Marine Pollution Bulletin 156 (2020) 111244

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Application of microbial network analysis to discriminate environmental heterogeneity in Fildes Peninsula, Antarctica

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ARTICLE INFO

Keywords:
Planktonic microbes
Environmental heterogeneity
Illumina MiSeq
Co-occurrence networks
Bioassessment
Antarctica

ABSTRACT

In order to determine the practicability of developing a protocol for bioassessing polar marine environment based on network analysis, microplankton communities and co-occurrence patterns at Ardley Cove and Great Wall Cove (King George Island, Antarctica) were studied in January 2016 through high-through sequencing. The spatial patterns and significant differences between community structures in two coves clearly reflect those in environmental heterogeneity. Moreover, both coves had their discriminated network structure and keystones. Then multivariate analyses to quantify the relationship between environmental variation and planktonic microbes response, give further evidence that nitrate and temperature, alone or in combination with other several parameters, structuring the communities respectively indeed. This study presents the first detailed description on co-occurrence networks between microbes and local environmental parameters in Antarctic coastal water. These findings suggest that co-occurrence networks based on planktonic microbes have the robust potential to assess environmental heterogeneity in polar marine ecosystem.

1. Introduction

As the most remote region on Earth, Antarctica plays an essential role in the climate system. The scientific significance of Antarctica has been recognized in the fields of biology and chemistry (Padeiro et al., 2016). The Western Antarctic Peninsula (WAP) is undoubtedly the most extreme and dynamic area in Antarctica, and has experienced significant warming over the past 50 years (Alcamán-Arias et al., 2018). In recent decades, WAP and its offshore islands have been subjected to increasing human activities, resulting in substantial environmental impacts (Bargagli, 2008; Lu et al., 2012). As a pivotal component of Antarctic marine food webs, the response of microbial communities to these intense environmental variations in polar oceans is important (Padeiro et al., 2016).

Microbes are extremely diverse in different ecosystems, and play a crucial role in global biogeochemical cycles (Freimann et al., 2013; Wu et al., 2017; Mo et al., 2018). It has been shown that microbial community structure is sensitive to environmental variability, and its diversity is commonly used as a bioindicator of ecological functioning (Jiang et al., 2014a, 2014b; Liu et al., 2019; Yang et al., 2019). For example, recent studies have demonstrated that physical, chemical, and biological properties of water remarkably affect the structure of microbial communities (Karimi et al., 2017). However, basic and detailed information about microbial communities in many parts of the polar oceans remains scarce (Luria et al., 2014). Previous studies have shown that planktonic eukaryotes and bacteria can be used to interpret integrated physicochemical variations in changing environments (Jiang et al., 2011, 2014a, 2014b, 2016; Xu et al., 2017; Yang et al., 2019). Although most studies on the spatial variability of polar marine microbial communities have focused on either the bacterial or eukaryotic component (Diíez et al., 2004; Ghiglione and Murray, 2012; Winter et al., 2013; Lee et al., 2014; Wang et al., 2019), there are limited studies on both bacteria and eukaryotes (Luria et al., 2014). The entire microbial community (bacteria and eukaryotes) and its relationships with the native environment should be considered simultaneously in order to develop an integrated vision of the changing water quality.

Microbial community structure is affected by local and regional physicochemical conditions (Buchan et al., 2014; Mikhailov et al., 2019). Co-occurrence network analysis can reveal interspecific interactions within and between microbial communities. Analyses of the various microbial food webs in different habitats can reveal the relationship between microbial communities and the local environment (Karimi et al., 2017). Furthermore, next generation sequencing data can reveal correlation and co-occurrence patterns, which can provide an

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https://doi.org/10.1016/j.marpolbul.2020.111244
Received 22 November 2019; Received in revised form 26 April 2020; Accepted 4 May 2020
Available online 17 May 2020
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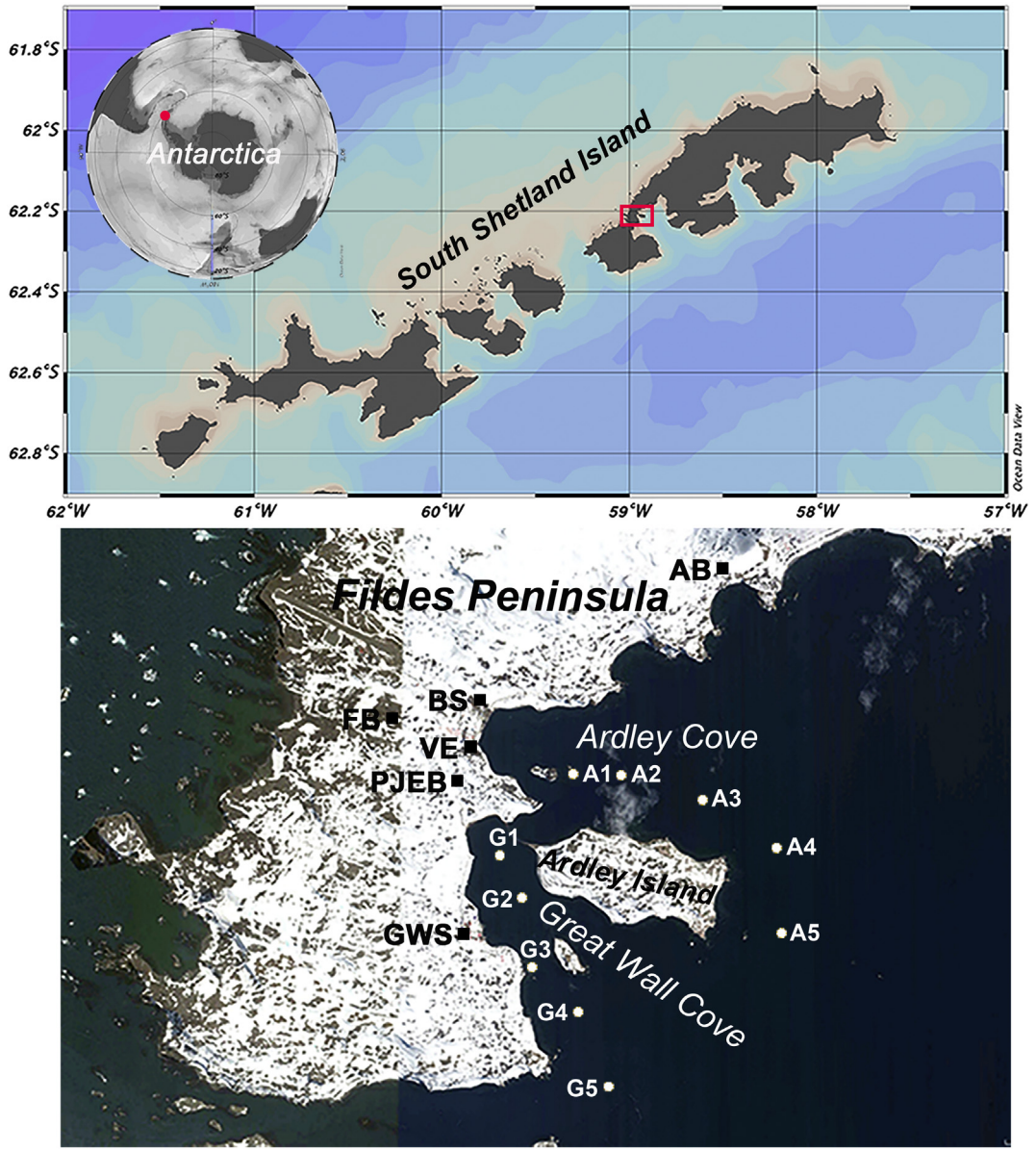

Fig. 1. Location of sampling stations in Ardley Cove and Great Wall Cove, Fildes Peninsula, King George Island, Antarctica. AB, Artiga Base; BS, Bellingshausen Station; FB, Frel Base; VLE, Villa Las Estrellas; PJEB, Prosefor Julio Escudero Base; GWS, Great Wall Station.

insight into the positive and negative interactions between species and environmental variables in the aquatic environment (Mo et al., 2018). However, co-occurrence patterns and linkages between microbes and environmental parameters in coastal seawaters of Antarctica have not yet been studied.

The main objectives of the present study were to: 1) exhibit spatial pattern of microbial community structure in response to environmental heterogeneity in coastal seawaters of two coves in the Fildes Peninsula, Antarctica; 2) reveal the co-occurrence patterns between microbes and abiotic variables in distinct environmental conditions; and 3) determine the feasibility of bioassessing polar environmental heterogeneity using network analysis based on marine microbial data.

2. Materials and methods

2.1. Study site and sampling

Samples (2 L) were collected at 10 stations, from different depths (0, 5, 10, 20, 30 m), from the coastal seawaters of the Ardley Cove and Great Wall Cove, Antarctica, in January 2016 (Fig. 1). Around Ardley Cove, there are several scientific stations, civilian settlements, and nearby marine bird settlements (i.e., the Ardley Island which is connected with peninsula to separate the two coves and has full of penguins in the northern side of island) (Fig. 1). However, there is only one scientific station around the Great Wall Cove (Fig. 1). At each sampling station, samples for nutrient measurements and biotic analyses were collected from different water depths using Niskin bottles. The collected water samples were prefiltered through a 200 μm sieve, followed by filtering through 0.22 μm filters (Whatman, USA) using a vacuum pump (< 20 cm Hg), for molecular analyses. The filtrates were stored at −80 °C.

Water temperature and salinity were measured using a YSI Model 30 system (Yellow Springs Instruments, Yellow Springs, USA). Nutrients, including nitrate (NO_3-N), nitrite (NO_2-N), silicate (SiO_3-Si), and phosphate (PO_4-P), were measured spectrophotometrically using a continuous flow autoanalyzer Scan^{++} (Skalar, the Netherlands), after filtering seawater through 0.45 μm cellulose acetate membrane filters (Whatman, USA), as described by Hansen and Koroleff (1999). The

dissolved oxygen (DO) concentration was determined by the Winkler titration method (Strickland and Parsons, 1972). The chlorophyll *a* (Chl *a*) concentration was estimated fluorometrically from 20 mL samples filtered through Whatman gF/F filters. The filtrates were ground in 90% acetone and maintained in the dark at −20 °C for 24 h. The fluorescence of the extract was measured with a 10-AU Field Fluorometer (Turner Designs, Sunnyvale, CA, USA).

2.2. DNA extraction and PCR amplification

DNA was extracted in a 1:1 phenol:chloroform mixture (Liu et al., 2019). Universal primers 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′) were used to amplify the V3-V4 region of the 16S rRNA gene (Otani et al., 2014), and the broad eukaryotic primers forward 3NDF (5′-GGCAAGTCTGGTGCCAG-3′) and V4 (5′-ACGGTATCT(AG)ATC(AG)TCTTCG-3′) were used for the V4 region of the 18S rRNA gene (Bråte et al., 2010).

For bacteria, the amplification reaction was performed in 20 μL final volume containing 12.4 μL sterile distilled water, 0.4 μL dNTPs (10 μM), 4 μL 5 × HF buffer, 1 μL of each primer (10 μM), 1 μL of the template, and 0.2 μL Phusion High-Fidelity DNA Polymerase (Thermo Scientific, Germany). The PCR conditions were as follows: 98 °C for 30 s, followed by 15 cycles of 98 °C for 5 s, 56 °C for 20 s, and 72 °C for 20 s, with a final extension step at 72 °C for 5 min.

For eukaryotes, PCR assays were performed in a triplicate 20 μL mixture containing 4 μL of 5× FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 mM), 0.4 μL of FastPfu Polymerase (Promega, USA), and 10 ng of template DNA. PCR amplification was performed using ABI GeneAmp® 9700 under the following conditions: 35 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, and final extension at 72 °C for 10 min.

2.3. High-throughput sequencing

PCR amplicons were checked by 2% agarose gel electrophoresis, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA), and quantified using QuantiFluor™-ST (Promega, USA). The concentrations of these purified DNA extracts were measured with a Qubit 2.0 fluorometer (Thermo Fisher Scientific Inc., USA). The purified amplicons were then pooled in an equimolar concentration for paired-end sequencing on an Illumina Miseq PE300 platform (http://www.illumina.com.cn/systems/miseq/workflow.aspx). Raw reads in the fastq files with low quality (Q < 20 or length < 200 bp) were discarded using QIIME (Version 1.17) (Caporaso et al., 2010). Tags were obtained by merging the paired reads according to their overlaps using COPE (Connecting Overlapped Pair-End, V1.2.3.3) (Liu et al., 2019), after cutting off the barcode and primer sequences. High quality pair-wise sequences were obtained employing the following standards: (i) bases with ASCII value below 33 were screened out; (ii) a minimum overlap of 19 bp between reads was ensured; (iii) no more than one mismatch was accepted while cutting off the primer sequences. Operational taxonomic unit (OTU) clustering was performed at a minimum sequence similarity of 97%, using QIIME (Version 1.8.0) (Caporaso et al., 2010). Chimeric sequences were screened out using UCHIME (Edgar et al., 2011). Each representative OTU obtained after clustering was compared against the Silva (SSU115) 18S rRNA database, with a confidence threshold of 70% for taxonomic affiliations.

2.4. Statistical analysis

Non-parametric species richness indices, including ACE and Chao, and diversity indices (Shannon and Simpson) were analyzed in *R* software, based on the OTU dataset of relative abundances (Dixon, 2003). Multivariate analyses were conducted using the PRIMER v7.0 package (Clarke and Gorley, 2015) and PERMANOVA+ for PRIMER (Anderson and Lochery, 2008). The spatial environmental variation in the two coves was summarized using principal components analysis (PCA) based on log-transformed/normalized abiotic data from 37 samples, and differences among groups of samples were tested using permutational multivariate analysis of variance (PERMANOVA). The contribution of each OTU to the microbial communities was summarized by similarity percentage analysis (SIMPER). The differences among the microbial communities of the two coves and their relationships with the environmental variables were analyzed by redundancy analysis (RDA), and PERMANOVA was used to test differences between the sample clouds. To evaluate the biotic factors responsible for the variation in the biota data, and to select the best interpretation model, a DistLM (Distance-Based Linear Model) using Akaike's information criterion (AIC) was applied. Biota-Environment analysis (BIOENV) was used to explore potential multivariate correlations between biotic and abiotic data.

The "psych" *R* package was used to calculate all possible pairwise Spearman's rank correlations (*r*) between each pair of the OTUs (including most dominant 50 eukaryotic and most dominant 50 bacterial OTUs), and between the environmental variables and OTUs. Only statistically significant ($P < 0.01$) and strong ($r > 0.6$ or $r < -0.6$) correlations were incorporated into the network analyses (Barberán et al., 2012). Gephi version 0.9.1 was used for visualization of the modular analysis network.

3. Results

3.1. Environmental conditions

Supplementary Table S1 presents the environmental variables recorded in the Ardley Cove and Great Wall Cove in January 2016. Comparing the two coves, the seawater temperature was slightly higher at the Ardley Cove than at the Great Wall Cove, whereas the salinity, Chl *a*, DO and NH_4-N concentration were higher at the Great Wall Cove than at the Ardley Cove. The measurements of SiO_3-Si, NO_2-N, NO_3-N, and PO_4-P were notably higher at the Ardley Cove (Fig. S1).

Principal components analyses (PCAs) using the environmental dataset from 37 samples are shown in Fig. 2. The first PCA axis explained 44.5% of the total environmental variability, and segregated

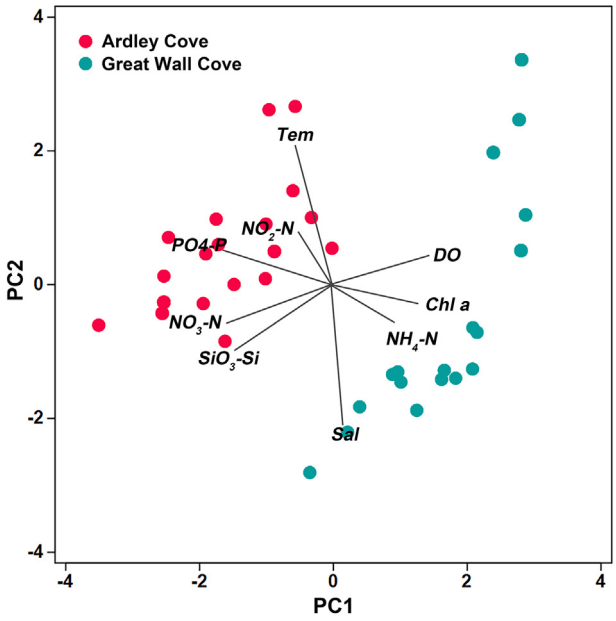

Fig. 2. Principal component analysis (PCA) based on log-transformed/normalized environmental variable data in Ardley Cove and Great Wall Cove. Chl *a*, Sal, Tem, DO, NH_4-N, NO_2-N, NO_3-N SiO_3-Si, and PO_4-P represent chlorophyll *a*, salinity, temperature, dissolved oxygen, ammonia, nitrite, nitrate, silicate, and phosphate, respectively.

the data points from the two coves in the plot. However, the second axis explained 27.0% of the total environmental variability, but failed to provide a further division (Fig. 2). Thus, the two principal components distinguished the environmental conditions of the two coves as two distinct groups. In the plot, temperature, SiO_3-Si, NO_2-N, NO_3-N, and PO_4-P were closely related in the samples of the Ardley Cove, whereas Chl *a*, DO, salinity, and NH_4-N showed a close relationship in the samples of the Great Wall Cove (Fig. 2). The PERMANOVA test revealed a significant difference between the sample clouds from the two coves (pseudo-F = 20.713, P = 0.001).

3.2. Spatial variation in taxonomic composition

Sequencing results of the 18S and 16S rRNA genes yielded 2,831,050 and 2,909,700 high-quality sequences, and 636 and 770 OTUs at 97% similarity level, respectively. The total number of OTUs (636 and 770) in the samples was roughly equivalent to the richness estimated by Chao1 (154–306 and 283–415, respectively) and ACE (148–299 and 288–417, respectively) indices (Tables S2 and S3). The rarefaction curves were nearly saturated for each sample (Fig. S2).

In case of eukaryotes, nine super groups (Alveolata, Stramenopiles, Hacrobia, Archaeplastida, Rhizaria, Opisthokonta, Amoebozoa, Apusozoa, and Palpitomonas) and 25 groups at the class or phylum level (e.g., Prasinophyceae, Dinoflagellata, Ciliophora, MASTs, Fungi, Bacillariophyta, etc.) were detected in the samples. The relative abundance of the OTUs is shown in Figs. 3A, B and S3A, B. At the supergroup taxonomic level, Alveolata and Stramenopiles appeared in all samples, and Stramenopiles dominated in most of samples. Archaeplastida and Hacrobia exhibited a higher relative abundance at the Great Wall Cove (Fig. S3A, B). At the group taxonomic level, Bacillariophyta predominated most of the samples from both the coves, with Ciliophora and Dinophyceae exhibiting higher relative abundance at the Ardley Cove, whereas the relative abundance of Prasinophyceae, *Amoebophyra*, and Haptophyta was higher at the Great Wall Cove than at the Ardley Cove (Fig. 3A, B).

In case of bacteria, the obtained taxonomic data covered a broad spectrum of known bacterial phyla. The dominant phyla in all samples belonged to Proteobacteria (Fig. S3C, D). In addition to the dominant phyla, numerous sequence reads related to Bacteroidetes were recorded in all 37 samples. At the family taxonomic level, *Rhodobacteraceae*, *Flavobacteriaceae*, and *Oceanospirillaceae* dominated at both the coves. *Rhodobacteraceae* predominated in the Ardley Cove samples, whereas *Oceanospirillaceae* predominated in the Great Wall Cove samples (Fig. 3C, D).

3.3. Spatial variation in community structure

Distance-based redundancy analysis (db-RDA) showing ordination was used to analyze spatial variation in the eukaryotic (Fig. 4A) and bacterial (Fig. 4B) communities, as well as their relationships with environmental variables, using Bray-Curtis similarities from square root transformed OTU abundance data. RDA analysis demonstrated that the eukaryotic and bacterial community compositions were significantly different between the two coves (PERMANOVA test, $P < 0.001$). Eukaryotic and bacterial community structures at the Ardley Cove were strongly associated with temperature and the NO_3-N, NO_2-N, SiO_3-Si, and PO_4-P concentrations (Fig. 4A, B).

In addition, SIMPER analysis revealed that species composition, in terms of both abundance and occurrence, differed between the two coves (Table S5). 14 eukaryotes and 17 bacteria were identified at the 70% cumulative contribution percentage level between the Ardley Cove and Great Wall Cove samples. These were the primary contributors to the dissimilarity of the two groups, due to their different abundance and/or occurrence in the Ardley Cove and Great Wall Cove samples (Table S5).

The environmental drivers of differences in the community structure were identified by DistLM analysis, and marginal and sequential tests were used separately to identify the abiotic variables that exerted a significant effect on the relative species abundances (Table S5). The marginal tests showed that nutrients, particularly NO_3-N, were significantly associated with eukaryotes and bacteria at the Ardley Cove ($P < 0.05$). NO_3-N was found to be the most important predictor variable for eukaryotes (AIC = 114.8, R^2 = 0.158) and bacteria (AIC = 106.88, R^2 = 0.350) in the sequential test (Table S5). In the case of the Great Wall Cove, the marginal tests showed that PO_4-P, SiO_3-Si, NO_3-N, DO, temperature, and salinity were significantly associated with eukaryote abundance ($P < 0.05$), whereas NO_3-N, SiO_3-Si, temperature, and salinity were significantly associated with bacteria abundance ($P < 0.05$). Temperature was found to be the predictor variable for both eukaryotes (AIC = 97.937, R^2 = 0.265) and bacteria (AIC = 101.97, R^2 = 0.153) in the sequential tests (Table S5).

Correlations between microbes and environmental parameters were established using the BIOENV analysis, and the subset of environmental variables that 'best' correlated with the biotic similarities was selected, as shown in Table 1. A combination of NO_3-N and NO_2-N with SiO_3-Si ($P < 0.05$) represented the best match for the spatial pattern of eukaryotic and bacterial communities at the Ardley Cove (Table 1). For eukaryote communities at the Great Wall Cove, the best variable was temperature (P = 0.001); however, no variable, alone or in combination, could be found to correlate with microbe similarities for bacteria ($P > 0.05$) (Table 1).

3.4. Co-occurrence of planktonic microbial communities

The interspecific interactions between each pair of microbes, and the relationships between microbes and environmental variables, were analyzed in detail at the OTU level, through networks based on the 50 most dominant bacterial and eukaryotic OTUs and all environmental variables at the two coves (Fig. 5A, B). The co-occurrence networks for the Ardley Cove and Great Wall Cove were constructed for significant ($P < 0.01$) and strong ($r > 0.6$ or $r < -0.6$) correlations between the relative abundances of bacteria and eukaryotes and the measured environmental variables (Fig. 5A, D). Co-occurrence networks for the two coves were markedly different. It was evident that correlations were fewer in the Ardley Cove samples than in the Great Wall Cove samples (Fig. 5A, D). In the Ardley Cove network, 269 edges were observed between each pair of nodes, whereas the Great Wall Cove network presented 382 edges. It was noteworthy that NO_3-N and temperature were the key environmental factors presenting the highest number of interactions at the Ardley Cove and Great Wall Cove, respectively (Fig. 5B and E). Moreover, OTU-14 represented the keystone species, depicted as largest nodes in the network, belonging to *Cryomorphaceae* of class Flavobacteriia, which was the major bacterial class in the Ardley Cove samples (Fig. 5C). Based on its interspecific interactions, OTU-14 was positively correlated with many bacteria and eukaryotes, including *Polaribacter* (OTU-8, OTU-363), SAR86 (OTU-13, OTU-38), *Porticoccaceae* (OTU-15, OTU-96), and *Amoebophyra* (OTU614, OTU523) (Fig. 5C). Furthermore, the keystone species in the Great Wall Cove samples was represented by OTU357 (belong to Picozoa) (Fig. 5F), which was positively correlated with numerous eukaryotic OTUs and negatively correlated with temperature and several bacteria (Fig. 5F).

4. Discussion

Previous studies have shown that microbial eukaryotes and bacteria play key roles in the Antarctic marine ecosystem, acting as the main food source and the primary contributors to energy fluxes into the microbial food loop (Smetacek and Nicol, 2005; Browning et al., 2014; Moreno-Pino et al., 2016; Zeng et al., 2014; Mo et al., 2018). Among the components of the ecosystem (i.e., physical, chemical, and biological), the biological component is particularly sensitive to perturbations

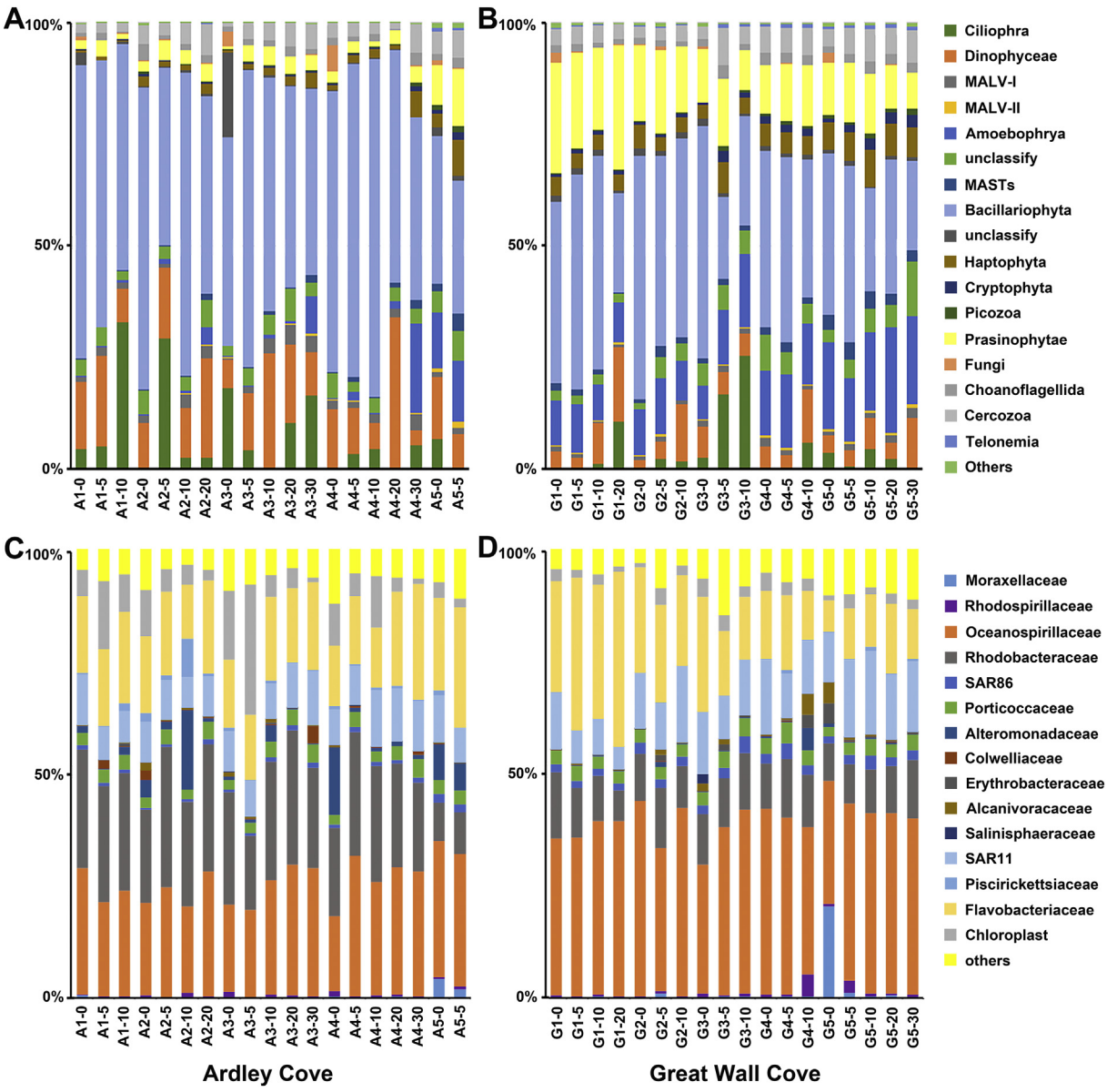

Fig. 3. Relative abundances of microbial eukaryotes (A) and bacteria (B) for 37 samples in Ardley Cove and Great Wall Cove.

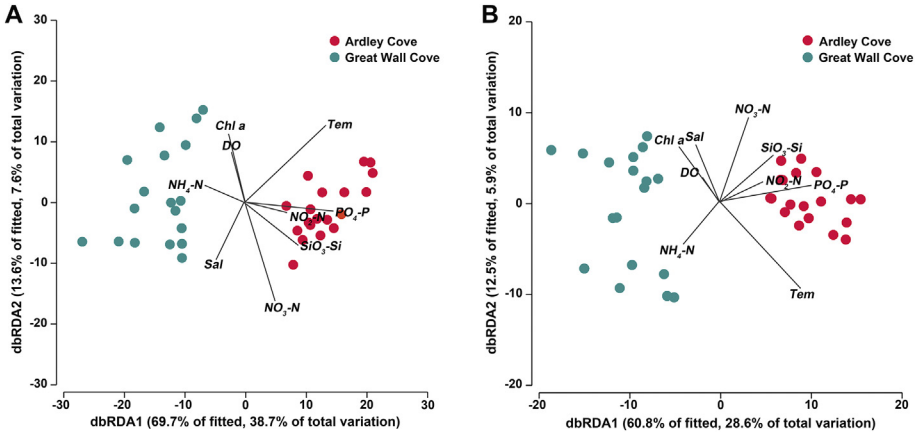

Fig. 4. Redundancy analysis (RDA) of microbial eukaryote (A) and bacteria (B) communities based on operational taxonomic units (OTUs). See Fig. 2 for abbreviations.

Table 1
Summary of results from biota-environment (BIOENV) analysis showing the best matches of combinations of environmental variables with variations in eukaryote and prokaryote abundances.

	ρ	Best combination of variables	*P*
Ardley Cove (eukaryote)	0.201	NO_3-N, NO_2-N, SiO_3-Si	0.011
Ardley Cove (prokaryote)	0.167	NO_3-N, NO_2-N, SiO_3-Si	0.003
Great Wall Cove (eukaryote)	0.381	Tem	0.001
Great Wall Cove (prokaryote)	0.096	Tem, SiO_3-Si	0.266

(Jiang et al., 2014a, 2014b; Liu et al., 2019; Wang et al., 2019; Yang et al., 2019). The small size, short generation time, rapid growth, and genetic plasticity of microbes render them capable of rapid adaptation to environmental changes (Bouchez et al., 2016; Karimi et al., 2017).

Previous studies have shown that the marine environment directly impacts the composition of planktonic microbial organisms (Blanchot et al., 2001; Jiang et al., 2016; Yang et al., 2019). In addition, the microplanktonic community has been used as a bioindicator for assessing environmental variations in marine ecosystems (Jiang et al., 2014a, 2014b; Liu et al., 2019). However, the traditional diversity indices (e.g., Shannon, Pielou's or Simpson) are not sensitive enough to indicate variations in water quality (Jiang et al., 2014a, 2014b), or to efficiently exhibit the relationship between community response and environmental changes (Karimi et al., 2017). Consequently, adopting a more integrative approach, which can precisely represent the complex associations between microbes and environmental conditions, beyond those shown by simple diversity indicators, is required. Thus, in the present study, integrated co-occurrence networks based on biotic and

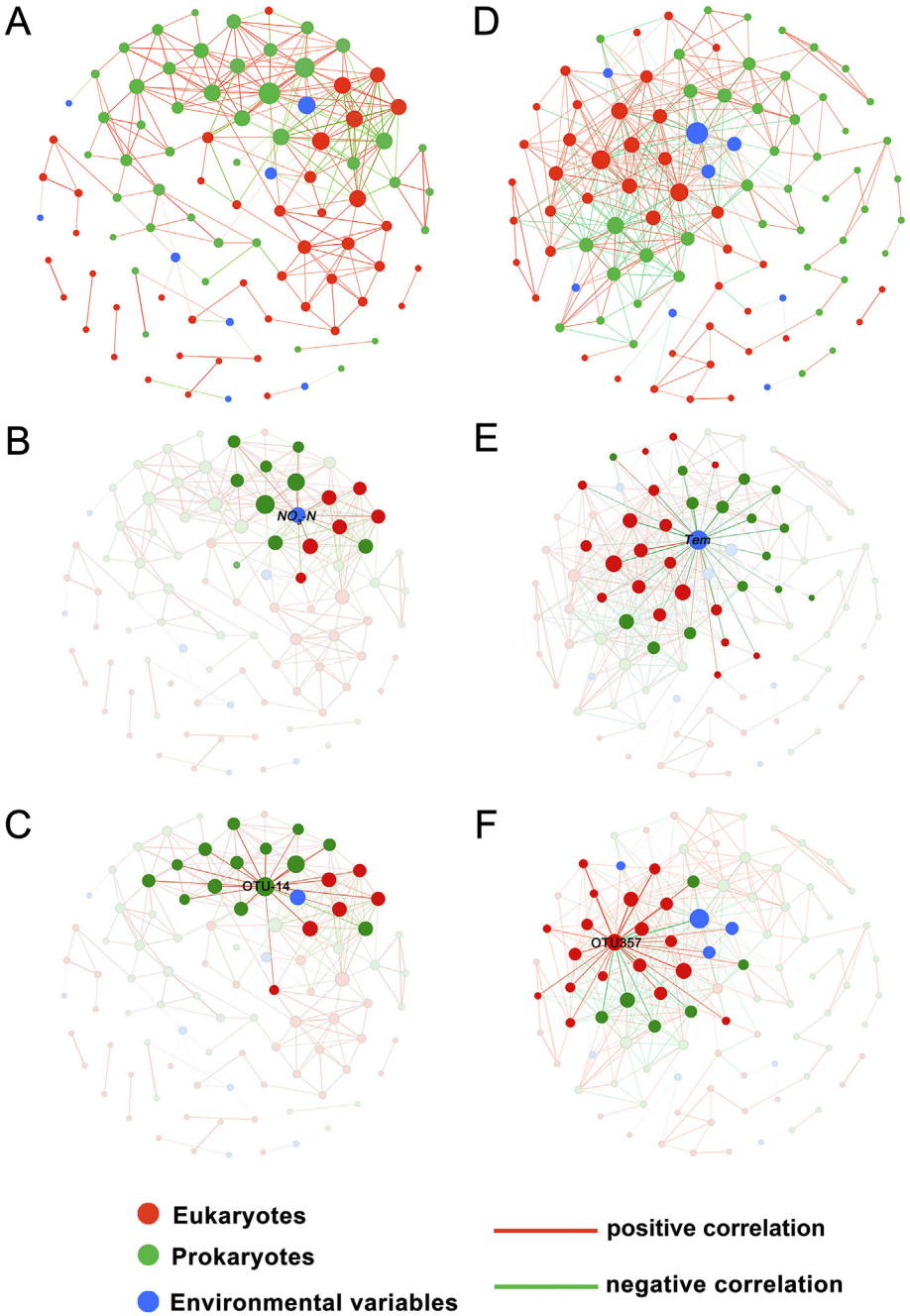

Fig. 5. Microbial co-occurrence networks for the most dominant 50 bacterial OTUs, most dominant 50 eukaryotic OTUs and eight environmental variables in Ardley Cove (A) and Great Wall Cove (D). The connections with key environmental variables (B, E) and keystone species (C, F) in the two coves highlight in the plots.

abiotic data were employed to reveal the response of the microplanktonic community to environmental changes in coastal Antarctic ecosystems.

Majority of interspecific interactions in the microbial communities can be observed or identified individually by uni- or multi-variate analyses (Jiang et al., 2016). However, there is a lack of a feasible tool for directly and simultaneously demonstrating such individual interactions. Co-occurrence networks have recently been shown to provide an integrated vision of all relationships existing between microbial organisms in a given environment (Karimi et al., 2017). In order to envision a complete picture of the microbial ecosystem, the associations between the microbes and influencing environmental factors must also be included while structuring the microbial networks. Therefore, co-occurrence networks based on interactions between biotic and abiotic components are essential, and can remarkably improve the accuracy of marine environmental heterogeneity bioassessments.

In recent years, the intensity of human activities in Antarctica, particularly in the WAP and its offshore islands, has increased, which has resulted in a substantial increase in environmental pollution, which has the potential to alter this pristine habitat (Bargagli, 2008; Lu et al., 2012; Padeiro et al., 2016). It was noteworthy that nutrient measurements were significantly higher at the Ardley Cove than at the Great Wall Cove in the present study, which may be attributed to the characteristics of the surrounding areas. During the austral summer, such as during the sampling period of this study (January 2016), water quality at the Ardley Cove is strongly affected by melting sea ice waters from the Collins Glacier (Moreno-Pino et al., 2016). In addition, this cove, which represents one of the only two civilian settlements on the entire Antarctic continent, experiences severe anthropogenic impacts because of the intense human activities and several scientific research stations situated here. Moreover, high amounts of particulate materials are released into the cove from nearby marine bird settlements in the Ardley Island. In comparison to the Ardley Cove, there is a lower effect of melting sea ice and human activities, with only one scientific station, in the Great Wall Cove. Therefore, environmental conditions, particularly nutrient concentrations, were remarkably different at the two coves (Luo et al., 2016). This indicates that significant environmental variation exists across this small spatial distance.

Previous studies have shown that in contrast to nutrient-replete coastal regions, where phytoplankton communities are dominated by larger-size classes, primary production in oligotrophic regions is largely attributed to smaller-sized picoplankton (Ciotti et al., 2002; Dong et al., 2018). This discrepancy may be due to the competitive advantage of large phytoplankton for growth in highly fluctuating nutrient environments (Malone, 1980), and the advantage of small phytoplankton in acquiring sparse nutrients in low-nutrient environments (Sherr et al., 2005). In the present study, larger-size classes, such as Bacillariophyta and Dinophyceae, exhibited a higher relative abundance in the Ardley Cove, whereas smaller-sized picoplankton (Prasinophyceae and *Amoebophyra*) represented the communities in Great Wall Cove by replacing the ecological niches of Bacillariophyta, Dinophyceae, and Ciliophora. It is known that phytoplankton abundance is controlled by several factors, such as temperature, nutrients, and community structure (Li et al., 2010). In addition, grazing pressure from microzooplankton (e.g., ciliates) can affect the phytoplankton size structure (Landry et al., 2009). In the Ardley Cove, diatoms and dinoflagellates bloomed under nutrient enrichment, whereas the picoplankton might have been predated upon by the abundant protozoans. Furthermore, the parasitic dinoflagellate *Amoebophyra* infects a broad range of hosts, including ciliates, radiolarians, and planktonic dinoflagellates, in eutrophicated coastal waters worldwide. *Amoebophyra* can infect specific types of bloom-forming marine dinoflagellates (Li, 2014). It is possible that the presence of *Amoebophyra* in the Great Wall Cove suppressed the blooms of dinoflagellates and other nano-/micro-size protists.

In their study of microbial communities below drifting sea-ice in the Arctic, de Sousa et al. (2019) showed that the co-occurrence patterns of a community may vary considerably with environmental conditions. Similarly, in the present study, the community structure of planktonic microbes in the two coves showed a clear distinction, which could be attributed to environmental heterogeneity. Nutrients had a greater influence on the bacterial and eukaryotic communities in the Ardley Cove than on those in the Great Wall Cove. We speculated that human activity and summer melting input have a stronger impact in the Ardley Cove, thereby shaping the microbial community structures. Moreover, DistLM analysis identified NO_3-N as the strongest influential factor at the Ardley Cove, whereas temperature was the most significant variable at the Great Wall Cove ($P < 0.05$). Furthermore, BIOENV analysis revealed significant linkages between spatial variations in the community structure and the environmental condition at each cove. The results showed that a combination of nutrients (NO_n-N and SiO_3-Si) best explained the spatial variation in the microbial community structure of the Ardley Cove. Conversely, only one variable (temperature) could explain the eukaryotic community structure at the Great Wall Cove, and no variable could be linked to the bacterial community.

The co-occurrence networks for the two coves based on Spearman's rank correlation results were structured, demonstrating the complex interspecific interactions and their relationships with specific environmental forcing factor. The main relationships in the Ardley Cove co-occurrence network were bacteria-bacteria/environmental variables. Several keystone bacteria might play mediatory roles in microbial food webs by connecting with other species or with the key environmental parameter, i.e., NO_3-N. It is possible that bacteria dominate the microbial communities at the Ardley Cove because of the sufficient nutrient supply. In previous studies, bacterial richness and community structure were shown to be strongly related to the physicochemical characteristics of water, particularly the nutrients (Wei et al., 2014; Ávila et al., 2017). In the Ardley Cove samples, bacteria contributed significantly to the microbial communities, and also exhibited strong relationships with NO_3-N and bacterivores. Flavobacteria, described as 'first responders' to phytoplankton blooms, break down complex organic matter by direct attachment and exoenzymatic attack on phytoplankton cells and phytoplankton-derived detrital particles (Williams et al., 2013). In the present study, the high abundance of diatoms may have led to Flavobacteria being the keystone species in the Ardley Cove co-occurrence network. However, in the Great Wall Cove network, the dominating relationships were eukaryote-eukaryote/environmental factors. At the Great Wall Cove, temperature was the key variable structuring the main linkages, and eukaryotes were probably the most important components of the microbial food web, which was notably different from the Ardley Cove. It is possible that lesser disturbance and lower nutrients in the Great Wall Cove may have resulted in the relationships between phytoplankton and herbivorous microzooplankton. Overall, the results of the present study revealed the relationship between microorganisms and environmental conditions in a typical polar coastal region with clear environmental heterogeneity. These findings suggest that integrated microbial co-occurrence networks can clearly reflect the influence of environmental stress on the composition and structure of microbial food webs, and thus, can potentially be used for the bioassessment of environmental conditions in the polar marine environment.

5. Conclusions

The present study demonstrates that planktonic microbial communities, which are significantly different between two Coves in Antarctica, could successfully reflect the polar coastal environmental heterogeneity. Multivariate correlation analysis showed that NO_3-N and temperature were the most important environmental variables at the Ardley Cove and Great Wall Cove, respectively. Moreover, comprehensive co-occurrence networks based on the micro-organisms and environmental variables precisely reflected the environmental heterogeneity. These networks provided an integrated vision of the regulation

of the bacterial and eukaryotic community structures by environmental parameters. Our findings provide insightful information on the structure of microbial food webs in polar ecosystems, and suggest that microbial co-occurrence networks can be employed as robust indicators for determining the impact of environmental heterogeneity on bioassessments of the polar oceans.

Data accessibility

Sequence data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) database (accession numbers, PRJNA574228; PRJNA574242).

CRediT authorship contribution statement

Qian Liu:Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing - original draft, Writing - review & editing.**Yong Jiang**:Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (NFSC) (No. 41676178); Marine S&T Fund of Shandong Province for Pilot National Laboratory for Marine Science and Technology (Qingdao) (Nos. 2018SDKJ0406-6, 2018SDKJ0104-4); the response and feedback of the Southern Ocean to climate change (RFSOCC2020-2025). We would like to thank Editage (www.editage.cn) for English language editing. And we greatly appreciate the editor and anonymous reviewers for their constructive comments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2020.111244.

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