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The diversity and biogeography of microeukaryotes in the euphotic zone of the northwestern Pacific Ocean

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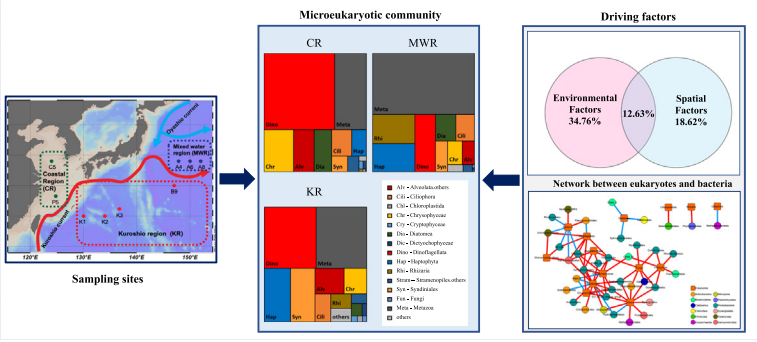
HIGHLIGHTS

- Microeukaryotic communities in the northwestern Pacific were investigated.
- The microeukaryotes were dominated by Metazoa and Dinoflagellata.
- The microeukaryotic biogeography were driven mainly by environmental factors.
- Temperature was the most important environmental factor.
- Bacteria presented biotic potential of shaping microeukaryotic biogeography.

GRAPHICAL ABSTRACT



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ABSTRACT

Microeukaryotes are the key ecosystem drivers mediating marine productivity, the food web and biogeochemical cycles. The northwestern Pacific Ocean (NWPO), as one of the world's largest oligotrophic regions, remains largely unexplored regarding diversity and biogeography of microeukaryotes. Here, we investigated the community composition and geographical distribution of microeukaryotes collected from the euphotic zone of three different regions in the NWPO using high-throughput sequencing of the 18S rRNA gene and quantified the contributions of environmental factors on the distributions of microeukaryotes. The relative abundance of different group taxa, except for Ciliophora, presented distinct patterns in each region, and Metazoa and Dinoflagellata dominated the community, contributing approximately half of reads abundance. Spatial and environmental factors explained 66.01% of community variation in the NWPO. Temperature was the most important environmental factor significantly correlated with community structure. Bacterial biomass was also significantly correlated with microeukaryotic distribution, especially for Dinoflagellata and Diatomea. Network analysis showed strong correlations between microeukaryotic groups and free-living bacteria and different bacterial taxa were correlated with specific microeukaryotic groups, indicating that their interactions enabled microeukaryotic groups to adapt to diverse environments. This study provides a first glance at the diversity and geographical distribution of microeukaryotes in the NWPO and sheds light on the biotic and abiotic factors in shaping the microeukaryotic community in the ocean.

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1. Introduction

Microeukaryotes (protists, fungi and small zooplankton) are vital to marine ecosystem, regulating marine productivity, the food web and biogeochemical cycles of various biogenic elements (Worden et al., 2015). Recently, more and more attention has been paid to protists for their high diversity and diverse trophic modes (Caron et al., 2008; Caron et al., 2012; Caron et al., 2016). In the epipelagic ocean, photosynthetic protists, like diatoms, dinoflagellates and certain haptophytes (coccolithophores), are the major contributors of primary production and construct the basement of the ecosystem, while high trophic protists, such as ciliates and flagellates, consuming bacteria and small protists are prayed by zooplankton (Sherr et al., 2007). Moreover, diverse mixotrophic protists also play important roles in balancing the ecosystem in the oligotrophic ocean (Unrein et al., 2014). This food-web transfer can fix CO_2 into particle organic carbon (POC) and sink down into the deep ocean to maintain the balance of the ocean system and carbon cycle (Worden et al., 2015). However, each group of microeukaryotes makes their own contribution to the balance of the ecosystem and dominates in distinct habitats (Grossmann et al., 2016). Recent studies demonstrate that microeukaryotic community structure is driven by biotic (e.g., prey availability, top-down grazing and bacterial effects) and abiotic (e.g., spatial factors, light, temperature, salinity and nutrients) factors (Sherr et al., 2007; Caron et al., 2016). Moreover, individual microeukaryotic taxa have distinct capabilities to adapt to different environmental factors, such as nutrients and temperature (Palenik, 2015; Rao et al., 2018). Understanding microeukaryotic diversity and group-specific interactions with environmental factors in different regions is critical to unveiling the ecological processes and mechanisms involved in maintaining the stability and function of the ecosystem (Naeem and Li, 1997).

High-throughput sequencing methods have enhanced our ability to assess the biodiversity of microeukaryotes and identify their ecological significance in the ocean (Bik et al., 2012). Large-scale and multi-sample datasets show that distribution patterns of protists are distinct between global ocean areas and different habitats (de Vargas et al., 2015; Grossmann et al., 2016). Growing evidence supports a long-tailed species abundance curve with a large number of rare species presenting in most ecosystems (Sogin et al., 2006; Fuhrman, 2009; Pedros-Alio, 2012; Lynch and Neufeld, 2015), however, microbial geographic patterns are shaped by different processes, like dispersal limitation and environmental stresses (Foissner et al., 2006; Hanson et al., 2012; Sul et al., 2013). Global-scale investigation proves that microbial community compositions are driven by spatial and environmental factors, especially temperature (Sunagawa et al., 2015; Villar et al., 2015). Spatial factors together with environmental factors also play important roles in structuring the microeukaryotic community (Zhang et al., 2018a; Zhang et al., 2018b). However, biotic factors are less frequently mentioned in previous microeukaryotic studies. Bacterial activity, such as material exchange, chemical communication and algicidal activity, should also be considered as important factors shaping microeukaryotic community structure in complex oceanic conditions (Caron et al., 2016). Direct associations between bacteria and protists have been proved by co-culture studies (Amin et al., 2015; Cruz-Lopez and Maske, 2016). Co-occurrence patterns in different marine regions can provide us a profound understanding of microbe-microbe interactions in natural environments (Fuhrman, 2009), and specific bacterial groups are tightly associated with phytoplankton bloom (Needham and Fuhrman, 2016). However, the interactions between microeukaryotes and bacteria in different natural habitats are still not well understood.

In the northwestern Pacific Ocean (NWPO), the environment is shaped by the Kuroshio and Oyashio currents which are driven by wind and possess different physical-chemical features (Qiu, 2001). The Kuroshio Current carries oligotrophic water at high temperature from the equator, while the Oyashio Current originating from the subarctic is characterized by high nutrient levels and low temperatures, and the confluence of them shapes the Kuroshio-Oyashio Transition Area which is also called the Mixed water region (Yasuda, 2003). These currents contribute a gradient of physics, chemistry and biology to the NWPO (Qiu, 2001) which provides an ideal area to study the interaction between microorganisms and environmental factors. Previous studies show that the different regions are dominated by different groups of phytoplankton (Selph et al., 2005; Kok et al., 2014; Kataoka et al., 2017). Recent studies focusing on either bacteria or picoeukaryotes show that temperature shapes community structures (Xia et al., 2017; Li et al., 2018; Wang et al., 2019). However, so far, we know little about the diversity and geographic distribution of microeukaryotes and their interactions with biotic and abiotic factors in this area.

In this study, we conducted a comprehensive study of the diversity of microeukaryotes in three different regions in the NWPO using high-throughput sequencing of the 18S rRNA gene. We assessed the contribution of spatial and environmental factors to shaping the geographic patterns of microeukaryotes. Furthermore, we assessed the biotic factors by constructing the co-occurrence network among microeukaryotes and free-living bacteria. The purpose of this study was to unveil eukaryotic microbial community structure in different habitats and to explore biotic and abiotic factors shaping community structure and distribution of microeukaryotes in the NWPO.

2. Materials and methods

2.1. Sampling and sample preparation

The survey was carried out in the northwestern Pacific Ocean (NWPO) from Mar. 30th to May. 6th 2015. Seawater samples were collected with Niskin bottles from the sea surface and the deep chlorophyll maximum (DCM) layer at nine sites except for site P5 which was sampled just one layer due to the strong mixing in the upper 30 m. Each sample was pre-filtered by 200 μm Bolting Cloth and filtered through the 1.6 μm GF/A membrane (142 mm diameter, Waterman) and then a 0.2 μm PC membrane (142 mm diameter, Millipore) by peristaltic pump (Flojet). 50 L to 500 L of seawater were filtered from each layer depending on microeukaryotes biomass: in the oligotrophic Kuroshio region, 500 L seawater was filtered due to the extremely low biomass, while in the coastal region, 50 L seawater was filtered due to the high biomass. Finally, 17 microeukaryotic samples (1.6–200 μm) were collected and each sample contained two replicates. Meanwhile, 17 free-living bacterial samples (0.2–1.6 μm) were collected for network analysis of microeukaryotic groups and bacteria. All samples were immediately frozen with liquid nitrogen and stored at −80 °C until processed.

2.2. Environmental parameters measurement

Temperature, salinity and oxygen concentration were obtained from CTD (conductivity-temperature-depth) profiler (SeaBird Electronics, Inc., Bellevue, WA, United States). Seawater samples were collected for nutrient analysis by filtering through GF/F membrane (47 mm diameter, Waterman). The nutrient concentrations were analyzed photometrically using an autoanalyzer (Model: SkalarSANplus). The analytical precision of NO_2^-, NO_3^-, PO_4^{3-}, and SiO_3^- were 0.1, 0.1, 0.05, and 0.2 μM. Bacterial abundance samples were collected by pre-filtering through a 1.6 μm GF/A membrane. Triplicate 2 mL seawater samples with 2% glutaraldehyde in 2 mL tubes were stored at −20 °C until processed. Bacterial cells were stained with SYBR Green I in anhydrous dimethyl sulfoxide and incubated in the dark for 15 min. Bacterial abundance was measured by a BD FACSAria Flow Cytometer (Becton Dickinson, USA) following the protocol described previously (Marie et al., 2001).

2.3. DNA extraction and sequencing

DNA of microeukaryotic samples (>1.6 μm) was extracted by improved extracting protocols (Yuan et al., 2015), owing to the complex cell covering of dinoflagellates and diatoms. Prokaryotic samples executed DNA were extracted by FastDNA SPIN extraction kit for soil (MP Biomedicals, Santa Ana, CA), according to the manufacturer's instructions.

The V4–V5 hypervariable region of eukaryotic 18S rDNA was amplified with Ek-NSF573 and Ek-NSR951 (378 bp) (Mangot et al., 2013). This primer couple was selected by an in silico approach with a suitable overlap to conform to the sequencing accuracy of Miseq platform (Bradley et al., 2016). All PCR reactions were carried out in 30 μL reactions with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs); 0.2 μM of forward and reverse primers, and about 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10s, annealing at 50 °C for 30s, and elongation at 72 °C for 60s, and finally, 72 °C for 5 min. A negative PCR control with no template DNA was included for the reactions. All amplicons were then sequenced on a single run using the Illumina MiSeq 2x300bp platform.

The V3–V4 hypervariable region of prokaryotic 16S rDNA was amplified with 341F (5′CCTACGGGRBGCASCAG-3′) and 806R (5′GGAC TACNNGGGTATCTAAT-3′) using the same protocol as eukaryotes. The primers can amplify both bacterial and archaea but with a bias for bacteria (Yu et al., 2005). All amplicons were sequenced on a single run using the Illumina HiSeq 2 × 250 bp platform.

2.4. Sequence assembly, clustering and annotation

Raw data were first separated to each sample by barcodes and then the barcodes and primer sequences were removed. Separated raw data were merged into raw tags using FLASH (V1.2.7, http://ccb.jhu.edu/software/FLASH/) (Magoc and Salzberg, 2011). Raw tags were filtered by quality filters processed using QIIME (V1.7.0, http://qiime.org/index.html) (Caporaso et al., 2010). Filtered tags were grouped into operational taxonomic units (OTUs) at 0.97 similarities with removal of chimera using USEARCH (version 7.1 http://drive5.com/uparse/) (Edgar, 2013). OTUs from 16s and 18s rDNA data were annotated using the RDP classifier (Cole et al., 2009) confronted against the Silva (release 128) (Quast et al., 2013) respectively by a confidence threshold of 0.7.

For analyses on higher levels of taxonomic group, the following taxa were selected: Alveolata. others, Ciliophora, Chloroplastida, Chrysophyceae, Cryptophyceae, Diatomea, Dictyochophyceae, Dinoflagellata, Haptophyta, Rhizaria, Stramenopiles. others, Syndiniales, Fungi, Metazoa. The group 'Alveolata. others' excluded Ciliophora, Dinoflagellata and Syndiniales. The group 'Stramenopiles. others' excluded Chrysophyceae, Diatomea and Dictyochophyceae. The OTUs ambiguously annotated or unclassified by one of the given taxonomic groups were shown as 'others'. Except for the multicellular groups of Metazoa, Fungi and others, the remaining groups were clustered into protists.

2.5. Statistical analysis

To ensure inter-sample comparability for our taxonomic diversity estimates and following statistical analyses, we utilized the QIIME software to subsample by randomly reducing the number of reads in each sample to the lowest number (19,793 sequence reads) of reads in any individual sample. Finally, the dataset retained 653,169 sequence reads for the entire community.

For alpha-diversity analysis, community diversity parameters (Shannon index) were calculated using the mothur software (http://www.mothur.org/wiki/Schloss_SOP#Alpha_diversity) (Schloss et al., 2011), and rarefaction curves were drawn by R (version 3.5.2). For beta-diversity analysis, non-metric multidimensional scaling (NMDS) analysis was operated in R based on Bray-Curtis similarity by '*vegan*' package. The Bray-Curtis similarity was derived via the vegdist function in '*Vegan*' package. An analysis of similarity (ANOSIM) was used to statistically test for significant differences in microeukaryotic communities in the three regions and two layers. Furthermore, the nonparametric Mann-Whitney *U* test and Kruskal-Wallis H test were calculated by '*stats*' package to test significant differences of microeukaryotic taxa among regions and layers.

Mantel tests were run in R to determine correlations between environmental factors and the microeukaryotic community (based on Bray-Cutis similarity). For environmental parameters (z-score-transformed), Euclidean distance matrices were calculated via the R base dist function. We quantified the relative effects of environmental and spatial factors in shaping the microeukaryotic community with variation partitioning analysis (VPA) based on redundancy analysis (RDA). A set of spatial variables was generated using principal coordinates of neighbor matrices (PCNM) analysis (Borcard and Legendre, 2002) based on the longitude and latitude of the sampling sites. Then, RDA was used to partition the variation of the community composition between the extracted PCNM spatial variables and environmental variables by 'vegan' package. VPA was performed using the "varpart" function of the vegan package which allows the total variation to be decomposed into fractions that indicate the importance of pure environmental variables, pure spatial variables, shared fraction and unexplained variation.

In order to demonstrate the relationship between eukaryotic groups and the environmental parameters, sparse partial least square (sPLS) was used as implemented in the R package '*mixOmics*' (Lê Cao et al., 2008). The sPLS was applied in regression mode, which could model a causal relationship between the lineages and the environmental traits, that is, PLS could predict environmental traits from lineage abundances by calculating correlation between two matrices constructed with eukaryotic relative abundance and environmental parameters. This approach enabled us to identify high correlations between certain lineages and environmental traits but without taking into account the global structure of the planktonic community.

Network analysis was conducted based on Spearman's correlations calculated using the rcorr function in the '*Hmisc*' package. Robust correlations were considered if Spearman's correlation coefficient (ρ) was >0.6 for positive or < −0.6 for negative and statistically significant ($p < 0.05$) (Barberán et al., 2011). Positive correlation could be cooperation, symbiosis or parasitism, and negative correlation means predation or competition. Networks were visualized using Cytoscape (version 3.7.1).

3. Results

3.1. Overview of the survey area

The survey was conducted in the NWPO from Mar. 30th to May. 6th 2015. The environmental parameters of the investigation area are shown in Table 1. Three regions: the coastal region (CR, including C5 and P5 sites), the Kuroshio region (KR, including K1, K2, K3 and B9 sites) and the mixed water region (MWR, including A4, A6 and A8 sites) were classified based on current flows (Fig. 1) and environmental factors (Table 1). Concentrations of nitrogen and phosphorus in the surface layer were much lower than that in the DCM layer, and the highest was observed in the DCM layer of the coastal C5 site. Concentration of chlorophyll *a* and bacterial abundance were higher in the DCM layer, except for bacterial abundance at A6 site. Temperature and salinity in the CR were obviously lower than that in the other two regions, but bacterial abundance and concentrations of chlorophyll *a* and oxygen were higher.

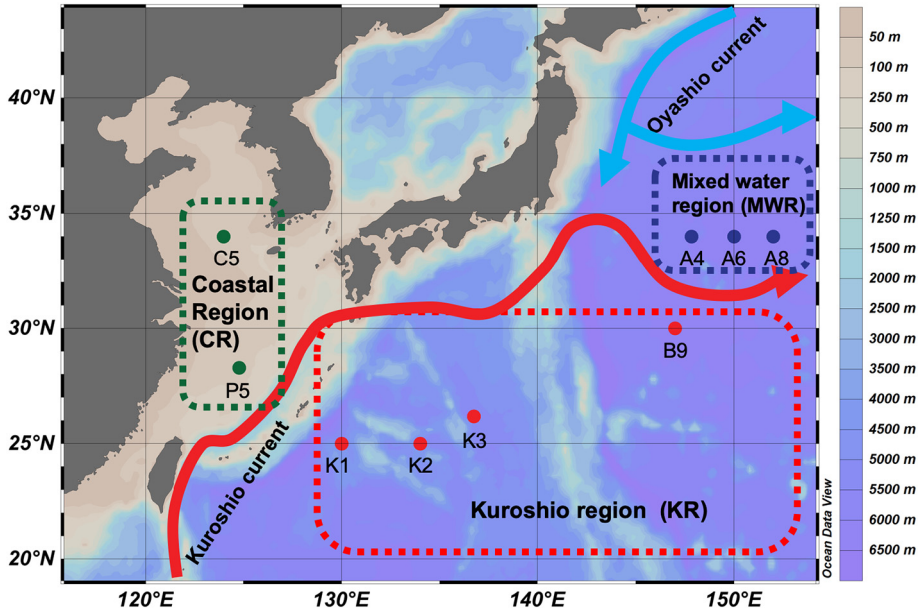

Fig. 1. Sampling sites and schematic illustration of the Kuroshio current and the Oyashio current.

3.2. Diversity and distribution of microeukaryotes

2,188,792 high-quality sequences were obtained from 33 samples and were clustered into 3036 operational taxonomic units (OTUs). The rarefaction curves were roughly saturated for all samples (Fig. 2A). In α diversity analysis, the Shannon index showed the diversity of each sample in the NWPO varied from 3.62 to 5.23 in the KR, from 2.94 to 4.55 in the MWR and from 2.38 to 3.72 in the CR (Table S1). Based on Mann-Whitney *U* test, the Shannon index of samples in the KR was significantly higher than that in the MWR ($P < 0.001$) and CR ($P < 0.01$) (Fig. 2B). In β diversity analysis, nonmetric multidimensional scaling analyses (NMDS) indicated that the community shifted across the three regions by sorting of sites (Fig. 2C). Analysis of similarity (ANOSIM) (Table 2) also showed that the communities of the three regions were significantly ($P < 0.01$) separated ($R = 0.596$). However, community dissimilarities between the two layers were 0.19 in the NWPO. Of the 15 most abundant taxa at class level, more taxa groups presented significant difference among different regions than two layers (Fig. S1, S2), indicating that the community difference was greater between regions than between water layers.

The relative abundances of microeukaryotic groups in the three regions exhibited different patterns (Fig. 3A). Protists accounted for 64% of total sequences, comprising 80%, 71% and 47% in the CR, KR and MWR, respectively. Metazoa and Dionflagellata were the most abundant groups in the investigation areas, and they accounted for more than half of the proportion of the community (35% and 23% of total sequence reads), however, their relative abundance differed among the three regions. Dinoflagellates were most abundant (44%) in the CR, while Metazoa had the highest proportion (52%) in the MWR. Dinoflagellata and Metazoa dominated in the KR and presented comparable proportions (27% and 26% respectively). Protistan composition of each site was relatively stable in the CR and KR but fluctuated in the MWR (Fig.3B). Dinoflagellata was the most abundant protist group in the CR and KR, but Haptophyta was more abundant in the KR and MWR than that in the CR. Diatomea contributed a little to the protist composition in the KR but its contribution was higher in the other two

Table 1
Sampling sites locations and environmental parameters.

Region	Station	Latitude (°N)	Longitude (°E)	Depth (m)	Temperature (°C)	Salinity (PSU)	Oxygen (mg/l)	Chlorophyll (μg/L)	BA (/L)	NO_2 (nM)	PO_4 (μM)	SiO_4 (μM)	NO_3 (μM)
CR	C5-S	34	124	5	13.43	32.91	8.63	0.19	1,289,806	6	0.04	0.43	0.07
	C5-D	34	124	30	9.31	32.90	9.33	2.69	1,813,807	301	0.24	4.50	5.11
	P5-S	28.29	124.78	5	17.51	33.73	9.23	5.80	1,995,198	25	0.07	3.36	0.03
KR	K1-S	25	130	5	23.24	34.89	4.67	0.01	256,543	UD	UD	UD	UD
	K1-D	25	130	60	21.97	34.87	6.29	0.93	768,711	24	0.01	1.18	0.07
	K2-S	25	134	5	24.58	35.19	4.79	0.02	302,876	15	0.11	9.92	0.22
	K2-D	25	134	85	20.19	34.90	6.56	0.29	608,536	59	0.13	14.10	0.55
	K3-S	26.8	136.73	5	23.25	34.77	5.12	0.01	576,059	12	0.01	0.32	0.08
	K3-D	26.8	136.73	80	19.97	34.87	6.67	0.52	804,833	34	0.15	1.31	1.70
	B9-S	30	147	5	20.26	34.82	4.77	0.06	176,553	14	0.14	22.38	0.13
	B9-D	30	147	60	18.00	34.83	7.31	0.66	552,371	17	0.13	20.90	0.12
MWR	A4-S	34	147.83	5	17.66	34.67	6.77	0.72	197,351	23	0.07	4.25	0.03
	A4-D	34	147.83	35	17.51	34.76	7.43	3.96	387,288	67	0.09	2.88	0.26
	A6-S	34	150	5	17.73	34.86	7.01	1.24	345,482	14	0.07	2.95	0.17
	A6-D	34	150	45	17.43	34.77	7.33	2.30	278,962	119	0.12	2.52	0.73
	A8-S	34	152	5	18.00	34.92	6.04	0.11	310,029	39	0.07	2.89	0.13
	A8-D	34	152	65	17.14	34.77	7.23	1.05	1,013,229	295	0.10	1.91	1.44

S, surface; D, DCM layer; BA, bacterial abundance; PSU, practical salinity unit; Chla, chlorophyll a. UD means the value is under the detection-limit.

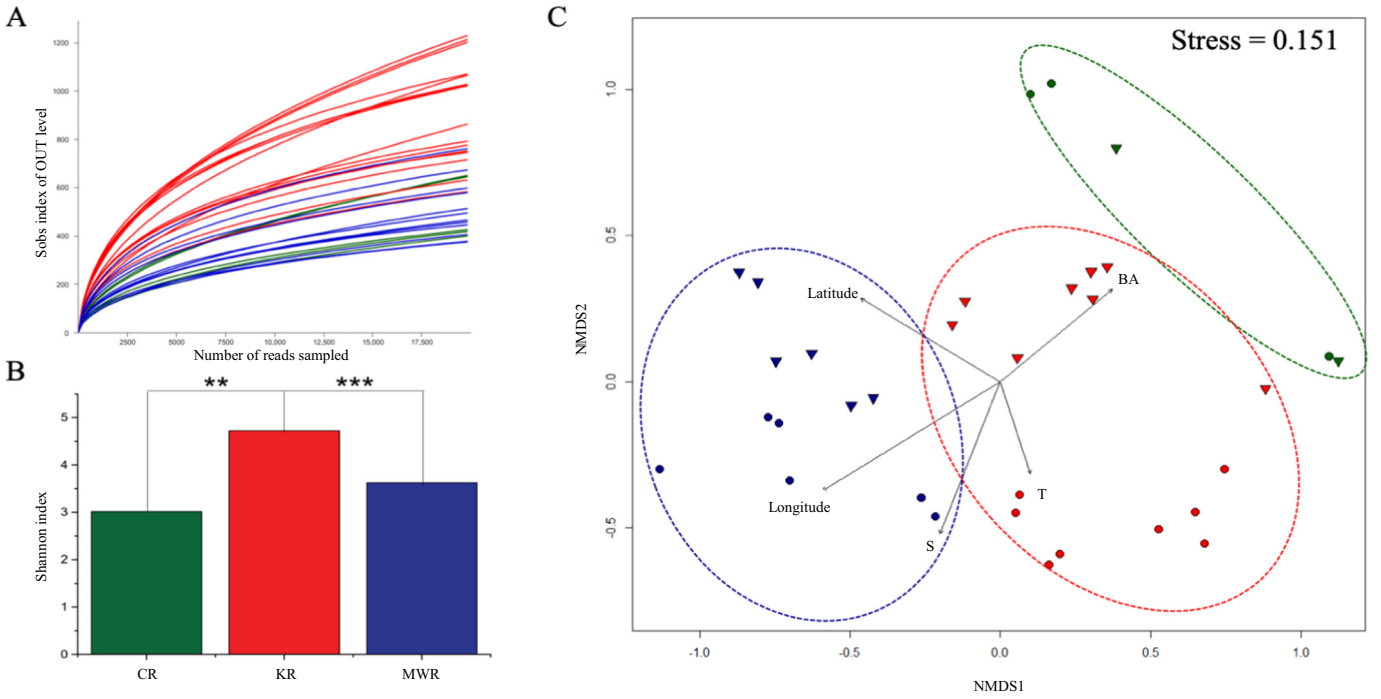

Fig. 2. Alpha and beta diversity analysis of eukaryotic composition among three regions. (A) rarefaction curves of similarity-based operational taxonomic unit (OTUs) at 97% sequence identity threshold, (B) difference significance of average Shannon index among three regions, (C) Non-metric multidimensional scaling ordinations (NMDS) for microeukaryote community and significantly correlated environmental parameters for sorting of sites (arrows). T: temperature; S: salinity; BA: bacterial abundance.

regions. Ciliophora contributed a similar proportion (4%) in the three regions.

3.3. Correlations of microeukaryotic communities with spatial and environmental factors

Spearman correlation comparing Bray-Cutis community similarity with geographic distance between samples presented significant negative correlations for the microeukaryotic community in the NWPO and KR with the correlation coefficients of −0.4997 ($P < 0.01$) and −0.3444 ($P < 0.01$), respectively (Fig. 4). However, in the CR and MWR, the correlation was not significant. Variation partitioning analysis showed that spatial and environmental factors together explained 66.01% of the entire microeukaryotic community variation and 12.63% of variation was jointly explained by both factors (Fig. 5A). The spatial factors (transformed into five spatial variables principal coordinates of neighbor matrices) explained 18.62% of pure variation ($P < 0.01$), while environmental factors explained 34.76% ($P < 0.01$). Within environmental factors, variation explained by abiotic factors (33.17% of pure variation) was higher than that explained by biotic factors (2.40% of pure variation) (Fig. 5B).

Table 2
Analysis of similarities (ANOSIM) of microeukaryotic communities among different layers and regions.

	Layers			Regions	
	R	P		R	P
Total	0.190**	0.002	Total	0.596**	0.001
CR	0.500	0.335	sur	0.686**	0.001
KR	0.314*	0.019	DCM	0.726**	0.001
MWR	0.654**	0.004			

The ANOSIM results are calculated using the Bray-Curtis similarity data estimated from 999 permutations.
* $P < 0.05$.
** $P < 0.01$.

Mantel test indicated that microeukaryotic community structure in the NWPO was significantly correlated with almost all environmental factors ($p < 0.01$) except for NO_2^- and SiO_3^- (Table 3). Microeukaryotic community was significantly correlated with temperature and salinity in the CR, while it was significantly correlated with all environmental factors except for nutrients in the KR. Microeukaryotic community in the MWR was significantly correlated with all the environmental factors except for salinity and presented high correlation with nutrients.

sPLS method was used to calculate the correlation between each microeukaryotic group distribution and each environmental factor in the NWPO. Heatmap results showed that the highest positive correlation was observed between Diatomea and NO_3^- concentration ($R = 0.73$) (Fig.6). Metazoa and Rhizaria were positively correlated with latitude and longitude, while Dinoflagellata, Dictyochophyceae and Syndiniales presented negative correlations, suggesting that distributions of different microeukaryotic groups had different geographic patterns. Temperature and salinity presented similar correlation patterns with different microeukaryotic groups. Chrysophyceae, Syndiniales and Dictyochophyceae preferred high temperature and salinity while Diatomea favored low temperature and salinity. Others presented high correlations with nutrients, such as nitrate, nitrite and phosphate. Diatomea and Dinoflagellata displayed positive correlations with concentrations of chlorophyll *a* and oxygen, indicating that they are the main photosynthetic groups contributing to oxygen generation in the ocean. Dinoflagellata ($R = 0.65$) and Diatomea ($R = 0.45$) presented highly positive correlations with bacterial abundance, while Metazoa exhibited a negative correlation.

3.4. Network analysis of microeukaryotic groups and free-living bacteria

16 s rRNA gene sequencing results of free-living bacteria (0.2–1.6 μm) collected from the surface and DCM layers at each site indicated different patterns of bacterial structure in the three regions (Fig. S3). Spearman correlation analysis denoted strong correlations between microeukaryotic groups and free-living bacteria (Fig. 7). 36 orders of bacteria were strongly correlated with 14 microeukaryotic groups, and

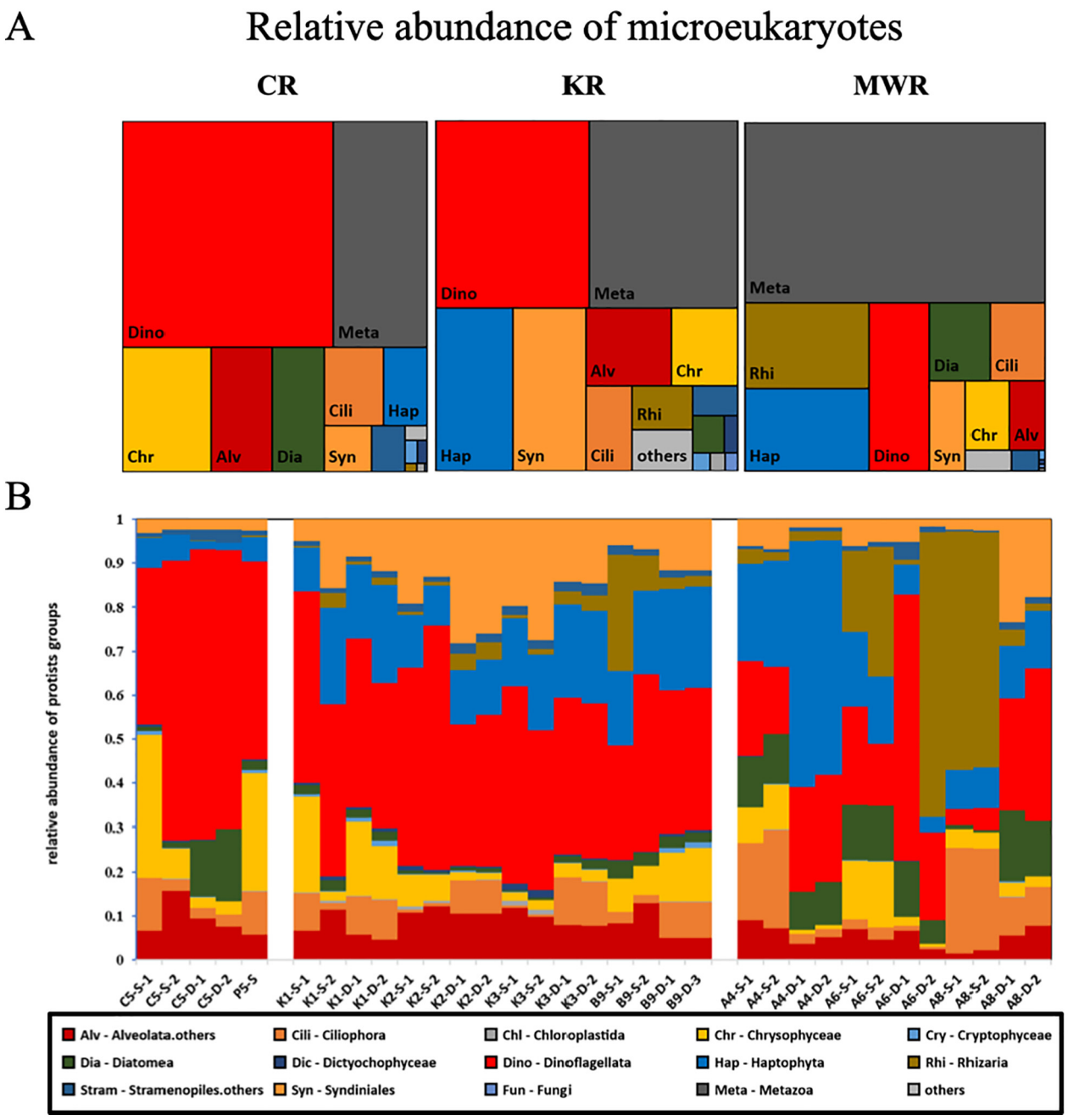

Fig. 3. Community composition of eukaryotic groups and protist groups (A) relative abundance of eukaryotic groups in three regions, (B) relative abundance of protists. Protists groups are all the groups except Fungi, Metazoa and others.

16 of them belonged to Proteobacteria. Almost all microeukaryotic groups were correlated with bacteria except for Cryptophyceae, and each group was associated with specific bacterial assemblage. Bacterial taxa were the most associated with Syndiniales (14 orders). Thiotrichales and Xanthomonadales were positively correlated with most microeukaryotic groups (five groups). Interestingly, some bacteria were specifically correlated with Diatomea, Chloroplastida, Rhizaria and Ciliophora.

4. Discussion

4.1. Microeukaryotic distribution in the NWPO

Studies have shown that microeukaryotes present different community compositions in different habitats (Grossmann et al., 2016; Massana et al., 2015; de Vargas et al., 2015) and our results also revealed the different diversity and structure of microeukaryotes among the three regions (Fig. 2, Table 2). Metazoa and Dionflagellate were the most abundant eukaryotic groups in our study, which was consistent with a previous study on global ocean scale (de Vargas et al., 2015). Although we used a 200 μm sieve to remove large zooplankton, Metazoa still accounted for most sequence reads of all microeukaryotes and particularly dominated in the MWR. A previous study shows that elongated species of small-size and eggs, spores or larvae of large-size zooplankton can pass through the 200 μm pores and contribute to the assemblage (Liu et al., 2017). The Oyashio current is a productive current carrying a high biomass of phytoplankton from the subarctic and contributes to a high biomass of zooplankton as latitude increases in the western Pacific Ocean (Taniguchi, 1972; Sakurai, 2007; Sun and Wang, 2017). Dinoflagellata was the second most abundant eukaryotic group in the

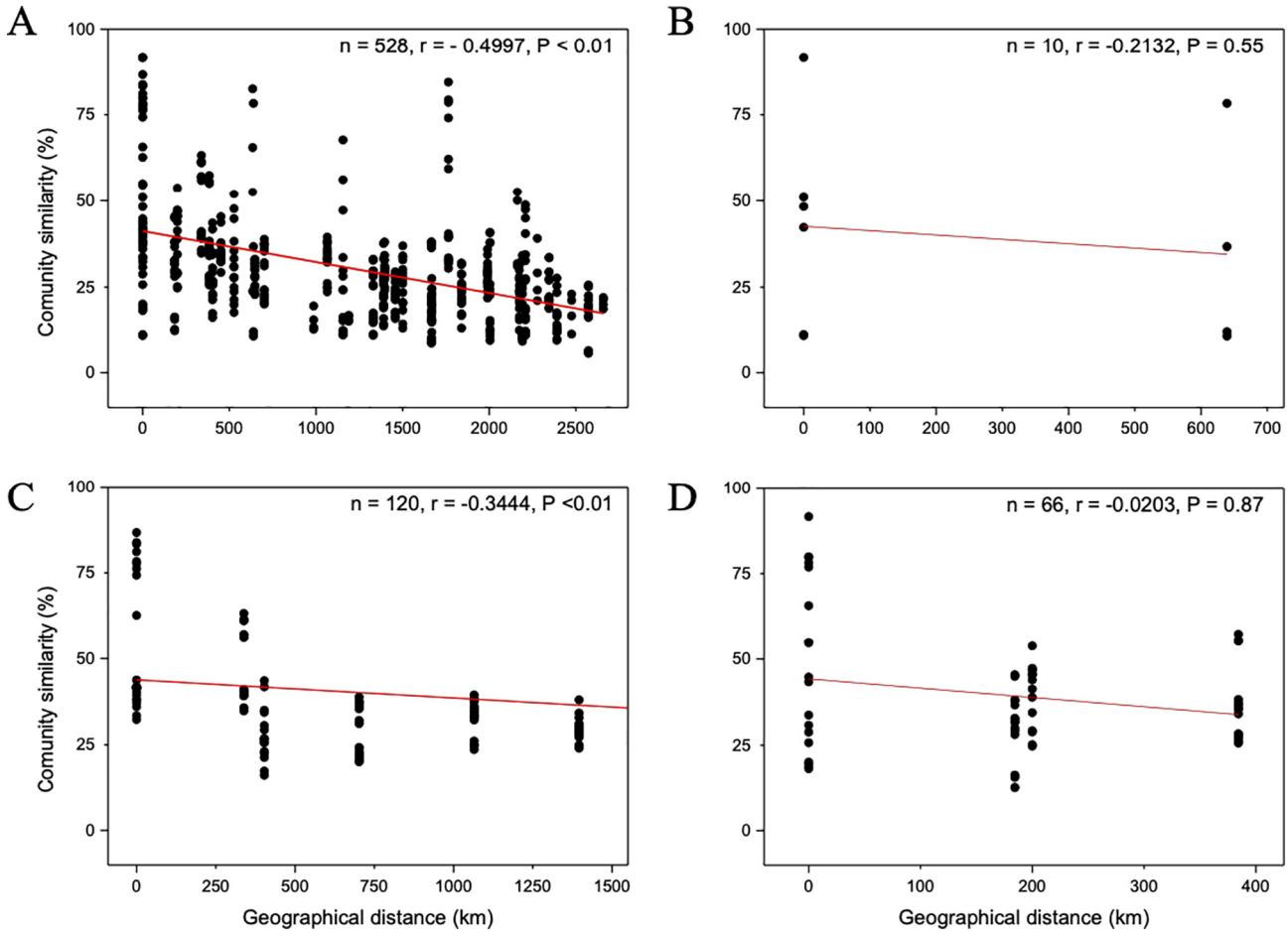

Fig. 4. Spearman's correlations between the Bray–Curtis similarity of microeukaryotic community and geographical distance between sampling sites in (A) NWPO, (B) CR, (C) KR and (D) MWR. n is the number of comparison and *P* values are indicated.

NWPO and dominated in the CR and KR. It is also reported that dinoflagellates dominate the phytoplankton community in these two regions (Kok et al., 2014; Liu et al., 2016). Previous studies show that diatoms prefer to live in coastal areas with high nutrient levels and low temperature (Smetacek, 2012), while haptophytes dominate in open ocean and oligotrophic ocean (Not et al., 2008; Unrein et al., 2014). Our study demonstrated these preferences: Diatomea constituted a high proportion in the CR and MWR, while Haptophyta was abundant in the KR and MWR. In addition, other groups also showed different distributions across three regions. Overall, these results indicated that microeukaryotes presented habitat-specific distributions in the NWPO.

4.2. Factors regulating geographical distribution of microeukaryotes

Dispersal limitation and environmental heterogeneity are the main factors shaping the distributions of microorganisms, and they generate a negative correlation between community similarity and geographic distance (distance-decay relationship) (Green and Bohannan, 2006; Hanson et al., 2012). Previous studies show that dispersal limitation caused by geographical distance is one of the important factors in shaping the microeukaryotic community in the ocean (Zhang et al., 2018a; Zhang et al., 2018b). We found that the microeukaryotic community presented a distance-decay relationship in each region which was strengthened as distance increased (Fig. 4). Limitation of microbial dispersal strengthened by increasing geographic distances has been reported (Nekola and White, 1999; Martiny et al., 2006). However, some studies show that environmental selection is also an important factor that strengthens the distance-decay relationship and eukaryotes are more likely to be affected by environmental factors than dispersal limitation (Hanson et al., 2012; Wu et al., 2017). In our study, environmental factors contributed more than spatial factors (34.76% compared with 18% of pure variation) (Fig. 5), indicating that environmental selection plays a more important role in shaping the microeukaryotic community than dispersal limitation in the NWPO.

Studies demonstrate that both environmental factors and geographical distance play important roles in driving community structure on a small scale (Horner-Devine et al., 2004; Martiny et al., 2006). However, our study showed that the factors shaping the microeukaryotic community in the KR were significantly different from that in the MWR (Table 3, Fig. 4). Community dissimilarity in the KR was significantly correlated with geographical distance, however, the distance-decay relationship in the KR was weaker than in the MWR (Fig. 4), suggesting that the distance impact in the KR was decreased by other factors. Meanwhile, all environmental factors showed a weak correlation with the microeukaryotic community in the KR. Hanson et al. (2012) report that dispersal may counteract microbial compositional differentiation and weaken the distance–decay relationship. It was postulated that the Kuroshio Current imposes strong dispersal activity on microorganisms and contributes to shaping the community in the KR. In contrast, the driving factors in the MWR presented another scenario where almost all environmental factors had significant correlations with the community, except for salinity (Table 3). These results indicate the importance of local environmental conditions in shaping the microeukaryotic community structure on a small scale.

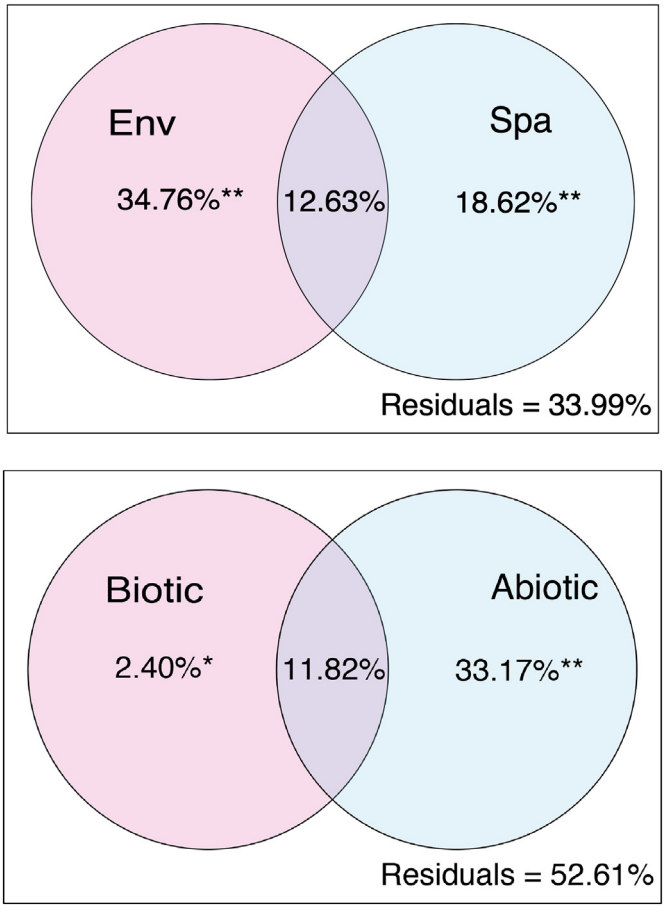

Fig. 5. Variation partitioning, showing the effects of spatial, environmental on the community composition of microeukaryotes in the NWPO. The percentage of variation were explained by each fraction, including pure, shared explained and unexplained variability. ANOVA permutation tests were calculated on the pure variation. ** $P < 0.01$. * $P < 0.05$.

4.3. Environmental factors shaping the microeukaryotic community

We used Mantel test to determine the impact of each environmental factor on shaping the microeukaryotic community. Among the factors, temperature had the highest impact on community (Table 3). Temperature is known as an important factor that may alter community composition and diversity and is also a stronger driver than other environmental factors in shaping microbial community composition by global investigation (Sunagawa et al., 2015). It is reported that temperature is the main factor driving picoeukaryotes and bacteria in the NWPO (Li et al., 2018; Wang et al., 2019). Studies indicate that more species diversity is present in low latitude ocean because high temperature leads to increasing productivity, metabolic rate and even speciation (Evans and Gaston, 2005; Fuhrman et al., 2008). In our study, we found that α diversities of the community were significantly higher in the warm KR than in the other two regions (Fig. 2B, table S1). For different taxa groups, temperature also showed high correlation with more groups than other factors. Among these correlations, temperature presented the highest negative correlation with Diatomea. A previous study shows that diatoms contribute more to phytoplankton community in the polar area than in the tropical and subtropical ocean (Malviya et al., 2016). A long-term study also demonstrates that diatoms prefer low temperature (Xiao et al., 2018), indicating that diatoms have a special strategy to adapt to low temperature. However, Chloroplastida, Syndiniales and Dictyochophyceae presented an opposite preference for temperature, while the remaining groups showed weak correlations with temperature. These results indicated that the adaptive capability of different groups to temperature is an important factor to determine the community distribution.

Inorganic nutrients are essential for growth and development of microorganisms and are thought to be important factors in shaping the phytoplankton community, and different microorganisms adapt to their optimal growth concentrations (Gregg and Casey, 2007; Follows and Dutkiewicz, 2011). Our results showed that only NO_3^- and PO_4^- were significantly correlated with the microeukaryotic community in the NWPO (Table 3). Distributions of phytoplankton groups exhibit distinct patterns under different nutrient limitations on a global scale for their specific strategies to utilize nutrients (Palenik, 2015). In our study, Diatomea was highly correlated with all nutrients, especially with NO_3^-. A previous study indicated that diatoms dominate in the early spring bloom, and they generally adapt to turbulent environments characterized by high levels of nutrients (Liess et al., 2009). Therefore, nutrients played an important role in shaping the communities of diatoms in the NWPO. A large-scale meta-analysis indicates that salinity is one of major determinants across different habitats (Lozupone and Knight, 2007). Our result showed that salinity presented a significant correlation with total community. Dinoflagellata and Diatomea presented a negative correlation with salinity, while Haptophyta presented a positive correlation. The former two groups are predominant in coastal ocean (Liu et al., 2016) which is easily affected by river input which contributes to a lower salinity. Meanwhile, haptophytes prefer to live in the open ocean, indicating that the salinity tolerance of microeukaryotes also impacts their distribution.

All microeukaryotic groups were more or less regulated by environmental factors, except for Ciliophora, Chloroplastida, Cryptophyceae and Fungi. Ciliophora are a free-living group with cilium that distribute in various habitats and are adaptive to environment (Foissner et al., 2009), which explains its even distribution in the NWPO (Fig. 3). Chloroplastida are an ancient eukaryotic group with smallest genome and perform simple function to adapt different environments (Leliaert et al., 2012). Cryptophyceae are mixotrophic, motile and low-light adapted with low metabolic rate, and their growth is less influenced by environmental changes (Morgan and Kalff, 1975; Klaveness, 1989; Litchman and Klausmeier, 2008). Fungi are the main decomposers in the ocean which are more likely to be correlated with other organisms than environmental factors (Hyde et al., 1998; Richards et al., 2012). Overall, the microeukaryotic community was more or less regulated by environmental factors. However, the response of each group to environmental factors was different due to their specific adaptation strategies. The molecular mechanisms involved in adaptation to varying environmental conditions needs further study.

Table 3
Mantel test for the correlation between community and environmental factors.

	Total	CR	KR	MWR
Env_distance	0.363**	0.622	0.201*	0.659**
Temperature	0.416**	0.636*	0.232*	0.717**
Oxygen	0.321**	0.109	0.299**	0.564**
Salinity	0.312**	0.156*	0.168*	0.267
BA	0.310**	0.045	0.223*	0.319*
Chlorophyll a	0.193**	0.119	0.324**	0.260*
NO_3^-	0.321**	0.679	0.036	0.572**
NO_2^-	0.103	0.668	−0.030	0.505**
PO_4^{3-}	0.192**	0.646	0.090	0.715**
SiO_4^{4-}	−0.079	0.242	0.110	0.241*

Env_distance, Euclidean distance of all environmental variables between sampling sites; BA, bacterial abundance. The significances are tested based on 999 permutations.
* $P < 0.05$.
** $P < 0.01$.

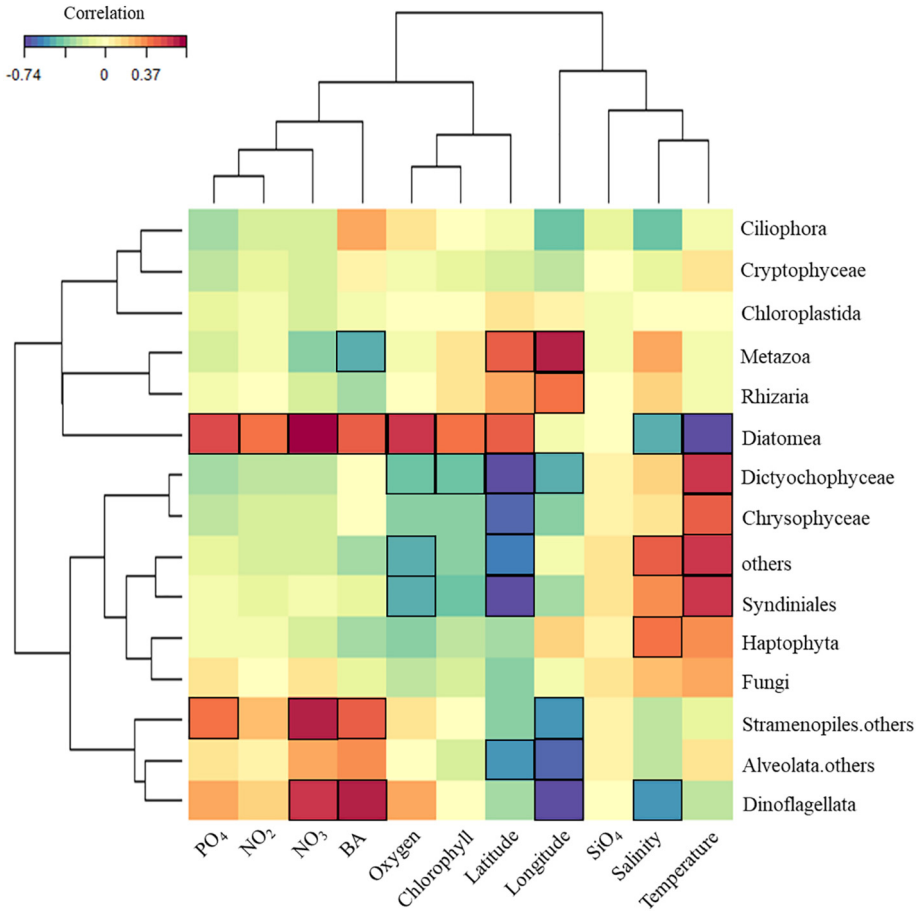

Fig. 6. Clustered heat map depicting correlations between eukaryotic groups and environmental parameters. Strong correlation is highlighted ($R > 0.4$ or < -0.4). BA: bacterial abundance.

4.4. Interaction between microeukaryotic groups and free-living bacteria

Although spatial and environmental factors explained 66.01% of variation in total, 33.99% of variation was still unexplained and might be caused by unmeasured environmental and ecological factors. Studies indicate that the interactions, for example, mutualism, cross-feeding, competition, parasitism, predation and allelopathy among microbes are also responsible for community structure (Lima-Mendez et al., 2015; Caron et al., 2016). Recent studies demonstrate that phytoplankton exhibits a stronger relationship with bacteria than with environmental factors (Pearman et al., 2016; Needham and Fuhrman, 2016). In our study, bacterial abundance was significantly correlated with the microeukaryotic distribution in the NWPO (Table 3) and highly correlated with specific groups (Fig. 6). Previous studies reveal that the growth of diatoms and dinoflagellates relies on bacteria through important materials exchange (Aziz et al., 2010; Tang et al., 2010; Amin et al., 2015; Cruz-Lopez and Maske, 2016). We also observed that these two groups were positively correlated with bacterial abundance, indicating that bacteria played important roles in shaping distribution of specific microeukaryotic groups in the natural environment across different habitats. Previous studies show that phytoplankton exhibit high selection on bacterial species (Hendrik et al., 2002; Sapp et al., 2007), which was also presented in our study, that each group of microeukaryotes interacted with a specific group-assembly of bacteria.

Dinoflagellates are an important group of protists with a wide distribution and strong adaptive ability (Gómez, 2012). The relative abundance of dinoflagellates reaches 40% of protists on average, ranging from 18% to 67% on the global scale (Le Bescot et al., 2016). Our results showed that Dinoflagellata was the most abundant protist in the NWPO, contributing the highest proportion of protists with a percentage ranging from 22.4% to 55.9% across the three regions. It is reported that more than half (58%) of dinoflagellates are heterotrophic species in the ocean and pray on other small organisms like bacteria and even small protists (Gómez, 2012), and they also need vitamin B1 and B12 produced by bacteria to support high community abundance (Tang et al., 2010). In our study, dinoflagellates exhibited the highest correlation with bacterial abundance, indicating a strong reliance on biomass of bacteria. We also found that Dinoflagellata were positively correlated with several different bacterial groups (Fig. 7), suggesting that dinoflagellates preferred to pray on bacteria and/or utilize their metabolites with a wide selection.

Diatoms contribute 20% of global primary productivity and produce large quantities of dissolved organic matter (DOM) (Armbrust, 2009; Myklestad, 2000). Interactions between diatoms and bacteria are well studied, and heterotrophic bacteria can utilize the DOM secreted by diatoms, while diatoms also need the heteroauxin or vitamins from bacteria to support growth (Amin et al., 2012). Our results showed that the distributions of diatoms presented high correlations with bacterial abundance, suggesting the interaction between diatoms and bacteria. Furthermore, we observed specifically negative correlations of Diatomea with Sphingomonadales, Order_II and Caldilineales (Fig. 7). A previous study shows that bacteria can present algicidal activity specific for diatoms (Paul and Pohnert, 2011). These results suggested that diatoms required high abundance of bacteria to support growth, however, and were easily affected by specific bacterial groups.

Co-occurring bacteria stimulating or inhibiting colony formation of the choanoflagellate *Salpingoeca rosetta* has been reported (Woznica et al., 2016), which may influence the development of multicellularity

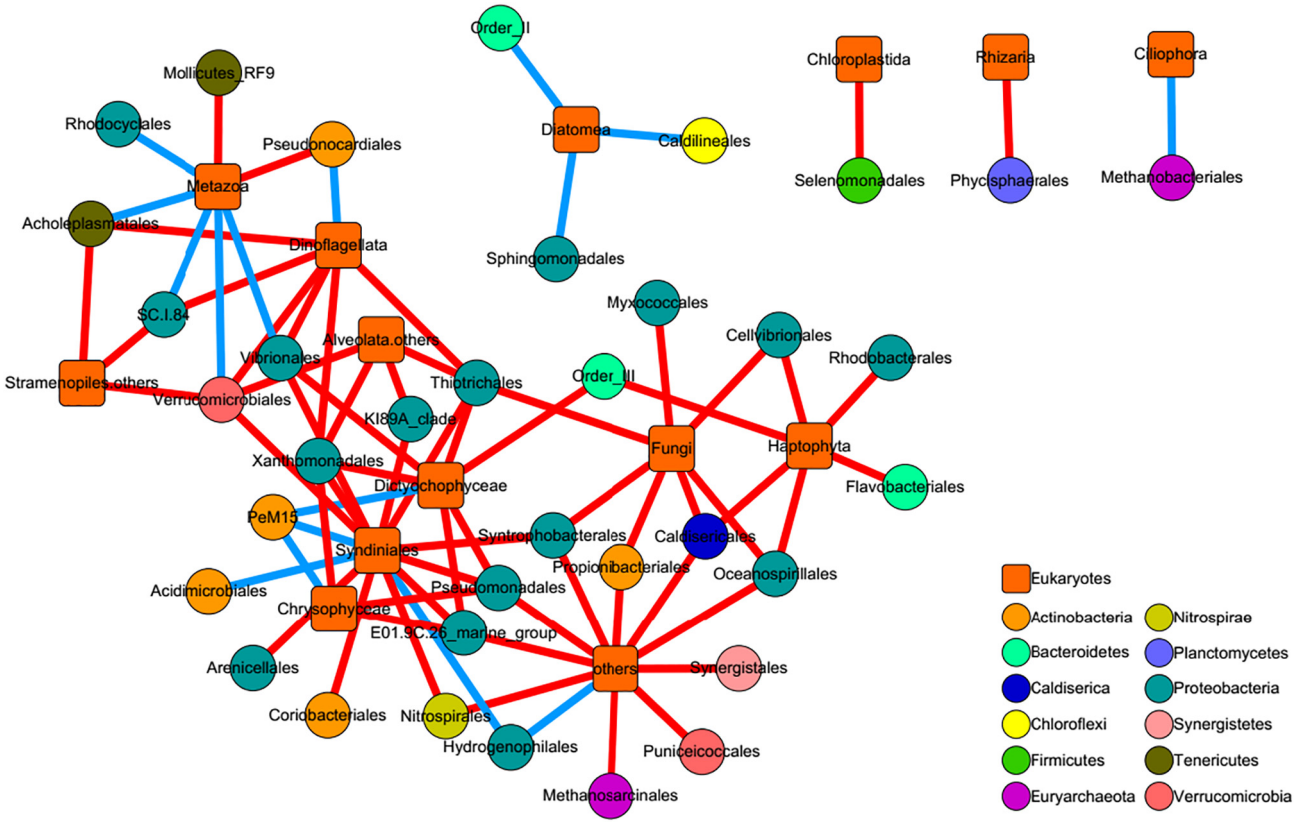

Fig. 7. Network depicting correlations between eukaryotic groups and bacterial groups. Red edges denote a strong positive correlation ($r > 0.6$) while blue edges characterize a strong negative correlation ($r < -0.6$).

in the super-groups such as Metazoa and Fungi. We also found that several orders of bacteria were significantly correlated with these two groups. Overall, each microeukaryotic group showed a high correlation with specific groups of free-living bacteria, suggesting a special association among different groups of microbes. However, field-based studies complemented with laboratory simulations are required to understand the various influences of associated bacteria on microeukaryotic physiology, such as those related to toxin production, organic matter production and recycling, and algal bloom formation.

5. Conclusion

Our results indicated that both diversity and composition of the microeukaryotic community differed among the three different regions of the NWPO. A distance-decay relationship for microeukaryotes was observed in the NWPO and was strengthened by environmental selection. Geographical distance had a significant impact on shaping community structure on a large scale, while environmental factors played important roles on a small scale, especially for dynamic environments. Temperature was the main abiotic factor regulating diversity and composition of the microeukaryotic community in the NWPO, and in each region. Bacterial abundance was significantly correlated with distribution of the microeukaryotic community, especially with Dinoflagellata and Diatomea. However, they presented different interactions with free-living bacteria revealed by co-occurrence network analysis. Dinoflagellates preferred to pray on bacteria and utilize their metabolites with a wide selection. Diatoms were sensitive to environmental factors and were vulnerable to negative effects caused by specific bacterial groups. Although interactions between the microeukaryotic community and biotic and abiotic factors across different habitats were unveiled in our study, metabolic activity influenced by different factors and utilization mechanisms of exchanging materials still need to be explored in future study.

Data availability

All raw sequence data from this study were deposited in the GenBank's Sequence Read Archive (SRA) database (http://www.ncbi.nlm.nih.gov/) under the accession number SRP (BioProject Accession PRJNA476806 and BioSample accession 18 s for SAMN06246921–SAMN06246956, 16s for SAMN10963348- SAMN10963364).

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.134289.

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