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Survey of Bacterial Phylogenetic Diversity During the Glacier Melting Season in an Arctic Fjord

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Abstract

To understand bacterial biogeography in response to the hydrographic impact of climate change derived from the Arctic glacier melting, we surveyed bacterial diversity and community composition using bacterial 16S rRNA gene metabarcoding in the seawaters of Kongsfjorden, Svalbard, during summer 2016. In the present study, bacterial biogeography in the Kongsfjorden seawaters showed distinct habitat patterns according to water mass classification and habitat transition between Atlantic and fjord surface waters. Moreover, we estimated phylogenetic diversity of bacterial communities using the net relatedness, nearest taxon, and beta nearest taxon indices. We found the influence of freshwater input from glacier melting in shaping bacterial assemblage composition through the stochastic model. We further evaluated bacterial contributions to phytoplankton-derived dimethylsulfoniopropionate (DMSP) using a quantitative PCR (qPCR) measurement with demethylation (*dmdA*) and cleavage (*dddP*) genes of two fundamentally different processes. Our qPCR results imply that bacterial DMSP degradation follows the Atlantic inflow during summer in Kongsfjorden. These findings suggest that the Atlantic inflow and glacial melting influence bacterial community composition and assembly processes and thus affect the degradation of phytoplankton-derived organic matter in an Arctic fjord.

Keywords Arctic glacial fjord · Kongsfjorden · Bacterial biogeography · Stochastic-deterministic balance · SAR11 · Phytoplankton-derived organic matter

Introduction

Kongsfjorden is an open glacial fjord system on the northwestern Spitsbergen (Svalbard) and is used as a monitoring site suitable for climate-related changes and their possible impacts on biodiversity in the Arctic region [1]. The effects of the glacial melting on biological and physiochemical environments in the Arctic fjord were previously investigated in Kongsfjorden [1–3] and the influence of hydrography on the seasonal biological processes was carefully discussed [3]. The hydrography of Kongsfjorden is controlled by the water mass balance of Atlantic inflow, Arctic waters, and glacial melting through the cross-shelf exchange. Generally, the environmental heterogeneity in Kongsfjorden responds to the effects of both Atlantic inflow and glacier melting [4], and a strong stratification of water columns occurs after the spring phytoplankton bloom [3]. A semi-persistent cyclonic circulation, located near the mouth of Kongsfjorden, is driving the inflow of Atlantic water toward the inner fjord during summer season (June to September) [5]. Given the hydrography of

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Kongsfjorden, the transition of bacterial habitats should correlate to the mixing of the water masses originating from the Atlantic water inflow and glacier freshwater runoff in summer, as has been shown for an intermediate mixing layer along with stratified water columns in the western Arctic Ocean [6].

The five dominant water masses (surface water, intermediate water, transformed Atlantic water, Atlantic water, Arctic water) are classified according to the basic tool for water mass identification such as the temperature-salinity diagram [4]. The biodiversity of Kongsfjorden seems to be influenced by both the intensity of Atlantic inflow and the freshwater input from glacial melting [1]. However, the effects of these two major environmental processes on the phylogenetic diversity of bacterial communities and their ecological significance are still unknown. In the light of its specific ecological characteristics, Kongsfjorden may provide fundamental insight into the phylogenetic responses of bacterial communities to climate change in the Arctic region. Thus, we addressed this question by estimating phylogenetic diversity (community structure and turnover) of local bacterial communities using the Net Relatedness Index (NRI), Nearest Taxon Index (NTI), and β-Nearest Taxon Index (βNTI) [7, 8]. In particular, the βNTI was used to predict the relative influence of deterministic and stochastic processes in microbial assemblages [8, 9]. Stochastic and deterministic models have been broadly established in microbial ecology [8–14]. In the deterministic model, assembly processes of communities are influenced by abiotic factors and species interaction (antagonistic and synergistic). On the other hand, assembly processes of the stochastic model are unpredictably influenced by changes in the ability to disperse (species movement or population size). Determining the influence on deterministic and stochastic processes in Kongsfjorden could provide a clue to answer the question "what underlying factors (biotic, abiotic, or dispersal) represent the assembly process of bacterial communities during the glacier melting season in Arctic fjords?"

In addition to the phylogenetic survey, we also looked at highly relevant microbial processes in the marine food web system. Given the ecological interactions between bacteria and phytoplankton [15], phytoplankton blooms select specific bacterial assemblages in Arctic glacial fjords [16]. In general, phytoplankton blooms in Arctic glacial fjords including Kongsfjorden occur during spring (April to May) and are controlled by light transmissibility together with nutrient supply and the inflow of Atlantic water [17–19]. After spring season, the water columns in Kongsfjorden become strongly stratified due to thermocline formation with temperature increase. The glacial meltwater discharge during summer results in sediment-laden plumes and suppresses both the salinity level and the light permeability of surface waters. This reduction in light intensity has implications on phytoplankton growth. In this seasonal shift from spring to summer, shallow surface waters are characterized by high chlorophyll *a* (ChlA) concentrations from the spring phytoplankton bloom, and phytoplankton-derived organic matter depleted by heterotrophic bacteria after the early spring phytoplankton bloom in Kongsfjorden [3]. Consequently, phytoplankton-derived organic matter from the blooming event attracts heterotrophic bacteria and further affects bacterial community composition [20]. On the other hand, the glacial meltwater may have a direct influence on the assembly of bacterial communities in Kongsfjorden. For example, the habitat transition of bacterial communities in the brackish zone was explained by the salinity change during sea-ice melting in the Arctic Ocean [21]. Thus, the combined effects of primary phytoplankton productivity and brackish zone (freshwater input from melting glaciers) on bacterial communities deserve much consideration in an Arctic glacial fjord.

Notably, bacteria play a major role in transforming phytoplankton-derived organic matter such as dimethylsulfoniopropionate (DMSP) that can be converted to substantial nutrients or a climatically important gas, dimethyl sulfide (DMS) [22]. The diverse sources and sinks of DMSP and their complex interactions with bacterial life in the ocean have been investigated because the fate of DMSP is recently known as a dynamic component of global climate change [23, 24]. Bacterial degradation of DMSP involves the cleavage and the demethylation pathways. The cleavage pathway leads to the formation of DMS, and the demethylation pathway produces methanethiol (MeSH). Recent metagenomic surveys have increased our understanding of bacterial DMSP degradation in the ocean [25–30]. Concerns about the biological contribution to DMSP have increased with the occurrence of phytoplankton blooms in the Arctic region [31–33]. However, information on the key bacterial population responsible for ecologically significant DMSP degradation in the bacterial assemblages of an Arctic glacial fjord are still limited.

We primarily aimed (1) to understand whether water mass dynamics shape bacterial biogeography, (2) to estimate phylogenetic diversity of bacterial communities under the influence of freshwater input from glacier melting, and (3) to evaluate bacterial DMSP degradation during the post-phytoplankton bloom period in Kongsfjorden. We surveyed bacterial biogeography (diversity and community composition) using a bacterial 16S rRNA gene metabarcoding approach. Furthermore, two specific enzymes, *dmdA* (demethylation) and *dddP* (cleavage) genes, involved in bacterial DMSP pathways were evaluated by a quantitative PCR (qPCR) method.

Materials and Methods

Sample Collection

To encompass the entire area of Kongsfjorden from the surface to bottom waters, 22 sampling stations (11 surface layers and 11 vertical water columns) were established based on bathymetry and currents at surface and subsurface layers [5]. A total of 83 seawater samples were collected from the 22 sites using a conductivity-temperature-depth (CTD) rosette system equipped on MS Teisten in July 2016 in Kongsfjorden. Of the 22 sampling sites, sample collection from the 11 surface water sites was done at 1 m depth (n = 11), whereas seawaters (n = 72) of the 11 vertical water column sites were collected from surface (1 m depth) to bottom layers at specific depth intervals. That is, the data set includes 22 surface seawater samples from the entire area of Kongsfjorden at 1-m depth (horizontally collected), plus 61 seawater samples from variable depths below 1 m (vertically collected). The 83 seawaters were classified into the known water mass types based on the temperature-salinity characteristics [4].

One liter of seawater at each sampled depth (n = 83) was immediately passed through 0.2-μm-pore membrane filters (Merck, Darmstadt, Germany) after sample collection, and then the filters were stored in a deep-freezer (− 80 °C) at Dasan Station located in Ny-Ålesund (Spitsbergen) before further environmental DNA (eDNA) extraction. Temperature and salinity were characterized using sensors contained in the CTD system during sample collection. The concentrations of nutrients (PO_4, NH_4, NO_2, NO_3, and SiO_2) at all of the sampled depths were estimated using a QuAAtro autoanalyzer (Seal Analytical, Norderstedt, Germany), and ChlA was also measured according to the previous description [34]. Sample information of depths, nutrients, ChlA, etc. was listed in the supplementary data (as Excel file).

DNA Extraction and Sequencing

eDNAs were extracted from the frozen filters using PowerWater DNA Isolation Kit (MoBio Laboratories, CA, USA) and measured using the Quant-iT PicoGreen dsDNA Reagent (Molecular Probes, OR, USA). The extracted eDNAs can represent DNAs from non-isolated organisms (herein larger than 0.2-μm diameter) in 1 l of seawater. eDNAs were amplified with bacterial 16S rRNA gene primers (V3–V4 region, Table S1) and then sequenced using the Illumina MiSeq platform (Macrogen, Seoul, South Korea) through two-step PCRs (amplicon and index PCR). PCR conditions and program, as well as primer sequences for the construction of the MiSeq library, were set according to Illumina's instruction manual [35]. The initial PCRs were carried out for eDNAs in triplicates using the KAPA HiFi Hotstart ReadyMix PCR kit (KAPA Biosystems, MA, USA) with Illumina's amplicon primer set. The initial PCR amplicons were purified using the QIAquick PCR Purification Kit (Qiagen, CA, USA), and used as templates for the index PCRs according to Illumina's protocol. Concentrations of the index PCR products were measured by Qubit 2.0 Fluorometer (Invitrogen, CA, USA) following amplicon purification. The purified amplicons were all mixed in equimolar amounts and subjected to sequencing. All sequencing reads obtained were submitted to the European Nucleotide Archive (https://www.ebi.ac.uk/ena) under the accession number ERP114510.

Sequence Data Processing

Sequences (n = 1,880,124) were quality-filtered (singleton removal and correction of amplification and sequencing errors), clustered into 97% operational taxonomic units (OTUs, n = 1537), and classified against the Silva.seed (v132) database in Mothur (v.1.40.5) [36] following the MiSeq SOP [37]. Bacterial alpha diversity (species richness) was determined by abundance-unweighted species richness indices (Chao1 and ACE), which were calculated with the 1537 OTUs using the "summary.single" command in Mothur. Beta diversity of bacterial OTUs was calculated by both Mothur and R, described below.

Phylogenetic Diversity with OTUs

Phylogenetic community structure was estimated using NRI, NTI, and βNTI (Table S2) with Phylocom [38], using the 175 most abundant OTUs. Briefly, a Newick-formatted tree was first generated based on the selected 175 OTUs using the "tree.shared" command with default settings in Mothur. The Newick format phylogenetic tree and relative abundance data were used as input for Phylocom. NRI and NTI were calculated with the "comstruct" command, and βNTI was calculated with the "comdistnt" command with the abundance-weighted distance in a phylogeny shuffle null model (900 randomizations). NRI and NTI describe two phylogenetic structuring patterns such as (1) phylogenetic over-dispersion (NRI and NTI < 0), with a high influence of biological interaction (probiosis and competition), and (2) phylogenetic clustering (NRI and NTI > 0) caused by deterministic habitat specificity [39, 40]. Furthermore, βNTI was applied to determine the relative influence of stochastic and deterministic assembly processes in the bacterial community. For example, a significant deviation in inter-sample phylogenetic distance ($|\beta NTI| > 2$) indicates the dominance of deterministic processes, while a non-significant deviation ($|\beta NTI| < 2$) indicates dominance of stochastic processes [8, 9].

Real-time Quantitative PCRs

Bacterial 16S rRNA and DMSP degradation (*dmdA* and *dddP*) genes were quantified using SYBR Premix Ex Taq™ with a Thermal Cycler Dice Real-time System (Takara Bio Inc., Shiga, Japan) according to the manufacturer's specifications. Information on used primer sets and their specific annealing temperatures for the qPCRs refer to Table S1. The quantification standard for the 16S rRNA gene consisted of tenfold serial dilution of a known amount of genomic DNA of *Escherichia coli*. To construct serially diluted standard curves of DMSP degradation genes, PCR products for *dmdA* and *dddP* genes from eDNAs were cloned into the PCR 4.0 vector using the TOPO TA Cloning kit (Invitrogen, CA, USA), and their copy numbers were quantified based on the length of the inserted amplicons and their concentrations. qPCRs for the target genes in samples and standards were performed in triplicates with the following conditions: initial denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 20 s, specific annealing of each target gene for 30 s, and elongation at 72 °C for 30 s. At the end of each run, a dissociation melt curve of the PCR product was determined to verify amplicon specificity.

Statistical Analyses

Statistical analysis and its visualization were performed using various packages in R program version 3.5.3 (https://www.R-project.org). Briefly, principal component analysis (PCA) using the prcomp function and correlation analysis using the cor function were applied from the base package [41]. Permutational multivariate analysis of variance (PERMANOVA) using the adonis function and non-metric multidimensional scaling (NMDS) using the metaMDS function were applied from the vegan package [42] and calculated with Bray-Curtis distance. In particular for PERMANOVA, the pairwise multiple comparison (post hoc) was further carried out with Bonferroni method in the pairwisea.adonis function [43]. In addition, the envfit function was used to fit environmental factors (Temp., Salinity, eDNA, PO_4, NH_4, NO_2, NO_3, and SiO_2) onto the NMDS ordination. The values in indicator species analysis were calculated using the indval function of the labdsv package [44]. Bacterial beta diversity was determined by NMDS with (i) 1537 OTUs in Mothur and (ii) only using the relative abundance of the top 175 OTUs in R. The statistical significance for the separation among water masses in NMDS was confirmed by analysis of molecular variance (AMOVA) using the "amova" command in Mothur and by PERMANOVA in R.

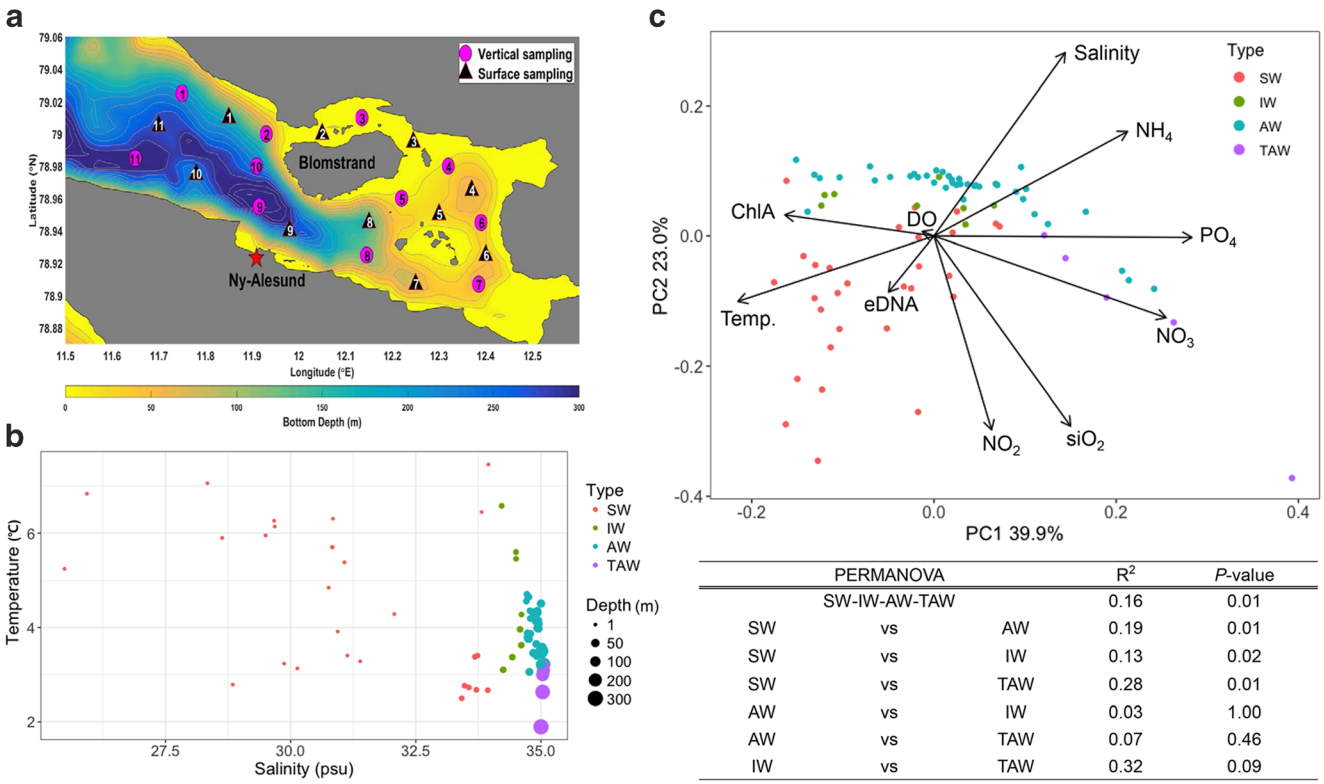
Results

Identification of Water Masses

A total of 83 seawater samples were collected from 22 sites (11 surface water sites and 11 vertical water column sampling sites), encompassing the entire area of Kongsfjorden from the surface to bottom waters (Fig. 1a). In the Kongsfjorden seawater samples ($n = 83$), water mass types were clearly classified according to the temperature-salinity reference [4] (Fig. 1b and Fig. S1). Kongsfjorden waters were classified into four water mass types: surface water (SW, $n = 29$), intermediate water (IW, $n = 8$), Atlantic water (AW, $n = 41$), and transformed Atlantic water (TAW, $n = 5$), which indicates a mixing layer between AW and Arctic-type water originating outside of the fjord [4]. SW samples were environmentally different from the other water mass types, as revealed by PCA and PERMANOVA of various environmental variables (such as temperature, salinity, PO_4^{3-}, NH_4^+, NO_2^-, NO_3^-, SiO_2, DO, ChlA, and eDNA) (Fig. 1c). For example, SW samples were clearly separated by salinity, NO_2^-, and SiO_2 in the PC2 axis (23.0% variation). In particular, salinity in SW (median 30.85 psu) was lower than in other waters (IW: median 34.50 psu, AW: median 34.89 psu, and TAW: median 35.03 psu) in box plot analysis (Fig. S2). PERMANOVA showed the significant difference among water masses (SW-IW-AW-TAW; $P < 0.05$), and the post hoc PERMANOVA analysis revealed pairwise comparisons of SW with IW, AW, and TAW were significantly different ($P < 0.05$) (Fig. 1c).

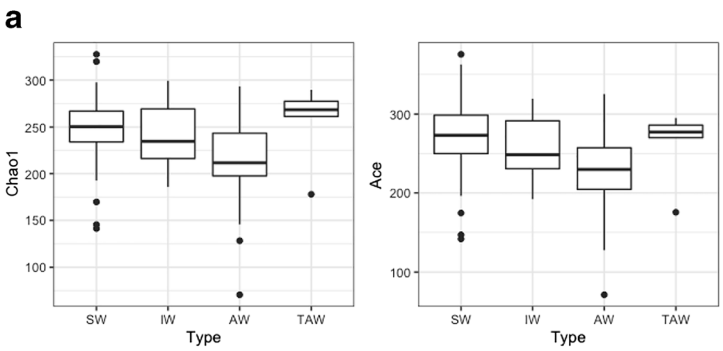
Bacterial Biogeography

A total of 1,880,124 bacterial 16S rRNA gene sequences were obtained from Kongsfjorden waters ($n = 83$; $22{,}653 \pm 3758$ reads per sample) and the number of sequences in each water mass and sample was descripted in Fig. S3. Sequences were clustered at 97% similarity level as OTUs, and a total 1537 OTUs were analyzed for bacterial diversity (alpha and beta). Alpha diversity showed a significant difference among water masses (SW-IW-AW-TAW; $P < 0.05$) and particularly between SW and AW ($P < 0.05$). Median values of alpha diversity were higher in SW (Chao1: 250 and ACE: 273) than that in AW (Chao1: 212 and ACE: 230) and seem to gradually decrease from SW to AW via IW (Chao1: 235 and ACE: 249) (Fig. 2a). Indices of alpha diversity were not remarkably correlated with environmental variations (Fig. 2b), indicating a weak influence of the measured environmental factors on bacterial diversity change. In contrast to the alpha diversity, beta diversity patterns on NMDS (Fig. 3a) showed a more apparent separation of bacterial communities into their water mass type. AMOVA showed a significant difference among water masses ($P < 0.01$). Between-group differences were



PERMANOVA			R^2	*P*-value
SW-IW-AW-TAW			0.16	0.01
SW	vs	AW	0.19	0.01
SW	vs	IW	0.13	0.02
SW	vs	TAW	0.28	0.01
AW	vs	IW	0.03	1.00
AW	vs	TAW	0.07	0.46
IW	vs	TAW	0.32	0.09

Fig. 1 **a** Sampling stations, **b** water mass classification by TS reference in Kongsfjorden (Fig. S1), and **c** environmental heterogeneity in PCA and PERMANOVA of Kongsfjorden waters. Water masses were classified into surface water (SW), Atlantic water (AW), intermediate water between SW and AW (IW), and transformed Atlantic water between AW and Arctic water (TAW)



PERMANOVA			Chao1		Ace	
			R^2	*P*-value	R^2	*P*-value
SW-IW-AW-TAW			0.07	0.01	0.08	0.01
SW	vs	AW	0.13	0.01	0.12	0.02
SW	vs	IW	0.01	1.00	0.01	1.00
SW	vs	TAW	0.02	1.00	0.02	1.00
AW	vs	IW	0.05	0.62	0.04	0.91
AW	vs	TAW	0.06	0.59	0.03	1.00
IW	vs	TAW	0.05	1.00	0.03	1.00

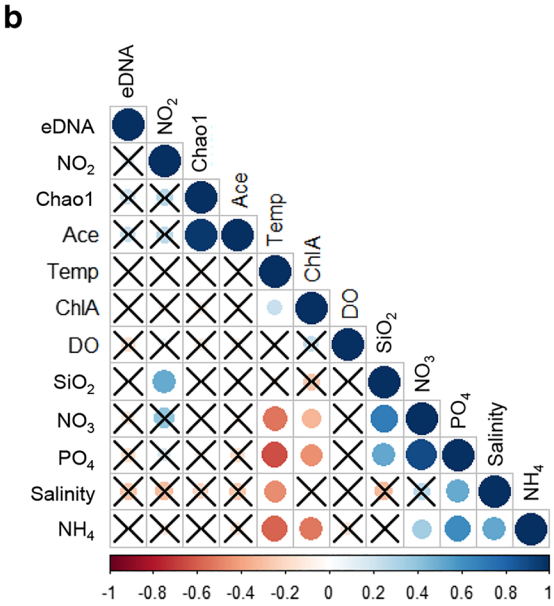
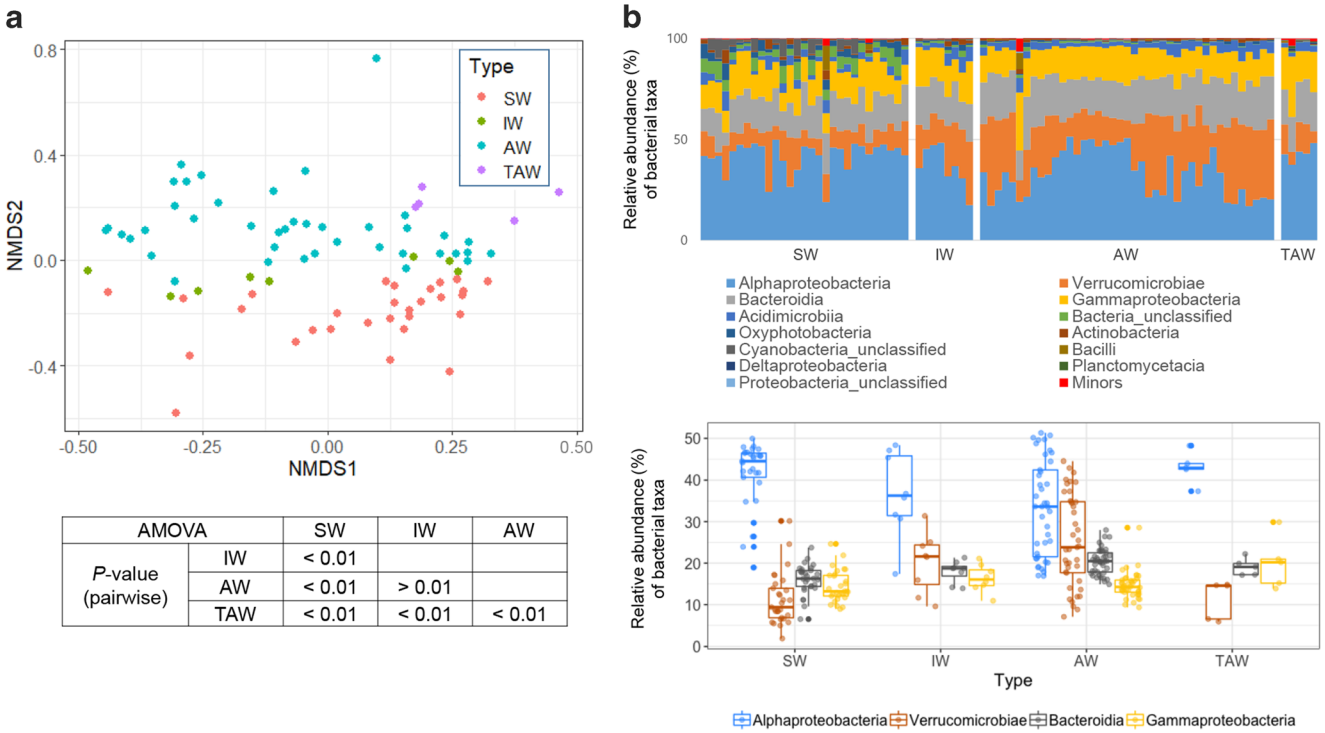


Fig. 2 **a** Alpha diversity represented by unweighted species richness estimators (Chao1 and Ace) in Kongsfjorden waters and **b** its correlation with water mass properties using Spearman rank correlation. Non-significant correlations ($P > 0.05$) were marked with "X" symbol. In the alpha diversity, a significant difference among water masses and between water masses was determined by PERMANOVA ($P < 0.05$)



AMOVA		SW	IW	AW
P-value (pairwise)	IW	< 0.01		
	AW	< 0.01	> 0.01	
	TAW	< 0.01	< 0.01	< 0.01

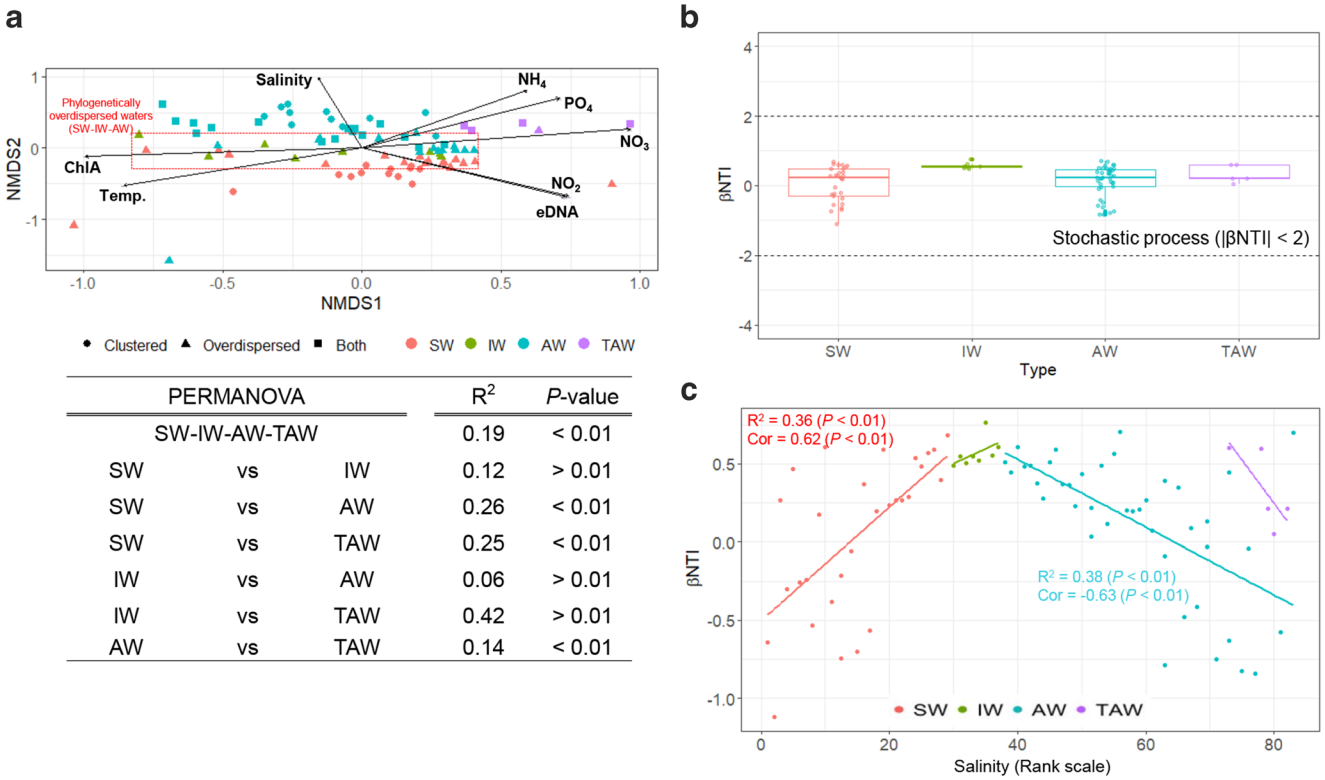
Fig. 3 **a** Beta diversity calculated by Bray-Curtis distance with a total OTUs ($n = 1537$) among Kongsfjorden water samples and **b** bacterial community composition and relative abundance of major taxa at the class level. Bacterial taxa with a lower relative abundance, less than 1% in the sample, were assigned as minors. Relative abundance values of bacterial taxa in each sample were shown in the supplementary data (excel file). In AMOVA, there was a significant difference among bacterial habitats of each water mass (SW-IW-AW-TAW; $P < 0.01$), and a significant difference in water mass pairs was determined by a P value ($P < 0.01$)

particularly pronounced in pairwise comparisons between SW, AW, and TAW ($P < 0.01$), whereas IW samples represented intermediate community states not distinguishable from AW.

The bacterial communities in Kongsfjorden waters consisted of a few major taxa at class level such as Alphaproteobacteria, Verrucomicrobiae, Bacteroidia, and Gammaproteobacteria (Fig. 3b), which were previously recorded as dominant heterotrophic bacteria in an Arctic fjord [45]. The relative abundances of these major taxa among Kongsfjorden waters were variable, and the abundance of Alphaproteobacteria was remarkably high (Fig. 3b). In particular, the distribution of Alphaproteobacteria showed a gradual decreasing pattern from SW to AW as similar to the trend of species richness indices. Most of the Alphaproteobacteria sequences in this study were assigned to the SAR11 clade and Rhodobacterales, the common ancestor of Roseobacter clade (RCA) (Fig. S4), as shown by a recent survey [46] using 16S rRNA gene sequencing data.

Phylogenetic Diversity

Top 175 most abundant OTUs (99.03% of total sequences) were selected for the phylogenetic survey (NRI, NTI, and βNTI) after removing singleton OTUs ($n = 1362$) containing only one sequence. Before the phylogenetic survey, beta diversity of the selected 175 OTUs was projected on NMDS (Fig. 4a) to confirm whether the selected OTUs are representative of the population as a whole and showed a similar trend of AMOVA using 1537 OTUs (Fig. 3a). In the beta diversity of 175 OTUs, NMDS showed water mass separation on the ordination. In particular among the measured environmental factors, salinity (R^2: 0.41, $P < 0.01$; Table S3) seemed to be particularly strongly related to the community separation among the water masses. In PERMANOVA of 175 OTUs (Fig. 4a), water mass separation among SW, IW, AW, and TAW was significantly different ($P < 0.01$), but pairwise comparisons of IW with other water masses were not significant ($P > 0.01$). To determine if bacterial communities are primarily shaped by biotic (over-dispersed) or abiotic (clustered) factors, the beta diversity of 175 OTUs was combined with phylogenetic structuring using NRI and NTI. Most Kongsfjorden waters were classified into the over-dispersed type (NRI and NTI < 0, $n = 40$), followed by clustered type (NRI and NTI > 0, $n = 23$) and ambiguous type (both over-dispersed and clustered, $n = 20$) (Fig. S5). In particular, all IW samples ($n = 8$) were assigned to the over-dispersed type together with some samples of SW ($n = 17$), AW ($n = 14$), and TAW ($n = 1$) and surrounded by the over-dispersed SW and AW samples. Interestingly, the phylogenetically over-



PERMANOVA			R^2	*P*-value
SW-IW-AW-TAW			0.19	< 0.01
SW	vs	IW	0.12	> 0.01
SW	vs	AW	0.26	< 0.01
SW	vs	TAW	0.25	< 0.01
IW	vs	AW	0.06	> 0.01
IW	vs	TAW	0.42	> 0.01
AW	vs	TAW	0.14	< 0.01

Fig. 4 **a** Beta diversity using Bray-Curtis distance with abundant OTUs ($n = 175$) among Kongsfjorden water samples in NMDS and PERMANOVA among water masses and between water mass pairs. **b** Patterns of βNTI among water masses with upper (βNTI = + 2) and lower (βNTI = − 2) significance thresholds, indicated by horizontal dashed lines, and (**c**) their relationship with salinity change. In addition, the envfit function was used to find important environmental factors in the NMDS ordination, and only significant variables ($P < 0.01$) are displayed

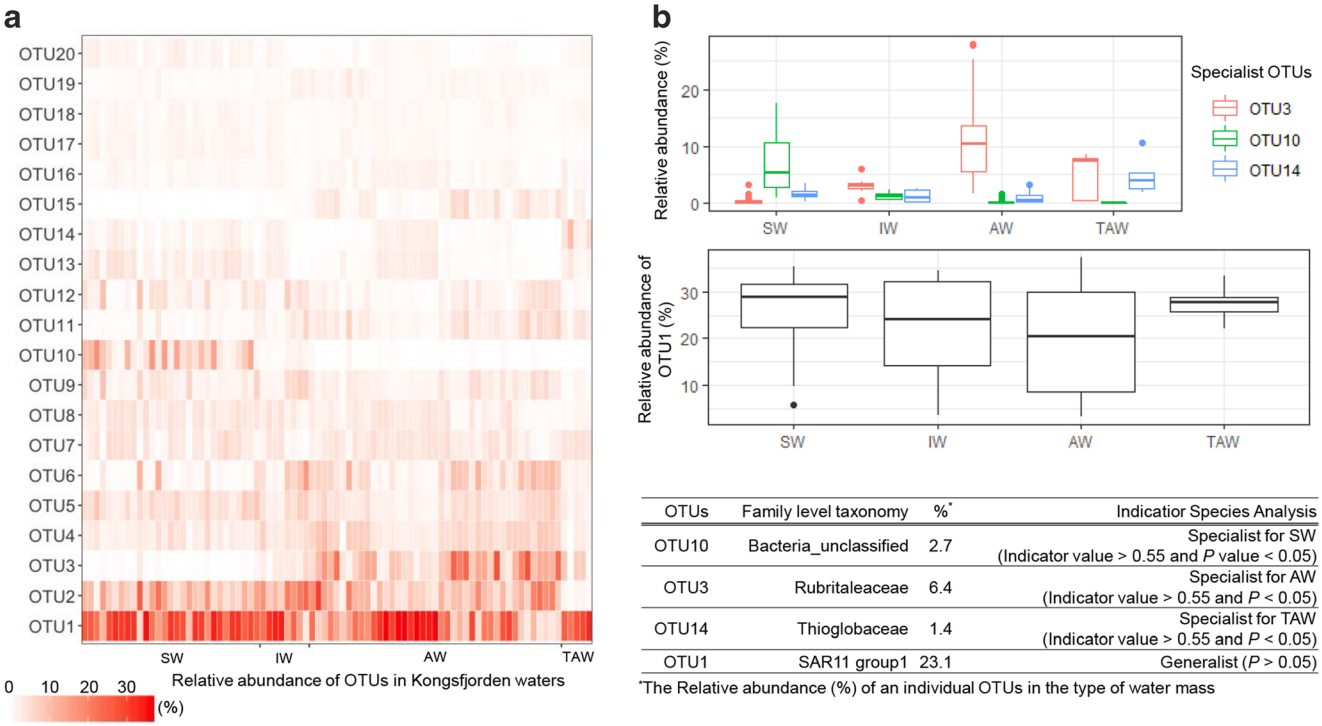
dispersed waters among SW, IW, and AW (the dashed-line box in Fig. 4a) seem to be influenced by salinity.

To determine if the assembly mechanisms at work in Kongsfjorden are best explained by deterministic or stochastic processes (e.g., dispersal, drift), βNTI values were calculated across all pairwise community comparisons [8, 9]. The βNTI values were in the range from − 2 to + 2 (Fig. 4b), indicating that the assembly of bacterial communities is primarily dominated by stochastic processes, and correlated with only salinity in environmental parameters of Kongsfjorden waters (Table S4). The relationship between the βNTI value and the salinity gradient suggested the influence of the mixing between Kongsfjorden water masses on the phylogenetic turnover. The variation of βNTI was significantly correlated with salinity change for SW (cor: 0.62, R^2: 0.36) and AW (cor: − 0.63, R^2: 0.38) (Fig. 4c). Interestingly, we found that the salinity gradients of SW and AW contrastingly converge in IW and this βNTI pattern is similar to the phylogenetic pattern from SW to AW via IW in Fig. 4a.

Of the total OTUs ($n = 1537$), the top 20 most abundant OTUs (78.32% of total sequences) were selected after removal of minor OTUs with a frequency below 1%. The distributions of the 20 major OTUs in Kongsfjorden waters were visualized in a heatmap (Fig. 5a). The heatmap visualization showed a variable distribution of OTU1, the most predominant and ubiquitous in Kongsfjorden waters. The OTU1 was taxonomically identified as *Candidatus* Pelagibacter (known as SAR11 clade), which is a dominant group of heterotrophic bacteria in the ocean [47]. An indicator species analysis using the major 20 OTUs was applied to determine a specialist (a representative OTU for each water mass type). We determined three significantly representative OTUs (OTU10, OTU3, and OTU14) with the highest indicator value (indicator value > 0.55 and $P > 0.05$) for SW-, AW-, and TAW-specificity (Fig. 5b), whereas the most abundant and ubiquitous OTU1 among the non-significant OTUs ($P < 0.05$) was considered to be a generalist (non-habitat specific for water mass type) in the indicator species analysis [48].

Bacterial DMSP Degradation

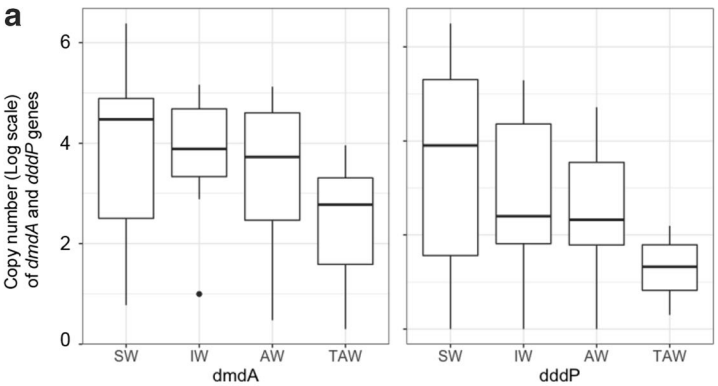
Abundances of genes encoding bacterial DMSP demethylation (*dmdA*) and DMS production (*dddP*) in Kongsfjorden waters were estimated by PERMANOVA and box plot analysis (Fig. 6a). PERMANOVA revealed that there was a significant difference in *dddP* gene abundance among water masses ($P < 0.05$), in which the only pairwise between SW and AW has a significance ($P < 0.05$), but *dmdA* gene is not significant among water masses ($P > 0.05$). Nonetheless,



OTUs	Family level taxonomy	%*	Indicator Species Analysis
OTU10	Bacteria_unclassified	2.7	Specialist for SW (Indicator value > 0.55 and *P* value < 0.05)
OTU3	Rubritaleaceae	6.4	Specialist for AW (Indicator value > 0.55 and *P* < 0.05)
OTU14	Thioglobaceae	1.4	Specialist for TAW (Indicator value > 0.55 and *P* < 0.05)
OTU1	SAR11 group1	23.1	Generalist (*P* > 0.05)

*The Relative abundance (%) of an individual OTUs in the type of water mass

Fig. 5 **a** Heatmap visualization of top 20 OTUs and **b** boxplot analysis, taxonomic identification, and species indicator value of specialist (water-specific) and generalist (non-specific) OTUs for the four water types. Water-specific OTUs (the highest indicator value and $P > 0.05$ in each water mass type) were surveyed using the species indicator analysis. The values of relative abundance and species indicator for the 20 OTUs were listed in the supplement (Excel file) and Table S5, respectively



PERMANOVA			dmdA		dddP	
			R^2	*P*-value	R^2	*P*-value
SW-IW-AW-TAW			0.02	0.08	0.06	0.00
SW	vs	AW	0.02	1.00	0.06	0.02
SW	vs	IW	0.01	1.00	0.03	1.00
SW	vs	TAW	0.05	0.66	0.08	0.10
AW	vs	IW	0.01	1.00	0.02	1.00
AW	vs	TAW	0.02	1.00	0.03	1.00
IW	vs	TAW	0.08	1.00	0.13	0.93

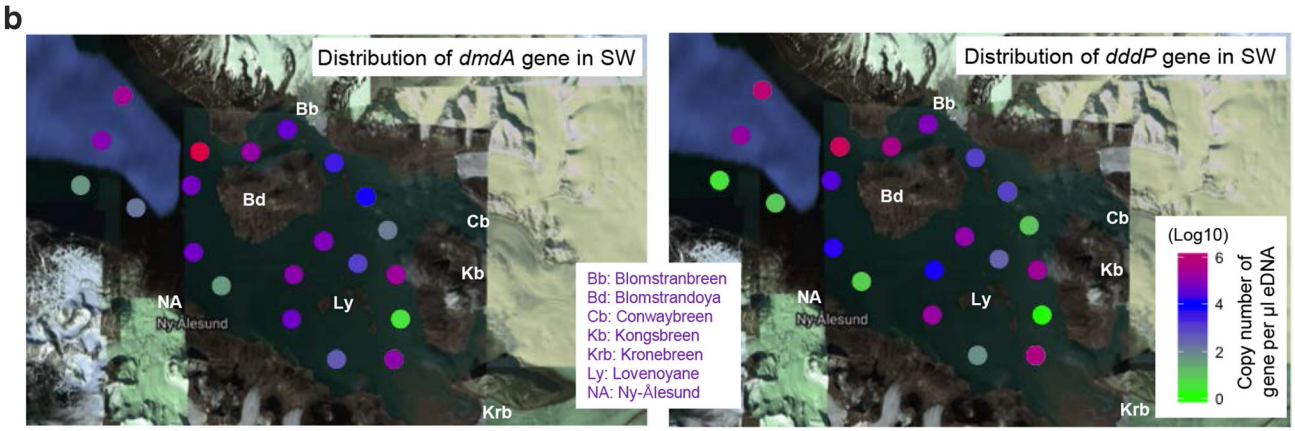


Fig. 6 **a** Distribution of *dmdA* and *dddP* genes in Kongsfjorden water masses estimated by box plot analysis and PERMANOVA and **b** within SW (1-m depth) by the spatial visualization using the ggmap package [49] in R

median values of copy number in *dmdA* and *dddP* seem to gradually decrease from SW (*dmdA*: 29,983 and *dddP*: 5760) to AW (*dmdA*: 4652 and *dddP*: 134) via IW (*dmdA*: 8071 and *dddP*: 253), indicating that dominance of the bacterial DMSP degradation in summer surface waters. Particularly, the distribution of *dmdA* gene within SW was spatially similar to that of *dddP* (Fig. 6b). For example, both genes occurred much more frequently in the area around Ly (the islands of Lovénøyane in the central part of Kongsfjorden) and through the narrow channel between Bd (Blomstrandhalvøya) and Bb (the glacier Blomstrandbreen), compared with the adjacent coastal area of NA (Ny-Ålesund).

The successional growth of marine heterotrophic bacteria correlated positively with phytoplankton biomass during bloom, but there is often a final decoupling toward its end [15]. Indeed, the distribution of ChlA concentrations (phytoplankton biomass) in Kongsfjorden waters was not similar to bacterial abundance during summer assuming post-phytoplankton bloom conditions (Fig. S6). Given that phytoplankton-derived DMSP provides carbon and sulfur nutrients for heterotrophic bacterial growth [24, 26], it is not surprising that the distributions of the genes encoding two specific enzymes, *dmdA* (demethylation) and *dddP* (cleavage), involved in bacterial DMSP catabolism correlated significantly to the pattern of bacterial DNA variation in Kongsfjorden waters (Fig. S6).

The distribution of *dmdA* and *dddP* genes was compared with the relative abundance of major bacterial taxa (SAR11, Verrucomicrobiales, Flavobacteriales, and Rhodobacterales) in Kongsfjorden waters (Fig. S7). Among these taxa, only SAR11 showed a positive correlation with the DMSP genes and a stronger relationship with the abundance of *dmdA* (cor: 0.61, R^2: 0.36, $P < 0.01$) than *dddP* (cor: 0.58, R^2: 0.32, $P < 0.01$) in Kongsfjorden waters. This correlation is more clearly explained by the population dynamics of OTU1 with *dmdA* (cor: 0.61, R^2: 0.37, $P < 0.01$) and *dddP* (cor: 0.58, R^2: 0.33, $P < 0.01$) in Fig. 7. As shown above, the OTU1 was taxonomically identified as SAR11 clade (hereafter SAR11-affiliated OTU). It was believed that SAR11 clade bacteria possess *dmdA* gene, but not *dddP* gene [24].

Discussion

Bacterial Biogeography and Hydrography in Kongsfjorden During Summer

The present study suggests that patterns of bacterial biogeography (diversity and community composition) in summer waters in Kongsfjorden are related to the salinity change derived from the Arctic glacier melting. In our results, Kongsfjorden waters harbored a simple community composition, which consisted of a few major bacterial taxa, and their population showed distinguishable variation according to the water mass type. In particular to the major bacterial OTUs, the specialists for SW (OTU10), AW (OTU3), and TAW (OTU14), which would thrive in relatively homogenous environments, showed water mass-specific distribution (Fig. 5b). On the other hand, the survival and growth of generalist SAR11-affiliated OTU would be best adapted to the environmental heterogeneity in the observed water samples [50]. Moreover, the patterns of alpha (Fig. 2a) and beta (Fig. 3a) diversity support a gradual change in bacterial communities from SW to AW via IW samples under stochastic processes (Fig. 4c). We suggest that the transition of bacterial communities between SW and AW is driven by the hydrography of Kongsfjorden.

The hydrography in Kongsfjorden is influenced by the meltwater discharge from tidewater glaciers (Kongsbreen and Kronebreen). The cold freshwater input from glacial melting (Fig. S8) causes a decrease in salinity and temperature and an increase in sediment particles in the inner fjord region [2]. The strong currents driven by glacial meltwater plumes in the front of Kongsbreen and Kronebreen result in the upwelling of subsurface waters [2, 5]. The plume-driven upwelling may provide nutrients into surface water, which may enhance not only primary production but also bacterial production. Indeed, the relative abundance of bacterial populations was conserved along the vertical water columns in the front of Kongsbreen and Kronebreen (ST6 and ST7 in Fig. S9) supporting the notion that bacterial biogeography is mediated by the plume-driven upwelling. In contrast, the difference between TAW and the other water masses seems to be due to the hydrographic influence from Arctic water in the outer fjord (Fig. S1).

Phylogenetic Assembly Processes in the Kongsfjorden Bacterial Community

The OTU-based phylogenetic modeling (Fig. 4a) suggests that the assembly of bacterial communities in IW is mediated by both SW and AW under a strong biological interaction (probiosis and competition) rather than abiotic factors as implied from the patterns of the bacterial diversity (alpha and beta). In particular, the gradual shift of βNTI in SW and AW with the salinity gradient indicates the water mixing between SW and AW. Moreover, their further conversion to IW suggests bacterial dispersal (immigration and emigration) mediated by water mass mixing between SW and AW. Taken together with these phylogenetic diversity patterns, the hydrographic influence on Kongsfjorden waters seems to affect the assembly processes of the bacterial community. In addition, we found that the most common phylogenetic structuring pattern was the over-dispersed type and that stochastic community assembly processes remained dominant in Kongsfjorden waters during summer. Under these circumstances, the relative abundance of bacterial populations is likely to be influenced by dispersal ability under weak influence of

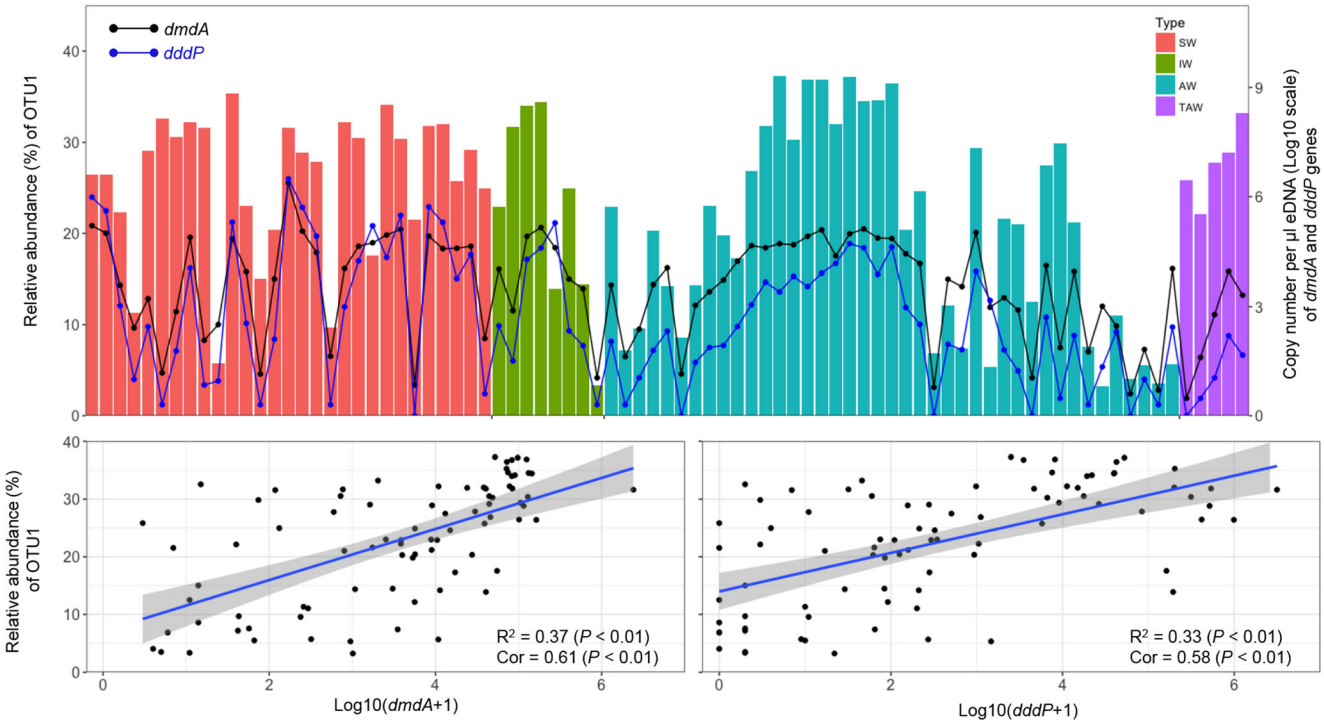


Fig. 7 Comparison between distribution of *dmdA* and *dddP* genes and relative abundance of OTU1 in Kongsfjorden waters

deterministic processes and/or high immigration rate as described by the neutral theory [51]. The water mass mixing under the post-phytoplankton bloom conditions may be a driving force leading to the assembly of phylogenetically less clustered bacterial communities through stochastic processes. This assumption implies that the relative influence of stochastic processes might be changed after the early spring phytoplankton bloom given the progressive succession in the conceptual model [9].

Notably, the composition of bacterial communities is influenced by phytoplankton bloom dynamics [15], and in particular, the SAR11 clade and RCA, the most predominant heterotrophic Alphaproteobacteria in the ocean, are known as prevalent taxa in the Arctic fjords [46, 52–55]. It was believed that RCA clade bacteria possess both demethylation and cleavage genes, while SAR11 bacteria carry only demethylation genes [24]. In the present study, the SAR11 clade was more predominant during summer than the RCA, a key player in marine bacterial communities during DMSP-producing phytoplankton blooms [56, 57]. A recent study of the seasonal dynamics of bloom-associated bacterial populations in a coastal region showed seasonal specificity of the RCA clade, which was abundant only in spring and rare or absent in the other seasons [48]. As described above, the diminishing of the bloom-associated RCA in Kongsfjorden waters after the early spring phytoplankton bloom may be not surprising, but the key bacterial groups affecting the DMSP cleavage pathway during this time remain unknown.

Bacterial DMSP Degradation Under Post-phytoplankton Bloom Conditions

It is known that dinoflagellates, one of the most significant phytoplankton groups producing DMSP [58], are still dominant during summer in Kongsfjorden [1]. In our results, marine heterotrophic Alphaproteobacteria dominated the post-phytoplankton bloom communities. Notably, the fundamentally different *dmdA* and *dddP* (demethylation and cleavage) genes in bacterial DMSP pathways occur frequently in marine Alphaproteobacteria [27, 59–63]. We surveyed bacterial DMSP degradation using the *dmdA* and *dddP* genes from the surface to bottom waters throughout the entire Kongsfjorden. It is found that the degradation of phytoplankton-derived DMSP is genetically available during summer in Kongsfjorden waters. Although the occurrence of DMSP degradation genes including *dmdA* and *dddP* was previously investigated in Kongsfjorden [30], the previous survey was limited to a few small and localized sampling points. Here, we suggest that the inflow of AW into Kongsfjorden and the resulting glacial melting may have led to the strong correlation between the dominating SAR11-affiliated OTU1 and *dmdA* gene, and further affect the spatially biased distribution of DMSP degradation genes in Fig. S10. Indeed, cyclonic circulations at the mouth of Kongsfjorden support the inflow of subsurface waters (20–30 m, AW), which trigger plume-driven upwelling in the front of Kongsbreen and Kronebreen at the inner fjord (Fig. S8). We assumed that the nutrient-rich plume should facilitate heterotrophic

bacterial growth and metabolic activity including DMSP degradation. Furthermore, the facilitated DMSP degradation at the inner fjord may be dispersed to outside the fjord through the surface current.

In conclusion, we demonstrated the use of a metabarcoding approach to monitor the phylogenetic diversity in Kongsfjorden. Information on the coincident bacterial populations and the assembly processes involved in shaping community composition may provide fundamental knowledge regarding the ecological system [8–10]. Our main findings are that bacterial biogeography and assembly processes are strongly influenced by the hydrography of Kongsfjorden and that the presence of two fundamentally different pathways in phytoplankton-derived DMSP degradation is linked to both AW inflow and glacial melt in Kongsfjorden. In addition to the distribution of DMSP degradation genes, understanding of bacterial phylogenetic diversity in Kongsfjorden may facilitate assessment and tracking of the rates, magnitude, and ecological significance of bacterial metabolism mediated with the phytoplankton bloom of an Arctic glacial fjord.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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