EFFECT OF PLANTING LOTUSES ON THE COMMUNITY STRUCTURE OF PLANKTONIC EUKARYOTES IN A SHALLOW POND OF A SUBTROPICAL URBAN PARK IN CHINA

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Abstract. Although the planktonic eukaryotic community (PEC) is an important component of freshwater ecosystems and plays an important ecological role, the impact of artificial water ecological modification on PEC has not been fully evaluated. To analyze the impact of lotus planting on the structure of PEC in urban park ponds, in this study, we analyzed the differences in PECs in different regions (artificial fountain region, potted lotus region, and direct lotus planting region) of an urban pond in subtropical China before and after lotus planting in the short term (~10 d) and for more than a month. Our results indicated that more than a month after planting lotuses, there were evident differences in the compositions of PECs in the three regions. Although there was no significant difference in operational taxonomic unit number and Chao1 indices before and after planting lotuses, planting lotuses significantly increased the Shannon index of the PECs. Moreover, different planting modes of lotuses can alter the structure of PECs after more than a month of growth, and planting lotuses can continuously strengthen the interconnection of various organisms in the PECs. These results expand our understanding of the impacts of artificial ecological modifications on the structure of PECs in urban ponds.

Keywords: *artificial ecological modification, freshwater, aquatic microbial ecology, microbial interactions, Shannon index*

Introduction

Planktonic eukaryotic communities (PECs) are comprised of diverse organisms, such as ciliates and flagellates, fungi, phytoplankton, and zooplankton, all of which play central roles in energy flow and material cycle function in aquatic ecosystems (Li et al., 2022; Xu et al., 2022; Astorg et al., 2023). Predatory zooplankton channel carbon and nutrients from prokaryotes and other microbes to higher trophic levels and regulate water clarity, energy, and nutrients in freshwater ecosystems (Carpenter et al., 1985; Bock et al., 2018). Li et al. (2023a) found that eukaryotic biodiversity has a stronger effect on ecosystem functions (represented by ecosystem metabolism and denitrification rate) than bacteria after long-term drought in artificial stream channels for 25 and 90 days of drought, both followed by 20 days of rewetting. However, despite the fact that the composition and function of PECs in oceans (de Vargas et al., 2015; Carradec et al., 2018; Sieracki et al., 2019), lakes (Bock et al., 2018; Wan et al., 2023), reservoirs (Zheng et al., 2020), and rivers (Li et al., 2022, 2023b) have been widely studied, the effects of artificial water

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ecosystem renovation on PECs in freshwater ecosystems are very scarce (Ni et al., 2010; Li et al., 2022).

Urbanization and economic development pose a challenge to the aquatic ecological environment of cities (Tian et al., 2021). In the past few decades, China has experienced rapid urbanization, resulting in severe pressure on the urban water ecosystem, such as a shortage of water resources, eutrophication of water bodies, and water pollution (Han et al., 2019). To balance the relationship between the urban development and the urban water ecosystem, “water ecological civilization” was proposed by the Ministry of Water Resources of China in 2013, which refers to the harmonious development of water environment and human activities (Tian et al., 2021). Although urban water ecological civilization has been extensively evaluated in different regions of China (Su et al., 2018; Han et al., 2019; Hartog, 2021; Tian et al., 2021), 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 PECs have not been fully evaluated.

Usually, aquatic plants, such as canna (*Canna generalis*) and lotus (*Nelumbo nucifera*), are planted in lakes or ponds in urban wetland parks to enhance their ornamental value. To analyze the impact of lotus planting on the structure of PEC in urban park ponds, in this study, we analyzed the differences in PECs in different regions (artificial fountain region, potted lotus region, and direct lotus planting region) of an urban pond in subtropical China before and after lotus planting in the short term (~10 d) and more than a month.

Materials and Methods

Study area and sample collection

Artificial renovation was carried out from February 10th to March 28th, 2023, with pond renovation from February 10th to March 10 and lotus planting from March 26th to 28th in the urban pond (113.51 E, 22.16 N; Zhuhai, China). The average water depth of the pond was approximately 0.6 m. The elevation of the pond was 38.0 m. The watershed area was approximately 0.99 hm^2. More than 30 varieties of *Nelumbo nucifera*, *Nelumbo lutea*, *Nymphaea tetragona*, *Nymphaea alba*, *Nymphaea mexicana*, and *Nymphaea lotus* were planted. The water samples were collected from ten sampling sites on February 2nd, April 18th, and May 22nd, 2023. Among the sampling sites, W1, W2 and W3 were in the artificial fountain region did not plant lotuses; W4, W5 and W6 were in the treatment 1 region, where lotuses were potted; W7, W8 and W9 were in the treatment 2 region, where lotuses were directly planted; and W10 was in the drainage channel (*Figure 1*). In the potted lotuses region, each lotus plant was firstly planted in a cement flowerpot with a diameter of approximately 30 cm, and then the flowerpots were transplanted to the region. The spacing between flowerpots of potted lotuses was approximately 20 cm. The planting density of lotuses in the treatment 2 region was approximately 3 $plants/m^2$.

Triplicate water samples were collected from approximately 0.2 m below the water surface at each sampling site using a 5-L Niskin bottle, mixed, and immediately stored in sterile sampling bags (LABPLAS, Canada). The samples were then transferred to the laboratory on ice. Approximately 500-mL of water from each sample was filtered through polycarbonate membranes with 0.22 μm of aperture (Millipore, USA). The filters were stored at -80 °C for DNA extraction. Residual water samples were used to determine the physical and chemical factors.

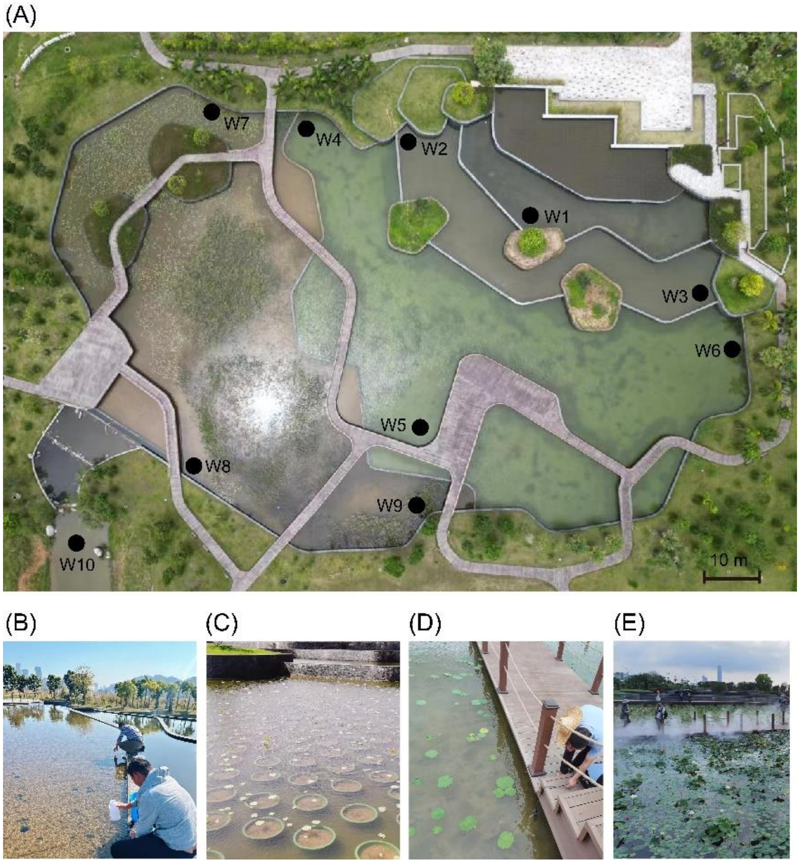

Figure 1. *Distribution of sampling sites (A) and sampling site photos (B-D). (B), Sampling photo before treatment; (C) and (D), photos of potted and directly planted areas within 10 days after treatment, respectively; (E) photo of the lotus pond after more than one month of treatment. W1-W3 were in the artificial fountain region did not plant lotuses; W4-W6 were in the treatment 1 region, where lotuses were potted; W7-W9 were in the treatment 2 region, where lotuses were directly planted; and W10 was in the drainage channel*

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 (DOP) 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) using a microplate reader (ThermoFisher Scientific, USA) according to a previously described method (Vinten et al., 2011). 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 (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N), total nitrogen (TN), total phosphorus (TP), and SiO_3-Si (Si) were determined using a flow injection water quality analyzer (Skalar, Netherlands). The permanganate index (COD_{Mn}) and phosphate (PO_4-P) content were determined according to standard methods (Huang, 2000). The concentration of un-ionized ammonia (NH_3) was calculated according to a previously described method (Zou and Cheng, 2002).

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Analysis of PEC composition

Water microbial DNA was extracted using a FastDNA Spin kit for soil (MP, Eschwege, Germany). The V4 region of the ribosomal small subunit (SSU) rRNA gene was amplified using the eukaryotic primer pair TAReuk454FWD1 (5′-CCAGCASCYGCGGTAATTCC-3′) and TAReukREV3 (5′-ACTTTCGTTCTTGATYRA-3′) with a 12-nt sample-specific barcode sequence at the 5’ end of the TAReuk454FWD1 primer (Fu et al., 2023), according to a previously described method (Lejzerowicz et al., 2015; Schön et al., 2021). 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 0.9.5 package (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 1.0.12 package (Kolde, 2019) in R 4.2.3. Differences were considered statistically significant at $P < 0.05$.

Results

WT gradually increased in the order of sampling times during the study, whereas there was no significant difference in WT among different treatment regions ($P > 0.05$; *Appendix 1A*). Within ten days after planting lotuses, the difference patterns of water Cond, Sal, and TDS in different regions of the pond did not change, but after more than one month, the difference among the regions in the three indicators were decreased (*Appendix 1B-D*). However, planting lotuses changed the water pH differences among the various regions of the pond in a short period of time (~10 days) and eliminated the water pH differences in the long term (more than a month) (*Appendix 1E*). Directly planting lotuses (T2) significantly reversed water DO in the short term ($P < 0.05$; *Appendix 1G*) and significantly increased water NH_4-N concentration ($P < 0.05$; *Appendix 1N*). Moreover, both potting (T1) and direct planting (T2) lotuses significantly increased water PO_4-P, TP, TN, NH_4-N, and Turb in the short term, whereas significantly decreased water NO_3-N concentration ($P < 0.05$; *Appendix 1*).

A total of 2,155,891 merged effective sequence tags were obtained from the 30 water samples. To exclude the impact of sequencing depth on the results, 29,822 tags were randomly resampled from each sample for subsequent analyses. The PCoA results showed that before planting lotuses (Feb 2nd), the compositions of the PECs in the artificial fountain region were evidently different from those in the planned lotus planting regions, whereas there was no significant difference between the two lotus planting regions. In the short term after planting lotuses (~10 d, Apr 18th), the compositions of PECs were more similar than those before planting lotuses. However, more than a month after planting lotuses (May 22nd), there were evident differences in the compositions of PECs in the artificial fountain region, treatment 1 region, and treatment 2 region (*Figure 2A*). Although there was no significant difference in OTU number and Chao1 indices before and after planting lotuses ($P \geq 0.05$; *Figure 2B and 2F*), planting lotuses significantly increased the Shannon index of the PECs ($P < 0.05$; *Figure 2D*). Moreover, generally, the OTU number, Shannon, and Chao1 indices of PECs in drainage channels were significantly higher than those in the pond ($P < 0.05$; *Figure 2C, 2E, and 2G*).

Taxonomic assignment results indicated that Opisthokonta was the major eukaryotic group in the PECs and contained over half of the sequences (*Figure 2H*). *Plumatella* sp. BZ-34 was the most abundant species in the communities, followed by *Berthella californica* (*Figure 2I*).

LEfSe results indicated that before planting lotuses, only *Plumatella* sp. BZ 34, Euglenea, and Euglenophyceae were significantly enriched in the artificial fountain region (control region), and Excavata was significantly enriched in the Treatment 1 region (|LDA| > 2; *Figure 3A*). Only *Pseudomuriella* sp. Itas 9/21 14-1d, *Desmodesmus pirkollei*, *Desmodesmus*, Opisthokonta, and Sphaeropleales was significantly enriched in the Treatment 1 region in the short term after planting lotuses (~10 d, |LDA| > 2; *Figure 3B*). However, more than a month after planting lotuses (May 22nd), *Hexarthra intermedia brasiliensis*, *Planctonema* sp. M110_1, *Oocystaceae* sp. GSL021, Flosculariacea, Euglenophyceae, Euglenea, Chlorophyta, Archaeplastida, and SAR were significantly enriched in the Treantment 1 region, whereas Euglenida, Spirotrichea, and Ciliophora were significantly enriched in the Treatment 2 region (|LDA| > 2; *Figure 3C*). These results indicated that different lotus planting modes can alter the structure of PECs after more than a month of growth.

Co-occurrence network analysis showed that the average degree of the network before planting lotuses was 2.341, whereas it increased to 4.807 and 5.424 in the short term after planting lotuses and more than one month after planting lotuses, respectively (*Figure 4*). The density of the network before planting lotuses was 0.059, whereas it increased to 0.086 and 0.094 in the short term after planting lotuses and more than one month after planting lotuses, respectively (*Figure 4*). These results indicated that planting lotuses can continuously strengthen the interconnection of various organisms in PECs.

db-RDA results showed that WT, Chla, COD_{Mn}, Cond, Sal, TDS, SPM, PO_4-P, TP, ORP, and TN significantly correlated with the PECs (*Figure 5A*). Furthermore, Spearman’s correlation analysis showed that Cond, Sal, TDS, COD_{Mn}, and SPM exhibited similar correlations with major planktonic eukaryotic species, and their correlations with these planktonic eukaryotic species were opposite to those of WT, ORP, PO_4-P, TP, and TN (*Figure 5B*). Detailly, *Berthella californica*, *Plumatella* sp. Bz-34, and some unidentified species were significantly positively correlated with Cond, Sal, TDS, COD_{Mn}, and SPM (Spearman correlation coefficient > 0.6 and $P < 0.05$), whereas significantly negatively correlated with WT, DOP, PO4-P, TP, TN, and Chla (Spearman correlation coefficient < -0.6 and $P < 0.05$; *Figure 5B*).

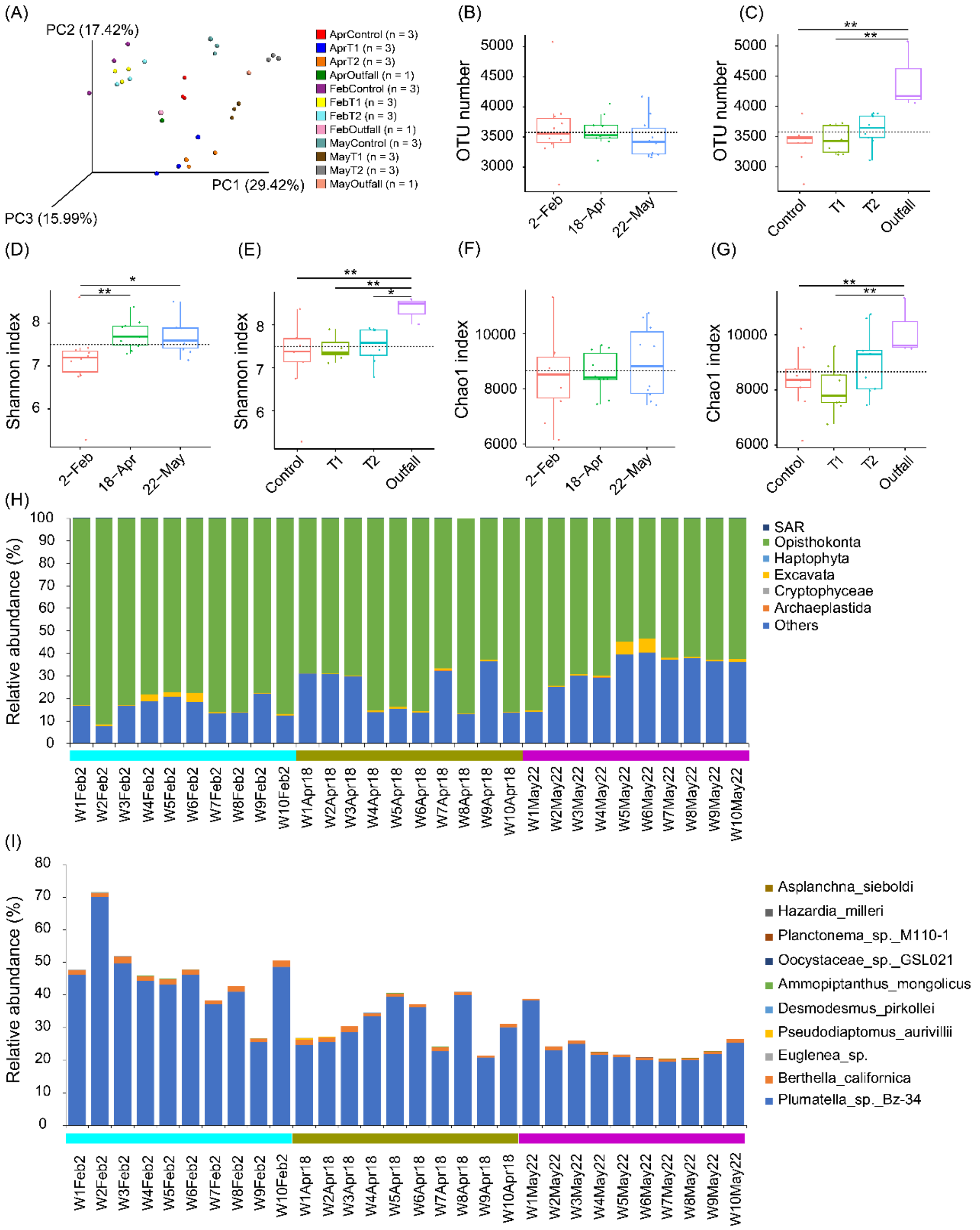

Figure 2. *Changes in the structure and diversity of planktonic eukaryotic communities (PECs) in an urban pond before and after planting lotuses. (A) Principal co-ordinate analysis; (B) difference in OTU numbers of the PECs before and after planting lotuses; (C) difference in OTU numbers of the PECs among different regions in the pond; (D) difference in Shannon index of the PECs before and after planting lotuses; (E) difference in the Shannon index of the PECs among different regions in the pond; (F) difference in Chao1 index of the PECs before and after planting lotuses; (G) difference in Chao1 index of the PECs before and after planting lotuses; (H) relative abundances of the main taxonomic groups in the PECs; (I) relative abundances of the top 10 species in the PECs. W1-W3 were in the artificial fountain (Control) region did not plant lotuses; W4-W6 were in the treatment 1 (T1) region, where lotuses were potted; W7-W9 were in the treatment 2 (T2) region, where lotuses were directly planted; and W10 was in the drainage channel. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$*

Asplanchna sieboldi, *Planctonema* sp. M110-1, and uncultured *Halteria* sp. were significantly positively correlated with WT and DOP (Spearman correlation coefficient > 0.6 and $P < 0.05$), whereas they were significantly negatively correlated with Cond, Sal, and TDS (Spearman correlation coefficient < -0.6 and $P < 0.05$; *Figure 5B*). Moreover, *Actinastrum hantzschii*, *Oocystis* sp. AN 2/29-4, *Planctonema* sp. M110-1, and some unidentified species were significantly positively correlated with *Chla* (Spearman correlation coefficient > 0.6 and $P < 0.05$), whereas *Berthella californica*, *Plumatella* sp. Bz-34, and an unidentified species were significantly negatively correlated with *Chla* (Spearman correlation coefficient < -0.6 and $P < 0.05$; *Figure 5B*).

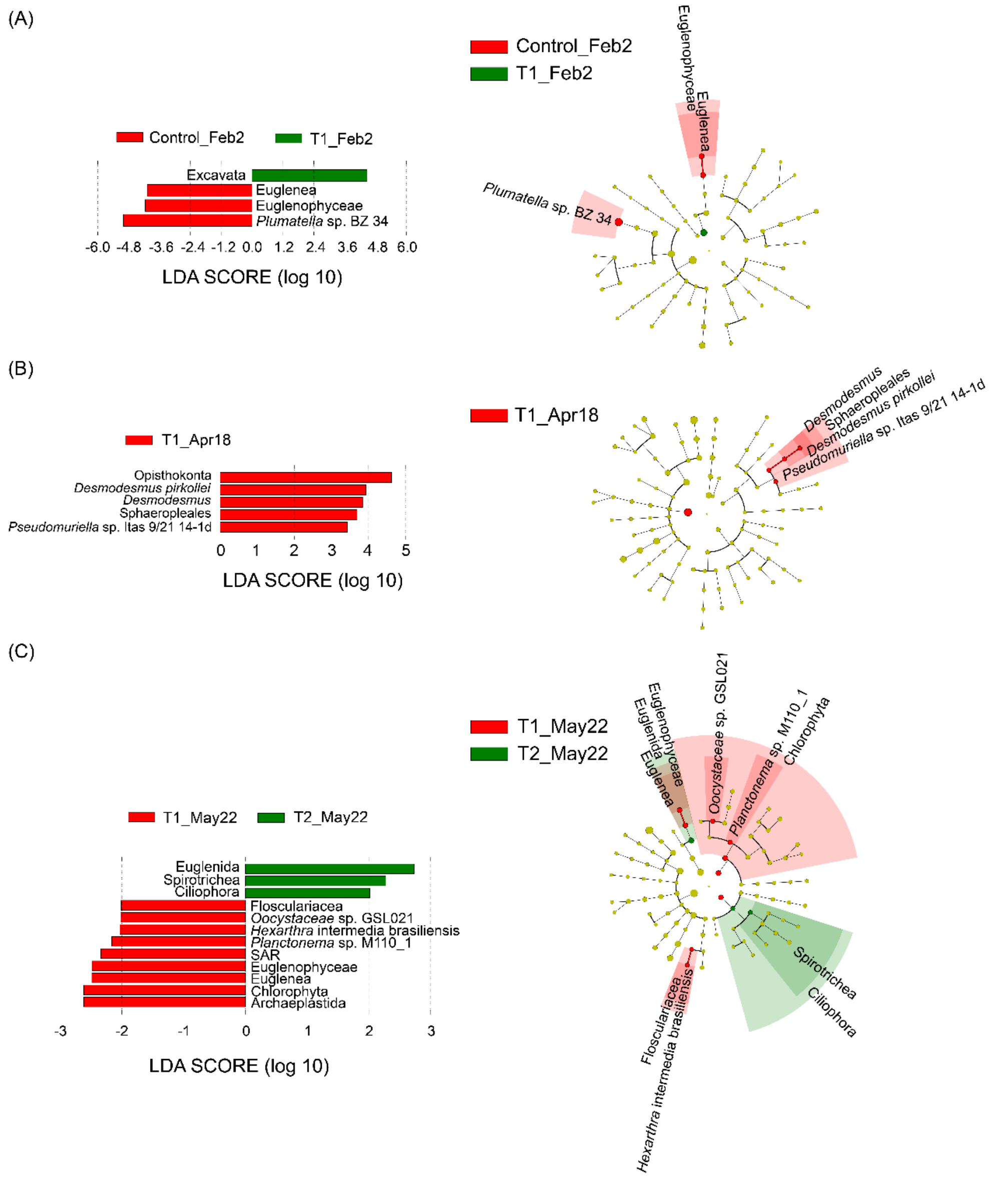

Figure 3. *Significantly different taxa of planktonic eukaryotic communities in the urban pond before (A) and after (B and C) planting lotuses. (B) in the short term after planting lotuses (~10 days); (C) more than a month after planting lotuses. Lotuses were potted in the treatment 1 (T1) region, whereas lotuses were planted directly in the treatment 2 (T2) region. Significantly different taxa were detected using linear discriminant analysis (LdA) effect size (LEfSe). The taxa with LDA > 2 or LDA < -2 were considered significantly different*

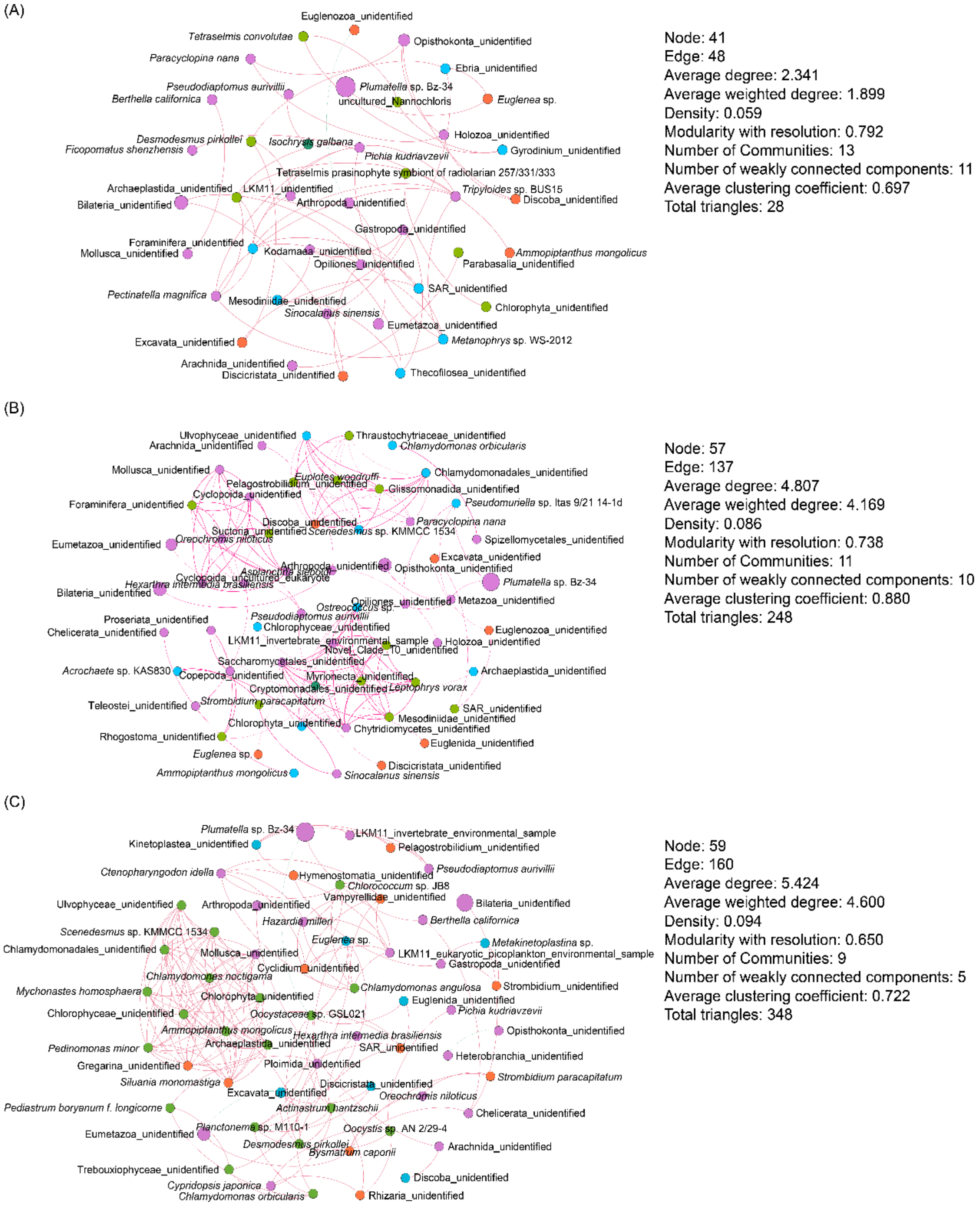

Figure 4. *Co-occurrence networks based on Spearman correlation coefficients indicate the strengths of microbial interactions in planktonic eukaryotic communities in an urban pond before (A) and after (B and C) planting lotuses. B, in the short term after planting lotuses (~10 d); C, more than a month after planting lotuses. Correlations with |Spearman correlation coefficient| > 0.6 and P < 0.05 were considered effective*

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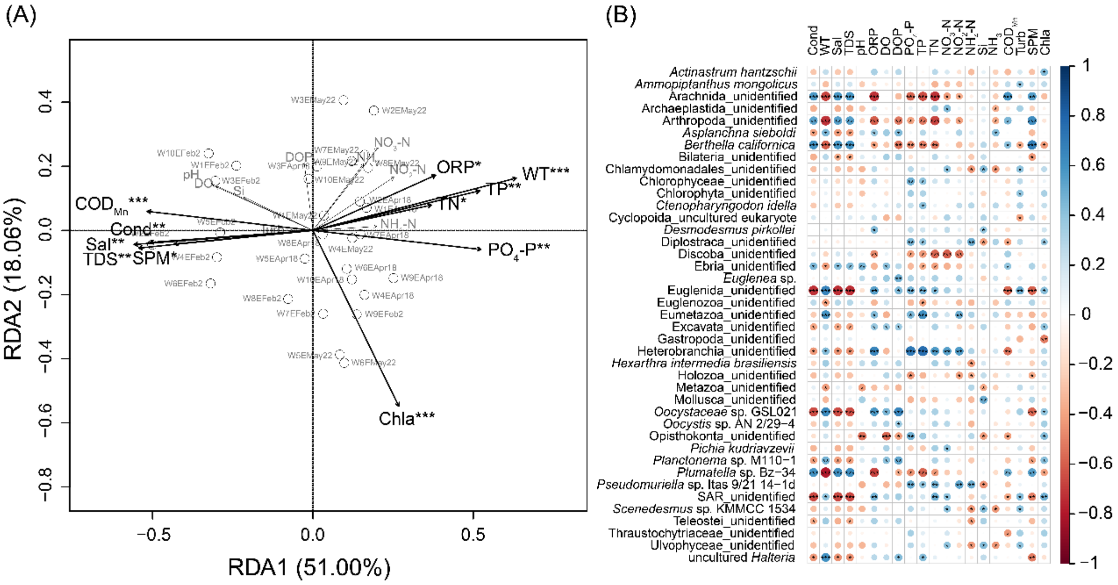

Figure 5. *Distance-based redundancy analysis (A) and bubble chart (B) show the correlations between major planktonic eukaryotes and water parameters. Correlations were measured using Spearman's correlation coefficient. Correlations with |Spearman correlation coefficient| > 0.6 and p < 0.05 were considered as effective. WT, water temperature; Cond, conductivity; Sal, salinity; TDS, total dissolved solids; ORP, oxidation-reduction potential; DO, dissolved oxygen; DOP, dissolved oxygen saturation; TP, total phosphorus; TN, total nitrogen; Si, SiO_3-Si; COD_{Mn}, permanganate index; Turb, turbidity; SPM, suspended particulate matter; Chla, chlorophyll-a. * P < 0.05; ** P < 0.01; *** P < 0.001*

Discussion

Many urban wetland parks have been built to increase the urban water ecological civilization indices, and aquatic plants are usually planted in lakes or ponds in urban wetland parks to enhance their ornamental value (Pham et al., 2023; Yang et al., 2023). However, the impact of this artificial ecological modification, which alters the ecosystem of lakes or ponds in urban wetland parks, on planktonic eukaryotes has received little attention. Our results indicated that more than a month after planting lotuses, there were evident differences in the composition of PECs in the planting lotus regions. Moreover, different planting modes of lotuses can alter the structure of PECs after more than a month of growth, and planting lotus can continuously strengthen the interconnection of various organisms in the PECs.

The diversity and stability of PECs are affected by several environmental factors. Geng et al. (2022) reported that physical and chemical indicators, nutritional parameters, and light effects explained >70% of zooplankton community dynamics, compared with 37% for phytoplankton. They also found that phytoplankton was primarily influenced by nutrients, water depth, and pH. Similarly, the diversity and stability of zooplankton exhibited physical and chemical parameter responses, and the influence of nutrients also played a significant role (Geng et al., 2022). Zhao et al. (2020) found that annual average total abundances decreased by 86% for phytoplankton and 78% for zooplankton, while concentrations increased 1.8 times for total nitrogen and 2.7 times for total phosphorus in a 13-year study sampled plankton and water quality from a man-made lake monthly, and concluded that the decline in plankton abundance was largely attributed to the predation of zooplankton on phytoplankton, fish predation on both phytoplankton and zooplankton, and linkages with the decline in water salinity, pH, and the increase in clarity. The decline in plankton abundance did not result from long-term changes in nutrient concentrations. Our results indicated that although there was no significant difference in OTU number and Chao1 indices before and after planting lotuses, planting lotuses significantly increased the Shannon index of the PECs. Moreover, planting lotuses can continuously strengthen the interconnections of various organisms in PECs.

Brozoans are suspension feeding, almost entirely colonial invertebrates that inhabit all types of aquatic environments, and *Plumatella* species are common freshwater species (Wood and Okamura, 2004; Saadi et al., 2022). For instance, *Plumatella mukaii* has been recognized in eastern Asia and western South America (Wood, 2001). *Plumatella geimermassardi* has also been detected in Britain, Ireland, and continental Europe (Wood and Okamura, 2004). In the present study, *Plumatella* sp. BZ-34 was the most abundant species in the communities, followed by *Berthella californica*, despite *Berthella californica* being a widespread species of heterobranch sea slug distributed across the North Pacific Ocean from Korea and Japan to the Galapagos Islands (Ghanimi et al., 2020).

Water environmental indicators are the main factors that affect the composition and interactions of PECs. For instance, Huang et al. (2022) reported that the species composition of phytoplankton in Xinmiao Lake is closely related to environmental factors; total salt, WT, and TP mainly affected the abundance of dominant species, and total salt, TP, and 5-day biochemical oxygen demand were the main factors affecting the biomass of dominant species. Zheng et al. (2020) found that Secchi depth and DO significantly affected PECs in 2017, whereas WT, TN, and Chla significantly affected PECs in 2018 in the Danjaingkou Reservoir in China. However, only WT and DO significantly affect PECs in June in the Xijiang River in China (Liu et al., 2021). Our results indicated that WT, Chla, COD_{Mn}, Cond, Sal, TDS, SPM, PO_4-P, TP, ORP, and TN were significantly correlated with PECs in the lotus pond. These results indicate that the range of each physical and chemical indicator varies in different water environments, resulting in varying impacts of these indicators on the PECs. Therefore, it is necessary to review numerous research reports on the impact of water environmental indicators on PECs to obtain a consistent conclusion that each aquatic physicochemical indicator has a consistent impact on PECs.

Although the SILVA dataset is widely used for taxonomic assignment in eukaryotic community analysis through high-throughput sequencing (Banerji et al., 2018; Ortiz-Álvarez et al., 2018; MaNeil et al., 2022), owing to the lack of strict classification (i.e., phylum, class, order, family, genus, and species levels) in eukaryotic species, there are many additional classification levels, such as subclass, superorder, and suborder, which results in uneven taxonomic assignment results; therefore, manual sorting is required.

PECs are an important component in the food chain that connects planktonic prokaryotes and aquatic insects and vertebrates, and they play a “top-down” effect in controlling planktonic prokaryotes and algae bloom (Li et al., 2020). Our results indicated that both potting (T1) and direct planting (T2) lotuses significantly increased water PO_4-P, TP, TN, NH_4-N, and Turb in the short term, whereas significantly decreased water NO_3-N concentration. Moreover, planting lotuses can continuously strengthen the interconnection of various organisms in PECs. Further research is needed to determine whether these changes increase the risk of algae bloom in the pond and reduce its ecological function, considering urban ponds are typically of anthropogenic origin and have typically been constructed for purposes other than biodiversity conservation, such as flood prevention, sediment capture, water purification, and aesthetics (Hill et al., 2018).

Conclusions

More than a month after planting lotuses, there were evident differences in the composition of PECs in the planting lotus regions. Different planting modes of lotuses can alter the structure of PECs after more than a month of growth, and planting lotuses can continuously strengthen the interconnection of various organisms in the PECs. These results expand our understanding of the impact of artificial ecological modification on the structure of PECs in urban ponds.

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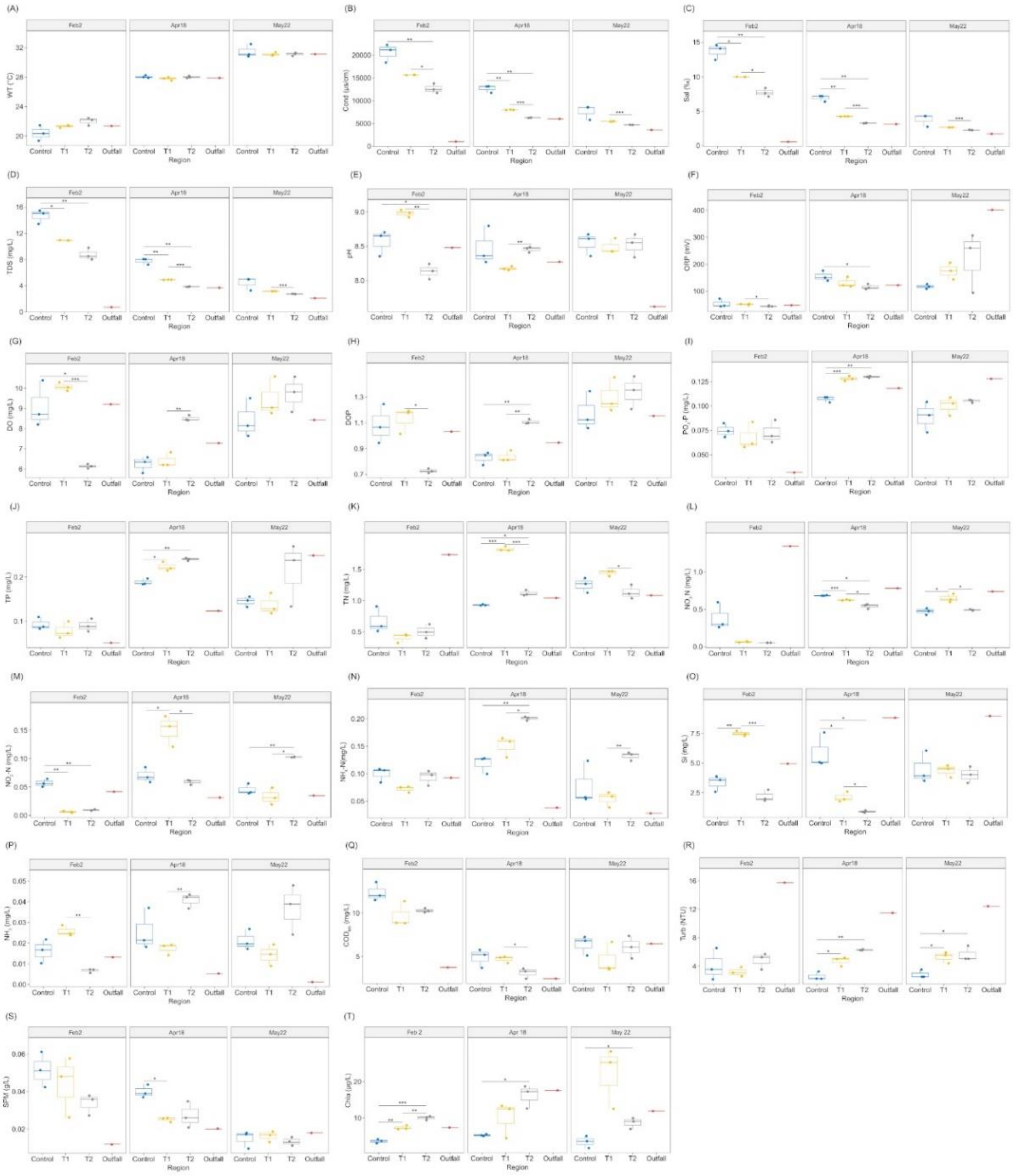
APPENDIX



Appendix 1. *Changes in water quality parameters before and after planting lotuses. (A) water temperature (WT); (B) conductivity (Cond); (C) salinity (Sal); (D) total dissolved solids (TDS); (E) pH; (F) oxidation-reduction potential (ORP); (G) dissolved oxygen (DO); (H) dissolved oxygen saturation (DOP); (I) PO_4-P; (J) total phosphorus (TP); (K) total nitrogen (TN); (L) NO_3-N; (M) NO_2-N; (N) NH_4-N; (O) SiO_3-Si (Si); (P) NH_3; (Q) permanganate index (COD_{Mn}); (R) turbidity (Turb); (S) suspended particulate matter (SPM); (T) chlorophyll-a (Chla). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$*

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