

EFFECT OF PLANTING LOTUSES ON PLANKTONIC PROKARYOTES IN A SHALLOW LAKE OF A SUBTROPICAL URBAN PARK IN CHINA

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Abstract. Although urban water ecological civilization has been extensively evaluated in different regions of China, and many urban wetland parks have been built to increase urban water ecological civilization indices, the effects of the construction and renovation of freshwater ecosystems on microbiota have not been fully evaluated. To analyze the impact of lotus planting on the structure of microbiota in urban park lakes, in this study, we analyzed the differences in microbiota in different regions (artificial fountain region, potted lotus region, and direct lotus planting region) of an urban lake in subtropical China before and after lotus planting in the short term (about 10 d) and after more than a month. Our results showed that planting lotuses significantly reduced the relative abundances of water *Jannaschia*, *Fontibacter*, and *Anaerospira* comparing with the control, and that of *Lutimonas* in the sediment microbiota in the long term (more than 1 month). Moreover, after planting lotuses, the connection between prokaryotes in the sediment microbiota gradually weakened.

Keywords: artificial ecological modification, freshwater microbiota, lotus, community ecology, lake ecosystem, co-occurrence

Introduction

Freshwater ecosystems have played a crucial role in the history of humankind, providing essential goods and services for our survival and well-being (Wantzen et al., 2016; Vári et al., 2022). However, these ecosystems are facing significant threats, with a global area decline by 64% from 1997 to 2011, and for Europe by 50% from 1970 to 2008 (Costanza et al., 2014; IPBES et al., 2018; Gozlan et al., 2019). The rapid urbanization and economic development pose a challenge to the ecological health of freshwater systems in cities (Tian et al., 2021). In recent decades, China has experienced rapid urbanization, leading to severe pressure on urban water ecosystems, including water resource shortage, eutrophication, and pollution (Han et al., 2019). In response to this issue, the Ministry of Water Resources of China proposed the concept

of “water ecological civilization” in 2013, which aims to achieve a harmonious balance between urban development and the health of water environments (Tian et al., 2021).

The microbiota present in various freshwater ecosystems play a crucial role in global energy fluxes and biogeochemical processes (Shen et al., 2019; Sagova-Mareckova et al., 2021), for instance, in nitrogen (N) cycling in freshwater ecosystems (Dai et al., 2021; Liu et al., 2023a). According to Shen et al. (2019), planktonic microbiota in eutrophic ecosystems have a higher potential for aerobic carbon fixation, fermentation, methanogenesis, anammox, denitrification, and sulfur mineralization compared to mesotrophic-oligotrophic ecosystems. However, they have a lower potential for aerobic respiration, CO oxidation, nitrogen fixation, and assimilatory sulfate reduction. As a result, the bacterial eutrophic index has been proposed as a potential measure for evaluating freshwater quality (Sagova-Mareckova et al., 2021; Ji et al., 2020). Additionally, microbiota also play a significant role in maintaining the health of aquatic animals (Huang et al., 2021a; Sehnal et al., 2021).

The impact of urban water ecological civilization has been extensively evaluated in various regions of China (Tian et al., 2021; Han et al., 2019; Su et al., 2018; Den Hartog et al., 2021). Many urban wetland parks have been constructed to improve the indices of urban water ecological civilization. However, the effects of these construction and renovation projects on microbiota have not been fully evaluated. Typically, aquatic plants such as canna (*Canna generalis*) and lotus (*Nelumbo nucifera*) are planted in urban wetland parks to enhance their ornamental value. In this study, we aimed to analyze the impact of lotus planting on the structure of microbiota in an urban park lake in subtropical China. We compared the differences in microbiota in different regions of the lake (artificial fountain region, potted lotus region, and direct lotus planting region) before and after lotus planting, both in the short term (~10 d) and after more than a month.

Materials and methods

Study area and sample collection

The study area and sample treatment method were the same as those used in the previous study of Li et al. (2024). The urban park is located in Hengqin Island, Zhuhai City, China. It has an elevation of 38 meters and a lake water area of about 10,000 m². The lake (113.51 E, 22.16 N) in the park has several artificial pond foundations that divide the water area into five sections, with the water eventually flowing into a river channel from the fifth section. Artificial renovation was conducted from February 10th to March 28th, 2023, with lake renovation taking place from February 10th to March 10th and lotus planting occurring from March 26th to 28th. Over 30 varieties of lotus plants, including *Nelumbo nucifera*, *Nelumbo lutea*, *Nymphaea tetragona*, *Nymphaea alba*, *Nymphaea mexicana*, and *Nymphaea lotus* were planted. Water (W) samples were collected from ten sampling sites on February 2nd, April 18th, and May 22nd, 2023. These sites included W1, W2, and W3 in the artificial fountain region without lotus plants (C; about 2800 m² of surface area); W4, W5, and W6 in the treatment 1 region (T1; about 4600 m² of surface area), where lotuses were potted; W7, W8, and W9 in the treatment 2 region (T2; about 2600 m² of surface area), where lotuses were directly planted; and W10 in the drainage channel (Fig. 1; Li et al., 2024). The spacing between flowerpots of potted lotuses was about 20 cm, and a cement flowerpot with a diameter of about 30 cm was used in the T1 region. The planting density of lotuses in the T2 region was about 3 plants per m².

Triplicate water samples were collected from each sampling site, approximately 0.2 m below the water surface using a 5-L Niskin bottle. The samples were mixed and immediately stored in sterile sampling bags (LABPLAS, Canada). They were then transported to the laboratory on ice. Approximately 500-mL of water from each sample was filtered through polycarbonate membranes with an aperture of 0.22 μm (Millipore, USA). The filters were stored at -80°C for DNA extraction. Any remaining water samples were used to determine the physical and chemical factors. Additionally, sediment (S) samples were collected from 0–5 cm using a cylindrical sampler and transported to the laboratory in ice bags. They were then stored at -20°C . The sampling sites were divided into different regions: S1, S2, and S3 were in the artificial fountain region without lotus plants (C); S4, S5, and S6 were in the treatment 1 region (T1), where lotus plants were potted; S7, S8, and S9 were in the treatment 2 region (T2), where lotus plants were directly planted; and S10 was in the drainage channel (*Fig. 1*; Li et al., 2024).

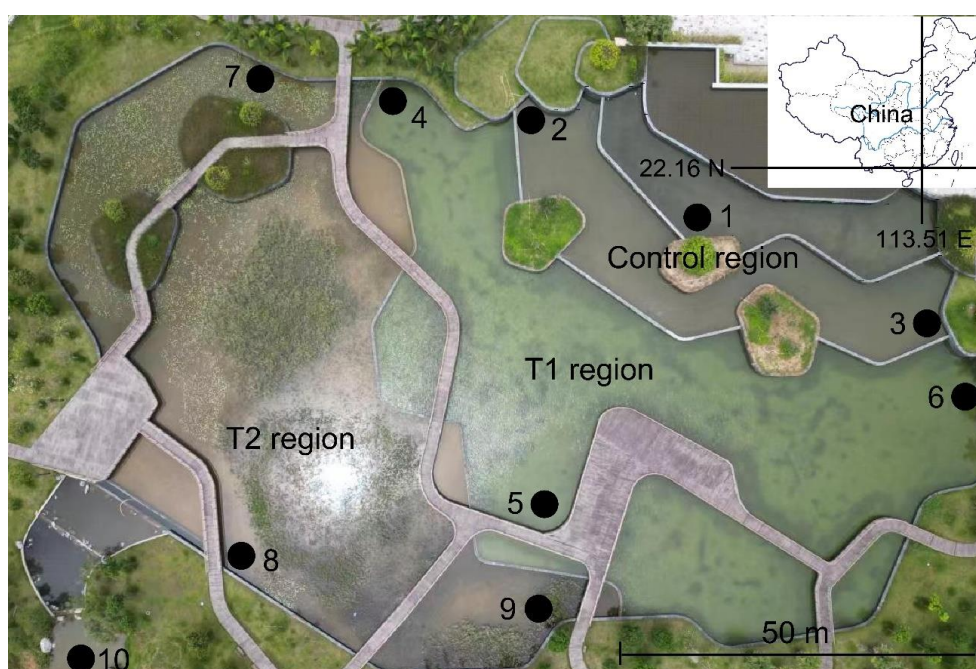


Figure 1. Distribution of sampling sites. Control region was the artificial fountain region did not plant lotuses. Lotuses were potted in the T1 region. Lotuses were directly planted in the T2 region

Determination of water physical and chemical indicators

The water temperature (WT), pH, salinity (Sal), dissolved oxygen (DO), oxidation-reduction potential (ORP), conductivity (Cond), and total dissolved solids (TDS) were measured using a smart portable multi-parameter water quality analyzer (YSI, USA). The dissolved oxygen saturation (DO%) was calculated according to the National Environmental Protection Standards of the People's Republic of China (HJ 506-2009). Approximately 500-mL of water was filtered using a Whatman GF/C filter membrane and used to measure the chlorophyll-a content (Chla) according to a previously described method (Vinten et al., 2021). Turbidity was determined according to the international standard method (ISO 7027-1:2016). Suspended particulate matter (SPM) was determined as described previously (He et al., 2017). The concentrations of

ammonium (NH₄-N), nitrate (NO₃-N), nitrite (NO₂-N), total nitrogen (TN), total phosphorus (TP), and SiO₃-Si were determined using a flow injection water quality analyzer (Skalar, Netherlands). The permanganate index (COD_{Mn}) and phosphate (PO₄-P) content were determined according to standard methods (Huang et al., 2000). The concentration of un-ionized ammonia (NH₃) was calculated according to a previously described method (Zou and Cheng, 2002).

Analysis of PEC composition

Water and sediment DNA was extracted using a FastDNA Spin kit for soil (MP, Eschwege, Germany). The V4-V5 region of the prokaryotic SSU rRNA gene was amplified using the eukaryotic primer pair 515F (5'-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') (Huang et al., 2021b) with a 12-nt sample-specific barcode sequence at the 5' end of the 515F primer (Fu et al., 2023), according to a previously described method (Huang et al., 2021b). The amplification products were purified using a gel recovery kit (Axygen, CA, USA) and sequenced on the HiSeq sequencing platform at Guangdong Meilikang Bio-Science Ltd. (Foshan, Guangdong, China).

The raw reads were merged using FLASH version 1.2.8 (Magoč and Salzberg, 2011) and quality-controlled using QIIME 1.9.0 (Caporaso et al., 2010). Chimeric sequences were detected and removed using UCHIME 4.2.40 (Edgar et al., 2011) before further analysis. The remaining effective sequences were clustered into operational taxonomic units (OTUs) with 97% identity using UPARSE 7.0.1090 (Edgar, 2013). The taxonomy of each OTU was assigned using RDP classifier 2.2 (Wang et al., 2007) with the SILVA 132 dataset. The OTU number, Shannon, and Chao1 indices of each PEC were calculated using QIIME 1.9.0.

Data analysis

A Kruskal-Wallis rank sum test with Dunn's post-hoc test was performed using R 4.2.3 (R Core Team, 2022) with the FSA package version 0.9.5 (Ogle et al., 2023). Principal co-ordinate analysis (PCoA) based on weighted UniFrac distance was conducted using QIIME 1.9.0. Linear discriminant analysis (LDA) effect size (LEfSe) was conducted using the Galaxy platform (<http://huttenhower.sph.harvard.edu/galaxy>). A distance-based redundancy analysis (db-RDA) was performed using the R vegan package (Dixon, 2003). A heatmap profile was drawn using the pheatmap version 1.0.12 package (Kolde, 2019) in R 4.2.3. Differences were considered statistically significant at $P < 0.05$.

Results

Overall, total of 3,453,073 effective sequences were obtained from the 60 samples. Finally, 18,060 effective sequences were randomly resampled from each sample for further analysis. Total of 81,504 OTUs were detected from these effective sequences. PCoA results based on the OTU compositions showed that water and sediment microbiota were divided into two groups, and water microbiota were divided into three sub-groups according to sampling times, whereas sediment microbiota were not divided into different sub-groups (Fig. 2A). Moreover, obvious differences in the water microbiota structure before and after planting lotuses were exhibited, whereas there was no difference detected in the sediment microbiota (Fig. 2A). The OTU number of water

and sediment microbiota in the T2 region before lotus planting, as well as the Chao1 index of sediment microbiota, were significantly higher than those in the Control and T1 regions ($P < 0.05$), and these differences were not significant after lotus planting ($P > 0.05$; Fig. 2B, C). Shannon index did not show significant differences ($P > 0.05$; Fig. 2D). The Good's coverage of sediment microbiota in the T2 region before lotus planting was significantly lower than those of the Control and T1 regions ($P < 0.05$), and the difference was not significant after lotus planting ($P > 0.05$; Fig. 2E).

As PCoA results, UPGMA results based on the OTU compositions also showed that water and sediment microbiota were divided into two groups, except S1Feb and S1Feb samples (Fig. 3). Moreover, the samples exhibited a clustering trend according to sampling times (Fig. 3). At the phylum level, Proteobacteria, Bacteroidetes, Cyanobacteria, Planctomycetes, Acidobacteria, Firmicutes, Actinobacteria, Chloroflexi, Gemmatimonadetes, Spirochaetes, Chlorobi, Nitrospirae, Fusobacteria, WS3, Verrucomicrobia, and OP8 dominated the microbiota (Fig. 3).

At the genus level, water microbiota of the control, treatment1, and treatment2 regions before planting lotuses were clustered separately according to the dominant genus compositions (Fig. 4A). The relative abundances of *Fontibacter* and *Idiomarina* in the T1 region were significantly higher than those in the control (Fig. 4B, H). The relative abundances of *Maribacter* and *Candidatus Aquiluna* in the T2 region were significantly higher than those in the control (Fig. 4C, K). Moreover, the relative abundance of *Marivita* in the T1 region was significantly lower than that in the control (Fig. 4G).

In the short term (~10 days) after planting lotuses, the water microbiota of the control, treatment1, and treatment2 regions were not clustered separately according to the dominant genus compositions (Fig. 5A). The relative abundances of *Aquirestis*, *Rhodobacter*, *Rheinheimera*, and *Halomonas* in the T2 region were significantly higher than those in the control ($P < 0.05$; Fig. 5B–D, J). The relative abundances of *Aquiflexum*, and *Polaribacter* in the T1 region were significantly higher than those in the control ($P < 0.05$; Fig. 5E, F). The relative abundance of *Limnohabitans* in the T1 region were significantly higher than that in the control ($P < 0.05$; Fig. 5G). Moreover, the relative abundance of *Pseudidiomarina* in the T2 region was significantly lower than that in the control ($P < 0.05$; Fig. 5H).

In the long term (more than one month) after planting lotuses, water microbiota of the control, T1, and T2 regions were clustered separately according to the dominant genus compositions (Fig. 6A). The relative abundances of *Anaeromyxobacter* and *Hydrogenophaga* in T2 region were significantly higher than those in the control (Fig. 6J, K). The relative abundances of *Flavobacterium* and *Lewinella* in the T2 region were significantly higher than those in the T1 region (Fig. 6B, F). Moreover, planting lotuses significantly reduced the relative abundances of *Jannaschia*, *Fontibacter*, and *Anaerospira* comparing with the control (Fig. 6C, G, M).

Based on the dominant genus compositions, sediment microbiota before planting lotuses were not clustered according to the control, T1, and T2 regions (Fig. 7A). The relative abundances of *Geobacter* and *Gallionella* in the T2 region were significantly higher than other regions (Fig. 7B, C). The relative abundance of *Dechloromonas* in the T2 region was significantly higher than that of the control (Fig. 7D). The relative abundances of *Fontibacter*, *Microcystis*, and *Aquiflexum* in the T1 region were significantly higher than those in the T2 region (Fig. 7E–7G). The relative abundance of *Paracoccus* in the T2 region was significantly lower than that in the control (Fig. 7H). The relative abundance of *Fluviicola* in the T2 region was significantly higher than that in the T1 region (Fig. 7I).

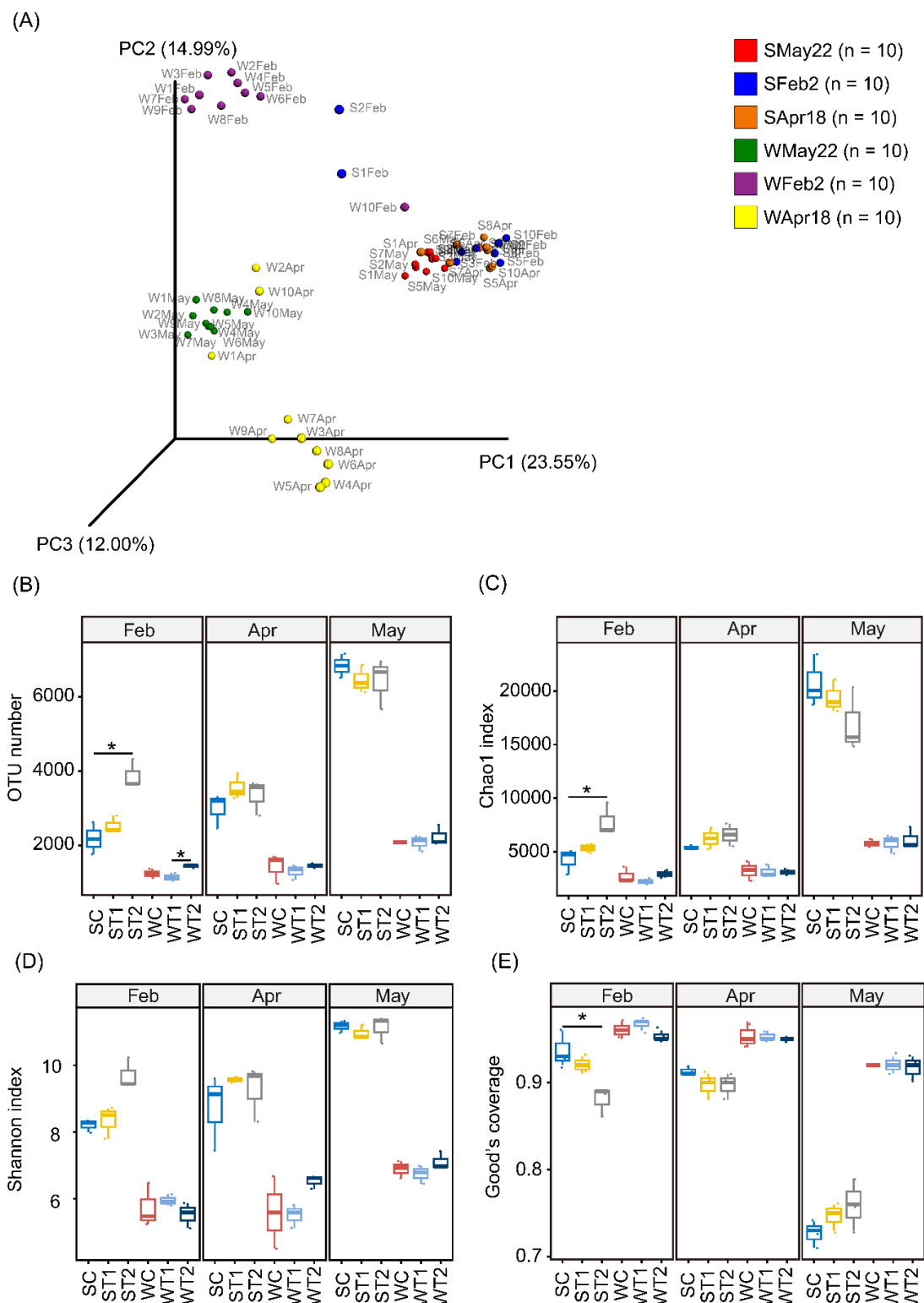


Figure 2. PCoA profile (A) and boxplots (B-F) shown the overall differences of freshwater and sediment microbiota structure and α -diversity indices. (B) OTU number; (C) Chao1 index; (D) Shannon index; (E) Simpson index; (F) Good's coverage. The capital S and W at the beginning of the sample names represent sediment and water samples, respectively. C, T1, and T2 indicate the samples collected from the region did not plant lotuses (W1-W3), the region potted lotuses (W4-W6), and the region directly planted lotuses (W7-W9), respectively. OTU, operational taxonomic unit. * $P < 0.05$

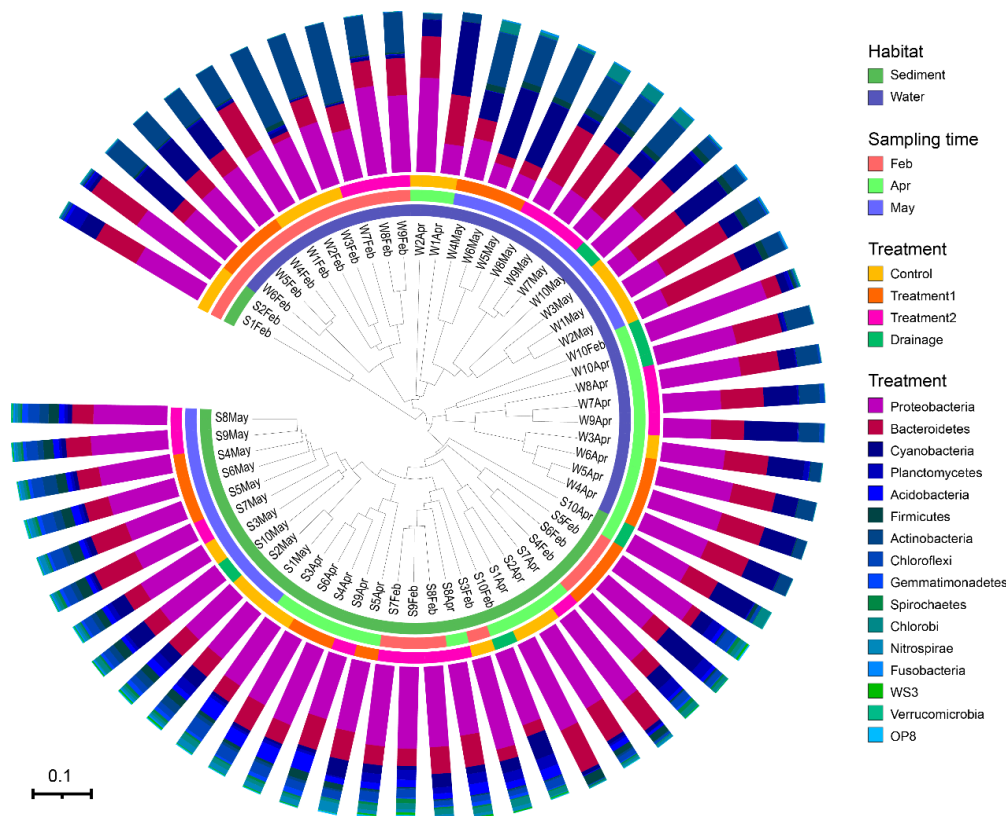


Figure 3. Composition of dominant phyla in the freshwater and sediment microbiota in a lotus-planting lake

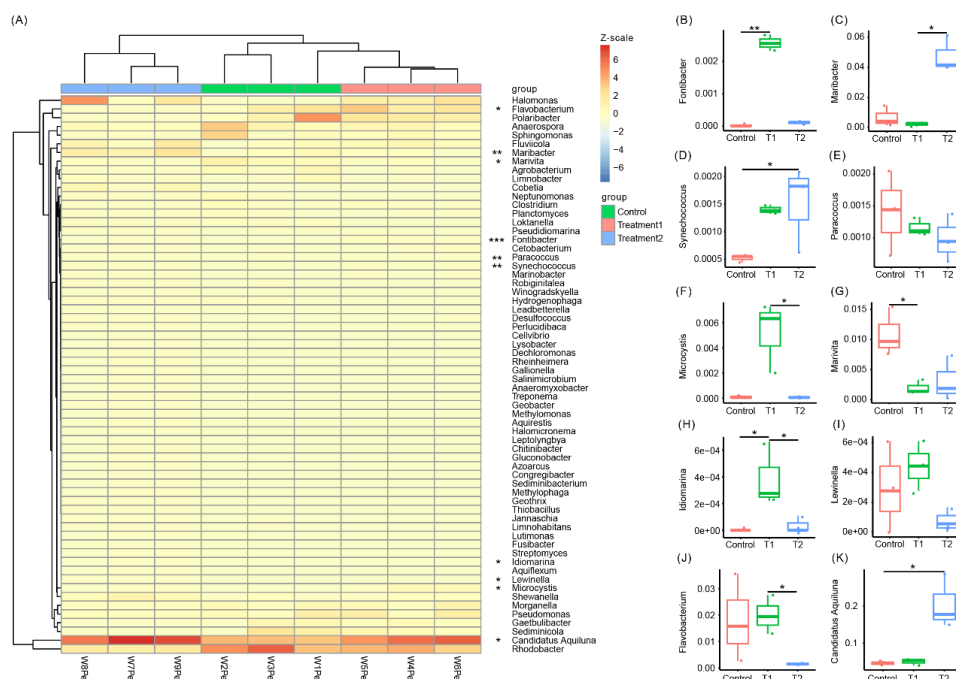


Figure 4. Heatmap profile (A) and boxplots (B-K) shown the differences of relative abundances of major dominant genera in freshwater microbiota collected in February. Control, region did not plant lotuses (W1-W3); T1, treatment 1 region where lotuses were potted (W4-W6); T2, treatment 2 region where lotuses were planted directly (W7-W9). * $P < 0.05$; ** $P < 0.01$

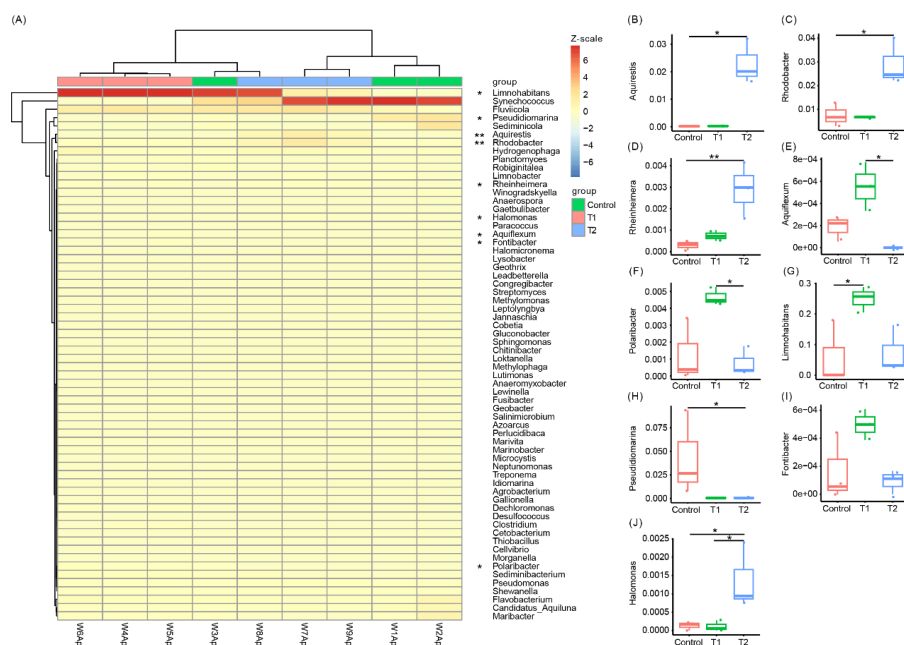


Figure 5. Heatmap profile (A) and boxplots (B-K) shown the differences of relative abundances of major dominant genera in freshwater microbiota collected in April. Control, region did not plant lotuses (W1-W3); T1, treatment 1 region where lotuses were potted (W4-W6); T2, treatment 2 region where lotuses were planted directly (W7-W9). * $P < 0.05$; ** $P < 0.01$

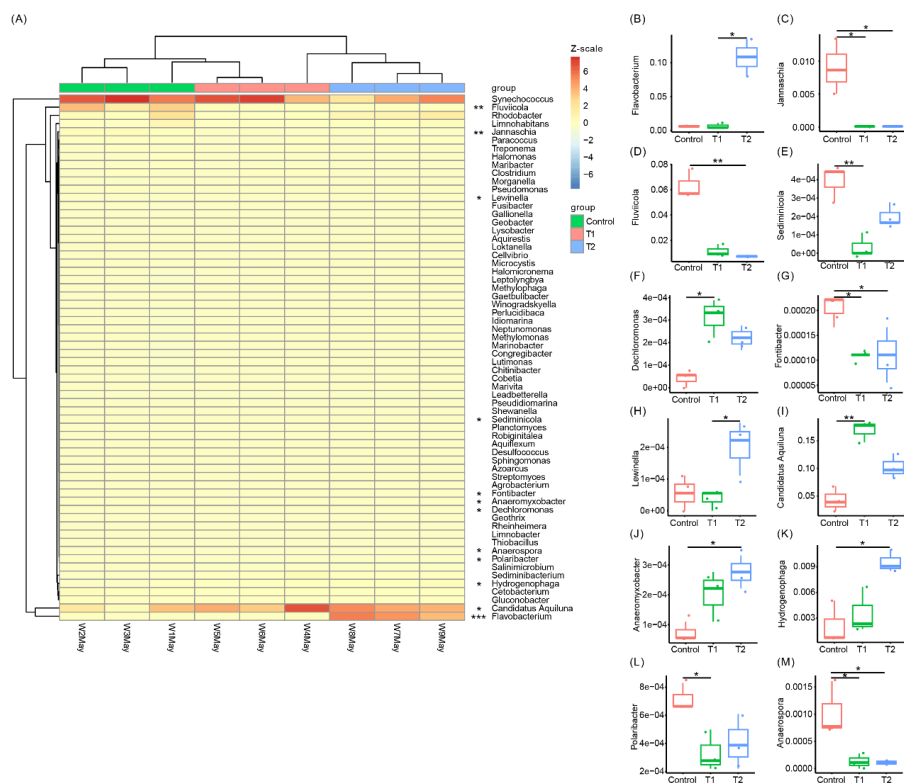


Figure 6. Heatmap profile (A) and boxplots (B-K) shown the differences of relative abundances of major dominant genera in freshwater microbiota collected in May. Control, region did not plant lotuses (W1-W3); T1, treatment 1 region where lotuses were potted (W4-W6); T2, treatment 2 region where lotuses were planted directly (W7-W9). * $P < 0.05$; ** $P < 0.01$

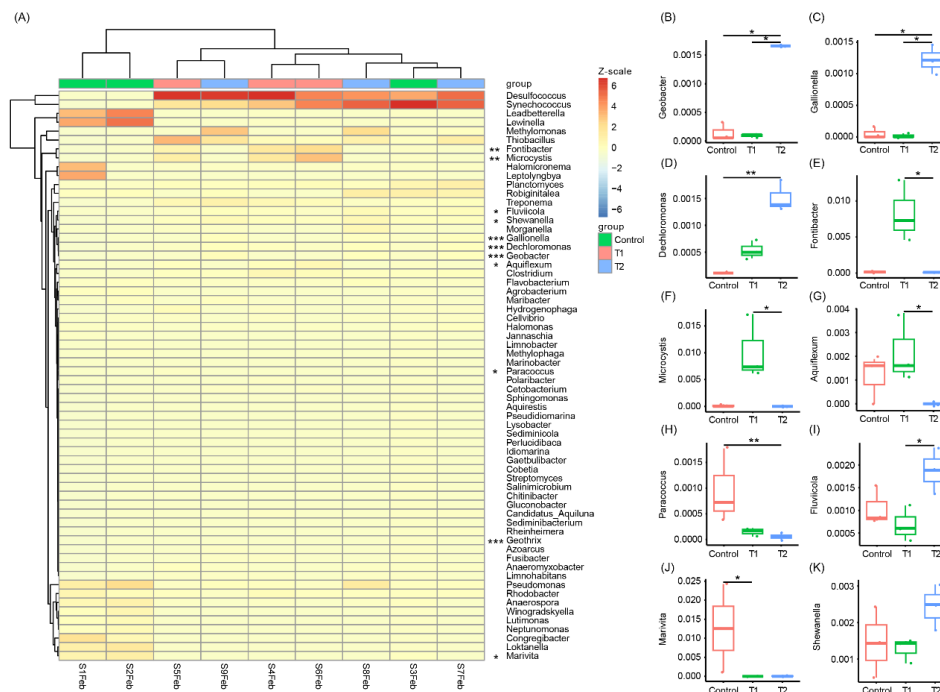


Figure 7. Heatmap profile (A) and boxplots (B-K) shown the differences of relative abundances of major dominant genera in sediment microbiota collected in February. Control, region did not plant lotuses (S1-S3); T1, treatment 1 region where lotuses were potted (S4-S6); T2, treatment 2 region where lotuses were planted directly (S7-S9). * $P < 0.05$; ** $P < 0.01$

In the short term (about 10 days) after planting lotuses, sediment microbiota were also not clustered according to the control, T1, and T2 regions (Fig. 8A). The relative abundances of *Candidatus Aquiluna* and *Thiobacillus* in the T1 region were significantly higher than those in the T2 region (Fig. 8B, C). Moreover, the relative abundances of *Aquiflexum* and *Lewinella* in the T1 region were significantly higher than those in the control (Fig. 8D, E).

In the long term (more than one month) after planting lotuses, sediment microbiota were still not clustered according to the control, T1, and T2 regions (Fig. 9A). The relative abundances of *Marinobacter* and *Fusibacter* in the T2 region were significantly higher than those in the control ($P < 0.05$; Fig. 9B, J), whereas the relative abundances of *Congregibacter*, *Halomicronema*, and *Synechococcus* in the T2 region were significantly lower than those in the control ($P < 0.05$; Fig. 9D, F, H). The relative abundance of *Robiginitalea* in the T1 region was significantly lower than that in the control ($P < 0.05$; Fig. 9C). Moreover, planting lotuses significantly reduced the relative abundances of *Lutimonas* in the sediment microbiota ($P < 0.05$; Fig. 9G).

Spearman correlation analysis of dominant genera in the water and sediment microbiota and water physicochemical indicators divided these dominant genera into three groups: Group 1, genera with similar correlations with the water physicochemical indicators, which was contained *Anaeromyxobacter*, *Azoarcus*, *Cetobacterium*, *Dechloromonas*, *Desulfococcus*, *Gluconobacter*, *Hydrogenophaga*, *Limnohabitans*, *Salinimicrobium*, *Sediminibacterium*, and *Treponema*; Group 2, genera with opposite correlations with the water physicochemical indicators, which was contained *Agrobacterium*, *Morganella*, *Sediminicola*, and *Sphingomonas*; and Group 3, other genera (Fig. 10).

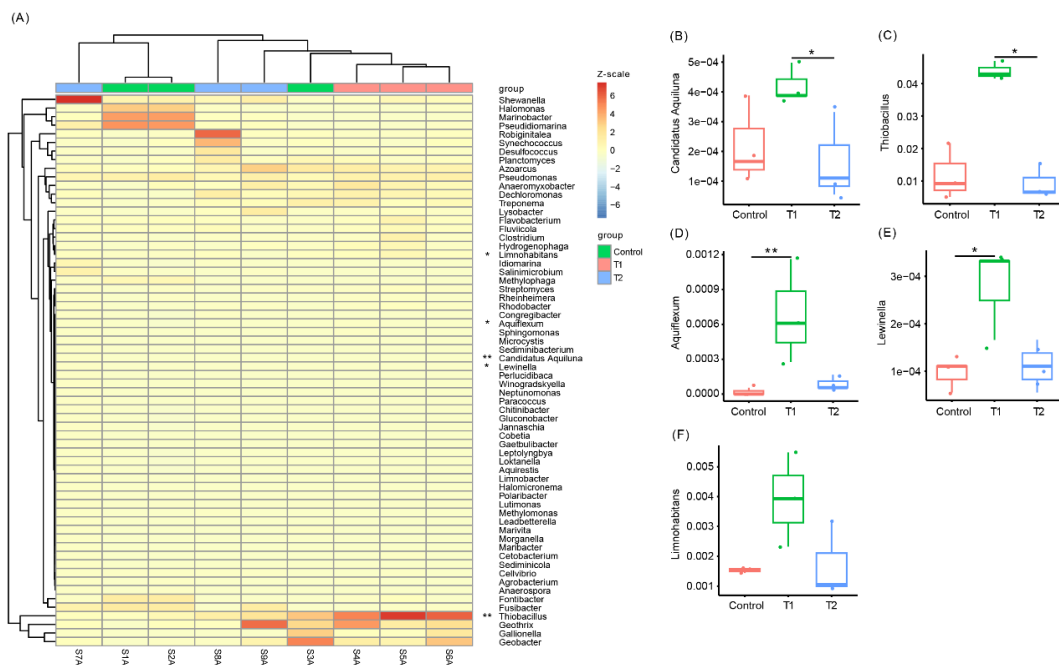


Figure 8. Heatmap profile (A) and boxplots (B-K) shown the differences of relative abundances of major dominant genera in sediment microbiota collected in April. Control, region did not plant lotuses (S1-S3); T1, treatment 1 region where lotuses were potted (S4-S6); T2, treatment 2 region where lotuses were planted directly (S7-S9). * $P < 0.05$; ** $P < 0.01$

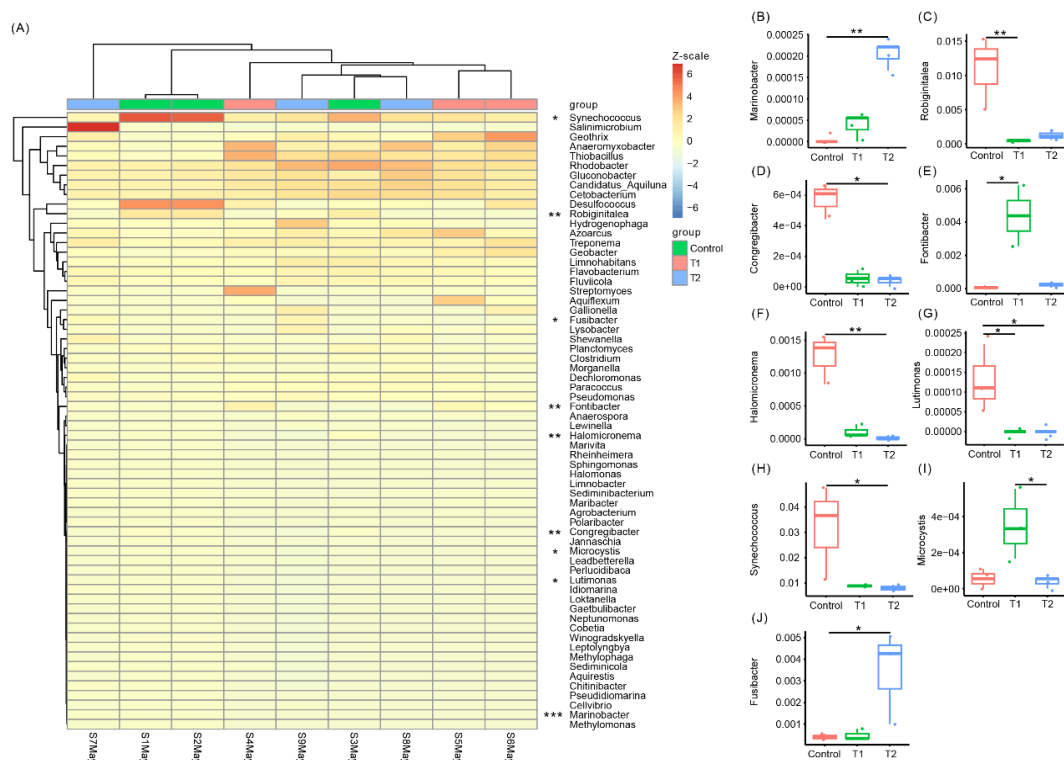


Figure 9. Heatmap profile (A) and boxplots (B-K) shown the differences of relative abundances of major dominant genera in sediment microbiota collected in May. Control, region did not plant lotuses (S1-S3); T1, treatment 1 region where lotuses were potted (S4-S6); T2, treatment 2 region where lotuses were planted directly (S7-S9). * $P < 0.05$; ** $P < 0.01$

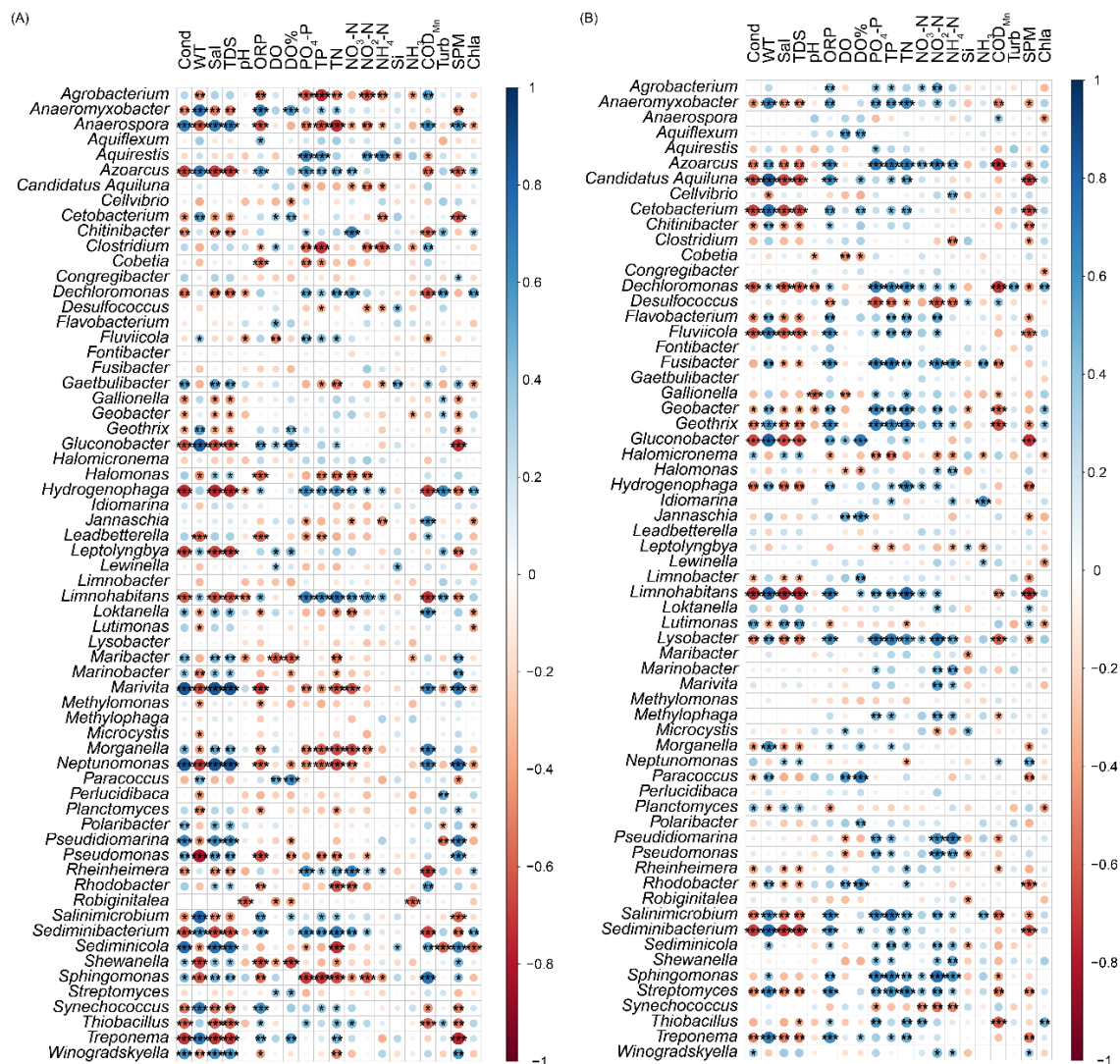


Figure 10. Correlation between major bacterial genera in freshwater (A) and sediment (B) microbiota and physical and chemical parameters. Cond, conductivity; WT, water temperature; Sal, salinity; TDS, total dissolved solids; ORP, oxidation-reduction potential; DO, dissolved oxygen; DO%, dissolved oxygen saturation; TP, total phosphorus; TN, total nitrogen; NH₃, un-ionized ammonia; CODMn, permanganate index; Turb, turbidity; SPM, suspended particulate matter; Chla, chlorophyll-a content. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Especially, the relative abundance of water *Agrobacterium* was significantly negatively correlated with WT, ORP, PO₄-P, TP, TN, NO₂-N, NO₄-N, and NH₃, and significantly positively correlated with COD_{Mn}, whereas the relative abundance of sediment *Agrobacterium* was significantly positively correlated with ORP, PO₄-P, TP, NO₃-N, and NO₂-N ($P < 0.05$; Fig. 10). The relative abundances of both water and sediment microbiota were significantly positively correlated with WT, ORP, TP, and TN, and significantly negatively correlated with Cond, Sal, TDS, and SPM ($P < 0.05$; Fig. 10). The relative abundances of both water and sediment *Azoarcus* were significantly positively correlated with WT, ORP, PO₄-P, TP, TN, and NO₃-N, and significantly negatively correlated with Cond, Sal, TDS, COD_{Mn}, and SPM ($P < 0.05$; Fig. 10). The relative abundances of water and sediment *Cetobacterium* were significantly positively

correlated with WT, and DOP, and significantly negatively correlated with Cond, Sal, TDS, and SPM ($P < 0.05$; Fig. 10). The relative abundances of both water and sediment *Dechloromonas* were significantly negatively correlated with Cond, Sal, TDS, pH, and COD_{Mn}, and significantly positively correlated with PO₄-P, TP, TN, NO₃-N, Turb, and Chla ($P < 0.05$; Fig. 10). The relative abundances of both water and sediment *Desulfococcus* were significantly positively correlated with Si, and significantly negatively correlated with TP, NO₂-N, and NH₄-N ($P < 0.05$; Fig. 10). The relative abundances of both water and sediment *Gluconobacter* were significantly positively correlated with WT, ORP, DO, DO%, and TN, and significantly negatively correlated with Cond, Sal, TDS, and SPM ($P < 0.05$; Fig. 10). The relative abundances of both water and sediment *Hydrogenophaga* were significantly positively correlated with ORP, TP, TN, NO₃-N, and NO₂-N, and significantly negatively correlated with Cond, Sal, TDS, and SPM ($P < 0.05$; Fig. 10). The relative abundances of both water and sediment *Limnohabitans* were significantly positively correlated with WT, ORP, PO₄-P, TP, TN, NO₃-N, and NO₂-N, and significantly negatively correlated with Cond, Sal, TDS, COD_{Mn}, and SPM ($P < 0.05$; Fig. 10). The relative abundances of both water and sediment *Salinimicrobium* were significantly positively correlated with WT, ORP, and TN, and significantly negatively correlated with Cond, Sal, TDS, and SPM ($P < 0.05$; Fig. 10). The relative abundances of both water and sediment *Sediminibacterium* were significantly positively correlated with WT, ORP, TP, and TN, and significantly negatively correlated with Cond, Sal, TDS, and SPM ($P < 0.05$; Fig. 10). The relative abundances of both water and sediment *Treponema* were significantly positively correlated with WT, ORP, DOP, and TN, and significantly negatively correlated with Cond, Sal, TDS, and SPM ($P < 0.05$; Fig. 10). The relative abundance of water *Morganella* was significantly positively correlated with Cond, Sal, TDS, and COD_{Mn}, and significantly negatively correlated with WT, ORP, PO₄-P, TP, TN, NO₃-N, and NO₂-N, whereas that of sediment *Morganella* was significantly positively correlated with WT, ORP, DOP, and TP, and significantly negatively correlated with Cond, Sal, TDS, and SPM ($P < 0.05$; Fig. 10). The relative abundance of water *Sediminicola* was significantly positively correlated with Cond, Sal, TDS, Si, COD_{Mn}, and SPM, and significantly negatively correlated with WT, PO₄-P, TN, Turb, and Chla, whereas that of sediment *Sediminicola* was significantly positively correlated with WT, ORP, PO₄-P, TP, TN, NO₂-N, and NH₄-N, and significantly negatively correlated with Si ($P < 0.05$; Fig. 10). The relative abundance of water *Sphingomonas* was significantly positively correlated with Cond, Sal, TDS, COD_{Mn}, and SPM, and significantly negatively correlated with WT, PO₄-P, TP, TN, NO₃-N, NO₂-N, NH₄-N, whereas that of sediment *Sphingomonas* was significantly positively correlated with WT, ORP, PO₄-P, TP, TN, NO₃-N, NO₂-N, and NH₄-N, and significantly negatively correlated with COD_{Mn} ($P < 0.05$; Fig. 10). These results indicated that although not absolute, in most cases the water genera in the Group 1 were significantly positively correlated with WT, and N and P nutrients, and significantly negatively correlated with Cond, Sal, TDS, COD_{Mn}, and SPM, whereas the water genera in the Group 2 was significantly negatively correlated with WT, and N and P nutrients, and significantly positively correlated with Cond, Sal, TDS, and COD_{Mn}.

Discussion

Lakes serve as important “hotspots” for carbon and nutrient cycling in the landscape, receiving nutrients from surrounding terrestrial ecosystems (Cotner et al., 2010; Butman

et al., 2016; Linz et al., 2018). The health of urban water bodies is crucial for human society, especially in the face of expanding human populations and anthropogenic stressors (Xiao et al., 2021). Microorganisms play a critical role in the transformations and cycling of biologically active elements in freshwater ecosystems (Newton et al., 2011). This key role is not only due to their function as primary degraders and mineralizers of organic compounds, but also their biomass production and trophic coupling to eukaryote predators, which have a profound impact on elemental fluxes and water quality in the ecosystem (Newton et al., 2011; Sagova-Mareckova et al., 2021). Conversely, water quality can also influence the community structure of aquatic microorganisms, which in turn affects their elemental cycling functioning (Yan et al., 2018). Our preliminary research has shown that planting lotuses can alter the physical and chemical indicators of water in an artificial landscape lake, ultimately impacting water quality (Li et al., 2024). Additionally, our previously study found that although planting lotuses did not significantly alter the water physical properties in various regions of the pond in the short-term, such as WT, Cond, Sal, and TDS, it did affect nutrient concentrations (Li et al., 2024). In this study, our results indicated that most dominant genera in water and sediment microbiota were significantly correlated with water physicochemical indicators (Fig. 10). Moreover, our results indicated that planting lotuses significantly reduced the relative abundances of *Jannaschia*, *Fontibacter*, and *Anaerospora* comparing with the control.

The concentration of nitrogen and phosphorus is currently a key concern for freshwater monitoring in China, mainly because they are important nutritional factors that trigger freshwater algal blooms (Cotner et al., 2010; Linz et al., 2018; Su, 2021). Phosphorus deficiency is one of the main factors limiting the growth of aquatic microbiota (Cotner et al., 2010). Our previously study results indicated that water PO₄-P and TP concentrations in the lake were significantly increased after planting lotuses in a short-term (~10 days) (Li et al., 2024). *Anaerospora hongkongensis* was sensitive to trophic variation and thus could be used as eco-markers (Li et al., 2024). Bao and Li (2017) report a stable consortium, HJ-4, composed of *Anaerospora hongkongensis* (85%) and facultative anaerobe, *Comamonadaceae* (15%), which can process ferrihydrite reduction coupled to anaerobic ammonium oxidation driven by sulfur redox cycling. Li et al. (2017) found excessive input of phosphorus significantly affects microbial Fe(III) reduction in flooded paddy soils by changing the abundances and community structure of *Clostridium* and *Geobacteraceae*. The relative abundance of *Aquirestis* was correlated with water quality improvement (Li et al., 2022). Plisova et al. (2005) reported a highly active alkaline phosphatase from the marine bacterium *Cobetia*. However, in this study, our results indicated that the relative abundances of water *Agrobacterium*, *Anaerospora*, *Clostridium*, *Cobetia*, *Leadbetterella*, *Marivita*, *Morganella*, *Neptunomonas*, and *Sphingomonas* significantly negatively correlated with water PO₄-P and TP concentrations, and the relative abundances of water *Aquirestis*, *Azoarcus*, *Dechloromonas*, *Fluviicola*, *Hydrogenophaga*, *Limnohabitans*, *Rheinheimera*, *Salinimicrobium*, and *Sediminibacterium* significantly positively correlated with water PO₄-P and TP concentrations (Fig. 10A).

Microbial interaction is an important prerequisite for maintaining the stability of microbiota structure and function (Zhou et al., 2022). Therefore, the interaction between microorganisms in a single habitat have been extensively studied (Milici et al., 2016; Gao et al., 2022; Codello et al., 2022; Ma et al., 2023). However, there is still insufficient research on the interactions between microorganisms in different habitats.

In this study, the water and sediment dominant genera were divided into three groups and although not absolute, in most cases the water genera in the Group 1 were significantly positively correlated with WT, and N and P nutrients, and significantly negatively correlated with Cond, Sal, TDS, COD_{Mn}, and SPM, whereas the water genera in the Group 2 was significantly negatively correlated with WT, and N and P nutrients, and significantly positively correlated with Cond, Sal, TDS, and COD_{Mn}. These results implied that the sediment microbiota may be an important supplement to the microorganisms involved in nutrient metabolism in water bodies.

Cluster analysis based on microbiota structure can be used to assess the impact of external factors on microbiota structure, such as season, geography, and human activities (Cotner et al., 2010; Liao et al., 2017; Liu et al., 2023b). Our cluster analysis, based on the dominant genus compositions, revealed that planting lotuses did not significantly affect the community structure of dominant genera in the pond sediment microbiota. Additionally, the structure of dominant genera in the sediment microbiota in each region was more consistent with that of water microbiota. Before lotus planting, there were differences in the structure of the water microbiota among regions of the pond, but these differences disappeared approximately 10 days after planting lotuses. However, after about 1 month, differences in the water microbiota structure reappeared, although the dominant genera with significant differences among regions were different from those before lotus planting. These results indicated that the impact of lotus planting on the water microbiota was greater than that on the sediment microbiota. Furthermore, the water microbiota structure may have been homogenized in the short term due to disturbance from lotus planting, whereas the long-term impact of lotus planting resulted in changes to the water microbiota structure.

Freshwater microbiota is an important factor affecting the microbiota and health of freshwater insects and vertebrates (Sehna et al., 2021; Zeng et al., 2020; Padeniya et al., 2022). Moreover, regional water microbiota was also connected with human longevity and welfare (Wu et al., 2021). However, in this study, we did not analyze the impact of changes in lake water and sediment microbiota caused by planting lotuses on human welfare. Further study about the impact should be conducted, especially to assess whether it increase the distribution and transmission of opportunistic pathogens.

Conclusions

Planting lotuses significantly reduced the relative abundances of water *Jannaschia*, *Fontibacter*, and *Anaerospira* comparing with the control, and that of *Lutimonas* in the sediment microbiota in the long term (more than 1 month). Moreover, after planting lotuses, the connection between prokaryotes in the sediment microbiota gradually weakened.

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REFERENCES

- [1] Bao, P., Li, G. X. (2017): Sulfur-driven iron reduction coupled to anaerobic ammonium oxidation. – *Environmental Science & Technology* 51: 6691-6698. DOI: 10.1021/acs.est.6b05971.
- [2] Butman, D., Stackpoole, S., Stets, E., McDonald, C. P., Clow, D. W., Striegl, R. G. (2016): Aquatic carbon cycling in the conterminous United States and implications for terrestrial carbon accounting. – *Proceedings of the National Academy of Sciences* 113: 58-63. DOI: 10.1073/pnas.1512651112.
- [3] Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenkov, T., Zaneveld, J., Knight, R. (2010): QIIME allows analysis of high-throughput community sequencing data. – *Nature Methods* 7: 335-336. DOI: 10.1038/nmeth.f.303.
- [4] Codello, A., Hose, G. C., Chariton, A. (2022): Microbial co-occurrence networks as a biomonitoring tool for aquatic environments: a review. – *Marine and Freshwater Research* 74: 409-422. DOI: 10.1071/MF22045.
- [5] Costanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S. J., Kubiszewski, I., Farber, S., Turner, R. K. (2014): Changes in the global value of ecosystem services. – *Global Environment Change* 26: 152-158. DOI: 10.1016/j.gloenvcha.2014.04.002.
- [6] Cotner, J. B., Hall, E. K., Scott, J. T., Heldal, M. (2010): Freshwater bacteria are stoichiometrically flexible with a nutrient composition similar to seston. – *Frontiers in Microbiology* 1: 132. DOI: 10.3389/fmicb.2010.00132.
- [7] Dai, X., Feng, J., Chen, Y., Huang, S., Shi, X., Liu, X., Sun, Y. (2021): Traditional Chinese medicine in nonalcoholic fatty liver disease: molecular insights and therapeutic perspectives. – *Chinese Medicine* 16: 68. DOI: 10.1186/s13020-021-00469-4.
- [8] Den Hartog, H. (2021): Engineering an ecological civilization along Shanghai's main waterfront and coastline: evaluating ongoing efforts to construct an urban eco-network. – *Frontiers in Environmental Science* 9: 639739. DOI: 10.3389/fenvs.2021.639739.
- [9] Dixon, P. (2003): VEGAN, a package of R functions for community ecology. – *Journal of Vegetation Science* 14(6): 927-930.
- [10] Edgar, R. C. (2013): UPARSE: highly accurate OTU sequences from microbial amplicon reads. – *Nature Methods* 10: 996-998. DOI: 10.1038/nmeth.2604.
- [11] Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., Knight, R. (2011): UCHIME improves sensitivity and speed of chimera detection. – *Bioinformatics* 27: 2194-2200. DOI: 10.1093/bioinformatics/btr381.
- [12] Fu, C., Ni, J., Huang, R., Gao, Y., Li, S., Li, Y., Li, J., Zhong, K., Zhang, P. (2023): Sex different effect of antibiotic and probiotic treatment on intestinal microbiota composition in chemically induced liver injury rats. – *Genomics* 115: 110647. DOI: 10.1016/j.ygeno.2023.110647.
- [13] Gao, C., Xu, L., Montoya, L., Madera, M., Hollingsworth, J., Chen, L., Purdom, E., Singan, V., Vogel, J., Huttmacher, R. B., Dahlberg, J. A., Coleman-Derr, D., Lemaux, P. G., Taylor, J. W. (2022): Co-occurrence networks reveal more complexity than community composition in resistance and resilience of microbial communities. – *Nature Communications* 13: 3867. DOI: 10.1038/s41467-022-31343-y.
- [14] Gozlan, R. E., Karimov, B. K., Zadereev, E., Kuznetsova, D., Brucet, S. (2019): Status, trends, and future dynamics of freshwater ecosystems in Europe and Central Asia. – *Inland Waters* 9: 78-94. DOI: 10.1080/20442041.2018.1510271.
- [15] Han, H., Li, H., Zhang, K. (2019): Urban water ecosystem health evaluation based on the improved fuzzy matter-element extension assessment model: case study from Zhengzhou

- City, China. – Mathematical Problems in Engineering 2019: 1-14. DOI: 10.1155/2019/7502342.
- [16] He, Q., Qiu, Y., Liu, H., Sun, X., Kang, L., Cao, L., Li, H., Ai, H. (2017): New insights into the impacts of suspended particulate matter on phytoplankton density in a tributary of the Three Gorges Reservoir, China. – Scientific Reports 7: 13518.
- [17] Hu, S., He, R., Wang, W., Zhao, D., Zeng, J., Huang, R., Duan, M., Yu, Z. (2021): Composition and co-occurrence patterns of *Phragmites Australis* rhizosphere bacterial community. – Aquatic Ecology 55: 695-710. DOI: 10.1007/s10452-021-09855-4.
- [18] Huang, R., Crowther, T. W., Sui, Y., Sun, B., Liang, Y. (2021b): High stability and metabolic capacity of bacterial community promote the rapid reduction of easily decomposing carbon in soil. – Communications Biology 4: 1376. DOI: 10.1038/s42003-021-02907-3.
- [19] Huang, X. F. (2000): Survey, Observation and Analysis of Lake Ecology. – China Standard Press, Beijing, pp. 1-242.
- [20] Huang, Z., Hou, D., Zhou, R., Zeng, S., Xing, C., Wei, D., Deng, X., Yu, L., Wang, H., Deng, Z., Weng, S., Ning, D., Xiao, C., Yan, Q., Zhou, J., He, Z., He, J. (2021a): Environmental water and sediment microbial communities shape intestine microbiota for host health: the central dogma in an anthropogenic aquaculture ecosystem. – Frontiers in Microbiology 12: 772149. DOI: 10.3389/fmicb.2021.772149.
- [21] IPBES (2018): IPBES Guide on the Production of Assessments. – IPBES Secretariat, Bonn.
- [22] Ji, B., Liang, J., Chen, R. (2020): Bacterial eutrophic index for potential water quality evaluation of a freshwater ecosystem. – Environmental Science and Pollution Research 27: 32449-32455. DOI: 10.1007/s11356-020-09585-4.
- [23] Kolde, R. (2019): Implementation of heatmaps that offers more control over dimensions and appearance. – DOI: 10.32614/CRAN.package.pheatmap.
- [24] Li, L., Qu, Z., Jia, R., Wang, B., Wang, Y., Qu, D. (2017): Excessive input of phosphorus significantly affects microbial Fe(III) reduction in flooded paddy soils by changing the abundances and community structures of *Clostridium* and *Geobacteraceae*. – Science of the Total Environment 607-608: 982-991. DOI: 10.1016/j.scitotenv.2017.07.078.
- [25] Li X., Wang, W., Liu, W., Wu, Q., Peng, W., Zhou Y., Mo J., Liu. Q. (2024): Effect of planting lotuses on the community structure of planktonic eukaryotes in a shallow pond of a subtropical urban park in China. – Applied Ecology and Environmental Research 22: 883-847. DOI: 10.15666/2201_833847.
- [26] Li, Z., Liu, Y., Xie, J., Wang, G., Cheng, X., Zhang, J., Sang, C., Liu, Z. (2022): Impact of microecological agents on water environment restoration and microbial community structures of trench system in a Baiyangdian wetland ecosystem. – Journal of Applied Microbiology 132: 2450-2463. DOI: 10.1111/jam.15238.
- [27] Liao, J. L., Cao, X. F., Wang, J., Zhao, L., Sun, J., Jiang, D., Huang, Y. (2017): Similar community assembly mechanisms underlie similar biogeography of rare and abundant bacteria in lakes on Yungui Plateau, China. – Limnology and Oceanography 62: 723-735. DOI: 10.1002/lno.10455.
- [28] Linz, A. M., He, S., Stevens, S. L. R., Anantharaman, K., Rohwer, R. R., Malmstrom, R. R., Bertilsson, S., McMahon, K. D. (2018): freshwater carbon and nutrient cycles revealed through reconstructed population genomes. – PeerJ 6: e6075. DOI: 10.7717/peerj.6075.
- [29] Liu, B., Wang, Y., Zhang, H., Zhou, Y., Zhang, C., Yang, N., Wang, W. (2023b): The variations of microbial diversity and community structure along different stream orders in Wuyi Mountains. – Microbial Ecology 86: 2330-2343.
- [30] Liu, X., Pan, B., Huang, Z., Liu, X., He, H., Zhu, X. (2023a): Anthropogenic land uses drive shifts in denitrification-related microbiota in freshwater river ecosystems. – Land Degradation & Development 34: 269-282. DOI: 10.1002/ldr.4458.

- [31] Ma, Y., Huang, L., Zhang, W. (2023): Environment drives the co-occurrence of bacteria and microeukaryotes in a typical subtropical bay. – *Journal of Oceanology and Limnology* 41: 2292-2308. DOI: 10.1007/s00343-022-2268-x.
- [32] Magoč, T., Salzberg, S. L. (2011): FLASH: fast length adjustment of short reads to improve genome assemblies. – *Bioinformatics* 27: 2957-2963. DOI: 10.1093/bioinformatics/btr507.
- [33] Milici, M., Deng, Z. L., Tomasch, J., Decelle, J., Wos-Oxley, M. L., Wang, H., Jáuregui, R., Plumeier, I., Giebel, H.-A., Badewien, T. H., Wurst, M., Pieper, D. H., Simon, M., Wagner-Döbler, I. (2016): Co-occurrence analysis of microbial taxa in the Atlantic Ocean reveals high connectivity in the free-living bacterioplankton. – *Frontiers in Microbiology* 7: 649. DOI: 10.3389/fmicb.2016.00649.
- [34] Newton, R. J., Jones, S. E., Eiler, A., McMahon, K. D., Bertilsson, S. (2011): A guide to the natural history of freshwater lake bacteria. – *Microbiology and Molecular Biology Reviews* 75: 14-49. DOI: 10.1128/MMBR.00028-10.
- [35] Ogle, D. H., Doll, J. C., Wheeler, A. P., Dinno, A. (2023): FSA: simple fisheries stock assessment methods. – <https://CRAN.R-project.org/package=FSA>.
- [36] Padeniya, U., Davis, D. A., Wells, D. E., Bruce, T. J. (2022): Microbial interactions, growth, and health of aquatic species in biofloc systems. – *Water* 14: 4019. DOI: 10.3390/w14244019.
- [37] R Core Team (2022): R: A Language and Environment for Statistical Computing. – R Foundation for Statistical Computing, Vienna. <https://www.R-project.org/>.
- [38] Sagova-Mareckova, M., Boenigk, J., Bouchez, A., Cermakova, K., Chonova, T., Cordier, T., Eisendle, U., Elersek, T., Fazi, S., Fleituch, T., Frühe, L., Gajdosova, M., Graupner, N., Haegerbaeumer, A., Kelly, A.-M., Kopecky, J., Leese, F., Nöges, P., Orlic, S., Panksep, K., Pawlowski, J., Petrusek, A., Piggott, J. J., Rusch, J. C., Salis, R., Schenk, J., Simek, K., Stovicek, A., Strand, D. A., Vasquez, M. I., Vrålstad, T., Zlatkovic, S., Zupancic, M., Stoeck, T. (2021): Expanding ecological assessment by integrating microorganisms into routine freshwater biomonitoring. – *Water Research* 191: 116767. DOI: 10.1016/j.watres.2020.116767.
- [39] Sehna, L., Brammer-Robbins, E., Wormington, A. M., Blaha, L., Bisesi, J., Larkin, I., Martyniuk, C. J., Simonin, M., Adamovsky, O. (2021): Microbiome composition and function in aquatic vertebrates: small organisms making big impacts on aquatic animal health. – *Frontiers in Microbiology* 12: 567408. DOI: 10.3389/fmicb.2021.567408.
- [40] Shen, M., Li, Q., Ren, M., Lin, Y., Wang, J., Chen, L., Li, T., Zhao, J. (2019): Trophic status is associated with community structure and metabolic potential of planktonic microbiota in plateau lakes. – *Frontiers in Microbiology* 10: 2560. DOI: 10.3389/fmicb.2019.02560.
- [41] Su, Y. (2021): Revisiting carbon, nitrogen, and phosphorus metabolisms in microalgae for wastewater treatment. – *Science of the Total Environment* 762: 144590. DOI: 10.1016/j.scitotenv.2020.144590.
- [42] Su, Y., Gao, W., Guan, D., Su, W. (2018): Dynamic assessment and forecast of urban water ecological footprint based on exponential smoothing analysis. – *Journal of Cleaner Production* 195: 354-364. DOI: 10.1016/j.jclepro.2018.05.184.
- [43] Tian, P., Wu, H., Yang, T., Jiang, F., Zhang, W., Zhu, Z., Yue, Q., Liu, M., Xu, X. (2021): Evaluation of urban water ecological civilization: a case study of three urban agglomerations in the Yangtze River economic belt, China. – *Ecological Indicators* 123: 107351. DOI: 10.1016/j.ecolind.2021.107351.
- [44] Vári, Á., Podschun, S. A., Erős, T., Hein, T., Pataki, B., Iojă, I.-C., Adamescu, C. M., Gerhardt, A., Gruber, T., Dedić, A., Ćirić M., Gavrilović, B., Báldi, A. (2022): Freshwater systems and ecosystem services: challenges and chances for cross-fertilization of disciplines. – *Ambio* 51: 135-151. DOI: 10.1007/s13280-021-01556-4.

- [45] Vinten, A. J. A., Artz, R. R. E., Thomas, N., Potts, J. M., Avery, L., Langan, S. J., Watson, H., Cook, Y., Taylor, C., Abel, C., Reid, E., Singh, B. K. (2011): Comparison of microbial community assays for the assessment of stream biofilm ecology. – *Journal of Microbiological Methods* 85: 190-198.
- [46] Wang, Q., Garrity, G. M., Tiedje, J. M., Cole, J. R. (2007): Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. – *Applied and Environmental Microbiology* 73(16): 5261-5267.
- [47] Wantzen, K. M., Ballouche, A., Longuet, I., Bao, I., Bocoum, H., Cissé, L., Chauhan, M., Girard, P., Gopal, B., Kane, A., Marchese, R. M., Nautiyal, P., Teixeira, P., Zalewski, M. (2016): River culture: an eco-social approach to mitigate the biological and cultural diversity crisis in riverscapes. – *Ecohydrology & Hydrobiology* 16: 7-18. DOI: 10.1016/j.ecohyd.2015.12.003.
- [48] Wu, L., Xie, X., Zhang, J., Ding, Y., Wu, Q. (2021): Bacterial diversity and community in regional water microbiota between different towns in world's longevity township Jiaoling, China. – *Diversity* 13: 361. DOI: 10.3390/d13080361.
- [49] Xiao, K., Dai, X., Chen, X., Bao, P., Wang, K. (2021): Editorial: the interplay between freshwater microorganisms and water quality. – *Frontiers in Environmental Science* 9: 784784. DOI: 10.3389/fenvs.2021.784784.
- [50] Yan, L., Zhang, S., Lin, D., Guo, C., Yan, L., Wang, S., He, Z. (2018): Nitrogen loading affects microbes, nitrifiers and denitrifiers attached to submerged macrophyte in constructed wetlands. – *Science of the Total Environment* 622-623: 121-126. DOI: 10.1016/j.scitotenv.2017.11.234.
- [51] Yu Plisova, E., Balabanova, L.A., Ivanova, E.P., Kozhemyako, V.B., Mikhailov, V. V., Agafonova, E.V., Rasskazov, V.A. (2005): A highly active alkaline phosphatase from the marine bacterium *Cobetia*. – *Marine Biotechnology* 7: 173-178. DOI: 10.1007/s10126-004-3022-4.
- [52] Zeng, A., Tan, K., Gong, P., Lei, P., Guo, Z., Wang, S., Gao, S., Zhou, Y., Shu, Y., Zhou, X., Miao, D., Zeng, F., Liu, H. (2020): Correlation of microbiota in the gut of fish species and water. – *3 Biotech* 10: 472. DOI: 10.1007/s13205-020-02461-5.
- [53] Zhou, R., Wang, H., Wei, D., Zeng, S., Hou, D., Weng, S., He, J., Huang, Z. (2022): Bacterial and eukaryotic community interactions might contribute to shrimp culture pond soil ecosystem at different culture stages. – *Soil Ecology Letters* 4: 119-130. DOI: 10.1007/s42832-021-0082-6.
- [54] Zou, L. Y., Cheng, X. C. (2002): Water quality evaluation index and conversion method of UIA. – *Fisheries Science* 21(2): 42-43.