• Article •

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Spatial heterogeneity of a microbial community in Kongsfjorden, Svalbard during late summer 2006 and its relationship to biotic and abiotic factors

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Abstract The 16S and 18S ribosomal ribonucleic acid genes of microbial organisms collected from the contrasting environments (temperature, salinity, silicate, phosphate and nitrate, p <0.05) of the inner and outer basins of Kongsfjorden (Spitsbergen, Arctic) were studied using polymerase chain reaction-denaturing gradient gel electrophoresis(DGGE) fingerprinting. Comparison of the microbial fingerprints and the physicochemical parameters revealed that molecular methodology exhibited a greater sensitivity. Sequences obtained from bacterial DGGE were affiliated with four main phylogenetic groups of bacteria:Proteobacteria(Alpha, Beta and Gamma), Bacteroidetes, Verrucomicrobia and Cyanobacteria. The relationships between the genotype distribution of these microbes and associated biotic/abiotic factors, revealed by canonical correspondence analysis, showed that Station 1 at 30 m (outer fjord) was grouped separately from the other sites. This difference could be a consequence of the thermocline and base of the euphotic layer at this depth where the Atlantic and Arctic-type waters overlapped.

Keywords **Microbial community, Genetic diversity, Kongsfjorden, Arctic fjord**

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0 Introduction

Kongsfjorden, a glacial fjord in the Arctic, is located along the northwest coast of Spitsbergen in the Svalbard Archipelago. The seasonal hydrography of this region is dominated by the balance of Atlantic, Arctic and glacial-melting waters, which substantially impact on both the physical and biological variation in this fjord system[1]. A rapid and overwhelming intrusion of Atlantic water across the shelf and into the fjord occurs during midsummer, and the fjord water switches from being Arctic dominant to Atlantic dominant[2]. The Atlantic water, which carries relatively warm and salty water into the fjord system, strongly alters the species composition towards more boreal species. In contrast, the glacial input and distance from the coast tend to make the inner part of the fjord assume more Arctic-like characteristics. The strong environmental gradients from the inner to the outer fjord may induce large changes in community composition and abundance in different regions of the fjord[3–4] and it is therefore worthwhile to develop methods to monitor the coastal ecosystem, thereby allowing predictions of the potential effects of these gradients on marine ecosystems to be made.

In Kongsfjorden, nano-phytoplankton and pico-phytoplankton represented about 92.36% of the total biomass in 2005 (He Jianfeng, unpublished). Many studies have firmly established the importance of microorganisms (organisms ≤ 20 μmol/L in size; i.e, bacteria and protozoa)

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in energy flow and nutrient cycling in marine planktonic ecosystems[5–7]. Microbial organisms exhibit complex and sensitive responses to environmental stimuli that are manifested through changes at the individual, population, and community levels of organization. Therefore, changes in the microbial community are reflected in relatively rapid shifts in density and species composition[8]. DNA fingerprinting methods, such as polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (PCR-DGGE), have provided us with new opportunities to better understand the temporal dynamics and spatial variation of microbial communities. DGGE analysis banding patterns for highly diverse microbial communities reflect the number and relative abundance of dominant ribosomal ribonucleic acid (rRNA) or functional deoxyribonucleic acid (DNA) types in a sample and thus allow comparisons to be made between communities. After pattern digitization, the coefficients of similarity between banding patterns of different gel strips can be calculated. This allows for dendrograms to be generated and subsequent grouping of samples according to similarities in their microbial community profiles. Analysis of the 16S rRNA gene via PCR-DGGE has been one of the most popular methods for examining prokaryotic community diversity[9–10].

Savin (2004) compared eukaryotic plankton diversity as measured by morphological and DGGE fingerprinting methods, and Terrado et al. (2008) reported that most components of the eukaryotic community varies positively with the environment based on DGGE and microscopic analyses[11–12]. More recently, both bacterial and eukaryotic planktonic organisms were studied using PCR-DGGE, and cluster analysis of the data indicated that DGGE fingerprinting could group communities on the basis of environmental habitats[13].

The objective of our study was to determine the depth profile and distribution of microbial diversity in the outer and inner fjords. In addition, comparative analysis was conducted to explore the relationships between genetic diversity and environmental factors (including both abiotic and biotic parameters).

1 Materials and methods

1.1 Sampling site and procedures

Oceanographic data and water samples were collected on 17 August 2006 at two sampling sites; one of which was in the outer fjord (Station 1 or K1), and the other was in the inner fjord (Station 5 or K5) (Figure 1). Water samples for determining physicochemical characters and microbial abundance were collected with a 2.5 L Niskin water sampler at 0, 5, 30, 100, 150 and 200 m depths at Station 1. The deepest sample taken at Station 5 was at 40 m because of the water column depth. The deepest sample taken at Station 5 was at 40 m because of the water column depth.

Sea-water samples for DNA analysis were collected directly from the Niskin bottles and placed into clean bottles that had been rinsed with acid and then with Milli-Q water, followed by three rinses with sample water prior to filtering. The microbial biomass was collected through a 47mm diameter, 0.2 μmol/L pore size Waterman membrane filter, after being passed through a 50 μmol/L mesh prefilter. Microbial samples were collected by filtering 300-800 mL of seawater under < 5 mm Hg pressure. Filters were frozen at –80℃ in lysis buffer (40 mmol/L EDTA, 50 mmol/L Tris-HCl, 0.75 M sucrose) until nucleic acid was extracted.

Samples (100 mL) for nutrient analysis were filtered through 0.45 μmol/L pore-size GF/F filters, before being fixed with HgCl solution and stored at –20℃. The concentration of nitrate, phosphate, and silicate were measured using a continuous flow nutrient analyzer [Skalar San++, Skalar UK (Ltd), York, UK] over the duration of one month.

1.2 Microbes counting procedures

4’-6-Diamidino-2-phenylindole-stained bacterial cells were counted at ×1000 using epifluorescence microscopy (Nikon Japan). Protists (< 20 μmol/L) were counted at ×1000 and > 20 μmol/L protists at ×160－400, as described by Sherr et al.[14]. Autotrophs were distinguished from heterotrophs by the auto-fluorescence of chlorophyll *a* using different filters. Diatoms and ciliates were distinguished from other protists by morphological analysis.

1.3 DNA extraction and PCR amplification

DNA was extracted according to the method described by Luo[15]. Bacterial and eukaryotic 16S and 18S rRNA genes were amplified with specific primers[16-18] (Table 1). PCR mixtures (50 μL) contained 1×buffer, 200 μmol/L of each deoxynucleoside triphosphate (TAKARA), 1.5 mmol/L $MgCl_2$ (TAKARA), 0.3 μmol/L of each primer, 2.5 U of Taq DNA polymerase (TAKARA) and approximately 50 ng of template DNA. PCR amplification of the bacteria 16S rRNA gene involved an initial denaturation step at 95℃ for 10 min and 10 touchdown cycles of denaturation at 95℃ for 45 s, a step at the annealing temperature (Table 1) for 1 step at 94℃ for 130 s, followed by 35 cycles of denaturation at 94℃ for 30 s, annealing at 56℃ for 45 s, and extension at 72℃ for 130 s. During the last cycle of both assays, the time of the extension step was increased to

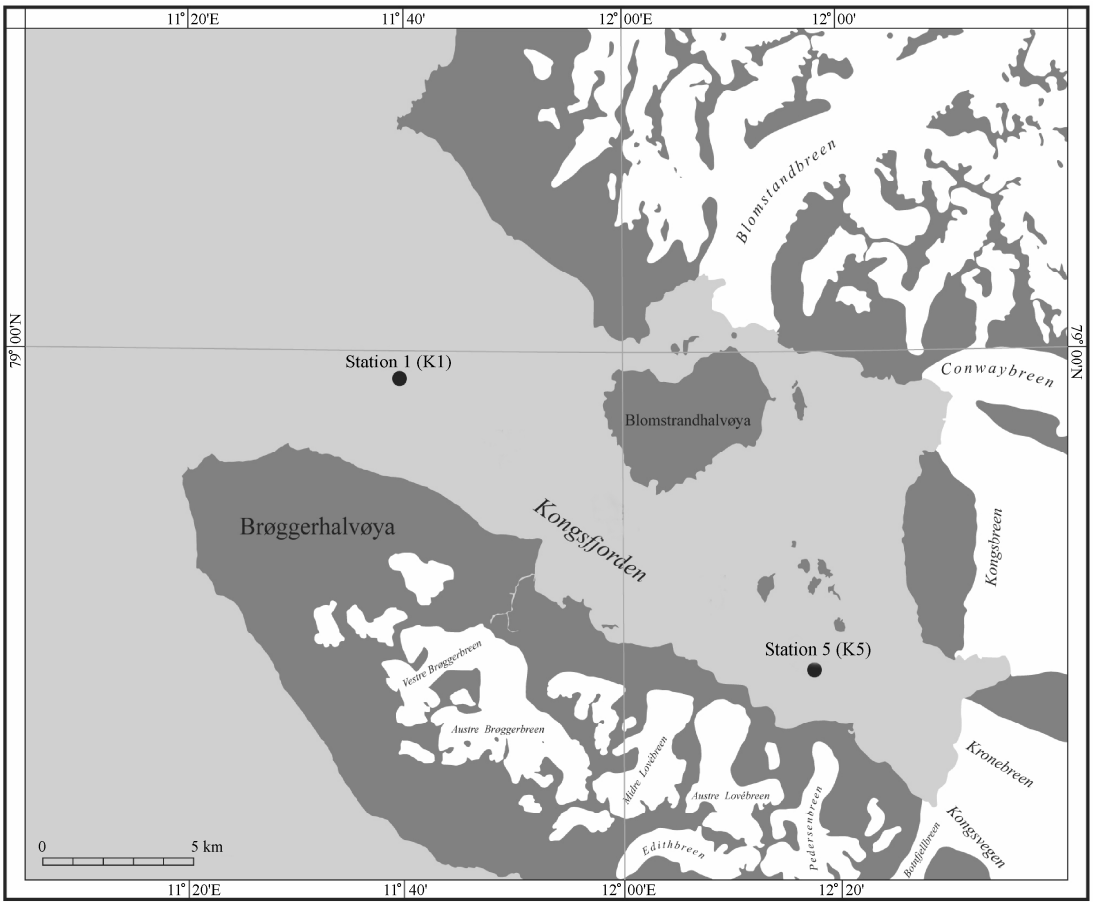

Figure 1 Map of sample stations in Kongsfjoren.

10 min. PCR product aliquots were electrophoresed in a 0.8% agarose gel, and then stained with ethidium bromide.

1.4 DGGE analysis and statistical analysis

DGGE was performed using a DCode Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA). Electrophoresis was performed with 0.16 mm-thick 6% polyacrylamide gels (ratio of acrylamide to bisacrylamide, 37.5:1) submerged in 1×TAE buffer (40 mmol/L Tris, 40 mmol/L acetic acid, 1 mmol/L EDTA; pH 7.4) at 60℃. Approximately 800 ng to 1 μg of each PCR product was applied to individual lanes in the gel. Denaturants of 40%–60% and 25%–50% (100% denaturant was defined as 7 M urea and 40% deionized formamide) were applied to separate the 16S and 18S rRNA genes, respectively. Electrophoresis was performed at 160 V for 5 h for the 16S rRNA gene and at 100 V for 16 h for the 18S rRNA gene. After electrophoresis, gels were stained with 3×GelRedTM (Biotium). Gel images were captured with a Gel Doc 2000 Gel documentation system (Bio-Rad, Hercules, CA, USA) and digitized using Quality One Software version 4.6 (Bio-Rad).

Table 1 Primer sequences and annealing temperatures used in PCR amplification

Primer	Oligonucleotide sequence (5'-3')	Annealing temperature	Specificity	Reference
534R	CCGTCAATTCMTTTRAGTTT	65–55℃ (10 cycles[b]) then 55℃ (20 cycles)	Bacteria	16
341F[a]	CCTACGGGAGGCAGCAG			
Euk 1A	CTGGTTGATCCTGCCAG	56℃	Eukarya	17
Euk516 A	ACCAGACTTGCCCTCC			18

a The GC clamp sequence is CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG.

b With the temperature decreasing 1℃ for each cycle.

Scanned DGGE profile patterns were checked manually. Each band was related to a single population and considered to be one operational taxonomic unit (OTU). OTUs were used as surrogates for the predominant "biodiversity units". For sample comparisons, band-match analysis was performed. The presence or absence of comigrating bands, independent of intensity, was converted to a binary (0/1) matrix. The Dice similarity matrix was used to conduct the unweighted pair-group analysis using arithmetic average (UPGMA) clustering. A dendrogram was then constructed from the UPGMA F values using Quality One Software version 4.6 (Bio-Rad). The Dice similarity coefficient (SD) was used to represent similarities between pairs of samples.

To test whether weighted-averaging techniques or linear methods were appropriate, detrended correspondence analysis (DCA) was performed using CANOCO for Windows 4.53 (Biometris, The Netherlands). The longest gradients resulting from DCA were 2.584 based on DGGE profiles, which did not indicate a clear linear or unimodal relationship[16]. We therefore performed canonical correspondence analysis (CCA) to compare species-environment correlations as described by Lepš and Šmilauer[19]. An automated forward selection was used to analyze inter-sample and interspecies distances. The variance inflation factor (VIF) of environmental variables was also calculated. Variables displaying a VIF value greater than 20 were excluded from CCA analyses, assuming collinearity existed between the respective variable and other variables included in the examined dataset. For all community ordination analyses, biplot scaling was used.

1.5 Bacterial DNA sequencing and phylogenetic analysis

Prominent DGGE bands were excised, eluted, repeatedly cleaned with additional DGGE gels, and reamplified using the primers 341F (without the GC-clamp) and 534R. The products were checked by electrophoresis on a 0.8% (w/v) agarose gel. Sequencing was performed using an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing primers were M13F and M13R, which targeted the plasmid.

Sequence data were checked for the presence of PCR-amplified chimeric sequences using the RDP Check-Chimera program. Alignments against the most closely related species, identified by BLAST searching the NCBI database, and known taxonomic sequences were conducted using ClustalW, version 1.74. Alignment files were edited with BioEdit, version 5.0. Phylogenetic analysis of the edited alignments was carried out using the PHYLIP package for Linux version 3.67 with evolutionary distances calculated by the Jukes-Cantor algorithm. Tree topology was inferred using the neighbor-joining method. Bootstrap analyses of 1000 replicates were used to estimate the reproducibility of tree topologies, and values over 50% are shown in bold on the tree reconstructions. Comparisons of sequence similarity were made using DNADIST within the PHYLIP software package.

The sequences reported in this paper have been deposited in the GenBank database under accession numbers GU167954-GU167973.

2 Results

2.1 Environmental parameters and main microbe groups

The abiotic factors (temperature, salinity, silicate, phosphate and nitrate) at Station 1 were significantly different from those at Station 5 (p <0.05) (Table 2). Briefly, the concentrations of phosphate at Station 5 were higher than those at Station 1, whereas, temperature, salinity, and the concentrations of silicate, and nitrate were comparatively lower. Water mass characteristics at the two stations also differed. A sharp halocline divided the water column in the Station 1 into upper brackish and lower marine water columns. Halocline depth ranged 20–40 m. Salinity above the halocline averaged 32.18 PSU, and below, averaged 34.83 PSU (Figure 2).

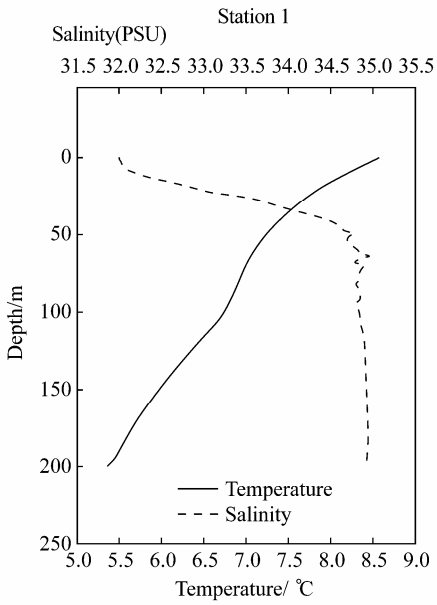

Figure 2 Salinity (PSU) and temperature (℃) profiles at the Station 1.

The biotic factors examined in this study were divided into five main groups within the 13 samples [bacteria,

autotrophic microflagellates (AMF), heterotrophic microflagellates (HMF), diatoms, ciliates]. Among all groups, bacteria accounted for 98.7% of the total number of organisms, whereas AMFs and HMFs accounted for 0.45% and 0.77%, respectively. Diatoms and ciliates accounted for only 0.03% and 0.002%, respectively. The vertical distribution of microbes at Station 1, except for diatoms, was approximately homogeneous (Figure 3a). The lowest abundances were all recorded at 30 m. In contrast, the vertical distribution of microbes at Station 5 was complex (Figure 3b). The abundance of bacteria at Station 1 was significantly lower than at Station 5 ($p < 0.05$).

Table 2 Comparison of environmental variables between Station 1 and Station 5

Variables	Station 1						Station 5							*p*
	0 m	5 m	30 m	100 m	150 m	200 m	0 m	2 m	5 m	10 m	20 m	30 m	40 m	
Temperature/℃	10	8.38	7.62	6.74	5.97	5.36	5.43	5.37	5.35	5.34	5.31	5.3	5.2	0.009
Salinity /PSU	31.9	33.22	33.79	34.84	34.92	34.99	30.05	30.02	30.07	31.5	32.25	33.01	33.37	0.007
Silicate/(μmol/L)	1.41	1.13	1.62	4.94	5.33	5.55	1.3	1.15	1.02	1.04	1.17	1.93	2.2	0.04
Phosphate/(μmol/L)	0.24	0.25	0.26	0.3	0.3	0.28	0.35	0.33	0.37	0.44	0.46	0.62	0.74	0.009
Nitrate /(μmol/L)	0.95	0.85	2.15	10.19	12.02	13.86	0.21	0.29	0.31	0.67	1.15	3.11	4.07	0.046

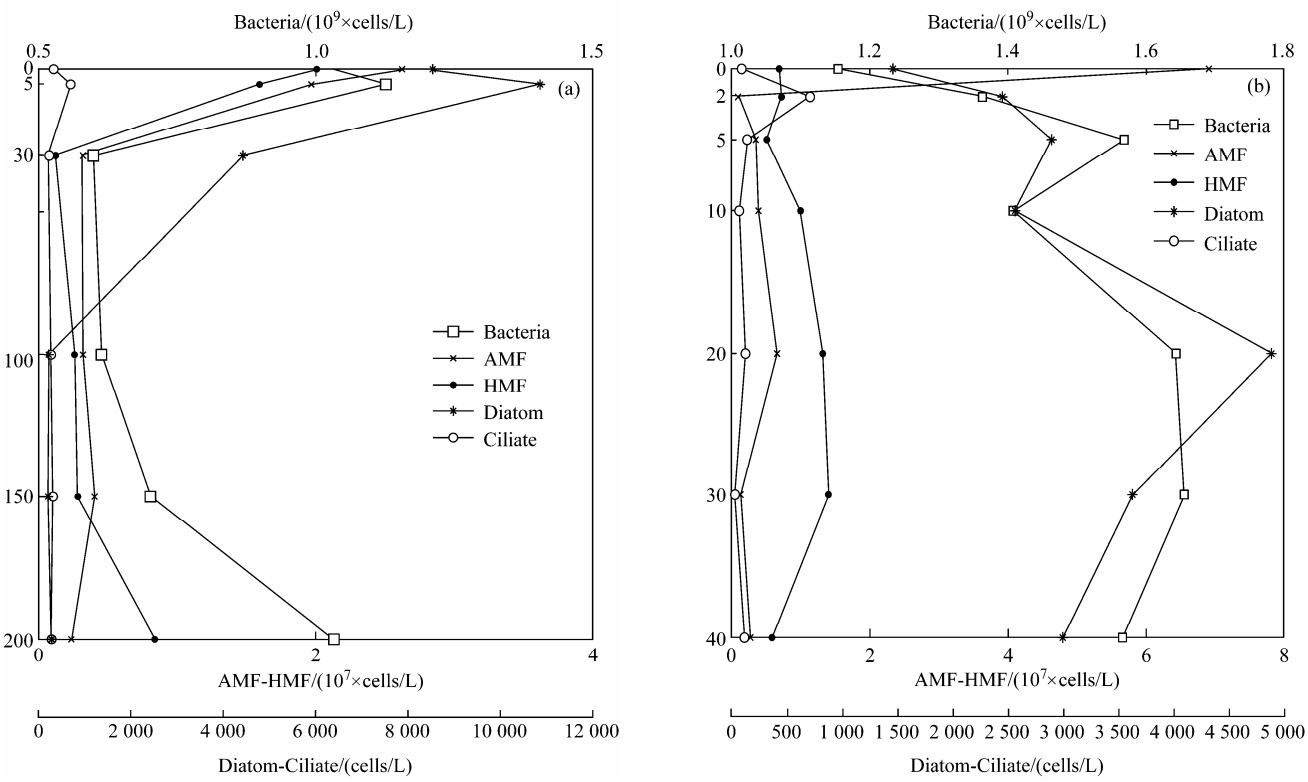

Figure 3 The depth profile of microeukayotic and bacterial cell counts, including autotrophic microflagellates (AMF), heterotrophic microflagellates (HMF), microdiatoms, microciliates in Station 1(a) and Station 5(b).

2.2 DGGE profiles of the bacterial and microeukaryotic plankton community

A typical DGGE profile image is shown in Figure 4. The number of bands was greater for the 16S rRNA gene than for the 18S rRNA gene, which indicated that the number of bacterial taxa detected in the samples was greater than those of the microeukaryotic taxa. Thirty different 16S rRNA

gene OTUs (Figure 4a) and 24 different 18S rRNA gene OTUs (Figure 4b) were detected in the DGGE analysis. The number of OTUs detected per site ranged from 6 to 30, and only five of them were common to all of the samples investigated. Two microeukaryotic OTUs were common to all 13 samples, and only three (12.5%) were restricted to single samples.

UPGMA clustering based on the Dice similarity matrix of DGGE fingerprints (Figure 5) showed that the microbial communities at Station 1–100 m and Station 1–150 m were first grouped into a cluster with the highest Dice similarity (0.090). Samples from all sites were separated into three distinct clusters with an SD=0.37, indicating that the microbial community structure differed significantly among the three clusters. Microbial communities of Station 1–100 m, Station 1–150 m and Station 1–200 m were more similar to Station 5 than to the other depths at Station 1.

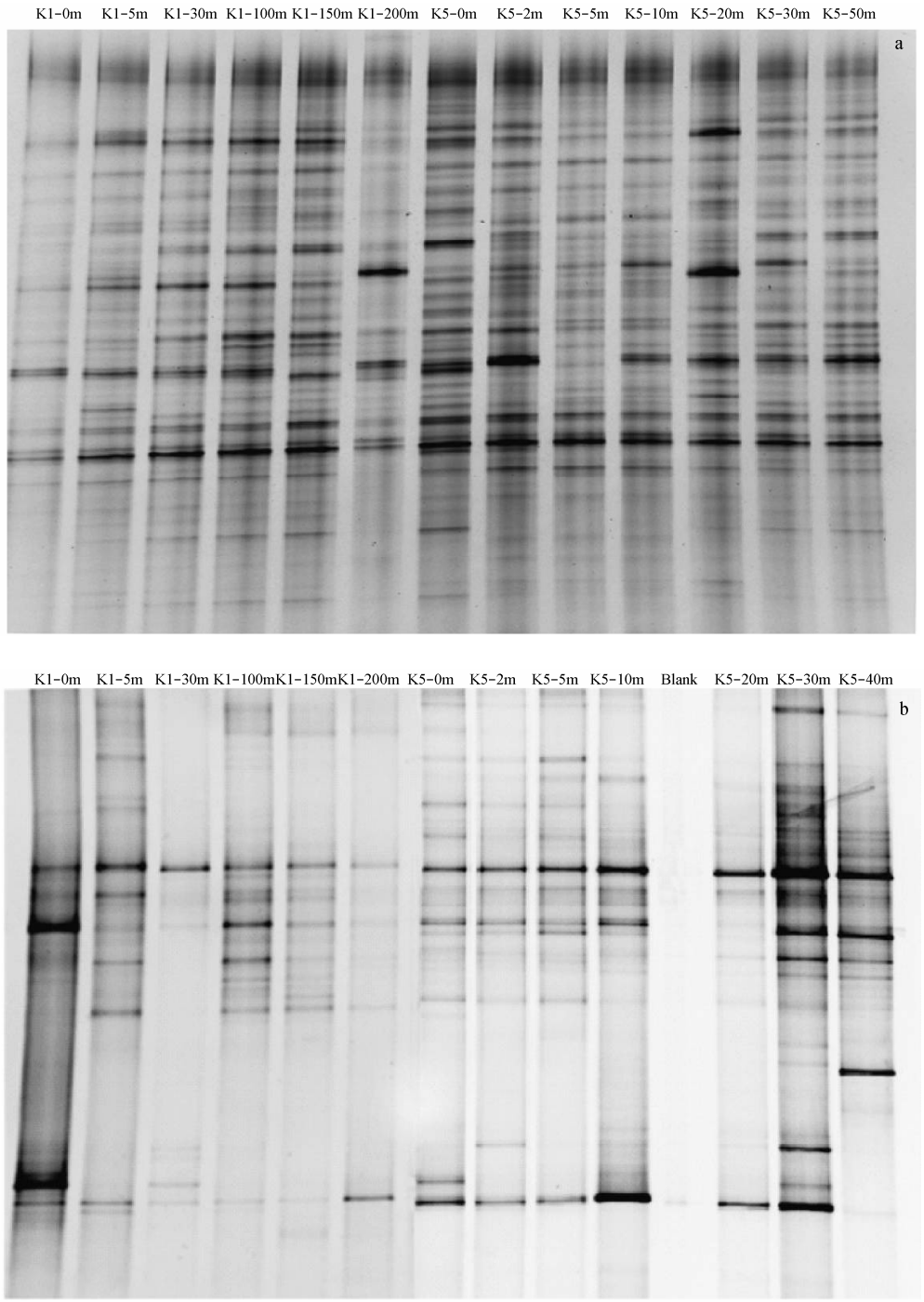

Figure 4 DGGE patterns for the 16S rRNA (a) and 18S rRNA (b) genes amplified from samples collected in August 2006. Each band was considered as an OTU.

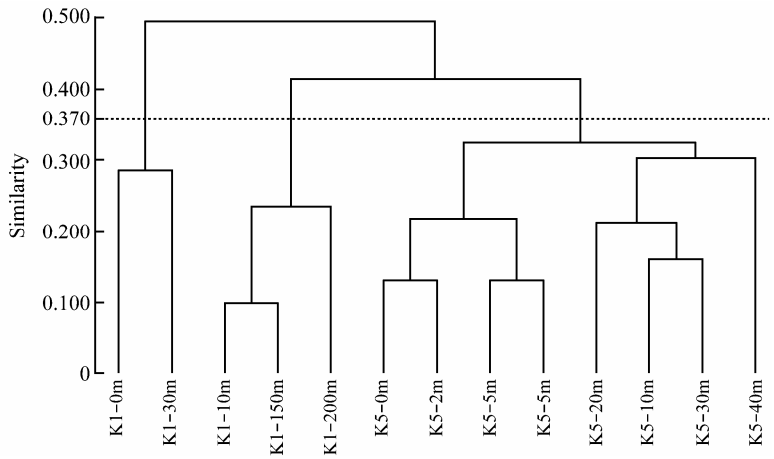

Figure 5 Group relationships of the 13 sampling sites. UPGMA clustering on the basis of the DGGE fingerprints.

2.3 Phylogenetic diversity of bacteria

The most prominent bacterial DGGE bands (n=22) were excised and sequenced. Sequence data revealed the presence of four putative divisions: Proteobacteria (Alpha, Beta, Gamma), Bacteroidetes, Verrucomicrobia and Cyanobacteria. Most sequences were related to the members of phyla Proteobacteria and Bacteroidetes. BLAST analyses identified the closest relatives of the species from which sequenced bands were derived from (Table 3), generally revealing high similarity (96%–100%) to bacterial 16S rRNA gene sequences in GenBank. The neighbor-joining tree of the Alphaproteobacteria revealed that KB21 clustered with the Roseobacter group and KB25 grouped with the SAR11 phylotypes. Gammaproteobacteria were separated into SAR 86 (KB1) and Psudomonadale (KB10). KB8, KB12 and KB13 sequences clustered with the Flavobacteria (Figure 6).

Table 3 Relatedness of bacteria in Kongsfjorden to known organisms

Clone Number	Phyla (and class for Proteobacteria)	Closest Match (show environments for them)	Identity/%	Accession number
KB1	Gamma proteobacterium	Uncultured gamma proteobacterium clone NABOS_FLbact33	100	EU544713
KB2	Verrucomicrobia	Uncultured bacterium clone ST-1K-33	100	DQ449538
KB3	Beta proteobacterium	Uncultured beta proteobacterium clone D03RT-RampDay9	99	GQ242766
KB4	Alpha proteobacterium	Alpha proteobacterium IMCC10404	100	FJ532499
KB7	Gamma proteobacterium	Uncultured gamma proteobacterium clone M05b26.10	100	EF486528
KB8	Bacteroidetes	Flavobacteriaceae bacterium MOLA 32, MOLA:32	99	AM990808
KB10	Gamma proteobacterium	Pseudomonas sp. PR3-14	100	FJ889638
KB11	cyanobacterium	Uncultured cyanobacterium clone Dpcom288	100	DQ881210
KB12	Bacteroidetes	Uncultured Bacteroidetes bacterium isolate DPEUO1	99	EF127653
KB13	Bacteroidetes	Uncultured Flavobacteriaceae bacterium clone PEACE2006/69-P3	99	EU39453
KB18	Beta proteobacterium	Uncultured beta proteobacterium MoDE-9	100	AF419359
KB19	Alpha proteobacterium	Uncultured alpha proteobacterium clone F2C35	99	AM279198
KB20	Verrucomicrobia	Uncultured bacterium clone ST-1K-33	100	DQ449538
KB21	Alpha proteobacterium	Uncultured Roseobacter sp. clone Arctic96A-1	100	AF35323
KB22	Alpha proteobacterium	Uncultured bacterium clone S23-1703	100	EF573604

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Clone Number	Phyla (and class for Proteobacteria)	Closest Match [show environments for them]	Identity (%)	Accession number
KB23	Bacteroidetes	Uncultured Bacteroidetes bacterium clone PLY-P1-17	98	AY354711
KB23	Bacteroidetes	Uncultured Bacteroidetes bacterium clone PLY-P1-17	98	AY354711
KB24	Alpha proteobacterium	Uncultured alpha proteobacterium clone JL-BS-J16	100	AY664257
KB25	SAR11 alpha proteobacterium	Uncultured SAR11 cluster alpha proteobacterium clone HF4000-37C10	99	EU361476
KB26	Alpha proteobacterium	Uncultured alpha proteobacterium clone M05b038.13	96	EF486532
KB27	Algae (Chloroplasts)	Uncultured phototrophic eukaryote clone JL-WNPG-T36	98	AY664132

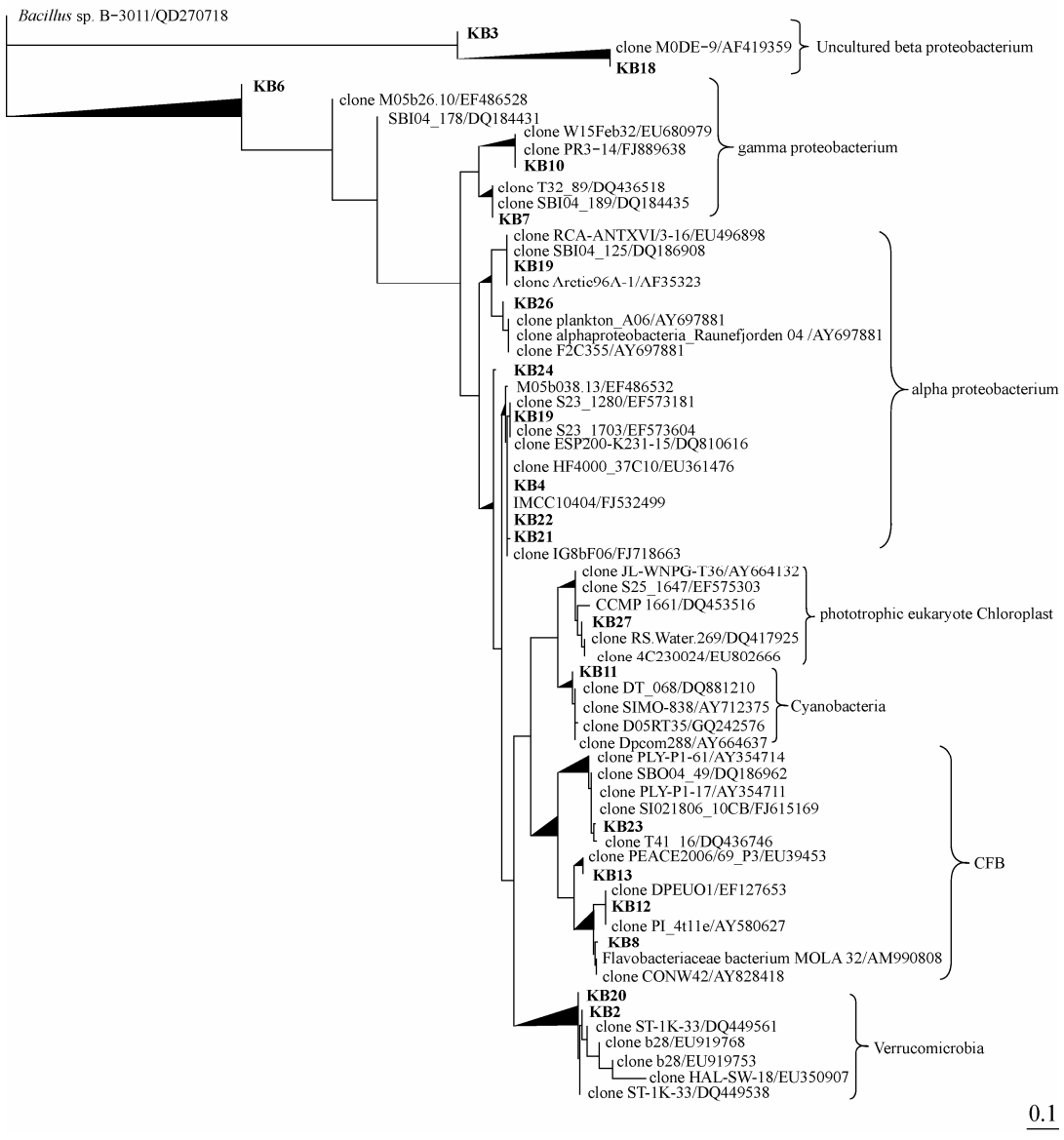

Figure 6 Phylogenetic tree of Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Cyanobacteria, phototrophic eukaryote chloroplast, Verrucomicrobia, and members of Bacteroidetes. GenBank accession numbers are given in parentheses. Bootstrap values above 50% are indicated in bold.

2.4 Bacterial community ordination analysis based on 16S rRNA gene sequence data

To obtain a detailed analysis of the bacterial community and the factors that influence distinct phylotypes, CCA of bacterial phylotypes and classical factors (abiotic and biotic factors) was performed. The ordination analysis of the bacterial community was conducted using the main groups of microbes based on cell counts, salinity, temperature, and nutrients (nitrate, phosphate, and silicate) as explanatory variables. CCA revealed high values of the variance inflation factor VIF (> 20) for the diatom and ciliate variables indicating collinearity with other variables. These data were therefore excluded from CCA. Significant correlation with the sample variation was observed for three factors (temperature, phosphate, and AMF) based on the 5% level in a partial Monte Carlo permutation test (Table 4).Both correlation coefficients and conditional effects are shown in Table 4. It is apparent that temperature had the strongest influence, followed by phosphate and AMF. CCA with interspecies distances was also conducted to calculate the influence of the environmental variables on specific phylotypes. Biplot scaling revealed five groups of phylotypes and two bands that could not be grouped with the other bands (Figure 7). Group I was mainly influenced by AMF. Phylotypes that positively correlated with temperature and salinity were grouped within group II. Group III did not show any correlation with any variable included in our dataset. The abundance of AMF was significantly correlated with the phylotypes of group V.

Table 4 Correlation coefficients and conditional effects of selected environmental variables produced by CCA and bacterial DGGE profile analysis

Community analysis, CCA	Environmental variables	Correlation coefficients		Conditional effects	
		Axis 1	Axis 2	P-value	F-factor
DGGE interspecies distances	Temperature /℃	0.9348	0.1256	0.002	16.33
	Phosphate-phosphorus /(μmol/L)	− 0.7367	0.2863	0.002	5.40
	AMF /(cells/L)	0.6745	− 0.3783	0.005	3.89

CCA, canonical correspondence analysis; DGGE, denaturing gradient gel electrophoresis; AMF, autotrophic microflagellates.

2.5 Genetic diversity of the microbial community in relation to biotic and abiotic factors

CCA analysis was performed using the abiotic variables of salinity, silicate, phosphate, and nitrate and the biotic variables of density (for bacteria, diatoms and ciliates), AMF, HMF and microbial OTU composition. Diatom variables were omitted because of a high variance of VIF. In the analysis, the first two axes were represented by 72.7% of the cumulative variance of the OTU-environment relationship. The CCA ordinations showed that the distribution of the microbial community was correlated primarily with salinity and the concentration of silicate for bacterial densities (Figure 8). Briefly, the concentration of salinity and the abundance of bacteria were positively (r =0.4432) and negatively (r= − 0.3955) correlated with the first CCA axis, respectively. The concentration of silicate was negatively (r= − 0.3812) correlated with the second axis (Table 5). In general, all sites except Station 1–30 m were separated on the first ordination axis (Figure 8). Station 1 sites were also generally distributed in the first and fourth quadrants, whereas the depths at Station 5 were generally distributed in the second and third quadrants.

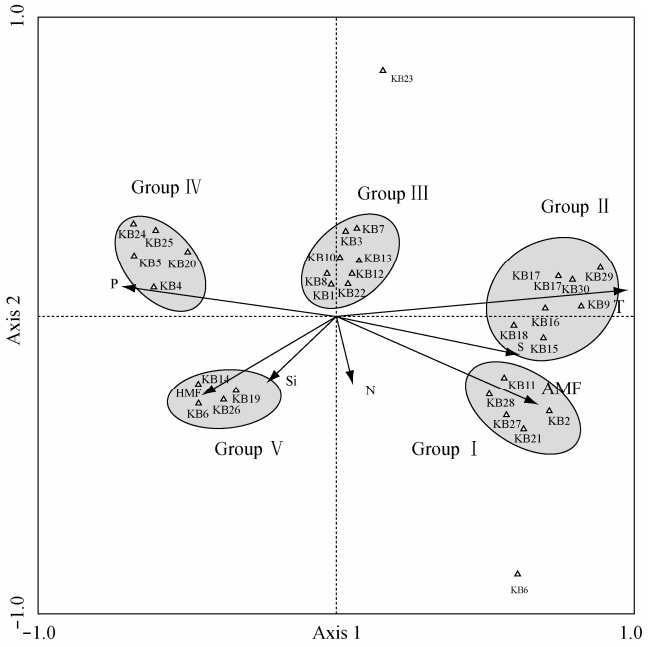

Figure 7 Biplot of interspecies distances; CCA of DGGE fingerprints of the bacterial community using autotrophic microflagellate (AMF) and heterotrophic microflagellate (HMF) cell counts, salinity (S), temperature (T), and the nutrients, nitrate (N), phosphate (P), and silicate (Si). Triangles with numbers and letters indicate sequenced bands. Arrows indicate the direction of increasing values of the respective variable, and the length of the arrows indicates the degree of correlation of the variable with community data. Significant values are indicated by bold arrows, and groups I, II, III, IV and V of phylotypes are indicated by a gray background.

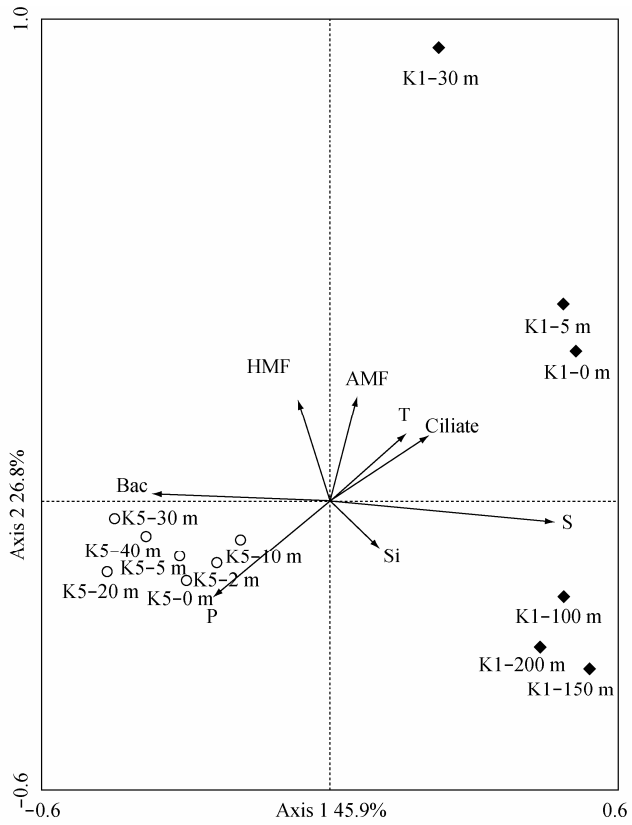

Figure 8 CCA ordination of the microbial community composition, as revealed by PCR-DGGE fingerprinting. Arrows indicate the direction of increasing values of the respective variable, and the length of arrows indicates the degree of correlation of the variable with community data. Significant values are indicated by bold arrows. AMF: autotrophic microflagellates; HMF: heterotrophic microflagellates; Bac: bacteria; S: salinity; T: temperature; N: nitrate; P: phosphate; Si: silicate.

3 Discussion

Traditionally, organic matter, salinity and temperature have been used to test the homogeneity of an environmental habitat. In recent years, analysis of partial regions of the rRNA gene has been used for the characterization of communities of microorganisms. rRNA gene sequences contain both conserved regions, which are useful for primer design, and variable regions, which can be used to distinguish differences in species and genotypes. Due to several favorable features (such as small size, short generation time, high sensitivity, comprehensive response to environmental conditions and importance in microbial food webs in high latitude coastal shelf), microbial organisms are ideal subjects for monitoring the conditions of aquatic environments. PCR-DGGE analysis of the microbial community produces a complex profile that could be used as surrogates for the relative abundance of dominant population, as well as the diversity of environmental microbial communities. However, most studies to date have focused on the prokaryotic microorganisms (e.g., bacterioplankton) with much less attention paid to the eukaryotes. Yu et al. (2008) reported that PCR-DGGE appeared to be appropriate for diversity characterization of the plankton community, as it is more canonical, Furthermore, Boon et al. (2000) revealed that the DGGE technique seemed to be more sensitive than the physicochemical approach for characterizing the homogeneity of an environmental habitat [20–21].

The water mass in Kongsfjorden during summer is a mixture of onshore transported warm and saline Atlantic water, colder and fresher Arctic-type water on the shelf and freshwater (glacier melt, calving, precipitation) [1]. The hydrological condition in Station 1, which could be presented outer part of shelf, was deeply affected by the intrusion of Atlantic and Arctic-type water [2]. The environmental characteristics of Station 5 were significantly different from those at Station 1 due to the inflow of glacial melt water [4]. DGGE patterns of the 16S rRNA and 18S rRNA genes showed high variation in band numbers, position and band intensity (Figure 4) and the UPGMA clustering results revealed that the microbial communities collected from the thirteen sites were clustered into three groups at the position of S_D=0.37 (Figure 5). Station 1–0 m, Station 1–5 m, and Station 1–30 m were generally considered to be at the same trophic level and subdivided into two groups on the basis of genetic diversity analysis. The intrusion of water flow and wind forcing create differences between the outer and inner parts of the shelf. These factors not only influence the levels of salinity

Table 5 Weighted correlation matrix showing the relationships between microbial OTU axes and variables.

Community analysis, CCA	Environmental variables	Correlation coefficients		Conditional effects
		Axis 1	Axis 2	P-value
DGGE intersample distances	Salinity /PSU	0.4432	– 0.053	0.001
	Bacteria /(cells/L)	– 0.3955	0.0187	0.002
	Silicate /(μmol/L)	0.3687	– 0.3812	0.004

CCA, canonical correspondence analysis; DGGE, denaturing gradient gel electrophoresis.

and nutrient, but also transport allochthonous microorganisms, especially bacteria, into the shelf. Subsequently, the ecosystem in Station 1 is potentially very complicated. As a reflection of the integrative ecological effects, biomonitoring methods are generally considered more acceptable than physicochemical methods for estimating long-term environmental conditions.

As discussed above, PCR-DGGE is an acceptable method for examining community composition and reflecting spatial variations. In our study, CCA was applied to further explore the correlation between microbial OTU composition and classical factors (both abiotic and biotic). The results clearly showed that the factors strongly correlated with the first two axes differed among the sites sampled. Salinity and silicate concentrations, together with bacteria parameter, showed a comparatively stronger contribution to the OTU composition. In addition, salinity was the major environmental factor that determined microbial community composition at Station 1–100 m, Station 1–150 m and Station 1–200 m. The concentration of silicate at these three sampling sites was also higher than at the other sites. These data suggest that salinity and silicate should be considered as initial determinative factors in studying the microbial community in the deep-water column. However, all of the sites except Station 1–30 m were generally separated on the first ordination axis (Figure 8). Station 1–30 m exhibited the lowest bands for 18S rRNA gene, relatively lower ones for 16S rRNA gene and the lowest numbers of main groups of microbes. The same results were concluded from the analysis of clone library analysis with 18 rRNA genes [15]. The 30 m depth NPK57 library also contained poor genotype diversity, with only one *Thalassiosira* clade (42% of all 26 sequences) and one *Calanus* clade (Metazoan). The Station 1–30 m was situated at the halocline and the bottom of the euphotic layer (data not shown) [4]. The Atlantic and Arctic-type water at this site potentially overlap, causing the remarkable decrease in genetic diversity and the numbers of microbes.

Interspecies distance analysis revealed the influence of different factors on specific phylotypes. An influence of temperature and salinity was observed for phylotypes detected in Station 1 and suggested that these factors contributed to a specific 'Atlantic and Arctic-type water' community. In addition, it should be noted that the Betaproteobacteria (KB3) are rare in the marine pelagic environment and are found predominantly in freshwater and coastal areas [22–23]. It could be concluded that this phylotype was detected due to the inflow of glacial meltwater. The phylotypes retrieved in this study belonged mainly to the Alphaproteobacteria. Within this group, band KB21 clustered with the sequence RCA-ANTXXIII/7-H315 (AM279203) retrieved from the Southern Ocean and RCA11-2 (AM279204) retrieved from the South Atlantic [24–25]. Both of those bacteria belonged to the Roseobacter-clade-affiliated (RCA) cluster, which appears to be of particular significance in the polar region [25]. Members of this cluster can conduct aerobic anoxygenic photosynthesis, oxidize carbon monoxide, and produce the climate-relevant gas dimethylsulfide through the degradation of algae osmolytes[26]. Sequence KB21 (RCA) in our CCA analysis had a significant positive relationship with the abundance of AMF and a negative relationship with the concentration of phosphate (Figure 7). Giebel (2009) also found a significant negative correlation between the dynamics of this cluster and phosphate, and in several cases, the vertical patterns of the RCA cluster exhibited maxima at the same depths as those of Chl *a* [24].

DNA-based methods provide more definitive information (such as diversity, evolution and ecology) on natural communities. Community level analysis using these methods is also a fast, easy, reliable, and inexpensive method to obtain scientifically sound results. Our results revealed that the assessment of spatial heterogeneity of a microbial community, with respect to genetic diversity, could be more sensitive technique to monitor the environmental conditions of aquatic habitats, and the dynamics of the aquatic ecosystem, than using traditional methods. However, all of these methods each have limitations and it may therefore be worthwhile to combine the use of fingerprinting techniques with traditional methods. To better understand how the principal environmental factors impact on the genetic diversity of a microbial community, further study should focus on the identification of specific populations or taxonomic groups and the assessment of their responses to natural or artificial environment disturbances.

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