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Bacterial communities along stratified water columns at the Chukchi Borderland in the western Arctic Ocean

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ABSTRACT

An expedition of the IBRV *ARAON* took place in the Arctic Ocean during the summer of 2010. To investigate the hydrographic features and bacterial variations in water columns, we categorized 16 water samples collected from distinct water masses at the Chukchi Borderland in the western Arctic Ocean. Bacterial diversity, relative abundance, and community composition were determined based on a pyrosequencing approach, and their relationship with water mass properties was considered. *Alphaproteobacteria* (43.2%), *Gammaproteobacteria* (16.7%), *Flavobacteria* (13.7%), and *Deltaproteobacteria* (12.0%) were the most common bacteria found in all samples, and the relative abundance of these predominant taxa represent the population dynamics of bacterial communities in different water masses (from the euphotic to the sub-euphotic zone) in the Arctic Ocean. Furthermore, the relative abundance of *Alphaproteobacteria* and its subgroup, SAR11 group I, were significantly related to depth change in water columns, suggesting that environmental heterogeneity caused by changes in depth may play an important role in bacterial population dynamics. In this study, bacterial communities in the Arctic Ocean exhibit biogeographic patterns according to the type of water mass. The halocline layer between the Pacific winter water and Atlantic water exhibits a variation in the composition of bacterial communities, which may be influenced by mixing of Pacific winter water and Atlantic water.

1. Introduction

Marine microbial ecology is currently an important research topic owing to the role of microbes in the marine ecosystem (DeLong et al., 2006; Field et al., 1997; Fuhrman and Davis, 1997; Fuhrman et al., 2008; Giovannoni et al., 1996; Giovannoni and Stingl, 2005; Hansman et al., 2009; Hewson et al., 2006; Karner et al., 2001; Pham et al., 2008; Pommier et al., 2007; Teira et al., 2006). Studies on marine bacterial ecology in the Arctic have been much less frequent than those in temperate oceans because of the difficulty in accessing Arctic areas. Recently, however, several reports on the distribution, structure, and abundance of bacterial communities have described interactions with oceanographic traits in the Arctic Ocean (Bowman et al., 2012; Comeau et al., 2011; Han et al., 2014; Kirchman et al., 2010; Winter et al., 2013; Zeng et al., 2013).

The Arctic Ocean is hydrographically complex and considered a double estuary with inflows of Pacific (through the Bering Strait) and Atlantic (through the Fram Strait) water (Carmack, 2007; Yamamoto-Kawai et al., 2008). A northward Pacific-origin current enters into the Chukchi Sea and mixes with the northern Atlantic water. The mixed water masses exhibit distinctive characteristics in terms of temperature, salinity, density, dissolved oxygen (DO), and nutrient characteristics that depend on the seafloor topography. These properties are useful to study ocean characteristics, and they directly distinguish water mass structure and its distribution. In most oceans including the Arctic, temperature–salinity (*T–S*) diagrams segregate the water mass into fine scales, but other important properties can also be used to characterize water masses that are not obvious in *T–S* diagrams (Emery, 2001).

From an ecological standpoint, an ecosystem consists of discrete areas that are defined as patches (Pickett, 1985). Each patch has a local community that represents a given environment. We hypothesized that this ecological concept could aid in understanding bacterial distribution across stratified water columns in the Arctic Ocean. Galand et al. (2009) have reported that Arctic water masses harbor distinct bacterial communities, suggesting that the water mass could be a key factor in microbial biogeography because the environmental heterogeneity in terms of water mass properties may favor a specific bacterial community.

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We tested this hypothesis by collecting water samples along a transect line at the Chukchi Borderland in the western Arctic Ocean from the continental shelf of Chukchi Sea to the Northwind Ridge near the Chukchi Plateau. Our study was designed to encompass a bacterial biogeographic change along water columns that ranged from euphotic to deep sub-euphotic zones, defined by photosynthetically active radiation (PAR), a measure of sunlight. We then conducted pyrosequencing, targeting the V1–V3 region of the bacterial 16S rRNA gene to shed light on bacterial communities in water samples collected. The major aim of the current study was to understand bacterial diversity of water columns in the Arctic Ocean. This study also considered the possibility of water mass identification by using biological properties such as composition and abundance of the bacterial community.

2. Materials and methods

2.1. Sample collection and sequencing

The IBRV *ARAON* expedition to the Pacific sector of the Arctic Ocean took place in July 2010, during which 16 seawater samples were obtained from water columns. The samples were collected using a conductivity–temperature–depth (CTD) rosette system (Sea-Bird, SBE-911plus) and immediately passed through 3-μm-pore membrane filters (ADVANTEC, Japan) to separate eukaryotes, followed by filtration using 0.2-μm-pore membrane filters (ADVANTEC) to capture prokaryotic bacteria. The samples were then stored in a deep-freezer (−80 °C) aboard the IBRV *ARAON* and transported to our laboratory on ice. DNA extraction, amplification of the prokaryotic 16S rRNA gene (V1 to V3), and pyrosequencing using a 454 GS FLX Titanium Sequencing System were performed as described previously (Han et al., 2014). All the pyrosequencing reads obtained were submitted to the Sequence Read Archive (SRA) under accession number ERP003637. Temperature, salinity, DO, fluorescence, PAR, and light transmission at all of the sampled depths were characterized using a variety of sensors contained in the CTD rosette system.

2.2. Sequence analyses

In total, 60,259 sequencing reads were obtained from the 16 samples after a filtering process (ChunLab, Republic of Korea, http://www.chunlab.com) was conducted. In brief, sequencing reads from the different samples were separated with unique barcodes. Then, the barcode, linker, and PCR primer sequences were removed from the original sequencing reads. The resulting sequencing reads were processed further for quality trimming and chimera removal. The checked sequences were taxonomically assigned using the EzTaxon-e Database (http://eztaxon-e.ezbiocloud.net) (Kim et al., 2012). The operational taxonomy units (OTUs) were defined with the MOTHUR program version 1.29 (Schloss et al., 2009) and used for the subsequent alpha diversity analysis.

In the alpha diversity analysis, the species richness was estimated based on the ACE (Abundance-base Coverage Estimator) and Chao1 indices, and species evenness was calculated with the Shannon and Simpson indices (Shaw et al., 2008). Principal coordinates analysis (PCoA) was used to visualize data similarities in beta diversity using the Fast UniFrac software (Hamady et al., 2009).

2.3. Statistical analyses

Principal Component Analysis (PCA) was performed to search a single axis, accounting for variation of water mass properties by using the Canoco 5 program (ter Braak and Šmilauer, 2012). Further statistical analyses were performed to investigate the relationship between the bacterial populations and water mass properties. The sequences were normalized before the statistical analyses. In brief, the relative abundance of each population was calculated, and the minor populations with a lower relative abundance of less than 1% in each sample were excluded from further analyses. To verify the link between the water mass properties and the major bacterial populations along the water columns, we performed a nonparametric correlation analysis using the Spearman's rho test. To identify tendencies toward a relationship between the major populations and the water mass properties, we first considered the effect of the property on the population abundance. All the water mass properties were inputted together to form a stepwise linear regression model, and the data were checked for normality (normal p–p plot), autoregression (Durbin–Watson), and outliers (standardized residual) with regression diagnostics. All the statistical analyses were performed using the SPSS program (version 16.0, SPSS Institute, Cary, NC, USA).

Table 1
Water mass properties and geological information in samples.

Station	Sample	Water mass[Ψ]	Date [mon/day/yr]	Latitude [N°]	Longitude [E°]	Depth [m]	Temp. [°C]	Salinity [psu]	DO [mg/l]	Fluor. [mg/m^3]	PAR [W/m^3]	Sigma-t [kg/m^3]	X-miss [%]
S1	S1_10	PWW	7/20/2010	73.13	−168.95	10	−1.5	30.3	16.4	0.5	256.1	24.4	98.642
	S1_30	PWW	7/20/2010	73.13	−168.95	30	−1.5	32.3	15.7	1.6	22.6	26.0	92.092
	S1_50	PWW	7/20/2010	73.13	−168.95	50	−1.7	33.1	11.1	1.2	0.2	26.7	91.673
S2	S2_10	SMLW	7/21/2010	73.51	−166.99	10	−1.4	28.9	13.4	0.5	118.1	23.2	98.687
	S2_40	PWW	7/21/2010	73.51	−166.99	40	−1.5	31.8	12.6	1.3	4.7	25.6	95.690
	S2_80	PWW	7/21/2010	73.51	−166.99	80	−1.6	32.8	9.5	0.6	0	26.4	97.267
S3	S3_90	IW1	7/23/2010	73.75	−167.03	90	−1.7	32.9	11.0	0.6	0	26.4	98.139
	S3_145	IW2	7/23/2010	73.75	−167.03	145	0.2	34.6	8.3	0.6	0	27.8	86.162
S4	S4_1800	AW	7/25/2010	75.00	−160.00	1800	−0.3	34.9	9.1	0.5	0	28.1	99.977
S5	S5_500	AW	7/26/2010	75.03	−159.47	500	0.7	34.8	9.4	0.3	0	27.9	99.953
	S5_1000	AW	7/26/2010	75.03	−159.47	1000	0	34.9	9.5	0.3	0	28.0	99.904
	S5_1600	AW	7/26/2010	75.03	−159.47	1600	−0.3	34.9	9.2	0.3	0	28.1	99.966
S6	S6_50	PSW	7/31/2010	75.98	−156.44	50	0	30.2	12.1	0.5	12.1	24.2	98.903
	S6_300	AW	7/31/2010	75.98	−156.44	300	0.2	34.6	8.7	0.2	0	27.8	99.948
	S6_500	AW	7/31/2010	75.98	−156.44	500	0.8	34.8	9.4	0.1	0	27.9	99.965
	S6_800	AW	7/31/2010	75.98	−156.44	800	0.2	34.9	9.6	0.1	0	28.0	99.991

[Ψ] SMLW: Surface mixed layer water; PSW: Pacific summer water; PWW: Pacific winter water; IW1: Intermediate water (boundary depth of PWW); IW2: Intermediate water (boundary depth of AW); AW: Atlantic water.

3. Results

3.1. Hydrographic features

The Arctic water masses at lower depths were segregated into cold-fresh, cold-saltier, and warm-saltiest water. The hydrographic features of the sampling stations in this study were summarized in Table 1 and Fig. 1. Depth correlated significantly with temperature ($r=0.532$, $p<0.05$) and salinity ($r=0.957$, $p<0.01$), as well as with several other properties of the water mass: DO ($r=-0.796$, $p<0.01$), fluorescence ($r=-0.688$, $p<0.05$), PAR ($r=-0.864$, $p<0.01$), and density ($r=0.957$, $p<0.01$). The water samples collected were projected onto vertical profiles of temperature and salinity (Fig. S1). The classification of the sampled water masses based on temperature and salinity was presented in a *T–S* diagram (Fig. 2). Consistent with previous studies (Carmack et al., 2008; Coachman and Barnes, 1961; Shimada et al., 2001; Steele et al., 2004), our results classified the samples into four types of water masses: (1) SMLW (surface mixed layer water; S2_10), (2) PSW (Pacific summer water; S6_50), (3) PWW (Pacific winter water; S1_10, S1_30, S1_50, S2_40, S2_80, and S3_90), and (4) AW (Atlantic water; S3_145, S4_1800, S5_500, S5_1000, S5_1600, S6_300, S6_500, and S6_800). These water masses can be more clearly characterized by their physical traits. In particular, light irradiation (i.e., PAR value) was measured at sampled depths. A PAR value of zero identifies samples from the euphotic zone (S1_10, S1_30, S1_50, S2_10, S2_40, and S6_50), whereas other values represent the deep sub-euphotic zone (S2_80, S3_90, S3_145, S4_1800, S5_500, S5_1000, S5_1600, S6_300, S6_500, and S6_800). Fluorescence was used to determine the subsurface chlorophyll maximum (SCM) layer within the PWW, and only S1_30, S1_50, and S2_40 were included in this layer. Marked changes in the DO and light transmission were observed between S3_90 and S3_145, which refer to the Pacific and Atlantic halocline in the *T–S* diagram (Fig. 2).

3.2. Bacterial diversity and taxonomic classification

Beta diversity analysis using PCoA (Fig. 3) revealed that some of the samples exhibited mismatches with their own water masses, although most of the samples within individual water masses of the *T–S* diagram (Fig. 2) were clustered in the same PCoA. For example, two types of intermediate water (IW) were detached from the PWW and AW clusters: IW1 (boundary depth of PWW; S3_90) and IW2 (boundary depth of AW; S3_145), respectively.

To compare bacterial diversity among different water masses, we estimated alpha diversity indices (species richness and evenness) (Table 2). The AW and SMLW samples exhibited the highest and lowest indices, respectively, for both species richness and evenness. A comparison of the Pacific water samples showed that PSW harbored more diversity than PWW. Overall, the SMLW and PSW, which were differentiated in the *T–S* diagram and the PCoA,

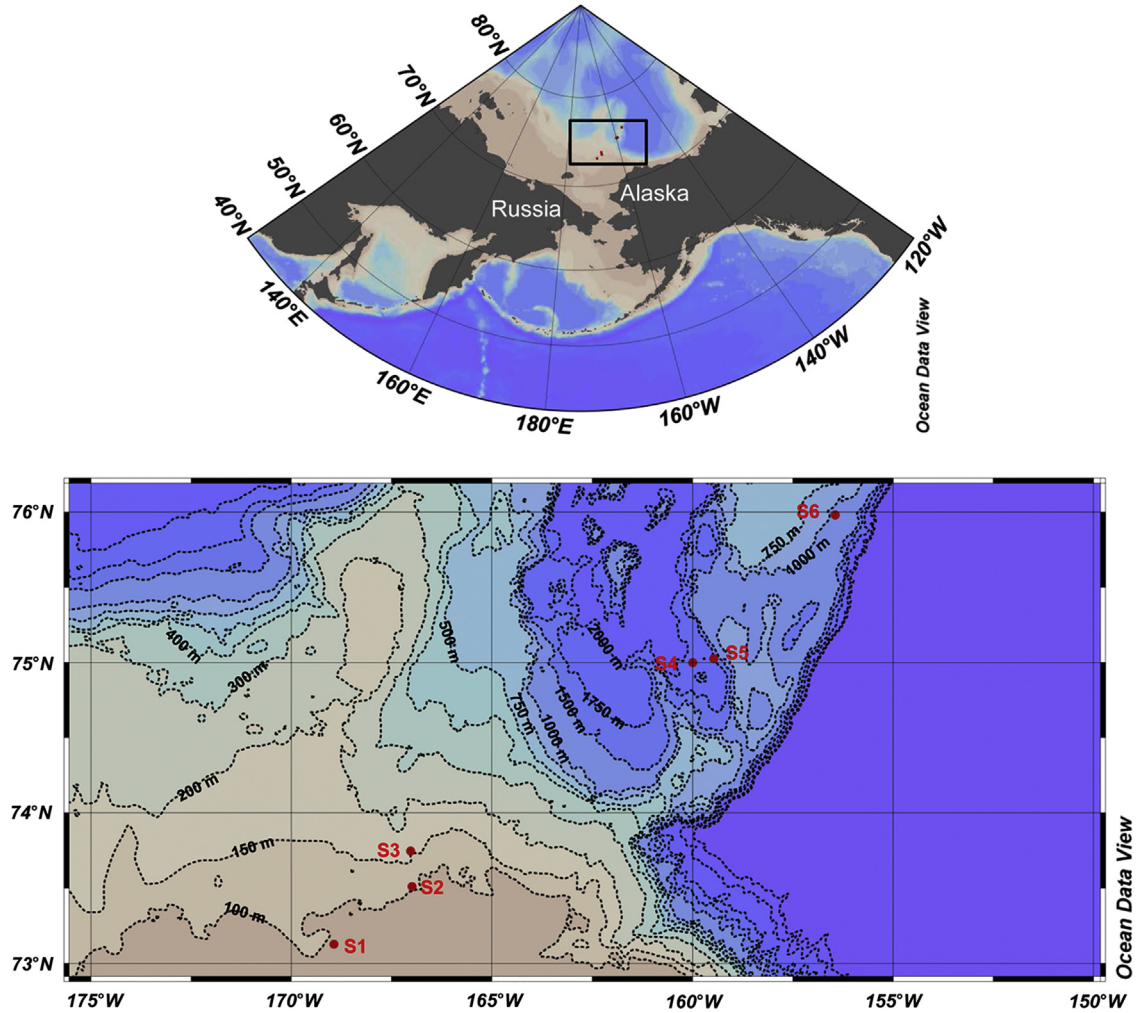

Fig. 1. Sampling locations in the Chukchi Sea in 2010. Closed red circles denote the sampling stations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

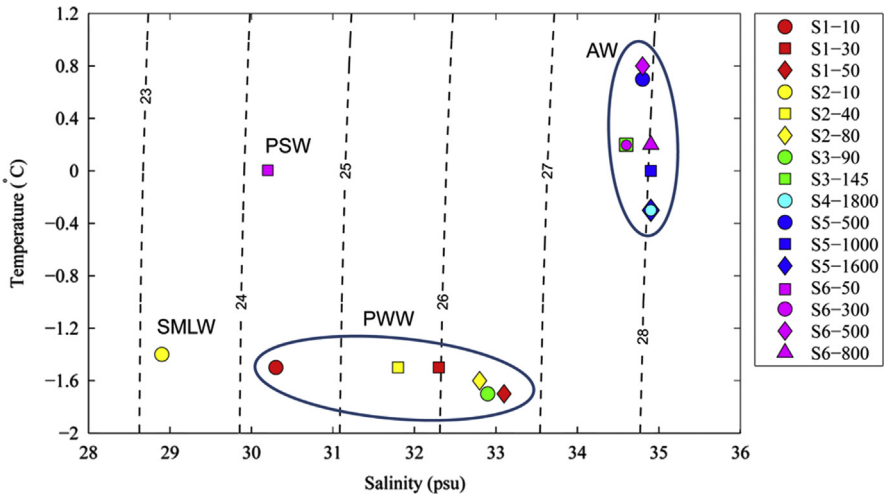

Fig. 2. Temperature–salinity (*T–S*) diagram. Temperature and salinity at sampled depths were used to identify water masses. The dashed line indicates the isopycnal curve (lines of equal density) determined by the interaction of temperature and salinity.

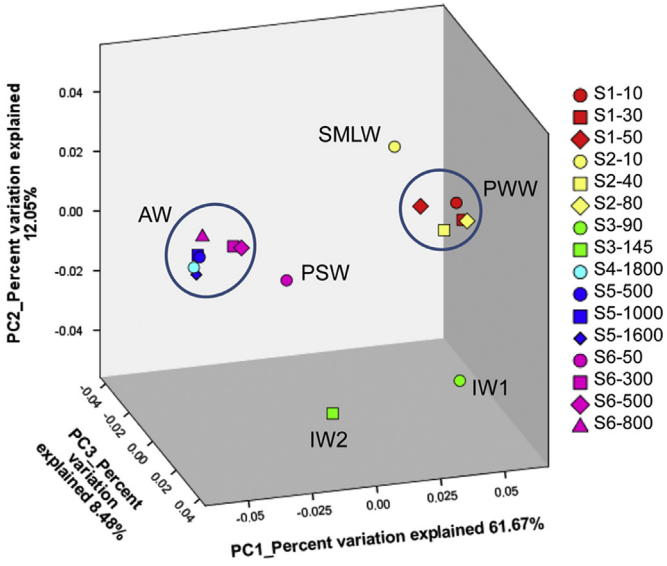

Fig. 3. Beta diversity analysis. Beta diversity of bacterial communities is expressed through Principal Coordinates Analysis (PCoA) and represents the differences among samples. Comparison of the variation between samples is explained along the PC1 (61.67%), PC2 (12.05%) and PC3 (8.48%) axes. Ellipses were drawn manually to aid in the visualization of results. AW: Atlantic water; IW1: intermediate water of PWW; IW2: intermediate water of AW; PSW: Pacific summer water; PWW: Pacific winter water; SMLW: surface mixed layer water.

were also differentiated from the other samples. In addition, alpha diversity indices showed that bacterial diversity was lower in the PWW compared with that in AW. By contrast, the diversity in both IW1 and IW2, located between PWW and AW, were distinct from those in PWW and AW but relatively similar to each other.

PCA was used to analyze variation of water mass properties in the samples (Fig. 4). In the PCA ordination, the eigenvalues for axes 1 and 2 were 0.9875 and 0.0125, respectively, which explained the variation at 98.75%. In addition, axis 1 was primarily defined by depth, whereas axis 2 was mostly related to PAR. PCA revealed that most samples of the sub-euphotic zone, clustered into AW in the *T–S* diagram and PCoA, were spatially distributed along axis 1, whereas most samples of the euphotic zone, apart from S1_10 and S2_10, which positioned along axis 2, were tightly grouped. These PCA patterns suggest that samples correlated significantly with depth in the sub-euphotic zone.

Table 2
Alpha diversity analysis. Species richness is expressed by the Chao1 and Abundance-base Coverage Estimator (ACE) indices, and denotes the number of taxa in a community. Species evenness denotes the measure of the relative abundance of taxa and is expressed by the Shannon and Inverse Simpson indices.

Groups[Ψ]	Species richness[*]		Species evenness[*]	
	Chao1	ACE	Shannon	Inverse Simpson
SMLW	139	219	1.5	2.1
PSW	267	257	3.3	12.5
PWW	173 ± 48	216 ± 75	2.2 ± 0.1	3.6 ± 0.5
IW1	147	172	2.7	7.7
IW2	158	204	2.7	7.4
AW	436 ± 125	558 ± 183	3.3 ± 0.4	7.4 ± 2.1

[*] Average value and standard deviation.
[Ψ] SMLW: Surface mixed layer water (S2_10); PSW: Pacific summer water (S6_50); PWW: Pacific winter water (S1_10, S1_30, S1_50, S2_40, and S2_80); IW1: Intermediate water (boundary depth of PWW, S3_90); IW2: Intermediate water (boundary depth of AW, S3_145); AW: Atlantic water (S4_1800, S5_500, S5_1000, S5_1600, S6_300, S6_500, and S6_800).

In this study, most of the sequences were assigned to the phylum *Proteobacteria* (Table S1). The majority of bacterial sequences were assigned as *Alphaproteobacteria* at class level, followed by *Gammaproteobacteria*, *Flavobacteria*, *Deltaproteobacteria*, SAR406, and SAR202. The composition of bacterial communities at our research area (Chukchi Sea) was similar to that reported in a previous study undertaken in the Canadian Basin, Eurasian Basin, and Baffin Bay (Galand et al., 2009). Although the relative abundance of the predominant bacterial populations varied in water samples, their relative abundance seemed to be similar within the clustered water mass (Fig. 5 and Table S2). Among these major populations, the relative abundance of *Alphaproteobacteria* exhibited a positive correlation with that of *Flavobacteria* ($r=0.529$, $p<0.05$), whereas the relative abundance of *Deltaproteobacteria* inversely correlated with that of both *Alphaproteobacteria* ($r=-0.832$, $p<0.01$) and *Flavobacteria* ($r=-0.682$, $p<0.01$) (Table S3). These correlations between the predominant bacterial populations were clarified further in the composition of bacterial communities across types of water mass (Fig. 5). *Alphaproteobacteria* was higher in SMLW, PSW, and PWW, but relatively lower in IW1, IW2, and AW. By contrast, *Gammaproteobacteria* was relatively higher in the samples of IW1 and IW2 as compared to the others. Interestingly, the relative abundance of *Flavobacteria* was the highest in PWW and IW1, but the relative abundance of *Deltaproteobacteria*, SAR406, and SAR202

was the highest in only AW. These patterns were similar to those of the composition of bacterial communities at the family level (Fig. S2 and Table S4).

The predominant populations at class level, apart from SAR406 and SAR202, were ubiquitous in all of the water masses tested. These populations consisted of a majority subpopulation at the family level. For example, *Alphaproteobacteria* was mostly represented by sequences belonging to SAR11 group I (family level). The relative abundance of SAR11 group I showed that the conserved proportion in PWW gradually decreased with depth (Fig. S2). In contrast, *Gammaproteobacteria* sequences were more diverse. The most abundant subpopulation of *Gammaproteobacteria* sequences belonged to *Ruthia* (family level), which contains free-living chemoautotrophs (Newton et al., 2007). Other abundant subpopulations of *Gammaproteobacteria* sequences belonged to the uncultured clusters AY552545 and AM402959 (family level). The AY552545 cluster consisted of marine bacteria producing proteorhodopsin (PR), a bacterial photoactive proton pump (Sabehi et al., 2004), and was mostly found in both SMLW and PSW. The AM402959 cluster was first sequenced from a bacterial endosymbiont of bivalves (Rodrigues et al., 2013), and found in both IW1 and IW2 in the present study. The majority of *Deltaproteobacteria* sequences were assigned to SAR324 at the family level, and most of *Flavobacteria* sequences were assigned to *Flavobacteriaceae* (family level). Overall, the bacterial distributions at the class level were similar to those of subpopulations at the family level (Figs. 5 and S2). However, most of these subpopulations (family level), with the exception of SAR11 group I, were relatively less ubiquitous or dominant in all samples. Thus, the frequency of major bacterial populations at the class level and SAR11 group I, the majority subpopulation of *Alphaproteobacteria*, were analyzed further using a rank correlation test to avoid statistical bias.

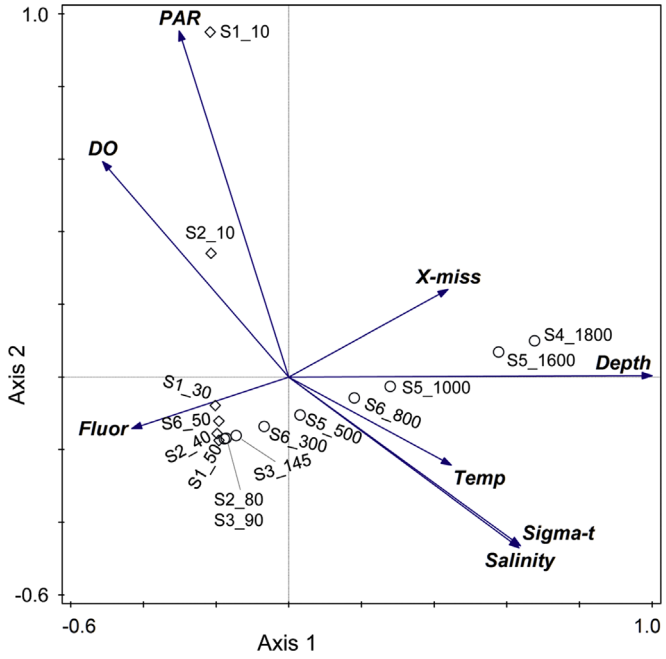

Fig. 4. Principal component analysis (PCA) ordination diagram. The diamonds indicate samples in the euphotic zone, and the circles indicate sub-euphotic samples.

3.3. Correlation between major bacterial populations (class level) and properties of water mass

The relationship between the relative abundance of predominant bacterial populations and properties of water mass was explored in this study. To test which water mass property relates significantly to

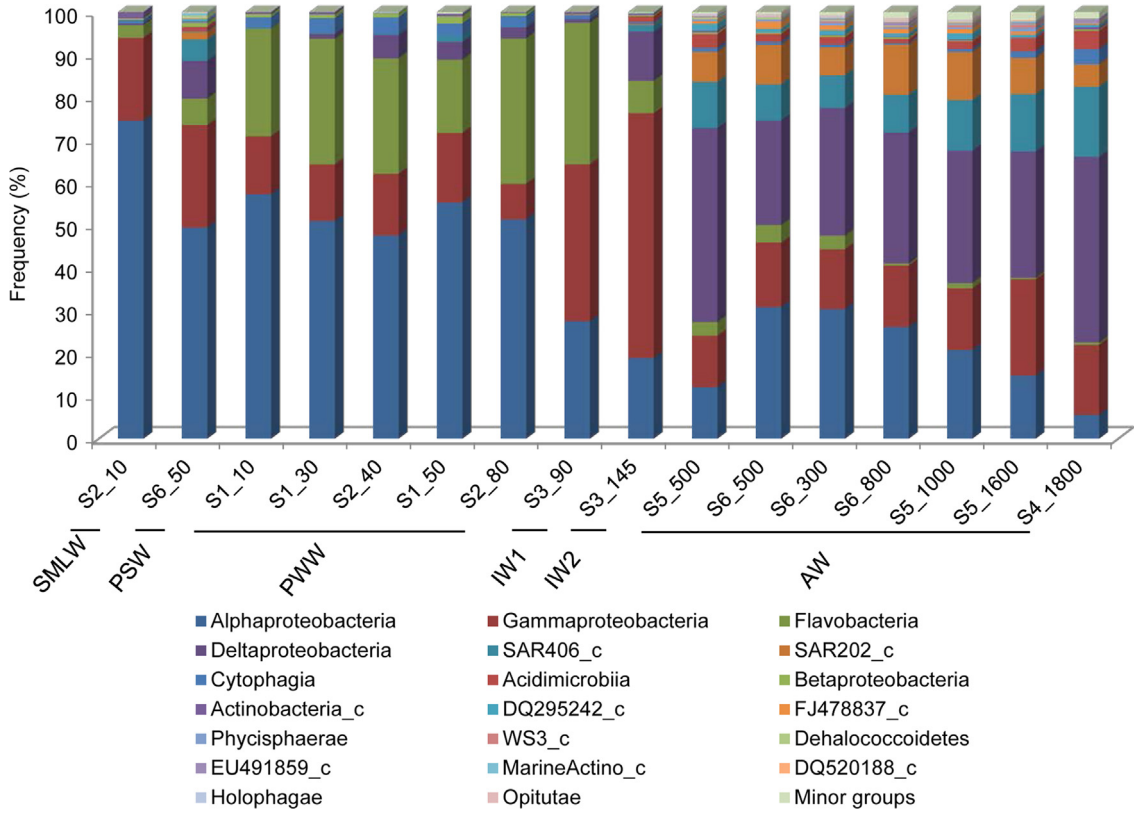

Fig. 5. Bacterial community composition in the samples (class level). The minor groups denote the sum of rare populations that were arbitrarily defined as having a frequency $< 1\%$ within a sample. The percentages of all taxa and their sequence numbers are listed in Table S2.

the distribution of bacterial population, we performed a nonparametric correlation analysis between the predominant bacterial populations (*Alphaproteobacteria*, *Gammaproteobacteria*, *Flavobacteria*, *Deltaproteobacteria*, and SRA11 group I) and water mass properties in all samples. With the exception of *Gammaproteobacteria*, the populations tested exhibited significant correlation with most of the properties (Table S5). Among them, the relative abundance of *Alphaproteobacteria* exhibited the clearest linear correlation with depth (correlation coefficient = −0.88, $p < 0.01$) and density (sigma-t; correlation coefficient = −0.85, $p < 0.01$) as compared to the other properties (Fig. 6). The relative abundance of *Deltaproteobacteria* also appeared to be dependent on depth (correlation coefficient = 0.87, $p < 0.01$) and density (correlation coefficient = 0.83, $p < 0.01$), but this population seemed to lean more towards deeper sub-euphotic waters (Fig. S3A). Similarly, *Flavobacteria* exhibited a bias toward the euphotic zone (Fig. S3B), and *Gammaproteobacteria* correlated relatively less linearly with the tested properties (Fig. S3C). Therefore, we excluded these biased data in the subsequent regression analysis to avoid erroneous statistical conclusions.

The causality associated with the strong correlation of the selected population and its subpopulation (*Alphaproteobacteria* and SAR11 group I) with the water mass properties was estimated through a linear regression analysis. The relative abundance of *Alphaproteobacteria* was explained by depth alone, with an accuracy of 82.2% ($p < 0.01$; Fig. S4A). As expected, SAR11 group I, the subpopulation of *Alphaproteobacteria*, also correlated with depth alone (correlation coefficient = −0.83, $p < 0.01$; Table S6) and exhibited the clearest linear correlation with this property (Fig. 7). The linear correlation analysis revealed that depth alone explained the distribution of SAR 11 group I, with 83.4% accuracy ($p < 0.01$; Fig. S4B).

4. Discussion

The stratified structure of the Arctic water column is complex. SMLW is strongly affected by melting sea-ice and river discharge during summer. Beneath the mixed layer, the influx of Pacific water is significant, and the highest and lowest temperatures indicate PSW and PWW, respectively. AW flows below PWW. Our samples were collected along water columns and represent the oceanographic traits of PSW, PWW, AW, and SMLW. These water masses, identified by a *T–S* diagram, coincided with the bacterial diversity patterns determined through PCoA, with the exception of the IW1 (S3_90) and IW2 (S3_145) samples. That is, although most samples of PWW and AW were clearly differentiated by their bacterial diversity patterns, the boundary layers (IW1 and IW2) between PWW and AW showed a distinct difference in bacterial diversity from that predicted by water mass identification. Mechanically, PWW is considered to influence AW (Aagaard et al., 1981). Woodgate et al. (2005) suggested that diapycnal mixing at near-freezing layer (herein designated as PWW) near 33.1 psu and the lower layer of the halocline near 34.3 psu and −0.6 °C (herein designated as AW) usually occurred at depths of 120–180 m in the western Arctic Ocean. Therefore, we assumed that the mismatched pattern of the IW1 and IW2 in the PCoA and *T–S* diagram as well as the marked changes in DO and light transmission, represent an intermediate mixing layer between PWW and AW rather than an experimental bias. These findings indicate that the bacterial communities are differentiated based on the properties of water mass, and that the boundary layers of both PWW (IW1) and AW (IW2) represent a bacterial community whose composition is influenced by the intermediate nature of the water mass.

Bacterial diversity in the Arctic euphotic zone could be affected by melting sea-ice. The alpha diversity indices showed that the diversity of the SMLW sample was markedly lower than that of the other water masses, which implies that the input of fresh water from melting sea-ice or river discharge directly or indirectly influences bacterial diversity. Similarly, the difference in alpha diversity between PWW and PSW may also be caused by freshwater input. However, the deeper sub-euphotic waters (AW) in the water columns exhibited higher alpha diversity indices as compared to those from the other waters samples from the euphotic zone. Thus, the higher alpha diversity and the variation in composition of bacterial communities in response to the depth gradient in the sub-euphotic zone suggest that the bacterial diversity of the Arctic sub-euphotic zone is not homogenous.

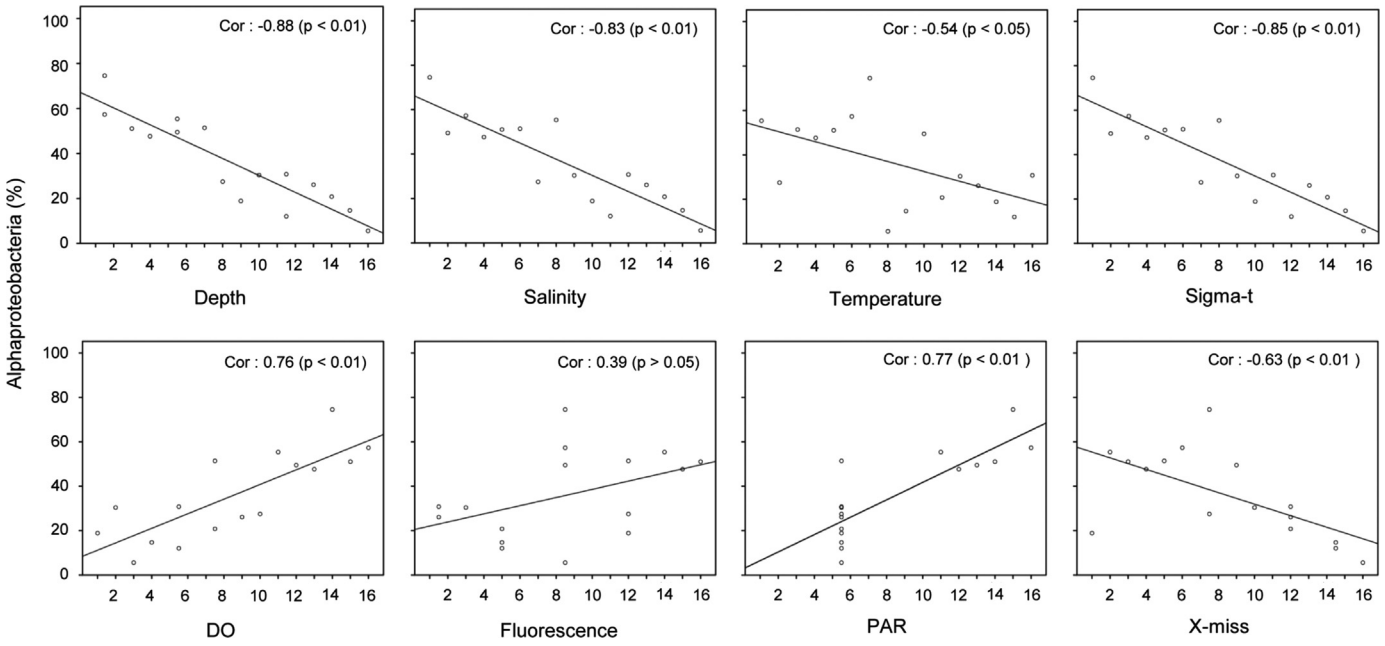

Fig. 6. The relative abundance of *Alphaproteobacteria* correlated with water mass properties in the samples. The correlation coefficients (Cor) and associated *p*-values (listed in Table S5) are shown for *Alphaproteobacteria*. The *x*-axis scale assigns 1 to the lowest-ranking of property.

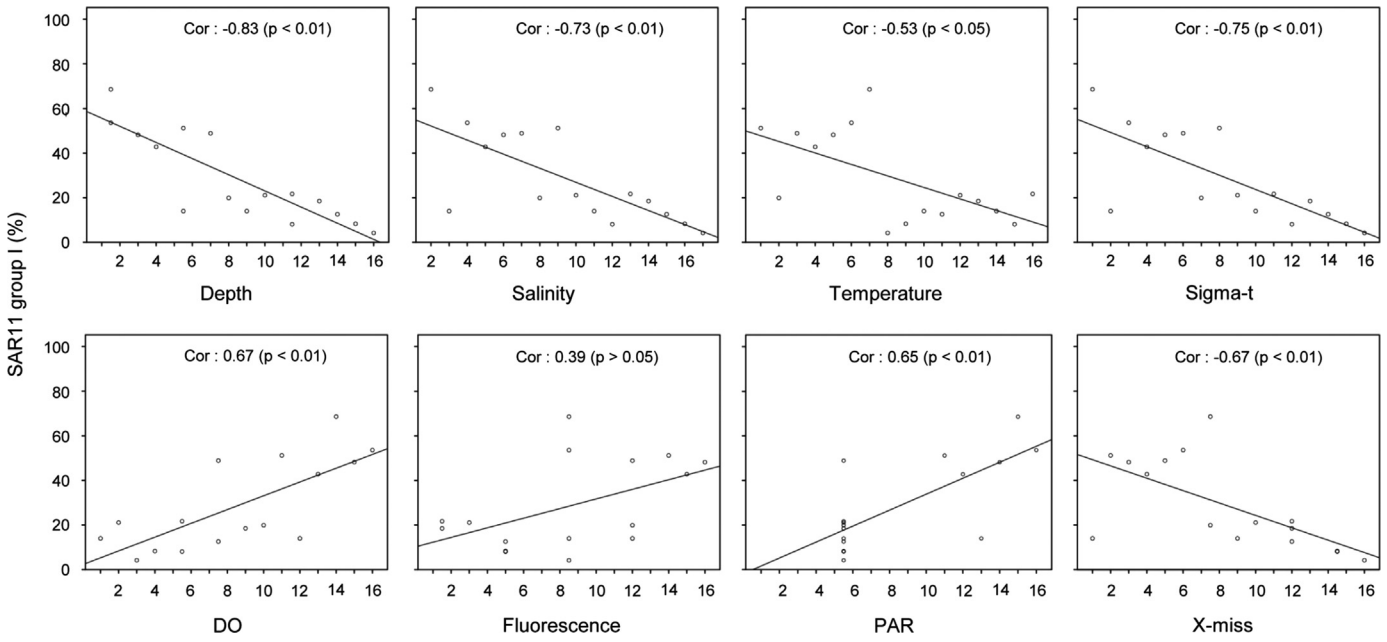

Fig. 7. The relative abundance of SAR11 group I correlated with water mass properties in the samples. The correlation coefficients (Cor) and associated *p*-values (listed in Table S6) are shown for SAR11 group I. The *x*-axis scale assigns 1 to the lowest-ranking of property.

Arctic water columns have a bacterial biogeography, and the bacterial distribution of that can be explained by the Arctic Ocean hydrography (Galand et al., 2009). According to documented bacterial communities in the Arctic Ocean, *Proteobacteria* is the most dominant, and both the gamma and alpha subtypes predominate in the euphotic zone (Bowman et al., 2012; Collins et al., 2010; Comeau et al., 2011; Galand et al., 2008; Han et al., 2014; Kirchman et al., 2010; Pommier et al., 2007), as shown in the present study. However, *Gammaproteobacteria* is more prevalent in surface water near coastal areas (Kellogg and Deming, 2009; Malmstrom et al., 2007), and *Alphaproteobacteria* is more predominant far from the coast (Bano and Hollibaugh, 2002; Collins et al., 2010; Galand et al., 2008; Han et al., 2014; Kirchman et al., 2010).

Furthermore, Zeng et al. (2013) have documented the occurrence of *Actinobacteria* and *Cyanobacteria*, which were previously reported in polar freshwater environments of the Arctic (Jungblut et al., 2010; Larose et al., 2010; Waleron et al., 2007) as well as in sea-ice (Bowman et al., 2012; Han et al., 2014) in the euphotic zone. These populations, however, were not primarily observed in the current study; this could be because the samples analyzed in Zeng et al.'s study were obtained closer to coastal areas, unlike the samples analyzed in our study. Therefore, we assumed that the patterns of variations in *Proteobacteria* subtypes and the presence of *Actinobacteria* and *Cyanobacteria* might be significant for pelagic bacterial communities affected by the river inflow along the arctic coastline from melting ices.

In this study, the variation in bacterial diversity between PWW and AW was explained by the results obtained from taxonomic analysis. The taxonomic classification confirmed that the structure of the bacterial community along water columns exhibits biogeographic patterns in response to the types of water mass, which most likely acts as a physical barrier that limits dispersal and curbs an assembly of bacterial communities on a macroscopic scale. For example, the predominance of the major populations (*Alphaproteobacteria*, *Gammaproteobacteria*, *Flavobacteria*, and *Deltaproteobacteria*) was variable along the water column, but their relative abundance seemed to be conserved within clustered waters (PWW or AW). This result indicates that PWW and AW each have a local bacterial community, supporting the hypothesis that different water masses harbor different bacterial communities (Galand et al., 2009).

Microscopically, our results also suggest that the interaction of bacterial communities in euphotic and sub-euphotic zones is linked by an individual dispersal capability of the bacterial population. For example, we found the gradual decrease of SAR11 group I, a major subpopulation of *Alphaproteobacteria*, from PWW to AW, which could be explained by its dispersal limitation between water masses. Generally, bacterial communities from the upper euphotic zone are composed of phototrophs and heterotrophic bacteria such as SAR11 group I. On the contrary, the sub-euphotic zone harbors different communities including chemotrophs (Hansman et al., 2009; Herndl et al., 2008), as demonstrated in the present study. We assumed that SAR11 group I is the most predominant in the arctic euphotic zone and it disperses freely into the deeper sub-euphotic zone through the water mass barrier, and may be restricted in the dark zone (unfavorable habitat), as shown in the current study. Galand et al. (2009) have suggested that sinking particles with attached microbes may reach greater depths through water mass barriers, supporting our hypothesis. Similarly, the dispersal of various planktons, including bivalve larvae, is freely and continually, but limited at favorable areas for viable populations (Darling et al., 2004; Sexton and Norris, 2008). This process may account for the specific presence of AM402959, a representative bacterial endosymbiont of bivalves, and the *Ruthia* cluster, composed of organisms known as free-living chemoautotrophs, in the intermediate layers (IW1 and IW2) between PWW and AW. Moreover, we found biological evidence for the intermediate-mixing layers (IW1 and IW2) between PWW and AW, suggested previously by Arctic oceanographic groups (Aagaard et al., 1981; Shimada et al., 2005; Weingartner et al., 1998; Woodgate et al., 2005).

The correlation between major bacterial populations and water mass properties was investigated to explain which environmental factors affected bacterial distributions; the correlation was estimated using a linear regression analysis. Only *Alphaproteobacteria* and its major subpopulation, SAR11 group I, were considered in the regression analysis for statistical reasons. Although other major populations were not tested in the regression analysis owing to their frequency bias, we assume that *Alphaproteobacteria*

and its subpopulation represent a general trend of population dynamics in bacterial communities along water columns because of their dominance in the general bacterial community. In our results, the population dynamics was explained by strong linear correlations of the relative abundance of *Alphaproteobacteria* with both depth and water density. However, the linear regression analysis revealed that among the water mass properties tested, depth was the only causal factor for the variation of *Alphaproteobacteria*. This result was also confirmed by the population dynamics of SAR 11 group I. Thus, we hypothesize that environmental heterogeneity caused by the depth change may play a key role in defining the ecological niche of SAR11 group I.

We developed a ˋsinking bacterial hypothesis´ for population dynamics of SAR11 group I from the following arguments. First, the deeper the depth, the harsher the environment because depth is a proxy for light irradiance and barometric pressure. Microbes including SAR11 group I might lose their viability when exposed to a harsh environment, characterized by low temperature, absence of photosynthesis, lack of nutrients or some combination thereof. Second, the relative abundance of SAR11 group I sharply decreased from halocline, formed by dense water sinking toward the deeper layers of the ocean (Jones et al., 1995). The distribution of SAR11 group I, more abundant in the euphotic zone than in the sub-euphotic zone, may be in large part due to the dense water sinking. Third, proteorhodopsin (PR) phototrophy might provide the energy required for survival during periods of low nutrient concentration (González et al., 2011) observed in oceanic environments. SAR11 clade encodes PR (Giovannoni et al., 2005; Rappé et al., 2002); however, its light-to-biochemical energy conversion, enabled by the PR photosystem for biosynthetic purposes (Martinez et al., 2007), is ineffective in the deeper, sub-euphotic zone. Thus, the population dynamics of SAR11 group I from PWW to AW supports the sinking bacterial dispersal hypothesis. Our hypothesis also applies to the populations dynamics of *Flavobacteriaceae* between PWW and AW. *Flavobacteriaceae* is prevalent in the euphotic zone, and contains bacteria that encode PR (Gómez-Consarnau et al., 2007; Kirchman et al., 2010) as SAR11 clade. The energy-yielding system of both SAR 11 group I and *Flavobacteriaceae* may be either lost or weakened in the sub-euphotic zone, and these bacteria ultimately become extinct when exposed to increased barometric pressure. By contrast, SAR324 and SAR406 groups are abundant in the lower surface layer of water columns on a global scale (Gallagher et al., 2004; López-García et al., 2001; Pham et al., 2008; Wright et al., 1997) and in the deeper sub-euphotic zone in the Arctic. Their characteristics of growth, metabolism, or both, may allow these otherwise nonculturable, barophilic populations to overcome extinction in the deeper sub-euphotic zone.

In conclusion, we investigated stratified water columns primarily consisting of Pacific and Atlantic water masses in the western Arctic Ocean to estimate the spatial variations in the composition of bacterial communities along water columns. In this study, we did not consider the inter-species competition within bacterial communities or between bacterial and plankton communities. However, we assumed that the predominant bacterial populations are less likely to be influenced by phytoplankton dynamics because we found no variation in the bacterial populations within PWW samples, which includes the subsurface chlorophyll maximum layer (a favorable habitat for phytoplankton). Arctic bacterial communities exhibit biogeographic patterns related to the type of water mass. The ecological patterns of bacterial communities in deep water might be influenced by the hydrographic characteristics of the Arctic.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dsr2.2015.01.018.

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