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Article

Mycoplanktonic Community Structure and Their Roles in Monitoring Environmental Changes in a Subtropical Estuary in the Beibu Gulf

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Abstract: Mycoplankton are an important component of marine ecosystems and play a key role in material cycling and energy flow in marine ecosystems. In this study, high-throughput sequencing of the 18S rDNA gene was employed to investigate the community structure of mycoplankton during summer and winter and their response to environmental changes in the Dafengjiang River estuary in the Beibu Gulf, Guangxi. The mycoplanktonic community was generally dominated by Ascomycota, Basidiomycota, and Cryptomycota. However, there were significant seasonal differences in the α-diversity of the mycoplanktonic community ($p < 0.05$). Random forest modeling also revealed that *Paramicrosporidium*, *Parengyodontium*, *Arthrinium*, *Paramycosphaerella*, *Pestalotia*, and *Talaromyces* were the most effective bioindicators of environmental changes. Spearman's correlation analysis and distance-based redundancy analysis suggested that the trophic status, chemical oxygen demand, dissolved oxygen, and salinity were the key environmental factors regulating the mycoplanktonic community structure. Variation partitioning analysis also found that nutrient levels were the main driver of the β-diversity of the mycoplanktonic community, showing a greater effect than the water quality parameters. In conclusion, this study revealed the mycoplanktonic community structure and its key drivers in the Dafengjiang River estuary, thus providing a theoretical reference for ecological environmental monitoring and resource management in the Beibu Gulf.

Keywords: Dafengjiang River; Beibu Gulf; mycoplankton; environment monitoring; 18S rRNA gene

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1. Introduction

With continued economic development and usage of marine and coastal resources, climate change has become a global environmental concern [1]. It has multiple profound effects on marine ecosystems, greatly altering the natural microbial diversity and community structure [2]. Marine mycoplankton (also known as planktonic fungi) play a fundamental role in the marine ecosystem structure as key participants in a number of biogeochemical cycles [3], particularly the decomposition of detritus or phytoplankton-derived organic matter [3,4]. For example, basidiomycetes and ascomycetes can degrade the shells and burrow linings of some marine animals, suggesting that they may be involved in the carbon cycle [5]. In addition, following their infection of phytoplankton species, marine mycoplankton can become a valuable food source for zooplankton, while the fungal

community is also known to process the organic matter created during dinoflagellate blooms through parasitism and saprophytism. These geobiochemical roles are likely to influence other microbial groups (i.e., protists and bacteria) via the microbial loop [6]. Thus, the relationship between the diversity of marine mycoplankton and environmental conditions and subsequent changes in their community structure have the potential to be used to assess environmental changes in marine ecosystems.

Previous studies have reported that the presence and levels of various nutrients in seawater, such as nitrates, nitrites, orthophosphates, and silicic acid, affect the community structure of marine mycoplankton. For example, Taylor et al. found that the availability of nitrogen was the main environmental driver of the coastal planktonic fungal community structure in the western English Channel [7]. In addition, Wang et al. reported that dissolved inorganic phosphorus, silicate, and inorganic nitrogen strongly affected the planktonic fungi community structure in the Bohai Sea, China [8]. Recent studies have emphasized the diversity and ecological importance of marine mycoplankton in estuaries. For example, Burgard et al. found that terrestrially influenced low-salinity environments significantly influenced the fungal communities in a Delaware River estuary and bay [9], while Wang et al. investigated three coastal sites off the South China Sea (the Pearl River estuary, Shenzhen Bay, and Daya Bay) [8] and found that their marine fungal communities were unprecedently diverse, with the ubiquitous dominance of Dikarya and the occasional dominance of Glomeromycota, Mucoromycota, Mortierellomycota, and Chytridiomycota. The salinity and nitrate levels were the key factors that determined the β-diversity of these fungal communities. Rojas-Jimenez explored the diversity and composition of the fungal communities along a salinity gradient (from 3 to 34 psu) in the Baltic Sea using 18S rRNA gene sequence analysis and found clear differences [10]. Recently, Zhao et al. revealed that environmental perturbation in the Beibu Gulf, China, significantly affected the planktonic fungal community and constituent taxa [11]. However, seasonal changes in marine mycoplankton, the key environmental drivers of their community structure, and their potential as indicators in the assessment of environmental disturbances in estuary ecosystems are still poorly understood.

To enhance the understanding of the environmental factors that regulate the diversity and ecological roles of mycoplankton in response to climatic changes and anthropogenic actions in subtropical estuaries, the present study analyzed seawater samples from the Dafengjiang River estuary in the Beibu Gulf during summer and winter using 18S rRNA high-throughput sequencing. The main goals of the study were to (a) evaluate changes in the community structure of marine mycoplankton between seasons, (b) elucidate the potential relationships between the diversity of the mycoplanktonic community and various environmental factors, and (c) identify key planktonic fungal indicators that could be employed to assess environmental changes in this subtropical estuary. The findings from this investigation thus provide new knowledge for the analysis of the impacts of environmental disturbance on mycoplanktonic communities in subtropical estuary ecosystems and the biogeochemical processes that they are involved in.

2. Materials and Methods

2.1. Study Sites and Experimental Design

The sampling sites were located in the Dafengjiang River estuary in the Beibu Gulf, China (Figure 1). Surface water samples (0.5 m deep) obtained from 15 sites (S1–S15) were collected during summer (July) and winter (December) in 2018. At each site, five water samples were collected from a 5 m × 5 m area (five-point sampling method) using 5 L Niskin bottles. Following collection, the samples were stored on ice for transport to the laboratory. For nutrient and chlorophyll *a* (Chl-*a*) analysis, 500 mL of each sample was filtered through a 0.45 μm filter (Millipore Corporation, Billerica, MA, USA). For DNA extraction, 3 L of the surface seawater samples was first filtered through a 200 μm polycarbonate filter (Nuclepore, Whatman, Florham Park, NJ, USA) to remove debris and

larger organisms and then through a polycarbonate membrane with a pore size of 0.22 μm (Millipore Corporation, Billerica, MA, USA). The 0.22 μm membranes were then stored at −20 °C for subsequent analysis.

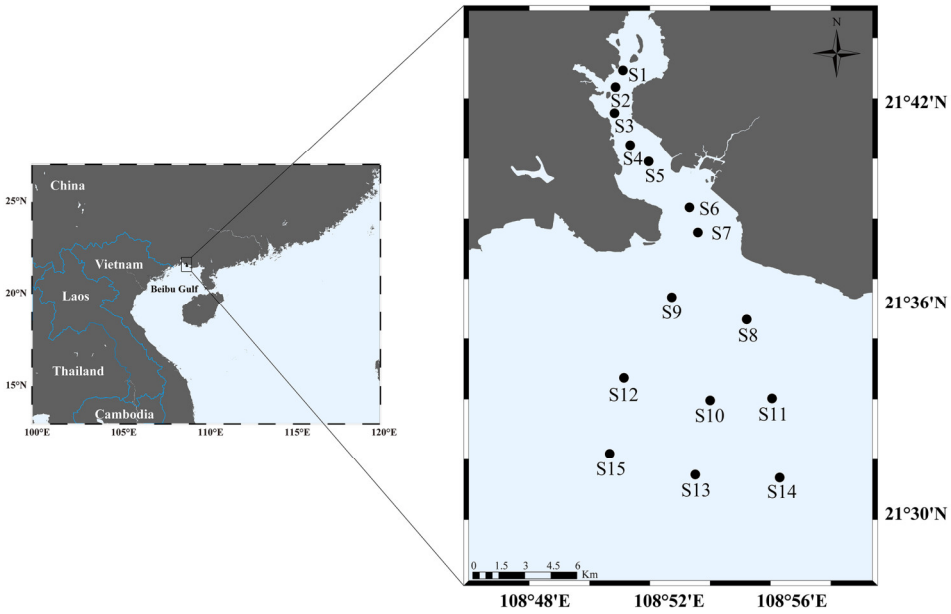

Figure 1. Location of the sampling sites in the Dafengjiang River estuary.

2.2. Environmental Factor Measurements

Temperature, salinity, pH, and dissolved oxygen (DO) were measured using a multiprobe portable meter (556 MPS; YSI, Yellow Springs, OH, USA). The concentrations of phosphate–phosphorus (PO_4^{3-}-P), total phosphorus (TP), nitrite–nitrogen (NO_2^--N), nitrate–nitrogen (NO_3^--N), ammonium–nitrogen (NH_4^+-N), and total nitrogen (TN) were determined using a continuous flow analyzer (Seal-AA3, Norderstedt, Germany). The Chl-*a* concentration was determined using spectrophotometry [12]. The total organic carbon (TOC) was measured using a TOC-VCPH Analyzer (Shimadzu, Kyoto, Japan). The chemical oxygen demand (COD) was measured using the alkaline $KMnO_4$ method. The dissolved inorganic nitrogen (DIN) was calculated as the sum of NO_2^--N, NO_3^--N, and NH_4^+-N, while the dissolved inorganic phosphorus (DIP) was taken to be PO_4^{3-}-P [13]. The Chinese eutrophication status index [13–15] was employed as the eutrophication index (EI) for this study and calculated as EI = (DIN × DIP × COD × 10^6)/4500.

2.3. DNA Extraction and PCR Amplification

Environmental DNA was extracted from the membranes using a DNeasy Power Water Kit (QIAGEN, Germantown, MD, USA) following the manufacturer's protocols and stored at −80 °C. The V4 region (~380 bp) of 18S rRNA was PCR-amplified using dual-bar coded primers TAReuk454FWD1(5′-CCAGCASCYGCTAGTAATTCC-3′) and TAReukREV3(5′-ACTTTCGTTTGATYRA-3′) [16]. PCR was performed using a 20 μL mixture containing 10 μL of 2×Taq PCR Mastermix (TIANGEN, Beijing, China), 1 μL of each primer (10 mM), 1 μL of template DNA (~50 ng), and 7 μL of double-distilled H_2O. Amplification was conducted using the following cycling parameters: initial denaturation at 98 °C for 2 min, followed by 10 cycles of 98 °C for 10 s, 53 °C for 30 s, and 72 °C for 30 s, then 15 cycles of 98 °C for 10 s, 48 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 2 min. After the quality of PCR products was verified using 1% agarose gel electrophoresis and a Nanodrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA), the samples were prepared using a TruSeq DNA kit (Illumina, San Diego, CA, USA) following the manufacturer's instructions.

2.4. High-Throughput Sequencing and Taxonomic Annotation

The purified library was prepared in accordance with Illumina library preparation protocols and then sent to Majorbio Biotech (Shanghai, China) for sequencing on an Illumina MiSeq platform. Raw sequences were processed and checked using Qiime2 to remove sequences with primer mismatches or a length of <275 bp, low-quality reads (with quality scores of <30), and barcode sequences [17]. Chimeric sequences were detected and eliminated using UCHIME [18]. Qiime2 was also employed for the de novo clustering of operational taxonomic units (OTU) based on a 97% sequence identity. The representative sequence for each OTU was annotated using the Silva database [19]. Only sequences belonging to the kingdom Fungi were retained. All sequence data have been deposited in GenBank under the accession number SUB11951013.

2.5. Statistical Analysis

Statistical analysis was conducted on the R platform (http://www.r-project.org/ accessed on 18 January 2019). Differences in the physicochemical factors between sampling sites were analyzed using one-way analysis of variance (ANOVA) with Tukey's HSD test. Richness, Shannon, Chao1, Simpson, and Good's coverage [20,21] indices were calculated using the vegan package, with the Shannon index used as a measure of the α-diversity. Bray–Curtis distance-based redundancy analysis (db-RDA) was conducted using vegan to visualize the mycoplanktonic β-diversity and its correlation with the environmental factors. Random forest analysis was conducted to identify the important indicator taxa using the random forest package. Correlations were calculated using the Spearman's rank method with the psych package. Variation partitioning analysis (VPA) was conducted using vegan.

3. Results

3.1. Nutrient Levels at Sampling Locations

Figure 2 presents the results of the ANOVA analysis used to detect significant variation in the parameters between summer and winter. The results showed that temperature, salinity, DO, NO_2^--N, NH_4^+-N, DIP, Chl-*a*, COD, TP, and EI differed significantly ($p < 0.001$) between the seasons, while the changes in NO_3^--N, DIN, and TN were not significant ($p > 0.05$). The average temperature of the samples from the Dafengjiang River estuary in summer and winter was 29.46 °C and 19.07 °C, respectively. When moving from S1 to S15, the levels of TN, NO_3^--N, DIN, TOC, and COD gradually decreased, while the salinity increased. The salinity during summer varied greatly, ranging from 17.37 to 30.89 psu, with an average of 24.8 psu, while it had a range of 23.9–30.4 mg/L in winter, with an average of 27.7 mg/L. NO_2^--N levels gradually decreased from S1 to S8 in summer and were significantly higher than in winter. The EI gradually decreased from S1 to S15 and exhibited a significant seasonal difference ($p < 0.05$). The EI in summer was >9 at sites S1–S6, and 1–9 at sites S7–S15, while the EI in winter was 1–9 at all sites. These findings indicate that, when moving from S1 to S15, the eutrophic status of the seawater gradually decreased and it was higher in summer than in winter.

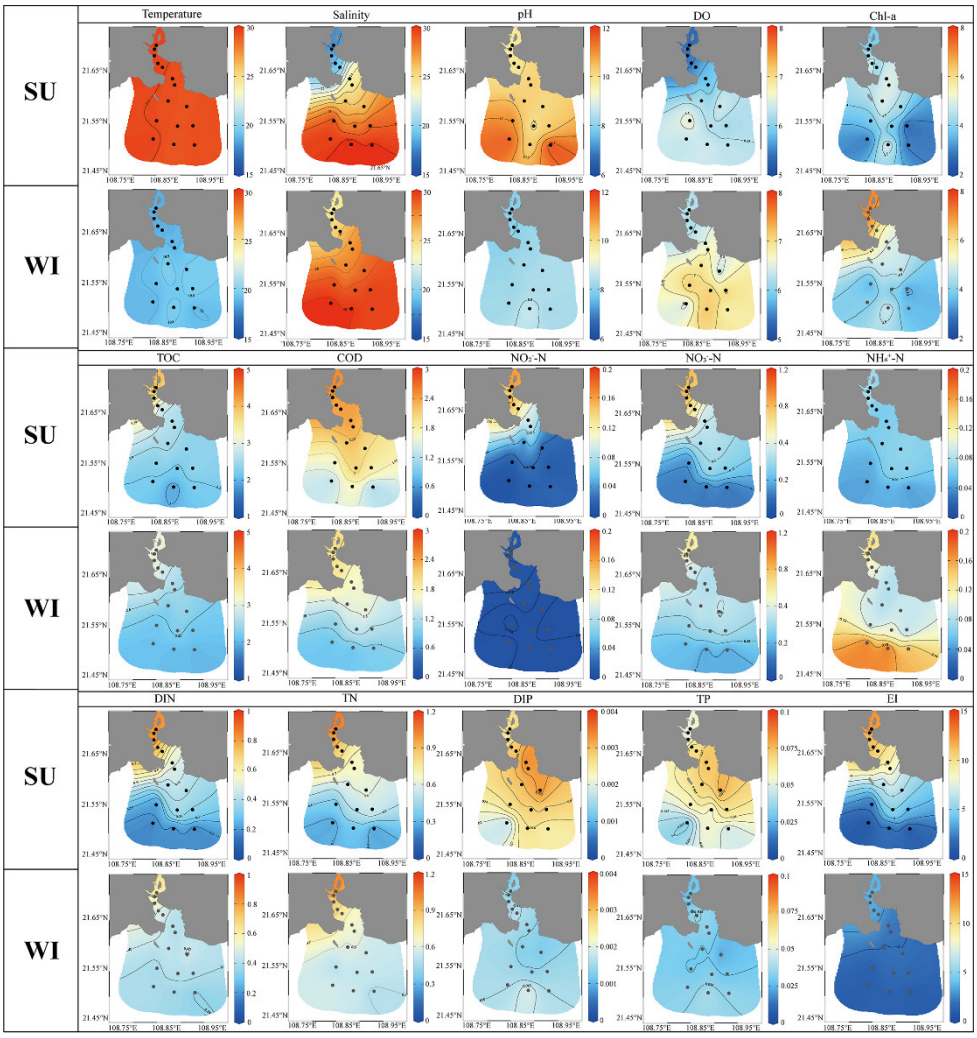

Figure 2. Environmental factors in summer and winter in the Dafengjiang River estuary. SU, summer; WI, winter. The environmental parameters in summer and winter (mean with SD) are provided in Supplementary Table S1.

3.2. Community Composition and Seasonal Diversity of Mycoplankton

After quality control and the removal of non-fungal genetic sequences, a total of 1432 OTUs were identified, belonging to 7 phyla, 27 classes, 74 orders, 119 families, and 151 genera. Good's coverage was greater than 92.14% for each sample, suggesting that the vast majority of the mycoplanktonic taxa were recovered.

The marine mycoplanktonic community in the Dafengjiang River estuary was dominated by Basidiomycota and Ascomycota, with a relative abundance of 81.9% and 15.0%, respectively (Figure 3), while unannotated sequences only accounted for 0.10% of the community. The three most dominant groups were the same in summer and winter (Basidiomycota, Ascomycota, and Cryptomycota), but there were seasonal differences in their relative abundance (54.30%, 39.29%, and 5.40% in summer and 88.37%, 9.28%, and 2.12% in winter, respectively). This indicated that there were seasonal differences in the composition of the marine mycoplanktonic community.

The richness, Shannon, and Chao1 indices for the mycoplanktonic community differed significantly between seasons ($p < 0.001$), but the Simpson index did not show a significant difference ($p > 0.05$; Figure 4). The richness and Chao1 indices were higher in summer than in winter, while the Shannon index (α-diversity) was lower in summer than in winter.

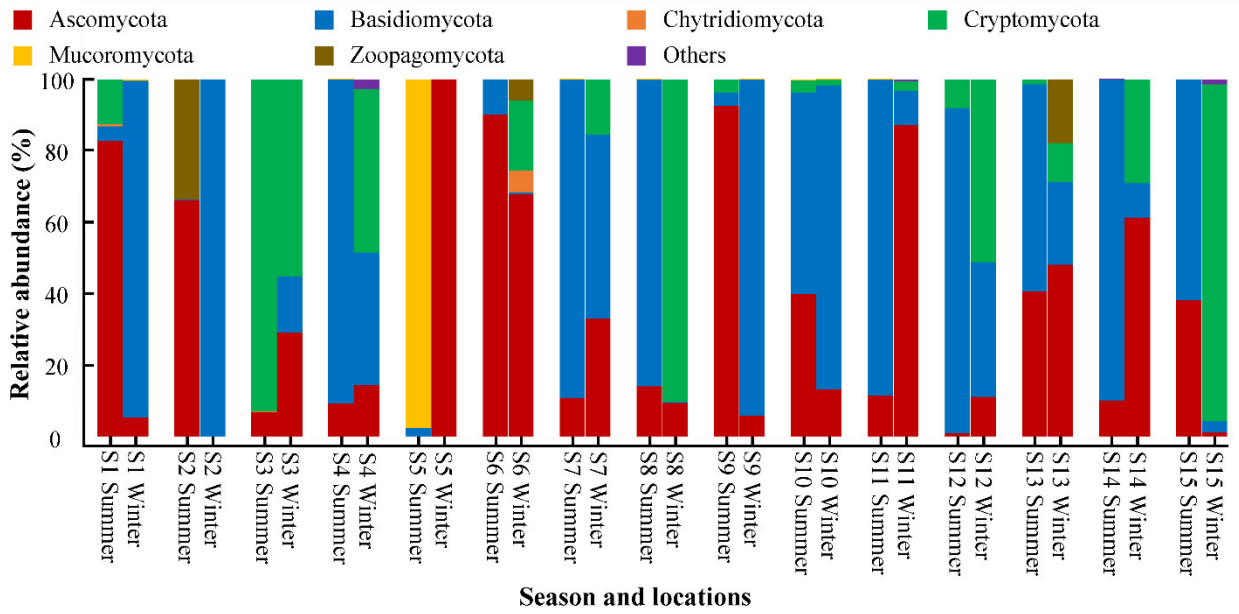

Figure 3. Relative abundance of mycoplankton in summer and winter in the Dafengjiang River estuary (phylum level).

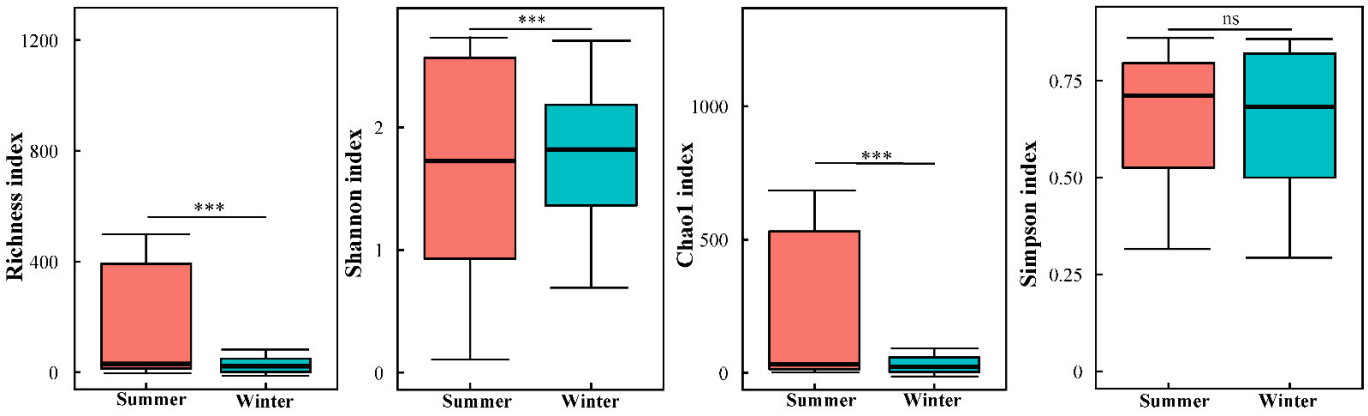

Figure 4. The α-diversity indexes including richness, Shannon, Chao1, and Simpson for two season groups. The differences between pairs of two groups were tested by the Wilcoxon test. ns, not significant. ***: $p < 0.001$.

3.3. Indicator Mycoplankton Taxa for Seasonal Environmental Changes

The random forest method was used to identify the most important mycoplankton taxa for the classification of the seasonal samples. As shown in Figure 5, *Paramicrosporidium*, *Parengyodontium*, *Arthrinium*, *Paramycosphaerella*, and *Pestalotia* had the highest Gini values of the 25 most important indicator genera. Spearman's correlation analysis revealed that these 25 indicator genera were significantly correlated with multiple environmental factors. For example, *Paramicrosporidium* was positively correlated with pH ($p < 0.01$) and negatively correlated with temperature, DIP, and TP ($p < 0.05$). *Parengyodontium* was positively correlated with temperature, NO_2^--N, NO_3^--N, DIN, DIP, TOC, COD, and TN ($p < 0.05$), and negatively correlated with salinity, pH, and DO ($p < 0.05$). *Phomopsis* was positively correlated with temperature, pH, DIP, COD, and TP ($p < 0.05$), but negatively correlated with NH_4^+-N ($p < 0.05$). *Aspergillus* showed positive correlations with salinity, pH, and DO ($p < 0.05$). Additionally, the 25 indicator genera were frequently correlated with temperature, DIP, TP, DO, and NH_4^+-N.

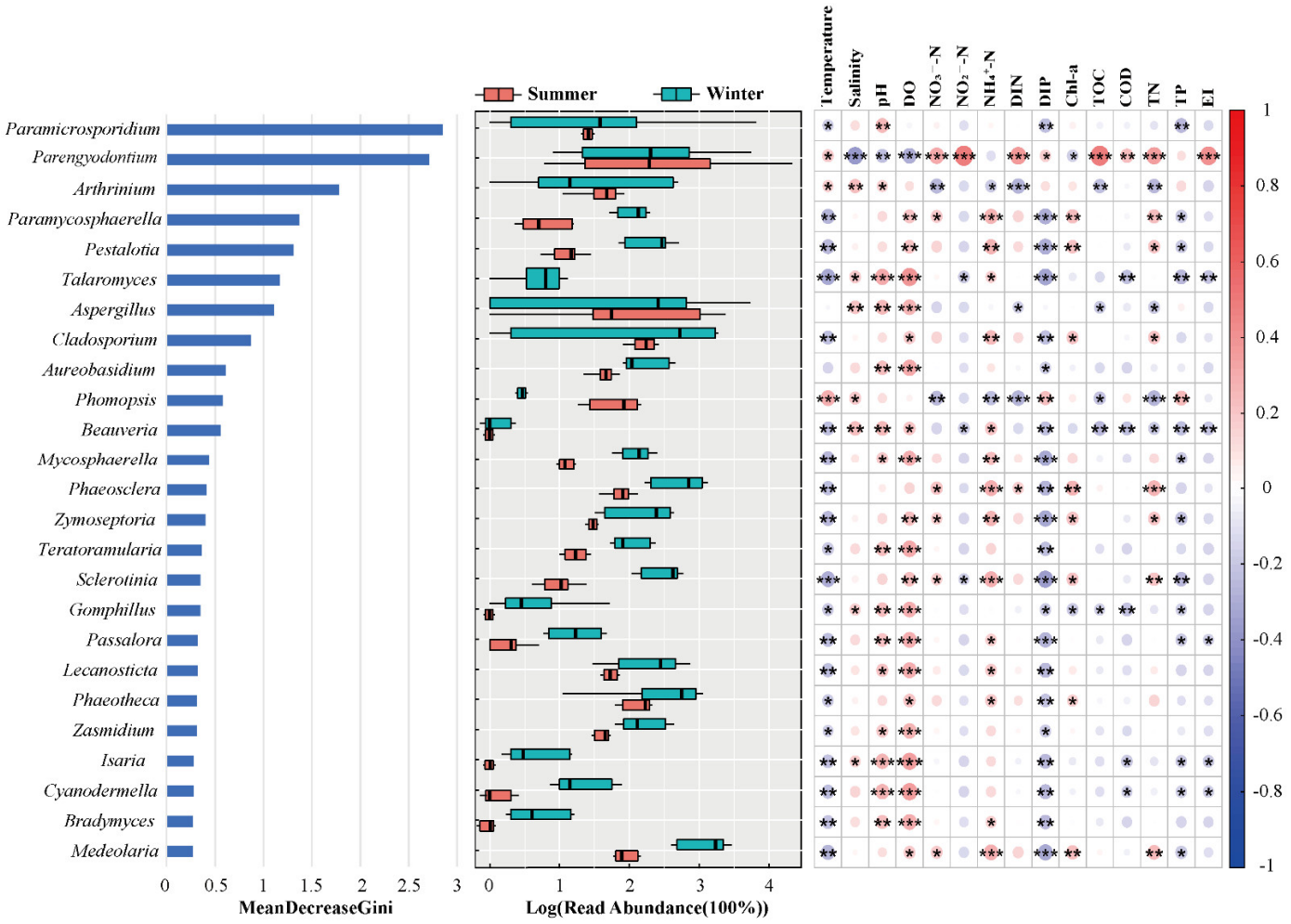

Figure 5. Random forest classification of the top 25 important genera. Left: The top 25 taxa analyzed using the Gini index, representing the importance of each genus in distinguishing different groups. Middle: Read abundances of the top 25 genera. Right: Pearson correlations between the relative abundances of the top 25 genera and environmental and nutrient factors. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

3.4. Multiple Drivers Affecting the Mycoplanktonic Community between Seasons

Spearman's rank correlation was conducted to determine the effects of the environmental factors on the α-diversity (indicated by the Shannon index) of the mycoplanktonic community (Table 1). The results showed that α-diversity was positively correlated with salinity and TP ($p < 0.05$) and negatively correlated with NO_3^--N, DIN, TOC, and TN ($p < 0.05$). The taxonomic diversity (indicated by the richness index) of the marine mycoplanktonic community was positively correlated with TN ($p < 0.05$) and negatively correlated with NO_3^--N, salinity, and DIN ($p < 0.05$).

The impact of the environmental parameters on the mycoplanktonic community structure and distribution was investigated using db-RDA. The results revealed that multiple factors were strongly correlated with the mycoplanktonic community (Figure 6). EI, COD, DO, and salinity had stronger effects on mycoplanktonic β-diversity than did the other factors. VPA was also conducted to analyze the contribution of water quality parameters (temperature, salinity, pH, DO, Chl-*a*, and COD) and nutrient levels (NO_2^--N, NO_3^--N, NH_4^+-N, DIN, TN, DIP, TOC, and TP) to mycoplanktonic β-diversity (Figure 7). The nutrient and water parameters explained more than 90% of the variation in the mycoplanktonic community in both seasons, but they explained only 44% of the community variation when all samples were analyzed together. In addition, although the explanation

power of the nutrient and water characteristics was similar, the effect of nutrient levels was higher than that of water quality parameters in both seasons and across all samples.

Table 1. Correlations between the Shannon α-diversity index and environmental factors.

	Shannon Index	Richness
Temperature	−0.09	0.02
Salinity	0.26 **	−0.17 *
pH	0.18 *	0.20 *
DO	0.18 *	0.15
Chl-*a*	0.05	−0.16
TOC	−0.29 ***	−0.08
COD	−0.20 *	0.15
NO_2^--N	0.03	0.02
NO_3^--N	−0.37 ***	−0.25 **
NH_4^+-N	−0.11	0.05
DIN	−0.33 ***	−0.19 *
TN	−0.28 ***	0.19 *
DIP	0.15	−0.14
TP	0.25 **	0
EI	−0.13	−0.05

Note: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

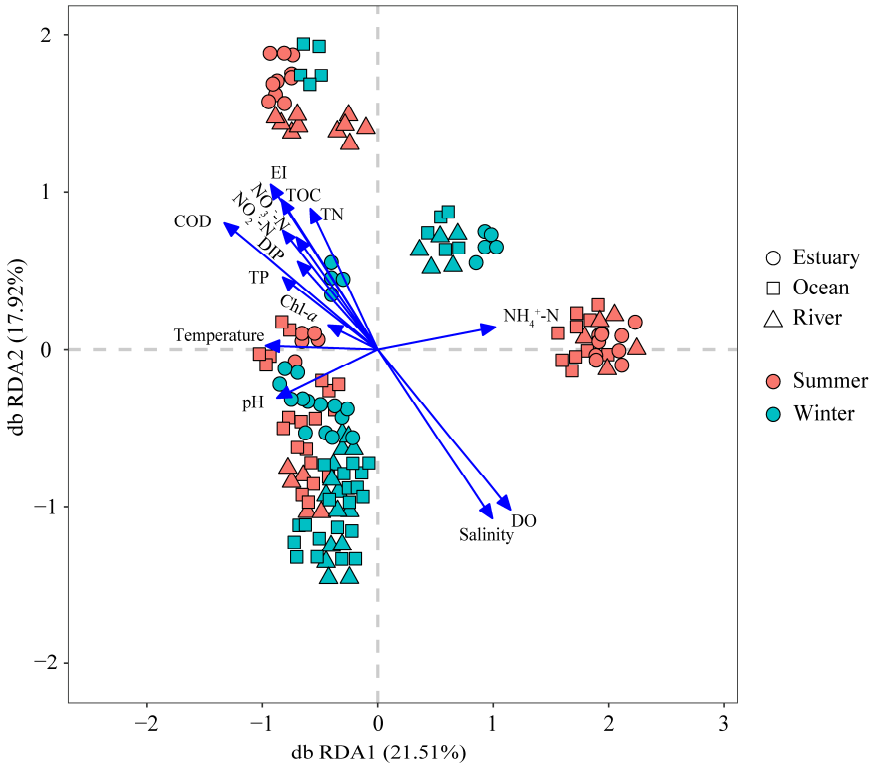

Figure 6. Distance-based redundancy analysis (db-RDA) based on the Bray–Curtis distance of the mycoplanktonic community in relation to the environmental factors.

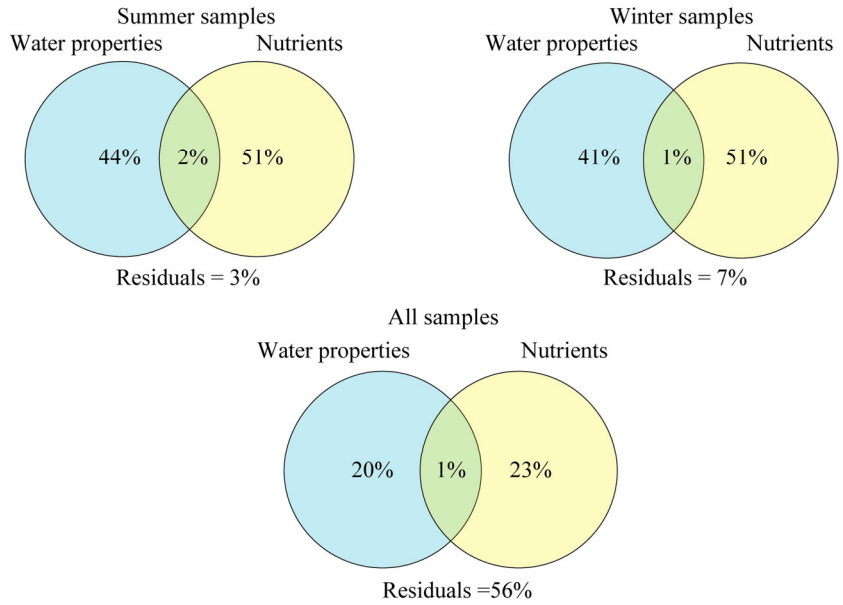

Figure 7. Variation partitioning analysis of the effects of water quality parameters (temperature, pH, salinity, Chl-*a*, DO, and COD) and nutrient levels (NO_2^--N, NO_3^--N, NH_4^+-N, DIN, TN, DIP, TOC, and TP) on the mycoplanktonic community composition in seawater from the Dafengjiang River estuary area.

4. Discussion

4.1. Composition and Diversity of the Mycoplanktonic Community in the Dafengjiang River Estuary

This study investigated the seasonal variation in mycoplanktonic diversity in a subtropical estuary ecosystem. Basidiomycota and Ascomycota collectively accounted for more than 96% of the mycoplanktonic community in terms of abundance. This dominance of Basidiomycota and Ascomycota is consistent with the results reported for other marine ecosystems, including the western English Channel [7], Pearl River estuary [8], and Baltic Sea [10]. Our study thus provides evidence that Basidiomycota and Ascomycota are also the dominant planktonic fungal groups in subtropical marine ecosystems.

We found that the α-diversity was significantly lower in summer than in winter ($p < 0.05$; Figure 4), indicating that the environmental conditions in winter were more suitable for higher taxonomic diversity and evenness within the mycoplanktonic community. This is consistent with the findings of Duan et al. [22] from an analysis of the seasonal patterns of planktonic fungi at a temperate coastal ocean site. However, another study in the Yellow Sea of China suggested that winter seawater samples had lower levels of fungal α-diversity [23]. It is possible that the unique climatic conditions and anthropogenic influences in different regions lead to distinct forms of seasonal heterogeneity in the marine fungal community; in particular, changes in precipitation between seasons can cause fluctuations in the freshwater input to estuarine areas, thus affecting the mycoplanktonic community.

4.2. Indicator Mycoplankton Taxa for Environmental Changes in the Dafengjiang River Estuary

Aquatic microorganisms can respond rapidly to changes in the environment and play an important role in biological monitoring as indicator taxa for the detection and evaluation of environmental disturbances in aquatic ecosystems [24–26]. In the present study, *Paramicrosporidium*, *Parengyodontium*, *Arthrinium*, *Paramycosphaerella*, and *Pestalotia* were the five genera most sensitive to environmental changes in the Dafengjiang River estuary (Figure 7). The ability of these fungi to indicate environmental changes may be related to their characteristics. For example, *Arthrinium* exhibited a negative correlation

with pH ($p < 0.05$) and a positive correlation with temperature ($p < 0.05$; Figure 7). The sensitive response of *Arthrinium* to environmental factors is consistent with the findings of Li et al. [26]. In addition, we found that the genus *Pestalotia* was positively correlated with TN and NH_4^+-N ($p < 0.05$). It may be that an increase in marine nitrogen promotes the proliferation of brown algae, which acts as a host for *Pestalotia* [27]. In summary, these indicator species may facilitate the assessment of global climate change and its subsequent ecological effects on estuary ecosystems.

4.3. Key Environmental Factors Affecting the Mycoplanktonic Community

Our results indicated that the Dafengjiang River estuary is seriously polluted by nutrients, including carbon, nitrogen, and phosphorus (Figure 2). In the Dafengjiang River estuary, the trophic status in summer and winter decreased when moving from the inner estuary to the outer estuary, suggesting that the high nutrient levels were mainly from terrestrial sources such as domestic sewage discharge, mariculture (shrimp and shellfish farming), port construction, and agricultural fertilizer [28,29].

Seasonal environmental changes in the Dafengjiang River estuary may also impact the diversity and composition of the mycoplanktonic community in a number of ways. We found that TOC was one of the key environmental factors affecting the α-diversity of the mycoplanktonic community in the estuary (Table 1). Marine planktonic fungi play a key role in decomposing organic matter [4,30], which may explain why TOC is the major factor affecting their α-diversity. In addition, mycoplanktonic α-diversity and richness were negatively correlated with NO_3^--N ($p < 0.05$; Table 1), indicating that diversification and reproduction within the mycoplanktonic community deplete nitrogen levels.

The db-RDA results indicated that the EI, COD, DO, and salinity were the key drivers affecting the variation in the mycoplanktonic community in the Dafengjiang River estuary (Figure 6). The correlation between the EI and the mycoplanktonic community is consistent with previous studies, which have reported that the diversity of mycoplankton is closely related to the nutrient levels in coastal waters [8,11,31,32]. In addition, the COD is an important index used to evaluate organic pollutants and has a significant impact on the fungal community composition in coastal ecosystems [8,11]. Our findings again highlight the important role of the fungal community as decomposers in the metabolism of marine organic matter [30]. Similar to our results, DO was identified as one of the major factors regulating the fungal community in the East China Sea [33]. Our results indicated that salinity was also an important factor affecting the seasonal dynamics of the planktonic fungal community in the subtropical estuary. These findings suggest that the trophic status and water quality parameters have a significant influence on the mycoplanktonic community in subtropical estuaries. Thus, we conducted VPA to determine their contribution to the β-diversity of this community (Figure 7), with the results suggesting that nutrient levels are the main driver of β-diversity. Our results support the view that coastal eutrophic pollution can alter the structure and diversity of mycoplanktonic communities, thus potentially leading to ecological damage from harmful algal blooms, hypoxia, and acidification [7,11,34–37].

5. Conclusions

This study studied the seasonal patterns of the marine mycoplanktonic community in the Dafengjiang River estuary. It was found that this community was dominated by Basidiomycota and Ascomycota, while there were significant differences in α-diversity between summer and winter. *Paramicrosporidium*, *Parengyodontium*, *Arthrinium*, *Paramycosphaerella*, and *Pestalotia* can be used as the main indicators reflecting changes in the marine environment. The trophic status, COD, DO, and salinity were the key drivers affecting variation in the mycoplanktonic community in the subtropical estuary, while nutrient levels had the strongest effect on the β-diversity. This study fills a gap in the knowledge on marine mycoplankton in the Dafengjiang River estuary, contributes to the understanding of the diversity of this plankton community in the Beibu Gulf, and provides a scientific

basis for understanding the environmental factors that regulate the diversity and ecological roles of mycoplankton in response to climate change and anthropogenic effects in subtropical estuaries.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/jmse10121940/s1, Table S1: Environmental parameters in summer and winter (mean with SD).

Author Contributions: J.H., conceptualization, methodology, writing—review and editing, supervision; H.Z., conceptualization, methodology; S.Y., writing—review and editing; X.Q., writing—review and editing; N.L. (Nengjian Liao), writing—review and editing; X.L., writing—review and editing; Q.W., writing—review and editing; W.L., writing—review and editing; G.J., conceptualization, supervision; N.L. (Nan Li), conceptualization, supervision; P.W., conceptualization, methodology, formal analysis, investigation, data curation, visualization, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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