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Benthic Microbes on the Shore of Southern Lake Taihu Exhibit Ecological Significance and Toxin-Producing Potential Through Comparison with Planktonic Microbes

Qihang Zhao [1], Bin Wu [2], Jun Zuo [1], Peng Xiao [1], He Zhang [1], Yaping Dong [2], Shuai Shang [2], Guanning Ji [2], Ruozhen Geng [2],* and Renhui Li [1],*

[1] National and Local Joint Engineering Research Center of Ecological Treatment Technology for Urban Water Pollution, Zhejiang Provincial Key Lab for Water Environment and Marine Biological Resources Protection, Wenzhou University, Wenzhou 325035, China; qhzhao0087@163.com (Q.Z.); jzuo@wzu.edu.cn (J.Z.); pxiao@wzu.edu.cn (P.X.)

[2] Research Center for Monitoring and Environmental Sciences, Taihu Basin & East China Sea Ecological Environment Supervision and Administration Authority, Ministry of Ecology and Environment of the People's Republic of China, Shanghai 200125, China

* Correspondence: geruozh@163.com (R.G.); renhui.li@wzu.edu.cn (R.L.)

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Abstract: Water quality and aquatic ecosystems along lakeshores are vital for ecological balance and human well-being. However, research has primarily focused on plankton, with benthic niches being largely overlooked. To enhance understanding of benthic microbial communities, we utilized 16S and 18S rRNA sequencing alongside multivariate statistical methods to analyze samples from the shoreline of Lake Taihu in Huzhou City, Zhejiang Province. Our results reveal a marked difference in species composition between benthic and planktonic microorganisms, with benthic cyanobacteria predominantly comprising filamentous genera like *Tychonema*, while 95% of planktonic cyanobacteria were *Cyanobium*. The β-diversity of benthic microorganisms was notably higher than that of planktonic counterparts. The neutral community model indicated that stochastic processes dominated planktonic microbial assembly, while deterministic processes prevailed in benthic communities. Null models showed that homogeneous selection influenced benthic community assembly, whereas planktonic communities were affected by undominated processes and dispersal limitation. Network analysis indicated that planktonic networks were more stable than benthic networks. Importantly, dominant benthic cyanobacterial genera posed potential toxin risks, highlighting the need for enhanced monitoring and ecological risk assessment. Overall, these findings enhance our understanding of benthic and planktonic microbial communities in lakeshores and offer valuable insights for aquatic assessment and management in eutrophicated environments.

Keywords: lakeshore; high-throughput sequencing; benthic microbes; planktonic microbes; community composition; ecological health assessment

1. Introduction

Lakeshore is a critical component of the natural ecological space of lakes, which has multiple functions, such as flood control, ecology, society, and economy; thus, strengthening the management and protection of the lakeshore is an important task for a lake ecosystem [1]. Studies on water quality and aquatic ecosystems along lakeshores are essential for preserving these valuable natural resources, maintaining ecological balance and enhancing human well-being [2]. However, previous studies on the ecology of lakeshores have predominantly focused on plankton [3–5] and riparian benthic communities have been largely overlooked. Further investigations are needed to assess whether these communities have significant effects on the ecology of the lakeshore.

Microbes primarily consist of planktonic and benthic species with unique habitat preferences. Planktonic microorganisms primarily inhabit aquatic systems and demonstrate pronounced dispersal abilities, whereas benthic microorganisms adhere to substrates, such as soil, rocks, and sediments, migrating within a limited range [6]. The benthic microbial community plays a crucial role in assessing aquatic ecosystems. These organisms are very sensitive to changes in water quality and are often utilized as biological indicators to evaluate the health status of water bodies [6]. A great deal of previous research has elucidated the species composition, diversity, and community structure of planktonic microorganisms in various aquatic systems [7–12]. However, there remains a notable knowledge gap concerning riparian benthic microbial communities that are closely associated with lake environments. Some studies have shown that benthic biofilm communities can support specific microbial taxa with potential functional roles and exhibit notable compositional differences from those of the surrounding environment [13,14]. Research has indicated that the diversity and composition of rhizosphere microorganisms are influenced by plant species and soil properties. Plants selectively affect the assembly of rhizosphere bacterial communities to acquire specific microbial functional traits necessary for their health [15]. Therefore, elucidating the composition and diversity of riparian biofilm microbial communities is crucial for a comprehensive understanding of regional ecosystem structure and function, offering valuable biological indicators for ecological assessment and environmental management [16].

Defining the mechanisms of community assembly and their potential ecological functions is a central focus in microbial ecology [17–19]. Deterministic processes derived from niche theory and stochastic processes based on neutral theory are complementary mechanisms in microbial community composition [20,21]. Deterministic processes grounded in niche theory suggest that community structure is primarily shaped by interspecies interactions and environmental selection [22,23]. In contrast, neutral theory posits that community structure is shaped by birth, death, migration, and dispersal limitations, emphasizing a stochastic balance between losses and gains among taxa [24]. Although numerous studies have investigated the assembly mechanisms and driving factors of planktonic microbial communities in various aquatic systems [12,25–27], understanding of the assembly mechanisms of benthic microbial communities remains incomplete.

Microbial interactions, including competition, parasitism, and symbiosis, form complex ecological networks in aquatic ecosystems [28,29]. Potential species interactions are crucial for maintaining the stability of community structure and ecological functions [23]. Recently, co-occurrence networks have been widely utilized to assess interspecies interactions among microorganisms [30,31]. The topological properties of community networks, along with metrics, such as robustness and vulnerability, are used to evaluate network stability [32]. Numerous studies have reported on the spatiotemporal dynamics of planktonic and benthic microbial networks and their responses to environmental stress. For instance, Mo et al. observed that an increase in salinity leads to a decrease in the stability of microbial networks [33]. Another study demonstrated that hydrological disturbances reduce the stability of planktonic and benthic microbial networks in high-altitude floodplains [6]. Additionally, He et al. found that microbial network stability is greater in underground than aboveground habitats across various environments [34]. However, many questions about how microbial communities assemble and coexist in unique habitats remain unanswered. For instance, we do not know of any study that has revealed differences in co-occurrence network patterns between benthic microbes on the shore and planktonic microbes in the water column.

To address the lack of understanding regarding the differences in community structure between benthic microbes and planktonic microbes in aquatic environments, we performed a comprehensive analysis of benthic and planktonic microbial communities at five locations along the lakeshore in the south of Lake Taihu as a case study. To investigate the diversity, species composition, co-occurrence patterns, and community composition of both the prokaryotic and eukaryotic microbial communities, 16S rRNA and 18S rRNA sequencing was used. The aim of this study was to test the following hypotheses: (1) Benthic cyanobacteria differ markedly from planktonic in species composition and diversity. (2) Deterministic processes have a greater influence on benthic microbial communities, whereas stochastic processes predominantly drive planktonic microbial communities. (3) Different co-occurrence patterns drive the stability and ecological functions of benthic and planktonic microbial communities. The findings enhance our comprehension of benthic microbial communities and will help to refine the assessments of environmental health conditions.

2. Methods

2.1. Study Area and Sampling Site

Lake Taihu, located in the center of the Taihu basin, which includes Suzhou City, Wuxi City, and Changzhou City in Jiangsu Province and Huzhou City in Zhejiang Province, is the largest lake in the basin and the third largest freshwater lake in China (after Lake Poyang and Lake Dongting). It is a typical shallow lake and an important water source and water resource regulation and storage center in the Yangtze River Delta. Lake Taihu covers an area of 2338 square kilometers, with a length of 68.5 km from north to south, an average width of 34 km from east to west, and a total length of 436 km of coastline [7]. In this study, surface water samples (0.5 m below the surface) and benthic samples (0.1–0.3 m below the surface) were collected from five sites along the shoreline in the south of Lake Taihu, Huzhou City, Zhejiang Province, China (Figure 1a). At each sampling site, we collected 2–3 benthic samples (Figure 1b). A total of 18 samples were collected, including 5 water samples and 13 benthic samples.

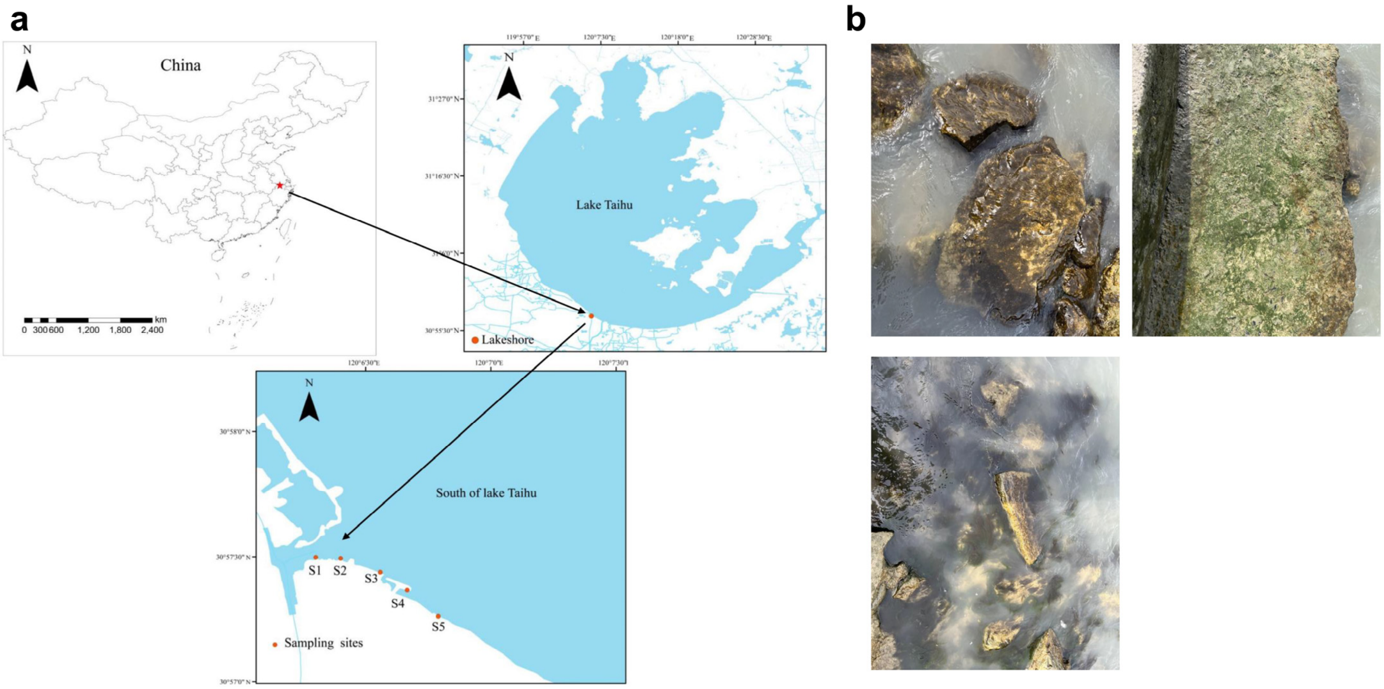

Figure 1. (**a**) Sampling points of the lakeshore in the Taihu basin. (**b**) Representative images of different habitats during sampling.

2.2. Field Investigation and Physicochemical Indexes

Water samples at 0.5 m depth were collected [35], and the collection of benthic samples was carried out by scraping the algae mats attached to the surfaces of rocks of the lake with a ring knife, placing them in clean blank plates, sealed with sealing film, marking and placing them in an ice box. The total area of benthic samples collected at each site was not less than 100 cm^2. These samples were frozen in liquid nitrogen and stored in an −80 °C freezer for subsequent extraction of environmental sample DNA [27]. Dissolved oxygen (DO), water temperature (WT), and pH were measured directly on-site with HQ2200 portable handheld meters (Hach Company, Loveland, CO, USA). Total dissolved solids and electrical conductivity were assessed using a DDBJ-350F portable conductivity meter (Leici Instruments Co., Ltd., Shanghai, China). Environmental parameters, such as total nitrogen and total phosphorus were measured following previous study [27].

2.3. DNA Extraction, PCR, and Sequencing

The total DNA was extracted using the FastDNA® Spin Kit (MP Biomedicals, Santa Ana, CA, USA), adhering to the manufacturer's instructions. To identify DNA sequences, PCR was conducted to amplify the 16S rRNA gene (targeting prokaryotes) and the 18S rRNA gene (targeting eukaryotes). For bacterial analysis, the variable V3-V4 region of the 16S rRNA gene was amplified using the forward primer 341F (5′-CCTAYGGGRBGCASCAG-3′) and the reverse primer 806R (5′-GGACTACNNGGGTATCTAAT-3′) [27]. The eukaryotic community was studied through high-throughput sequencing of the V9 region of the 18S rRNA gene. For this analysis, the forward primer 1380F (5′-GTACACACCGCCCGTC-3′) and reverse primer 1510R (5′-TGATCCTTCTGCAGGTTCACCTAC-3′) were utilized for eukaryotic gene sequencing, as described by [33]. The PCR procedure began with an initial denaturation at 98 °C for 1 min, then proceeded with 30 cycles consisting of 10 s at 98 °C, 60 s at 50 °C, and 30 s at 72 °C. A final extension at 72 °C for 10 min was performed after amplification. PCR products from triplicate reactions for each sample were combined and subjected to gel purification [33]. PCR products were purified using the FastPure Gel DNA Extraction Mini Kit (Nanjing Vazyme Biotech, Nanjing, China), according to the manufacturer's instructions. The purified DNA samples were kept at −20 °C and dispatched to a company for sequencing using the Novaseq PE250 platform from Illumina (San Diego, CA, USA).

2.4. Bioinformatic Processing

The demultiplexing of high-throughput raw data based on different primer tags and barcodes was performed using Mothur v1.45 software [36]. To ensure the accuracy of subsequent analyses, USEARCH v11 software was utilized to remove primers, HiSeq adapters, and low-quality sequences from the raw data. The DADA2 module within the QIIME 2 software package was employed for error rate estimation, ASV threshold evaluation, and chimera removal, yielding ASV information [37]. For the 16S rRNA gene, the taxonomic annotation of bacterial ASV representative sequences was performed using the SILVA v138.1S database, while the taxonomic annotation of eukaryotic microbial ASV representative sequences was conducted using the PR2 v2.10 database [38,39]. For normalization, non-target ASVs were removed from both bacterial and eukaryotic microbial communities. The ASV table for bacteria and eukaryotic microorganisms was normalized according to the minimum number of reads for each sample's respective community using the vegan package in R v4.2.3. In all samples, high-quality sequences were obtained from 16S rRNA and 18S rRNA sequencing, yielding 10,055 and 6235 sequences, respectively. These sequences were clustered into 8490 and 4454 ASVs, respectively, at a 97% similarity level.

2.5. Community Assembly Analysis

To determine the impact of stochastic processes on microbial communities, the neutral community model (NCM) was employed. The R^2 value represents the overall goodness of fit of the NCM, with a higher R^2 suggesting that community assembly is more influenced by stochastic processes [40]. The β-nearest taxon index (βNTI) was calculated using the R packages picante and vegan to quantitatively describe the contributions of different ecological processes to microbial community structure. A βNTI value between −2 and 2 indicates that community assembly occurs through a stochastic process. Conversely, a βNTI value less than −2 or more than 2 suggests that the assembly is driven by deterministic processes [27,41]. Additionally, the R package iCAMP was used to perform null model analyses based on phylogenetic bins and quantify the relative importance of various ecological processes. iCAMP demonstrates exceptional accuracy, sensitivity, precision, and specificity in simulating communities, outperforming community-based methods by

10–160% [42]. It is suitable for studying microbial community assembly processes across various ecosystems, including but not limited to grasslands, forests, marine, and freshwater ecosystems. It is particularly well-suited for analyzing high-throughput sequencing data, such as 16S rRNA gene or metagenomic data. Specifically, observed taxa were first classified into different bins based on their phylogenetic relationships. For each bin, phylogenetic diversity was analyzed using βNRI under a null model, and beta diversity of abundance was analyzed using the modified Raup–Crick index (RC). For example, percentages of pairwise comparisons with $\beta\text{NRI} < -1.96$ and $\beta\text{NRI} > 1.96$ were considered indicators of homogeneous and heterogeneous selection, respectively. Comparisons with $|\beta\text{NRI}| \leq 1.96$ and $\text{RC} < -0.95$ were categorized as homogeneous dispersal, whereas $|\beta\text{NRI}| \leq 1.96$ and $\text{RC} > 0.95$ indicated dispersal limitation, and $|\beta\text{NRI}| \leq 1.96$ and $|\text{RC}| \leq 0.95$ were indicative of drift [42,43].

2.6. Co-Occurrence Networks

Based on the molecular ecological network analysis pipeline (MENAP), we constructed microbial co-occurrence networks to elucidate interspecies interactions among microorganisms across different habitats [44,45]. In this study, only amplicon sequence variants (ASVs) present in ≥20% of the samples were used to compute the Pearson correlation coefficient matrix for each group. Additionally, 1000 random networks corresponding to the empirical network were generated using this platform to assess the significance of the empirical network. Network topological features, such as the total number of nodes and edges, average degree, and average clustering coefficient, were calculated by MENAP after the network construction [46]. The data for nodes and edges were imported into Gephi 0.9.2 for network visualization https://gephi.org/ (accessed on 1 September 2024). Furthermore, we utilized the R package “igraph” to calculate network robustness and vulnerability in order to evaluate the stability of the networks [47].

2.7. Statistical Analyses

Using the “vegan” package in R version 4.2.3, we analyzed the α-diversity of microbial communities by calculating the Shannon–Wiener index, Pielou’s evenness, and the richness. The β-diversity of microbial communities was analyzed by calculating the Bray–Curtis and Jaccard dissimilarity indices [48,49]. We used the “adespatial” package in R to decompose the β-diversity of microbial communities into turnover components and richness difference components [50]. Additionally, the R package “spaa” was utilized to determine the Levins’ niche breadth of microbial communities [51]. Tukey’s post hoc test was applied for significance testing of mean comparisons for α, β, and niche breadth. Statistical significance was considered at $p < 0.05$. Non-metric multidimensional scaling (NMDS) based on Bray–Curtis distance was applied to identify similarities in microbial communities between different habitats. Finally, an analysis of similarities (ANOSIM) was used to assess the statistical significance of microbial community variation between habitats. Furthermore, the R package “LinkET” was used for Mantel analysis to reveal the relationships between microorganisms and environmental factors [52].

3. Result

3.1. Structure, α-Diversity, and Niche Breadth of Prokaryotic and Eukaryotic Microbial Communities

At the phylum level, the top four dominant phyla in the benthic microbial communities were Proteobacteria (32.9%), Cyanobacteria (22.6), Bacteroidota (16.7%), and Firmicutes (15.5%) (Figure 2a). In contrast, the predominant phyla in planktonic microbial communities were Actinobacteriota (36.1%), Proteobacteria (27.8%), Bacteroidota (18.5%), and Cyanobacteria (9.7%) (Figure 2a). The proportion of Cyanobacteria was notably higher in benthic microbial communities compared to planktonic communities (Figure 2a). At the genus level, the top three genera in benthic prokaryotic communities were *Exiguobacterium* (14.8%), *Acinetobacter* (14.8%), and *Tychonema* (12.6%) (Figure 2c). In planktonic communities, the major genera were *CL500-29*, *hgcl*, and *Cyanobium* (Figure 2c). Among eukaryotic microbes, Metazoa was clearly the dominant phylum across different habitats, with a higher proportion in benthic (48%) compared to planktonic (28%) communities (Figure 2b). Additionally, Gyrista (benthic, 13%; planktonic, 20%) and fungi (benthic, 11%; planktonic, 8.2%) were present in significant proportions across different habitats (Figure 2b). Notably, Chlorophyta had a higher proportion in planktonic (16%) compared to benthic communities (6%) (Figure 2b). The α-diversity of microbial communities in different habitats and taxa was assessed using the Shannon–Wiener index, Pielou's evenness, and the richness. Our results indicated no significant distinctions between the three indices for different taxa across habitats (Figure S1a,b).

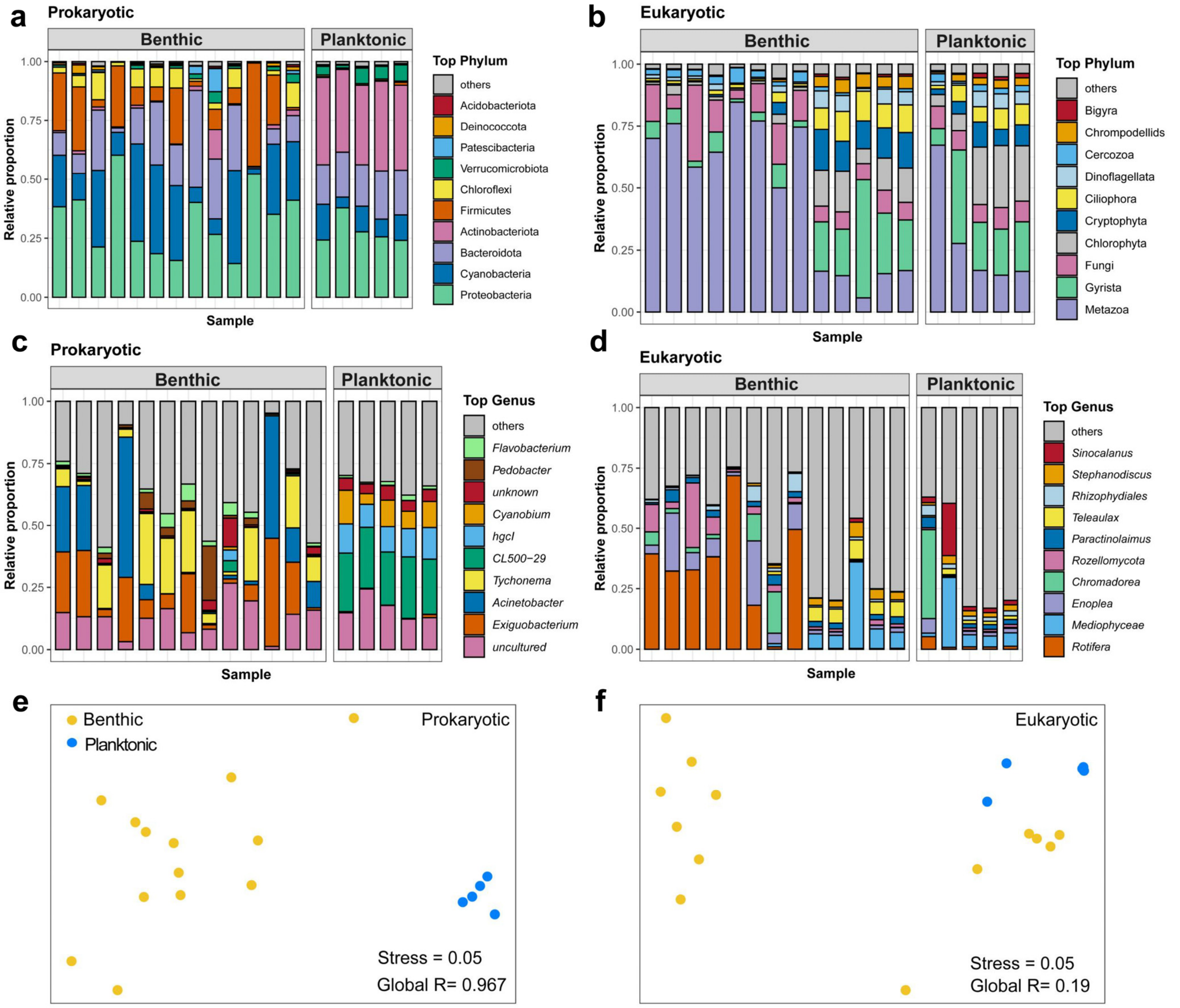

Figure 2. (**a**,**b**) Prokaryotic and eukaryotic community composition at the phylum, respectively. (**c**,**d**) Prokaryotic and eukaryotic community composition at the genus level, respectively. (**e**,**f**) NMDS plot based on Bray–Curtis distance showing the variations in beta diversity of prokaryotic and eukaryotic microbial communities across different habitats.

NMDS analysis revealed the significant separation of microbial communities between benthic and planktonic environments (Figure 2e,f). Furthermore, prokaryotic community composition exhibited more pronounced variation across habitats (ANOSIM: r = 0.967, $p = 0.001$) compared to eukaryotic communities (ANOSIM: r = 0.19, $p = 0.001$) (Figure 2e,f).

We used niche width (Levins) indices to compare community structures across different habitats and taxa. The results indicate the significantly higher niche width of benthic eukaryotic microorganisms compared to planktonic microorganisms. This suggests that the benthic eukaryotic microorganisms exhibit more specific environmental requirements and specialized functions in their ecological niche or play a more distinct role within the ecosystem. No significant difference was observed among prokaryotic microorganisms (Figure 3d).

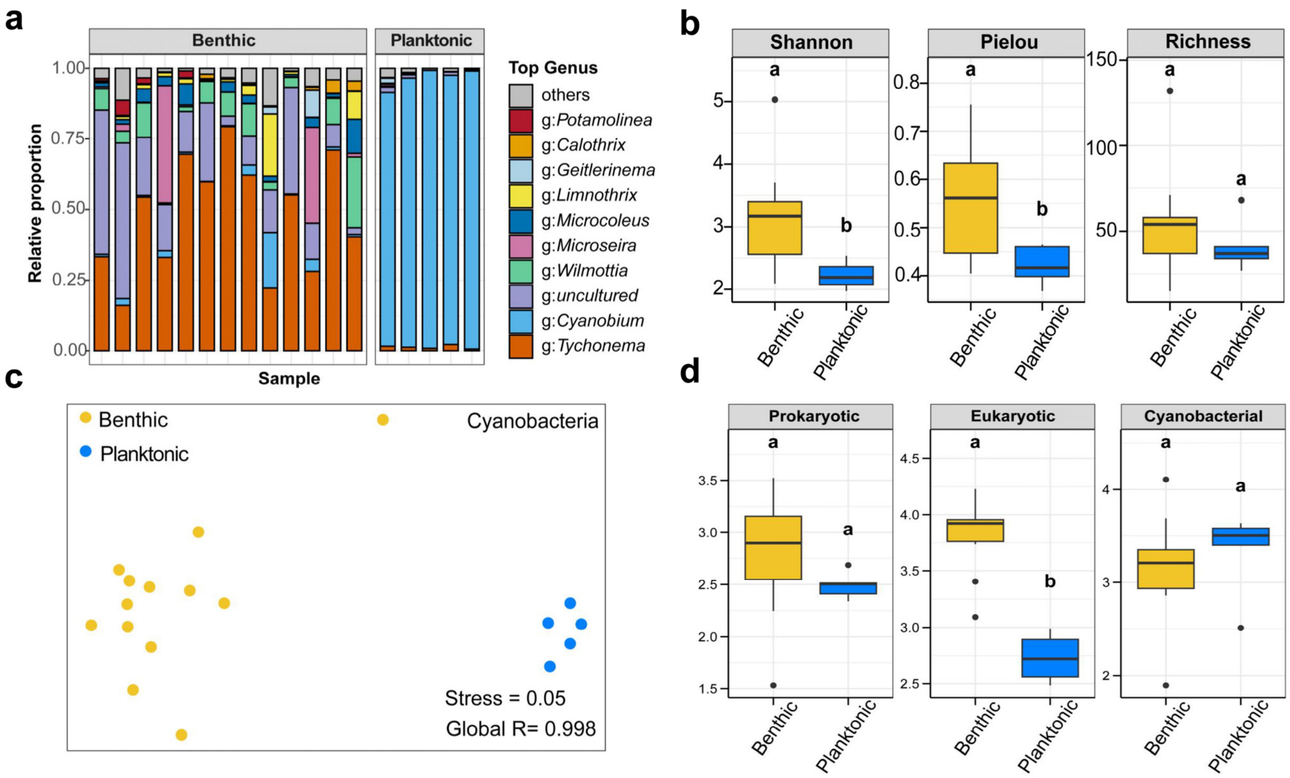

Figure 3. (**a**) *Cyanobacteria* community composition at the genus level. (**b**) α-diversity of cyanobacteria community across different habitats. The different letters in the figure indicate significant differences among components, while the same letters denote no significant differences between groups. (**c**) NMDS plot based on Bray–Curtis distance showing the variations in beta diversity of cyanobacteria microbial communities across different habitats. The yellow dots represent benthic microorganisms, while the blue dots represent planktonic microorganisms. (**d**) Niche breadth among the three taxa in the two habitats. The different letters in the figure indicate significant differences among components, while the same letters denote no significant differences between groups.

The physicochemical properties of different sampling sites are summarized in Table S1. Mantel analysis further revealed the impact of environmental conditions on microbial community composition (Figure S2). The results indicated that Chl-*a*, pH, and SD were significant factors affecting benthic eukaryotic microorganisms (all $p < 0.05$), while DTN was a significant environmental factor influencing planktonic eukaryotic microorganisms ($p < 0.05$). Additionally, NO_3-N was the only factor with a significant impact on planktonic prokaryotic microorganisms ($p < 0.05$).

3.2. Structure, α-Diversity, and Niche Breadth of Cyanobacterial Communities

Based on 16S rRNA sequencing, cyanobacterial sequences were clustered into 395 ASVs representing 48 genera. Specifically, *Tychonema* was the dominant genus in benthic cyanobacteria communities, comprising 48% of the genera (Figure 3a). Other major genera in other benthic communities included *Wilmottia*, *Microcoleus*, *Microseira*, and *Limnothrix*. In contrast, *Cyanobium* was the predominant genus in planktonic cyanobacterial communities, accounting for up to 95% (Figure 3a). The α-diversity results showed that Shannon–Wiener and Pielou's evenness were significantly higher in benthic communities compared to planktonic communities, with no significant differences in richness across habitats (Figure 3b). NMDS analysis revealed significant variations in cyanobacterial community similarity across different habitats (ANOSIM: r = 0.998, p = 0.001). DO and NO_3-N were significantly related to benthic and planktonic cyanobacterial communities, respectively (both $p < 0.05$; Figure 3c). Additionally, no distinct differences were found between the ecological niches of benthic and planktonic cyanobacterial communities (Figure 3d).

3.3. *β-Diversity of Prokaryotes, Eukaryotes, and Cyanobacteria*

Bray–Curtis and Jaccard dissimilarities were used to assess the β-diversity of microbial communities from different habitats and taxa. With the exception of eukaryotic microorganisms, for which Bray–Curtis dissimilarity did not show significant differences across habitats, all other data indicated that the β-diversity of benthic microbial communities was notably higher than that of planktonic communities (Figure 4a–c). Furthermore, by decomposing β-diversity, we discovered that species turnover was the predominant factor explaining distinctions in bacterial and eukaryotic microbial community compositions across both habitats, contributing 53% (benthic) and 83% (planktonic) in bacterial communities and 75% (benthic) and 78% (planktonic) in eukaryotic communities (Figure 5). In contrast, the contribution of nestedness to β-diversity was relatively minor, accounting for 47% (benthic) and 17% (planktonic) and 25% (benthic) and 22% (planktonic) communities (Figure 5). Similarly, species turnover had a significantly greater influence than nestedness in shaping cyanobacterial communities (Figure 5). Interestingly, we observed that all groups showed an increase in species turnover and a decrease in nestedness as the habitat shifted from benthic to planktonic.

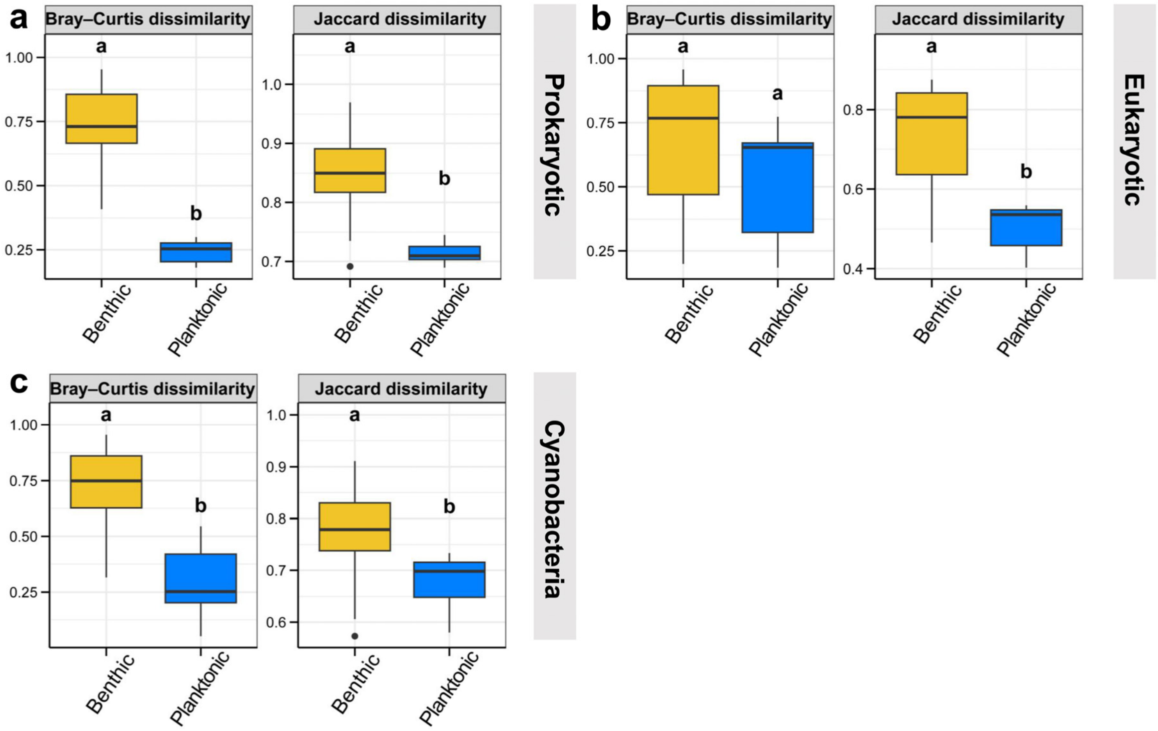

Figure 4. β-diversity based on Bray–Curtis and Jaccard distance showing the variations of (**a**) prokaryotic (**b**) eukaryotic and (**c**) cyanobacteria communities across different habitats. The different letters in the figure indicate significant differences among components, while the same letters denote no significant differences between groups.

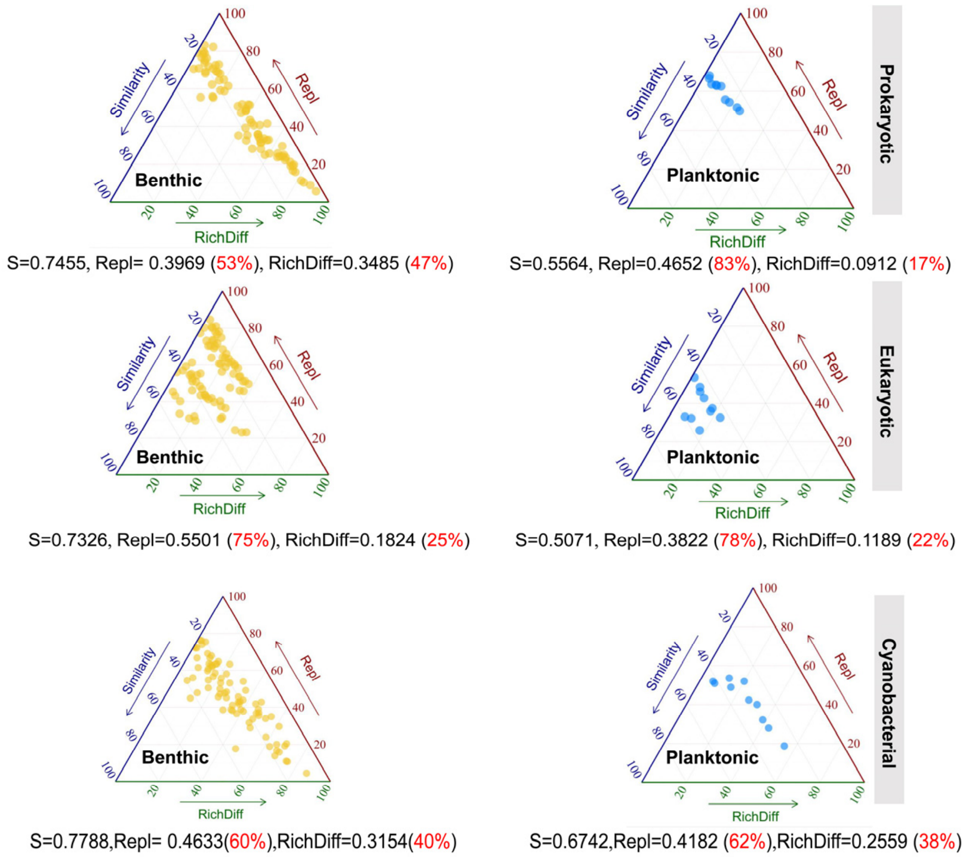

Figure 5. Decomposing β-diversity reveals the contributions of turnover and nestedness to β-diversity. Repl: turnover; RichDiff: nestednessThe yellow dots represent benthic microorganisms, while the blue dots represent planktonic microorganisms.

3.4. Community Assembly Governing Microbial Community Patterns Across Different Habitats

NCM effectively explained the role of stochasticity in microbial community assembly under contrasting habitats (Figure 6). In benthic organisms, the model explained 50.6% and 48.5% of community variations for prokaryotic and eukaryotic microorganisms, respectively (Figure 6a,b). For planktonic microorganisms, the explained variations were notably increased in prokaryotic (64.9%) and eukaryotic (71.8%) communities (Figure 6c,d). Additionally, the migration rates (Nm) of planktonic communities (prokaryotes: 5116; eukaryotes: 7664) were higher than those of benthic communities (prokaryotes: 673; eukaryotes: 2282) (Figure 6). These results suggest that stochastic processes have a greater impact on the variation in planktonic communities compared to benthic communities.

Furthermore, a phylogenetic distance-based null model was used to access the relative importance of various ecological processes in the assembly of microbial communities (Figure 7). The βNTI values for the two planktonic microbial community groups were predominantly within the range of −2 to 2, whereas a considerable proportion of βNTI values in benthic communities exceeded 2 (Figure 7a). These findings suggest that deterministic processes played a more significant role in the assembly of benthic communities compared to planktonic communities for both prokaryotic and eukaryotic microorganisms. Additionally, in benthic habitats, deterministic homogeneous selection predominantly drove the turnover of prokaryotic (52.9%) and eukaryotic (37.6%) communities (Figure 7b,c). In contrast, in aquatic environments, prokaryotic and eukaryotic microbial community assembly was primarily governed by stochastic undominated processes (52.6%) and dispersal limitation (55.6%), respectively (Figure 7d,e).

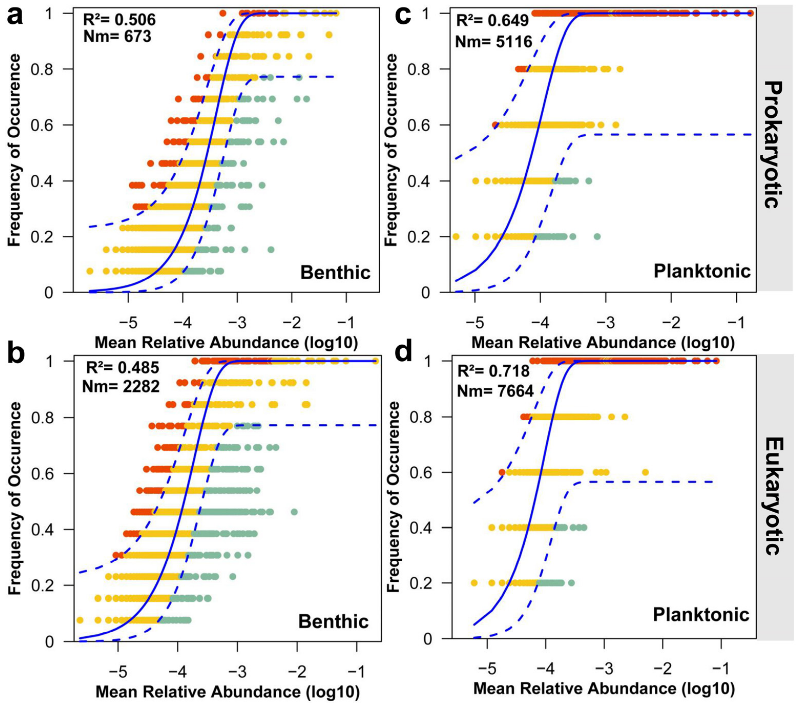

Figure 6. The contribution of stochastic processes to the assembly of (**a**,**b**) benthic and (**c**,**d**) planktonic microbial communities during different habitats. R^2 represents the fit to the neutral model and Nm indicates the immigration rate of metacommunity. The points falling above and below the 95% confidence interval are colored red and green, respectively, and those within the interval are colored yellow. Solid line represents the best-fitting neutral model; dashed lines represent the 95% CIs around the best-fitting neutral model.

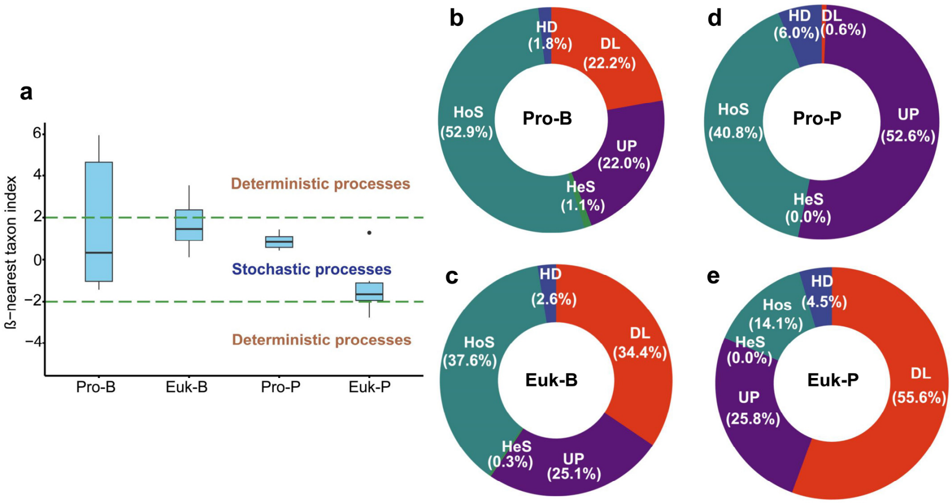

Figure 7. Microbial community assembly driven by different ecological processes. (**a**) Beta-nearest taxonomic index of microbial communities in different sample groups. (**b**–**e**) The percentage contribution of individual processes to community assembly. Pro-B: prokaryotic benthic; Euk-B: eukaryotic benthic; Pro-P: prokaryotic planktonic; Euk-P: eukaryotic planktonic; HoS: homogeneous Selection; Hes: heterogeneous Selection; DL: dispersal Limitation; HD: homogenizing Dispersal; UP: undominated process. The βNTI value between −2 and 2 indicates that community assembly occurs through a stochastic process. Conversely, the value less than −2 or more than 2 suggests that the assembly is driven by deterministic processes.

3.5. Network Pattern of Microbial Community Across Different Habitats

Four global networks were constructed based on microbial datasets from two different habitat types (Figure 8a). The properties of the networks are summarized in Tables S2 and S3. All networks exhibited power law distributions (R^2 = 0.774–0.869), indicating that the networks exhibited scale-free and non-random structures (Tables S2 and S3). Compared to their corresponding random networks, all empirical networks displayed higher average clustering coefficients and average path lengths, suggesting they possess “small-world” and modular characteristics. The planktonic microbial prokaryotic and eukaryotic networks comprised 438 nodes and 871 edges and 422 nodes and 939 edges, respectively (Figure 7b), while the benthic microbial prokaryotic and eukaryotic networks consisted of 165 nodes and 221 edges and 125 nodes and 463 edges, respectively (Figure 8b). These findings are indicative of more potential interactions between microbial communities in planktonic habitats than between those in benthic habitats. The modularity indices of benthic microbial networks were lower than those of planktonic communities (Figure 8b).

To assess the robustness of microbial networks across different habitats, natural connectivity analyses were performed to identify differences between the two groups (Figure 8c). Connectivity refers to the degree to which nodes (microbial species) in a network are linked through edges (interactions). Robustness, in this context, describes the ability of a microbial network to maintain its function and structure despite disturbances, such as the removal of nodes. In prokaryotic groups, the natural connectivity of planktonic networks was higher than that of benthic microbial communities. In eukaryotic groups, natural connectivity in benthic microbial networks declined sharply with increasing node removal, whereas natural connectivity in planktonic networks did not decline notably until the proportion of removed nodes reached 0.4 (Figure 8c). These results indicate that microbial networks in benthic habitats are less robust than those in planktonic habitats. Furthermore, in both habitat types, benthic networks exhibited higher vulnerability compared to planktonic networks (Figure 8d). Vulnerability refers to the susceptibility of a network to disintegration or functional loss when subjected to disturbances. These findings demonstrate that benthic microbial networks are more unstable compared to planktonic microbial networks.

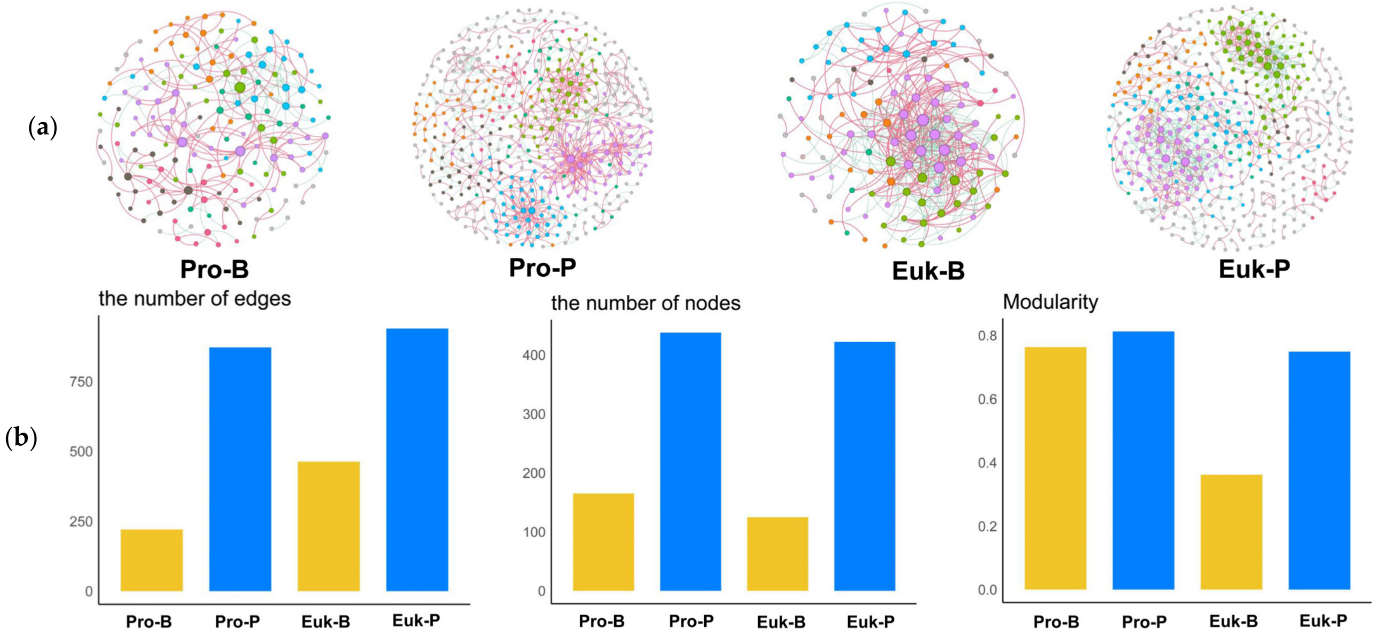

Figure 8. *Cont.*

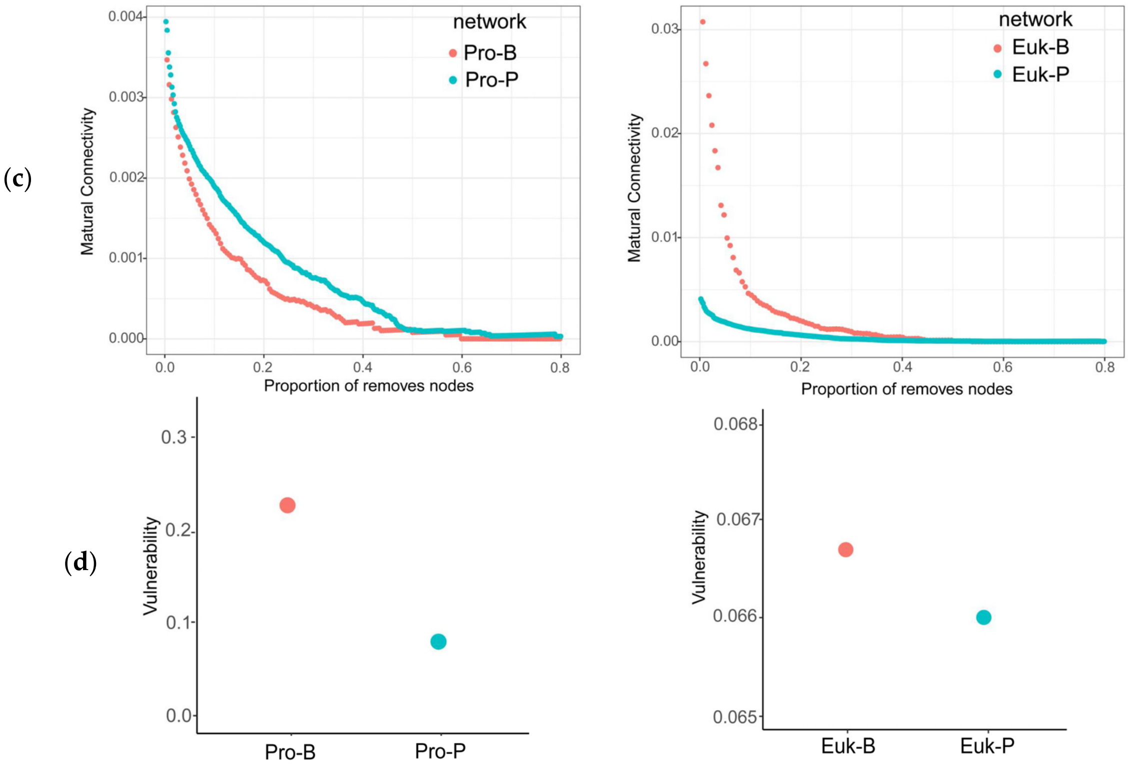

Figure 8. Ecological network patterns of microbial communities during different habitats. (**a**) Visualization of constructed microbial networks in the two habitats across different taxa. (**b**) Comparison of network complexity based on edge, node, and module analysis between the two habitats across different taxa. (**c**) Network robustness indicated by natural connectivity. (**d**) Network vulnerability assessed by measuring the maximum node vulnerability in each network. Pro-B: prokaryotic benthic, Pro-P: prokaryotic planktonic, Euk-B: eukaryotic benthic, Euk-P: eukaryotic planktonic.

4. Discussion

4.1. Diversity and Structure of Benthic and Planktonic Microbial Communities

Similar to planktonic microorganisms, benthic microorganisms have significant impacts on biogeochemical processes within riverine ecosystems, including nutrient cycling and pollutant degradation [53,54]. Consistent with our predictions, the results of this study revealed significant differences in species composition between benthic and planktonic microorganisms (Figure 2a–d). Specifically, Actinobacteriota was more prevalent in planktonic microbial communities compared to benthic communities, whereas Proteobacteria was more abundant in benthic microorganisms (Figure 2a). Proteobacteria typically possess diverse metabolic pathways, enabling them to adapt to various environmental conditions, including the rapid utilization of organic matter [55]. This may confer a competitive advantage in benthic environments. In contrast, Actinobacteriota tend to inhabit planktonic environments, where they perform specific ecological functions, such as producing secondary metabolites that include antibiotics. These functions might not be necessary in benthic environments but are beneficial for their survival in the intense microbial competition that occurs in planktonic habitats [56]. Additionally, some groups within Proteobacteria preferentially form biofilms, which enable them to better anchor and survive in benthic environments [57]. Among eukaryotes, Metazoa were significantly dominant in both habitats, with a more pronounced dominance in benthic environments (Figure 2b). Two factors can explain this phenomenon. Metazoa in benthic environments may have easier access to nutrients on the substrate because they can directly obtain the required nutrients from the attached surface. In planktonic environments, Metazoa need to actively search for food resources [58]. In contrast, Metazoa attached to solid surfaces benefit from greater physical protection, which reduces their risk of predation. This protective advantage may contribute to their better survival and reproduction in benthic environments [58]. Gyrista occupied a significant proportion of the species composition in both habitats (Figure 2b), suggesting that different groups within Gyrista may have undergone niche differentiation based on their physiological adaptations. Some groups may be more adapted to living on solid surfaces, such as adhering to the bottom of water bodies or rock surfaces, while others may be more inclined towards a planktonic existence in the water column [59].

In the present study, distinct differences in species composition at the genus level across different habitats were evident in the phylum Cyanobacteria. NMDS results strongly confirmed this finding (Figure 3a,c). *Tychonema* was the most dominant genus among benthic cyanobacteria (Figure 3a). *Tychonema* is found in mildly eutrophic lakes and serves as an indicator of water quality [60]. Additionally, species of the genus *Wilmottia* have been found in a variety of environments, including freshwater, wet soil, and tree bark, suggesting that these species may have a broad ecological niche adaptability [61]. The genus *Microcoleus* has also been reported in benthic environments [62]. In planktonic environments, *Cyanobium* constituted up to 95% of the community (Figure 3a). *Cyanobium* exhibits rapid growth and adaptability to varying environmental conditions, enabling it to proliferate quickly and become a dominant species in aquatic environments [63]. Additionally, *Cyanobium* is very resistant to adverse conditions and pollution, which may contribute to its dominance in aquatic environments [64]. Notably, the Shannon–Wiener index and Pielou's evenness of benthic cyanobacteria were significantly higher than those of planktonic cyanobacteria (Figure 3b). Benthic cyanobacteria may exhibit higher diversity due to their microhabitats, such as specific ecological niches provided by rock surfaces, whereas planktonic cyanobacteria may display lower diversity due to the homogeneous characteristics of the aquatic environment [65].

Furthermore, we observed that the β-diversity of benthic microorganisms is significantly higher than that of planktonic environments (Figure 4a–c). First, benthic microorganisms inhabit specific microhabitats, such as the surfaces of submerged plants. In addition, benthic microorganisms inhabit specific microenvironments, which, due to their unique physical and chemical properties, may support particular microbial taxa, thereby making spatial differences in community composition more pronounced [66]. Additionally, compared to the relatively homogeneous conditions in aquatic environments, benthic communities are likely exposed to more complex environmental conditions. This complexity promotes the differentiation of microbial community structures, thereby increasing β-diversity [67]. The complex internal structure and functional differentiation of biofilms confers protection to microorganisms exposed to environmental stress [68]. The analysis of β-diversity revealed that the proportion of species turnover was higher in planktonic microorganisms compared to those in biofilms, while benthic communities exhibited stronger nestedness (Figure 5). These findings may reflect that planktonic microorganisms are more influenced by environmental factors in aquatic systems, such as flow rate and temperature variations, which can lead to more frequent species turnover across different habitats [69,70]. In contrast, biofilm communities show more pronounced ordered differences in species richness, with some communities potentially being subsets of others, which could be related to habitat specificity or the adaptation of species to particular environments [69,70].

4.2. *Ecological Processes of Benthic and Planktonic Microbial Communities*

Revealing the differences in assembly mechanisms between benthic and planktonic microorganisms enhances the understanding of the responses of microbial communities to environmental changes. Our results indicated that stochastic processes dominate community assembly in planktonic microorganisms, while deterministic processes are crucial in benthic community assembly (Figures 6a–d and 7a–e). Several reasons may explain these findings. On the one hand, the abundance of resources in aquatic environments allows microorganisms to access necessary nutrients more easily, thereby reducing interspecies competition. Environmental disturbances can promote the influence of stochastic processes in aquatic environments. For instance, disturbances that include floods, droughts, or pollution can alter species composition, providing opportunities for invasion and colonization by other species [20]. In contrast, benthic microorganisms, which grow in specific microhabitats, face intense competition for nutrients, making deterministic processes more influential in their community assembly [71,72].

4.3. Co-Occurrence Patterns of Benthic and Planktonic Microbial Communities

Network analysis data suggest that both co-occurring benthic and planktonic microorganisms exhibit modular structures with non-random properties and display "small-world" characteristics (Figure 8a). We observed that the planktonic microbial network contained more nodes and edges compared to the benthic microbial network (Figure 8b), indicating a greater number of connections and relationships among planktonic microbes. Furthermore, the planktonic microbial network demonstrated higher robustness and lower vulnerability compared to the benthic network (Figure 8c,d), suggesting that it may be more stable. Several factors can explain this result. First, a decrease in microbial network complexity is likely to reduce network stability, consistent with core ecological theory that network stability is linked to network complexity [73]. Additionally, nutrient availability is a key driver of microbial community network structure [74]. In the current study, nutrient concentrations in aquatic environments were susceptible to external factors. For example, rainfall can contribute substantial amounts of nutrients, promoting dynamic modifications in planktonic microbial community structures [75]. However, resources in benthic habitats are limited compared to aquatic environments, and interspecies competition or antagonism may be more pronounced. More importantly, the stability of microbial communities relies on their resilience to external disturbance and ecological stochasticity [76]. Microbial demographic stochasticity enables them to serve as buffer pools against the impacts of disturbances on other species, thereby enhancing community stability [77]. Therefore, the reduction in the complexity of benthic networks can be attributed to a decrease in the proportion of stochastic processes (Figure 6).

4.4. Implications for Environmental Management

Since the Industrial Revolution, freshwater ecosystems have been affected by the compounded effects of human activities and climate change [78]. The overgrowth of harmful cyanobacteria in eutrophic lakes and reservoirs has accelerated and become a major environmental issue [79–81]. Harmful cyanobacterial blooms release toxins and odorous substances, which threaten human health and ecosystem services [82] (Xu et al., 2021). Over the past two decades, cyanobacterial blooms have been a major environmental issue in the Taihu Basin, causing significant harm to human health and ecological safety [82]. Therefore, methods for preventing and managing planktonic cyanobacterial blooms have become a research priority [83–86]. However, ecological risk assessments for benthic harmful cyanobacteria have not been previously performed in the Taihu Basin. In this study, the dominant benthic cyanobacteria species included *Tychonema*, *Wilmottia*, *Microcoleus*, *Limnothrix*, and *Geitlerinema*. Certain species within the genus *Tychonema* produce the neurotoxin anatoxin-a. For instance, monitoring of the Lech River in Germany detected benthic *Tychonema* mats that produced anatoxin-a [87]. Additionally, *Tychonema* species, capable of producing anatoxin-a that threaten human and animal health, were identified in Lake Garda in northern Italy [88]. Another study reported two microcystin-producing strains of *Wilmottia* isolated from Antarctic mats [89]. Similarly, some species within the genus *Microcoleus* produce microcystin [90]. *Limnothrix* and *Geitlerinema* can also produce toxins [91,92]. Recent groundbreaking research identified the potential impact of cyanobacterial toxins on human health through aerosolization [93]. Further investigations are needed to determine whether cyanobacterial toxins in aquatic environments are primarily contributed by benthic or planktonic cyanobacteria. More strikingly, a recent study reported the death of animals after consuming benthic cyanobacteria [94]. Therefore, it is imperative for environmental managers to enhance the monitoring of benthic algae and their toxins, especially in human activity-intensive areas, such as lakeshores. The timely detection of potential risks and the implementation of control measures can effectively protect human health. The additional knowledge of harmful benthic algae through the interdisciplinary collaboration and integration of knowledge from fields, such as ecology, environmental science, and public health, is necessary. These investigations should include the risk assessment of newly discovered toxins, the increased understanding of their potential impacts on human health, and the development of appropriate safety standards and guidelines.

5. Conclusions

This study systematically investigated the species composition, community assembly, and ecological interactions of benthic and planktonic microorganisms along a lakeshore zone of southern Lake Taihu. Our results indicate significant differences in species composition between benthic and planktonic microorganisms, with prokaryotes showing greater differences compared to eukaryotes. The β-diversity of benthic microorganisms was significantly higher than that of planktonic microorganisms. Stochastic processes predominated in the assembly of planktonic microbial communities, while deterministic processes were more prominent in the assembly of benthic communities. Furthermore, homogeneous selection dominated the assembly of benthic microbial communities, whereas undominated processes and dispersal limitation played significant roles in the assembly of planktonic communities. Planktonic microbial networks exhibiting greater robustness and lower vulnerability, indicating a greater resistance to environmental stress. Importantly, we found that the dominant genera of benthic cyanobacteria have significant toxin-producing potential, posing a risk to human health, which necessitates improved monitoring and more effective mitigation strategies. Our study highlights new findings on benthic microbial communities on the lakeshore, particularly focusing on potentially toxin-producing cyanobacteria. The findings emphasize the urgent need for the enhanced monitoring and management of the benthic cyanobacterial communities in such eutrophicated lakes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w16213155/s1. Table S1. Environmental characteristics of sampling sites in the lakeshore; Table S2. Global network properties of benthic microbial community co-occurrence and their random networks; Table S3. Global network properties of planktonic microbial community co-occurrence and their random networks; Figure S1. Shannon-Wiener index, Pielou's evenness and richness among the prokaryotic (a) and eukaryotic (b) in the two habitats; Figure S2. The impacts of environmental factors on microbial structure based on Mantel's test among the three taxa.

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