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Mitigation of Eutrophication in a Shallow Lake: The Influences of Submerged Macrophytes on Phosphorus and Bacterial Community Structure in Sediments

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Abstract: Remediating water eutrophication is critical for maintaining healthy and sustainable development of lakes. The aim of this study was to explore the seasonal variation in phosphorus (P) speciation and bacterial community structure in sediments of Qin Lake (Taizhou, Jiangsu Province, China) associated with the growth of submerged macrophyte *Vallisneria natans*. The differences in sediment bacterial diversity and community structure between *V. natans* growing and control areas were analyzed over a period of one year. The results showed that *V. natans* growth reduced the total P and organic matter contents of the sediments and increased the bioavailable iron (Fe) and Fe-bound P contents. The α-diversity of sediment bacteria was significantly higher in the presence of *V. natans* than in the controls during the vigorous plant growth stage. In the presence of *V. natans*, there was a higher relative abundance of Proteobacteria and lower relative abundances of Chloroflexi and Acidobacteria. The Fe(II) content in the sediment had a larger influence on the spatial distribution of bacterial communities than sediment Fe-bound P, organic matter, and Fe(II) contents. *V. natans* growth could reshape sediment bacterial community structure in the shallow lake, which, in turn, enhanced P immobilization in the sediments and thereby improved the water quality.

Keywords: submerged macrophytes; phosphorus speciation; bacterial community structure; lakes; seasonal variation

1. Introduction

Nutrient overloading is a major cause of water eutrophication and water quality decline in shallow lakes. Eutrophication is always followed by rapid growth of phytoplankton and other microorganisms, which will deteriorate water quality and subsequently destroy the lake ecosystem. Outbreaks of algal blooms could happen as a result of water eutrophication with serious consequences [1]. Therefore, eutrophication control is essential for maintaining healthy and sustainable development of lakes.

Phosphorus (P) is a vital and limiting nutrient, and P concentrations can serve as an indicator of lake water quality [2]. Endogenous P in lakes mainly comes from the accumulation and recycling of P in sediments, which play an essential role in the P cycling of shallow lakes [3]. An excess of endogenous P severely affects lake water quality in the long term, even after exogenous P is reduced [4]. Therefore, the retention of P by sediments is the key factor that needs to be addressed to control eutrophication and restore lake ecosystems [5]. In situ remediation using aquatic plants is an effective and environmentally friendly method of controlling lake eutrophication [6,7]. Submerged plants consume a large amount of nutrients during their growth, and thereby markedly decrease the nutrient (P and nitrogen (N)) levels in lakes. In addition, submerged plants can improve the bioavailability of organic matter and other nutrients in lake sediments by secreting oxygen and organic acids from their roots [8,9]. These plants also compete with phytoplankton and prevent sediment resuspension. Therefore, the growth of submerged plants ultimately influences the P cycle in lakes.

From a microbiological perspective, the growth of submerged plants in lakes evidently affects the structure and distribution of sediment microbial communities by altering the pH, dissolved oxygen, and organic matter levels in the lake environment [10,11]. For example, oxygen secretion from the roots of submerged plants can regulate oxygen concentrations in the sediments and affect the associated microbial communities. This, in turn, influences P speciation and biogeochemical cycling [12]. Therefore, the composition of rhizosphere bacterial communities associated with submerged plants is different from that of non-rhizosphere bacterial communities [13,14]. The effects of submerged plants on the environmental conditions and transport of nutrients in sediments depend on the plant species and growth stage. Therefore, the diversity of the associated rhizosphere bacterial communities can vary greatly [15]. Submerged plants reportedly release soluble organic carbon [16,17] and increase the rhizosphere microbial diversity [9]. However, some studies have found that the root exudates of submerged plants may decrease the rhizosphere bacterial diversity due to growth inhibition of specific taxa [14,18]. Therefore, the effects of submerged plants on lake sediment bacterial diversity and associated environmental factors need to be further explored.

Alternating oxidation-reduction (redox) reactions occur frequently at the sediment–water interface of shallow lakes. In particular, P and iron (Fe) undergo alternating deposition and dissolution at this interface. Based on several studies of P and Fe accumulation and release in the anaerobic bottom layer of lakes, it can be inferred that the release of P from lake sediments depends largely on the redox process of Fe [19,20]. Microorganisms play an essential role in the geochemical cycles of nutrient elements in lake sediments [21]. The submerged plant-microbe-sediment system, which is even more complicated with physical, chemical, and biological interactions, also has a profound influence on lake nutrient (especially P) cycles [22]. Submerged plants can enhance the oxidizing capacity of the rhizosphere microenvironment via root oxygen secretions, and thereby promote Fe oxidation. The resulting Fe oxides precipitate phosphate, leading to the immobilization of P in the sediments [7]. However, it remains unclear how the growth of submerged plants affects sediment microbial communities that are potentially involved in P cycling in the changing environment of lakes.

Vallisneria natans (Lour.) Hara is a perennial submerged macrophyte that commonly grows in the middle and lower reaches of the Yangtze River. Owing to its high adaptability and well-developed aerenchyma and root tissues, *V. natans* has become a pioneer species for the restoration of eutrophic lakes and efficiently removes P from water bodies [23]. The removal rate of total P by *V. natans* was found to reach 75% in a simulated eutrophication water ecosystem [24]. *V. natans* was planted in Qin Lake (a shallow freshwater lake in China) to improve the water quality over a decade. However, little is known about the response of sediment microbial communities to *V. natans* growth throughout the year in Qin Lake. Therefore, this study investigated the changes in sediment physicochemical properties, especially the formation of Fe-bound P, in Qin Lake during various growth stages of *V. natans* over one year. The effects of *V. natans* and sediment-associated environmental factors on the sediment bacterial communities were explored. The results of this study could reveal the dynamics of P and bacterial communities in shallow lake sediments as influenced by

V. natans and help to better control eutrophication caused by sedimental P release in Qin Lake.

2. Materials and Methods

2.1. Sampling Site

Qin Lake is located at the junction of Taizhou and Yancheng in central Jiangsu Province, China (Figure 1). The lake area has a subtropical monsoon climate. It is 1.4 km long from east to west and 1.5 km long from north to south, with a total area of approximately 2.3 km^2. The mean water depth of the lake is approximately 1.5 m, and the water pH is generally neutral. Most of the nearshore areas suffer high-level eutrophication; the water in these areas is turbid, and the transparency is only 25 cm. From the late 1980s to the beginning of this century, the total N and P concentrations in the lake water increased 4-fold and 17-fold, respectively. This nutrient increase coincided with serious eutrophication [25]. To mitigate lake eutrophication, the local government implemented a series of countermeasures, including the restoration of aquatic plant communities. A small part of the lake (approximately 5% of the main lake surface area) has undergone greening transformation to create a scenic lake. Of this transformed area, 70% is covered by aquatic plants (*V. natans*). To minimize interference from external factors, the sampling site was selected inside the scenic lake area where there is high biodiversity, far away from farmland and residential areas.

Figure 1. Locations of the study area and sampling site. (**A**) Taizhou in Jiangsu Province, China; (**B**) Qin Lake, with the sampling locations indicated by the red stars; and (**C**) *V. natans* growing area and control area within the sampling site.

2.2. Sample Collection

Sediment sampling was carried out in January, March, May, July, September, October, and December 2019. Six sampling locations were selected, three from the lake area planted with *V. natans* (32°37′27″ N–32°37′32″ N, 120°09′83″ E–120°09′89″ E), and three from the lake area without submerged plants (control; 32°37′32″ N–32°37′37″ N, 120°09′74″ E–120°09′81″ E). The sampling locations were spaced approximately 100 m apart. At each location, three surface sediment samples (0–15 cm) were collected using a Peterson sampler from a water depth of 1.5 m and mixed uniformly. A total of 42 sediment samples were collected throughout the year. At the time of sediment sampling, the pH and dissolved oxygen concentration of the overlying water (right above the water/sediment interface) were also measured on site immediately after collection.

The sediment samples were placed in a cooler and transported to the laboratory, where each sample was divided into three subsamples. One subsample was immediately stored in a refrigerator at −80 °C for microbial community analysis. The second subsample was vacuum freeze-dried at −50 °C for the determination of Fe content. The third subsample was air-dried, ground, passed through a sieve (mesh size 150 μm), and then stored at room temperature for two weeks before conventional physicochemical testing.

2.3. Physicochemical Analysis

The pH and dissolved oxygen concentration of the lake water samples were measured on site using a portable SX725 Portable pH/Dissolved Oxygen Meter (Shanghai San-Xin Instrumentation Inc., Shanghai, China). Fe was extracted from the freeze-dried sediment samples using 0.5 M HCl, and the content of bioavailable Fe, including Fe(II) and Fe(III), was determined using the *o*-phenanthroline spectrophotometry method [26]. The organic matter content was determined using the dichromate oxidation method. Briefly, the air-dried sediment was oxidized by potassium dichromate in the presence of H_2SO_4 with a controlled heating step (170–180 °C for 5 min). The excess of dichromate was titrated with 0.2 M $FeSO_4 \cdot 7H_2O$, and the organic matter content was calculated following an equation [27,28]. The total P content was determined using the molybdenum-antimony anti-spectrophotometric method [28] after digestion with $HClO_4$-H_2SO_4. The Fe-bound P was fractioned with the SMT (Standards, Measurements, and Testing Program of the European Commission) protocol [29] and determined also using the molybdenum-antimony anti-spectrophotometric method [28].

2.4. DNA Extraction, PCR Amplification, and Sequencing

Total DNA was extracted from 0.5 g of sediment samples using a PowerSoil DNA extraction kit (QIAGEN Inc., Valencia, CA, USA) following the manufacturer's instructions. The V3–V4 region of the bacterial 16S rRNA gene was amplified using the universal primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) [30]. After purification and quantification, PCR products were sent to Majorbio Biomedical (Shanghai, China), where they were sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) using a 464 bp paired-end protocol.

2.5. Data Analyses

The bioinformatic analysis of high-throughput sequence data was carried out online using the Majorbio I-Sanger Cloud Platform (http://www.i-sanger.com accessed on 15 January 2021). Clean sequences were clustered into operational taxonomic units (OTUs) according to a sequence similarity threshold of 97% [31]. Species annotation of the OTUs was performed using the SILVA bacterial 16S rRNA database (Release 132; http://www.arb-silva.de accessed on 17 January 2021). The bacterial taxonomic information was obtained for all samples, and the relative abundances of dominant taxa ($\geq$1% of total sequences at the phylum level and $\geq$2% of total sequences at the family level) were calculated. Bacterial α-diversity was estimated using the observed number of species (Sobs), abundance-based coverage estimator (ACE), and Shannon diversity index [32,33]. Functional genes and metabolic pathways (Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways) were predicted using Phylogenetic Investigation of Communities by Re-construction of Unobserved States (PICRUSt 1.1.0; https://github.com/picrust accessed on 27 January 2021).

Statistical analyses were carried out using IBM SPSS Statistics 26 (https://www.ibm.com/products/spss-statistics accessed on 17 March 2021). The differences in sediment/water physicochemical properties, bacterial α-diversity indexes, including Sobs, Shannon and ACE indexes, and dominant taxa abundances between samples were determined using one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests. A p value of less than 0.05 was considered to indicate statistical significance. Non-metric multidimensional scaling (NMDS) based on Bray–Curtis distances was used to visualize the similarities or differences in bacterial community composition between samples. Canonical correlation analysis (CCA) was used to analyze the relationship between bacterial community structure and sediment-associated environmental factors. The significance of the influence of environmental factors on the distribution of bacterial communities was tested using the envfit function, where a greater r^2 value indicates a larger influence. Both NMDS and CCA were carried out using R language (http://www.r-project.org/ accessed on 15 April 2021). The relationship between major bacterial phyla and associated environmental factors was determined based on Spearman's rank correlation.

3. Results

3.1. pH and Dissolved Oxygen Concentration in the Lake Water

In situ measurement results showed that pH values and dissolved oxygen concentrations were higher in water samples associated with *V. natans* than in the control samples (without *V. natans*) throughout the course of the year (Figure 2). With the exception of January and December, pH values were significantly higher in water samples with *V. natans* than those in the control samples ($p < 0.05$). In the presence of *V. natans*, the pH of the water samples decreased from January (8.20) to May (7.32) and then gradually increased, reaching a peak value (8.83) in September; from September onwards, pH varied slightly. These pH trends were also observed for the control samples, but the decrease in pH was more evident in the control samples than in the presence of *V. natans*; the lowest pH (6.54) occurred in May.

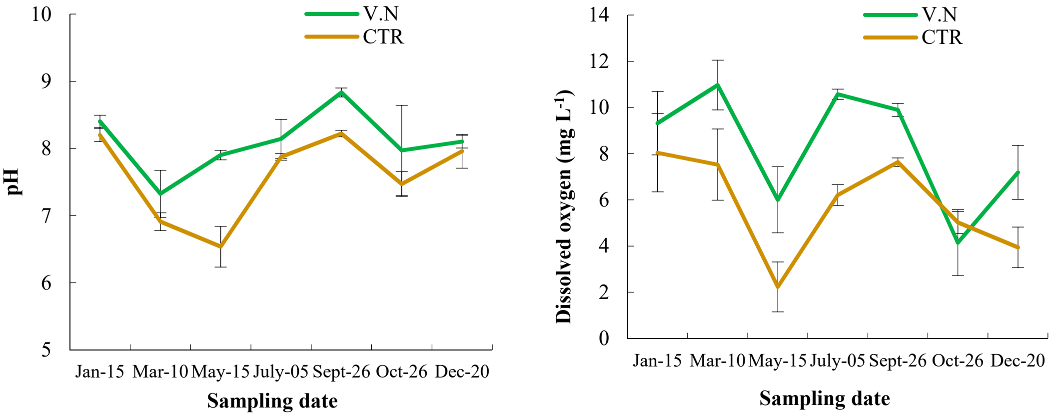

Figure 2. Variation in pH and dissolved oxygen concentration of lake water throughout one year in the presence (VN) and absence (CTR) of *V. natans*.

Similar to the trends in pH, the lowest dissolved oxygen concentrations occurred in May, both in water samples with and without *V. natans* (6.00 and 2.23 mg L^{-1}, respectively). The dissolved oxygen concentrations were similar in January and September for both sample types, ranging from 7.63 to 9.89 mg L^{-1}. The highest dissolved oxygen concentration (10.97 mg L^{-1}) was observed in water samples with *V. natans* in March, and this concentration was significantly higher than that of the control samples ($p < 0.05$).

3.2. Organic Matter, Fe, and P Contents in the Lake Sediment

The organic matter contents were markedly lower in sediment samples associated with *V. natans* than those in the control samples (Figure 3). In particular, these differences were found to be significant in July and September when the organic matter contents were 28.4% and 40.9% lower, respectively, in the *V. natans* samples ($p < 0.05$). In the presence of *V. natans*, the highest organic matter content (28.8 g kg^{-1}) was reached in March, and the lowest content (20.9 g kg^{-1}) was recorded in October.

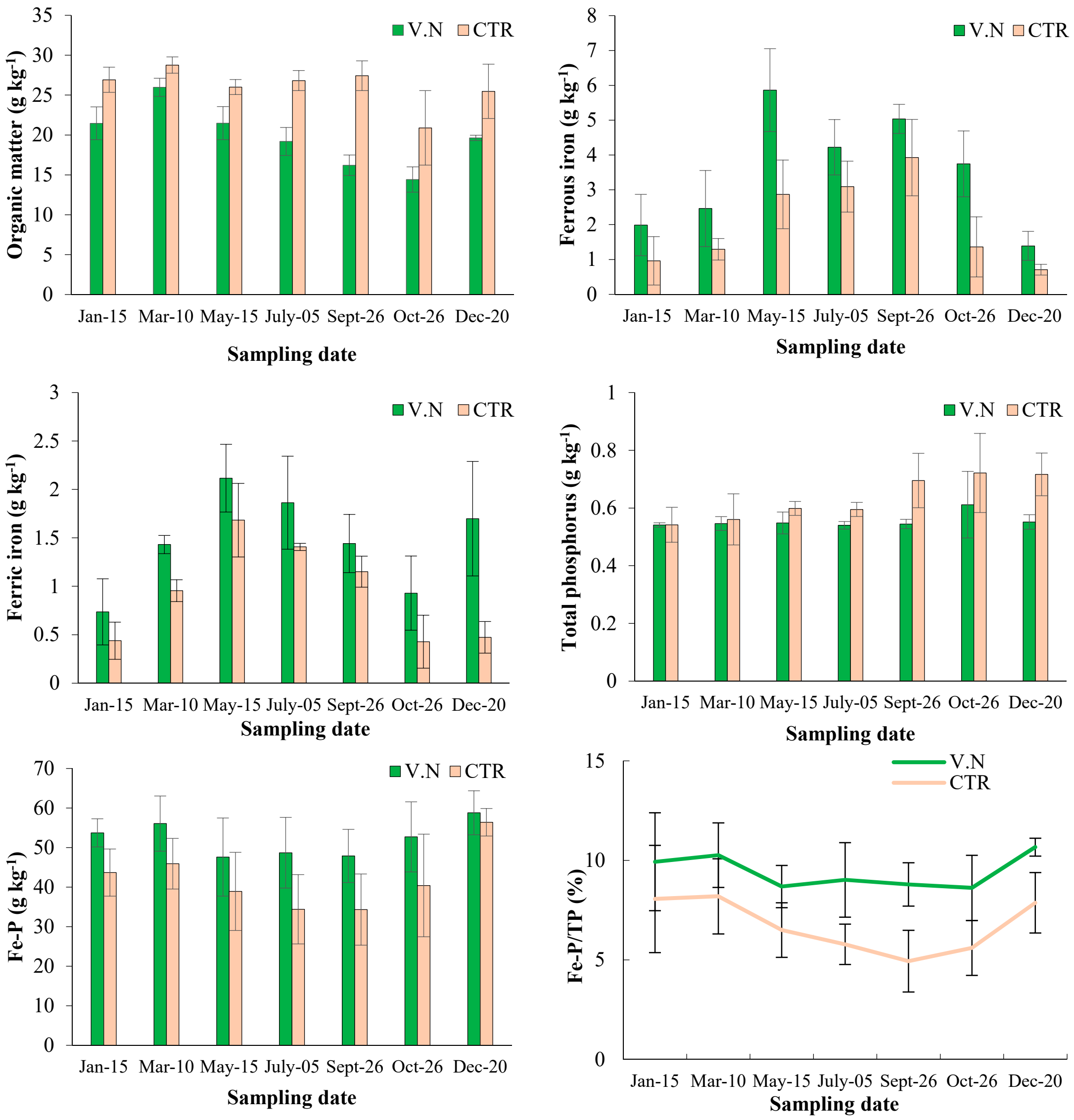

Figure 3. Variation in the organic matter, iron (Fe), and phosphorus (P) contents of lake sediment samples in the presence (VN) and absence (CTR) of *V. natans* over the course of one year. TP, total phosphorus; Fe-P, iron-bound phosphorus. Error bars indicate standard deviations of duplicate measurements.

The growth of *V. natans* led to significantly higher contents of bioavailable Fe in the sediment samples. The highest contents of both Fe(II) and Fe(III) occurred in the presence of *V. natans* in May. Overall, the bioavailable Fe contents first increased and then decreased over time, reaching higher values in summer than in winter.

Generally, the total P contents of sediment samples were lower throughout the year in the presence than in the absence of *V. natans*. Compared to the control, there was a significant decrease in the total P contents in May, when *V. natans* started to grow vigorously. Furthermore, a relative decrease of 23.1% was observed in December ($p < 0.05$). In contrast, the Fe-bound P contents were higher in the sediment samples with *V. natans* than in the control samples, and this difference was especially significant in September ($p < 0.05$). The Fe-bound P contents did not change markedly with season. Only a slight decrease in Fe-bound P contents occurred in the samples with *V. natans* during May and July, which corresponded to the fastest growth stage of *V. natans*. Moreover, the ratio of Fe-bound P to total P (Fe-P/TP) was markedly higher in the presence of *V. natans*. The largest difference in this ratio was observed in September, with the ratio being 43.9% higher in the presence of

V. natans. The Fe-P/TP ratio values remained relatively stable throughout the year, ranging from 4.9% to 10.6%.

3.3. Bacterial Community Composition in the Lake Sediment

3.3.1. Bacterial Community Diversity

High-throughput sequencing of the 42 sediment samples yielded a total of 2,273,130 effective sequences with a mean length of 425 bp. These effective sequences were classified into 16,235 OTUs, and the mean coverage was over 95%. The largest OTU number per sample (4600) was found in *V. natans*-associated sediments in May, whereas the smallest OTU number per sample (2420) was found in the control sample in September. In addition, 33.9%–35.4% of the total OTUs were shared between the two types of sample group in January, March, and May. The percentage of shared (common) OTUs started to gradually increase from 38.1% in July and reached 48.2% in December (Supplementary Figure S1).

Three α-diversity indexes were used to explore the effects of *V. natans* growth on bacterial species richness and evenness in the sediment samples (Table 1). The highest Sobs and Shannon index values were observed in *V. natans*-associated sediments in July (5077.3 and 7.32, respectively). The lowest Sobs and Shannon index values were obtained in *V. natans*-associated sediments in October (3096.3 and 6.49, respectively). There were significant differences in bacterial diversity between the two treatments according to the Sobs and ACE indexes in January, ACE index in September, and Shannon index in October. The results indicated that the bacterial richness of the control samples was relatively high in January. Meanwhile, the bacterial evenness of *V. natans*-associated sediments was the lowest in October. On the whole, the bacterial α-diversity (including species richness and evenness) tended to increase first and then decrease over time, reaching the highest levels in summer.

Table 1. Alpha-diversity of sediment bacterial communities estimated based on operational taxonomic units clustered at the 97% level.

Sampling Time	Treatment	Sobs	Shannon	ACE	Coverage
January	V.N	3582.3 b	6.67	4680.2	0.972
	CTR	4509.3 a*	7.15	6665.2	0.94
March	V.N	3698.3	7.01	4375.3	0.98
	CTR	4074.3	6.95	6216.0	0.95
May	V.N	3849.3	6.73	5602.9	0.96
	CTR	3162.5	6.37	4054.8	0.98
July	V.N	5077.3	7.32	7054.0	0.95
	CTR	4948.0	7.24	7172.5	0.95
September	V.N	4309.8	7.01	7053.4 a	0.95
	CTR	4163.8	7.01	6076.5 b	0.95
October	V.N	3096.3	6.49 b	5440.9	0.95
	CTR	3940.5	7.18 a	5138.7	0.96
December	V.N	3648.3	6.80	6562.4	0.94
	CTR	3835.3	6.92	6468.6	0.95

Note: V.N and CTR refer to sediment samples in the presence and absence of *V. natans*, respectively. *a and b in the same column indicate significant differences between the treatments at each sampling time by one-way ANOVA (Tukey, $p < 0.05$). The values without lowercase letter are not significantly different.

3.3.2. Bacterial Community Composition

The bacterial OTUs retrieved from the 42 sediment samples were classified into 3618 species, 1577 genera, 853 families, and 72 phyla. At the phylum level, the top 10 bacterial taxa in terms of relative abundance were Chloroflexi (22.6% of total sequences), Proteobacteria (20.9%), Acidobacteria (11.1%), Actinobacteria (5.9%), Desulfobacterota (5.8%), Bacteroidota (4.5%), Nitrospirota (3.7%), Gemmatimonadota (3.6%), Myxococcota (2.7%), and Firmicutes (2.2%; Figure 4A). The relative abundances of the dominant phyla in the bacterial community varied considerably with season. Proteobacteria, Chloroflexi, and Acidobacteria were dominant in January and March, accounting for >50% of the community. Chloroflexi was predominant in May, July, and September. In the later growth stages, the relative abundance of Proteobacteria became higher than that of Chloroflexi.

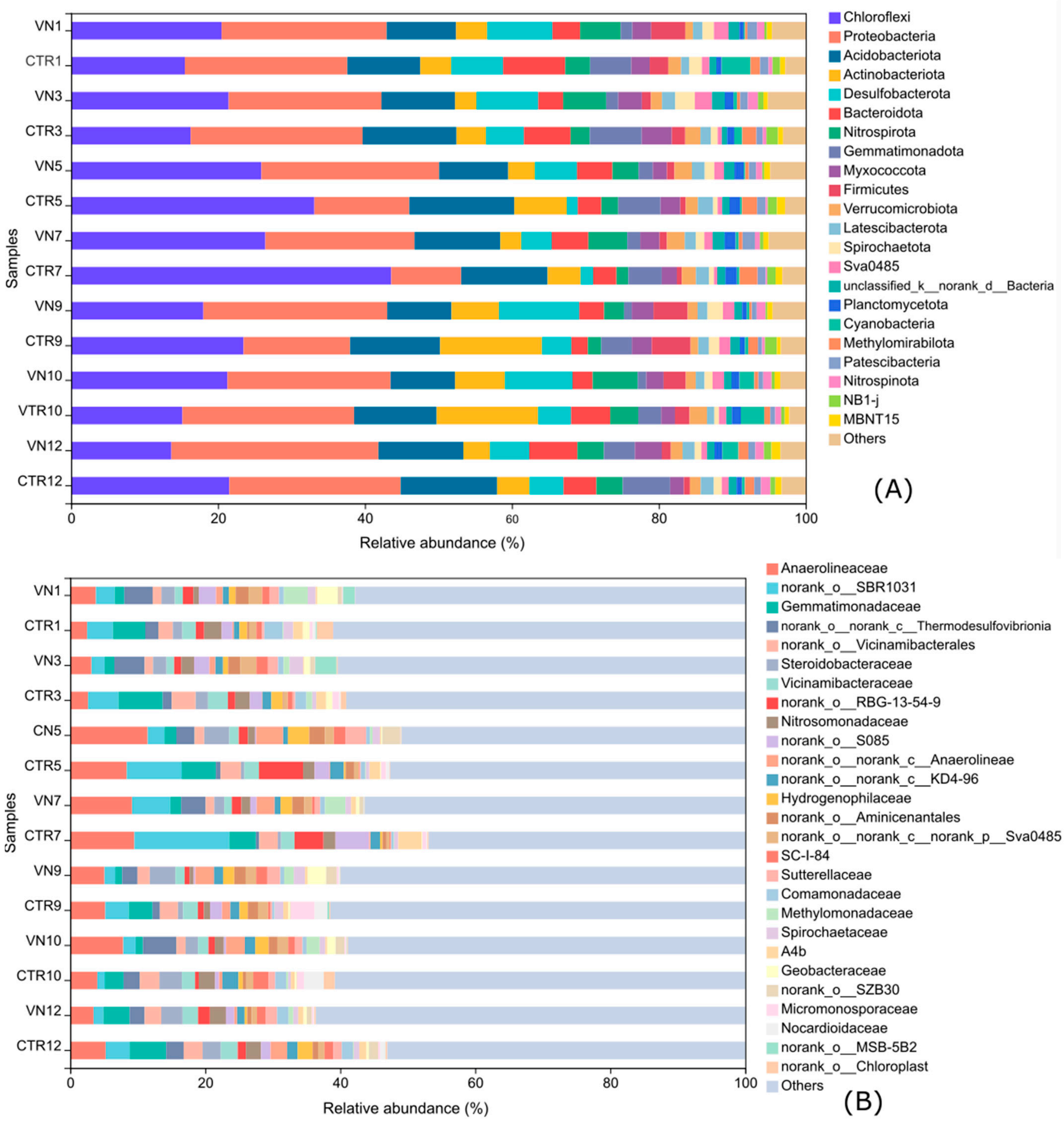

Figure 4. Bacterial community compositions at the (**A**) phylum (≥1% of total sequences) and (**B**) family (≥2%) levels, in lake sediment samples collected in the presence (VN) and absence (CTR) of *V. natans* over the course of one year. The numbers in the sample codes represent the month of sampling (i.e., 1, 3, and 5 represent January, March, and May, respectively).

Further, ANOVA was used to examine the differences in the relative abundances of dominant bacterial phyla between the two sediment sample types at different time points (Supplementary Figure S2). In January (before *V. natans* started to grow), there was little difference in the relative abundances of the top four phyla between the two treatments. However, the relative abundances of Bacteroidota and Gemmatimonadota were significantly lower, and the relative abundance of Nitrospirota was significantly higher in the presence of *V. natans* than in the control samples ($p < 0.05$). Moreover, many phyla significantly differed in their relative abundance between the two treatments in May. For example, the relative abundances of Proteobacteria and Desulfobacterota were significantly higher, and those of Acidobacteria, Actinobacteria, Gemmatimonadota, Myxococcota, and Methylomirabilota were significantly lower in the presence of *V. natans* than in the control samples ($p < 0.05$). In December, there were no significant differences in the relative abundances of dominant phyla between the two treatments.

At the family level, the most abundant bacteria were classified as Anaerolinenaceae (phylum Chloroflexi) and norank_o_SBR1031 (unclassified; Figure 4B). The sum of the relative abundances of these two families accounted for a higher percentage of the bacterial community in May and July than in the other seasons, and the highest value was found in the control samples (23.6%). The third to sixth most abundant families were Gemmatimonadaceae, norank_c_Thermodesulfovibrionia, norank_o_Vicinamibacterales, and Steroidobacteraceae. Of these, Gemmatimonadaceae and norank_o_Vicinamibacterales displayed significantly lower relative abundances, and norank_c_Thermodesulfovibrionia and Steroidobacteraceae generally showed higher relative abundances in *V. natans*-associated sediments than in the control samples. In addition, the relative abundance of Nitrosomonadaceae was lower, and the relative abundances of Hydrogenophilaceae and Geobacteraceae were higher in *V. natans*-associated sediments than in the control samples.

3.3.3. Differences in Bacterial Community Composition

To visualize the differences in bacterial community compositions, an NMDS plot was generated based on the relative abundances of bacterial OTUs (stress = 0.125; Figure 5). The community composition of *V. natans*-associated sediments was distinctly separate from that of the control samples over the study period. This indicated that the growth of *V. natans* considerably altered the composition of sediment bacterial communities. Among all the samples, the replicates of the same sample group were most closely distributed, and the community composition was most similar in the presence of *V. natans* during the vigorous growth stage (May and July). In contrast, larger differences were observed in the community composition of samples with and without *V. natans* during the remaining months.

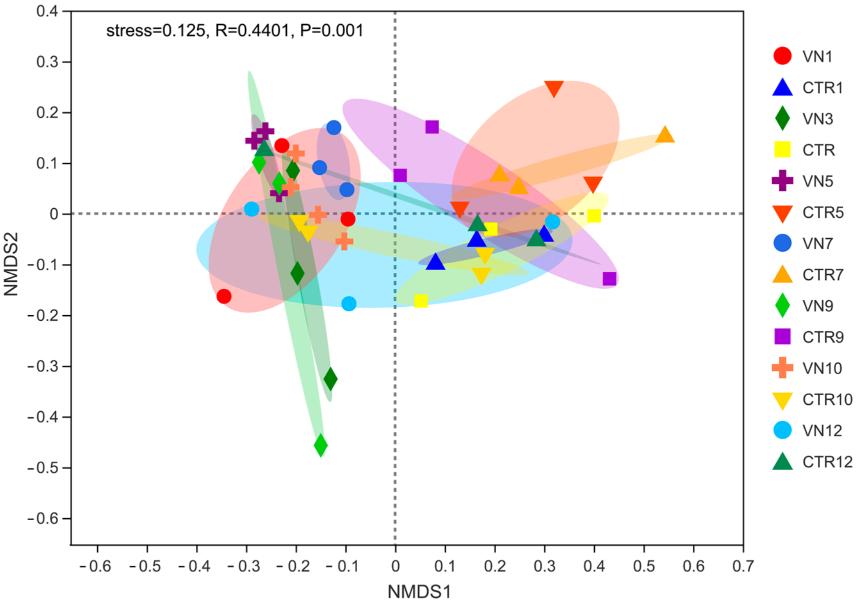

Figure 5. Non-metric multidimensional scaling (NMDS) ordination plot showing the distribution of bacterial communities in lake sediment samples collected in the presence (VN) and absence (CTR) of *V. natans* over the course of one year. The numbers in the sample codes represent the month of sampling (i.e., 1, 3, and 5 represent January, March, and May, respectively).

3.4. Relationship between Bacterial Communities and Environmental Factors

To determine the relationship between sediment bacterial community structure (at the OTU level) and associated environmental factors, CCA was performed. It was found that the first and second CCA axes explained 37.8% and 23.4% of the variation in the bacterial community structure, respectively (Figure 6).

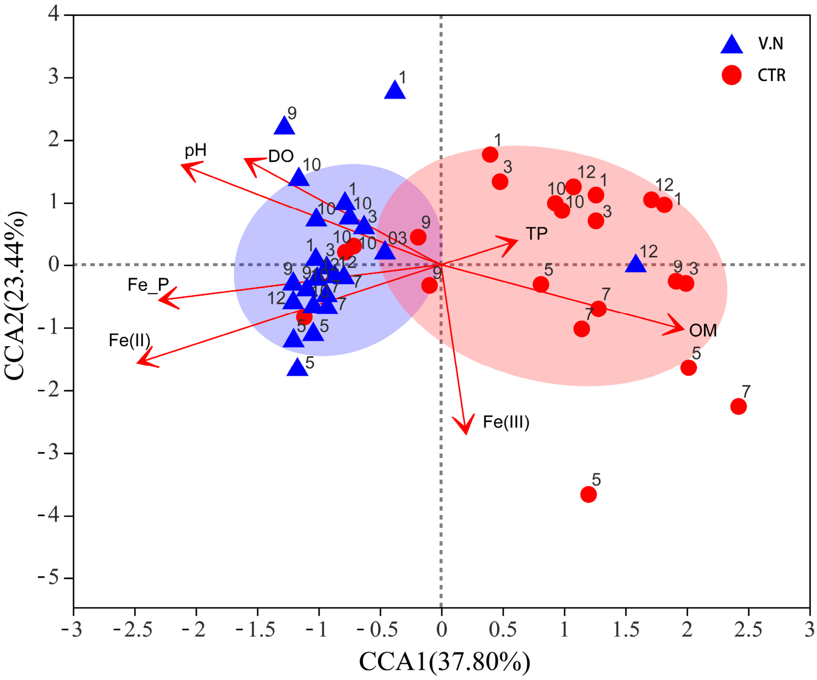

Figure 6. Canonical correlation analysis (CCA) of the relationship between operational taxonomic unit (OTU)-level bacterial community structure in sediments and the associated environmental factors in the lake. Samples were collected in the presence (V.N) and absence (CTR) of *V. natans*. pH, pH in overlying water; DO, dissolved oxygen in overlying water; OM, organic matter in sediment; TP, total phosphorus in sediment; Fe_P, iron-bound phosphorus in sediment; Fe(II), ferrous iron in sediment; and Fe(III), ferric iron in sediment.

Considering the length of the arrows, the sediment Fe(II) content had the largest influence on the distribution of bacterial communities in the samples ($p = 0.001$). The bacterial community structure of *V. natans*-associated sediments was significantly positively correlated with sediment Fe(II) and Fe-bound P contents, water pH, and dissolved oxygen concentrations. The bacterial community structure of the control samples was mainly influenced by the total P and organic matter contents in the sediment (Figure 6). Furthermore, envfit analysis revealed that among the main environmental factors tested, sediment Fe(II) and Fe(III), water pH, Fe-bound P, dissolved oxygen concentration, and organic matter had the most influence on the distribution of sediment bacterial communities ($p < 0.05$; Supplementary Table S1).

Subsequently, Spearman's rank correlation was used to explore the relationship between bacterial taxa (top 20 phyla) abundances and environmental factors. It was found that the relative abundances of the dominant bacterial phyla were most frequently correlated with water dissolved oxygen concentration and less frequently correlated with water pH and sediment Fe(III) and Fe-bound P contents (Figure 7).

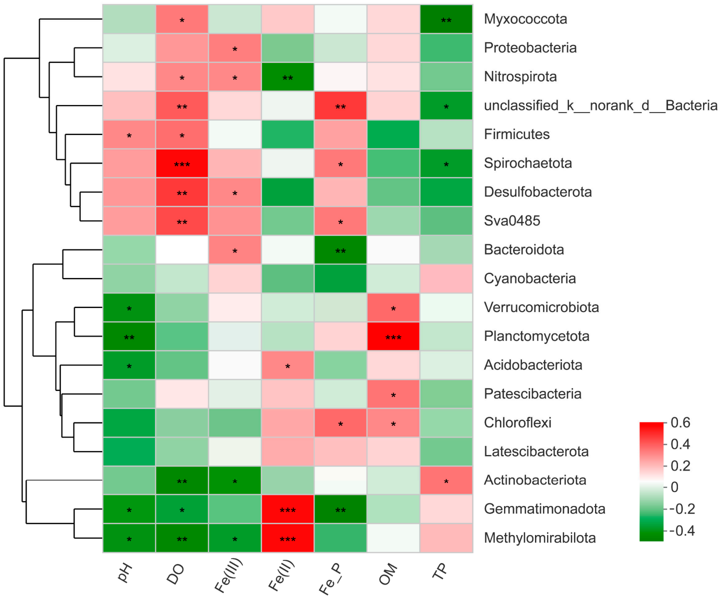

Figure 7. A heatmap showing the Spearman's correlation matrix between the relative abundances of dominant bacterial phyla in lake sediments and the associated environmental factors. DO, dissolved oxygen in overlying water; Fe(III), ferric iron in sediment; Fe(II), ferrous iron in sediment; Fe_P, iron-bound phosphorus in sediment; OM, organic matter in sediment; and TP, total phosphorus in sediment. *, $p < 0.05$; **, $p < 0.01$; *** $p < 0.001$ in Spearman's rank correlation analysis.

3.5. Prediction of Bacterial Functions

The potential functions of the bacteria identified in the sediment samples were predicted using PICRUSt analysis. There were no considerable differences between the two treatments in terms of the main functional pathways of the bacteria, including their metabolism, cellular processes, environmental information processing, and genetic information processing (Figure 8A). Further, the abundances of the main pathways related to energy metabolism were compared between the two treatments (Figure 8B). It was found that the relative abundances of functional pathways related to sulfur (S) metabolism, N metabolism, methane (CH_4) metabolism, carbon (C) fixation, and oxidative phosphorylation in *V. natans*-associated sediments were higher in January, March, and July, but lower in the remaining months, when compared to those of the control samples. With the exception of December, the abundances of pathways related to photosynthesis were higher in *V. natans*-associated sediments than in the control samples.

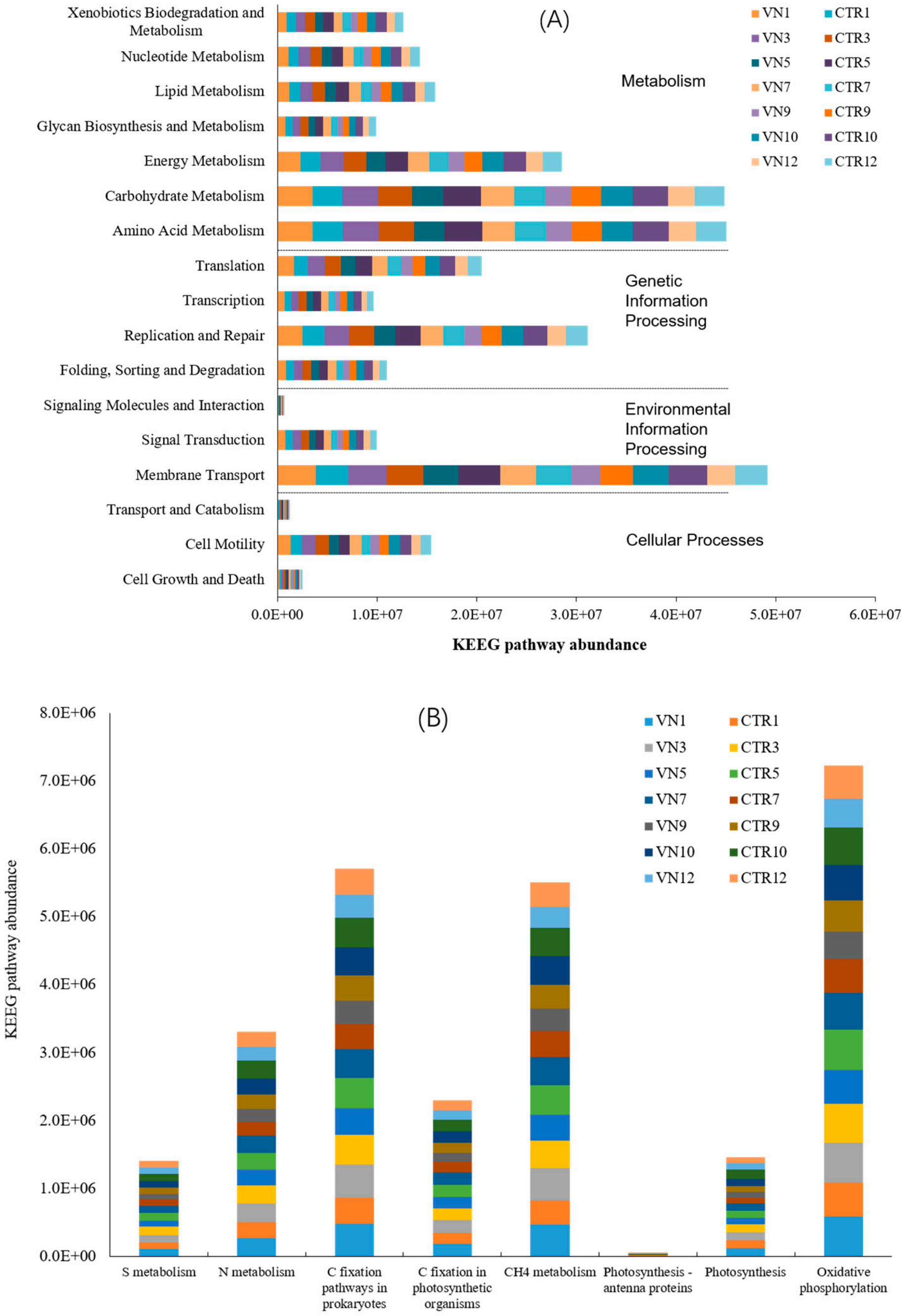

Figure 8. Kyoto Encyclopedia of Genes and Genomes (KEGG)-predicted metabolic pathways of category level 1 (Metabolism, Cellular processes, Organismal systems, and Genetic information processing) and the corresponding subcategories (level 2) (**A**) and energy metabolism pathways at level 3 (**B**) of the bacteria identified in lake sediments. Sediment samples were collected from sites with *V. natans* (VN) and without (CTR) *V. natans*. The numbers in the sample codes represent the month of sampling (i.e., 1, 3, and 5 represent January, March, and May, respectively).

4. Discussion

In recent years, the water quality of Qin Lake has considerably improved since the introduction of *V. natans* in a part of the lake area. In addition to improving lake water quality, the growth of *V. natans* has been found to increase the bacterial diversity and alter the bacterial community composition in the surface water of West Lake in Hangzhou, China [3]. However, most previous studies on the remediation effects of *V. natans* have been limited to a specific plant growth stage and have focused on lake water bodies. Here, P speciation in the sediments from Qin Lake was analyzed during the various growth stages of *V. natans* throughout one year. It was found that the growth of *V. natans* reduced the total P content and increased the percentage of Fe-bound P in the sediments. Fan et al. [34] also found that the bioavailable Fe and Fe-bound P contents in sediments were higher in a macrophyte-dominated bay than in a cyanobacteria-dominated bay of Tai Lake, Jiangsu Province, China.

In summer, the temperature rises, and the metabolism of various benthic microorganisms becomes vigorous in lakes. This increased microbial metabolism leads to enhanced reduction of Fe(III) which, in turn, causes the release of Fe/Al-bound P from the sediments [6]. Indeed, in the present study, both the Fe(II) and Fe(III) contents of sediment samples increased during the high-temperature season, whereas the Fe-bound P contents displayed a relative decrease in Qin Lake. Compared to the control, *V. natans* growth attenuated the decrease in the sediment Fe-bound P contents, thereby maintaining a relatively high proportion of Fe-bound P with respect to the total P. This result indicates that *V. natans* growth facilitates the immobilization of P in the sediment of the shallow lake. In addition, *V. natans* can take up a large amount of P during their vigorous growth stage. This P removal effect can be further enhanced by implementing harvesting and surface dredging [35]. Thus, *V. natans* can be managed to facilitate the remediation of eutrophic water bodies in Qin Lake and other similar lakes.

Based on high-throughput sequencing, it was found that the bacterial diversity of sediments markedly varied with season in Qin Lake. Overall, the bacterial diversity was higher in summer than in winter, especially for sediments in the *V. natans* growing area. There are two potential explanations for this diversity increase. First, in summer, the temperature rises to a level that may be suitable for supporting an increase in most microbial activities in the sediments. Second, *V. natans* plants grow fast and exhibit vigorous metabolic activities in summer; the roots develop rapidly and deliver large amounts of oxygen into the sediments, thus supporting a higher diversity of bacteria [6].

The NMDS analysis performed in this study revealed that the bacterial community composition of the sediments was markedly different between the Qin Lake areas with and without *V. natans*. Moreover, the sediment samples collected from sites in the presence of *V. natans* clustered closer together than those sampled from sites in the absence of the plant. *V. natans* plants possess a flourishing root system and well-developed aerenchyma. Therefore, *V. natans* can substantially change the oxygen state in the rhizosphere and efficiently absorb nutrients (such as N and P) and heavy metals (such as Cu, Pb, and Cr) from sediments [36,37]. Moreover, root exudates may change sediment pH and thus alter the rhizosphere environment. Together, these factors may explain the major difference in bacterial community composition between the lake sediments with and without *V. natans* observed in the present study. Similarly, Zhao et al. [14] observed considerable differences in the microbial community compositions between rhizosphere and non-rhizosphere sediments associated with three submerged plants, *Ceratophyllum demersum*, *Vallisneria spiralis*, and *Elodea nuttallii*, during microcosm culture.

In this study, Proteobacteria, Chloroflexi, and Acidobacteria were identified as the dominant bacterial phyla in the sediments from Qin Lake throughout the year. Of these, Chloroflexi was the most abundant phylum in May, July, and September, and its relative abundance was lower in the presence of *V. natans*. Chloroflexi comprises a group of bacteria capable of photosynthesis; they can fix CO_2 to generate energy and are strongly affected by light [38,39]. Light and temperature changes may be the primary causes of the increase in the relative abundance of Chloroflexi from May to September. Light and temperature are also potentially the dominant factors driving the seasonal variation in the bacterial community composition [15].

Members of Chloroflexi are commonly found in sediments of heavily polluted rivers, lakes, and oceans [40,41]. This may also explain the relatively low abundance of Chloroflexi in the Qin Lake area where *V. natans* grows and the water quality is favorable. The bacterial function prediction performed in this study indicated that the abundances of photosynthesis-related pathways were higher in sediments associated with *V. natans* than in the control samples. Planting *V. natans* in shallow lake ecosystems can effectively inhibit phytoplankton blooms, increase the transparency of the water body [6], and increase the amount of light reaching the sediments. These factors are conducive to the increased photosynthesis by sediment microbes.

In this study, the relative abundance of Proteobacteria in the sediment bacterial community was found to markedly increase during the vigorous growth stage of *V. natans*. The growth of *V. natans* consumes a large amount of nutrients and inhibits the release of nutrients from the sediments. This, in turn, greatly alters the microenvironment for bacterial growth. The increase in Proteobacteria abundance related to *V. natans* growth may even have resulted from the improved water quality [15].

Acidobacteria, which ranked third in terms of relative abundance in the sediment samples from Qin Lake, are involved in the degradation and transformation of multiple elements, such as the reduction of Fe under anaerobic conditions [42]. Members of Acidobacteria also make a major contribution to the P cycle [43]. In the present study, the relative abundance of Acidobacteria was lower in the presence of *V. natans* than in the control samples, especially during summer and autumn. This result may be associated with an increase in environmental pH due to the growth of *V. natans* [10].

At the family level, Anaerolinenaceae and norank_o_SBR1031 were the most abundant bacteria in the sediment samples. These two families accounted for higher proportions of the sediment bacterial community in the control samples in May and July than in the other samples. During this stage (May and July), the rapid growth of *V. natans* could shift the sediment environment to aerobic or microaerobic [44]. These conditions are unfavorable for the strict anaerobic growth of Anaerolinenaceae [45]. Compared to that of the control, the relative abundance of norank_c_Thermodesulfovibrionia was always higher in sediment samples associated with *V. natans* throughout the year. In addition to sulfate, sulfite, and thiosulfate, Desulfovibrio spp. can use Fe(III) as an electron donor [46]. Therefore, the relatively abundant norank_c_Thermodesulfovibrionia detected in our samples might also participate in Fe reduction in the sediments.

Owing to the effects of *V. natans* growth, the contents of bioavailable Fe, especially reduced Fe(II), were always higher in sediments associated with *V. natans* compared to those of the control samples during the different seasons. The organic matter content and composition, dissolved oxygen, and redox potential in the environment all affect the microbial community composition [9] and further influence the Fe redox process. During the one-year study period, the relative abundances of Proteobacteria and Desulfobacterota in the sediment samples were markedly higher in May than in the remaining months. Desulfobacterota can reduce sulfate to hydrogen sulfide, and the latter will bind to free Fe, thereby increasing the immobilization of Fe and releasing P from Fe-P [47]. As mentioned earlier, some sulfate-reducing bacteria also have the ability to reduce Fe(III) [48]. This might also explain the decrease of sediment Fe-P content in Qin Lake in May.

Many bacteria in environmental samples can simultaneously reduce Fe(III) when oxidizing organic C. The resulting reduced Fe can be utilized by various Fe-oxidizing bacteria, such as microaerobic and anaerobic Fe-oxidizing bacteria that are commonly found in the rhizosphere and surrounding environments [49]. Eventually, Fe-bound P precipitates are formed, thereby reducing the release of endogenous P from sediments. The relative abundance of Geobacteraceae, a typical family of Fe-reducing bacteria [50], was higher in the sediment samples from the *V. natans* growing area than in the control samples. The organic matter produced by the massive growth of *V. natans* would have supplied abundant C for these Fe-reducing bacteria, thereby facilitating the Fe–P turnover. Similarly, Hydrogenophilaceae appeared to be more abundant in *V. natans*-associated sediment samples from May to October. The Hydrogenophilaceae family contains the *Thiobacillus* genus, which harbors bacteria that oxidize reduced sulfur compounds [51].

Based on CCA, it was found that [Fe(II)], [Fe(III)] and water pH had a prominent influence on the distribution of sediment bacterial communities in Qin Lake. These environmental factors were also significantly correlated with the relative abundances of most dominant bacterial phyla detected in the sediments. It can be concluded that the growth of *V. natans* impacts the sediment bacterial community structure in Qin Lake by increasing water pH and dissolved oxygen concentration and reducing sediment total P content. The resulting changes in bacterial activities, in turn, alter Fe speciation by facilitating the formation of Fe-bound P in the sediments [6] and thereby reduce the release of endogenous P from the sediments. These effects of *V. natans* growth exhibit notable seasonal variation.

The prediction of potential functional pathways of microbial communities revealed that the abundance of pathways related to S, N, and CH_4 metabolism was lower in *V. natans*-associated sediments in May as compared to that of the control, indicating lower activities of the associated microorganisms. Previous studies have shown that methanogenic archaea are common in sediments [52], and CH_4 cycling processes are subject to large spatiotemporal heterogeneity [53], with high fluctuations in the abundance of methanotrophs [52]. Moreover, CH_4 dynamics have significant positive correlations with sediment total organic carbon, Fe(II), and Fe(III) contents [54], in addition to total phosphorus and soluble reactive phosphorus contents [55]. Therefore, future research should evaluate the archaeal communities associated with *V. natans* growth to further increase the understanding of microbial functions in P speciation in shallow lake sediments.

5. Conclusions

In this study, periodic sampling was carried out over a one-year period to evaluate the effects of submerged plants (*V. natans*) on P speciation and bacterial community structure in the sediments of a shallow lake. It was found that the growth of *V. natans* in the lake increased water pH and dissolved oxygen concentration. The growth of *V. natans* also reduced total P content and greatly increased the bioavailable Fe, organic matter, and Fe-bound P contents of the sediments. The sediment bacterial communities were dominated by Proteobacteria, Chloroflexi, and Acidobacteria. The presence of *V. natans* increased the relative abundance of Proteobacteria and decreased the relative abundances of Chloroflexi and Acidobacteria. The bacterial diversity increased during the vigorous growth stage of *V. natans*. Among the various environmental factors, sediment Fe(II) and Fe(III) contents had the greatest influence on the spatial distribution of bacterial communities in the sediments, followed by water pH. Vigorous growth of *V. natans* notably affected the sediment bacterial community structure in the shallow lake by changing the physicochemical properties of the water and sediment. The altered bacterial communities may drive the formation of Fe-bound P and thereby increase the immobilization of endogenous P in the sediments. The restoration effect of *V. natans* on eutrophic shallow lakes can be further enhanced by applying Fe flocculants and implementing appropriate dredging projects.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su13179833/s1, Figure S1: Venn diagram showing the distribution of operational taxonomic units between lake sediment samples in the presence (V.N) and absence (CTR) of *V. natans* over the course of one year, Figure S2: Bacterial phyla with significant difference in relative abundance between lake sediment samples with *V. natans* (V.N) and without *V. natans* (CTR) at different time points, Table S1: The relationship between sediment bacterial community structure and associated environmental factors by canonical correlation analysis (CCA) and envfit analysis.

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