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ORIGINAL PAPER

Water masses influence bacterioplankton community structure in summer Kongsfjorden

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Abstract

To ascertain the saying "Everything is everywhere, but the environment selects", it was imperative to find out the main factor influencing bacterioplankton composition at genus level of Kongsfjorden where was influenced both by glacier melting water and Atlantic water. Thus, bacterioplankton diversity was investigated using pyrosequencing. In addition, nutrients, chlorophyll *a*, in situ temperature and salinity were measured. There were seventeen of 33 identified genera with relative abundance > 0.1%. Redundancy analysis showed that 73.02% of bacterioplankton community variance could be explained by environmental parameters. Furthermore, most of the abundant genera demonstrated significant correlation with environment parameters revealed by correlation analysis. Moreover, phosphate, nitrate and Chl *a* concentration, and the abundance of top nine identified genera varied with water mass significantly as shown by analysis of variance. Our results supported the notion that environmental factors, especially water mass had significant effect on bacterioplankton distribution at genus level. Considering the high sensitivity to environmental change and low error rate in identification, bacterioplankton at genus level could be potential bio-markers for monitoring environmental changes.

Keywords Correlation · Bacterioplankton genus · Environmental parameters · Pearson · Spearman

Abbreviations

ANOVA	Analysis of variance
Chl *a*	Chlorophyll *a*
DCA	Detrended correspondence analysis
OTUs	Operational taxonomic units
PCR	Polymerase chain reaction
PCR-DGGE	Polymerase chain reaction-denaturing gel gradient electrophoresis
pRDA	Partial redundancy analysis
RDA	Redundancy analysis
SW	Surface water
TAW	Transformed atlantic water
TIW	Transformed intermediate water

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Introduction

Svalbard is located closely to the warming core area and experiencing sudden rise in temperature. In western Svalbard Area, ice-free period increased by 3.3 days per year, and the surface temperature increased 0.5°C during 1980–2010 (Kortscha et al. 2012). Kongsfjorden is considered as a local indicator for climate change, and it is an ideal natural laboratory in Arctic to study the impact of climate change on marine ecosystem. This fjord, located at Svalbard (79°N, 12°E), is influenced by both Atlantic Ocean and glacier waters. The outer part of Kongsfjorden is connected to the inflow of transformed Atlantic water, and variable climate signals in the West Spitsbergen Current have influenced this

fjord (Hop et al. 2012; Walczowski et al. 2012; Wassmann et al. 2015). Meanwhile, the inner part of Kongsfjorden is strongly influenced by the connected glaciers (e.g. glaciers Kongsbreen, Midtre Lovénbreen, and Austre Lovénbreen where the glacier runoff is about 25 ± 5 km^3 a^{-1}) (Hagen et al. 2003; Karner et al. 2013; Schellenberger et al. 2014). In addition, the sea ice melting water showed its impact on the water mass of this fjord (Gerland and Renner 2007), and regular winter ice cover has been rare since 2005/2006 (Cottier et al. 2007).

Bacterioplankton are important microbial communities in Polar Region, which experience extreme temperature and seasonal light variations. Though the abundance of bacterioplankton decreased from low latitude to high latitude, it has been estimated approximately at 10^8–10^9 cells/L in Kongsfjorden (Jankowska et al. 2005; Wang et al. 2009; Møller et al. 2011). Bacterioplankton, the key member of marine food web, are important in the microbial loop for their metabolic pathway in biogeochemical processes, such as carbon, nitrogen and phosphorus cycles (Falkowski et al. 1998). About 19% particulate organic carbon and 36% particulate nitrogen were accounted by bacteria in Kongsfjorden (Zhu et al. 2016). They are sensitive to environment change; however, the variance of bacterioplankton abundance and diversity could also exert a sustained influence on the micro-environment they thrived in, through microbial food web and metabolic pathway (Doney et al. 2012; Sunagawa et al. 2015).

The factors that influenced the bacterioplankton community structure have been reported before. These factors included seasonal changes, phytoplankton bloom, organic matters, the concentration of nutrients, and water depths (De Corte et al. 2011; Sunagawa et al. 2015; Luria et al. 2016; Sinha et al. 2016; Jain et al. 2019; Underwood et al. 2019). In Kongsfjorden, Bacteroidetes were found to be the most abundant bacterial group in spring season (Piquet et al. 2015), whereas Gamma-proteobacteria and Bacteroidetes were the dominant members in summer season (Zeng et al. 2013). The core taxa of particle-attached bacteria are formed by the members of Verrucomicrobia and Bacteroidetes, whose community composition is strongly influenced by carbohydrate content of particulate organic matter. However, the core taxa of free-living bacteria are the members of Alpha-proteobacteria and Gamma-proteobacteria, whose community composition is influenced by glacial meltwater influx and particulate organic carbon (Jain et al. 2019).

In fact, the factors affecting free-living bacteria composition are more complicated than particle-attached bacteria (Jain et al. 2019). The free-living bacterial community structure at the location near to Kongsbreen was also influenced by nitrite, phosphate and silicate, in addition to the glacial meltwater influx and particle organic carbon (Jain and Krishnan 2017; Jain et al. 2019). Besides, few studies focused on the influence of environment change on lower bacterioplankton taxonomy level (genus). This study was aimed to depict a clear picture of the bacterioplankton community diversity and structure from different taxonomy levels, and identify the main factor influencing their composition through the analysis of correlations with environment parameters at genus level in Kongsfjorden, Arctic.

Materials and methods

Study area and sampling

Samples were collected in Kongsfjorden, Ny-Ålesund (Svalbard, Norway, 78°56´N, 11°52´E) in July 2012 (Online Resource Fig. S1). A total of 14 samples from five stations were collected (Table 1). Sea water was collected using Niskin water bottle (KC-Denmark), and physical parameters (depth, temperature and salinity in situ) were determined with a Conductance–Temperature–Depth (SD204, SAIV A/S). For each sample, 2 L seawater was filtered using polycarbonate filters (47 mm diameter, 0.2 µm pore-size, Whatman), after prefilled by 3 µm pore size (47 mm diameter, Whatman). Samples were frozen at -80°C refrigerator immediately till further analysis.

Nutrients and Chlorophyll *a* determination

For each sample, 100 ml subsamples were filtered through 47 mm diameter Whatman GF/F filters, and the concentrations of inorganic nutrients (nitrate, nitrite, silicate and phosphate) were determined using autoanalyzer (Skalar San++, Netherlands), following previous methods (Grasshoff et al. 1999; He et al. 2012).

In addition, 500 ml subsamples were filtered using 47 mm diameter Whatman GF/F filters under low vacuum pressure and the contents of chlorophyll *a* (Chl *a*) were determined using a fluorometer (Turner Designs 10, USA) following the method of Parsons (1984) and He et al. (2012).

Total DNA extraction, amplification and sequencing

Total DNA was extracted using the modified cetyltrimethylammonium bromide method (Cao et al. 2019), and analyzed by agarose gel electrophoresis. The bacteria 16S rDNA V1-V3 hyper-variable regions was amplified using universal primer pairs, F8 (5′-*CCTATCCCCTGTGTGCCTTGGCAGTCTCAG*-AGAGTTTGATCCTGGCTCAG-3′) (Turner et al. 1999) and R533 (5′-*CCATCTCATCCCTGCGTGTCTCCGACTCAG*-NNNNNNNN-TTACCGCGGCTGCTGGCAC-3′) (Sun et al. 2014) (the adaptor sequence were italicized and sample-specific barcode sequences were indicated as NNNNNNNN). Polymerase chain reaction (PCR) was performed using 5–10 ng of genomic DNA in a reaction

Table 1 In situ temperature and salinity, nutrients concentrations in Kongsfjorden 2012

Station	Depth (m)	T (°C)	S (psu)	Nitrite (μmol/L)	Phosphate (μmol/L)	Silicate (μmol/L)	Nitrate (μmol/L)	Chl *a* (μg/L)
K1	0[1]	6.20	33.48	0.51	0.06	1.71	0.27	0.24
	30[2]	2.47	34.68	0.36	0.23	1.88	2.25	0.30
	50[2]	2.32	34.78	0.62	0.25	2.21	3.21	0.09
K2	0[1]	6.84	33.13	0.61	0.10	1.83	0.50	0.29
	30[2]	2.74	34.69	0.61	0.16	1.98	1.87	0.15
	50[2]	2.75	34.80	0.49	0.19	2.39	3.01	0.05
K3	0[1]	5.16	32.28	0.40	0.08	2.22	1.22	0.34
	30[2]	3.64	34.70	0.09	0.09	1.70	1.40	0.28
	50[2]	2.86	34.85	0.23	0.21	2.29	2.63	0.12
K4	0[1]	5.31	32.35	0.07	0.11	3.00	0.85	0.74
	20[3]	4.04	34.57	0.08	0.16	1.85	0.69	0.32
	50[2]	2.90	34.79	0.26	0.26	2.30	2.08	0.13
K5	0[1]	5.04	31.90	0.11	0.07	2.62	0.80	0.73
	20[3]	4.44	34.39	0.16	0.11	1.66	0.85	0.18
ANOVA								
F[4]		23.591	26.786	0.772	9.666	1.956	21.213	3.826
Sig.[4]		0.000**	0.000**	0.536	0.003**	0.185	0.000**	0.046*
F[5]		0.126	0.311	9.090	0.627	0.452	0.471	0.861
Sig.[5]		0.969	0.863	0.003**	0.655	0.769	0.756	0.522
F[6]		37.697	43.553	1.221	8.926	1.357	16.604	4.978
Sig.[6]		0.000**	0.000**	0.332	0.005**	0.297	0.000**	0.029*

Two-tailed test was selected. * $P < 0.05$, significant correlated. ** $P < 0.01$, very significant correlated

[1] The Surface Water (SW)

[2] The Transformed Atlantic Water (TAW)

[3] The Transformed Intermediate Water (TIW)

[4] Results of ANOVA calculated based on depth

[5] Results of ANOVA calculated based on stations

[6] Results of ANOVA calculated based on water mass

volume of 20 μL, which contain 5 × FastPfu Buffer 4μL, 2.5mM dNTPs 2μL, forward primers (5 μM) 0.8 μL, reverse primer (5 μM) 0.8 μL, FastPfu Polymerase 0.4 μL, BSA 0.2μL and ddH_2O up to 20 μL. The PCR conditions were as follows: initial denaturation at 95 °C for 2 min, 25 cycles as 95 °C for 30 s, 56.4 °C for 1 min, 72 °C for 30 s, and a final extension at 72 °C for 5 min. PCR products were purified using AxyPrep DNA purification Kit (Axygen, USA), and quantified with a Qubit® 2.0 Fluorometer (Life Technologies, USA). Pyrosequencing was performed using FLX Titanium Genome Sequencer (454/Roche Life Sciences, USA).

16S rDNA sequences data and statistical analyses

Mothur 3.1.2 (Schloss et al. 2009) was used to analyze 16S rDNA rough sequences. Reads were filtered as following parameters: length (400 ≤ length ≤ 650 bp), quality score (≥ 25), number of ambiguous bases (=0), and length of homopolymer runs (<8). Sequences were clustered at 97% similarity using Silva software (silva.seed_v119) (Pruesse et al. 2007) and used as reference sequences. Taxonomy was assigned to Operational Taxonomic Unit (OTU) according to Greengenes 13_5 database (McDonald et al. 2012). Sequences assigned as mitochondria and chloroplasts were deleted from the dataset for further analysis. Alpha-diversity indexes (ACE, Chao1, Shannon and invsimpson and Good's coverage) were also determined using Mothur.

R (Version 3.1.2) was used to construct Venn diagram, and perform Detrended correspondence analysis (DCA), redundancy analysis (RDA) and partial redundancy analysis (pRDA) with the vegan package (Oksanen et al. 2018). Pairwise comparisons of environmental factors and bacterial communities were performed with a color gradient denoting of Pearson and Spearman's correlation coefficients, including cluster analysis.

Analysis of variance (ANOVA) of bacterial communities and environmental factors among depth, stations and water masses, Pearson and Spearman analysis estimating significant differences and correlations between environment

parameters and bacterial communities and within them, were performed using SPSS 20.0 software (IBM SPSS Statistics).

The sampling location map was prepared using ODV 4.5 (https://odv.awi.de.). The taxonomy composition pie chart and radar graph were constructed using Excel (Microsoft, USA). All figures in this paper were prepared using Illustrator CS4.0 (Adobe, USA).

Results

Water mass

Three water masses were identified based on in situ salinity and temperature data according to previous report (Svendsen et al. 2002). The high-temperature–low-salinity Surface Water (SW), characterized by temperature higher than 5°C and salinity ranging from 31.90 to 33.48 psu; the low-temperature–higher salinity Transformed Atlantic Water (TAW), characterized by temperature ranging from 2.47 to 3.64 °C and salinity higher than 34.68 psu; and the Transformed Intermediate Water (TIW), characterized by temperature ranging from 4.04 to 4.44 °C and salinity ranging from 34.39 to 34.57 psu (Fig. 1a; Table 1).

The TAW, influenced by Atlantic water, was distributed from the deeper outer part toward the inner part of Kongsfjorden (deeper than 30 m); the TIW was mostly influenced by glacier runoff in summer, and distributed in the inner part of Kongsfjorden (K4-20 m and K5-20 m); and the SW was distributed throughout the surface of Kongsfjorden absorbing most of the sunlight.

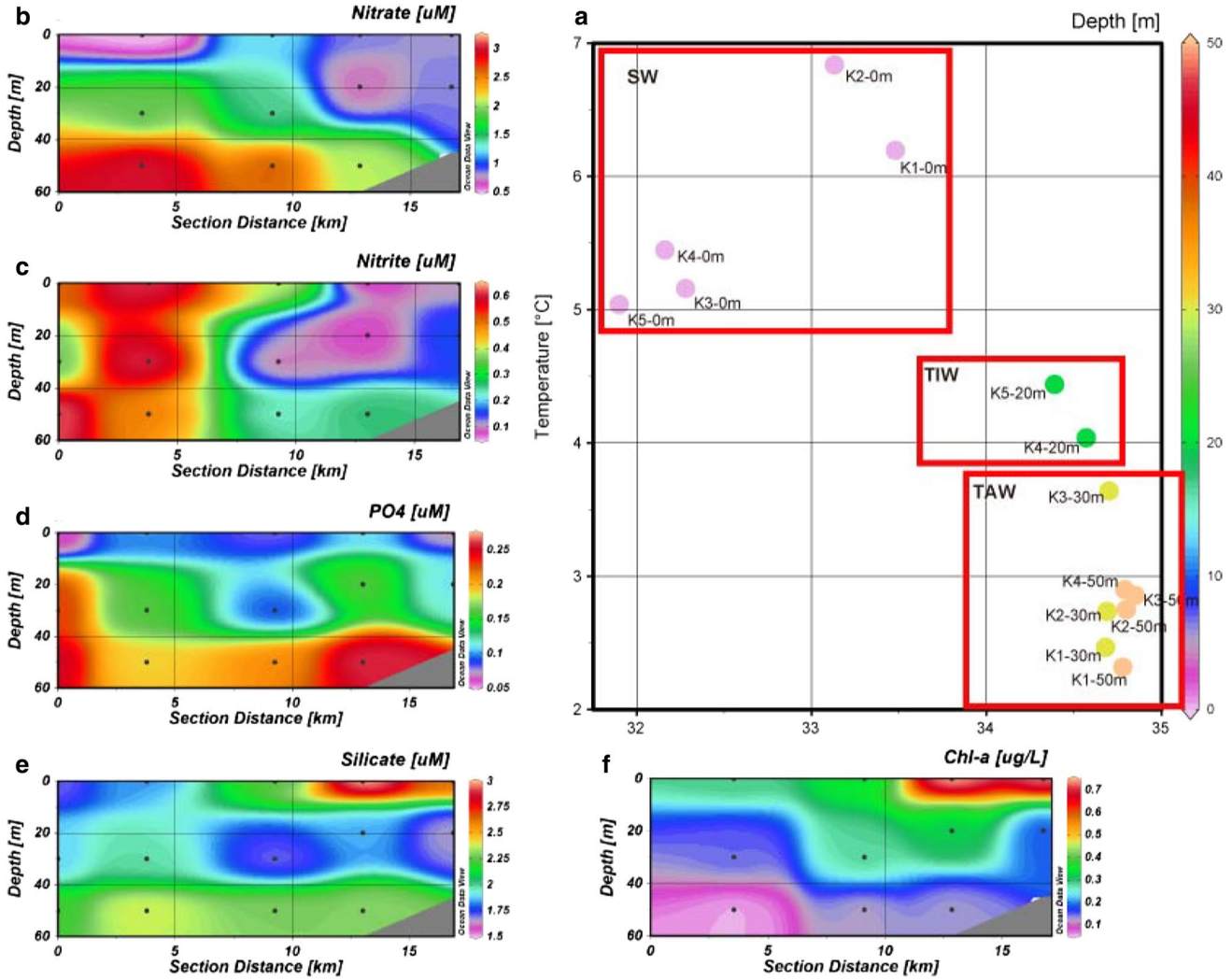

Fig. 1 Distribution of temperature (°C), salinity (psu), water masses and nutrient in sampling locations. SW: T > 5°C, 31.90 > S > 33.48; TAW: 2.47 °C > T > 3.46 °C; S < 34.68 psu; TIW: 4.04 °C < T < 4.44 °C, 34.39 < S > 34.57. The dots represent the samples obtained, and the colors correspond to the depths on the color bar

Nutrients

Nutrient concentration varied with respect to sampling station, depth, and water masses (Fig. 1; Table 1). The average concentration of nitrate increased from SW (0.084 µmol L^{-1}), TIW (0.135 µmol L^{-1}) to TAW (0.199 µmol L^{-1}). It also increased with the sampling depth with the highest concentration detected at 50 m of K1 station (K1-50 m, this abbreviation was used hereafter) (TAW) and lowest concentration detected at K1-0 m (SW) (Fig. 1b). Furthermore, the concentration of nitrite increased from inner to the outer part of Kongsfjorden with the minimum nitrite concentration detected at K4-0 m (SW) and maximum value was detected at K1-50 m (TAW) (Fig. 1c). Similarly, the phosphate concentration increased along the sampling depth with the maximum concentration detected at K4-50m (TAW), and minimum concentration at K1-0m (SW) (Fig. 1d). However, the concentration of silicate decreased from inner surface to the deeper and outer waters with the highest value detected at K4-0m (SW) and lowest value detected at K5-20 m (TIW) (Fig. 1e).

For Chl *a*, the average concentration was found to be 0.283 µg L^{-1} in summer Kongsfjorden. In contrast to nitrate and phosphate concentrations, Chl *a* concentration decreased from inner surface to the outer deeper waters of Kongsfjorden with the highest value detected at K4-0 m (SW) and lowest value at K2-50 m (TAW) (Fig. 1f).

Bacterioplankton diversity

In total, 34,644 sequences were classified into 1010 Operational Taxonomic Units (OTUs) after quality analysis and trimming of the original sequences at 97% similarity. The OTUs with single read were eliminated. Nearly half of the OTUs (46.06%) was found to be distributed throughout the three water masses. The unique OTUs in SW, TIW and TAW accounted for 9.70%, 2.48% and 17.13% of total OTUs, respectively (Fig. 2a). The mean reads of 14 samples were determined to be 2673 (from 1981 to 3389). The sequence data were submitted to the National Center for Biotechnology Information Sequence Read Archives under BioProject ID PRJNA299749.

The average good reads coverage was found to be 78.60% (75.53% to 80.04%). The highest alpha diversity index determined by ACE, Chao and Shannon were detected at K1-50 m, whereas the highest invsimpson index was detected at K5-0 m (Table 2). Minimum ACE and Chao1 index were detected at K3-0 m, and minimum Shannon and invsimpson indices were observed at K2-0 m.

Dominant bacterial communities

The most dominant bacterioplankton phylum was observed to be Proteobacteria, followed by Bacteroidetes, Actinobacteria, and Verrucomicrobia. Besides, Cyanobacteria, Firmicutes, Lentisphaerae, and groups OD1, SAR406, and TM7 were detected with minimal abundance (Online Resource Fig. S2).

Among the dominant bacterioplankton groups, Alphaproteobacteria included the most abundance OTUs (45.7% of total OTUs), followed by Bacteroidetes (23.45%), Gammaproteobacteria (10.88%) and Betaproteobacteria (7.82%). However, at the order level, Flavobacteriales (Bacteroidetes) were found to be the most abundant one (20.71% of total OTUs), followed by Rhodobacterales and Pelagibacterales (17.16% and 14.18% of the total OTUs, respectively) (Alphaproteobacteria) (Online Resource Fig. S2a), each of the above order possessed more than 10% of total reads (34.78%, 19.20% and 18.44% of total reads, respectively) (Online Resource Fig. S2b). Another 12 orders containing more than 1% of total OTUs, were Caulobacterales (3.71%) of Alphaproteobacteria, Burkholderiales (6.12%) of Betaproteobacteria, Oceanospirillales (4.59%), Alteromonadales (1.29%), Xanthomonadales (1.21%) of Gammaproteobacteria, Saprospirales (1.05%) of Bacteroidetes, Actinomycetales (1.45%) of Actinobacteria and Verrucomicrobiales (1.13%) of Verrucomicrobia. Moreover, the unclassified group of Alphaproteobacteria (8.14%), Gamma-proteobacteria (2.90%), and other Bacteroidetes (1.69%) contained OTUs higher than 1% (Online Resource Fig. S2a).

Within total sequences, 53.38% was classified into 33 identified genera and 17 of them were detected at > 0.1% of the total sequences. At genus level, genera *Pelagibacter* and *Octadecabacter* of Alpha-proteobacteria were most detected with the relative abundance > 10% of the total sequences. The genera *Ulvibacter* (Bacteroidetes), *Polaribacter* (Bacteroidetes), *Fluviicola* (Bacteroidetes), *Oceanibulbus* (Alpha-proteobacteria) were detected with relative abundance between 1–10% of the total sequences, respectively. Similarly, *Loktanella* (Alpha-proteobacteria), *Sediminicola* (Bacteroidetes), Candidatus *Portiera* (Gamma-proteobacteria), MB11C04 (Verrucomicrobia), HTCC2207 (Gamma-proteobacteria), Candidatus *Aquiluna* (Actinobacteria), *Pontirhabdus* (Bacteroidetes), *Erythrobacter* (Alpha-proteobacteria), *Pseudoruegeria* (Alpha-proteobacteria), *Xanthobacillum* (Bacteroidetes), *Coraliomargarita* (Verrucomicrobia) were detected with the relative abundance of 0.1–1% of the total sequences.

The dominant taxa showed different distribution patterns in current study. In SW, some genera showed different trend from outer to inner of the Kongsfjorden. The abundance of *Octadecabacter,* MB11C04 and *Pseudoruegeria* increased

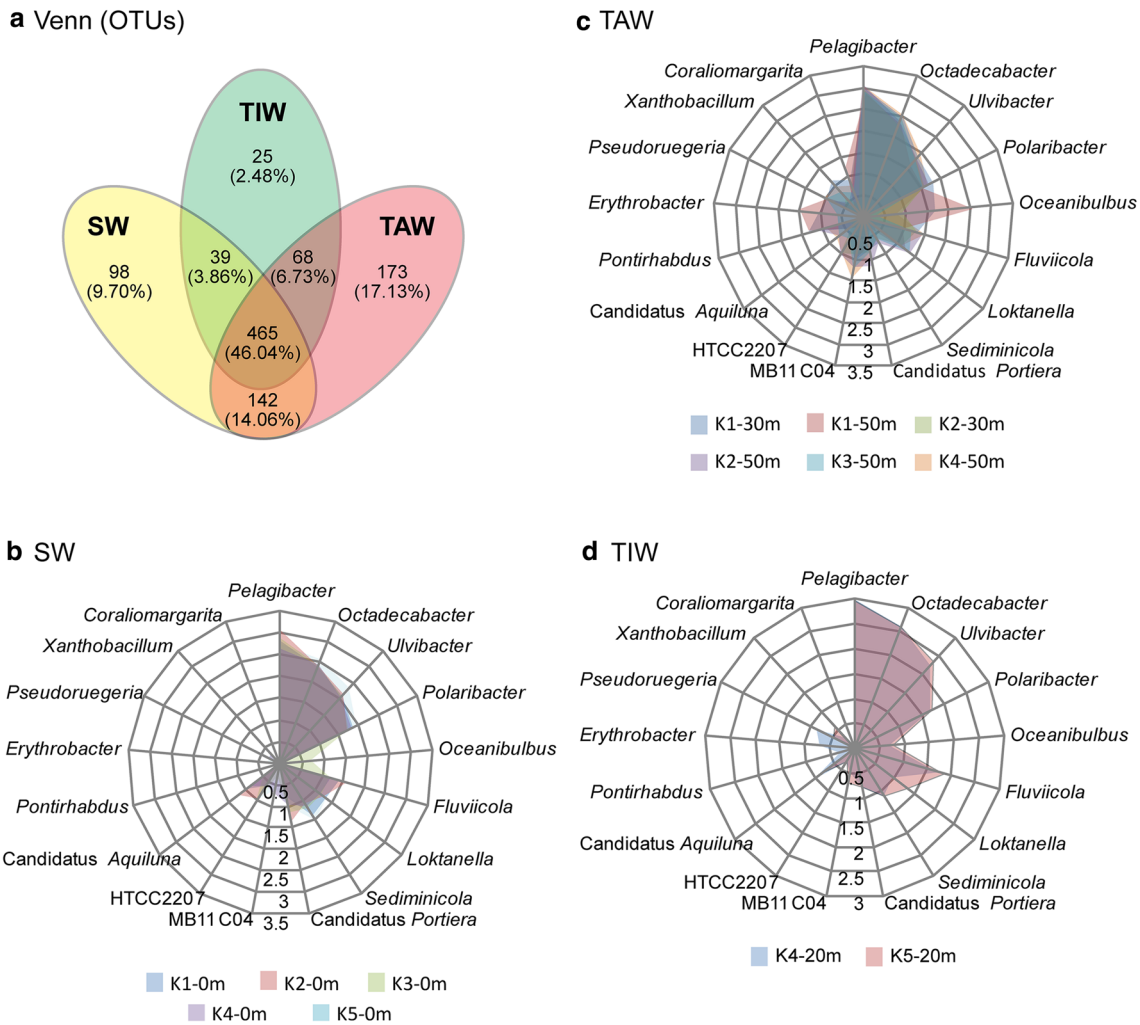

Fig. 2 Similarity in bacterioplankton composition among different water masses. **a** Venn diagrams representing the overlap of OTUs (at 3% sequence cutoff value) among water masses. **b–d** distribution of top bacterioplankton among water masses; the relative abundance was logarithmically transformed

from outer to inner of the Kongsfjorden (Fig. 2b, Table 3). Furthermore, the abundance of *Pelagibacter* and Candidatus *Portiera* was found to be decreased from outer to inner of the Kongsfjorden (Fig. 2b, Table 3), with the highest abundance of HTCC2207 detected at station K3 followed by station K2. In TAW, the genera *Pelagibacter*, *Octadecabacter*, *Fluviicola* and *Sediminicola* did not exhibit obvious difference among different sampling stations. For genera *Ulvibacter* and *Polaribacter*, the abundances in 30 m were higher than those of 50 m at K1 (Fig. 2c, Table 3). *Ulvibacter* biomass was found to be increased from outer to inner Kongsfjorden but *Polaribacter* biomass decreased at 30m and no obvious difference was detected at 50 m (Fig. 2c, Table 3). In TIW (K4-20m and K5-20m), five genera, *Ulvibacter*, *Polaribacter*, *Fluviicola*, MB11C04 and *Xanthobacillum* showed higher abundance in K5 station. Higher abundance of genera *Pelagibacter*, *Octadecabacter*, Candidatus *Aquiluna*, *Erythrobacter*, *Pseudoruegeria* and *Coraliomargarita* was observed at K4, while the other genera showed the similar abundance of K4 and K5 stations (Fig. 2d, Table 3). Furthermore, some genera with few reads were found to be unique to water mass. While *Erythrobacter*, *Polaromonas* and *Thiobacillus* were unique in SW, *Fluviicola*, *Planktotalea* and *Rhodoferax* were unique in TIW. Similarly, *Luteolibacter* and *Leeuwenhoekiella* et al. (15 genera) were unique in TAW (Online Resource Table S1).

Redundancy analysis (RDA) and partial redundancy analysis (pRDA)

The co-occurrence between environment and bacterioplankton communities was analyzed by RDA after DCA calculation (maximum axis length was 1.6026, which is < 3) (Lepx and Smilauer 2003).

Table 2 Estimate of coverage, phylotype richness and diversity for the bacterioplankton community

Label	Sample ID	Reads	OTU	ACE	Chao1	Shannon	Invsimpson	Coverage
0.03	K1-0m[1]	1981	347	6637.54	3039.76	4.91	19.78	0.7553
0.03	K1-30m[2]	2841	427	6737.51	3558.70	5.15	21.12	0.7849
0.03	K1-50m[2]	2557	412	7686.10	3726.20	5.20	26.14	0.7594
0.03	K2-0m[1]	2558	379	5965.64	2768.80	4.59	11.30	0.7969
0.03	K2-30m[2]	2233	393	6328.75	3180.27	4.87	13.52	0.7640
0.03	K2-50m[2]	3223	472	6804.34	3278.17	5.04	15.23	0.7956
0.03	K3-0m[1]	2267	386	4173.88	2350.37	4.94	20.46	0.7887
0.03	K3-50m[2]	2817	427	5713.27	3358.01	5.09	21.45	0.8004
0.03	K4-0m[1]	2125	363	4893.34	2579.28	5.10	33.04	0.7730
0.03	K4-20m[3]	3015	443	4994.40	3100.22	4.98	19.64	0.8072
0.03	K4-50m[2]	3389	476	6596.55	3334.41	5.08	18.52	0.8043
0.03	K5-0m[1]	2729	424	6602.27	3031.89	5.14	34.85	0.7931
0.03	K5-20m[3]	2909	434	7072.24	3501.73	4.98	19.84	0.7951
ANOVA								
F[4]		3.326	4.832	0.924	5.836	0.800	0.455	0.973
Sig.[4]		0.070	0.029*	0.468	0.017*	0.524	0.720	0.447
F[5]		0.263	0.263	3.644	0.812	1.290	2.070	1.650
Sig.[5]		0.894	0.894	0.056	0.551	0.351	0.177	0.253
F[6]		3.716	5.400	1.512	9.634	1.082	0.618	0.934
Sig.[6]		0.062	0.026*	0.267	0.005**	0.375	0.558	0.425

Two-tailed test was selected. * $P < 0.05$, significant correlated. ** $P < 0.01$, very significant correlated

[1] The Surface Water (SW)

[2] The Transformed Atlantic Water (TAW)

[3] The Transformed Intermediate Water (TIW)

[4] Results of ANOVA calculated based on depth

[5] Results of ANOVA calculated based on stations

[6] Results of ANOVA calculated based on water mass

RDA result indicated that 73.02% of the variance could be explained by environment parameters. Of these variances, 47.43% was caused by temperature, salinity and depth, 5.55% was caused by nutrients (nitrite, nitrate, ammonium, phosphate, silicate) and Chl *a*, and the rest (20.04%) could be attributed to the co-effect of temperature, salinity, depth, nutrients and Chl *a* (Table 4).

Analysis of variance (ANOVA)

The temperature, salinity, concentrations of nitrate, phosphate and Chl *a* were found to be changed significantly among the water masses (Table 1) as indicated by ANOVA results. Based on LSD method, multiple comparative analysis showed that both temperature and salinity of SW were significantly higher than those at other sampling depth. The nitrate concentration of SW and TIW were found to be significantly lower than that of TAW. Furthermore, phosphate concentration in SW was significantly lower than that in TAW, with the concentration in 20 m significantly lower than that in 50 m. Similarly, the concentration of Chl *a* in SW was significantly higher compared to that in TAW, with no significant differences observed among other depths (Table 1).

The abundance (total number of reads and OTUs of each sampling station) and alpha-diversity indices were not significantly varied among the sampling stations (Table 2). However, the number of OTUs and Chao index were significantly affected by sampling depth and water mass (Table 2) based on the ANOVA results. The number of OTUs and Chao index in 0 m were significantly lower than that in 20 m and 50 m. In addition, these two indices of SW showed significantly lower value than that of TAW and TIW. That may be caused by the nutrient difference.

For the dominant bacterioplankton genera, biomass varied with respect to sampling depth and water masses but not sampling stations (Table 3). There was significant difference among sampling depth and water masses for the distribution of genera, such as *Ulvibacter* and *Fluviicola* (>1%), *Sediminicola*, MB11C04, *Pseudoruegeria* and *Coraliomargarita* (each > 0.1%). However, only *Ulvibacter* exhibited significant difference among sampling stations (Table 3). Moreover, the distribution of genera *Loktanella* (>1%) showed significant difference among

Table 3 Distribution of the top seventeen bacterioplankton genera and ANOVA analyses

Sample ID	*Pelagibacter*	*Octadecabacter*	*Ulvibacter*	*Polaribacter*	*Oceanibulbus*	*Fluviicola*	*Loktanella*	*Sediminicola*	Candidatus-*Portiera*	MB11C04	HTCC2207	Candidatus*Aquiluna*	*Pontirhabdus*	*Erythrobacter*	*Pseudoruegeria*	*Xanthobacillum*	*Coraliomargarita*
K1-0m[1]	651	238	126	72	1	25	20	25	6	0	4	9	0	9	1	3	1
K1-30m[2]	906	296	73	63	47	20	2	5	6	11	4	3	21	11	9	13	8
K1-50m[2]	697	198	40	34	358	9	18	2	5	11	2	3	20	30	8	2	16
K2-0m[1]	1107	248	143	53	0	40	18	12	23	1	9	16	0	2	1	0	0
K2-30m[2]	903	182	64	37	8	19	13	4	7	8	10	2	3	1	3	4	2
K2-50m[2]	1234	249	72	53	1	28	24	5	14	15	10	5	13	1	7	3	5
K3-0m[1]	727	250	118	55	5	15	15	16	14	2	11	3	0	1	1	4	1
K3-50m[2]	866	301	64	36	2	15	23	4	7	17	4	5	4	4	9	6	4
K4-0m[1]	429	273	116	69	0	16	14	14	9	5	4	7	0	1	2	2	0
K4-20m[3]	941	400	178	50	5	54	9	13	6	3	2	10	2	5	7	2	1
K4-50m[2]	1115	351	96	47	6	31	13	0	9	28	9	6	8	1	10	9	6
K5-0m[1]	527	350	220	91	1	26	21	34	10	4	9	3	0	2	2	2	0
K5-20m[3]	900	396	226	55	6	73	21	13	6	5	2	6	0	1	3	4	0
F[4]	2.363	0.539	2.614	4.950	1.038	1.754	2.498	8.419	1.975	12.706	0.727	0.671	3.360	0.592	17.243	1.441	4.611
Sig.[4]	0.144	0.600	0.122	0.032	0.390	0.222	0.132	0.007**	0.189	0.002**	0.507	0.533	0.077	0.571	0.0015**	0.282	0.0385*
F[5]	1.098	4.410	4.768	0.961	1.064	1.512	1.015	0.977	1.635	0.223	1.771	0.435	1.439	3.549	0.424	0.408	1.390
Sig.[5]	0.420	0.036*	0.029*	0.479	0.434	0.286	0.454	0.471	0.256	0.918	0.228	0.780	0.306	0.060	0.787	0.798	0.320
F[6]	2.251	4.955	15.721	4.525	0.737	15.998	0.178	10.988	1.773	8.785	2.053	1.657	6.630	0.569	13.219	2.359	5.408
Sig.[6]	0.156	0.032*	0.001**	0.040*	0.503	0.001**	0.840	0.003**	0.219	0.006**	0.179	0.239	0.015*	0.584	0.002**	0.145	0.026*

Two-tailed test was selected. * $P < 0.05$, significant correlated. ** $P < 0.01$, very significant correlated

[1] The Surface Water (SW)

[2] The Transformed Atlantic Water (TAW)

[3] The Transformed Intermediate Water (TIW)

[4] Results of ANOVA calculated based on depth

[5] Results of ANOVA calculated based on sites

[6] Results of ANOVA calculated based on water mass

Table 4 RDA and pRDA analysis of co-occurrence between environment and bacterioplankton communities

RDA	Inertia	Proportion	Rank	
Total	1.27E+05	1.00E+00		
Constrained	9.25E+04	**7.30E-01**	Environment factors	
Unconstrained	3.42E+04	2.70E-01		
Inertia is variance				
Eigenvalues for constrained axes:				
RDA1	RDA2	RDA3	RDA4	RDA5	RDA6	RDA7	RDA8	RDA9
79,624	9585	2167	838	208	30	6	2	0
Inertia Proportion Rank (pRDA 1)								
Total	1.27E+05	1.00E+00						
Conditional	7.36E+04	5.81E-01	(essential environment factor)					
Constrained	7.03E+03	**5.55E-02**	(nutrients)					
Unconstrained	4.60E+04	3.63E-01	(Bacterioplankton genera)					
Inertia Proportion Rank (pRDA 2)								
Total	1.27E+05	1.00E+00						
Conditional	2.06E+04	1.63E-01	(nutrients)					
Constrained	6.01E+04	**4.74E-01**	(essential environment factor)					
Unconstrained	4.60E+04	3.63E-01	(Bacterioplankton genera)					

The key values appear in the text were highlighted with bold

20.04% = 73.02%-47.43%-5.55% (explained by nutrients, Chl *a* combined with temperature, salinity and depth)

sampling depth, while *Polaribacter* (>1%) and *Pontirhabdus* (>0.1%) showed significant difference among water masses. Besides, none of the genera with abundance higher than 10% showed significant difference among sampling depth, stations and water masses.

Correlation analysis among environment factors and bacterioplankton communities

Spearman and Pearson analysis showed similar results as ANOVA. It was observed that temperature and salinity in situ (key points in determining the water masses), and sampling depth exhibited high impact on the distribution of nutrients and dominant bacterioplankton genera (Fig. 3; Online Resource Table S2 and S3). Sampling depth showed significant positive correlation with phosphate and nitrate, and negative correlation with Chl *a*, whereas sampling station exhibited significant negative correlation with nitrite (Online Resource Table S2 and S3). The genera, such as *Ulvibacter*, *Polaribacter* and *Sediminicola*, showed significant or very significant negative correlation with salinity and depth. However, they exhibited significant or very significant positive correlation with temperature. Only genera *Octadecabacter* (very significant) and *Ulvibacter* (significant) showed positive correlation with sampling stations (Online Resource Table S2 and S3). Besides depth and water masses, most nutrients exhibited significant correlation with bacterioplankton genera. Phosphate showed significant positive correlation with *Oceanibulbus*, significant negative correlation with *Ulvibacter*, and very significant negative correlation with *Polaribacter* and *Sediminicola*. Furthermore, only nitrite showed very significant negative correlation with *Octadecabacter*. Similarly, silicate exhibited no significant correlation with any bacterioplankton genera (Online Resource Table S2 and S3).

Discussion

Kongsfjorden, a well-known glacial fjord system, is influenced by the Atlantic Water. A higher temperature was observed in the current study (2.86–6.84 °C) compared to previously recorded data. The temperature in upper 50 m waters ranged from 2 °C to 4 °C in July 2000 (Svendsen et al. 2002), and from – 1 °C to 6 °C in July 2011 (Ji et al., 2014). There was an empirical model indicating that increasing 1 °C will induce a net balance of − 0.7 m and − 0.55 m w.e. for glaciers Austre Brøggerbreen and Midre Lovénbreen (Lefauconnier et al. 1993). Temperature is one of the major factors influencing prokaryotic life (Winter et al. 2008; Sunagawa et al. 2015), especially in low-temperature regions. Since the glacier runoff constituted about 5% of the fjord water mass (Cottier et al. 2005), high temperature indicates that glacier runoff will have a significant influence

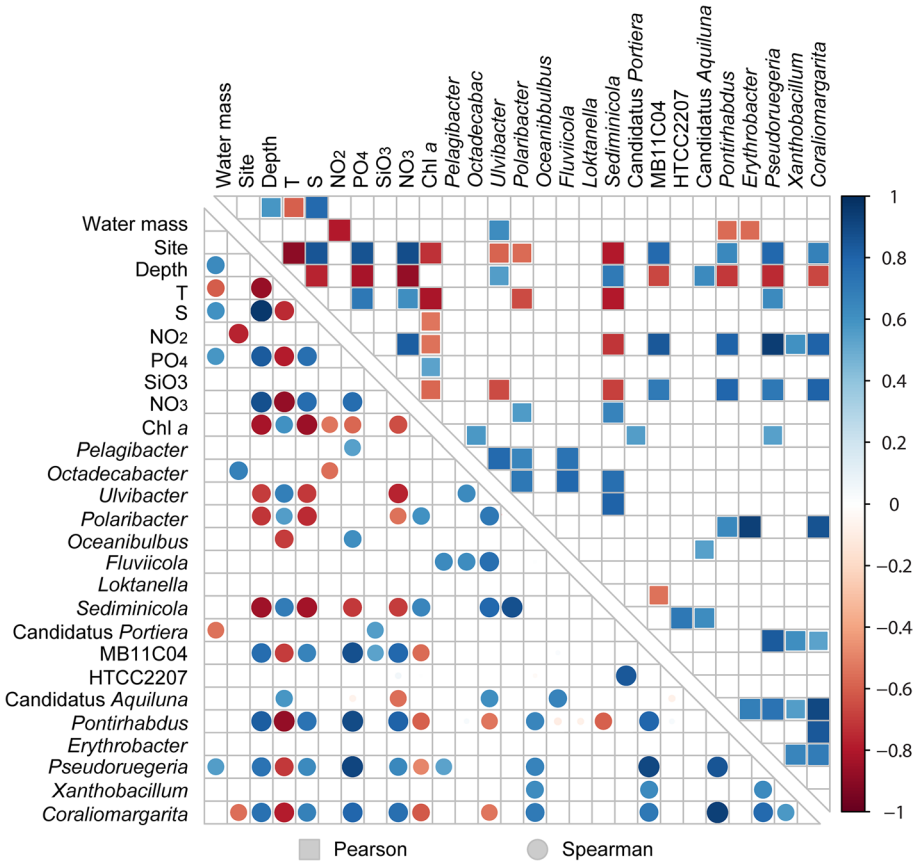

Fig. 3 Pairwise comparisons of environmental factors and bacterioplankton genera abundance with a color gradient denoting Pearson (block) and Spearman's (dot) correlation coefficients. The color and size indicate Pearson and Spearman correlation coefficients, respectively. Only significant correlations are shown ($P < 0.05$)

on Kongsfjorden bacterioplankton. Any small change in temperature might exhibit substantial changes in prokaryotic growth rates (Kirchman et al. 2009).

Chl *a* concentration was considered as an indicator for phytoplankton (Harding et al. 2016). The maximum average Chl *a* concentration in SW indicated relatively high biomass of phytoplankton, especially at K4-0 m and K5-0 m, which were mostly influenced by glacier runoff water. The growth of phytoplankton demonstrated that it consumed inorganic nutrients because relatively low nitrite, nitrate and phosphate values were observed in SW. Furthermore, the integration of terrestrial waters increased the silicate concentration, such as the water from Voitelva River (5.6 µmol L^{-1}) and Wexelva Streamlet (11.8 µmol L^{-1}) (Ji et al. 2014); however, phytoplankton in Kongsfjorden, such as diatom (the dominant group) would consume silicate in summer (Keck et al. 1999). This could explain the detection of higher concentration of Chl *a* and silicate; lower concentration of nitrite, nitrate and phosphate in SW. Moreover, the average concentration of nitrite, nitrate, phosphate and silicate were detected under the minimum threshold (inorganic nitrogen, 1.0 µmol L^{-1}; phosphate, 0.1 µmol L^{-1}; silicate, 2.0 µmol L^{-1}), limiting the growth of phytoplankton (Justić et al. 1995).

The water masses can greatly influence the nutrients concentration. A year-round observation showed that a rapid and overwhelming intrusion of Atlantic Water across the shelf and into the fjord resulted into intense seasonal variance during midsummer (Cottier et al. 2005). The average Chl *a* concentration decreased from SW (0.468 µg L^{-1}), TIW (0.250 µg L^{-1}) to TAW (0.160 µg L^{-1}) from inner surface water (K4-0 m and K5-0 m) to outer deeper water (K2-50 m which was influenced by Arctic Water). The average concentration of nitrate and phosphate increased from SW (0.084 µmol L^{-1}; 0.728 µmol L^{-1}), TIW (0.135 µmol L^{-1}; 0.770 µmol L^{-1}) to TAW (0.199 µmol L^{-1}; 2.350 µmol L^{-1}), respectively (Fig. 1b, d).

In this study, the bacterioplankton community composition was in agreement with the results obtained by Polymerase chain reaction-denaturing gel gradient electrophoresis (PCR-DGGE) and pyrosequencing, indicating that Alphaproteobacteria, Bacteroidetes and Gamma-proteobacteria were the dominant clades in summer Kongsfjorden (Qiao et al. 2015; Zeng et al. 2013). Our results are consistent with the previous study investigated by DEEG (Zeng et al. 2009). These data of current study and previous researches suggested that there was no obvious variance of dominant bacterioplankton community composition annually at the phylum level. Although, it was reported that Gamma-proteobacteria was the most dominant clade followed by Bacteroidetes based on isolation method (Sinha et al. 2016).

This difference would be caused by the selection of culture medium, in which the obtainable nutrition can stimulate or limit the growth of certain bacteria groups (Genevieve et al. 2019). With the development of culture-dependent methods, our understanding of bacterioplankton diversity and community composition comes to a new facet and a number of novel groups in marine environment have been revealed (Bowman et al. 2005; Zengler 2009). Meanwhile, the culture-dependent method provides more physiological and metabolic characteristics information about the bacteria isolations. Since there was no significant variance of dominant bacterioplankton composition at phylum level, the identified bacterioplankton genera with abundance of > 0.1% were considered using high-throughput sequencing and highly efficient annotation database. According to the "Everything is everywhere, but the environment selects" hypothesis, the abundance of identified genera exhibited different distribution trends among the identified water masses (Table 3). *Octadecabacter*, which had been reported from polar sea ice and water characterized by heterotrophic, psychrophilic, gas vacuolate (Gosink et al. 1997), showed relatively low abundance in TAW. That may be caused by the gas vesicles produced by the polar gas vacuolate strains, which help *Octadecabacter* rising up in the water column (Gosink et al. 1997). Meanwhile, the bacteria genera first reported from surface water or fresh water exhibited larger sequence numbers in SW, such as *Oceanibulbus* (>1%), *Loktanella*, and *Sediminicola* (Döbler et al. 2004; Trappen et al. 2004; Ivanova et al. 2005; Khan et al. 2006). Similar observation was made in a previous study in northern Baffin Bay (Fu et al. 2013). The structure of cultured Rhodobacterales was distinctive in different water masses where two ocean currents mixed in northern Baffin Bay (Fu et al. 2013).

The correlation analysis combined with environment parameters and the abundance of top 17 identified genera showed that 11 abundant identified genera exhibited significant correlation with at least two environment parameters. The abundance of *Sediminicola*, MB11C04, *Pontirhabdus*, *Pseudoruegeria* and *Coraliomargarita* showed significant correlation with phosphate and nitrate as demonstrated by Pearson and Spearman analysis. The genus *Sediminicola* can reduce nitrate (Khan et al. 2006; Hwang et al. 2015), so a significant negative correlation with nitrate was observed (Fig. 3). The genera *Pontirhabdu* and *Coraliomargarita* do not reduce nitrate to nitrite or nitrogen (Yoon et al. 2007; Yi et al. 2011; Zhou et al. 2019), and they show significant positive correlation with nitrate (Fig. 3). Though further research about how the nutrient influence the bacterioplankton abundance are in progress, our result has indicated that the nutrient concentration may promote/limit the abundance of the bacterioplankton genera. However, low-frequency significant correlation was observed among the abundant genera that only six appeared significant correlation with at least two abundant genera (Fig. 3, Online Resource Table S2 and S3). Results indicated that environment parameters highly influenced the bacterioplankton community structure than inter-correlation among bacterioplankton in summer Kongsfjorden. A previous study about Chukchi Borderland and surface water of Kongsfjorden also suggested that rare abundant phylotypes were apparently changed under environmental changes (Zeng et al. 2013). Though the abundance was only 0.1–1%, it can prove to be of great importance in the community functions (Hunter-Cevera et al. 2005).

Water mass also plays an important role in determining the bacterioplankton distribution and diversity at both order and genus level. The abundance and distribution of Rhodobacterales in an Arctic marine systems was determined by water mass (Fu et al. 2013). The abundance of 9 genera was significantly different among water masses according to ANOVA result using sequence data (Table 3). In TIW, the abundance of *Octadecabacter* and *Fluviicola*, which are typical sea-ice genera (Vollmers et al. 2013) and fresh water genus (O'Sullivan et al. 2005), was significantly higher than that in SW and TAW. However, the abundance of *Pontirhabdus*, first reported from sea water (Yi et al. 2011), was significantly higher in TAW than that in SW and TIW. This genus had not been observed in SW. Similarly, abundance of *Ulvibacter* and MB11C04 were significantly different among the three water masses.

Furthermore, the presence of unique OTUs in different water masses suggested their influence on bacterioplankton. The OTUs of genera *Leeuwenhoekiella*, *Luteolibacter*, and *Coraliomargarita* were detected only in TAW. Previous studies suggested that marine microbial communities in the Kongsfjorden–Krossfjorden system are shaped by both water mass origin (Atlantic, Arctic) and melting water input (Zeng et al., 2009; Piquet et al., 2011). The genera *Leeuwenhoekiella* (Nedashkovskaya et al. 2009), *Luteolibacter* (Jiang et al. 2012), and *Coraliomargarita* (Yoon et al. 2007; Zhou et al. 2019) are reported as isolated from surface water, fresh water or Arctic tundra. By transferring of melting water and being influenced by the Atlantic water, these genera came to deeper depth, such as TAW. When coupled with sampling depth, similar distribution was observed, exhibiting a visual influence of depth that was influenced by water masses. Our results supported the idea that water masses are determining the marine microbial communities at genus level. The increasing relative abundance of terrigenous bacteria, that an Alpha- and Gammaproteobacteria dominant community shifts to Cytophaga–Flavobacterium–Bacteroides-dominated community, further provided a secondary evidence that glacier runoff can influence bacterioplankton community structure (Piquet et al. 2011). The dominant bacteria phyla, Alpha-proteobacteria and Becteroidetes observed in this study are typical abundant bacteria in glacier or snow

melting water (Larose et al. 2010; Zeng et al. 2013). Sphingomonadales and Actinomycetales are the abundant groups in current study, which have been reported from glacier and snow melting water with relative abundance of 16.4% and 7.7%, respectively (Larose et al. 2010).

Conclusions

Due to its susceptibility to environment change, Kongsfjorden is an ideal natural laboratory to study and monitor bacterioplankton community and diversity variance. On comparing the data from the current study and previous researches, there was no obvious difference of dominant bacterioplankton community composition annually at the phylum level. This study focused on the correlations of water masses and bacterioplankton communities at genus level. On combining the analysis of RDA, ANOVA, Pearson and Spearman, it can be suggested that environment factors, especially water mass exhibited significant influence on bacterioplankton abundance. Furthermore, the genera with relative abundance ranged from 0.1–1%, such as *Pontirhabdus* are more sensitive to water mass that could be developed as the candidate bio-markers for monitoring environmental changes. Further research is required to improve our understanding of organic carbon fluxes of the whole Svalbard glacier meltwater.

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