-1}$. No significant differences in prokaryotic heterotrophic production between Kongsfjorden and Krossfjorden were detected (two-way ANOVA, $p = 0.258$).

Fig. 4 Relative contribution of CARD-FISH groups to the total number of DAPI-stained cells in Kongsfjorden during the spring bloom period versus the contribution of Leu-active members (taking up ^3H -leucine) to the total Leu-active bacterial community. Data of both depths and all stations in both Kongsfjorden and Krossfjorden are combined but separated according to early, mid-, and late spring periods. *Bacteroidetes*—black circles, *Roseobacter*—gray circles, *SAR86*—black triangles, *Betaproteobacteria*—white triangles, *bacteria*—squares

Bacterial community dynamics

A total of 130 bacterial fingerprints were obtained by DGGE. We identified 31 bacterial OTUs (band classes assigned using BioNumerics); the average number of OTUs per sample was 13, and the estimated Shannon diversity index (H) was 2.22. Most diverse bacterial band patterns ($H = 2.84$) were observed in samples from mid-spring at the central station (M) in Kongsfjorden (5-05-08-M0: date–location–depth), while the lowest diversity

Fig. 5 Heterotrophic prokaryotic production in $\text{ng C L}^{-1} \text{h}^{-1}$ of surface and 20-m-depth samples from the stations ocean, middle, and glacier in Kongsfjorden and Krossfjorden from April 14 to May 12, 2008. Error bars indicate standard deviation of measurements in triplicate

($H = 1.02$) was found at 20 m depth at the same station in early spring (14-04-08-M20). Pearson's similarity analysis of bacterial band patterns showed a variable community with low similarity (<25 %) between early to mid-spring and late spring bacterial fingerprints (data not shown).

Canonical correlation analysis (CCA) of the DGGE-generated bacterial 16S rRNA gene fingerprints revealed strong separation of the early to mid-spring samples taken in the year 2008 from the late spring samples taken in the year 2007 (Fig. 6). The bacterial communities of Kongsfjorden and Krossfjorden were clearly separated forming distinct clusters. The 95 % confidence ellipses show overlap within the Krossfjorden between the middle (KM) and near-glacier (KG) samples. The near glacier (G) and ocean site (O) overlap and take a central position in the plot, whereas the mid-Kongsfjorden samples (M) form a separated cluster. The bacterial community of site M in the year 2007 (late spring) correlated with the Si:N ratio, while exhibiting an inverse relation to the presence of diatom pigments and chl-*a*. The opposite trend was found for the bacterial communities of both Krossfjorden sites. ANOVA of the CCA showed that the variables that explained a significant part ($p < 0.001$) of the variation in the CCA of the bacterial fingerprints were time and phosphate concentration, explaining ~14 and 3.1 % of the observed variance in the bacterial data set, respectively. Significant contributions ($p < 0.01$) to the variation in bacterial communities were attributable to the presence of cyanobacteria (1.9 %), temperature (1.6 %), and NO_x (1.5 %), while the other measured factors did not significantly contribute to the observed variance. Thus, a major fraction of the variance (~70 %) could not be explained by the factors studied here.

Archaeal community dynamics

Pearson's similarity analysis of 134 DGGE-generated archaeal 16S rRNA gene fingerprints revealed that all fingerprints shared 60 % similarity (data not shown). We found an average of 15 OTUs and a Shannon diversity index of 2.65. The average diversity did not significantly differ between early to mid-spring ($H = 2.73$) and late spring ($H = 2.55$). Only Kongsfjorden surface and samples from Stations M and G in late June showed a marked reduction in the number of OTUs and in Shannon diversity index with the lowest archaeal diversity observed on June 19, 2007 ($H = 1.70$). Pearson's similarity analysis revealed little variability over time, rather some clustering according to sampling location (Kongsfjorden versus Krossfjorden).

CCA of the archaeal fingerprints revealed a similar separation of the archaeal community between the years 2007 and 2008 as observed for bacteria (Fig. 7). The archaeal community composition differed between the two fjords while the two stations within the fjords. KM and KG versus M and G clearly overlapped in their community composition. The ocean site (O) showed little overlap in the archaeal community composition with the other sites; however, a higher similarity in archaeal community composition at the Kongsfjorden stations was observed as compared to Krossfjorden. ANOVA analysis of CCA variance confirmed the importance of time and location as significant shaping factors explaining 6.7 and ~3.0 % of the observed variance. A relatively low explanatory power of phosphate concentration (2.2 %), temperature (1.8 %), and salinity (1.5 %) was found (data not shown). Nearly 84 % of the variance could not be explained by the variables analyzed here.

Discussion

Prokaryotic community dynamics at the onset of the spring bloom

Kongsfjorden and Krossfjorden springtime prokaryotic abundance, activity and heterotrophic prokaryotic production increased from early spring to mid spring to late spring. For logistic reasons, early spring to mid spring (2008) and mid spring to late spring (2007) samples were collected in two different, consecutive years. Therefore, it cannot be ruled out that the observed changes in microbial abundance, diversity, and activity between early and late spring samples were partly due to inter-annual variability in (the timing of) phytoplankton dynamics. However, as reported previously (Piquet et al. 2014), both years showed similar developments with respect to abiotic (irradiance attenuation, salinity, temperature, nutrients) as well as

Fig. 6 CCA plot generated from the 16S rRNA fingerprints of bacterial communities of early (April 9) to mid (May 12)-spring 2008 and late spring (May 22–June 26) 2007. *Left plot, blue arrows, and their lengths indicate direction and proportion to the correlation between the tested environmental variable and the ordination. OTUs are identified by their relative (%) migration distance in red. Right*

plot, for each sample bacterial fingerprints are shown as a symbol in the plot. Open squares are 2008 samples, open circles 2007 samples, color codes are given for each sampling location indicated by its name abbreviation (see Table 1). The percentages of inertia on dimensions 1 and 2 are 40.7 and 15.7 %, respectively, computed relative to the restricted inertia in the CCA

Fig. 7 CCA plot of the archaeal 16S rRNA fingerprints of early to mid-spring 2008 and late spring 2007. See legend to Fig. 6 for a description of the *symbols and arrows*. The percentages of inertia on

dimensions 1 and 2 are 31.4 and 16.6 %, respectively, computed relative to the restricted inertia in the CCA

biotic (phytoplankton biomass and composition revealed by HPLC-CHEMTAX) parameters. As a result, inter-annual variability is expected to be minor as compared to the

observed seasonal variability. Prokaryotic abundance ranged between 2.8 and 6.1×10^5 cells mL^{-1} , in agreement with prokaryote abundances found in the Canadian Arctic

(Sala et al. 2008) and Fram Strait (Seuthe et al. 2011) in the winter to spring transition period under pre-bloom conditions. As expected, the majority of the DAPI-stained cells hybridized to the general bacterial probes with similar fraction (75–87 %) as in a previous study performed in Kongsfjorden during the spring to summer transition of 2008 (June–July, 74–92 % of DAPI-stained cells were bacteria (De Corte et al. 2011).

The increase in prokaryotic abundance, bacterial activity, and productivity (Figs. 1, 2, 3) can be interpreted as a response to increased phytoplankton biomass in Kongsfjorden from the early to mid-spring period of the year 2008 (Piquet et al. 2014). Chlorophyll *a* concentrations increased in this period from below 0.4–2.5 $\mu\text{g L}^{-1}$ (Piquet et al. 2014). However, chl-*a* never reached values corresponding to a full spring bloom peak, which can reach around 12 $\mu\text{g L}^{-1}$ chl-*a* in the Kongsfjorden (Hop et al. 2002; Hodal et al. 2012). High concentrations of NO_x, PO₄, and Si measured in our early April (Piquet et al. 2014) correspond to Kongsfjorden values from March 2006 measured by Rokkan Iversen and Seuthe (2011), when the water column was replenished in nutrients by wintertime deep mixing. Hence, these inorganic nutrient concentrations correspond to a pre-phytoplankton bloom condition of the water column. At the same time, although post-winter water is replenished in nutrients, it may be depleted in bioavailable dissolved organic matter (DOM) required for heterotrophic bacterial growth, as shown by the low proportions of active bacterial cells taking up ³H-leucine in early April.

The class Bacteroidetes is mainly associated with high molecular and complex DOM (Riemann et al. 2000; Comeau et al. 2011) in marine as well as in freshwater environments (Kirchman 2002; Galand et al. 2008). The genus *Roseobacter* (member of the Alphaproteobacteria class) is found to be strongly associated with dimethyl sulfide/dimethylsulfoniopionate originating primarily from *Phaeocystis* sp. (Gonzalez et al. 2000; Ortega-Retuerta et al. 2008). Diatoms and *Phaeocystis* sp. constitute the typical Kongsfjorden spring bloom (Hop et al. 2002; Hodal et al. 2012; Hegseth and Tverberg 2013) and account for the largest fraction of the phytoplankton biomass from mid-April to mid-May (2008) (Piquet et al. 2014). In Arctic waters, DOM produced during the spring bloom is rapidly turned over in the spring–summer period by the microbial community (Osterholz et al. 2014). Dissolved organic compounds produced by diatoms form an important substrate for heterotrophic bacteria (Amin et al. 2012) and lead to a rapid increase in the number of active bacterial cells (Elifantz et al. 2007; Kirchman et al. 2007). Thus, we suggest that the increase in phytoplankton abundance (diatoms and *Phaeocystis* sp.) may have led to the strong increase in activity of Bacteroidetes,

Roseobacter, and other unidentified Bacteria. Increased activity within the bacterial domain could in addition to the *Roseobacter* clade members be caused by unprobed members of the Alphaproteobacteria known to respond to enhanced phytoplankton production (Elifantz et al. 2007). Alphaproteobacteria contributed a large portion (34 ± 19 %) of the Kongsfjorden prokaryotic community in June–July (De Corte et al. 2013). However, we cannot rule out that other groups not assessed here, or even cells from the same phylogenetic groups assessed in this study that are not efficiently hybridized by the available probes (e.g., De Corte et al. 2013) might account for a significant fraction of the active unidentified bacteria.

Prokaryote community dynamics during the late spring to post-spring bloom period

The chemical signature of the water column during late spring (May–June 2007) was indicative of post-bloom conditions (Piquet et al. 2014). The preceding bloom was primarily composed of *Phaeocystis* sp. and lacked the complementary diatoms typical for Kongsfjorden blooms (Hegseth and Tverberg 2013). Pigment analyses of the late spring phytoplankton revealed that they were dominated by pico- to nanoplankton, with a major contribution by haptophyte pigments (*Phaeocystis* sp.). Furthermore, cryptophytes, chlorophytes, and cyanobacterial pigments were found (Piquet et al. 2014). These groups are typical for post-bloom conditions, but are also known to be associated with low salinity caused by glacier meltwater input (Keck et al. 1999; Moline et al. 2004; Piquet et al. 2014). The prokaryotic community was highly active in late spring in both fjords at all locations and depths, and was dominated by active cells (Figs. 2, 3). The SAR86 cluster, belonging to the Gammaproteobacteria, was previously found to be associated with phytoplankton blooms (Gonzalez et al. 2000; Kirchman 2008). Although the abundance of SAR86 was generally too low to be considered responding to the *Phaeocystis* sp. bloom, its increase in single-cell activity coincides with the development of the phytoplankton bloom. Unidentified bacterial cells formed the major fraction of the active bacterial community after the bloom. Thus, further investigation will be essential to determine their identity and function.

The highest prokaryotic abundances were found in late spring under post-bloom conditions. It is expected that heterotrophic bacterial abundance should be controlled by flagellate grazing or viral lysis during this period (De Corte et al. 2011). However, under post-bloom conditions in Kongsfjorden, only a weak link was found between prokaryotic and viral communities, suggesting a limited control by viral lysis (De Corte et al. 2011). Rokkan Iversen and Seuthe (2011) found very low biomass levels

of bacterivorous predators in Kongsfjorden. Therefore, late spring conditions in Kongsfjorden and Krossfjorden may enable heterotrophic Bacteria to contribute significantly to complete remineralization of phototrophically generated biomass.

Environmental and biological control of prokaryotic dynamics

DGGE analysis revealed that the bacterial communities of Kongsfjorden and Krossfjorden differed strongly between the early bloom and the post-bloom period (Fig. 6), coinciding with the shifts in relative abundance and activity of specific bacterial groups as shown by FISH. Coordination analysis of the bacterial community composition also indicated a response to the phytoplankton bloom. Most of the variation between the bacterial assemblages was significantly explained by time. Since we have no long-term information on the microbial community composition and the potential occurrence of natural inter-annual variations, we cannot explain this observation by variations in the environmental parameters that led to variation in phytoplankton composition. Of the tested variables, temperature and salinity varied independently from phytoplankton growth, composition, or production. The factors correlating with the observed variation between the bacterial communities in the different years were phosphate concentration, N:P and Si:N ratios, and pigment abundance of haptophytes, cryptophytes, and cyanobacteria (Fig. 6, left panel). We conclude that differences between bacterial assemblages of the early bloom (early to mid-spring) and the post-bloom (late spring) (Fig. 6, right panel) were primarily related to biological processes associated with phytoplankton growth, and composition. Within the bacterial communities associated with the different bloom phases, we found further separation of the samples according to sampling location in agreement with previous results from spring–summer surface waters (Piquet et al. 2010). The late spring, post-*Phaeocystis* bloom conditions influenced the bacterial community at Station M at Kongsfjorden, which is probably provoked by nitrogen limitation as can be judged from the positive correlation with the Si:N ratio and negative correlation with N:P ratios. In contrast, Krossfjorden stations correlated positively with the presence of diatoms and chl-*a* concentration. Late June samples (year 2007) also showed an association with temperature. This is in agreement with hydrographic conditions measured in both fjords from 15 to the end of June; salinities dropped significantly in surface waters and resulted in a solar heating of the upper water column (Piquet et al. 2014). Surface salinity data indicated that substantial glacier meltwater influx started around June 7 from the Krossfjorden glacier and at June 14 from the

Kongsfjorden glacier. In the following sampling days, the effects of glacier meltwater extended to all other stations, which all experienced reduced salinities and increased temperatures. There was a response of the bacterial communities as shown by the meltwater influenced cluster, in which temperature explained most of the variation.

Dynamics in springtime bacterial communities only revealed minor shaping effects by glacier meltwater in late spring–early summer. When both spring periods were clustered, we found no clear effects of environmental variables related to glacier melting. Possibly the shaping power of glacier meltwater, as expressed by salinity, was subtle in spring, in comparison with the shaping power of variables related to phytoplankton, sample location, and temperature. During the late spring to early summer period of the year 2005, at the onset of the glacier melt period, bacteria identified as Betaproteobacteria, typically found in freshwater, were retrieved from Kongsfjorden surface waters (Piquet et al. 2010). This suggested that glacier meltwater was feeding the Kongsfjorden system with bacteria known to be associated with freshwater in temperate and cold water systems (Kirchman et al. 2005; Galand et al. 2008). In the Canadian Arctic, Betaproteobacteria abundance decreased with distance from the freshwater source (Garneau et al. 2005). We expected to find higher relative abundances of freshwater bacteria in surface samples at the near-glacier stations. However, in the present study, Betaproteobacteria abundances and activity were low in all our samples, showing no trend between sampling locations or period. This provides additional support for the limited shaping effect by glacier meltwater, neither through reduced salinity nor through the input of non-marine bacterial strains, at least in the spring season.

Dynamics in archaeal communities

In early and late spring, 25 and 13 % of DAPI-stained cells, respectively, were not bacteria as revealed by CARD-FISH. It would be tempting to conclude that these percentages represent the archaeal fraction of the prokaryotic community. However, it is likely that not all cells are hybridizable for a variety of reasons, including inefficient permeabilization of cell walls (Teira et al. 2004) and incomplete coverage of target organisms by the probe. Thus, this unhybridized DAPI-stained cells might be bacteria as well as archaea. Archaea are known to contribute a small but significant fraction of the prokaryotic community in Arctic and Antarctic wintertime surface waters, decreasing in relative abundance toward summer (Murray et al. 1998; Alonso-Sáez et al. 2008, 2012). Archaea only contributed 1.6 % of the Kongsfjorden prokaryotic community in the spring–summer period (De Corte et al. 2011). As we collected samples earlier in spring, when the water

was in a pre-bloom state and resembled wintertime water column structure and nutrient state, we speculated that we would encounter a higher relative archaeal abundance than reported by De Corte et al. (2013). Archaeal communities of Kongsfjorden and Krossfjorden showed a lower variability compared to the bacterial community as revealed by the high similarity (>40 %) shared between archaeal fingerprints (compared to <25 % for bacteria) from all stations, locations, times, and depths. A decrease in archaeal diversity might be expected in response to increased phytoplankton and bacterial activity. However, the archaeal community remained highly diverse and similar in all samples. Only in late June (year 2007) when salinity was low due to glacier meltwater inflow, the archaeal community was less diverse forming a separate cluster in Pearson's similarity analysis.

CCA of the archaeal communities also revealed the temporal separation between the two spring periods and the sampling sites (Fig. 7, right panel). Sites within both fjords (M&G, KM&KG) overlap in community composition while the community at the Ocean (O) station is closely related to that present at Kongsfjorden stations. Biotic factors such as phytoplankton abundance did not significantly contribute to the observed variance, leaving the abiotic variables as main drivers of archaeal community composition (Fig. 7, left panel). Taken together, in spring, Kongsfjorden and Krossfjorden harbor a stable and resilient archaeal community with little variation in time and space. The variation found in the archaeal community is apparently caused by the influence of glacier meltwater varying among different fjords.

Our data suggest that if intrusions of Atlantic water and glacier meltwater inflow become more frequent and more intense in the future, shifts in archaeal communities and a generally lower contribution of archaea to the prokaryotic community can be expected.

Conclusion

Despite similar exposure to glacier meltwater, on the one hand, and warm Atlantic marine water, on the other hand, Kongsfjorden and Krossfjorden differ in hydrographic conditions and were differentially affected by the contrasting gradients. Archaeal communities were affected only marginally by altered hydrographic conditions, mostly caused by differences in water mass origin. Both archaeal and bacterial communities clustered according to seasonal and spatial factors. However, whereas bacterial communities appear to be indirectly influenced by abiotic factors through the phytoplankton composition, the archaeal community appears directly influenced by abiotic factors. Springtime bacterial communities changed in composition

and activity due to altered hydrographic conditions and glacier meltwater input, which, in turn, provoked changes in phytoplankton assemblages. Consequently, climate-driven changes might affect prokaryotic communities of the fjords differentially, with more alterations expected for Kongsfjorden bacterial and archaeal communities. In addition, future studies should also consider the sediment microbial communities of both fjords, which have been reported to contain a rich microbial diversity (Tian et al. 2009). To better understand the dynamics of polar ecosystems, long-time monitoring is essential. A multi-disciplinary approach that includes broad-scale, year-round sampling of multiple stations and depths of physical and biogeochemical parameters and metabolic rate measurements combined with state-of-the-art -omics might help to better understand the dynamics of coastal Arctic systems and, based on this knowledge, forecast their future developments.

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