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Bacterioplankton community structure in the Arctic waters as revealed by pyrosequencing of 16S rRNA genes

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Abstract Fjords and open oceans are two typical marine ecosystems in the Arctic region, where glacial meltwater and sea ice meltwater have great effects on the bacterioplankton community structure during the summer season. This study aimed to determine the differences in bacterioplankton communities between these two ecosystems in the Arctic region. We conducted a detailed census of microbial communities in Kongsfjorden (Spitsbergen) and the Chukchi Borderland using high-throughput pyrosequencing of the 16S rRNA gene. *Gammaproteobacteria* and *Bacteroidetes* were the dominant members of the bacterioplankton community in Kongsfjorden. By contrast, the most abundant bacterial groups in the surface seawater samples from the Chukchi Borderland were *Alphaproteobacteria* and *Actinobacteria*. Differences in bacterial communities were found between the surface and subsurface waters in the investigation area of the Chukchi Borderland, and significant differences in bacterial community structure were also observed in the subsurface water between the shelf and deep basin areas. These results suggest the effect of hydrogeographic conditions on bacterial communities. Ubiquitous phylotypes found in all the investigated samples belonged to a few bacterial groups that dominate marine bacterioplankton communities. The sequence data suggested that changes in environmental conditions result in abundant rare phylotypes and reduced amounts of other phylotypes.

Keywords Bacterioplankton community · Pyrosequencing · Kongsfjorden · Chukchi Borderland

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Introduction

Planktonic marine bacteria are abundant (typically 10^8–10^9 cells l^{-1}) in the Arctic region (Jiang et al. 2005; Kirchman et al. 2007; Sala et al. 2010). These microorganisms, which are an important food source in marine food webs, play crucial roles in organic matter cycling (Azam et al. 1983; Naganuma et al. 2006). It is important to determine which bacterial groups dominate marine bacterioplankton because abundant groups may be proportionally more influential in carbon cycling and other biogeochemical

processes (Cottrell and Kirchman 2000a). Understanding why particular bacteria dominate microbial communities is a fundamental ecological question (Cottrell and Kirchman 2000a). Culture-independent studies are essential in determining the biodiversity of marine bacterial communities, because only a small fraction of naturally-occurring bacterial assemblages can be cultured with currently available methods (Amann et al. 1995). The 16S rRNA gene is an excellent phylogenetic marker that can be used in identifying which bacterial taxa are present in a given sample (Pace 1997). Several groups have studied the 16S rRNA gene sequences in marine bacterioplankton communities using Sanger sequencing; they also revealed that *Gammaproteobacteria*, *Alphaproteobacteria* and *Bacteroidetes* dominate the bacterioplankton communities in the Arctic waters (Alonso-Sáez et al. 2008; Bano and Hollibaugh 2002; Garneau et al. 2006; Malmstrom et al. 2007; Zeng et al. 2009) .

However, descriptions of most marine bacterioplankton communities remain incomplete because traditional culture-independent molecular identification methods have high cost and low throughput, thereby rendering many taxa, especially rare marine bacterial taxa, undetectable. The possible key species that may be present at low or high numbers but perform indispensable functions for the community should be identified clearly in order to obtain a more detailed description of marine bacterioplankton community structure (Hunter-Cevera et al. 2005). Organisms mustering 0.1 % prevalence, including nitrogen fixers, are of great importance in the functioning of the community (Hunter-Cevera et al. 2005). Recently, massively parallel pyrosequencing technologies, such as the Roche 454 GS FLX, have been developed. These technologies enable metagenomic and metagenetic analyses in a manner that exceeds the capacity of traditional Sanger sequencing-based approaches by several orders of magnitude, with the possibility of detecting even very rare phylotypes (Tedersoo et al. 2010). Pyrosequencing has been used to enumerate and contrast marine microbial diversity in the Arctic waters (Bowman et al. 2012; Comeau et al. 2011; Kirchman et al. 2010). Studies have shown that the abundant, large phylogenetic groups represented in the pyrosequence data are generally similar to those reported by previous studies in the Arctic (Alonso-Sáez et al. 2008; Bano and Hollibaugh 2002; Garneau et al. 2006; Malmstrom et al. 2007; Zeng et al. 2009).

The heterogeneous nature of high-latitude seas (Carmack and Wassmann 2008) implies that the structure and function of marine ecosystems may differ between geographical areas throughout the pan-Arctic region. As an example of typical Arctic marine ecosystems, Kongsfjorden and the Chukchi Borderland represent a glacial meltwater-influenced fjord ecosystem and a sea ice-associated pelagic ecosystem, respectively (Coupel et al. 2012; Svendsen et al. 2002). Kongsfjorden is an Arctic fjord on the western side of Spitsbergen (Fig. S1; Jones 2001). It is influenced by mild temperatures mediated by the inflow of transformed Atlantic water as well as by meltwater of glacial origin. The input of sediment-rich glacial meltwater leads to decreased salinity, increased turbidity and decreased light penetration in the fjord during summer (Piquet et al. 2010). Mixing of warm Atlantic water with glacial freshwater and enhanced sediment concentration are important determinants for bacterioplankton growth and species composition of West Spitsbergen fjords. In contrast, about 600 miles north of the Bering Strait, the Chukchi Borderland is at the entrance to the Arctic Ocean (Fig. S1; Jones 2001) and is known as a complex area of tortuous topography in the northern Chukchi Sea (Hall 1990). Waters from the Pacific and the Atlantic meet and interact in this adjoining region of ridges and deep-sea plateaus. The Atlantic waters are warmer, saltier and deeper. They have made their way anticlockwise around the Arctic Ocean, hugging the continental slope (http://psc.apl.washington.edu/HLD/CBL/CBL.html). The Pacific waters flowing northward through the Bering Strait are colder and fresher and carry a rich nutrient load (Cooper et al. 1997). Similar to the northern Bering Sea, sea ice cover is one of the several physico-chemical properties that vary dramatically with the seasons in this area. Seasonal ice melt could induce changes both in the community structure and physiological activity of bacterioplankton in the underlying seawater (Grzymski and Murrsay 2007; Sala et al. 2010). In the current study, we employed 454 pyrosequencing of the 16S rRNA gene to enumerate and contrast marine bacterial diversity in the two Arctic regions, which exhibit distinct hydrogeographic characteristics at a greater resolution than previous studies. We used the community structure data to examine differences in bacterial content between the surface seawater (10 m depth) strongly influenced by sea ice melting and the subsurface seawater (80–100 m depth)

in the Chukchi Borderland. We also explored changes in the abundance and rarity of the bacterial phylotypes in the investigated Arctic regions.

Materials and methods

Sampling and bacterial counts

The locations, sample dates, and biogeochemical properties of the samples analyzed in this study are summarized in Table 1 and Fig. S1 (Jones 2001). Water samples in the Chukchi Borderland were collected using a conductivity temperature depth (CTD)-rosette system during the first Korean Arctic cruise. Seawater in Kongsfjorden was sampled at a depth of approximately 1 m from the top water layer. Microorganisms present in the sample were collected by filtration of 1.5–2 l of water onto 0.2 μm pore-sized Nuclepore filters (Whatman). Bacterial abundance was determined using the 4′,6-diamidino-2-phenylindole (DAPI) staining protocol (Porter and Feig 1980).

DNA extraction, amplification and sequencing

The DNA extraction method was previously described by Bosshard et al. (2000) and Bano and Hollibaugh (2000). The V1–V3 region of the 16S rRNA gene was amplified using the forward primer 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer 533R (5′-TTACCGCGGCTGCTGGCAC-3′) and pyrosequenced using a Roche 454 Genome Sequencer FLX Titanium platform at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China, as previously described (Wu et al. 2012).

Sequence analyses

Previous studies described sources of errors in 454 sequencing runs. Hence, the valid reads should comply with the following rules: each pyrosequencing read containing a primer sequence should be ≥150 bp in length, must have no ambiguous bases, must match the primer and one of the used barcode sequences, and should present at least a 75 % match to a previously determined 16S rRNA gene sequence. Under these conditions, 10,281 of 59,326 unique tags were rejected and were excluded from classification. Tag sequences found in this study were deposited at the European

Table 1 Summary of samples for community structure

Sample	Sampling area	Date	Sampling depth (m)	Latitude	Longitude	Temperature (°C)	Salinity (psu)	δ_t (kg m^{-3})	Chlorophyll (mg m^{-3})	DO (mg l^{-1})	Prokaryotes (10^8 cells l^{-1})
K2s	Chukchi Borderland	2010-07-22	10	73°30.71N	166°59.25W	−1.4	28.6	23.2	0.5	13.4	5.3
K2b	Chukchi Borderland	2010-07-22	80	73°30.71N	166°59.25W	−1.6	32.6	26.4	0.6	10.2	14.2
K5s	Chukchi Borderland	2010-08-05	10	76°0.14N	159°0.8W	−1.2	25.6	20.8	0.2	12.4	4.9
K5b	Chukchi Borderland	2010-08-05	100	76°0.14N	159°0.8W	−1	32.1	25.8	0.1	10.3	4.0
St.1	Kongsfjorden, Spitzbergen	2011-07-11	1	78°59.28N	11°39.56E	6.3	32.1	25.2	0.2	ND	18.4
St.5	Kongsfjorden, Spitzbergen	2011-07-06	1	78°54.33N	12°17.57E	5.0	31.8	25.1	0.7	ND	32.6

ND no data

Nucleotide Archive (ENA) under the accession number ERP001390 (https://www.ebi.ac.uk/ena/data/view/ERP001390).

Diversity and community structure analyses

Analysis was conducted using the microbial ecology community software program Mothur (Schloss et al. 2009). Sequences were aligned and compared with the Bacterial SILVA database (SILVA version 106; http://www.arb-silva.de/documentation/backgroud/release-106/). Representative sequences for shared operational taxonomic units (OTUs) as defined by 100 % (unique), 97, 95, and 90 % similarities were obtained. Rarefaction analysis and Good's coverage for the six libraries were also determined. Heatmap figures and Venn diagrams were generated using custom Perl scripts. A canonical correspondence analysis (CCA) was performed to analyze the variation of communities in the two Arctic locations and their relationships with environmental variables using Canoco 4.5 (ter Braak and Šmilauer 2002).

Results

We examined 16S rRNA gene fragments ("tags") from microbial communities in surface (1–10 m depth) and subsurface (80–100 m depth) waters of various Arctic regions in the summer season (Table 1). A total of 49,045 valid reads and 1,955 OTUs (at the 97 % level, corresponding to taxonomically valid species) were obtained from the 6 samples through 454 pyrosequencing analysis. An average of 8,174 tags was obtained for each bacterial community (Table 2). The number of different bacterial sequences ("ribotypes") decreased substantially when placed into groups that shared ≥97, ≥95, or ≥90 % similarity. Good's coverage estimations revealed that 96.3–97.8 % of the species (at the 97 % level) were obtained in all of the samples. However, rarefaction curves suggest that the sequencing effort was not large enough to capture the complete diversity of these communities, specifically because the curves did not level off (the slope did not go to zero) with increasing sample size (Fig. S2).

Taxonomic composition

All sequences were classified from phylum to genus according to the Mothur program using the default setting. These sequences/OTUs were assigned to 23 different phyla or groups. The two fjord seawater libraries (St.1 and St.5) showed similar taxonomic compositions. However, the four pelagic libraries from the Chukchi Borderland showed dissimilar 16S rRNA profiles in terms of phylum level distributions (Fig. 1). A total of 9 phyla were determined from bacterial communities in Kongsfjorden, whereas 11–19 phyla were found in the samples from the Chukchi Borderland. The K5b (pelagic subsurface water) library included the maximum number of phyla (19), where *Proteobacteria*, *Actinobacteria*, and *Planctomycetes* were the most important groups, accounting for 81.59 % of the reads. The K5s (surface water at the same station; 15 phyla) library was numerically dominated by *Proteobacteria*, *Actinobacteria*, *Cyanobacteria* and *Bacteroidetes*, representing 97.67 % of the reads. The libraries from station K2 in the Chukchi Borderland showed relatively simple diversity at the phylum level, with 13 phyla and 11 phyla for K2s and K2b, respectively. *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* represented 92.57 % of the reads in the K2s (surface water) library, whereas *Proteobacteria*, *Cyanobacteria*, *Bacteroidetes*, and *Actinobacteria* accounted for 98.56 % of the reads in the K2b (subsurface water) library. The K2b library also contained the highest percentage of *Cyanobacteria* (37.64 %) and the lowest number of phyla (11) among the four pelagic libraries from the Chukchi Borderland. *Proteobacteria* and *Bacteroidetes* dominated the two fjord seawater libraries, accounting for 99.65 and 98.9 % of the reads in the St.1 and St.5 libraries, respectively.

Proteobacteria (31.72–86.87 %) was the most abundant phylum across all the investigated samples.

Table 2 Summary of sequence numbers for the 6 bacterial communities in the Arctic waters

Category	Average	SD
Read length	426	84.9
Total tags per sample	8,174	662
Different sequences per sample	996	299
Coverage	0.972	0.005
≥97 % clusters per sample[a]	469	159
≥95 % clusters per sample[a]	326	127
≥90 % clusters per sample[a]	199	94

[a] Groups formed by sequences sharing ≥97, ≥95, or ≥ 90 % similarity

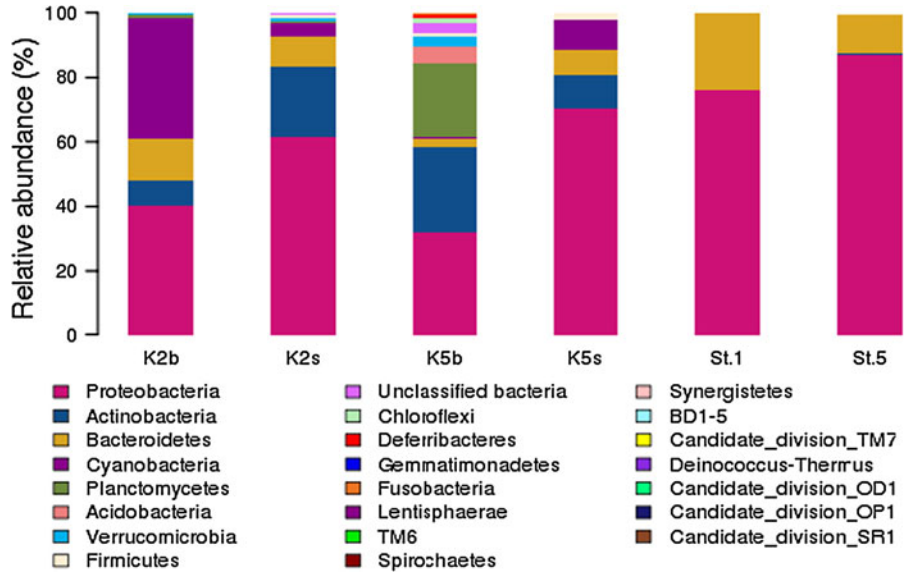

Fig. 1 Bacterial composition of the different communities. Relative read abundance of different bacterial phyla within the different communities

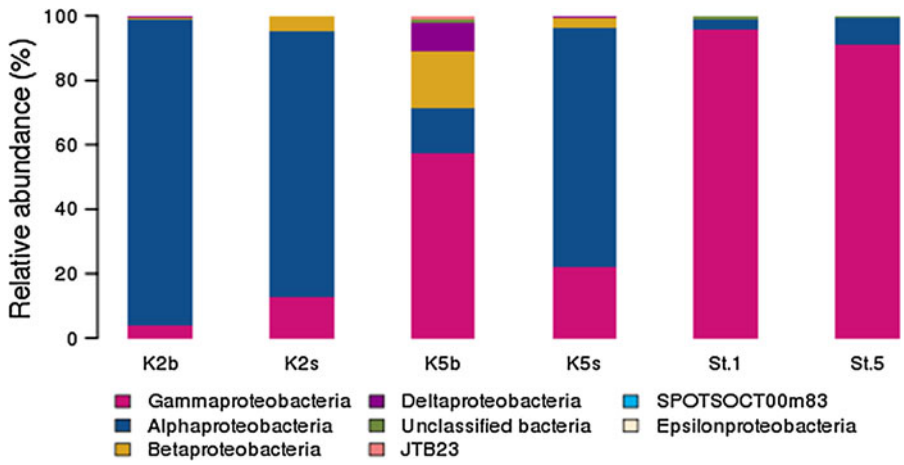

Fig. 2 Composition of Proteobacteria in the different communities. Relative read abundance of different Proteobacteria classes within the different communities

However, distributions among the proteobacterial classes exhibited differences among the 4 pelagic water samples (K2s, K2b, K5s, and K5b), as well as between the pelagic and fjord waters (St.1 and St.5) (Fig. 2). *Alphaproteobacteria* was the most abundant group in K2b, K2s and K5s, accounting for 94.95, 82.51 and 74.22 % of the *Proteobacteria*-related reads, respectively. By contrast, *Gammaproteobacteria* was the most important group in St.1, St.5 and K5b, accounting for 95.7, 91.09 and 57.6 %, respectively. *Gammaproteobacteria* was the second most abundant group in K2b (3.87 %), K2s (12.68 %) and K5s (21.83 %), whereas *Alphaproteobacteria* was the second most abundant group in both fjord libraries (3.17–8.19 %). *Betaproteobacteria* was the second most important group in K5b (17.6 %) and *Deltaproteobacteria* was exclusively abundant in K5b (9.04 %).

The 10 most abundant OTUs within the different samples were determined to further understand the important bacteria (Fig. 3). The most abundant OTUs associated with the K2s library were the sequences related to *Rhodobacteraceae* (39.39 %; including *Sulfitobacter* and *Octadecabacter*), *Actinobacteria* (15.18 %; including *Actinomycetales* and *Acidimicrobiaceae*) and *Cyanobacteria* (2.40 %). The K2b library was dominated

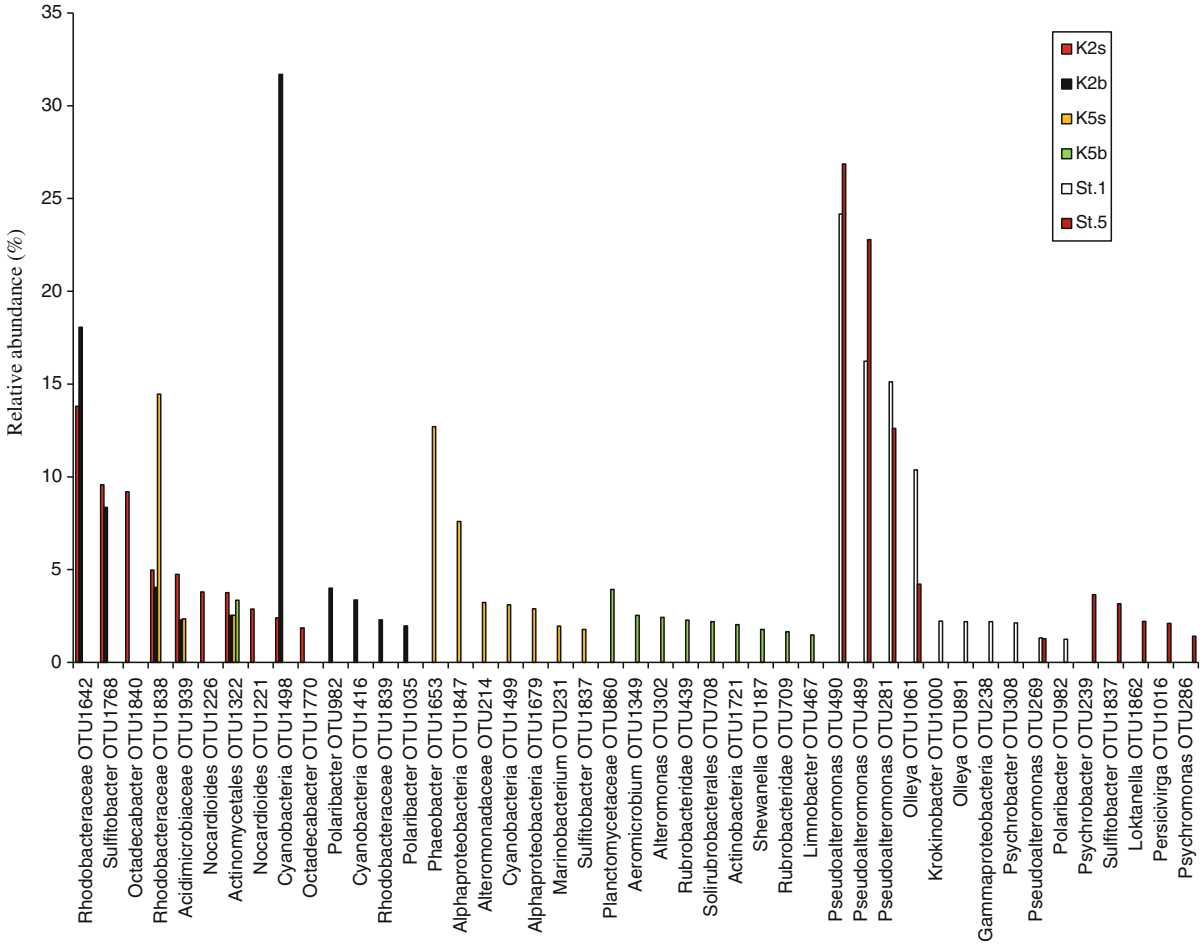

Fig. 3 The 10 most abundant bacterial OTUs in different samples. OTUs were identified using 97 % cutoffs

by *Cyanobacteria* (35.05 %), *Rhodobacteraceae* (32.74 %; including *Sulfitobacter*), *Actinobacteria* (4.82 %; including *Actinomycetales* and *Acidimicrobiaceae*), and *Polaribacter* (5.97 %). The K5s library was dominated by sequences related to *Rhodobacteraceae* (28.95 %; including *Phaeobacter* and *Sulfitobacter*), unclassified *Alphaproteobacteria* (10.48 %), *Alteromonadaceae* (5.17 %; including *Marinobacterium*), *Cyanobacteria* (3.11 %), *Actinomycetales* (2.54 %) and *Acidimicrobiaceae* (2.34 %). The most abundant sequences in the K5b library were those related to *Planctomycetaceae* (3.93 %), *Actinobacteria* (14.05 %; including *Actinomycetales*, *Rubrobacteridae* and *Solirubrobacterales*), *Alteromonadales* (4.2 %; including *Alteromonas* and *Shewanella*) and *Limnobacter* (1.47 %). *Rhodobacteraceae* OTU1838 and *Acidimicrobiaceae* OTU1939 were common in K2s, K2b and K5s, whereas *Actinomycetales* OTU1322 was observed in all libraries from the Chukchi Borderland.

Compared with the libraries from the Arctic pelagic waters, the two fjord libraries contained different dominant OTUs. The St.1 library was dominated by *Gammaproteobacteria* (61.15 %; including *Pseudoalteromonas* and *Psychrobacter*) and *Flavobacteriaceae* (16.04 %; including *Olleya*, *Krokinobacter* and *Polaribacter*). The St.5 library was numerically dominated by sequences related to *Gammaproteobacteria* (68.59 %; including *Pseudoalteromonas*, *Psychrobacter* and *Psychromonas*), *Flavobacteriaceae* (6.32 %; including *Olleya* and *Persicivirga*), and *Rhodobacteraceae* (5.37 %; including *Sulfitobacter* and *Loktanella*). In addition, two fjord libraries shared most of the dominant OTUs, including *Pseudoalteromonas* OTUs 490, 489, 281 and 269, as well as *Olleya*

OTU1061. In addition, two dominant OTUs were found in both pelagic and fjord libraries, including *Polaribacter* OTU982 in K2b and St.1, as well as *Sulfitobacter* OTU1837 in K5s and St.5.

Bacterial community comparison

Community richness was higher in surface waters of the investigated Chukchi Borderland than Kongsfjorden for all definitions of OTU (Table S1). In addition, the pelagic community in surface water appeared more diverse than the fjord community by both Shannon's index of diversity and Simpson's index. However, compared with the other 3 pelagic samples, subsurface water sample K2b from the Chukchi Borderland showed similar richness and diversity to the 2 fjord samples for all definitions of OTU.

Hierarchically clustered heatmap analysis based on the bacterial community profiles revealed that the 2 fjord samples formed a cluster separated from the cluster consisting of 4 pelagic samples (Fig. S3). In addition, pelagic samples K2s and K2b were grouped together first and then clustered with K5s and K5b.

CCA was used to perform ordination in order to analyze the variation of communities in the two Arctic locations at the species level (97 % similarity) as well as their relationships with environmental variables. Consistent with the hierarchically clustered heatmap analysis, CCA (Fig. 4) exhibited that the bacterial community compositions in the fjord samples (St.1 and St.5) were significantly different from the open ocean samples. In addition, subsurface water sample K5b from the Chukchi Borderland showed a significantly different phylogenetic community composition from the other surface and subsurface water samples collected from the same area. In the CCA ordination, the eigenvalues for axes 1 and 2 were 0.874 and 0.664, respectively. CCA suggested that bacterial communities sampled from Kongsfjorden were significantly correlated with temperature and bacterial abundance in seawater.

The species shared among these communities were determined through a Venn diagram at a 3 % distance level to compare the relationships among these communities in detail (Fig. S4). The number of species shared between the K2s and K2b communities was 161. That is, 56.6 % of the OTUs in the subsurface water K2b library were present in the surface water K2s library. The most abundant OTUs shared by the two

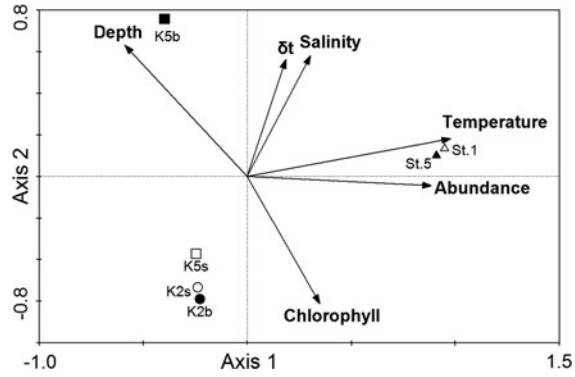

Fig. 4 CCA ordination diagram showing 6 samples collected from two Arctic locations with respect to environmental variables

libraries belonged to *Sulfitobacter*, *Rhodobacteraceae*, *Actinomycetales*, *Acidimicrobiaceae*, *Polaribacter* and *Cyanobacteria*. At station K5, 21.7 % of the OTUs in the subsurface water K5b library were present in the surface water K5s library. OTUs belonging to *Pelagibacter*, *Aeromicrobium*, *Actinomycetales*, *Alteromonas*, and *Alteromonadaceae* were common in both communities. In addition, 34.1 % of the OTUs in the K2s library were shared by the K5s library, whereas 29.9 % of the OTUs in the K2b library were shared by the K5b library. The most abundant OTUs shared by the K2s and K5s libraries were clustered within *Octadecabacter*, *Rhodobacteraceae*, *Balneatrix*, *Alteromonadaceae*, *Actinomycetales*, *Acidimicrobiaceae* and *Cyanobacteria*. In the K2b and K5b libraries, the most abundant OTU belonged to *Actinomycetales*. A total of 49 common species were observed in all the Chukchi Borderland samples, including the dominant *Actinomycetales* OTU1322. The St.1 library shared 47.9 % of the OTUs with the St.5 library, which was dominated by *Pseudoalteromonas* and *Olleya* (Fig. 3). The Kongsfjorden and the Chukchi Borderland shared a total of 67 OTUs affiliated to the genera *Loktanella*, *Octadecabacter*, *Phaeobacter* and *Sulfitobacter* within the *Alphaproteobacteria*, the family *Comamonadaceae* within the *Betaproteobacteria*, the genera *Alteromonas*, *Glaciecola*, *Methylophaga*, *Pseudoalteromonas* and *Pseudomonas* within the *Gammaproteobacteria*, the genera of *Flavobacterium*, *Maribacter*, *Olleya*, *Polaribacter*, *Porphyromonas*, *Psychroserpens*, *Ulvibacter* and *Winogradskyella* within the Bacteroidetes, the genera *Nocardioides* and *Rhodoglobus* within the Actinobacteria, and the genus *Roseibacillus* within the

Table 3 Shared species found in all 6 Arctic seawater samples

Species (phylum)	Shared reads					
	K2s	K2b	K5s	K5b	St.1	St.5
Alteromonadaceae OTU214 (Gammaproteobacteria)	132	6	249	40	3	3
Pseudoalteromonas OTU281 (Gammaproteobacteria)	3	3	3	1	1,156	963
Microbacteriaceae OTU1326 (Actinobacteria)	112	2	40	7	6	31
Microbacteriaceae OTU1331 (Actinobacteria)	20	6	87	12	1	2
Acidimicrobiaceae OTU1939 (Actinobacteria)	409	145	181	24	2	1
Polaribacter OTU982 (Bacteroidetes)	129	253	56	1	96	14
Rhodobacteraceae OTU1642 (Proteobacteria)	1,187	1,142	7	2	2	6
Sulfitobacter OTU1768 (Alphaproteobacteria)	823	528	27	3	6	11
Octadecabacter OTU1840 (Alphaproteobacteria)	791	41	73	1	1	1

Verrucomicrobia. Among those OTUs, 9 OTUs were common in all the 6 investigated samples (Table 3), accounting for 19.0 % of the total pyrosequencing reads.

Discussion

Compared with pelagic Chukchi Borderland samples K2s and K5s, the Kongsfjorden samples had higher cell abundance (Table 1) but a lower degree of bacterial diversity and richness (Figs. 1, 2; Table S1). Similar results were also observed in the Chukchi Borderland sample K2b, suggesting a link between high bacterial abundance and relatively low bacterial diversity in marine environments. The high bacterial abundance in Kongsfjorden can be attributed to the increase in temperature and the freshwater input from the melting glaciers which consequently influences critical hydrological properties with important biological implications and contributes terrestrial nutrients and microorganisms to the fjord (Hop et al. 2002; Weslawski et al. 2000). In addition, the low diversity found (Table S1) in the coastal samples is probably due to the proliferation of specific bacterial groups associated with release of dissolved organic matter after a phytoplankton bloom (De Corte et al. 2011, 2013). Compared with the entrance station St.1, the inner station St.5 in Kongsfjorden showed lower salinity but higher bacterial abundance (Table 1), indicating the influence of freshwater input on marine ecosystems in the fjord. Known for their ability to degrade organic matter (Battin et al. 2001; Cottrell and Kirchman 2000b; Kirchman 2002; Riemann et al. 2000), *Gammaproteobacteria* and *Bacteroidetes* were the most dominant bacterial groups, accounting for 96 and 91 % of the reads in St.1 and St.5 libraries, respectively. This result suggests the existence of rich nutrients suitable for heterotrophic bacterial growth in the seawater. OTUs belonging to the genera *Pseudoalteromonas* and *Olleya* represented more than 69 and 67 % of the reads in the St.1 and St.5 libraries, respectively. These data demonstrate a link between the preponderance of special phylotypes and relatively low diversity in marine bacterioplankton. The dominant *Pseudoalteromonas* and *Olleya* OTUs showed significant sequence similarity (values ranging from 99.1 to 100 %) to clones or strains from marine environments, including sea ice, seawater and sediment (as indicated by GenBank descriptions). In addition, an unexpected difference in bacterioplankton community composition in Kongsfjorden was observed between the data of the current and those of previous studies (Zeng et al. 2009). This finding revealed that *Alphaproteobacteria* and algae (chloroplasts) constituted two dominant fractions in surface water. The bacterial community in Kongsfjorden varies over time and appears to be correlated with the inflow of glacial meltwater and phytoplankton bloom (Piquet et al. 2010).

Compared with the fjord bacterial community, the pelagic bacterial community revealed strong variations in community composition. This means that *Alphaproteobacteria* and *Actinobacteria* accounted for 72 and 62 % of the reads in the K2s and K5s libraries, respectively. This result indicated that the

differences in bacterial community composition can be associated with hydrogeographic conditions (Figs. S1, S5; Jones 2001). The SAR11 clade and the *Rhodobacterales* of *Alphaproteobacteria* can represent as much as 30–50 % of bacteria in the ocean surface waters (Giovannoni et al. 2005). A previous study reported that *Alphaproteobacteria* is dominated by the SAR11 clade in the western Arctic Ocean (Kirchman et al. 2010). By contrast, in our work *Rhodobacteraceae* accounted for 46.7–34.4 % of the reads in the K2s and K5s libraries, respectively, whereas the SAR11 clade represented only 0.9–15.4 % of the reads in the two surface water libraries. This discrepancy reflects the changes over time in the bacterial communities associated with hydrographic parameters and nutrients in seawater. Given the limitations of high-throughput sequencing (Jones 2010) and various rRNA operon copy numbers per bacterial genome (Klappenbach et al. 2000), abundances estimated from pyrosequencing data should be confirmed by quantitative methods such as fluorescence in situ hybridization (Kirchman et al. 2010). Differences in relative abundance determined by FISH and clone library have been reported in the surface waters of the Chukchi Sea (Malmstrom et al. 2007). As determined by FISH, the well-known cosmopolitan SAR11 clade makes up 25 % of the prokaryotic communities. However, this clade only represents 7 % of the clone sequences in the clone library.

At the two pelagic stations (i.e., K2 and K5) the percentage of *Proteobacteria* in the bacterioplankton community decreased with increasing water depth (Fig. 1). However, this should be further confirmed. K2 also showed similar community composition between the surface and subsurface waters. However, station K5 showed a distinct community composition between the surface and subsurface waters (Figs. 1, 2). In addition, sample K5b formed a separate branch in the cluster generated by the 4 pelagic samples (Fig. S3). Salinity values (Table 1) indicated that the surface water in the Chukchi Borderland was more significantly influenced by sea ice melting compared with the subsurface water at a depth of 80–100 m. Complete sea ice melting can dilute the concentration of surface water nutrients (Gradinger 2009), thereby affecting algal and bacterial growth in seawater. Station K2 (143 m depth) is located on the Chukchi shelf. The upper waters of the Chukchi Borderland are primarily influenced by nutrient-rich Pacific waters entering through the Bering Strait (Rella and Uchid 2011), thus, this may contribute to a higher bacterial abundance (Table 1) but lower species richness and diversity (Table S1) in sample K2b than other samples in the same region. By contrast, station K5 (2,543 m depth) is located in the deep Amerasian Basin, where waters from the Pacific and Atlantic Oceans meet and interact (Rella and Uchid 2011). Among the 10 most abundant bacterial phylotypes in pelagic samples K2s, K2b, and K5s, *Rhodobacteraceae* OTU1838, *Acidimicrobiaceae* OTU1939 and *Actinomycetales* OTU1322 were the most common (Fig. 3), sharing 98.5–99.7 % sequence similarity to the bacterial clones in the northern Bering Sea (Zeng et al. 2012), indicating the influence of Pacific-derived waters on bacterial community composition in the upper waters of the Chukchi Borderland. Members of *Planctomycetes*, *Verrucomicrobia*, *Acidobacteria*, *Chloroflexi* and *Deferribacteres*, widely distributed in marine environments, were exclusively abundant in sample K5b. The distinct bacterial community composition of sample K5b may thus reflect the additional influence of Atlantic-derived waters on the microbial community in deeper waters of the Chukchi Borderland.

The Chukchi Borderland and Kongsfjorden represent two typical Arctic marine ecosystems, namely, pelagic and fjord. The surface water in these two regions is significantly influenced by sea ice melting or freshwater input in the summer season. Distinct bacterioplankton community compositions have been observed between these two regions in this study, suggesting that the two communities in coastal and open ocean areas are influenced by different controlling factors. Bacterioplankton community in open ocean can be influenced by hydrographic conditions, while coastal bacterioplankton community can be influenced by temperature and nutrients. At the same time, ubiquitous phylotypes were observed in all the investigated samples (Table 3). They all belonged to a few abundant bacterial groups dominating marine bacterioplankton communities, including *Rhodobacteraceae*, *Bacteroidetes* and *Actinobacteria*. However, the abundance of these phylotypes changed dramatically in the Arctic regions examined. For example, *Acidimicrobiaceae* OTU1939 was abundant in the Chukchi Borderland, but it became a rare phylotype in Kongsfjorden. By contrast, the rare phylotype *Pseudoalteromonas* OTU281 in the Chukchi Borderland was the most abundant phylotype in Kongsfjorden.

The sequence data in this study support the "seed bank" hypothesis (Pedrós-Alió 2006), which states that rare but cosmopolitan phylotypes may serve as a reservoir of phylotypes that can initiate the development of new communities when environmental conditions change. Moreover, the current data argue against the previous report (Kirchman et al. 2010), which states that the abundance and rarity of phylotypes in the Arctic regions do not change.

High-throughput pyrosequencing has been used to examine marine bacterial communities in the polar region (Bowman et al. 2012; Comeau et al. 2011; Kirchman et al. 2010). Compared with the results (three to four phyla) based on Sanger sequencing-based analysis of 16S rRNA gene clone libraries (Zeng et al. 2013), higher diversity at the phylum level was detected in the surface water of the Chukchi Borderland in this current study, although both approaches revealed a similar composition of the major bacterial groups. However, defining a species with 3 % dissimilarity in the comparison of 16S rRNA gene sequences (Stackebrandt and Goebel 1994), the sequencing effort in this study was not large enough to capture the complete diversity of these communities. Due to the low numbers of samples that were processed in the current study, especially in the Chukchi Borderland, more data using this approach should be gathered to yield more insights into the structure of oceanic bacterial communities. In addition, large-scale sequencing of 16S rRNA in various marine environments can lead to intriguing observations that encourage future analyses.

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