sustainability

MDPI

Article

Abiotic and Biotic Effects on Microbial Diversity of Small Water Bodies in and around Towns

Chao Peng and Pingping Li *

College of Biology and the Environment, Nanjing Forestry University, Nanjing 210037, China; pengchao@njfu.edu.cn
* Correspondence: ppli@njfu.edu.cn

Abstract: Microbial communities play very important roles in pollutant treatment and absorption and material and energy cycling in wetlands. Among different wetland types, wetlands in and around towns are the most closely related to human life, but how human activities affect microbes in small water bodies has received little attention. In this study, nine small water bodies of three different landscape types were chosen in the Lishui District of Nanjing City, China. The microbial community characteristics of four different seasons were revealed by metagenomics in 2021, and the possible effects of abiotic and biological factors such as the effects of alien organisms on microbial communities were analyzed. The results showed significant differences in microbial community structure in different seasons and habitats. Abiotic and biological factors jointly affected the microbial communities, and the influence of water quality was greater than that of the habitat type and biological factors. This study shows that in addition to the water quality, the wetland biome structure, especially the abundance of alien species, may have an impact on microbial communities. The results emphasize that human activities such as land use and the introduction of alien species have significant impacts on the ecosystem structure and function.

Keywords: microorganism community; wetland; metagenomic; alien species; diversity

check for updates

Citation: Peng, C.; Li, P. Abiotic and Biotic Effects on Microbial Diversity of Small Water Bodies in and around Towns. *Sustainability* **2023**, *15*, 8151. https://doi.org/10.3390/su15108151

Academic Editors: Wen Xiong, Peter A. Bowler, Zhongxin Wu, Xiaoyu Li and Dongkui Gao

Received: 8 April 2023
Revised: 1 May 2023
Accepted: 10 May 2023
Published: 17 May 2023

1. Introduction

In aquatic ecosystems, there are many microorganisms with different functions carrying out metabolic activities and community succession, and the compositions of microbial communities in different ecological habitats are obviously different [1]. There are many factors affecting the diversity of microbial communities, among which nutrients in water are widely considered, and the community structure of planktonic bacteria changes with the increase in nutrients in the ecosystem [2–4]. Freshwater bodies can form particularly stable states at either high or low nutrient load levels, and they differ from each other in ecological function [5,6]. Despite the understanding of the impacts of the nutrition status, our knowledge regarding numerous other factors that have effects on the arrangement and operational framework of microbial communities is still unclear.

With increasing economic and population growth, more artificial lakes have emerged in the process of residential settlement development, and whether these seminatural, semiartificial, or completely artificial water bodies can continuously and stably provide a comfortable and sustainable ecosystem and environmental space has become a new scientific and social issue. Differing from natural lakes, artificial lakes (especially artificial lake in downtowns) have the characteristics of a single ecosystem, small inflow, poor water quality, low water exchange rate, and complex pollution sources, and their water environmental pollution problems are far more prominent than those of natural lakes [7,8]. Especially in the early days of a new artificial lake, there is less sediment at the bottom of the lake, and under the influence of continuous human activities, it is difficult to rapidly improve the water pollution levels. Corresponding studies have shown that nutrients such as nitrogen and phosphorus in a natural lake bottom are more likely to enter a relatively stable material cycle under the action of microorganisms [9,10]. Previous studies have also shown that the continuous interaction between artificial water bodies and microorganisms during construction determines the final homeostasis pattern [11–13]. Therefore, by determining the microbial community structure and function in downtown and surrounding small water bodies, especially small water bodies prone to eutrophication, we can understand the impact of other disturbances caused by human activities on the microbial diversity of water bodies in addition to eutrophication.

The employment of the 16S rRNA gene/ARISA method revealed that specific phylotypes varied independently of the entire bacterial community dynamics [14]. Metagenomics involves characterizing the total microbial community DNA (which includes prokaryotes, viruses, and eukaryotes) in an environment [15]. Metagenomics technology can directly analyze the genetic information of microorganisms in environmental samples without relying on cultivation, thus bypassing the bottleneck whereby most microorganisms are difficult to cultivate [16,17]. With the rise of metagenomics technology, in addition to the taxonomic composition, the analysis of the functional composition of the genes of the environmental microbial community is realized, and even the reconstruction of multiple near-complete microbial genome information is realized.

In this study, the microbial community structure and function of nine small urban water bodies with different human disturbance intensities and habitat types were studied by metagenomics. We also investigated the status of higher plants and fish in the water body while measuring its microbial characteristics, focusing on the types and numbers of alien species and discussing the influence of abiotic factors and biological factors on microbial communities.

2. Materials and Methods

2.1. Study Sites and Sample Collection

To study the biodiversity and functional differences of small water bodies under different urbanization levels and human activities, a total of 9 small urban artificial lakes or wetland water bodies around cities were selected for a comparative study in Lishui District, Nanjing City, China (Table 1, Figure 1). The common features of these water bodies include the following: (1) the water area is 0.2–0.3 km^2, with an average depth of 1–2 m; (2) the shores of the water bodies are not hardened; (3) there is no paved road for motor vehicles within 200 m of the shoreline. According to the different surrounding habitats and land use types, the types of wetlands were further divided into vegetation regions, agricultural regions, and artificial lakes downtown.

Table 1. Geographic information and habitat types for the study sites.

Region	Code	Longtitude	Latitude	Habitat Type
Dongpin town	DP	119.064	31.705	Downtown
Zhetang New District	ZT1	118.984	31.739	Downtown
Zhetang New District	ZT2	118.990	31.731	Vegetation
Baima town	BM	119.148	31.635	Farm land
Yongyang town	YY1	119.042	31.625	Farm land
Yongyang town	YY2	119.112	31.679	Vegetation
Shiqiu town	SQ1	118.986	31.643	Farm land
Shiqiu town	SQ2	118.957	31.637	Downtown
Jingqiao town	JQ	119.089	31.512	Vegetation

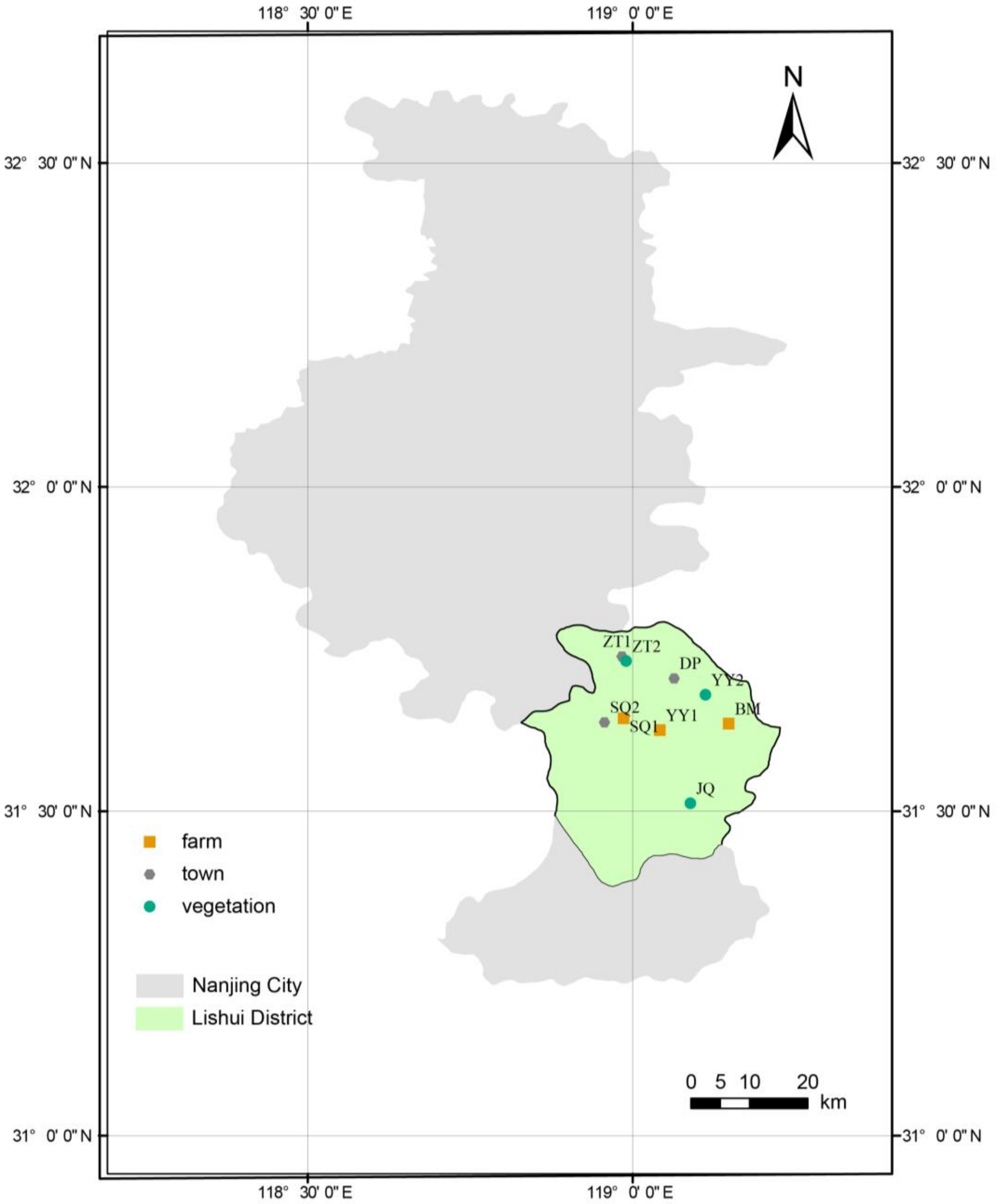

Figure 1. Schematic diagram of study area and sample site.

In this study, the aquatic microbial community and biodiversity were surveyed for 9 target water bodies in four seasons of 2021. Water samples were collected and measurements were taken simultaneously on the first day of the survey in each season. The water samples were randomly collected from 3 sampling points in different areas of the water body without vegetation cover and dead branch and leaf interference. Here, 500 mL of water was collected 4 times and mixed into 2 L to be measured.

2.2. Water Quality Measurement and Biodiversity Survey

A total of 17 water parameters were measured. The water depth was determined using a water gauge (less than 1 m) or a Speedtech handheld portable sounder (greater than 1 m). The in situ determination of the water temperature, dissolved oxygen, pH, and conductivity was performed using a handheld multiparameter water quality analyzer (YSI ProQuatro). The total suspended solids, volatile suspended solids particles, total nitrogen (TN), total organic carbon (TOC), total phosphorus (TP), chemical oxygen demand (COD), biochemical oxygen demand (BOD), and chlorophyll were measured following Rice et al.'s protocol [18].

The fish community was sampled using several different sampling tools including handheld scoop nets (handle 1.5 m, net diameter 0.5 m, mesh 0.05 cm), using the same fishing effort. A 200 m section of the shore edge of the sample point was the collection site, and the collection time was 15 min. A flow gillnet (double stream gillnet, 10 m long, 2.5 m high, mesh 0.1 cm) was set in the lake bay and placed for 11–12 h. Cages were (5 × 0.5 × 0.5 m, mesh 0.025 cm) generally placed at water depths of 0.8–1.5 m for 12–13 h. Fish samples were collected for identification and measurement; except for a few samples kept in 4% formalin solution and brought back to the laboratory, most of the other fish were replaced in the water body.

The quadrat method was used to study the populations of herbaceous plants [19]. Dimensions of 2 m × 2 m, 1 m × 1 m, and 0.5 m × 0.5 m were measured for emergent plant communities, floating leaf plant communities, and submerged plant communities respectively. Generally, where the water depth was less than 80 cm, the sample was collected directly by hand, and when the water depth was higher than 80 cm, it was collected by a Peterson mud picker. All aquatic plants in the square were collected by hand or using a mud picker and brought back to the laboratory to identify species and clean up the plants. The species number, average height, and phenology were recorded on site. For the identification of plant samples, please refer to [20].

2.3. Microbe Community and Metagenomic Data

All water samples were filtered using a sequential 120 μm Nylon membrane and 0.22 μm pore size polycarbonate (PC) membrane. The volume of the filtered water was not less than 1 L, and all 0.22 μm membranes were used for DNA extraction.

Metagenomic library sequencing was carried out at Shanghai Major Biotechnology Co., Ltd. (Shanghai, China) using the Illumina NovaSeq sequencing platform for high-throughput sequencing, and the library was established as a PE150 double-ended library. No less than 20 GB of data per sample was generated during sequencing.

The sequence readouts were assembled using the metagenomic assembly software MEGAHIT (v1.0.6), and reads <500 bp were filtered out [21]. Prodigal software was used to predict open reading frames (meta mode) in assembled contig sequences. To obtain a nonredundant gene set, CD-HIT was used to remove redundancy from the predicted gene sequence, with a similarity cutoff of 0.95 [22]. The sequence data were compared with constructed nonredundant gene sets using Bowtie software (v 1.1.2) to calculate the abundance of individual genes in different samples [23]. Samtools software (version 0.1.19) was used to convert SAM files to BAM files and sort them [24].

Diamond software was used for species annotation based on coding sequences (CDSs) [25]. The predicted protein sequences were aligned to a nonredundant protein database (NR), and the classification information for each CDS was obtained using the MEGAN6 LCA-based blast2lca program. Combined with the TPM data of the genes, the abundance of each sample at different classification levels, such as the phylum, class, and order, was calculated.

Using a Protein Basic Alignment Search Tool (BLASTP)-based search (BLAST v2.2.28+, http://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 9 December 2022), nonredundant genomes were compared to the KEGG GENES database with similarity cutoffs of 1×10^{-5} [25]. The KEGG Orthology-based annotation system v2.0 was used for the functional annotation of the BLASTP results. Amino acid sequences were also compared to the Carbohydrate Active Enzyme Database (http://www.cazy.org/, accessed on 12 December 2022) to predict all enzymes.

2.4. Data Analysis

R tools were used to visualize the microbial data, including their relative abundance (RA) at different taxonomic levels. The diversity level of each point was characterized by α

diversity indices (Shannon and Simpson indices). To determine the β diversity, first the "Hellinger" transformation of the microbial community read count matrix was performed at the taxonomic level and then the Bray–Curtis dissimilarity matrix was calculated for nonweighted group average method (UPGMA) hierarchical clustering and a principal coordinate analysis (PCoA). The correlation between bacterial community composition and environmental factors were assessed using a redundancy analysis (RDA), and the chi-square test was used to compare the abundance differences of various bacterial species or metabolic functions between the two samples ($p < 0.05$). A multivariate analysis of differences in the compositions of ecosystem communities in different groups ($p < 0.05$) was used to determine whether the ecosystem community composition of different groups was significantly different ($p < 0.05$). The Wilcoxon rank sum test was used to determine significant differences in gene abundance for specific metabolic pathways between groups.

To explore the influence of aquatic biological factors on microbial communities, a model of the impacts of biological and abiotic factors on microbial diversity was established using the PC values of the dimensionality reduction in fish and plant communities at various points; the relative abundance and importance of alien species and species diversity as biological factors; and the dimensionality reduction PC values for the habitat, temperature, and water quality as abiotic factors. First, the microbial community structure and diversity were used as the dependent variables and other factors were used as independent variables for the linear fitting. Independent variable filtering was performed via full subset regression and automatic model filtering (dredge function). K-means clustering, a machine-learning-based method, was used to verify whether the characteristics of the fish community, flora, and microbes in the same habitat were closer [26]. Considering that abiotic factors such as the habitat, temperature, and water quality also have an impact on biological factors, a structural equation model (SEM) path analysis was carried out on the basis of the linear model, and the abiotic factors were used as exogenous variables and the biological factors were used as endogenous variables to analyze the main influencing factors.

The above statistical analysis was performed in R version 4.1.2 working environment, with the R packages vegan [27], psych [28], pheatmap [29], leap [30], NbClust [31], MuMIn [32], and lavaan [33].

3. Results

3.1. Water Quality

The average water depth of the nine sites in this study was 1.38 m. The survey was conducted over four quarters, with winter surveys conducted in January, with water temperatures between 3.8 and 6.5 °C at the sites, and average water temperatures of 17.6 and 17.5 °C in spring and autumn, respectively, and above 30 °C in summer. The dissolved oxygen was negatively correlated with the temperature, with the highest dissolved oxygen rate being found in winter, averaging 12.03 mg/L, and the lowest values in summer, ranging between 2.7 and 8.7 mg/L. Except for JQ, the autumn water body pH was 6.81, and all water bodies at the survey site were weakly alkaline (Table S1). The average TN of each water body was 1.61 mg/L. Overall, the TN was lower during the abundant water period, while the content was higher in the autumn and winter dry period. The average TP of each point was 0.073 mg/L. The average TOC of the nine study sites was 11.723 mg/L, of which the average content of dissolved organic carbon components (DOC) was 5.882 mg/L, and the DOC content was the main determinant of the water color. The average chlorophyll a content in each water body was 24.01 μg/L, which was the main indicator of planktonic algae in the water body, indicating that there was a certain degree of eutrophication in the water body (Table S1). Only one of the highly correlated water quality parameters was kept for the subsequent analysis (Figure S1).

3.2. Fish and Wetland Plant Diversity

A total of 3650 fish from 39 species were caught in this survey, belonging to 6 orders, 16 families, and 34 genera (Table S2). Among them, cyprinids accounted for an absolute advantage, accounting for 61.53% of the total fish. Carps comprised the dominant family, accounting for 58.97% of the total fish (Figure S2). In this survey, the main exotic fish were *Gambusia affnis*, *Oreochromis niloticus*, and *Hypostoms plecostomus*.

A total of 70 species of aquatic plants were collected in this survey, belonging to 3 orders, 6 orders, 30 families, and 55 genera. Among them, there were 2 species of ferns, 35 species of monocots, and 33 species of dicots (Table S3). A total of 15 species of exotic aquatic plants were found in this survey (Tables S3 and S4).

3.3. Microbe Community

Details of the obtained sequencing data are shown in Figure S3 and Table S5. The sequencing produced more than 50,000,000 clean reads. The total length of the sample composition of scaffolds averaged 366,246,514 bp, the number averaged 112,937, and most of the samples exceeded 100,000 scaffolds in quantity and 3348 bp in average length. The scaffold N50 was between 2805 and 5597 and had an average length of 3657 bp (Table S5).

At the phylum level, Proteobacteria predominated in the vast majority of samples, with relative abundances exceeding 50%, except for YY2 summer samples with a higher proportion of cyanobacteria (Figure 2a). At the order level, Burkholderiales had the highest relative abundance (Figure 2b), which has been identified as a keystone species in several different studied ecosystems, mainly including pathogenic bacteria (e.g., Bordetella, Ralstonia, Oxalobacter) and Burkholderia, which are common in terrestrial ecosystems.

The Shannon diversity index values of microbial communities varied from 1.265 (DP autumn) to 3.789 (BM autumn) for the different quarters of the 9 lakes in this study, while the Pielou uniformity index values varied from 0.293 (DP autumn) to 0.796 (BM autumn) (Table S5, Figure S4).

The PCA of the microbial communities showed that the sites had large seasonal variations (Figure S5). Among them, the seasonal variation in DP was the smallest, while JQ, SQ2, YY1, and YY2 all showed greater differences between summer and other seasons. ZT1 and ZT2 showed higher β diversity levels in different seasons (Figure S5). The community similarity between summer and autumn points was higher, except for DP, BM, and JQ. In winter and spring, different habitat types showed certain similarities (Figure S6).

According to the KEGG database tertiary classification, the water samples in this study contained a total of 160 gene-coding functional pathways, which fell into six categories: cellular processes, environmental information processing, genetic information processing, human diseases, metabolism, and organic systems. Among them, the most abundant were ribosomes, ABC transporters, and two-component systems (Figure S7).

3.4. Effects of Environmental and Biological Factors on Microbial Community Structure

The multivariate ANOVA revealed significant differences in microbial communities in different seasons and habitats, and the differences in community composition in the different seasons were more significant (F = 2.666, $p < 0.01$) (Table 2). Specifically, the microbial communities differed significantly between summer and winter and spring, while the differences between winter and spring and between summer and autumn were not significant, consistent with the pattern shown in the previous section (Tables 2 and 3, Figure S8). There were significant differences between agricultural land water bodies and downtown artificial water bodies, while the differences between water bodies of vegetation regions and the other two habitats were not significant (Tables 2 and 3, Figure S8).

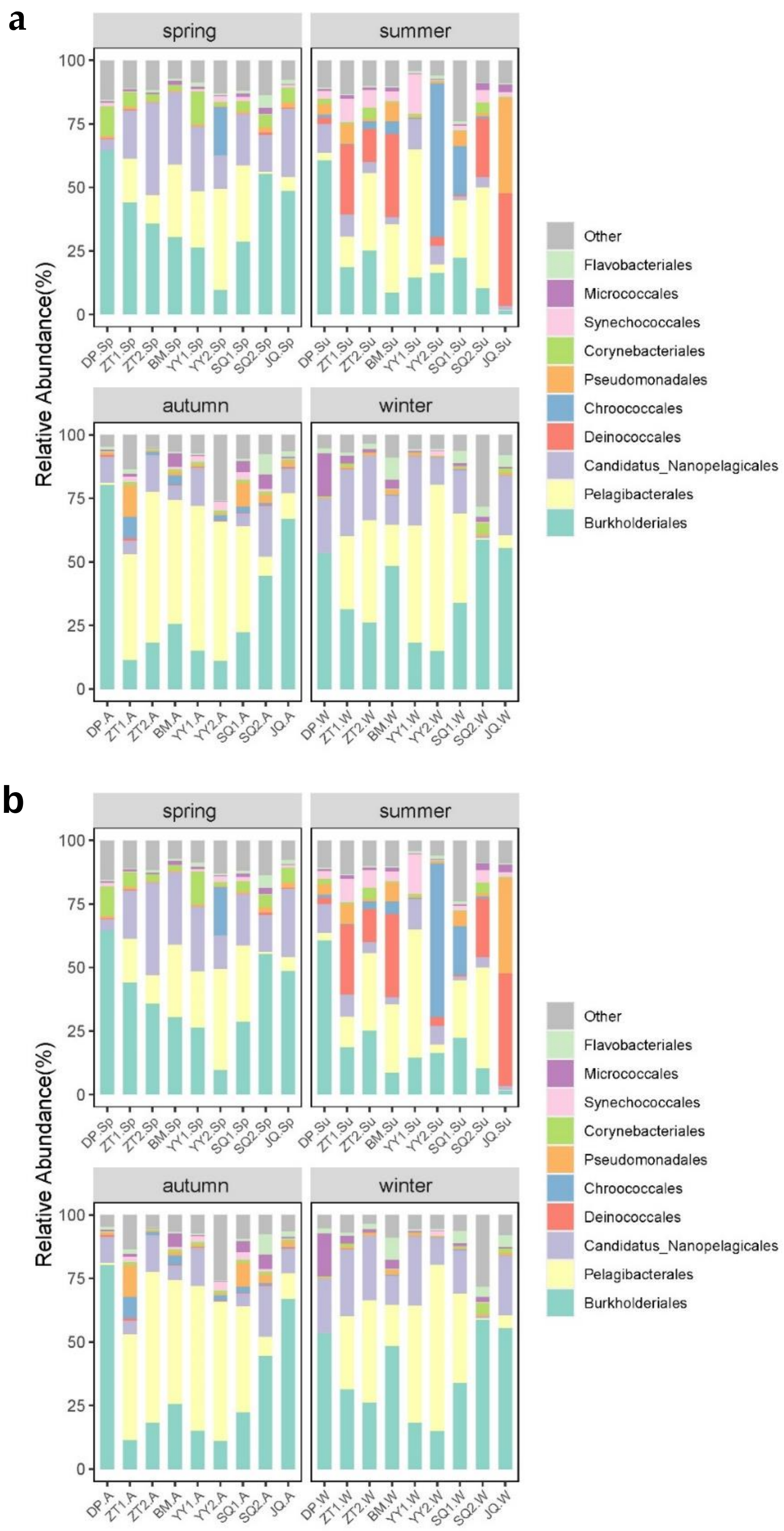

Figure 2. Microbe community structure at the phylum (**a**) and order (**b**) levels.

Table 2. A permutation multivariate analysis of variance for microbial communities in different seasons and habitats.

	DF	**SS**	**F**	**R^2**	**P**	**Sig.**
habitat	2	0.541	1.988	0.102	0.046	*
time	3	1.088	2.666	0.205	0.001	***
habitat:time	6	0.420	0.514	0.079	0.988	
Residuals	24	3.265	0.614			
Total	35	5.314				

DF, degree of freedom; SS, sum of squares; Sig. * $0.01 < p < 0.05$, *** $p < 0.001$.

Table 3. A pairwise comparison of permutation multivariate analyses of variance in different seasons and habitats of microbial communities.

			F	**R^2**	**P**	**Sig.**
win	vs.	Spr	0.830	0.049	0.412	
win	vs.	Sum	4.838	0.232	0.002	**
win	vs.	Aut	1.432	0.082	0.194	
Spr	vs.	Sum	5.129	0.242	0.001	***
Spr	vs.	Aut	2.214	0.121	0.063	
Sum	vs.	Aut	1.958	0.109	0.07	
town	vs.	vegetation	1.816	0.076	0.128	
town	vs.	farm	3.614	0.141	0.02	*
vegetation	vs.	farm	0.502	0.022	0.869	

Sig. * $0.01 < p < 0.05$, ** $0.001 < p < 0.01$, *** $p < 0.001$.

The Shannon diversity for the different seasons was highest in summer, followed by spring, autumn, and winter, but the differences in diversity levels between them were not significant (Figure 3a). Compared with the seasonal trend of the community structure, summer and spring were closer to spring and more obvious in autumn at the level of diversity, indicating that there was a certain continuity in the succession from summer to autumn in the species composition. The level of microbial diversity was highest in agricultural land areas, followed by towns and vegetation areas (Figure 3b).

The RDA showed that the water quality indicators measured in this study explained 27.6% of the differences in community structure (Figure 4a). The four water quality indices of the TN, TP, chlorophyll content, and BOD were retained after variable selection, indicating that their impact on microbial communities was the most direct and significant (Figure 4b). The explanatory degree of the community structure of the retained variables was still 25.7%, which was less different than that of the full model (Figure 4b).

The regression analysis of microbial diversity using water quality indicators showed that the temperature, conductivity, chlorophyll content, and particulate matter concentration significantly affected the level of microbial α diversity (Figure S9). The full subset regression analysis showed that the temperature, pH, and particulate matter content contributed the most directly to diversity levels (Figure 5a), and the general linear models constructed by the three variables reached an R^2 of 0.482 for the diversity levels (Figure 5b).

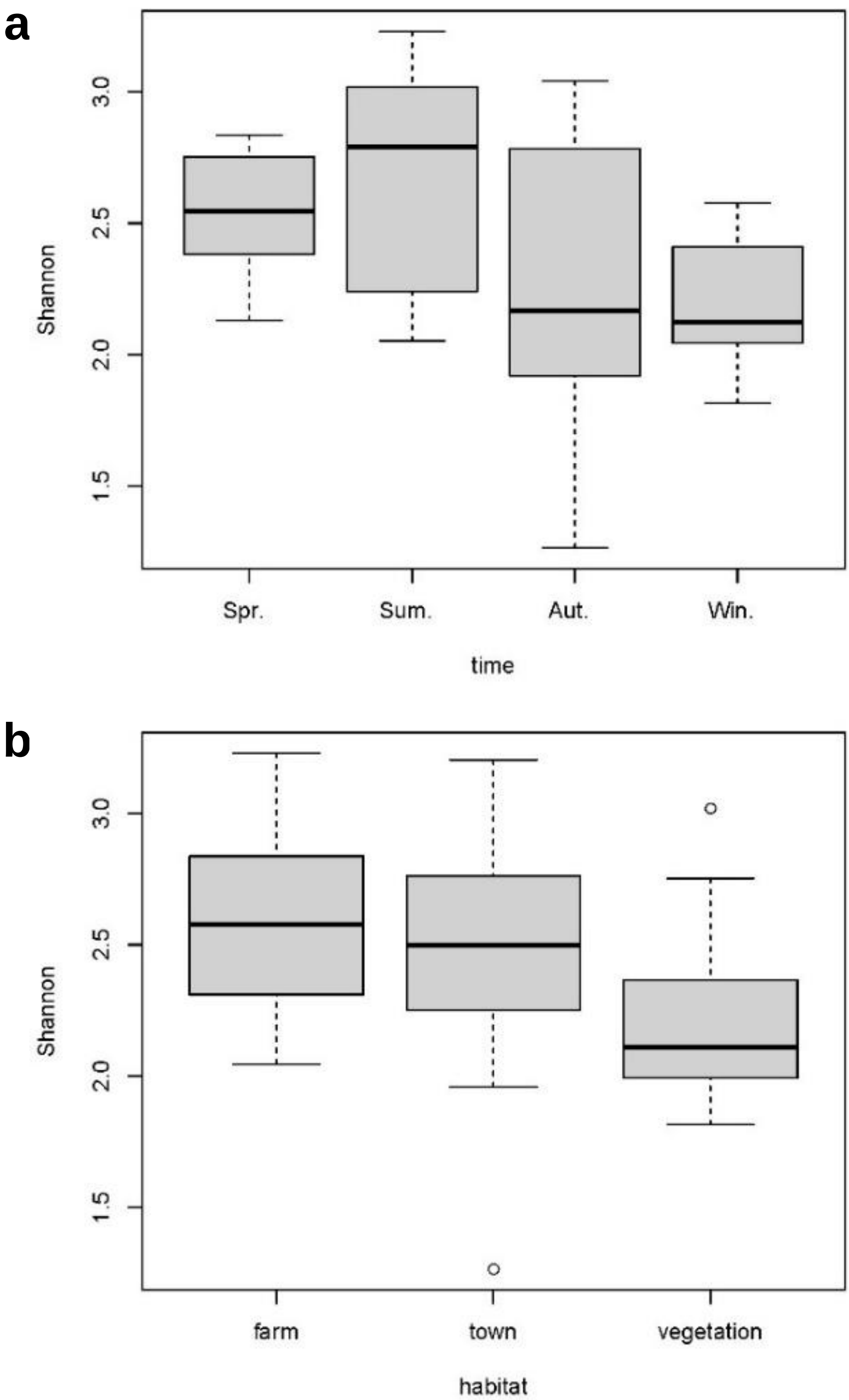

Figure 3. Differences in microbial diversity in ponds with different sample times (**a**) or habitats (**b**).

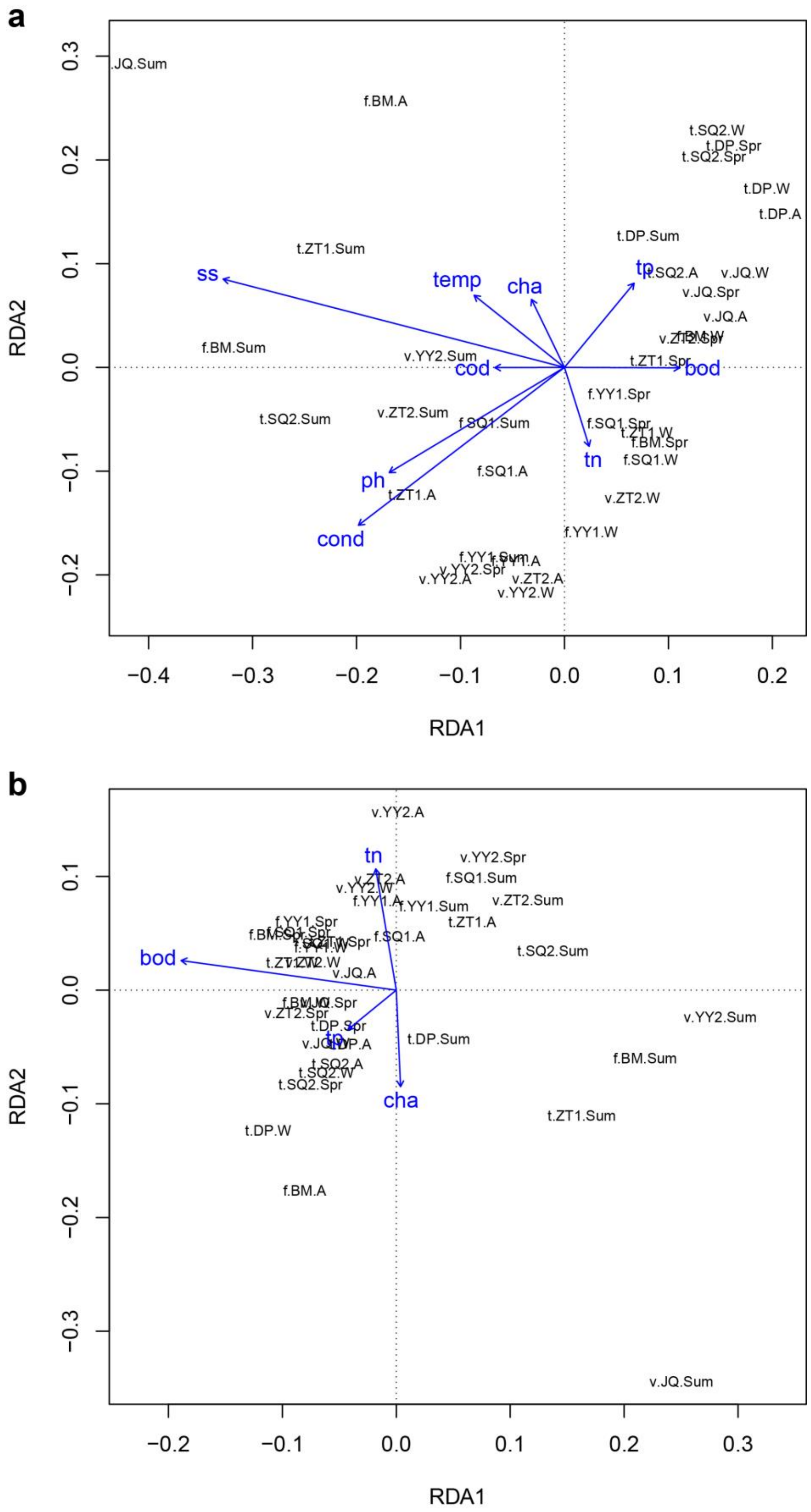

Figure 4. The RDA analysis of the impact of water quality on microbial communities, (**a**) full model, (**b**) factors selected using Akaike's information criterion as the selection criterion.

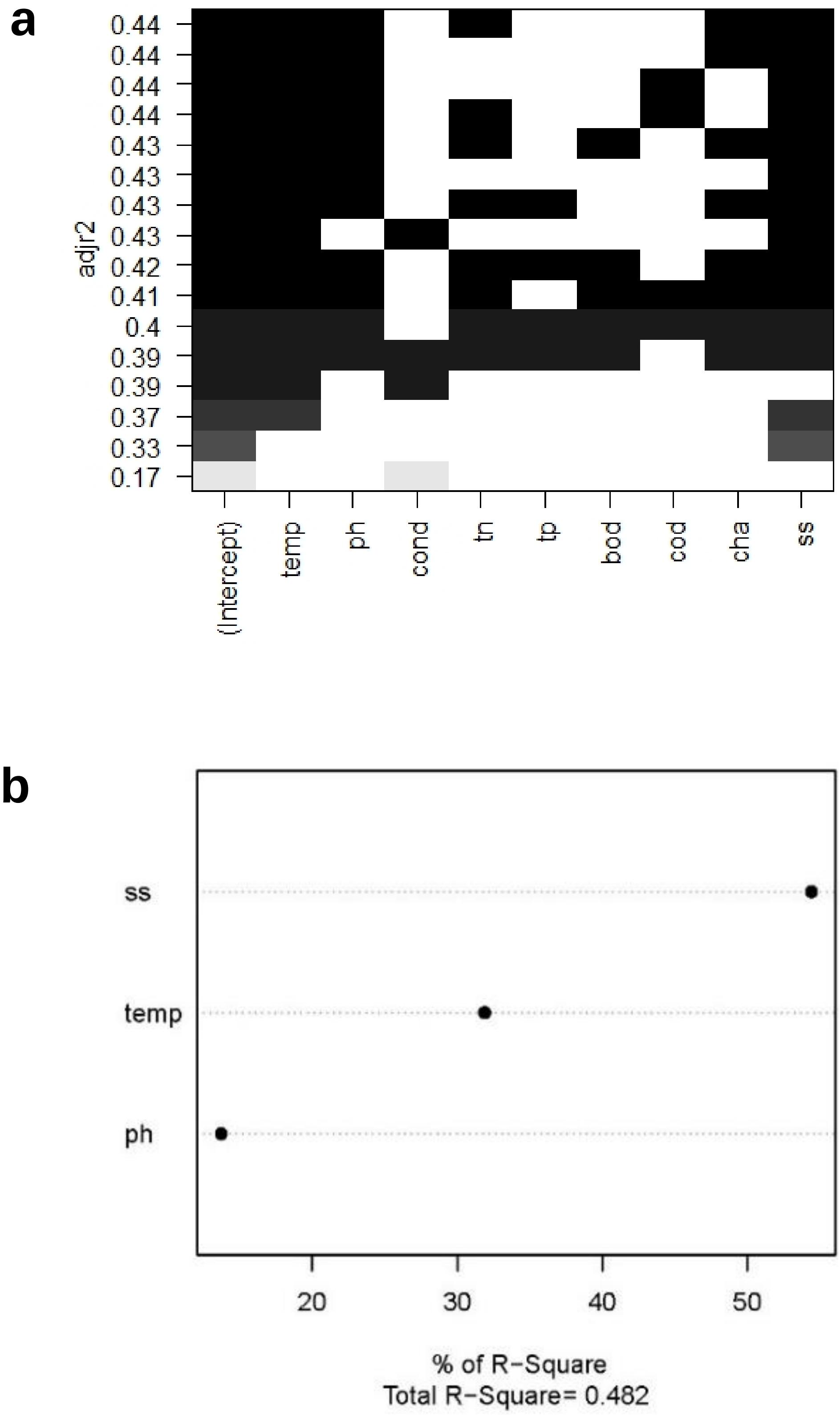

Figure 5. Full subset regression for the impacts of water quality variables on microbial diversity (**a**) and relative weights of water quality indicators that significantly affect microbial diversity (**b**).

Abiotic and biological factors jointly affected the diversity of the microbial communities, and water quality + fish community + habitat explained 53.3% of the differences in the microbial communities, while water quality + wetland plant community + wetland plant alien species abundance explained 56.9% of the level of microbial diversity (Table 4).

Among them, the water quality had the greatest impact, showing significant contributions to different microbial diversity indicators, which was greater than other abiotic and biotic factors (Figure S10). The k-means results also support the characteristics of the fish community, wetland community, and microorganism community, which were clustered according to habitat type (Figure S11).

Table 4. Linear models of abiotic and biotic factors on microbial diversity.

Model	Freedom	AICc	adj.R^2
dependent variable: MC			
WQ + FC + Hbt	6	84.3	0.533
dependent variable: MD			
WQ + PC + PA	5	79.6	0.569

Hbt: habitat type; WQ: water quality; FC: fish community; PC: wetland plant community; PA: alien wetland plant abundance; MC: microbial community; MD, microbial alpha diversity; AICc: Akaike's information criterion.

The comparative fit index (CFI) and Tucker–Lewis index (TLI) of the SEM for the microbial community structure and diversity were greater than 0.9, indicating that the SEM fitting effect was good. The analysis showed that the habitat and season did not directly affect the microbial communities, and the direct effects of fish communities on the microbial community structure were not significant. The influence of the water quality was significant among the variables. For the microbial α diversity, the wetland biomes and alien species abundance had significant direct effects (Figure 6).

The comparative fit index (CFI) and Tucker–Lewis index (TLI) of the SEM for the microbial community structure and diversity were greater than 0.9, indicating that the SEM fitting effect was good. The analysis showed that the habitat and season did not directly affect the microbial communities, and the direct effects of fish communities on the microbial community structure were not significant. The influence of the water quality was significant among the variables. For the microbial α diversity, the wetland biomes and alien species abundance had significant direct effects (Figure 6).

3.5. Discussion

Previous studies have shown that Proteobacteria, cyanobacteria, actinomycetes, Bacteroides, and Jumicromycetes are the most typical microbial groups in lake water [34,35]. In the lakeside wetlands of large natural lakes with different climates, such as Wuliangsu and Poyang Lakes, Proteomycetes showed obvious dominance, indicating that they had good tolerance to different ecological environments in their natural habitats. Actinobacteria is another dominant taxon, with relative abundances ranging from 8.76% (BM summer) to 44.04% (DP winter) and being more dominant in winter and spring. Actinomycetes are Gram-positive bacteria that are widely distributed in nature and closely related to human production and life [36], and can be important features of small lakes regionally. Due to the strong growth of algae in summer, the relative abundance of cyanobacteria in summer is higher than in other seasons, except for in DP and JQ, and a considerable proportion of cyanobacteria are present in summer, indicating that they may have a negative impact on water bodies in summer. In addition, certain proportions of cyanobacteria also existed in the autumn ZT1 and YY2 sites, and the negative effects caused by them may last for a long time.

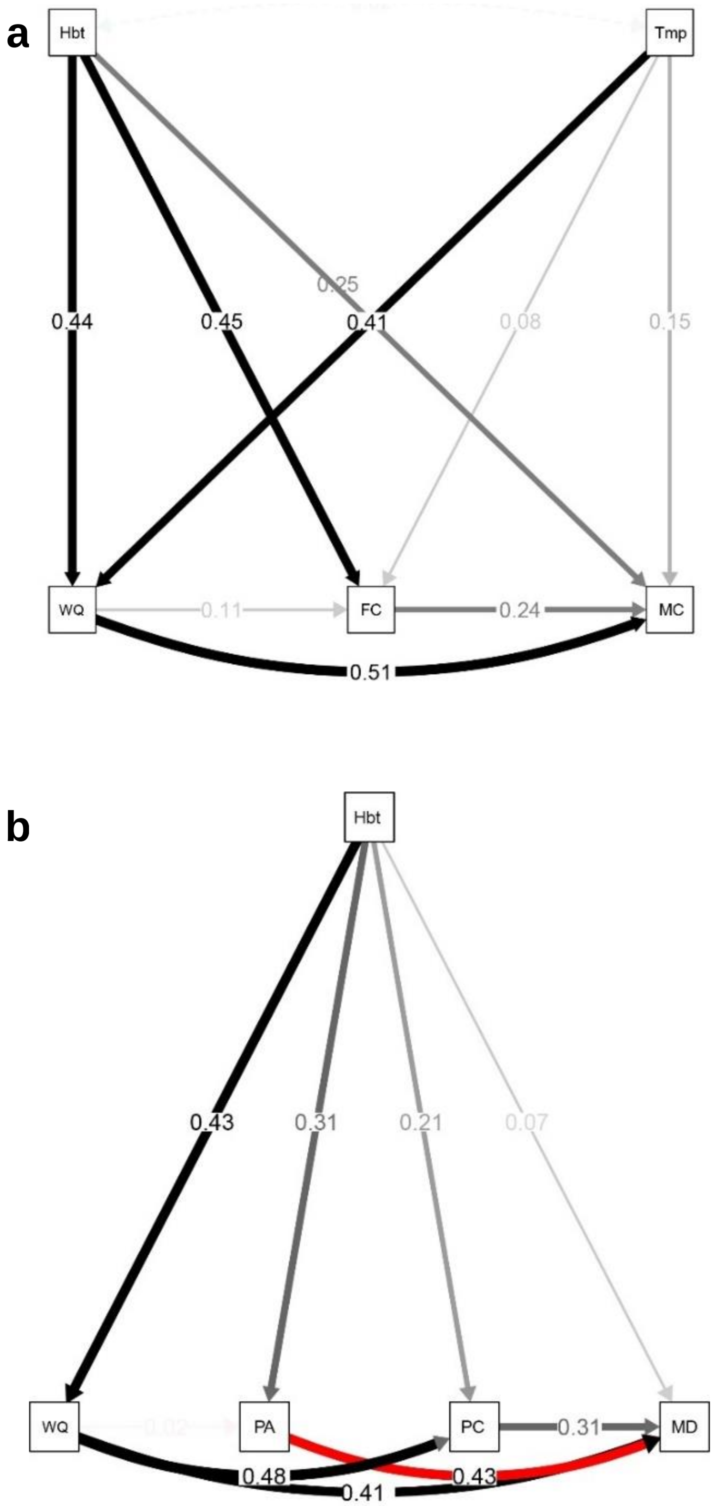

Figure 6. A structural equation model analysis of the effects of biotic and abiotic factors on microbial community (**a**) and microbial diversity (**b**). Red arrow indicates negative effect. (Hbt: habitat type; Tmp: temperature; WQ: water quality; FC: fish community; PC: wetland plant community; PA: alien wetland plant abundance; MC: microbial community; MD, microbial alpha diversity).

Pelagibacterales and Candidatus Nanopelagicales also had relatively high relative abundance rates at the order level. Pelagibacterales belongs to the α Proteobacteria, one of the most abundant orders of bacteria in oceans and freshwater lakes. Several downstream phylogenetic lineages of Pelagibacterales show obvious correlations with physicochemical properties in aquatic environments and have high spatiotemporal distribution polymorphisms [37]. Pelagibacterales showed higher relative abundance rates in autumn and winter, while the DP and JQ sites were significantly less abundant in this order than the other sites. Candidatus Nanopelagicales are widespread taxa based on 16 actinomycete ACL family bacteria isolated by Neuenschwander et al. [38]. Not only does this order have a tiny cell size, but it is also one of the most streamlined free-floating microorganisms, with a very small genome size (1.2–1.4 Mbp) and low genomic GC content. The bacteria were isolated from Zurich, a deepwater lake in oligo-meso-nutrient Switzerland. However, the water bodies in this study have different degrees of eutrophication, indicating that the ecological characteristics of such bacteria need more research.

The microbial community research on downtown and surrounding water bodies in China is still mainly focused on large urban inner lakes [39,40]. Shi et al. studied Nanhai Lake, the largest lake in Baotou, Inner Mongolia, and found that the microbial community of eutrophicated urban lakes was significantly affected by the TN, TP, and pH, and seasonal changes may be the main factors leading to changes in microbial community structure [41]. The water quality is an important factor affecting the structure of microbial communities in small water bodies, and the distribution of microorganisms is significantly affected by the content of nutrients and organic matter, which may be more pronounced in medium-nutrient water [42,43]. In the urban water system, the abundance of Proteobacteria was highest in the river reach with highest nutrient concentration [35]. This study also shows that the microbial communities of lakes and wetlands in cities in the lower reaches of the Yangtze River that are affected by humans are closely related to water quality. The TP may affect the community dominance of algae, especially cyanobacteria, and affect the similarity of microbial communities between different water bodies. The TN and BOD in the water body are important factors that cause differences in water quality in different water bodies, which jointly determine the abundance of actinomycetes and Proteobacteria among the dominant taxa in this region.

In addition to water pollution, human activities can also affect wetland microorganisms in other ways, such as via hydrological connectivity, water system degradation, emerging contaminants, and selective pressures caused by human activities [44–46]. In the present study, farmland water bodies and downtown water bodies were affected by human activities in different ways, leading to obvious differences in microbial communities between two types of habitats. When eutrophication is present in freshwater bodies, the diversity pattern of plankton microbial communities may be related to the nutrient concentrations [35,47,48]. However, in the present work, the nutrient levels had a significant effect on the microbial community structure, and the effect on the α diversity patterns was not significant, which may be due to eutrophication occurring at different sites. This study revealed that suspended particulate matter plays an important role, the concentration of particulate matter promotes the diversity of microorganisms, and the content of particulate matter in the water bodies of different habitat types is also more obvious, which may be related to different human activities, such as tourism and agriculture [49,50]. In this study, the concentration of particulate matter in agricultural land and downtown water was higher, which formed a pattern in which the diversity and uniformity of microbial species in these two habitats were higher than those in vegetation areas. Ray et al. determined that the removal of solids from culture water significantly ($p \leq 0.01$) reduced the abundance rates of nematodes, rotifers, cyanobacteria, and bacteria, indicating the importance of particulate matter in water bodies for microbial diversity [51].

In addition to water pollution, the introduction and invasion of alien species are also challenges for wetland ecosystems [52,53]. The original intention of the manual intervention in urban water bodies is often to improve the living environment, but there is often a lack of attention paid to the control of the alien species, and many exotic plants (such as *Iris pseudacorus,* etc.) have been intentionally or unintentionally introduced into local wetlands [54,55]. These alien species enter urban parks, rapidly multiply, and crowd out native indigenous species, leading to the homogenization of these aquatic species, potentially further affecting the ecological function of water bodies [56,57]. The results in the present study showed that the richness of alien plant species was much higher than that of fish populations, which may be closely related to the construction of wetland parks and landscaping. We found that biological factors may also affect the microbial diversity of small water bodies, and the abundance of exotic plants in wetlands is one of the main factors. It is well known that alien plants may become a potential threat and cause damage to ecosystems [58,59], while in the present study, we found that nonnative plant populations may impact the microbial community and function of water bodies. Invasive plants have previously been found in terrestrial ecosystems to affect soil chemistry and microbial communities [60], but they have rarely been reported before in aquatic ecosystems. Alien organisms may change local microbial communities by affecting the properties of local conditions [61,62] and altering the phyllospheric and rhizosphere microbial characteristics [63–65]. Although exotic fish species are relatively small, species such as mosquito fish and tilapia have high abundance rates in all water bodies, and the risk of potential biological invasion by alien fish may be higher. Empirical studies have supported the effects of exotic fish on microorganisms in water bodies [66], and in this study, the impact of the fish population composition on small water bodies may have been indirect, such as by disturbing the suspension of the particulate matter in the ponds.

The present work highlighted that different degrees of disturbance patterns of human activities had significant impacts on the water quality, aquatic biodiversity, and microbial communities in the downtown and surrounding small water bodies. Compared with small natural wetlands, there are significant differences in the structures of aquatic biological communities in small water bodies around towns and agricultural areas, mainly in the species richness and abundance of exotic species. We also found that small water bodies in and around towns have low fish diversity rates and homogeneous wetland plant communities, which may be due to the use of similar wetland management strategies, such as landscaping. The impacts on microbial communities are mainly through the properties of water bodies and the structure of wetland plant communities. The results implied that in the management of small water bodies, it is important to reduce the introduction of exotic wetland plants and strengthen the regulation of the fish community structure.

This study was carried out on a regional scale, and it is representative of the cities in the middle and lower reaches of the Yangtze River, while the numbers of habitat types and studied sites were limited. More samples with different land use patterns should be considered in further research to help us deeply understand the impacts of human activities on small water bodies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15108151/s1, Table S1. Statistical values of water quality of 9 urban ponds at Lishui, Nanjing; Table S2. List of fish species at the sites investigated in this study; Table S3. List of wetland plant species in this study; Table S4. Important values of dominant wetland plants in each urban ponds at Lishui, Nanjing; Table S5. Statistics of metagenomic splicing results and alpha diversity of each sample; Figure S1. Correlation between water quality parameters; Figure S2. Composition of fish community at each study site; Figure S3. Sequencing data of microbial metagenomes; Figure S4. Microbial alpha diversity in each sample; Figure S5. PCA of microbial community; Figure S6. Heatmap of microbial community similarity; Figure S7. Heat map of gene abundance similarity of KEGG metabolic pathway in different samples; Figure S8. PCoA of microbial community based on bray distance; Figure S9. Linear relationship between water quality and microbial Shannon diversity; Figure S10. Contribution of biotic and abiotic factors to the linear model of microbial diversity (WQ: Water Quality, FC: Fish Community, PC: wetland Plant Community, PA: Alien wetland Plant abundance); Figure S11. The cluster plot based on k-means analysis on organism and microbial community, the habitat type of each sample is labelled.

Author Contributions: Conceptualization, P.L.; methodology, C.P.; validation, P.L.; formal analysis, C.P. and P.L.; investigation, C.P.; writing—original draft preparation, C.P. and P.L.; writing—review and editing, C.P. and P.L.; supervision, P.L.; funding acquisition, P.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

Data Availability Statement: All DNA sequences have been deposited into NCBI under project number PRJNA751434.

Acknowledgments: The authors thank members of Li Lab for help with the field data and for their suggestions. We are grateful to the anonymous referees for their helpful comments on earlier versions for this paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Blazewicz, S.J.; Barnard, R.L.; Daly, R.A.; Firestone, M.K. Evaluating rRNA as an indicator of microbial activity in environmental communities: Limitations and uses. *ISME J.* **2013**, *7*, 2061–2068. [CrossRef]
2. Acharya, K.; Blackburn, A.; Mohammed, J.; Haile, A.T.; Hiruy, A.M.; Werner, D. Metagenomic water quality monitoring with a portable laboratory. *Water Res.* **2020**, *184*, 116112. [CrossRef]
3. Ezzariai, A.; Hafidi, M.; Khadra, A.; Aemig, Q.; El Fels, L.; Barret, M.; Merlina, G.; Patureau, D.; Pinelli, E. Human and veterinary antibiotics during composting of sludge or manure: Global perspectives on persistence, degradation, and resistance genes. *J. Hazard. Mater.* **2018**, *359*, 465–481. [CrossRef]
4. Newton, R.J.; Jones, S.E.; Eiler, A.; McMahon, K.D.; Bertilsson, S. A guide to the natural history of freshwater lake bacteria. *Microbiol. Mol. Biol. Rev.* **2011**, *75*, 14–49. [CrossRef]
5. Czekalski, N.; Sigdel, R.; Birtel, J.; Matthews, B.; Bürgmann, H. Does human activity impact the natural antibiotic resistance background? Abundance of antibiotic resistance genes in 21 Swiss lakes. *Environ. Int.* **2015**, *81*, 45–55. [CrossRef] [PubMed]
6. Zhao, D.; Cao, X.; Huang, R.; Zeng, J.; Shen, F.; Xu, H.; Wang, S.; He, X.; Yu, Z. The heterogeneity of composition and assembly processes of the microbial community between different nutrient loading lake zones in Taihu Lake. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 5913–5923. [CrossRef] [PubMed]
7. Wei, J.; Wang, M.; Chen, C.T.; Wu, H.M.; Lin, L.; Li, M. Seasonal succession of phytoplankton in two temperate artificial lakes with different water sources. *Environ. Sci. Pollut. Res.* **2020**, *11*, 42324–42334. [CrossRef]
8. Bakr, N.; Abd El-kawy, O.R. Modeling the artificial lake-surface area change in arid agro-ecosystem: A case study in the newly reclaimed area, Egypt. *J. Environ. Manag.* **2020**, *271*, 15. [CrossRef]
9. Holker, F.; Vanni, M.J.; Kuiper, J.J.; Meile, C.; Grossart, H.P.; Stief, P.; Adrian, R.; Lorke, A.; Dellwig, O.; Brand, A.; et al. Tube-dwelling invertebrates: Tiny ecosystem engineers have large effects in lake ecosystems. *Ecol. Monogr.* **2015**, *85*, 333–351. [CrossRef]
10. Mao, R.; Zhang, X.H.; Song, C.C.; Wang, X.W.; Finnegan, P.M. Plant functional group controls litter decomposition rate and its temperature sensitivity: An incubation experiment on litters from a boreal peatland in northeast China. *Sci. Total Environ.* **2018**, *626*, 678–683. [CrossRef]
11. Zhang, C.; Xu, P.; Wang, X.C.; Xu, L. Bacterial viability and diversity in a landscape lake replenished with reclaimed water: A case study in Xi'an, China. *Environ. Sci. Pollut. Res.* **2020**, *27*, 32796–32808. [CrossRef]
12. Zhang, H.; Wang, Y.; Chen, S.; Zhao, Z.; Feng, J.; Zhang, Z.; Lu, K.; Jia, J. Water bacterial and fungal community compositions associated with urban lakes, Xi'an, China. *Int. J. Environ. Res. Public Health* **2018**, *15*, 469. [CrossRef]
13. Zhang, X.; Xian, Y.; Wang, L.; Wang, D. Behaviour and habitat selection of Yangtze finless porpoises in Dongting Lake, China, and the adjacent waters: Impact of human activity. *Pak. J. Zool.* **2013**, *45*, 635–642.
14. Newton, R.J.; Kent, A.D.; Triplett, E.W.; McMahon, K.D. Microbial community dynamics in a humic lake: Differential persistence of common freshwater phylotypes. *Environ. Microbiol.* **2006**, *8*, 956–970. [CrossRef]
15. Staley, C.; Sadowsky, M.J. Application of metagenomics to assess microbial communities in water and other environmental matrices. *J. Mar. Biol. Assoc. UK* **2016**, *96*, 121–129. [CrossRef]
16. Handelsman, J.; Rondon, M.R.; Brady, S.F.; Clardy, J.; Goodman, R.M. Molecular biological access to the chemistry of unknown soil microbes: A new frontier for natural products. *Chem. Biol.* **1998**, *5*, R245–R249. [CrossRef]
17. Amann, R.I.; Ludwig, W.; Schleifer, K.-H. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* **1995**, *59*, 143–169. [CrossRef]
18. Rice, E.W.; Bridgewater, L.; Association, A.P.H. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association: Washington, DC, USA, 2012.
19. Fang, J.; Wang, X.; Shen, Z.; Tang, Z.; He, J.; Yu, D.; Jiang, Y.; Wang, Z.; Zheng, C.; Zhu, J.; et al. Methods and protocols for plant community inventory. *Biodivers. Sci.* **2009**, *17*, 533. [CrossRef]
20. Chen, Y.; Ma, X.; Du, Y.; Feng, M.; Li, M. *The Chinese Aquatic Plants*; Henan Science and Technology Press: Zhengzhou, China, 2012.
21. Li, D.; Liu, C.-M.; Luo, R.; Sadakane, K.; Lam, T.-W. MEGAHIT: An ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics* **2015**, *31*, 1674–1676. [CrossRef]

22. Fu, L.; Niu, B.; Zhu, Z.; Wu, S.; Li, W. CD-HIT: Accelerated for clustering the next-generation sequencing data. *Bioinformatics* **2012**, *28*, 3150–3152. [CrossRef] [PubMed]
23. Langmead, B. Aligning short sequencing reads with bowtie. *Curr. Protoc. Bioinform.* **2010**, *32*, 11–17. [CrossRef] [PubMed]
24. Li, H.; Handsaker, B.; Wysoker, A.; Fennell, T.; Ruan, J.; Homer, N.; Marth, G.; Abecasis, G.; Durbin, R.; Subgroup, G.P.D.P. The sequence alignment/map format and SAMtools. *Bioinformatics* **2009**, *25*, 2078–2079. [CrossRef] [PubMed]
25. Buchfink, B.; Xie, C.; Huson, D.H. Fast and sensitive protein alignment using DIAMOND. *Nat. Methods* **2015**, *12*, 59–60. [CrossRef]
26. Tsui, T.-H.; van Loosdrecht, M.C.M.; Dai, Y.; Tong, Y.W. Machine learning and circular bioeconomy: Building new resource efficiency from diverse waste streams. *Bioresour. Technol.* **2023**, *369*, 128445. [CrossRef] [PubMed]
27. Oksanen, J.; Blanchet, F.G.; Kindt, R.; Legendre, P.; Minchin, P.R.; O'hara, R.B.; Simpson, G.L.; Solymos, P.; Stevens, M.H.H.; Wagner, H. Package 'Vegan': Community Ecology Package. 2017. Available online: https://github.com/vegandevs/vegan (accessed on 8 March 2022).
28. Revelle, W. Package 'Psych', v 2.2.3. 2015. Available online: https://personality-project.org/r/psych/ (accessed on 8 March 2022).
29. Kolde, R.; Kolde, M.R. Package 'Pheatmap'. 2018. Available online: https://cran.r-project.org/web/packages/pheatmap/index.html (accessed on 8 March 2022).
30. Specht, A.T.; Li, J. LEAP: Constructing gene co-expression networks for single-cell RNA-sequencing data using pseudotime ordering. *Bioinformatics* **2016**, *33*, 764–766. [CrossRef]
31. Charrad, M.; Ghazzali, N.; Boiteau, V.; Niknafs, A. NbClust: An R Package for Determining the Relevant Number of Clusters in a Data Set. *J. Stat. Softw.* **2014**, *61*, 1–36. [CrossRef]
32. Bartoń, K. Package "MuMIn": Multi-Model Inference, v 1.46.0. 2022. Available online: https://cran.r-project.org/web/packages/MuMIn/index.html (accessed on 11 October 2022).
33. Rosseel, Y. lavaan: An R package for structural equation modeling. *J. Stat. Softw.* **2012**, *48*, 1–36. [CrossRef]
34. Ren, L.; He, D.; Xing, P.; Wang, Y.; Wu, Q. Bacterial diversity and ecological function in lake water bodies. *Biodivers. Sci.* **2013**, *21*, 421–432. [CrossRef]
35. Wang, H.; Liu, X.; Wang, Y.; Zhang, S.; Zhang, G.; Han, Y.; Li, M.; Liu, L. Spatial and temporal dynamics of microbial community composition and factors influencing the surface water and sediments of urban rivers. *J. Environ. Sci.* **2023**, *124*, 187–197. [CrossRef]
36. Allen, M.A.; Goh, F.; Burns, B.P.; Neilan, B.A. Bacterial, archaeal and eukaryotic diversity of smooth and pustular microbial mat communities in the hypersaline lagoon of Shark Bay. *Geobiology* **2009**, *7*, 82–96. [CrossRef]
37. Herlemann, D.P.R.; Woelk, J.; Labrenz, M.; Jürgens, K. Diversity and abundance of "Pelagibacterales" (SAR11) in the Baltic Sea salinity gradient. *Syst. Appl. Microbiol.* **2014**, *37*, 601–604. [CrossRef] [PubMed]
38. Neuenschwander, S.M.; Ghai, R.; Pernthaler, J.; Salcher, M.M. Microdiversification in genome-streamlined ubiquitous freshwater Actinobacteria. *ISME J.* **2018**, *12*, 185–198. [CrossRef]
39. Tao, X. Analysis of Water Quality and Bacterial Diversity Based on PCR-DGGE on East Lake, Nanhu Lake, Tangxun Lake in Wuhan City. Master's Thesis, South-Central University for Nationalities, Wuhan, China, 2016.
40. Zou, S.-J.; Yin, L.-Q.; Zhao, B.-L.; Chen, P.-P.; Wei, P.-S.; Ma, X.-F. Bacterioplankton community structure characteristics in Liangzi lake and Houguan lake, Wuhan. *J. Hydroecol.* **2021**, *42*, 33–41.
41. Shi, D. Microbial Community Structure of Eutrophic Urban Lake and Influence Factors. Master's Thesis, Inner Mongolia University of Science and Technology, Baotou, China, 2021.
42. Wu, X.; Xi, W.; Ye, W.; Yang, H. Bacterial community composition of a shallow hypertrophic freshwater lake in China, revealed by 16S rRNA gene sequences. *FEMS Microbiol. Ecol.* **2007**, *61*, 85–96. [CrossRef] [PubMed]
43. Buosi, P.R.B.; Pauleto, G.M.; Lansac-Tôha, F.A.; Velho, L.F.M. Ciliate community associated with aquatic macrophyte roots: Effects of nutrient enrichment on the community composition and species richness. *Eur. J. Protistol.* **2011**, *47*, 86–102. [CrossRef]
44. Paver, S.F.; Newton, R.J.; Coleman, M.L. Microbial communities of the Laurentian Great Lakes reflect connectivity and local biogeochemistry. *Environ. Microbiol.* **2020**, *22*, 433–446. [CrossRef] [PubMed]
45. Wang, R.; Xu, S.; Jiang, C.; Zhang, Y.; Bai, N.; Zhuang, G.; Bai, Z.; Zhuang, X. Impacts of human activities on the composition and abundance of sulfate-reducing and sulfur-oxidizing microorganisms in polluted river sediments. *Front. Microbiol.* **2019**, *10*, 231. [CrossRef] [PubMed]
46. You, X.; Wang, S.; Li, G.; Du, L.; Dong, X. Microplastics in the soil: A review of distribution, anthropogenic impact, and interaction with soil microorganisms based on meta-analysis. *Sci. Total Environ.* **2022**, *832*, 154975. [CrossRef]
47. Dai, Y.; Yang, Y.; Wu, Z.; Feng, Q.; Xie, S.; Liu, Y. Spatiotemporal variation of planktonic and sediment bacterial assemblages in two plateau freshwater lakes at different trophic status. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 4161–4175. [CrossRef] [PubMed]
48. Zeng, J.; Jiao, C.; Zhao, D.; Xu, H.; Huang, R.; Cao, X.; Yu, Z.; Wu, Q.L. Patterns and assembly processes of planktonic and sedimentary bacterial community differ along a trophic gradient in freshwater lakes. *Ecol. Ind.* **2019**, *106*, 105491. [CrossRef]
49. Bhatta, R.; Ghimire, P.S.; Paudyal, R.; Tripathee, L.; Gurung, S.; Regmi, D.; Joshi, R.; Sharma, C.M. Inorganic components in lake waters in the Third Pole. In *Water Quality in the Third Pole: The Roles of Climate Change and Human Activities*; Sharma, C.M., Kang, S., Tripathee, L., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 239–259.
50. Li, D.-K.; Zhuo, J.; Wang, Z. Effect of human activities and climate change on the water surface area of Hongjiannao Lake. *J. Glaciol. Geocryol.* **2009**, *31*, 1110–1115.

51. Ray, A.J.; Seaborn, G.; Leffler, J.W.; Wilde, S.B.; Lawson, A.; Browdy, C.L. Characterization of microbial communities in minimal-exchange, intensive aquaculture systems and the effects of suspended solids management. *Aquaculture* **2010**, *310*, 130–138. [CrossRef]
52. Simberloff, D.; Barney, J.N.; Mack, R.N.; Carlton, J.T.; Reaser, J.K.; Stewart, B.S.; Tabor, G.; Lane, E.M.; Hyatt, W.; Malcom, J.W.; et al. U.S. action lowers barriers to invasive species. *Science* **2020**, *367*, 636. [CrossRef]
53. Xiong, W.; Xie, D.; Wang, Q.; Wang, H.; Wu, Z.; Sun, H.; Li, T.; Bowler, P.A. Non-native species in Poyang Lake Basin: Status, threats and management. *Aquat. Invasions* **2023**, *18*, 119–134. [CrossRef]
54. Wang, H.; Xiao, K.; Wu, Z.; Chen, J.; Xiong, W.; Wang, Z.; Wang, Q.; Zhu, H.; Bowler, P.A. Delta arrowhead (*Sagittaria platyphylla*) in the Yangtze River: An invasive aquatic plant and the potential ecological consequences. *Bioinvasions Rec.* **2020**, *9*, 618–626. [CrossRef]
55. Xiong, W.; Wang, H.; Wu, Z.; Xiao, K.; Li, T.; Bowler, P.A. Ecological and economic impacts of alien invasive yellow flag (*Iris Pseudacorus* l.) in China. *Sustainability* **2023**, *15*, 5905. [CrossRef]
56. Pearse, W.D.; Cavender-Bares, J.; Hobbie, S.E.; Avolio, M.L.; Bettez, N.; Chowdhury, R.R.; Darling, L.E.; Groffman, P.M.; Grove, J.M.; Hall, S.J. Homogenization of plant diversity, composition, and structure in North American urban yards. *Ecosphere* **2018**, *9*, e02105. [CrossRef]
57. Liu, M.; Clarke, L.J.; Baker, S.C.; Jordan, G.J.; Burridge, C.P. A practical guide to DNA metabarcoding for entomological ecologists. *Ecol. Entomol.* **2020**, *45*, 373–385. [CrossRef]
58. Xiong, W.; Tao, J.; Liu, C.; Liang, Y.; Sun, H.; Chen, K.; Cheng, Y.; Chen, Y. Invasive aquatic plant (*Alternanthera philoxeroides*) facilitates the invasion of western Mosquitofish (*Gambusia affinis*) in Yangtze River, China. *Aquat. Ecosyst. Health Manag.* **2019**, *22*, 408–416. [CrossRef]
59. Simberloff, D.; Holle, B.V. Positive interactions of nonindigenous species: Invasional meltdown? *Biol. Invasions* **1999**, *1*, 21–32. [CrossRef]
60. Jacobs, K.; Conradie, T.; Jacobs, S. Microbial communities in the Fynbos region of South Africa: What happens during woody alien plant invasions. *Diversity* **2020**, *12*, 254. [CrossRef]
61. Zedler, J.B.; Kercher, S. Causes and consequences of invasive plants in wetlands: Opportunities, opportunists, and outcomes. *Crit. Rev. Plant Sci.* **2004**, *23*, 431–452. [CrossRef]
62. Shang, S.; Hu, S.; Liu, X.; Zang, Y.; Chen, J.; Gao, N.; Li, L.; Wang, J.; Liu, L.; Xu, J.; et al. Effects of *Spartina alterniflora* invasion on the community structure and diversity of wetland soil bacteria in the Yellow River Delta. *Ecol. Evol.* **2022**, *12*, e8905. [CrossRef]
63. Rizzo, L.; Fraschetti, S.; Alifano, P.; Pizzolante, G.; Stabili, L. The alien species *Caulerpa cylindracea* and its associated bacteria in the Mediterranean Sea. *Mar. Biol.* **2016**, *163*, 4. [CrossRef]
64. Ge, Y.; Wang, Q.; Wang, L.; Liu, W.; Liu, X.; Huang, Y.; Christie, P. Response of soil enzymes and microbial communities to root extracts of the alien *Alternanthera philoxeroides*. *Arch. Agron. Soil Sci.* **2018**, *64*, 708–717. [CrossRef]
65. Wang, X.; Wang, X.; Wang, W.; Wang, J.; Yu, F. Effects of invasive plant diversity on soil microbial communities. *Diversity* **2022**, *14*, 992. [CrossRef]
66. Mieczan, T.; Płaska, W.; Adamczuk, M.; Toporowska, M.; Bartkowska, A. Effects of the invasive fish species ameiurus nebulosus on microbial communities in peat pools. *Water* **2022**, *14*, 815. [CrossRef]