Journal of Microbiology (2021) Vol. 59, No. 4, pp. 376–388
DOI 10.1007/s12275-021-0635-5
eISSN 1976-3794
pISSN 1225-8873

Different distribution patterns of microorganisms between aquaculture pond sediment and water[§]

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(Received Dec 3, 2020 / Revised Jan 18, 2021 / Accepted Jan 19, 2021)

Aquatic microorganisms in the sediment and water column are closely related; however, their distribution patterns between these two habitats still remain largely unknown. In this study, we compared sediment and water microeukaryotic and bacterial microorganisms in aquaculture ponds from different areas in China, and analyzed the influencing environmental factors as well as the inter-taxa relationships. We found that bacteria were significantly more abundant than fungi in both sediment and water, and the bacterial richness and diversity in sediment were higher than in water in all the sampling areas, but no significant differences were found between the two habitats for microeukaryotes. Bacterial taxa could be clearly separated through cluster analysis between the sediment and water, while eukaryotic taxa at all classification levels could not. Spirochaetea, Deltaproteobacteria, Nitrospirae, Ignavibacteriae, Firmicutes, Chloroflexi, and Lentimicrobiaceae were more abundantly distributed in sediment, while Betaproteobacteria, Alphaproteobacter, Cyanobacteria, Roseiflexaceae, Dinghuibacter, Cryomorphaceae, and Actinobacteria were more abundant in water samples. For eukaryotes, only Cryptomonadales were found to be distributed differently between the two habitats. Microorganisms in sediment were mainly correlated with enzymes related to organic matter decomposition, while water temperature, pH, dissolved oxygen, and nutrient levels all showed significant correlation with the microbial communities in pond water. Intensive interspecific relationships were also found among eukaryotes and bacteria. Together, our results indicated that eukaryotic microorganisms are distributed less differently between sediment and water in aquaculture ponds compared to bacteria. This study provides valuable data for evaluating microbial distributions in aquatic environments, which may also be of practical use in aquaculture pond management.

***Keywords*:** eukaryotic microorganisms, bacterial community, sediment, water, aquaculture pond

Introduction

Microorganisms are the most important colonizers and play crucial roles in the sediment and water of various natural water bodies (Massana and Logares, 2013; Segovia *et al.*, 2015; Wörmer *et al.*, 2019). For example, a great variety of bacteria are involved in the degradation of organic matter, and can significantly affect its preservation in sediments (Arnosti, 2011; Koho *et al.*, 2013). Bacteria have also been found to play important roles in nitrogen (see Kuypers *et al.,* 2018), phosphorus (McMahon and Read, 2013; Figueroa and Coates, 2017), and sulfur cycles (Jörgensen *et al.*, 2019), as well as other biogeochemical processes, in both sediment and water. Eukaryotic microorganisms, including protists, algae, unicellular fungi, and some small invertebrates, are also involved in a diverse set of ecological functions in water and sediment, e.g., by participating in biogeochemical cycles (Edgcomb *et al.*, 2011; Anderson *et al.*, 2013) and food network interactions (Massana, 2011; Grujcic *et al.*, 2018). However, most previous studies on microorganisms in water bodies have been limited to either sediment or the water column, probably due to the distinct difference between the two habitats, and this may have hampered our understanding of their distributions in these environments.

The water column and sediment constitute the two most important and closely related components of aquatic systems. Sediment usually acts as a sink/source of nutrients from/to the water column (Powers *et al.*, 2016; Pearce *et al.*, 2017; Shaughnessy *et al.*, 2019). It therefore seems logical to assume that microorganisms are exchanged along with nutrients between the sediment and water, resulting in similar microbiological communities between the two habitats. Indeed, some shared common members of bacterial (Ekwanzala *et al.*, 2017; Kumar *et al.*, 2019) and eukaryotic (Kosolapov *et al.*, 2017; Shi *et al.*, 2020) taxa have been identified between sediment and water samples, and sediment is believed to be an important source of bacteria (Fries *et al.*, 2008) and invertebrates (Wang *et al.*, 2020) to the water column. Meanwhile, distinct taxa and distribution patterns of microorganisms between sediment and the water column have also been found in these studies (Kumar *et al.*, 2019; Shi *et al.*, 2020; Wang *et al.*, 2020) and others (Wei *et al.*, 2016; Li *et al.*, 2018), indicating complex microbiological relationships between aquatic sediment and water.

Different microbial taxa seem to have specific preferences for sediment and water environments. For example, micro-

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[§]Supplemental material for this article may be found at http://www.springerlink.com/content/120956.

organisms involved in aerobic chemoheterotrophy and phototrophy are found to be prevalent in water (Kumar *et al.*, 2019; Ul-Hasan *et al.*, 2019), while those participating in organic matter decomposition and sulfur compound transformation are frequently detected in sediment (Mahmoudi *et al.*, 2017; Jörgensen *et al.*, 2019). In addition, a wide variety of environmental factors have been found to shape the distributions of microorganisms in water and sediment, such as water temperature (Quiroga *et al.*, 2013), pH (Wang *et al.*, 2015b), dissolved oxygen (DO) (Merlo *et al.*, 2014), nutrient level (Fodelianakis *et al.*, 2014; Zhao *et al.*, 2015), and sedimentary organic matter (Mahmoudi *et al.*, 2017; Dai *et al.*, 2018). Interactions among different microbial taxa have also been found to be an important factor contributing to their distribution patterns in sediment and water (Barberán *et al.*, 2012; Wei *et al.*, 2016). Understanding microbial community patterns and the factors that affect them is important for elucidating the relationships among microorganisms between aquatic sediment and water.

Pond aquaculture serves as the most important form of freshwater aquaculture in China, and accounts for more than half of the national freshwater aquaculture area (National Bureau of Fisheries and China Society of Fisheries, 2019). Sediment and water in aquaculture ponds are more closely related than in other aquatic environments, due to the activities of massive culture species and the process of mechanical agitation for aeration during the culturing of fish. However, although – because of their importance – microorganisms in the sediment and water of aquaculture systems have been reasonably well studied already (Guan *et al.*, 2020; Sun *et al.*, 2020), the characteristics and differences of their distributions between aquaculture pond sediment and water have seldom been analyzed; indeed, their distribution patterns remain largely unknown. Thus, in this study, we compared sediment and water microeukaryotic and bacterial microorganisms in different aquaculture ponds from three different aquaculture areas in China, and analyzed their environmental factors of influence as well as the interspecific relationships between different taxa. Our aim was to uncover the differences in the distribution patterns of eukaryotic and bacterial communities between the sediment and water column of aquaculture ponds, and reveal the key environmental factors driving the differences. The results provide valuable data for evaluating microbial distributions in aquatic sediment and water, which may also be of practical use in aquaculture pond management.

Materials and Methods

Sample collection and physicochemical analysis

Sediment and water samples were collected from *Ctenopharyngodon idellus* aquaculture ponds located in the aquaculture areas of Changjiang, Heilongjiang and Zhujiang in China, as described by Dai *et al.* (2018), between May and June in 2017. These ponds ranged from 7,000 to 27,000 m^2 in size and 1.5 to 3.5 m in depth, where *C. idellus* – intercropped with *Hypophthalmichthys nobilis* and *H. molitrix* – were raised for commercial use. Three ponds were sampled in each area. In each pond, surface sediment samples from three to five different sites were collected using a core sampler, and samples from the same pond were pooled and transferred into sterile plastic bags. Pond water samples at approximately 20 cm below the water surface were collected at each site using a water sampler according to previous studies (Wang *et al.*, 2020; Zheng *et al.*, 2020). Three replicates of water samples in each pond were mixed and combined as one. The pond sediment and water samples were kept on ice and transported to the laboratory within the same day as the sampling process. The in-situ water temperature, pH, and DO were measured by a handheld smarTROLL Multiparameter (In-Situ Inc.).

In the laboratory, 300 ml of water from each pond was filtered onto 0.2 µm polycarbonate membranes. The membranes and a part of the sediment samples from each pond were stored at -80°C until DNA extraction. The remaining samples were kept at 4°C for analysis. The concentrations of total phosphorus (TP), nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_4^+), total nitrogen (TN), and chlorophyll-a (*chla*) in water samples were measured according to standard methods (China Environmental Protection Agency, 2002). Sediment chemical characteristics, including pH, TN, TP, NO_2^-, NO_3^-, NH_4^+, total organic carbon (TOC), dissolved organic carbon (DOC), microbial biomass carbon, and potential nitrification rates were measured according to our previous study (Dai *et al.*, 2018). In addition, sediment enzymes including β-galactosidase, urease, acid phosphatase, and arylsulfatase were also determined, to evaluate the level of activity of organic matter transformation in pond sediment according to Bowles *et al.* (2014). The average values of pond sediment and water physicochemical parameters in each sampling area are shown in Supplementary data Tables S1 and S2, respectively.

Nucleic acid extraction and quantitative PCR

The water membranes and approximately 1 g of wet sediment were used for DNA extraction with the E.Z.N.A.^{TM} Water DNA Kit (Omega) and DNeasy PowerSoil Kit (Qiagen), respectively, following the manufacturers' instructions. The extracted genomic DNA for sediment and water was examined for purity by electrophoresis on 1.0% (w/v) agarose gels, and the concentrations were determined with a Nanodrop 2000 spectrophotometer (Thermo Scientific).

The bacterial and fungal abundance in pond sediment and water was estimated by quantitative PCR (qPCR) with primers that target the 16S and Internal Transcribed Specer rRNA (ITS rRNA) genes. The primers and reaction conditions for qPCR are provided in Supplementary data Table S3. The standard plasmids used for quantification were obtained from pure clones of 16S and ITS rRNA gene amplifications from the pond samples. The amplification efficiency was calculated to be 102.7 ($R^2 = 0.999$) and 95.2% ($R^2 = 0.998$) for bacteria and eukaryotes, respectively. A 10 µl PCR reaction system was used for each sample, which contained 5 µl 2× SYBRGreen qPCR Master Mix, 0.2 µl each primer (10 µM), and 1.0 µl template DNA. Different dilutions of DNA templates and melting curve analysis were used to detect potential inhibition of co-extracted compounds, and no significant inhibitions were found. The qPCR reactions were performed in triplicate for each sample.

MiSeq pyrosequencing

Preparation of the bacterial and eukaryotic sequence library and the subsequent Illumina MiSeq sequencing were performed by GENEWIZ Inc. (Suzhou). The V3 and V4 regions of bacterial 16S rDNA were amplified with primers containing sequences “CCTACGGRRBGCASCAGKVRVGAAT” and “GGACTACNVGGGTWTCTAATCC”, and the V7 and V8 regions of eukaryotic 18S rDNA were amplified with primers containing sequences “CGWTAACGAACGAG” and “AICCATTCAATCGG”. The primers were attached to adaptor sequences for Illumina MiSeq sequencing. PCR products from different aquaculture pond samples were quantified with a Qubit 2.0 Fluorometer (Invitrogen), and were used to prepare the bacterial and eukaryotic libraries. DNA libraries were then combined and loaded onto an Illumina MiSeq instrument (Illumina) for pair-end sequencing following the manufacturer’s instructions.

Reads from the 16S and 18S MiSeq sequencing were filtered and assembled with Quantitative Insights into Microbial Ecology (QIIME, version 1.9.1) software (Caporaso *et al.*, 2010), and the reads shorter than 200 bp and with a quality score < 25 were removed. The high quality sequences were then clustered into operational taxonomic units (OTUs) using VSEARCH 1.9.6 (at 97% sequence identity) against the Silva 128 database (Quast *et al.*, 2013), with a confidence level of 80%. The representative sequence of each OTU was selected for taxonomic analysis using the RDP (Ribosomal Database Program) classifier (Cole *et al.*, 2014), and the community composition of each sample was evaluated based on the numbers of different taxonomic levels.

Data analysis

Randomly selected bacterial and eukaryotic sequences were used for α- and β-diversity analyses with QIIME. The Chao 1 estimator, Shannon, Simpson index, and Good’s coverage were calculated using the QIIME workflow, and the rarefaction curves were graphed with R 3.3.1 (R Core Team, 2016). Analysis of similarities (ANOSIM) based on the Bray-Curtis algorithm and principal coordinate analysis (PCoA) based on weighted unifrac phylogenetic distances were performed using R to measure the community similarity between different samples. Canonical correlation analysis (CCA) and redundancy analysis (RDA) between microbial communities and the environmental factors were performed with the vagan package (Oksanen *et al*., 2018) in R. The CCA, RDA and heatmap results were plotted using the ggplot2 package (Wickham, 2009) in R.

The microbial difference on different taxonomic levels between aquaculture pond sediment and water were characterized by Linear Discriminant Analysis (LDA) Effect Size (LEfSe) analysis (Segata *et al.*, 2011) through an online toolkit (http://huttenhower.sph.harvard.edu/galaxy). To reveal the interspecific relationships, co-occurrence networks of prevalent OTUs in aquaculture ponds were constructed with Gephi 0.9.1 (Bastian *et al.*, 2009), based on calculation of Spearman correlations (with $r > 0.6$, and $p < 0.05$). Differences in physicochemical properties and microbial gene numbers of different samples were evaluated using one-way analysis of variance and the Student’s t-test using IBM SPSS 19.0 (IBM Corp. Armonk). The relationships between the microbial community and environmental factors were analyzed with Pearson correlation analyses in SPSS.

Nucleotide sequence accession number

The raw MiSeq pyrosequencing data have been deposited in the NCBI Sequence Read Archive database under accession number SRP197144.

Results

Microbial community diversity and structure in sediment and water of aquaculture ponds

The differences in bacterial and fungal abundance between

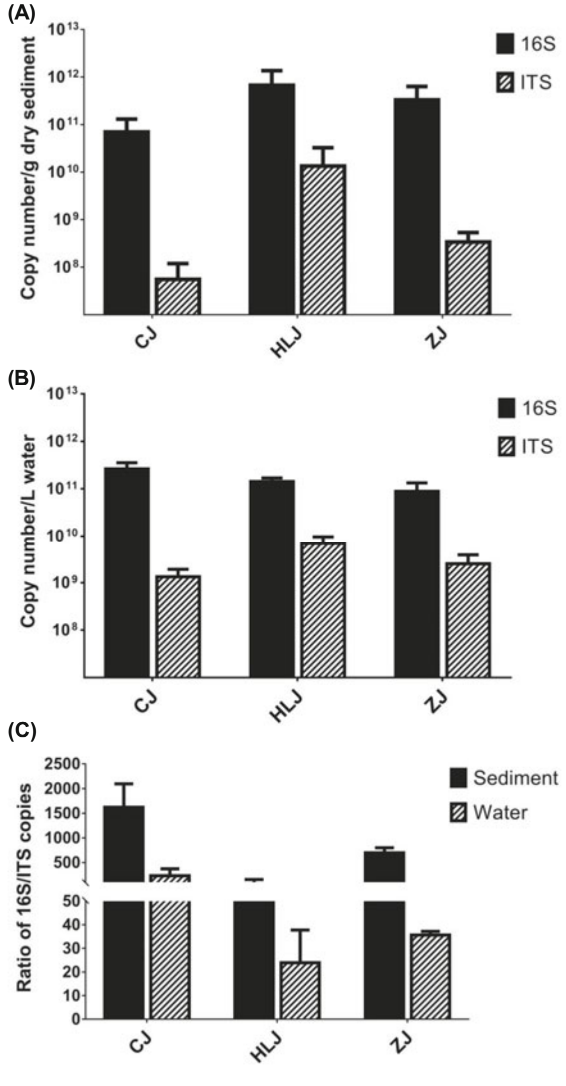

Fig. 1. Abundance of bacteria and fungi in aquaculture ponds of different areas estimated from 16S and ITS gene copy numbers. Bacterial and fungal gene copy numbers in pond sediment (A) and water (B), and the ratios of bacterial to fungal gene copies in pond sediment and water (C). CJ represents Changjiang, HLJ represents Heilongjiang, and ZJ represents Zhujiang – the locations where aquaculture ponds were sampled.

Table 1. **Microbial diversity indices for samples from different aquaculture ponds**

Sample type	Microbial type	Sample ID*	ace	chao1	shannon
Sediment	Bacteria	CJ	1697.83 ± 161.93	1697.70 ± 181.41	8.97 ± 0.46
		HLJ	1444.45 ± 164.13	1456.45 ± 151.04	8.71 ± 0.35
		ZJ	1523.78 ± 14.01	1529.11 ± 19.45	8.67 ± 0.06
	Eukaryotes	CJ	186.04 ± 39.72^{b}	192.62 ± 48.09^{b}	4.21 ± 0.80^{b}
		HLJ	331.27 ± 67.66^{a}	336.79 ± 64.04^{a}	5.55 ± 0.08^{a}
		ZJ	189.51 ± 8.95^{b}	187.81 ± 8.82^{b}	5.18 ± 0.53^{ab}
Water	Bacteria	CJ	726.49 ± 152.08^{ab}	742.09 ± 143.86^{ab}	6.61 ± 0.29^{ab}
		HLJ	666.96 ± 73.23^{b}	692.22 ± 74.69^{b}	6.13 ± 0.50^{b}
		ZJ	982.68 ± 172.91^{a}	989.75 ± 140.86^{a}	7.19 ± 0.18^{a}
	Eukaryotes	CJ	238.98 ± 22.05^{b}	247.61 ± 17.73	4.66 ± 0.31^{b}
		HLJ	289.19 ± 27.48^{ab}	279.95 ± 26.82	4.17 ± 0.27^{b}
		ZJ	326.55 ± 67.16^{a}	316.66 ± 63.21	5.40 ± 0.18^{a}

* CJ represents Changjiang, HLJ represents Heilongjiang, and ZJ represents Zhujiang – the locations where aquaculture ponds were sampled. Values were represented as mean ± SD, and different letters in the upper right-hand corner (a, b) indicate a significant difference ($p < 0.05$) between different sampling areas based on the analysis of variance.

sediment and water in different areas were compared based on 16S and ITS gene copy numbers (Fig. 1A and B). The bacterial abundance was significantly higher than the fungal abundance in both pond sediment and water ($p < 0.05$ and $p < 0.01$, respectively). In addition, the ratios of bacterial to fungal abundance were significantly higher in pond sediment than those in pond water ($p < 0.05$, Fig. 1C).

Rarefaction curves of sediment and water samples indicated sufficient sequencing depths (Supplementary data Fig. S1), and the sequencing coverage was found to be larger than 98.8% and 99.9% for bacterial and eukaryotic organisms, respectively. Bacterial richness (including ace and chao1 estimators) and diversity were much higher than those of eukaryotes in both sediment and water (Table 1). Bacterial richness and diversity in sediment were higher than in water in all the sampling areas; however, no significant difference was found between eukaryotes in the aquaculture pond sediment and water. Among the sampling areas, the richness and diversity of eukaryotes in sediment were higher in Heilongjiang's ponds than in the other areas ($p < 0.05$), but the richness and diversity of bacteria and eukaryotes in Heilongjiang's pond water were relatively lower, especially compared to Zhujiang's ponds, indicating a different microbial community diversity in aquaculture pond sediment compared to water. However, no significant differences were found between sediment bacteria in different areas.

To elucidate the microbial community structure and similarity in the sediment and water of aquaculture ponds, the number of bacterial and eukaryotic classification levels was counted and compared between the two habitats (Supple-

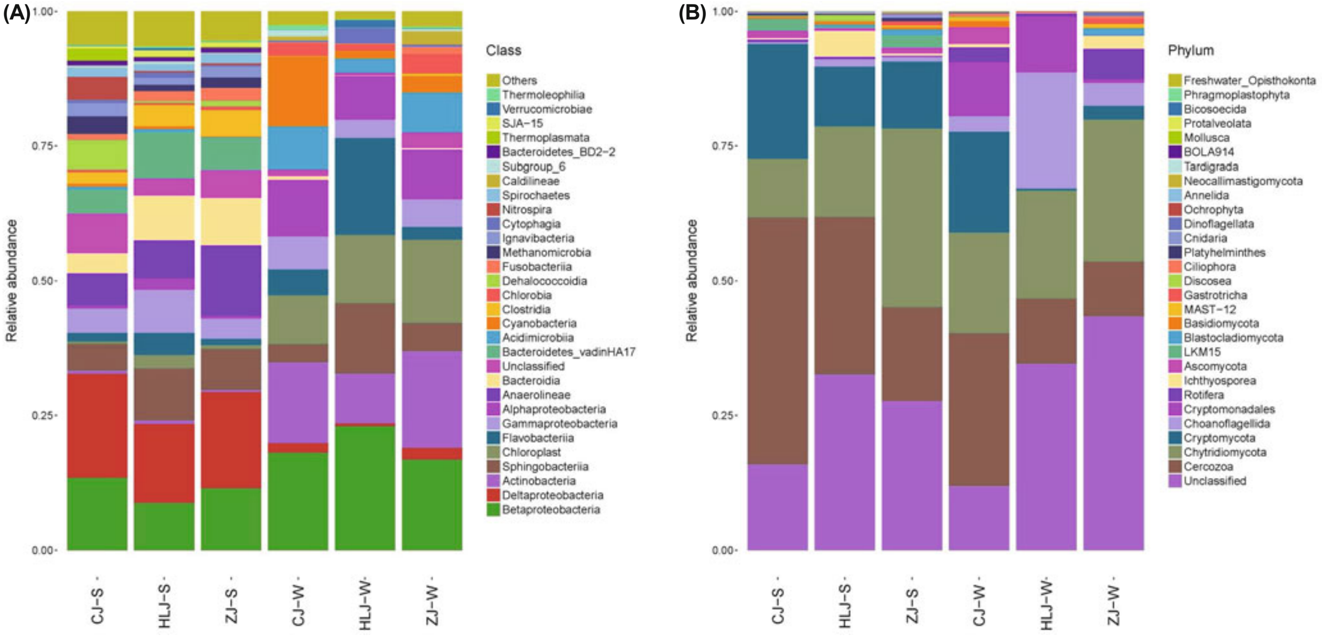

Fig. 2. **Bacterial (A) and eukaryotic (B) community composition at class/phylum level in aquaculture pond sediment and water.** Relative abundance was calculated based on the percentage of sequences detected for each taxa. CJ represents Changjiang, HLJ represents Heilongjiang, and ZJ represents Zhujiang – the locations where aquaculture ponds were sampled. "S" indicates sediment samples and "W" indicates water samples.

mentary data Table S4). For both bacteria and eukaryotes, most of the identified classification taxa were found to be the shared ones between the aquaculture pond sediment and water. However, few prevalent (with relative abundance > 1%) bacterial and eukaryotic classification taxa were found to be shared between the two habitats, especially at the OTU level.

The bacterial communities of the aquaculture pond sediment and water could be clearly separated through cluster analysis at all classification levels (from phylum to family) (Supplementary data Fig. S2), while eukaryotic taxa in the aquaculture pond sediment and water could not be separated at all classification levels (from phylum to family, Supplementary data Fig. S3). Meanwhile, the OTU-based PCoA analysis of the bacterial community also indicated a difference between aquaculture pond sediment and water samples (Supplementary data Fig. S4). Although samples from different areas could be separated (except one of the samples in Changjiang), differences between the sediment and water tended to surpass those between the sampling areas (Supplementary data Fig. S4). ANOSIM testing confirmed the significant difference of bacteria between the sediment and water samples ($R = 1$, $p = 0.001$), but differences between different areas were not significant. With respect to the eukaryotic community in

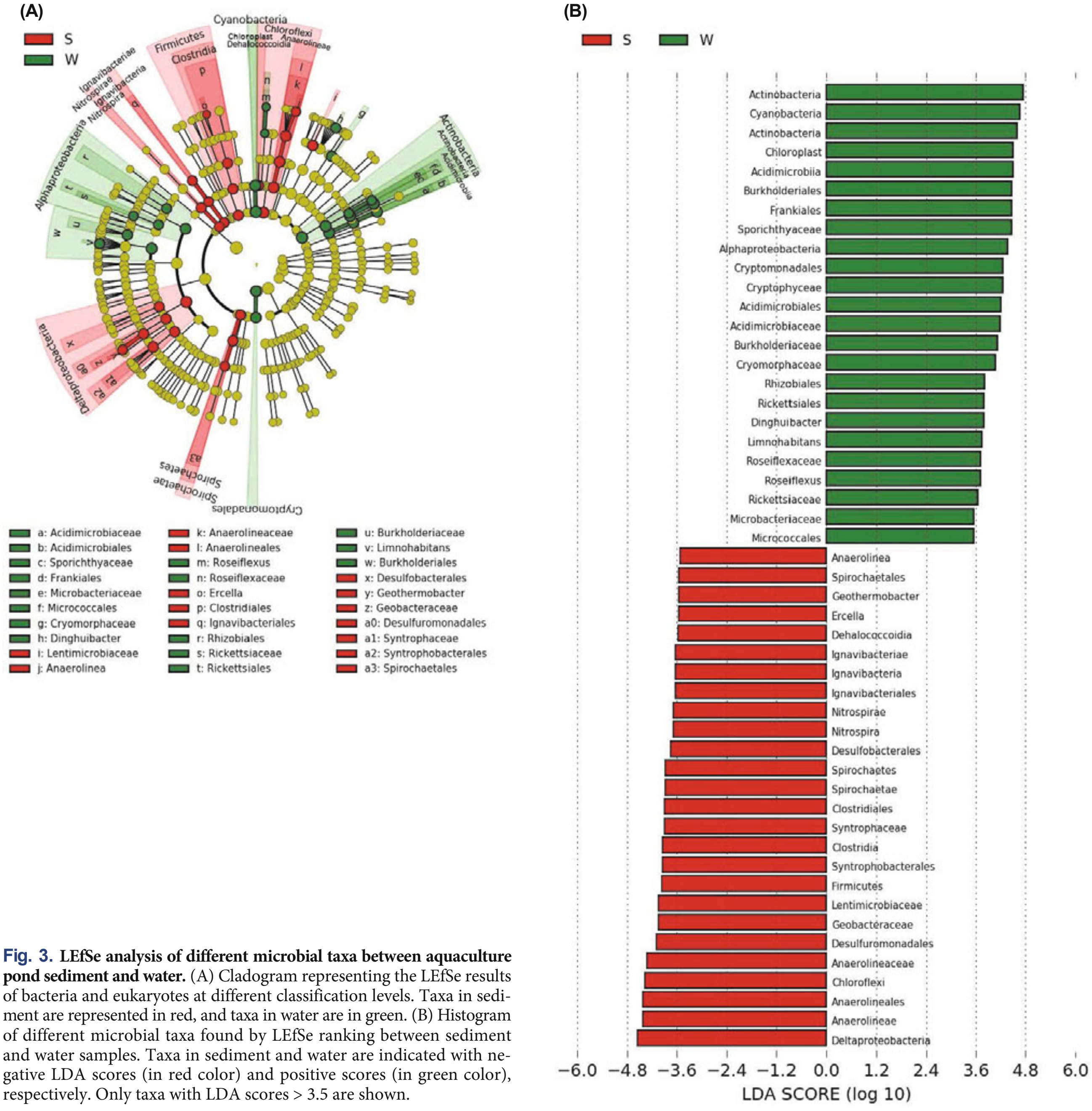

Fig. 3. LEfSe analysis of different microbial taxa between aquaculture pond sediment and water. (A) Cladogram representing the LEfSe results of bacteria and eukaryotes at different classification levels. Taxa in sediment are represented in red, and taxa in water are in green. (B) Histogram of different microbial taxa found by LEfSe ranking between sediment and water samples. Taxa in sediment and water are indicated with negative LDA scores (in red color) and positive scores (in green color), respectively. Only taxa with LDA scores > 3.5 are shown.

the aquaculture ponds, sediment, and water samples could be separated on OTU-based PCoA axes (Supplementary data Fig. S5), in contrast to the results based on different eukaryotic classification levels. In addition, a significant difference in eukaryotic OTUs was found between the sediment and water samples (ANOSIM test, $R = 0.5801$, $p = 0.001$); although, they were not evidently clustered into different branches (Supplementary data Fig. S4), indicating significantly different eukaryotic community structures between the aquaculture sediment and water.

Different microbial taxa between the sediment and water of aquaculture ponds

The most prevalent bacterial phylum in the aquaculture ponds was Proteobacteria, for both sediment and water samples (33.77–38.60% and 33.31–36.60%, respectively). However, the prevalent taxa at lower classification levels differed between sediment and water samples. Deltaproteobacteria were most prevalent in aquaculture pond sediment (14.59–19.25%), while in water Betaproteobacteria was the most prevalent class (16.82–23.02%). In addition, Betaproteobacteria (8.81–13.47%), Anaerolineae (5.95–13.10%), Sphingobacteriia (4.92–9.60%), and Bacteroidia (3.78–8.83%) were found to be abundantly distributed in pond sediment, while Actinobacteria (9.19–17.94%), Chloroplast (9.17–15.63%), Alphaproteobacteria (8.02–10.41%), and Flavobacteriia (2.35–17.91%) were highly abundant in pond water (Fig. 2A). Furthermore, Anaerolineaceae in Class Anaerolineae was the most prevalent bacterial family in sediment samples (5.95–13.10%), while Sporichthyaceae in Class Actinobacteria was most prevalent in water samples (6.81–12.68%).

For eukaryotes, Cercozoa were most prevalent in the aquaculture pond sediments (17.44–45.88%), followed by Chytridiomycota (10.75–33.11%), Cryptomycota (11.11–21.35%), Ichthyosporea (0.31–4.89%) and LKM15 (0.43–2.38%). In the aquaculture pond water, meanwhile, Chytridiomycota were the most prevalent eukarya (18.80–26.29%), followed by Cercozoa (10.10–28.20%), Choanoflagellida (2.99–21.51%), Cryptomycota (0.42–18.59%), and Cryptomonadales (0.61–10.34%) (Fig. 2B). Large quantities of eukaryotic sequences were not identified at lower classification levels in sediment and water samples owing to the lack of reference sequences, indicating largely unknown eukaryotic diversity in both aquaculture pond sediment and water. However, by comparing the identified eukaryotic taxa, relatively higher abundances of Ichthyosporea and Cercozoa taxa were found in sediment samples, including Abeoformidae (0–37.23% of the identified taxa) and Ebriacea (0–34.37% of the identified taxa). In water samples, meanwhile, more Choanoflagellatea and Rotifera taxa were detected, including Codonosigidae (12.07–79.52% of the identified taxa) and Ploimida (2.94–37.78% of the identified taxa).

LEfSe analyses based on bacterial and eukaryotic taxa at different classification levels were also performed to illustrate the differences in microbial taxa between aquaculture pond sediment and water (Fig. 3). Spirochaetea (including Spirochaetales), Deltaproteobacteria (including Syntrophaceae, Geothermobacter and Desulfobacterales), Nitrospirae (including Nitrospira), Ignavibacteriae (including Ignavibac-

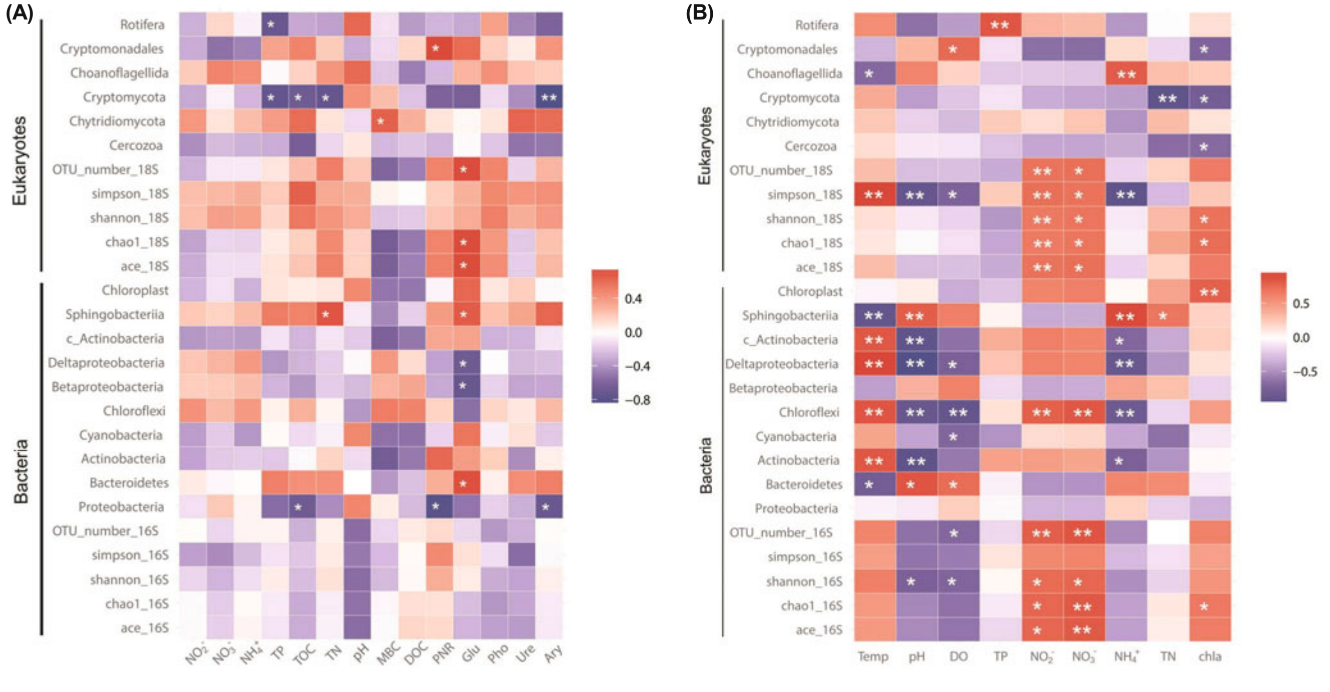

Fig. 4. Heatmaps showing the correlation between the bacterial and eukaryotic community and the environmental factors in aquaculture pond sediment (A) and water (B). The correlation analysis is based on all the pond samples from different areas. Values of correlation coefficients are indicated in color gradients. NO_2^-, nitrite; NO_3^-, nitrate; NH_4^+, ammonia; TP, total phosphorus; TOC, total organic carbon; TN, total nitrogen; MBC, microbial biomass carbon; DOC, dissolved organic carbon; PNR, potential ammonia oxidation; Glu, β-glucosidase; Pho, acid phosphatase; Ure, urease; Ary, arylsulfatase. * indicates correlation significant at $p \leq 0.05$, and ** indicates correlation significant at $p \leq 0.01$.

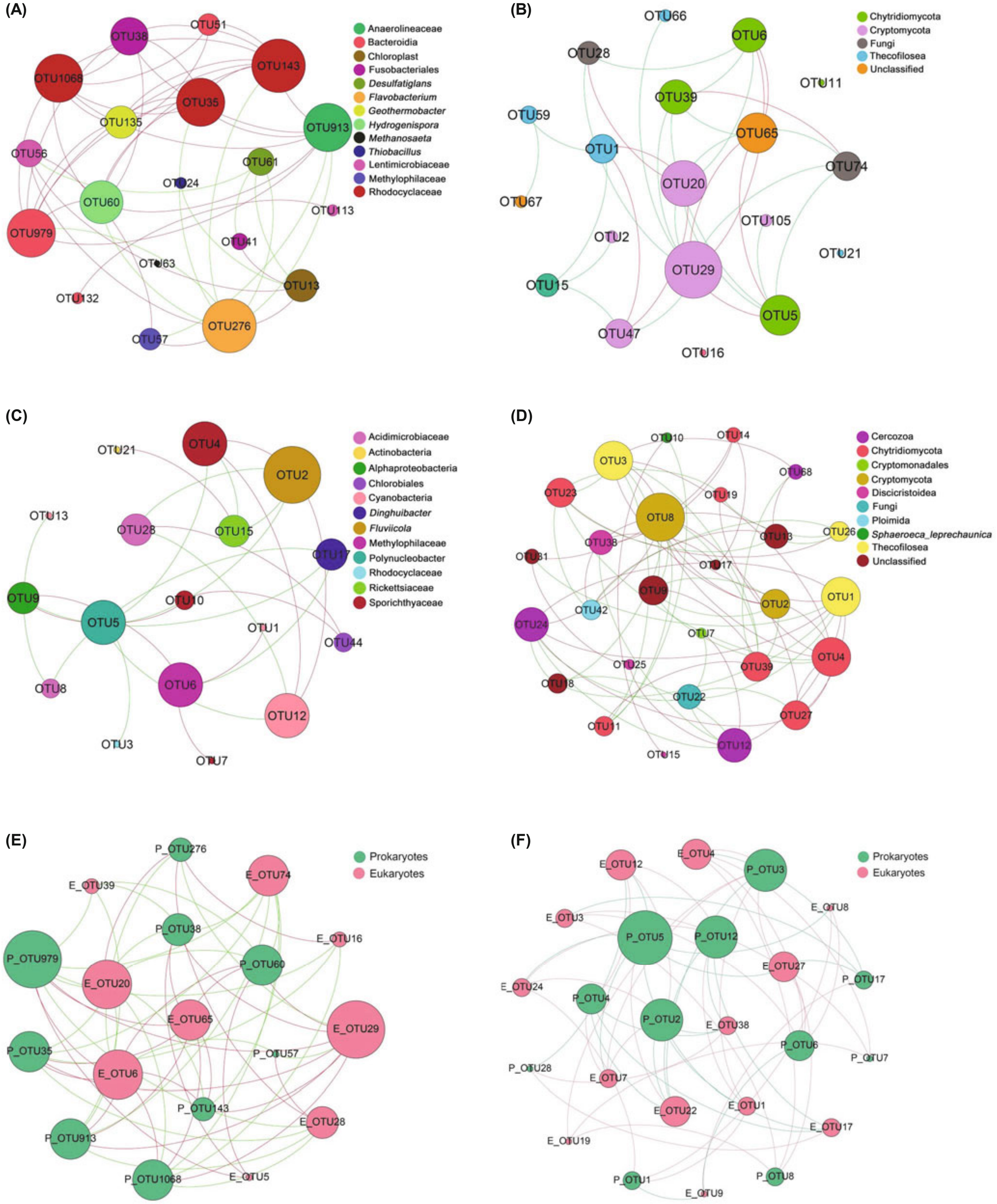

Fig. 5. **Co-occurrence networks of prevalent OTUs in aquaculture ponds.** (A) Bacterial and (B) eukaryotic co-occurrence networks in pond sediment. (C) Bacterial and (D) eukaryotic co-occurrence networks in pond water. Occurrence networks between bacteria and eukaryotes in (E) sediment and (F) water. Different colors of nodes indicate different taxonomies. Edges in red and green indicate positive and negative correlations among nodes, respectively. The sizes of nodes are proportional to the numbers of connections.

teria), Firmicutes (including Clostridiales), Chloroflexi (including Chloroplast and Anaerolineaceae), and Lentimicrobiaceae were found to be more abundantly distributed in sediment samples; whereas, Betaproteobacteria (including Limnohabitans and Burkholderiaceae), Alphaproteobacter (including Rickettsiaceae and Rhizobiales), Cyanobacteria (including Chloroplast), Roseiflexaceae (including Roseiflexus), Dinghuibacter, Cryomorphaceae, and Actinobacteria (including Microbacteriaceae, Sporichthyaceae and Acidimicrobiaceae) were more abundant in water samples. For eukaryotes, only Cryptomonadales were found to be differently distributed between sediment and water samples, with their abundance being higher in water than in sediment samples.

To further elucidate the microbial differences between aquaculture pond sediment and water, the most abundant bacterial and eukaryotic OTUs in sediment and water were compared. The majority of the most top abundant bacterial OTUs had absolute dominance in pond water, and their closest relatives were detected in the water column of similar freshwater habitats. Four of the most abundant bacterial OTUs were found to be more prevalent in pond sediment than in water, and their closest relatives were from anoxic water or sediment environments (Supplementary data Table S5). Two important bacterial taxa (OTU135 and OTU61), which belong to *Geothermobacter* and *Desulfatiglans*, respectively, were prevalently distributed in pond sediment. The majority of the most abundant eukaryotic OTUs were detected in both the pond sediment and water, and their relatives were from different habitats. OTU7 and OTU10 had significant dominance in pond water, and OTU5 and OTU6 had significant dominance in pond sediment, but their relatives could be detected from contrasting habitats (Supplementary data Table S6). In addition, an unclassified OTU (OTU9) was found to be among the most abundant eukaryotic taxa distributed in both pond sediment and water.

Relationships between environmental factors and microbial communities in the sediment and water of aquaculture ponds

The relationships of the bacterial and eukaryotic communities with environmental factors in both the sediment and water of aquaculture ponds were analyzed using Pearson correlation analysis. The eukaryotic OTU numbers, Chao1 and ace indexes, as well as Sphingobacteriia, Deltaproteobacteria, Betaproteobacteria and Bacteroidetes taxa abundances, were significantly correlated with the concentration of β-glucosidase in pond sediment ($p < 0.05$). Cryptomycota and Proteobacteria taxa abundances were significantly correlated with arylsulfatase concentrations in pond sediment ($p < 0.05$). However, few other correlations were found in the pond sediment (Fig. 4A). In the pond water, the most prevalent bacterial taxa were found to be significantly correlated with water temperature, pH and DO ($p < 0.05$). Eukaryotic and bacterial richness and diversity were significantly correlated with NO_2^- and NO_3^- concentrations in the water column ($p < 0.05$). In addition, Choanoflagellida and Sphingobacteriia, Actinobacteria, Deltaproteobacteria, and Chloroflexi were significantly correlated with NH_4^+ concentrations in the water column ($p < 0.05$), while Cryptomonadales, Cryptomycota, and Cercozoa, as well as the eukaryotic diversity, were significantly correlated with *chla* in the water samples ($p < 0.05$, Fig. 4B).

CCA and RDA analyses were also performed to reveal the relationships between environmental factors and the bacterial and eukaryotic communities in the sediment and water of aquaculture ponds. Arylsulfatase, TP, and urease were found to have significant effects on both bacterial and eukaryotic prevalent OTUs in pond sediment ($p < 0.01$). In addition, DOC and TOC were two other important contributors to the variations of bacterial and eukaryotic prevalent OTUs in the pond sediment ($p < 0.01$). TN and pH were the most important environmental factors for the bacterial and eukaryotic prevalent OTUs in the pond water. However, eukaryotic OTUs in pond water were less affected by environmental factors compared to the bacterial ones (Supplementary data Fig. S6). On the contrary, the bacterial phyla in the pond sediment and water were less affected by environmental factors, while the prevalent eukaryotic phyla were significantly affected by the TN, *chla*, water temperature, NH_4^+ and pH of the pond water ($p < 0.01$, Supplementary data Fig. S7).

Co-occurrence network analysis of microbial community in aquaculture pond sediment and water

Co-occurrence network analysis was conducted to reveal the potential interspecific relationships among bacteria and eukaryotes in aquaculture pond sediment and water. In pond sediment, Rhodocyclaceae, Bacteroidia, and Anaerolineaceae OTUs had wide correlations with other taxa, and most of them were positively correlated with other taxa. *Flavobacterium* (OTU276) also had wide correlations with other taxa, and most of the *Flavobacterium* OTUs were negatively correlated with other taxa in pond sediment (Fig. 5A). Correlations among eukaryotic OTUs in pond sediment were relatively weak. Cryptomycota and Chytridiomycota were the main eukaryotic taxa that had wide correlations with other taxa (Fig. 5B). In pond water, *Fluviicola*, Sporichthyaceae, Polynucleobacter, Methylophilaceae, and Cyanobacteria taxa had wide correlations with other taxa (Fig. 5C). The eukaryotic correlations in pond water were complicated. Most prevalent eukaryotic OTUs had wide correlations with other taxa. Cercozoa (including Thecofilosea) and Chytridiomycota were in the center of the network, and had the widest correlations with other prevalent eukaryotes in the pond water (Fig. 5D). There were also wide correlations between bacterial and eukaryotic OTUs in aquaculture pond sediment and water. In sediment, both the bacterial and eukaryotic OTUs were in the center of the network, and most of the prevalent eukaryotic OTUs (including OTU74, OTU28, OTU39, OTU20, and OTU16) had negative correlations with the bacterial OTUs (Fig. 5E). In water, however, bacterial OTUs were in the center of the network that connected different eukaryotes, and most of the prevalent bacterial OTUs (including OTU1, OTU3, OTU6, OTU7, and OTU8) had positive correlations with eukaryotic OTUs (Fig. 5F).

Discussion

Many previous studies have recognized the importance of microbiological relationships between aquatic sediment and water, and compared the distribution of microorganisms in sediment and water (Wei *et al.*, 2016; Ekwanzala *et al.*, 2017; Li *et al.*, 2018; Kumar *et al.*, 2019; Sun *et al.*, 2020). However, most such studies focused on prokaryotes (bacteria or archaea), with eukaryotic microorganisms rarely researched (e.g., Ul-Hasan *et al.,* 2019; Shi *et al.*, 2020), especially in aquaculture ponds. In this study, using aquaculture ponds from three leading freshwater aquaculture-producing areas in South and North China, we compared both bacterial and eukaryotic microorganisms in sediment and water samples, and analyzed the environmental factors of influence as well as the interspecific relationships between different taxa in the two habitats.

Greater abundance and diversity of bacteria than fungi in aquaculture pond sediment and water

In this study, a significantly higher abundance of bacteria than fungi were found in both aquaculture pond sediment and water. Aquaculture systems are considered to be ideal for bacterial growth owing to the high organic loads of feed debris, species excrements, and dead organisms (Nevejan *et al.*, 2018). Indeed, the bacterial abundances detected in our sampling ponds reached up to 1.47×10^{12} copies/g dry sediment and 3.68×10^{11} copies/L water, which are much higher than in other habitats in both sediment and water (Zhang *et al.*, 2015; Wei *et al.*, 2016; Shi *et al.*, 2019). However, the fungal abundances in aquaculture sediment and water (up to 3.56×10^{10} copies/g dry sediment and 8.79×10^{9} copies/L water, respectively) seem to be only slightly higher than, or even similar to, those in other environments – for example, when compared to the eukaryotic abundances in estuarine sediment and water (Shi *et al.*, 2020) or the fungal abundances in coastal mangrove sediment (Luo *et al.*, 2017). This indicates that fungal abundances might not be significantly enriched in aquaculture ponds, but the abundances could not be illustrated clearly owing to the limited number of similar studies on quantifying eukaryotes in aquatic environments.

Our study also found significantly higher diversities of bacteria than eukaryotes in both aquaculture pond sediment and water. By analyzing the classification taxa, bacterial communities in the aquaculture pond sediment were mainly dominated by Betaproteobacteria, Anaerolineae, Sphingobacteriia, and Bacteroidia, many of which have been reported to be highly active in the decomposition of organic matter (Horňák *et al.*, 2017; Gómez-Consarnau *et al.*, 2019; Moncada *et al.*, 2019; Yang *et al.*, 2020). By contrast, eukaryotic microorganisms in aquaculture pond sediment were dominated by Cercozoa, followed by Chytridiomycota, Cryptomycota, Ichthyosporea, and LKM15, which contain numerous groups of parasites and saprophytes (Glockling *et al.*, 2013; James *et al.*, 2013; Scholz *et al.*, 2016; Dumack *et al.*, 2020). These results suggest that the bacterial community in aquaculture pond sediment plays a primary role in the decomposition of organic matter. The significantly higher ratios of bacterial to fungal abundance in sediment than in water (the 16S/ITS ratios ranged from 41.38 to 1957.34 and 14.14 to 379.75 in pond sediment and water, respectively) also proved the importance of sediment bacteria in aquaculture ponds. On the other hand, Actinobacteria, Chloroplast, Alphaproteobacteria, and Flavobacteriia were prevalent in aquaculture pond water, which comprise a large number of phototrophic taxa (Gupta and Khadka, 2016; Miroshnikov *et al.*, 2019), while eukaryotic microorganisms in pond water were dominated by Chytridiomycota, followed by Cercozoa, Choanoflagellida, Cryptomycota, and Cryptomonadales, which contain a significant portion of non-photosynthetic taxa (Letcher and Powell, 2018; Wijayawardene *et al.*, 2018; Dumack *et al.*, 2020). Considering the superiority of bacteria in abundance and diversity, the bacterial community in the water column could be important in photosynthesis in aquaculture ponds.

Less different eukaryotic microorganisms than bacteria between aquaculture pond sediment and water

Previous studies have indicated a significantly higher diversity and richness of microorganisms in sediment than in water (Wei *et al.*, 2016; Kumar *et al.*, 2019; Ul-Hasan *et al.*, 2019; Shi *et al.*, 2020). In the present study, we found similar distribution patterns of the bacteria communities in the sediment and water of aquaculture ponds, with the bacterial richness and diversity in sediment being higher than in water in all the sampling areas. However, the eukaryotic differences between sediment and water were found to be less significant. Although different taxa were detected between aquaculture pond sediment and water, the eukaryotic richness (evidenced by the OTU number, Chao1 and ace indexes) and diversity in sediment and water were not significantly different, and the eukaryotic communities in sediment and water shared some similarities (e.g., Cercozoa, Chytridiomycota, and Cryptomycota taxa were prevalent in both habitats). Interestingly, the fungal phylum Cryptomycota were also found to be distributed similarly between sediment and water samples in a sea cucumber farming system (Guo *et al.*, 2015). Dispersal limitation is considered to be an important factor shaping fungal diversity patterns, in addition to environmental filtering (Crump *et al.*, 2012; Bahram *et al.*, 2015), possibly indicating that fungal taxa in aquatic environments would be less influenced compared to bacteria by the heterogeneity differences between sediment and water, considering the physical connection between these two habitats. In addition, different structuring processes have been found between bacteria and microbial eukaryotes inhabiting the same communities; specifically, bacteria and microbial eukaryotes have been found to be mainly structured by environmental selection and drift, respectively (Logares *et al.*, 2018). Thus, microeukaryotic communities might be more evenly distributed between the sediment and water of aquaculture pond.

LEfSe analysis also uncovered more different bacterial than microeukaryotic taxa between aquaculture pond sediment and water in our study. Spirochaetales, Geothermobacter, Desulfobacterales, Nitrospirae, Firmicutes, and Chloroflexi were found to be more abundant in aquaculture pond sediment than in water. These bacteria have also been found to be prevalent in sediment in previous studies (Wei *et al.*, 2016; Kumar *et al.*, 2019; Shi *et al.*, 2019), and many taxa therein are considered to be functional during the transformation of organic matter. For example, Spirochaetes have been found to contribute to organic matter decomposition (Benacer *et al.*, 2013; Hamdi *et al.*, 2015), and Chloroflexi taxa may play an important role in hydrocarbon degradation (Liu *et al.*, 2015). Functional taxa in Geothermobacter, Desulfobacterales, and Nitrospirae are important in the transformation of metal, sulfur compounds and nitrification (Wei *et al.*, 2016;

Kumar *et al.*, 2019), indicating active organic matter transformation in aquaculture pond sediment. In addition, in our study, Ignavibacteriae and Lentimicrobiaceae were also found to be more abundantly distributed in pond sediment. These taxa have been detected in sediments in previous studies, and might be involved in organic matter transformation (Gavrilov *et al.*, 2019; Zheng *et al.*, 2019). Alphaproteobacter, Cyanobacteria, and Actinobacteria were more abundant in the aquaculture pond water than in the sediment, in accordance with previous studies (Glockling *et al.*, 2013; Wei *et al.*, 2016; Kumar *et al.*, 2019). Besides, Betaproteobacteria, Roseiflexaceae, Dinghuibacter, and Cryomorphaceae were also more abundant in the pond water, indicating diverse bacterial communities between aquaculture pond sediment and water. By contrast, only Cryptomonadales microeukaryotes were found to be distributed differently between aquaculture pond sediment and water samples in our study. Cryptomonadales-related taxa have been reported to be important photosynthetic eukaryotes in near-surface waters (Kataoka *et al.*, 2017). The prevalence of Cryptomonadales in pond water may reflect their significant contribution to photosynthesis in aquaculture ponds.

Lesser influence of environmental factors on microorganisms in aquaculture pond sediment than water

Consistent with the above discussion, the microbial communities in sediment were significantly correlated with organic matter transformation (transforming enzymes) in the aquaculture ponds of our study, suggesting a high abundance of organic matter decomposing microorganisms in aquaculture pond sediment. Our previous study indicated significant correlations between ammonia-oxidizing microorganisms and the organic matter availability in aquaculture pond sediment (Dai *et al.*, 2018). From CCA and RDA analyses based on prevalent microbial taxa in this study, we found that most prevalent bacterial taxa were positively correlated with the levels of organic matter (in the form of DOC and TOC), and most belonged to groups active in the decomposition of organic matter (see Supplementary data Figs. S6 and S7 and the above discussion), which would contribute greatly to the correlation between the bacterial community in pond sediment and organic matter. Some prevalent eukaryotic microorganisms belonging to groups of saprophytes, which contribute to organic matter decomposition, were also found to be positively correlated with the levels of organic matter, while other eukaryotes (e.g., Rotifera and Cercozoa taxa) belonging to groups of predators on bacteria (Nevejan *et al.*, 2018) were negatively correlated with the levels of organic matter in the aquaculture pond sediment in our study (Supplementary data Figs. S6 and S7). These correlations may suggest the process of organic matter decomposition to be an important factor in structuring microbial communities in aquaculture pond sediment.

The level of correlation between microorganisms and environmental factors in the aquaculture pond water in our study was more significant than that in the sediment (Fig. 4). This result is consistent with previous studies, both of prokaryotes and microbial eukaryotes (Wei *et al.*, 2016; Shi *et al.*, 2020). In the present study, water temperature, pH, DO, and nutrient levels (TN, NO_2^-, NO_3^-, and NH_4^+) all showed significant correlations with bacterial and microeukaryotic communities in aquaculture pond water. Most bacterial taxa were found to be negatively correlated with DO in pond water, possibly due to their aerobic respiration during organic matter decomposition. The concentrations of TN, NO_2^-, and NO_3^-, meanwhile, were significantly positively correlated with microbial communities in pond water, indicating their preference for nutrient-enriched environments, and NH_4^+ concentrations were found to be negatively correlated with both the bacterial and microeukaryotic communities. Ammonia-nitrogen is frequently enriched in aquaculture ponds owing to the large quantities of massive organic matter decomposition (Dai *et al.*, 2018), and may inhibit on microorganisms when at high concentrations (Parker *et al.*, 2012; Rajagopal *et al.*, 2013; Wang *et al.*, 2015a). The negative correlation between ammonia-nitrogen and both bacteria and microbial eukaryotes may indicate an adverse effect of increased NH_4^+ concentrations on microbial communities in aquaculture pond water. In addition, *chla* was found to be significantly negatively correlated with some prevalent eukaryotic taxa, including Cercozoa, Cryptomonadales, and Cryptomycota, possibly indicating wide correlations between different taxa in aquaculture pond water.

Intensive interspecific relationships among eukaryotes and bacteria in aquaculture pond sediment and water

Co-occurrence network analysis was conducted in our study to reveal the possible interspecific relationships among bacterial and microeukaryotic communities in aquaculture pond sediment and water. Although microorganisms in pond sediment were found to be less influenced by environmental factors, significant correlations among different taxa were detected in the aquaculture pond sediment. Rhodocyclaceae, Bacteroidia, and Anaerolineaceae OTUs had widely positive correlations with other taxa, which contain numerous bacterial groups active in decomposing organic matter (see the above discussion), indicating the importance of nutrient-related taxa in the bacterial community of the aquaculture pond sediment. Interestingly, most OTUs of *Flavobacterium*, which genus are consistent of opportunistic pathogens, were negatively correlated with other taxa in pond sediment, which probably indicates that the interspecific relationships among microorganisms could be important in controlling pathogenic bacteria in aquaculture ponds. For eukaryotes, Cryptomycota and Chytridiomycota were the main taxa that had wide correlations with other taxa in pond sediment. These fungal groups are known to cover a great number of parasites to zooplankton and algae (Gleason *et al.*, 2017). Their wide relationships might indicate an important role of fungal parasitism in microeukaryotic communities in aquaculture pond sediment.

In aquaculture pond water, *Fluviicola*, Sporichthyaceae, Polynucleobacter, Methylophilaceae, and Cyanobacteria OTUs were found in this study to have wide correlations with other bacterial taxa. These taxa were also found to be more prevalent in pond water than in sediment (see the results above). Cercozoa and Chytridiomycota OTUs had the widest correlations with other prevalent eukaryotes in the pond water in this study, the groups of which, on the contrary, were found to be more prevalent in pond sediment, possibly indicating more taxa relationships in microeukaryotic than in bacterial communities between aquaculture pond sediment and water. Indeed, significant correlations between different microeukaryotic taxa were detected, especially in aquaculture pond water (Fig. 5), when compared to correlations between bacterial taxa. In addition, wide correlations between bacterial and eukaryotic OTUs in aquaculture pond sediment and water were also found in our study. In sediment, most of the prevalent eukaryotic OTUs had negative correlations with the bacterial OTUs (Fig. 5E), wherein the former mostly belong to fungal groups involved in parasitism, further indicating an important role of fungal parasitism in microbial communities in aquaculture pond sediment. In pond water, most of the prevalent bacterial OTUs had positive correlations with eukaryotic OTUs (Fig. 5F). These prevalent bacterial OTUs mainly belonged to taxa of Sporichthyaceae, Methylophilaceae, or Cyanobacteria, which were also found to have wide correlations with other bacterial taxa, suggesting intensive interspecific relationships among eukaryotes and bacteria in aquaculture ponds. However, to better understand the relationships between bacteria and microbial eukaryotes in aquaculture ponds, more precise studies are still needed.

To the best of our knowledge, this is the first study to have analyzed the differences in the distributions of bacterial and microeukaryotic communities between aquaculture pond sediment and water. By comparing sediment and water eukaryotic and bacterial microorganisms in different aquaculture ponds from three different aquaculture areas, we found that the bacterial abundance was significantly higher than the fungal abundance in both pond sediment and water, and the bacterial richness and diversity were much higher than those of eukaryotes in both sediment and water. We found distinct differences in the bacterial and microeukaryotic communities between sediment and water, but the differences in eukaryotic microorganisms between pond sediment and water were less significant compared to those of bacteria. Organic matter was the primary environmental factor involved in shaping microbial communities in pond sediment, while bacterial and microeukaryotic communities in pond water were influenced by numerous environmental factors, including water temperature, pH, DO, and nutrient levels (TN, NO_2^-, NO_3^-, and NH_4^+). In addition, co-occurrence network analysis revealed intensive interspecific relationships among eukaryotes and bacteria in aquaculture pond sediment and water. Considering the importance of microorganisms during the culturing of fish, the results of our study may provide valuable data on the microbial distributions in these aquatic environments, and may also be of practical use in aquaculture pond management.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (No.31902378), and funds from East-West Cooperation Project of Ningxia Hui Autonomous Region (2017BY087) and the Project of Agricultural Technology Demonstration and Service Support.

Conflict of Interest

The authors declare no conflict of interest.

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