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Submersed macrophytes Vallisneria natans and Vallisneria spinulosa improve water quality and affect microbial communities in sediment and water columns

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ABSTRACT

Healthy aquatic ecosystems are essential for human beings. However, anthropogenic activities severely worsen water quality. In this study, using assembling mesocosms, we developed an efficient and easy-to-handle method to monitor the water quality by measuring the electrical conductivity (EC) of water. Our data demonstrate that the growth of two submersed macrophytes, *Vallisneria natans* and *Vallisneria spinulosa*, improves water quality by decreasing EC. Furthermore, using high-throughput DNA sequencing, we analyzed the microbial community abundance and structure in sediment and water columns with or without plant growth. We generated 33,775 amplicon sequence variants from 69 samples of four sediment groups (BkM, CtM, VnR, and VsR) and three water column sample groups (CtW, VnW, and VsW). The results show that the relative abundance of bacteria was higher in the sediment than in the water column. Moreover, the diversity and composition of microbiomes were altered by *Vallisneria* spp. growth, and the α -diversity of the microbial communities also varied significantly with or without *Vallisneria* spp. growth for both the sediment and water columns.

1. Introduction

Aquatic ecosystems are essential for human society; however, industrial processes and other anthropogenic activities aggravate the pollution of rivers and lakes and severely worsen water quality, further decreasing its self-clarification [1-3]. Phytoremediation through aquatic macrophytes is a green and economic strategy that has proven promising for restoring water quality [4,5]. Aquatic macrophytes are macroscopic flora that can be separated into four groups: (i) emergent macrophytes, (ii) floating-leaved macrophytes, (iii) free-floating macrophytes, and (iv) submerged or submersed macrophytes [6,7]. Submersed macrophytes, as essential maintainers of low trophic levels, are essential in water ecosystems and crucial in nutrient recycling and promoting ecosystem biodiversity and self-clarification, thus reconstructing their health [8-12]. Submersed macrophytes stabilize freshwater ecosystems, promote the removal of pollution from the water environment, and improve water quality by (i) physically adsorbing the pollutants due to their large surface area, (ii) absorbing to support their growth, (iii) absorbing and degrading pollutants, and (iv) providing an adherent

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surface for the microbial community and producing adherent biofilms. Submersed plants and epiphytic biofilms provide an appropriate microenvironment for microbiomes, thereby removing pollution [13–18].

The physical, chemical, and biological components of aquatic ecosystems exhibit an intimate interdependence [12,19–24]. Submersed macrophytes and interacting microorganisms constitute the main components of the self-purification ability of water bodies. Aquatic plants not only generate oxygen through stem and leaf photosynthesis, creating an oxygen-rich environment in the water, but also recruit the microbial community with strong metabolic ability for organic/inorganic matter by providing carbon sources and other matter to support the microorganisms through secretion from stems, leaves, and roots. Microorganisms provide carbon dioxide and secondary metabolites to host plants and even inhibit the growth of harmful microorganisms, thereby promoting plant growth. Many studies have demonstrated that microbes and submersed macrophytes interact to improve water quality [13,18,25–28]. Microbial diversity affects water quality, and it is also affected by it; thus, microbial diversity can be a bioindicator in aquatic ecosystems [29–32]. Many studies have recently focused on microbiomes in sediments and epiphytic biofilms of urban black odorous ecosystems, as well as other aquatic ecosystems that contain submersed macrophytes [17,33–37]. However, most studies performed in natural water bodies may be affected by many known/unknown factors, and the microbial diversity affected by submersed macrophyte growth under laboratory conditions is rarely reported.

Vallisneria spp. are important submersed macrophytes that are widely used to improve the water quality of freshwater ecosystems and have attracted increasing attention in recent years [11,24,35,38–40]. *Vallisneria natans* and *V. spinulosa* are two species of the genus *Vallisneria* with similar morphologies. *V. spinulosa* is thought to be endemic to China and is a dominant submersed macrophyte in lakes of the middle-lower reaches of the Yangtze River, often coexisting with *V. natans*, while *V. natans* is a cosmopolitan species [41, 42].

In this study, we conducted mesocosm experiments to detect the changes in water quality caused by the growth of *V. natans* and *V. spinulosa*. Furthermore, using high-throughput DNA sequencing (HTS) technologies (also called next-generation sequencing (NGS) technologies), we analyzed the microbial community abundance and structure in sediments and water columns with or without plant growth. Our data showed that the diversity and composition of the microbiomes were altered by *Vallisneria* spp. growth.

2. Materials and methods

2.1. Plant materials

Nine plastic tanks (250 L, diameter = 80 cm, and height = 52 cm) were used for this comparative experiment, and each tank was filled with a ~20-cm layer of soil as sediment and ~30 cm of water. The soil used in this study was taken from the top 0–10 cm of soil from the Lushan Botanical Garden Poyang Lake Branch Garden, located in Weijia town, Jiujiang city, Jiangxi province, China (latitude $29^{\circ}40'25''N$, longitude $116^{\circ}5'4''E$). *V. natans* and *V. spinulosa* were collected from Changhuchi Lake, a shallow sub-lake of Poyang Lake, Jiangxi province. The seedlings were generated from the seeds and winter buds of *V. natans* and *V. spinulosa*, respectively, and approximately 10-cm high seedlings were planted in the tanks, with ~20 plants in each plastic tank. Nine 250-L plastic tanks were divided into three groups as follows: (i) without plants, (ii) planted *V. natans* (Vn), and (iii) planted *V. spinulosa* (Vs); each group included three replicates. All tanks were exposed to natural sunlight outside the lab, with little shading. During the experimental period, the light intensity ranged from approximately 5000 to 90,000 LUX. All the tanks were approximately 50 and 30 cm for *V. natans* and *V. spinulosa*, respectively.

2.2. Electrical conductivity (EC) assays

The EC of the water columns was measured using a portable HQ4200 pH/EC/TDS meter (HACH) according to the manufacturer's instructions. The EC of all nine plastic tanks (three groups) was measured weekly from 167 days (167d) after the plants had grown to 216 days (216d) when the leaves of these plants almost stopped elongating. Each group comprised three biological replicates.

2.3. Experimental Design and samples Collection

For bacterial community assays, 15 soil samples were collected from the soil described in the "plant materials" section (latitude $29^{\circ}40'25''N$, longitude $116^{\circ}5'4''E$) and named BkM (BkM1-BkM15) as the soil control; sediments samples were collected from *V. natans* (VnR) and *V. spinulosa* (VsR) root or mud without plant growth controls. Five sediments samples were collected from each plastic tank at different position using an autoclaved 50-mL tube, and marked as VnR (VnR1-VnR15), VsR (VsR1-VsR15) and CtM (CtM1-CtM15), respectively. The soil samples and sediment/mud samples were all treated as sediments in this study. Water column samples (referred to as water samples below) were taken from the three plastic tank groups: those planted with *V. natans* (Vn), *V. spinulosa* (Vs), or without plants, marked as VnW (VnW1-VnW3), VsW (VsW1-VsW3), and CtW (CtW1-CtW3), respectively. To prepare the water samples, 500 mL of water was taken from each tank and filtered with gauze and then with sterilized filter paper. Finally, the planktonic microorganisms in the water columns were harvested using an autoclaved vacuum filtration bottle with a 0.22-µm filter membrane (Millipore Filter Membrane, Aquo-system, 0.22 µm/50 mm). All the samples were stored at -80 °C until DNA isolation was performed.

2.4. DNA isolation

For DNA extraction from the sediment samples, 0.2 g (sediments) were transferred to a PowerBead tube and extraction was performed using the MagPure Stool DNA LQ Kit (Magen, Guangzhou, China) following the manufacturer's instructions. For the water samples (planktonic microorganisms), the filter membranes were cut, ground, and then transferred to a PowerBead tube, following the same protocol as for the sediment samples. Isolated DNA was checked for purity using a Nanodrop (Thermo Scientific, USA), and DNA concentrations were measured using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, MA, USA). DNA isolates were stored at -80 °C until sequencing.

2.5. PCR, amplicon library preparation and sequencing

The V3–V4 region of 16S rRNA gene was amplified with 12.5 ng of template DNA using the primers of 341F/805R (F-5'-CCTACGGGNGGCWGCAG-3'; R-5'-GACTACHVGGGTATCTAATCC-3') in a 30 μ L PCR reaction system. The PCR program was performed as follows: denaturation for 3 min at 95 °C; 25 cycles of denaturation at 95 °C for 30 s, at 55 °C for 30 s, and at 72 °C for 15 s; and then final extension at 72 °C for 5 min. The purified PCR production was then quantified using a Qubit 2.0 Fluorometer, and the sequencing libraries were built using the NEBNext® UltraTM II DNA Library Prep Kit for Illumina (New England Biolabs, MA, USA) following the manufacturer's instructions. The index codes have also been added. The library quality was assessed using a Qubit 2.0 Fluorometer. Finally, the library was sequenced on an Illumina NovaSeq 6000 platform at the Wuhan Benagen Technology Company Limited (Wuhan, China).

2.6. Data processing and Bioinformatic analysis

Cutadapt (v.3.5) software was used to identify and remove primer sequences, and length filtering was performed to obtain clean sequences. To identify feature sequences, that is, amplicon sequence variants (ASVs), DADA2 [43] was used to filter the raw data, splice paired-end reads, and remove chimeric sequences. The QIIME2 software was used to filter low-abundance ASVs (threshold 1) [44], and all samples were flattened with the minimum number of sequences. Subsequently, the bacterial ASVs were assigned to taxonomic groups using the SILVA database (https://www.arb-silva.de/) for species annotation. The α -diversity indices, including Shannon, Simpson, Chao1, and ACE, were calculated using the QIIME2 software. The β -diversity indices, including PCA, PCoA, NMDS and PLS-DA, were calculated using the R packages to investigate the community dissimilarities among the treatments. Furthermore, an analysis of similarities (ANOSIM) was conducted to examine the significance between groups and to determine the significance of the distance between and within groups. Significance was assessed using a one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. Linear discriminant analysis (LDA) effect size (LEfSe) was used to identify the biomarkers in the community. Most figures were prepared using the ImageGP web tool [45]. Bioinformatics analysis following the EasyAmplicon pipeline showed similar results [46].

3. Results

3.1. The growth of aquatic plants V. natans and V. spinulosa reduced EC of water columns

EC is a crucial indicator of several water quality variables. A lower EC indicates better water quality; moreover, EC can be easily measured at a low cost [47,48]. In this study, we used the EC index to reflect the water quality. The EC index of the different plastic tanks was measured every week from 167 to 216 d after the plants were planted. The average EC of the control, which excluded any aquatic plant growth, was approximately 300 µs/cm, whereas the ECs of the groups involving the growth of aquatic plants *V. natans* and *V. spinulosa* were approximately 150 µs/cm and from 140.2 to 209.4 µs/cm, respectively (Fig. 1). These data suggest that the



Fig. 1. Electrical conductivity assays with and without Vallisneria spp. growth. CK: without aquatic plant; V. natans: with Vallisneria natans plants; V. spinulosa: with Vallisneria spinulosa plants.

growth of the aquatic plants *V. natans* and *V. spinulosa* significantly decreased EC, indicating that *V. natans* and *V. spinulosa* purified the aquatic ecosystem where they grew. In addition, the data suggest that *V. natans* has a slightly greater water purification ability than *V. spinulosa*, with no significant difference as indicated by the *t*-test (data not shown).

3.2. The growth of aquatic plants V. natans and V. spinulosa affect the bacterial communities

For the bacterial community assays, 3,391,282 valid sequences were obtained from 69 samples belonging to four sediment groups (BkM, CtM, VnR, and VsR) and three water groups (CtW, VnW, and VsW), generating 33,775 ASVs (Supplementary Tables S1 and S2, and Supplementary Fig. S1). BkM, CtM, VnR, and VsR shared 766 core ASVs, while CtW, VnW, and VsW shared 90 (Supplementary Fig. S2). For the α -diversity of the bacterial community, Shannon index rarefaction curves for sediment and water column samples reached saturation rates, indicating that our data have a sufficient sequence covering most of the bacterial community (Supplement Fig. S3).

The Chao1 index, which represents community richness, ranged from 837 to 3028 and 211to 407 in the sediment and water samples, respectively (Supplementary Table S2, Fig. 2A–C). The Shannon index, representing community diversity, ranged from 8.459 to 10.735 and from 3.707 to 5.569 for the sediment and water samples, respectively (Supplementary Table S2, Fig. 2B–D). The mean α -diversity was much higher in the sediment than that in the water samples, suggesting that richness and diversity are lower in water than in sediment. We found a significant difference (p < 0.01 in the *t*-test) between the mud control group (CtM) and the groups with the aquatic plants *V. natans* or *V. spinulosa* (VnR or VsR) (Fig. 2A and B, and Supplementary Tables S2 and S3). In the two control groups, BkM (soil) and CtM (water), the Chao1 and Shannon indices of CtM were higher than those of BkM, suggesting that water promotes bacterial community richness and diversity. A significant difference (p < 0.01, *t*-test) in the Shannon index was obtained between the water control group CtW and the water sample VnW, which contained *V. natans* (Fig. 2D and Supplementary Tables S2 and S4). Together, our data indicate that *Vallisneria* spp. reduce bacterial community richness and diversity in sediment and water columns.

For β -diversity, unconstrained principal coordinate analyses (PCoAs) based on the Bray–Curtis distance were performed (Fig. 3).



Fig. 2. The α -diversity of Chao1 and Shannon indices of the sediment and water bacterial communities in different groups. A–B, sediments; C–D, water columns. BkM: soil samples control; CtM: sediment sampling from tanks without aquatic plant; VnR: sediment sampling from root of *V. natans* plants; VsR: sediment sampling from root of *V. spinulosa* plants; CtW: water column sampling from tanks without aquatic plant; VnR: water column sampling from tanks planted *V. natans*; VsW: water column sampling from tanks planted *V. natans*; VsW: water column sampling from tanks planted *V. natans*; VsW: water column sampling from tanks planted *V. spinulosa*.



Fig. 3. β-diversity based on the Bray–Curtis distance of bacterial communities of sediment and water columns in different groups. Unconstrained PCoA (for principal coordinates PCo1 and PCo2) with the Bray–Curtis distance showed that the root microbiota of the control groups was separate from that of the groups with *V. natans* or *V. spinulosa*. (A) Sediment; (B) Water column.

Sediments from the plant growth groups (VnR and VsR) were separated into soil and mud control groups (BkM and CtM) across the first principal component (PC1, 29.4 %). VnR, VsR, CtM, and BkM were separated across the second principal component (PC2: 8.42 %) (Adonis: $R^2 = 0.431$, p = 0.001) (Fig. 3A). For water samples, VnW and VsR were separated from the control CtW as the first principal component, which explained 30.93 % of the variation (PC1, 30.93 %), whereas the second principal component explained 20.23 % of the variation (PC2, 20.23 %) (Adonis, $R^2 = 0.447$, p = 0.001) (Fig. 3B). These data suggest that the largest source of variation in microbial communities is introduced by aquatic plant growth, and that different plants (*V. natans* and *V. spinulosa*) affect the variation in microbial communities.

3.3. Composition of bacterial community

The composition of the bacterial community was analyzed from phylum to family in all 69 samples, generating 58 phyla, 161 classes, 380 orders, 573 families, and 948 genera (Supplementary tables S5–S9). *Proteobacteria, Acidobacteriota, Planctomycetota, Verrucomicrobiota, Bacteroidota,* and *Cyanobacteria* were the dominant phyla in the sediment samples with *V. natans* or *V. spinulosa* (VnR and VsR, respectively), and all had a relative abundance of more than 5 % (Fig. 4A and Supplementary Table S10). *Proteobacteria* was the most dominant phylum, with 29 and 35.9 % abundance in VnR and VsR, respectively (Fig. 4A and Supplementary Table S10). In the mud control sample group (CtM), *Acidobacteriota, Proteobacteria, Planctomycetota, Chloroflexi,* and *Verrucomicrobiota* were the dominant phyla (CtM; Fig. 4A and Supplementary Table S10). Furthermore, our data showed that the abundance of *Proteobacteria, Bacteroidota,* and *Cyanobacteria* increased in the VnR and VsR groups when compared with that in the control sediments BkM and CtM, whereas the phyla *Acidobacteriota* and *Chloroflexi* were reduced (Fig. 4A and Supplementary Table S10). At the class level, *Gammaproteobacteria* and *Alphaproteobacteria* were the dominant classes in the VnR and VsR groups, and their relative abundance increased nearly two-fold compared with that in the CtM group (Supplementary Table S12).

In water samples with plant growth (VnW and VsW), the top four phyla, *Proteobacteria, Bacteroidota, Cyanobacteria,* and *Actinobacteriota,* comprised more than 90 % of the total phyla (Fig. 4B and Supplementary Table S11). Moreover, *Bacteroidota* and *Cyanobacteria* increased with plant growth, whereas *Actinobacteriota* and *Verrucomicrobiota* decreased (Fig. 4B and Supplementary Table S11).

Network analysis has been widely used to describe the composition of microbial communities and detect associations between microorganisms [49–51]. We performed network analysis with the top 50 genera for each group (Spearman's correlation coefficient r



Fig. 4. Histograms of the bacterial community at the phylum level. A, sediment samples; B, water column samples.

> 0.6, p < 0.05) (Supplementary Fig. S4 and Supplement Tables S14–S28). The clustering coefficient represents the complexity of the network and the strong interactions among microorganisms. For the sediment samples, the VsR group network showed the highest clustering coefficient (0.505), followed by the VnR group. The clustering coefficients for the water column samples were very high (0.959, 1, and 0.959 for CtW, VnW, and VsW, respectively) (Supplementary Fig. S4 and Supplementary Table S14). This indicates that microbial interactions in the water column are stronger than those in the sediment, and that *Vallisneria* spp. growth strengthens these interactions. The average shortest path length was higher in the VnR and VsR groups than in the CtM group (3.045, 2.876, and 2.709 for VnR, VsR, and CtM, respectively). The number of edges increased in VsR and decreased in VnR. However, in the water column samples, *V. natans* growth increased the number of edges, and *V. spinulosa* growth decreased this index (Supplementary Fig. S4 and Supplementary Table S14).

Linear discriminant analysis (LDA) effect size (LEfSe) was performed on genera with relative abundances of more than 0.1 % (LDA thresholds of four and three for sediment and water column samples, respectively). The LEfSe results suggest that in sediment samples, o_Cytophagales, g_Novosphingobium, and g_Arenimonas are biomarkers of the VsR group. In the VnR group, c_Planctomycetes,



Fig. 5. LEfSe analysis of the sediment (A) and water (B) column samples.

c_Cyanobacteriia, and f_Chitinophagaceae were identified as biomarkers. The biomarkers in the CtM group were g_Subgroup_7, f_Anaerolineaceae, and o_Myxococcales (Fig. 5A, Supplementary Fig. S5, Supplementary Table S29, and Supplementary Table S31). In the water column samples, g_Lacunisphaera, g_Allorhizobium_Neorhizobium_Pararhizobium_Rhizobium.s_bacterium, and g_Arcicella were biomarkers for the VsW group, and bacteria g_Flavobacterium.s_Flavobacterium_indicum, c_Cyanobacteriia, g_UKL13_1, f_Hyphomicrobiaceae, and g_Hydrogenophaga were biomarkers for the VsW group. The CtW group biomarkers were f_CandidatusMoranbacteria, g_CandidatusPlanktophila, g_Peredibacterstarrii, g_Polynucleobacteracidiphobus, g_Polaromonas, g_MWH_UniP1aquaticgroup, g_FukuN18freshwatergroup, g_SH3_11. s_bacterium_SH3_11, and g_Luteolibacter.s_Verrucomicrobia_bacterium (Fig. 5B, Supplementary Fig. S6, Supplementary Tables S30 and S32).

4. Discussion

Human society requires pure water for daily life, including direct and indirect consumption during industrial and agricultural processes. However, anthropogenic activities pollute the Earth's aquatic ecosystem, and this pollution worsens daily [1–3]. Using aquatic macrophytes to restore water quality is a green and economical biological method because these aquatic plants can promote biodiversity and the self-clarification of water ecosystems, thus reconstructing their health [4,5,8–11]. Several indices are commonly used to evaluate water quality, including total concentrations of P and N, pH, EC, and turbidity. Many types of pollution influence the EC, and the total ion concentration is correlated with the EC [52]. Moreover, EC measurements are easy to perform and relatively inexpensive [53]. In this study, we found that EC decreased with the growth of the aquatic macrophytes *V. natans* and *V. spinulosa*, providing direct evidence that their growth improves water quality (Fig. 1), making them useful for aquatic ecosystem conservation in the future.

We analyzed the sediment and water column microbial community abundance and structure using high-throughput DNA sequencing technologies. Bacterial abundance was much lower in the water columns, as indicated by the α -diversity of the bacterial community (Fig. 2), which was similar to that of previous reports [31,54,55], possibly because the sediments have more nutrition and oxygen than water, thus supporting an increased number and variety of bacteria. The microbial association and interactions were stronger in the water column than in the sediment, as shown by the network analysis data (Supplementary Fig. S4 and Supplementary Tables S14–S28), possibly because the water columns act as one unit, and the microorganisms interact and communicate freely, while in the sediment, microorganism interactions and communication are limited to numerous small units. The growth of *V. natans* and *V. spinulosa* reduced the Chao1 and Shannon indices of sediment and water samples (Fig. 2), suggesting that these submersed macrophytes may promote the growth of some microorganisms while inhibiting others, thereby reducing bacterial abundance and diversity. The β -diversity results of the microbial communities in the sediments and water columns significantly differed between the absence and presence of *Vallisneria* spp. (Fig. 3). In natural wild aquatic ecosystems, submersed macrophytes influence bacterial community composition in their rhizosphere sediments, epiphytic leaves, and surrounding water [56,57].

Microbial diversity is used as a bioindicator in aquatic ecosystems because it is affected by water quality [29,30]. Our data revealed differences in microbial diversity and water quality caused by submersed macrophyte growth. The submersed macrophytes improved water quality and affected microbial diversity, reconstructing the microbial community by recruiting specific microorganisms while inhibiting others; the aquatic plants and the interacting microorganisms improve water quality, mutually promoting each other [31, 32,38].

Proteobacteria, Acidobacteriota, Planctomycetota, Verrucomicrobiota, Bacteroidota, and Cyanobacteria were the dominant phyla in the VnR and VsR groups, whereas in the mud control sample group (CtM), Acidobacteriota, Proteobacteria, Planctomycetota, Chloroflexi and Verrucomicrobiota were the dominant phyla (Fig. 4A and Supplementary Table S10). Proteobacteria, Verrucomicrobiota, and Bacteroidota increased due to these Vallisneria spp. aquatic macrophytes, especially for α -proteobacteria and γ -proteobacteria, similar to a previous study [54]. It has been reported that different submersed macrophytes differ in the shapes of their interacting bacterial communities [32,54,55]. Our results showed that V. natans and V. spinulosa, highly related Vallisneria spp., differ in their microbial communities in sediment and water columns. Future research using metagenomics and metaculturomics techniques will provide more solid data to help us use different aquatic plants and their microbial communities to improve and protect aquatic ecosystems.

5. Conclusions

The growth of two submersed macrophytes, *V. natans* and *V. spinulosa*, improved the water quality as the EC index decreased. Furthermore, 16S rRNA amplicon analysis was used to analyze the microbial community abundance and structure in the sediment and water columns with or without plant growth. A total of 33,775 ASVs were generated from four sediment groups (BkM, CtM, VnR, and VsR) and three water column sample groups (CtW, VnW, and VsW). The diversity and composition of the microbiomes were altered by *Vallisneria* spp. growth in the sediment and water columns, and the relative abundance of bacteria was higher in the sediment than that in the water columns. The α -diversity of the microbial communities decreased due to submersed macrophytes in both sediment and water columns, and the β -diversity also varied significantly with or without *Vallisneria* spp. The relative abundances of *Proteobacteria*, *Bacteroidota*, and *Cyanobacteria* increased, whereas the phyla *Acidobacteriota* and *Chloroflexi* were reduced by *Vallisneria* spp. *Gammaproteobacteria* and *Alphaproteobacteria* were the dominant classes in *Vallisneria* spp. sediment groups. *Proteobacteria*, *Bacteroidota*, *Cyanobacteriota* and *Actinobacteriota* were the dominant phyla in water columns; *Bacteroidota* and *Cyanobacteria* were increased, whereas *Actinobacteriota* and *Verrucomicrobiota* were decreased by *Vallisneria* spp.

CRediT authorship contribution statement

Libing Liao: Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Deshui Yu:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Data curation, Conceptualization. **Lei Xu:** Investigation. **Qian Hu:** Investigation. **Tongjun Liang:** Resources. **Ludan Chen:** Resources, Investigation. **Qiuping Zhu:** Visualization, Resources, Investigation. **Songping Liu:** Investigation. **Aiwen Zhong:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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