



# Distribution Patterns of Microeukaryotic Community Between Sediment and Water of the Yellow River Estuary

Tian Shi<sup>1,2</sup> · Mingcong Li<sup>1,2</sup> · Guangshan Wei<sup>3,4</sup> · Jiai Liu<sup>1,2</sup> · Zheng Gao<sup>1,2</sup>

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## Abstract

Water and sediment have always been closely tied in aquatic systems. However, little information regarding the full extent of microeukaryotic composition in both the two habitats did we know especially in estuaries. In the present study, the microeukaryotic abundance, diversity, composition, and their response to environmental factors between sediment and water in the Yellow River Estuary (YRE) were investigated. The microeukaryotic 18S rRNA gene abundance ranged from  $1.03 \times 10^6$  to  $5.48 \times 10^7$  copies/g dry for sediment, and  $3.01 \times 10^4$  to  $1.25 \times 10^6$  copies/mL for water. The distribution patterns of eukaryotic microorganisms could be clustered into two different branches. And the compositions of microeukaryotes in the two habitats were distinct obviously. *Metazoa*, *Fungi*, *Streptophyta*, *Ochrophyta*, *Cercozoa*, and *Dinophyta* were more abundant in sediment. The dominant phyla in water were *Dinophyta*, followed by *Metazoa*, *Ochrophyta*, *Cryptophyta*, *Chlorophyta*, *Cercozoa*, *Fungi*, *Katablepharidophyta*, *Choanoflagellida*, and *Haptophyta*. Interestingly, the eukaryotic microorganisms detected in sediment were much less sensitive to environmental variables compared with water. Furthermore, their potential co-occurrence networks in particular were also discovered in the present study. As such, we have provided baseline data to support further research on estuarine microeukaryotes in both sediment and water, which was useful for guiding the practical application of ecosystem management and biodiversity protection.

## Introduction

Microbial eukaryotes (protists and single-celled fungi) play fundamental ecological roles as primary producers [1–3], consumers [4, 5], decomposers, saprotrophs and parasites [6, 7], as well as maintainers of biogeochemical cycles [8–11].

The previous evidence showed that microbial eukaryotes were involved in food network interaction in a rice field soil [12, 13] and Baltic Sea oxic-anoxic interface ecosystems [14, 15]. In addition, the symbiotic interactions and predatory might have a significant impact on biosphere and nutrient cycling [16–19]. Hence, eukaryotic microorganisms are crucial to maintain environmental ecological stability.

Both diverse biotic and abiotic factors had been proposed to interfere abundance and diversity of microeukaryotic communities [20, 21]. Some studies have revealed that abiotic factors can influence the distributions of microeukaryotic communities, such as pH value [22]; geographical distance [23]; depth [24]; phytoplankton taxa, and various nutrients [25, 26]. Wang et al. showed that the prokaryotic  $\beta$ -diversity was strongly influenced by spatial factors [27], while either the spatial factors or physicochemical factors being dominant were still controversial [28]. In addition, accumulating evidence had demonstrated that biotic factors also played a very important role in the distribution of microeukaryotes. Previous study revealed that ciliate communities were not only controlled by abiotic factors but also shaped by biotic factors [29, 30]. Instead of living apart, microbes interact to form ecological networks

Tian Shi and Mingcong Li have contributed equally to this work.

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✉ Zheng Gao  
gaozheng@sdau.edu.cn

- <sup>1</sup> State Key Laboratory of Crop Biology, Shandong Agricultural University, Tai'an 271018, China
- <sup>2</sup> College of Life Sciences, Shandong Agricultural University, Tai'an 271018, China
- <sup>3</sup> Key Laboratory of Marine Genetic Resources, Third Institute of Oceanography, SOA, Xiamen 361005, China
- <sup>4</sup> South China Sea Resource Exploitation and Protection Collaborative Innovation Center (SCS-REPIC), Sun Yat-Sen University, Guangzhou 510275, China

that perform ecosystem functions [31, 32]. Therefore, the expounding and predicting the variation in network structure is a high-priority issue of microbial ecology [33]. The microeukaryotic communities can be impacted by various factors, especially in complex environment; therefore, the effect of influences on eukaryotic microorganisms is still a question that need further investigation. The Yellow River Estuary (YRE), located at the interface of the Yellow River and the Bohai Sea, is a typical oxic/anoxic transition zone. Previous studies on estuarine microbes have mostly focused on single habitat, as sediment [34–36] or water [37–39]. Our previous study had reported the distinct distribution patterns of prokaryotic microorganism between sediment and water column of the Yellow River Estuary [40]. However, the study performed on the distribution of eukaryotic microorganisms between water and sediments needed further study. To fulfill our prime objective, the specific aims were to: (1) compare the different composition patterns of microeukaryotic community between sediment and overlying water, (2) reveal the key environmental factors influencing the distribution of eukaryotic microorganisms between sediment and water habitat, (3) investigate the co-existing eukaryotic microorganisms between water and sediment. The results will provide novel insights for comprehensively understanding of microeukaryotic communities, their relationships with environmental factors and assessing the eukaryotic ecology in estuary ecosystems.

## Material and Methods

### Sample Collection and Physicochemical Analysis

The details of the sampling collection and physicochemical characteristics have been described in our previous study and performed in Table S1 [40]. Briefly, surface sediment cores (0–5 cm) were collected at each site using a professional sediment sampler (Wildlife Supply Company, USA) and were transferred into sterile polypropylene bags (approximate 1 kg sediment each site). The overlying water samples were collected using a water sampler (Wildlife Supply Company, USA) at a depth of 0.5 m from each site. After being evenly mixed of three replicates, the water was transferred into sterile glass bottles and total of 3 L water was collected in each site. The sediment cores and water samples were transported on ice to the laboratory, immediately.

### DNA Extraction and Quantitative PCR

Extraction and purification of DNA from water and sediment samples for each site were extracted using the E.N.Z.A.<sup>TM</sup> Water DNA Kit and E.N.Z.A.<sup>TM</sup> Soil DNA Kit (Omega, USA) according to the manufacturer's instructions,

respectively. The extracts were quantified using Nanodrop 2000 Spectrophotometer (Thermo Scientific, USA) and the integrity of the extracts were assessed on a 1.0% (w/v) agarose gel. DNA extracts obtained from water and sediment samples were done in duplicate for parallelism and homogenized storing at  $-20\text{ }^{\circ}\text{C}$  for further analysis. The PCR primers and the amplification conditions were listed in Table S2. The standard curves were generated using serial dilutions of known copy number of plasmids containing the 18S rRNA gene fragments, exhibiting a linear response with a high correlation coefficient ( $R^2=0.995$ ), and these gene fragments were linear from gene  $8.1 \times 10^2$  to  $8.1 \times 10^8$  copies/ $\mu\text{L}$  for microeukaryotes ( $E=95.6\%$ ). In order to prove the validity, all experiments were performed based on a PCR assay of three replicates.

### DNA Pyrosequencing and Raw Pyrosequencing Reads Analysis

The pyrosequencing data were processed following a previous described study [40]. The primers and reaction conditions were illustrated in Table S2. Pyrosequencing was performed with a Quanti-Fluor<sup>TM</sup>-ST Fluorometer (Promega) on a Roche 454 GS FLX platform by the Majorbio Bio Tech Co. Ltd (Shanghai, China). Raw data were processed and analyzed using QIIME v.1.9.0 and other programs (e.g. Mothur v.1.34.4) [41, 42]. The processing of sequences included: quality filtered, chimera checked, clustering, singletons filtered. Quality filtering reserved sequences satisfied the following criteria: (i) quality score  $> 19$  when averaged across the read after trimming adapters and primers, (ii) no sequencing mismatches within the PCR primer regions, (iii) minimum sequence length of 300 bp and (iv) homopolymers smaller than six nucleotides. Primer sequences and barcode were also removed prior to further analysis. Above all, to compare the eukaryotic biodiversity by 454-pyrosequencing, the final sequences processed to the lowest read number was applied to the level of unity for normalizing the different sequencing depths.

### Analysis of Microeukaryotic Community

The estimator of microeukaryotic community Chao1 richness index, Shannon diversity index and Shannon indexes-based rarefactions were calculated using the Mothur software (Table 1 and Fig. S2). Heat maps and correlative clustering analyses were generated using Heatmap Illustrator (HemI) software. Canonical Correspondence Analysis (CCA) between microeukaryotic communities and the environmental factors was performed using CANOCO 5 software (Microcomputer Power, USA) on the strength of the result of detrended correspondence analysis (DCA) with 9999 Monte Carlo permutations for the significance

**Table 1** Microeukaryotic diversity estimators for samples in the Yellow River Estuary

Sample types	Sample ID	OTU	Chao1	Shannon
Sediment	SA	136	386	3.68
	SB	90	120	3.63
	SC	133	177	4.33
	SD	78	98	3.09
	SE	108	131	3.97
Water	WA	105	248	3.48
	WB	88	195	2.7
	WC	75	304	2.14
	WD	92	264	2.98
	WE	100	249	3.41

tests. Principal co-ordinates analysis (PCoA) was performed by R software (REF) based on Bray–Curtis distances. The co-occurrence networks among microeukaryotic microorganisms were constructed using Cytoscape version 3.5.1. Welch's *t* test was to check for significant differences of microeukaryotic community between sediment and water, using the software STAMP. The differences of microeukaryotic taxa between sediment and water were performed at different taxonomic levels based on the Linear Discriminant Analysis (LDA) Effect Size (LEfSe) method (<https://huttenhower.sph.harvard.edu/lefse/>) [43].

### Accession Number and Data Availability

The raw pyrosequencing data have been submitted to the Sequence Read Archive(SRA) of the NCBI under a specific accession number: SRP 057857.

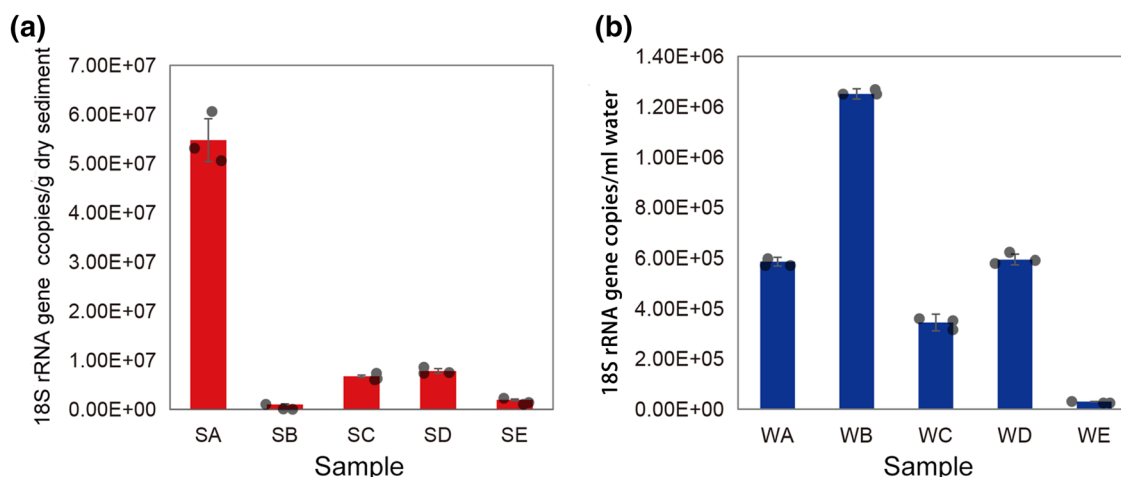
## Results

### Microeukaryotic Abundance in the Sediment and Water Habitats

Fluorescence quantitative PCR was used to detect the abundance of 18S rRNA genes of microeukaryotes in water and sediment habitats. The microeukaryotic 18S rRNA gene abundance in each sediment site ranged from  $1.03 \times 10^6$  to  $5.48 \times 10^7$  copies/g dry sediment ( $1.45 \times 10^7$  copies/g for average) (Fig. 1a and c). Nevertheless, microeukaryotic 18S rRNA gene abundance varied from  $3.01 \times 10^4$  to  $1.25 \times 10^6$  copies/mL water ( $5.61 \times 10^5$  copies/mL for average) (Fig. 1b and d). Comparing all the samples, sediment samples had highest abundance at site A, followed by D and C, with the lowest abundance at site E and B. Comparatively, in water samples, the highest abundance was at site B, followed by D, A, the lowest abundance being at site C and E.

### Microeukaryotic Diversity and Community Composition Between the Sediment and Water Habitats

In order to reveal the diversity of microeukaryotes in water and sediment, high-throughput sequencing of samples was performed. The Shannon index-based (Fig. S2) rarefaction curves indicated the sequencing depth was enough to evaluate the microeukaryotic diversity. Eukaryotic microorganisms in sediment and water samples performed by 454 high-throughput sequencing showed that the OTU number in sediment (78–136 OTUs) was higher than water (75–105 OTUs) in each site except D site. Chao1 index were higher in water compared with sediment samples except site A.



**Fig. 1** Histogram and jitter plot of 18S rRNA gene copies in sediment (a) and water (b), in which red color represents sediment, blue color represents water

Shannon diversity index was significantly higher in sediment than that in water (Wilcoxon rank-sum test,  $p=0.032$ ). It could be seen that diversity did not indicate the clear trend. The OTU-based PCoA results exhibited distinct distribution patterns of microeukaryotic community composition between sediment and water samples (Fig. S5). Although water sample E was separated from other four water samples, forming obvious outlier, sediment samples, and water samples could separate distinctly.

### Microeukaryotic Community Composition Between the Sediment and Water Habitat

To illuminate the microeukaryotic community dissimilarities and similarities between sediment and water habitats, microeukaryotic community compositions in the water and sediment samples were analyzed at different classification levels. From phylum to order level, the two habitats indeed had some differences. At the phylum level (Fig. 2), the sample WE was separated distinctly from the other samples (including samples SA to SE and WA to WD) and the other samples could also be clustered into two separate branches, a sediment branch (SA to SE) and a water branch (WA to WD). For community composition, *Metazoa* (0–45.3%), *Fungi* (23.15–47.15%), *Streptophyta* (1.1–35.2%), *Ochrophyta* (6.3–19.2%), *Cercozoa* (1.6–15.9%) and *Dinophyta* (1.65–78.02%) were more abundant in sediment than that of water. The dominant phyla *Dinophyta* (1.65–78.02%), followed by *Metazoa* (0–45.3%), *Ochrophyta* (1.4–20.3%), *Cryptophyta* (1.1–6.3%), *Chlorophyta* (1–9.1%), *Cercozoa* (1.1–4.7%), *Fungi* (0–8.5%), *Katablepharidophyta* (0.3–2.7%), *Choanoflagellida* (0–1.6%) and *Haptophyta* (0–1.6%) were more abundant in water. According to similarity analysis, the result showed that the two habitats had

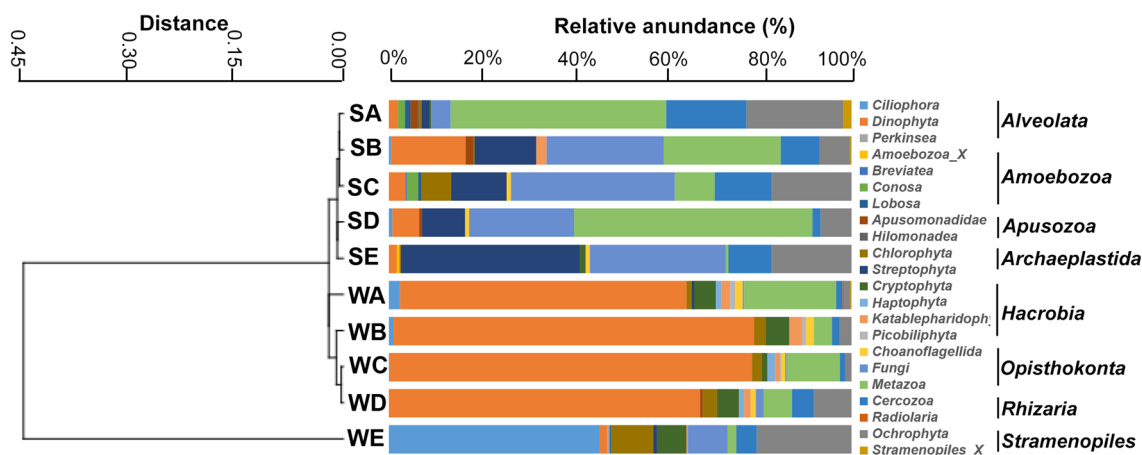
absolutely significant differences of microeukaryotic community composition (ANOSIM,  $p=0.027$ ).

### Differences of Microeukaryotic Community Composition Between Sediment and Water Habitats

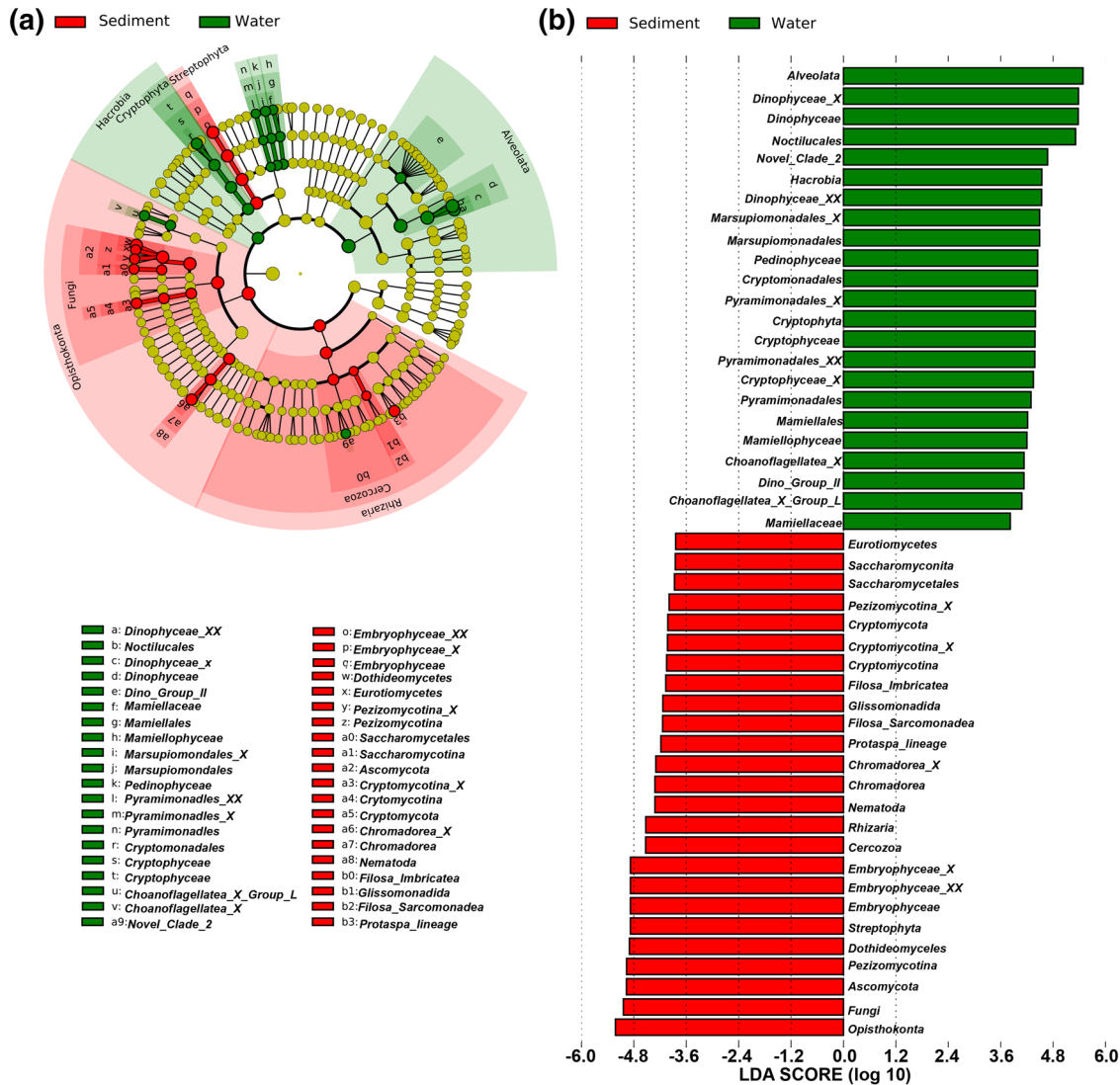
To manifest the detailed differences in the microeukaryotic communities between sediment and water at different levels, LEfSe analysis was conducted based on the data of eukaryotic microorganisms from super-phylum to genus level (Fig. 3). Compared to water, there were 25 taxa dominants in sediment ( $p<0.05$ ), mainly part of the super-phylum *Opisthokonta*, *Archaeplastida*, and *Rhizaria*. However, there were 23 dominant taxa in water, in which *Alveolata*, *Opisthokonta*, *Rhizaria*, and *Hacrobia* were the dominant super-phyla that contributed to the difference.

The results of Welch's t-test at 95% confidence intervals, (Fig. S6) on phylum and class level further corroborated the significant differences could be distinguished between sediment and water habitats ( $p<0.05$ ). *Fungi* and *Cercozoa* showed the absolute dominance in sediment than that of water, while *Cryptophyta* and *Dinophyta* were more abundant in water samples on phylum level. Moreover, *Astomycota* and *Fiosa-Imbricatea* were more abundant in sediment on class level, *Cryptophyceae* and *Dinophyteae* showed the absolute dominance in water samples.

The highest abundant OTU-based analysis was also carried out to further manifest the differences in microeukaryotic community between sediment and water (Table S3). Microeukaryotic dominant OTUs including OTU92 (12.7%), OTU95 (4.7%), OTU822 (3.0%), OTU145 (2.8%), and OTU464 (2.8%) had sheer dominance in sediment (Table S6a), representing *Dothideomycetes*, *Noctiluca\_scintillans*, *Coscinodiscus\_radiatus*, *Novel-Clade-4\_X*, and *Odontella\_sinensis*, respectively. The most abundant OTUs of eukaryotic microorganisms in



**Fig. 2** PCoA plots based on microeukaryotic Bray-Curtis distance metrics of sediment and water samples. Red color represents sediment samples, blue color represents water samples



**Fig. 3** LefSe analysis revealed the most significant ( $p < 0.05$ ) eukaryotic microbial taxa between sediment and water. **a** Taxonomic cladogram based on LefSe analysis of eukaryotic microorganisms from super-phylum to family level. Red color represents sediment-enriched taxa, green color represents water-enriched taxa and yellow color represents nonspecific taxa. **b** Histogram of LDA scores coupled with effect size for differentially abundant taxa between sediment and water. (Red) Sediment-enriched taxa with negative LDA scores, and (green) water-enriched taxa with positive scores. Only taxa meeting an LDA significant threshold of  $> 2$  are shown

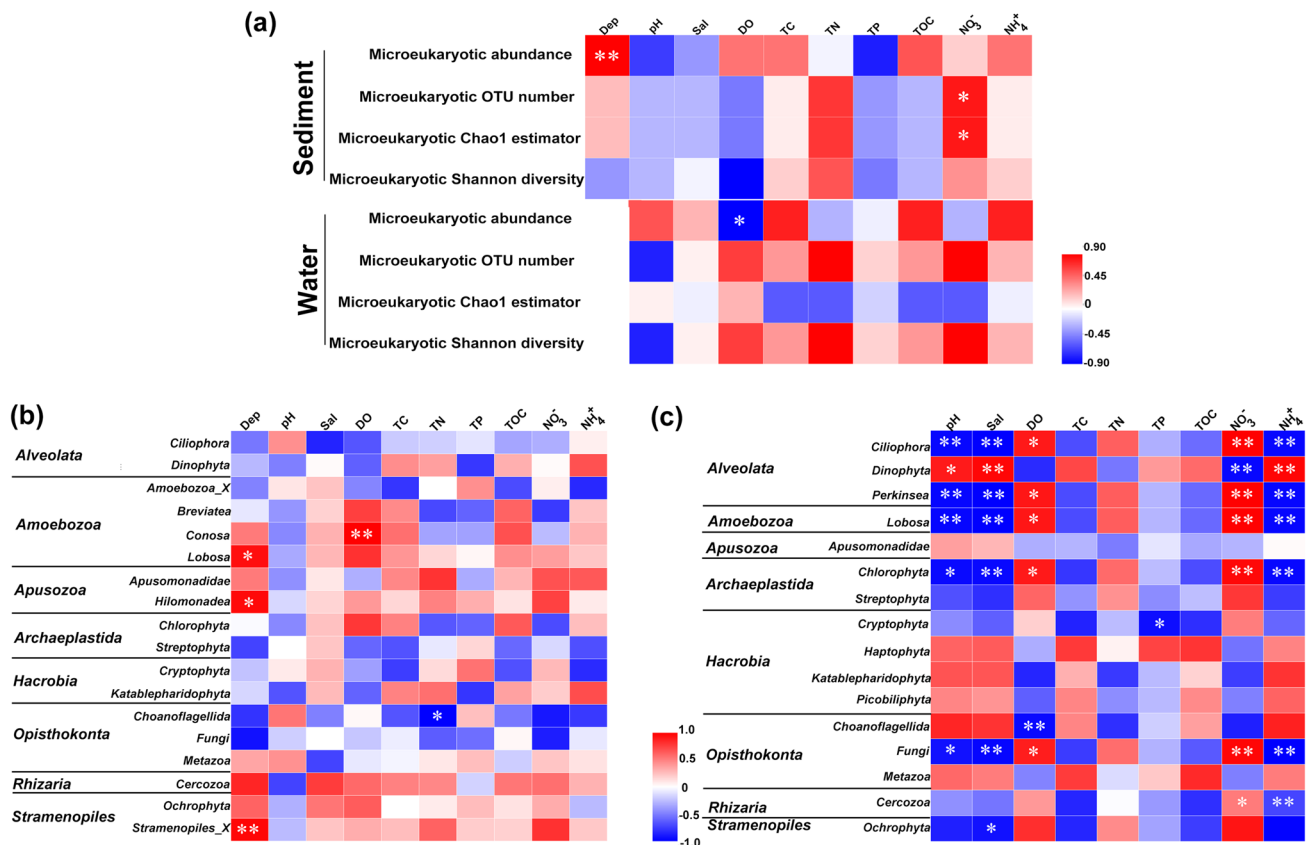
water (Table S6b) were OTU95 (40.6%), OTU482 (8.3%), OTU869 (5.8%), OTU676 (2.0%), and OTU134 (1.8%), representing *Noctiluca\_scintillans*, *Noctiluca\_scintillans*, *Tintinnidium\_sp.*, *Parallelostrombidium\_paralatum*, and *Dino-Group-I-Clade-4\_X\_sp.*, respectively. In summary, the microeukaryotic communities between water and sediment varied distinctly whether in diverse taxonomic classification or OTU level.

resents nonspecific taxa. **b** Histogram of LDA scores coupled with effect size for differentially abundant taxa between sediment and water. (Red) Sediment-enriched taxa with negative LDA scores, and (green) water-enriched taxa with positive scores. Only taxa meeting an LDA significant threshold of  $> 2$  are shown

### Physicochemical Variables Influencing Microeukaryotic Communities in Sediment and Water Habitats

Spearman correlation analysis was used to reveal the relationships between the alpha diversity of the microeukaryotic communities and environmental factors (Fig. 4). In sediment





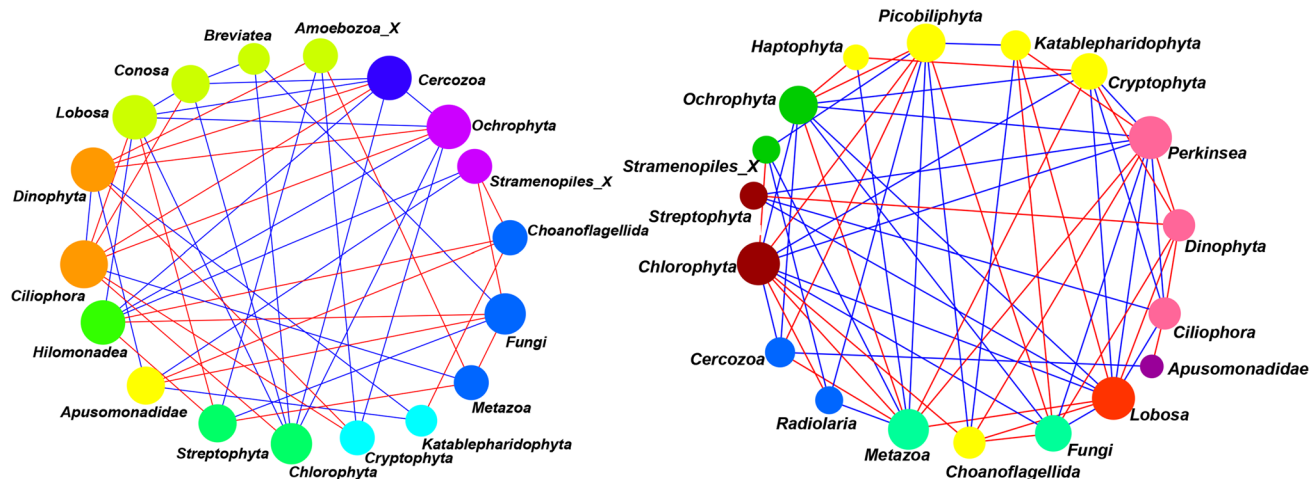
**Fig. 4** Spearman correlation heat maps of influential factors with a microeukaryotic abundance and diversity in sediment and water and Pearson correlation heat maps of influential factors with dominant taxa in **b** sediment and in **c** water samples. The values of Pearson's

(Spearman's) correlation coefficients are characterized by the scale bar. *Sal* salinity, *DO* dissolved oxygen, *TN* total carbon, *TC* total carbon, *TOC* total organic carbon, *TP* phosphorus

(Fig. 4a), the only remarkable ( $\rho = 0.975, p < 0.01$ ) positive correlation was detected between microeukaryotic abundance and depth; the OTU number ( $\rho = 0.9$ ) and Chao 1 ( $\rho = 0.9$ ) were significantly ( $p < 0.05$ ) and positively correlated with NO<sub>3</sub><sup>-</sup>, while no physical and chemical factors were significantly associated with Shannon diversity index (Table S3). Unlike sediment samples, the only factor that negatively and significantly correlated ( $\rho = 0.9, p < 0.05$ ) with microeukaryotic abundance in the water was dissolved oxygen (Fig. 4a). There was no significant ( $p > 0.05$ ) relationship among physicochemical factors and other diversity indexes (including Shannon diversity index, Chao1) and OTU number (Table S3). Pearson correlation between the microeukaryotic taxa and environmental factors revealed that few environmental factors were remarkably correlated with sediment microeukaryotic taxa (Fig. 4b), in which depth was the significant factor affecting much more taxa, while DO and TN were found to only exert significant effect on few taxa (Table S4). In the water samples (Fig. 4c), there were more statistically significant relationships between environmental variables and

microeukaryotic community, such as pH, salinity, dissolved oxygen, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, TP (Table S5).

Canonical Correspondence Analysis (CCA) was conducted to explain the relationships between measured factors and microeukaryotic community (Fig. S4), the first and second axis explained 25.9% and 25.2% of the variables in the sediment and 28.7% and 24.9% in the water samples, respectively. In sediment (Fig. S4a), no variables had remarkable effect on the microeukaryotic communities, while it revealed that more factors had significant correlations (Mantel test,  $p < 0.05$ ) with water microeukaryotic communities than that of sediment (Fig. S4b), of which TN was the most predominant factor ( $p < 0.05$ ) to regulate microeukaryotic community, then followed by pH, salinity, and nitrate content. Taken together, microeukaryotic taxa in water were more sensitive to the environmental factors compared with sediment.



**Fig. 5** Co-occurrence networks of dominant microeukaryotic phyla in sediment (**a**) and water (**b**) using Spearman analysis. Nodes of the same color indicate that they belong to the same taxa while different colors show variable taxonomies. Edges show positive or nega-

tive relations, in which red color represents negative and blue represents the positive correlations, respectively. Nodes with diverse size express the number of connections among the taxa

## Network Interactions of Eukaryotic Microbes in Sediment or Water Habitat

The network relationships among eukaryotic microorganisms were more complex in water, while relatively simple in sediment. In sediment habitat (Fig. 5a), the coexisting network analysis demonstrated that *Dinophyta*, *Ciliophora*, *Ochrophyta*, *Chlorophyta*, and *Fungi* were in the center of the network, which meant that they had extensive relationships with other taxa. Intriguingly, there were widely interphylum positive correlations with the *Breviatea*, while *Choanoflagellida* were universally negatively correlated with other taxa. In the water samples (Fig. 5b), *Cryptophyta*, *Chlorophyta*, *Perkinsea*, *Chlorophyta*, *Fungi*, *Lobosa*, *Picobiliphyta*, and *Metazoa* had the most relationships with other taxa. Equally intriguing was that *Dinophyta* was broadly positively correlated with other groups, while other taxa were more or less positively and negatively related.

## Discussion

Most previous reports on estuarine microbes were mainly focused on prokaryotic, especially bacterial communities [44–46], or single habitat, sediment [34–36] or water [37–39]. However, comparative research on eukaryotic microorganisms between water and sediments have rarely been explored with microbial molecular ecology technology especially in estuary habitat. Here, the study covering of the microeukaryotic abundance, diversity, community composition, and the relationships with environmental

factors in both sediment and water, were assessed by qPCR and pyrosequencing methods.

In the present study, the diversity was similar between the water and sediment bacterial community, and there was no clear trend in community diversity. Yong et al. [26] had reported that microeukaryotic communities in water habitat were more diverse than those in sediment. Some recent molecular investigation reported [45, 47] that protistan group has long been considered to be confined to the aquatic environment, and is also common in the terrestrial environment [48]. However, some studies had been proposed the opposite conclusion. Gong et al. [24] pointed that the diversity of microeukaryotes in water was higher than that of sediment, which might be due to the sampling depth. It has to be emphasized that by Shulze et al. [5] that the microeukaryotic communities in sediment have the highest diversity in the equatorial North Pacific, habitat type, water column depth, and sediment horizon should be responsible for this result. A study brought up by Chen et al. had been certified that sediments were more diverse than water, which should be attributed into environmental filtering and stochastic processes [49]. All the conclusions indicated that the influencing factors may be complex rather than single one. Furthermore, choosing the right annotation process and reference database has also proven to be critical because it largely limits the risk of mispricing. In addition, we also tried to compare the abundance of water and sediment, but due to the fact that we used two DNA-extraction kits to extract the water and sediment samples, which may generate artificial differences between these two compartment, we did not put this result.

The diverse distribution patterns of microeukaryotes between water and sediment could be attributed to many factors. The multiple statistical analysis performed in the present study highlighted the remarkable differences of microeukaryotic community were found between sediment and water. Some studies focused on microeukaryotic community also verified our view [24, 49–53]. Eukaryotic taxonomic groups associated with analogous environmental conditions contained some common parts [54], which were also reflected in our study (Table S7). An interesting finding was that *Metazoa* and *Ochrophyta* were discovered to overwhelmingly dominate in both sediment and water, and our results based on the OTU also showed that some taxa (*Dothideomycetes*) indeed could simultaneously adapt both the two habitats, which indicated that there might exist a little overlap between sediment and water due to their inseparable bond, and the existence of a connection between network structure and habitat preference, most eukaryotic microbial groups demonstrate adaptation to specific abiotic and biotic conditions even within similar habitats [55], which had been proposed by some other studies [49, 56, 57].

The distribution of eukaryotic microorganisms community had some similarity with bacteria/archaea community. Based on our previous study [40], prokaryotic (bacteria and archaea) performed higher abundance and diversity in the sediment than water, while eukaryotic microorganisms did not exhibit a clear trend of diversity between sediment and water habitat. Bacterial communities at different taxonomic levels were apparently distinct between the sediment and water, but archaeal communities were not. And the distribution patterns of eukaryotic microorganisms could be clustered into two different branches between the sediment and water. Our result showed that bacteria and eukaryotic microorganisms were more sensitive to habitat effects, unlike archaea.

In view of previous studies, we relied on an assumption that there were four mechanisms which can lead to the sensitivity of habitat, containing environmental factors, spatial effects, niche plasticity, co-occurrence relationships [40]. First, not only abiotic factors (including temperature, salinity, altitude; nutrients, and pollutions) [25, 44, 57–60] but also biotic factors [20, 30] had been previously proven to exert different degrees of effects on abundance and diversity of eukaryotic communities in different habitats. In addition, the unexplained variation might be related to complex environmental factors or other mechanisms not referred [61, 62]. Second, spatial effect and niche plasticity can also affect microeukaryotic distribution patterns. Concluded from the PCoA and clustering results (Figs. 1 and 3, Table 1), the microeukaryotic organisms between sediment and water community did cluster into two distinct branches. This was supported by some studies [21, 27, 63, 64]. Results in a Chinese subtropical river demonstrated by Wang et al. [21]

and Bohai sea by Xie et al. [65] confirmed our conjecture. Finally, both positive and negative microeukaryotic relationships were discovered in sediment and water of our study. A growing number of evidences demonstrated that the co-occurrence relationships among eukaryotes might be responsible for the community structure [27, 57, 66]. On the other hand, the co-occurrence or exclusion of phyla between trophic levels probably indicates that their OTU members have similar or opposing habitat preferences, as well as direct relationships between their members. Moreover, certain pairs of phyla (e.g. *Discoba* and *Chlorophyta*) exhibited both significant exclusion and co-occurrence between their members, indicating that the biological processes governing OTU assembly, such as the boundaries of ecological niches, were also influential at taxonomic levels below the phylum, as recently deduced from a bacterial and fungal cultivation experiment [55].

To sum up, microeukaryotic diversity, abundance, and distribution patterns in such closely interlinked sediment and overlying water were found in the YRE. Eukaryotic microorganisms in sediment were much less sensitive to environmental factors compared with water. Moreover, some potential relationships were also found among microeukaryotes.

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## Compliance with Ethical Standards

**Conflict of interest** The authors declare no conflict of interest.

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