



# Bacterial Communities and Their Predicted Functions Explain the Sediment Nitrogen Changes Along with Submerged Macrophyte Restoration

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## Abstract

Submerged vegetation biomass fluctuation usually occurs during the preliminary stage of vegetation restoration in shallow lakes, which impacts the final status and duration for achieving a macrophyte-dominant state. This study uncovered the sediment N characteristics and the sediment bacterial community and their predicted functions during the preliminary stage of vegetation recovery in the West Lake, a typical subtropical degenerated shallow lake in China. Results showed increased amounts of sediment TN and NH<sub>4</sub>-N, reaching 3425.76 and 345.5 mg kg<sup>-1</sup>, respectively, when the vegetation biomass decreased from its maximum to its minimum. The maximum concentration of sediment NH<sub>4</sub>-N reached 508.60 mg kg<sup>-1</sup> with the decline in vegetation, which might restrict further growth of the submerged macrophytes. The bacterial community structure during the high macrophyte biomass (HMB) period was distinct from that observed during the low macrophyte biomass (LMB) period. Specific taxa such as the phyla Chloroflexi and Acidobacteria and the genus *Anaerolineaceae* that are related to organic carbon degradation were significantly higher during the LMB period. Potential denitrifiers, such as *Lactococcus* and *Bacillus* genera decreased during the LMB period. Accumulation of sediment ammonia could be attributed to the enhanced production by assimilatory nitrate reduction, organic N degradation, and/or the decreased consumption by nitrification. Our findings highlight that the unstable preliminary stage of vegetation restoration brings drastic fluctuation of sediment N loading, of which NH<sub>4</sub>-N accumulation caused by bacterial communities prevents further growth of the submerged macrophytes. Therefore, extra management measures for the vegetation recovery areas should be taken to avoid excess NH<sub>4</sub>-N accumulation in sediments.

**Keywords** Lake restoration · Submerged macrophytes · Sediment · Nitrogen characteristics · Bacterial community · Predicted function

## Introduction

Restoration of eutrophic shallow lakes is a process during which lakes switch from a phytoplankton-dominated turbid

state to a macrophyte-dominated clear state [1, 2]. The decline of the submerged macrophytes usually indicates the degradation of water ecosystems, due to their positive feedback to the healthy state of shallow lakes [3]. Thus, a wide variety of restoration management efforts have been devoted to the restoration of aquatic vascular macrophytes, mainly by reducing external nutrient loading and biomanipulation [4–6]. However, undesirable results of either unstable plant succession or delayed macrophyte recolonization often occur during the preliminary stage of lake restoration. Ibelings et al. found that an alternative state, with clear (above charophytes) and turbid waters (deeper parts of the lake) existing side-by-side, occurred 7 years after nutrient reduction in Lake Veluwe (the Netherlands) [7]. Sondergaard et al. also found that the submerged macrophyte community experienced two rounds of flourishing-decline over 10 years and finally disappeared

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completely or appeared only in low densities [4]. This preliminary stage, characterized by uncertain vegetation existence, could be deemed as an unstable equilibrium state based on the theory of regime shift in lakes [1].

Recolonization and expansion of submerged macrophytes are determined by numerous factors, among which sediment conditions are important factors [8, 9]. In order to ensure successful plant recolonization, the content range of sediment nitrogen (N) has been tested. Previous studies revealed that *Hydrilla verticillata* and *Vallisneria natans* could be well nourished when total N (TN) concentrations in the sediment are around 1000–2000 mg kg<sup>-1</sup> [10, 11], and that inorganic nitrogen in sediments improved the growth of *Myriophyllum spicatum* when NH<sub>4</sub>-N concentrations were less than 200 mg kg<sup>-1</sup> [12]. However, NH<sub>4</sub><sup>+</sup> is also a paradoxical nutrient ion because high concentrations will lead to growth inhibition and oxidative stress for submerged macrophytes [12, 13]. In addition to the sediment conditions, submerged macrophytes interact with their environment during a preliminary stage, which could be positive or negative for their further development and reproduction [14].

Bacterial assemblage in sediment is usually affected by vegetation colonization or restoration, and has an impact on the sediment element cycle. Bacteria in vegetated sediments usually show significantly higher abundance of N-cycling genes than those in bare sediments [15, 16]. Another study showed that enhanced nitrification activity observed in the rhizosphere of the submerged macrophyte *Littorella uniflora* was due to ammonia-oxidizing archaea (AOA) enrichment [17]. Denitrification coupled to nitrification in the rhizosphere could be regulated by interactions between *Vallisneria spiralis* L. and rhizosphere microbes [18]. Previous studies on the feedbacks of microbial communities to colonization of submerged plants mainly focused on the effects of plant existence. However, a successful restoration aims at reaching a stable macrophyte-dominant state rather than their presence only [19].

During the vegetation restoration practice in West Lake (Hangzhou City, China), flourishing and subsequent decline in the standing crop of submerged macrophytes were observed. The declining vegetation community was not restored completely during the next growing season as we expected. Thus, this fluctuation was regarded as an unstable preliminary stage of plant restoration. It is hypothesized that (1) this unstable state may result in a drastic change in the lake's internal N loading, which will affect the vegetation community itself, and that (2) the structure and function of the microbial community in the sediment will change accordingly and involve in the process of sediment N transformation. This study explored the mechanism of the preliminary unstable stage from the perspective of sediment microbial ecology, providing evidence for accelerating the progress of lake restoration.

## Materials and Methods

### Study Sites and Sampling

The study area was located in Maojiabu (30° 14' N, 120° 07' E), a sub-lake of West Lake, Hangzhou City, China. The West Lake is a typical shallow lake and a tourist resort, with a water surface area of approximately 6.5 km<sup>2</sup> and a mean water depth of 2.3 m. It was listed as a World Heritage Site in 2011. Two pilot-scale restoration areas, M1 and M2, in southeast Maojiabu were set up in May 2014 by using protective enclosures. The geographic and eutrophic features of the restored areas are listed in Fig. S1 and Table S1. Planting techniques of the selected submerged macrophytes were recorded previously [20]. In brief, submerged species including *Hydrilla verticillata* (*H. verticillata*), *Vallisneria natans* (*V. natans*), *Myriophyllum spicatum* (*M. spicatum*), and *Ceratophyllum oryzetorum* (*C. oryzetorum*) were planted in the M1 and M2 areas. *Najas major* (*N. major*) and *Potamogeton crispus* (*P. crispus*) were not planted but spontaneously appeared inside the enclosures from August 2014 to October 2014 and from January 2015 to April 2015, respectively.

The sampling and measurement methods for macrophyte biomass were consistent with our previous study [20]. Four vegetation growth stages occurred during the study period. The propagation stage, from August to October 2014, was characterized by peak total macrophyte biomass. The decline stage, from October 2014 to January 2015, was characterized by a sharp decline in macrophyte biomass. The wintering stage, from January to April 2015, was characterized by slow decay of most of the macrophyte species and growth of *P. crispus*, a species that germinated and grew during winter. The reviving stage, from April to August 2015, was characterized by decay of the wintering species *P. crispus* and growth of other species with rising temperature.

Sediment sampling at M1 and M2 areas was conducted in August 2014, October 2014, January 2015, April 2015, and August 2015, in accordance with plant growth stages. When referred to sampling time, letters from A to E represent the five time series and the numbers 1 and 2 represent the two areas (e.g., A1 means samples taken from the M1 area in August 2014). A quadrat frame of 60 cm × 60 cm was used to delimit the sampling units in which the sediment samples were taken. The vegetated and the non-vegetated sediment samples were taken from three sampling units both inside and outside each enclosure. The upper layer of the sediment (0–10 cm) was collected using a Peterson sediment collector. Each sediment sample was divided into two subsamples, one stored at -80 °C and the other air-dried at room temperature.

## Measurement of the Sediment Nitrogen Forms

The air-dried sediment samples were ground into powder, and the rocks, spiral shells, and plant tissues were removed. Then, the powder was sieved through a 0.15- $\mu\text{m}$  mesh and collected for further analysis. TN was analyzed by the persulfate digestion method [21]. The methods for  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  analyses were modified as described by Nommik and Vahtras and Zhao et al., respectively [22, 23]. Briefly,  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  were extracted with 2 M KCl using a 1:5 sieved sediment sample to extractant ( $w/v$ ) ratio on a reciprocal shaker for 1 h. The extracts were filtered through 0.45- $\mu\text{m}$  filter papers and then analyzed using spectrophotometry with Nessler reagent and sulfamic acid for  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  measurement, respectively. Organic N (Org-N) was designated as TN minus the above two inorganic nitrogen compounds ( $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ ), considering that the content of  $\text{NO}_2\text{-N}$  in the sediments was usually very low [24]. Sediment pH was tested in 1:5 soil:water extract [25].

## Microbial Communities and Their Predicted Function

High throughput sequencing for 16S rDNA was carried out on an Illumina HiSeq 2500 platform. The microbial DNA was extracted by E.Z.N.A.<sup>TM</sup> Soil DNA Kit (Omega, USA), according to the manufacturer's instructions. After combining the DNA extracts of the triplicates for each sampling site, the bacterial hypervariable regions V4–V5 of the 16S rDNA gene were PCR-amplified using TransStart<sup>®</sup> FastPfu DNA Polymerase. The reaction mix (20  $\mu\text{l}$ ) contained 10 ng of template DNA, 250  $\mu\text{M}$  of dNTPs, 0.2  $\mu\text{M}$  of each primer, 0.4  $\mu\text{l}$  of FastPfu DNA Polymerase, and 4  $\mu\text{l}$  of  $\times 5$  TransStart<sup>®</sup> FastPfu Buffer. PCR included 27 cycles of 95  $^\circ\text{C}$  for 30 s, 55  $^\circ\text{C}$  for 30 s, and 72  $^\circ\text{C}$  for 45 s in a thermal cycler (ABI GeneAmp<sup>®</sup> 9700, USA). After purification and quantification of the PCR product, a DNA library was constructed. Subsequently, 250 bp paired-end sequencing was carried out on the Illumina HiSeq 2500 platform to generate the raw reads. Functional prediction was conducted via software the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt; <http://picrust.github.com>) [26].

## Data Analysis

One-way analysis of variance (ANOVA), supported by the software package PASW Statistics 18.0, was used to analyze the significance of the increased amount of each sediment nitrogen form caused by colonization of submerged macrophytes. Two-way ANOVA was used to assess the effects of sampling time and location (M1 and M2) on sediment nitrogen characteristics. The Student-Newman-Keuls (SNK) method was used for stepwise multiple comparisons.

Redundancy analysis (RDA), supported by the software package CANOCO 4.5 for Windows, was applied to analyze the relationships between the microbe taxa and sediment nitrogen characteristics. The RDA results are displayed as biplots, which depict the relationships between response and explanatory variables.

## Results

### Impact of Colonization of Submerged Macrophytes on Sediment Nitrogen Forms

The concentrations of all nitrogen forms were lower in the vegetated sites than in the corresponding non-vegetated sites at the beginning of the propagation stage. After 1 year, these concentrations all increased in the vegetated sites, gradually becoming higher than those in the non-vegetated sites. This pattern was more significant for M1 than for M2 (Table 1). Decreases in TN,  $\text{NH}_4\text{-N}$ , and Org-N were greater in M1 compared to M2 when the submerged macrophytes were flourishing (August 2014 and October 2014), and the increases in these three indices were greater in M1 compared to M2 when macrophyte biomass was relatively low (August 2015). Colonization of submerged macrophytes decreased the sediment TN by 70.30 and 16.51% and sediment  $\text{NH}_4\text{-N}$  by 67.17 and 11.46% in the M1 and M2 areas, respectively, during the vegetation-flourishing period. The sediment TN increased by 50.34 and 4.11% and sediment  $\text{NH}_4\text{-N}$  increased by 153.59 and 72.99% in the M1 and M2 areas, respectively, in association with the decline of the vegetation community.

**Table 1** Increasing amounts of sediment nitrogen forms in the vegetated sites compared to the non-vegetated sites at different growth stages of the submerged macrophyte community. The concentration units of TN,  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$ , and Org-N are  $\text{mg kg}^{-1}$  dry weight. A negative value in the table means that the average content of the indicator is lower in the vegetated site than that in the non-vegetated site

		TN	$\text{NO}_3\text{-N}$	$\text{NH}_4\text{-N}$	Org-N
M1	Aug 2014	-1980.46***	-1.36	-105.09*	-1879.69**
	Oct 2014	-1644.82**	-2.47	-40.98	-1483.47**
	Jan 2015	563.80	0.91	-31.93	589.15
	Apr 2015	432.65	9.97*	89.39	327.62
	Aug 2015	1445.30**	0.53	240.31*	1198.785*
M2	Aug 2014	-962.76*	-3.62	-10.27	-949.15*
	Oct 2014	-562.10	-2.07	-20.05	-540.26
	Jan 2015	-1620.56	1.51	3.80	-1626.15
	Apr 2015	-370.71	1.21	75.68	-447.87
	Aug 2015	239.81	-0.53	127.00*	113.05

Asterisks indicate significant differences between the discrepancy values \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

The increasing contents of the sediment TN and NH<sub>4</sub>-N for M1 reached 3425.76 and 345.5 mg kg<sup>-1</sup>, respectively, when the vegetation biomass changed from its maximum level to its minimum level.

### Comparison of Sediment Nitrogen Characteristics inside the two Enclosures

All the indices were significantly different by time periods and/or locations, except for NO<sub>3</sub>-N and %NO<sub>3</sub>-N (Table 2). As shown in Fig. 1a, in the propagation stage, the contents of TN in M1 were 4.81 and 3.49 times lower than those in M2, respectively. As time passed, TN gradually increased in M1, but did not change in M2, which narrowed the gap between M1 and M2. The content of Org-N showed a similar trend to that of TN (Fig. 1b). The content and percentage of NO<sub>3</sub>-N, which was a minor form of sediment inorganic nitrogen, showed no significant differences either between M1 and M2 areas or among the sampling times (Fig. 1c). NH<sub>4</sub>-N was the dominant form of sediment inorganic nitrogen, and the content increased gradually in both areas, despite no significant differences between M1 and M2 areas during the year. The content of NH<sub>4</sub>-N increased steadily in M1, whereas it only increased at the end of the reviving stage in M2 (Fig. 1d). For % NH<sub>4</sub>-N, the value was almost unchanged in both M1 and M2 areas as time passed, but was 1.07 times higher in M1 than in M2 on average, except in January 2015 (Fig. 2).

### An Overview of Sequencing Results and Microbial Community Structure

A total of 38,147 high-quality 16S rDNA sequence reads were obtained from the Illumina HiSeq platform. After equalizing the high-quality reads, the equalized reads were assigned to 2012 operational taxonomic units (OTUs) at 97% similarity. The OTUs with relative abundance greater than 0.5% were clustered into 13 phyla (including unclassified phyla), with

27.70% classified as Proteobacteria (Fig. S2), followed by Chloroflexi (21.28%), Firmicutes (13.06%), and Acidobacteria (9.64%). Rarefaction analysis based on the observed species and Shannon index indicated that sequencing for all of the communities investigated here reached near saturation (Fig. S3). Alpha diversity indices of observed species, Chao1, Shannon, and PD Whole Tree indices were 5.27–8.95, 905.85–2477.27, 806–2149, and 63.24–129.52, respectively (Fig. S4). There were no significant differences among groups ( $P > 0.05$ ) based on  $F$  test.

The dissimilarities of the sediment microbial communities for each sample were visualized using three multivariate analysis methods: principal component analysis (PCA), principal coordinate analysis (PCoA), and hierarchical clustering analysis. The first two axes totally explained 84.5 and 80.6% of the data variability in both PCA and PCoA. The two-dimensional plots showed a clear distinction of samples by time other than location. The microbial communities during the higher macrophyte biomass (HMB) period (for samples taken in Aug 2014 and Oct 2014) differed distinctly from those observed during the lower macrophyte biomass (LMB) period (for samples taken in Jan 2015, Apr 2015, and Aug 2015). Greater dissimilarity was observed among the samples during the HMB period than during the LMB period (Fig. 3). Average macrophyte biomass for these two periods was 954.7 and 504.3 g m<sup>-2</sup> FW [20]. Besides biomass, the species diversity of the macrophyte community was different between the two periods. The biomass of four species were evenly distributed during the HMB period, while only one or two species were dominant during the LMB period [20]. A similar result was found from hierarchical clustering analysis based on weighted UniFrac distance at the phylum level (Fig. 4). The cluster tree showed two distinct branches of samples for the HMB and LMB periods. The microbial community structure in the sediment samples was influenced by the biomass and diversity of the submerged macrophyte community, indicating that specific

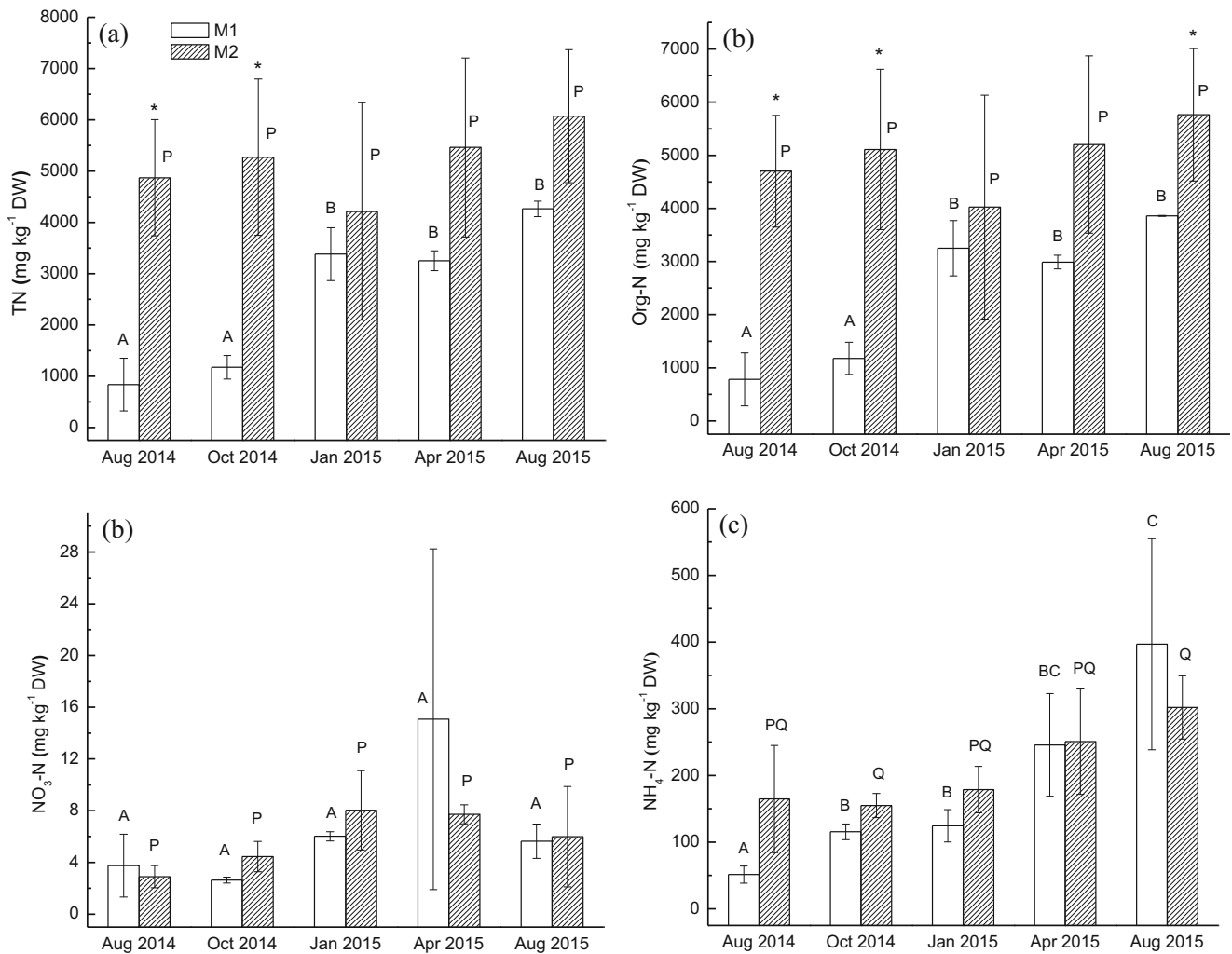
**Table 2** Two-way ANOVA showing the effects of sampling time and location on sediment nitrogen characteristics. Concentration units of TN, NO<sub>3</sub>-N, NH<sub>4</sub>-N, and Org-N are mg kg<sup>-1</sup> DW

	Time		Location		Time × location	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
TN	2.427	0.097	26.393***	0.000	1.847	0.176
NO <sub>3</sub> -N	2.999	0.056	0.239	0.632	1.040	0.421
NH <sub>4</sub> -N	10.646***	0.000	0.784	0.392	1.552	0.245
Org-N	1.943	0.159	26.41***	0.000	1.761	0.193
pH	1.271	0.327	5.315*	0.037	5.589**	0.007
%NO <sub>3</sub> -N	0.838	0.524	3.903	0.068	1.791	0.187
%NH <sub>4</sub> -N	1.299	0.321	12.919**	0.003	2.454	0.098
%Org-N	3.647*	0.036	30.236***	0.000	1.817	0.190

Asterisks indicate the significance of the  $F$  values

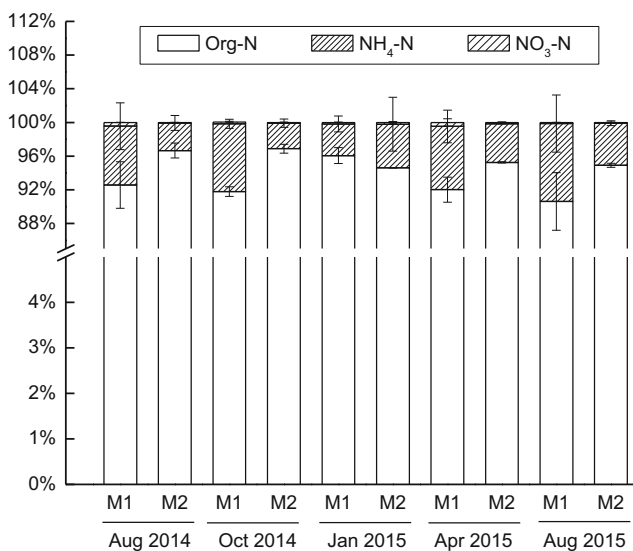
\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$





**Fig. 1** Contents of sediment nitrogen forms in the vegetated sites. Letters A, B, and C (P and Q) represent significant differences (one-way ANOVA,  $p < 0.05$ , LSD post hoc tests) among different time periods

for M1 and M2 areas. Asterisks indicate significant differences ( $p < 0.05$ ) between M1 and M2 areas during the same period



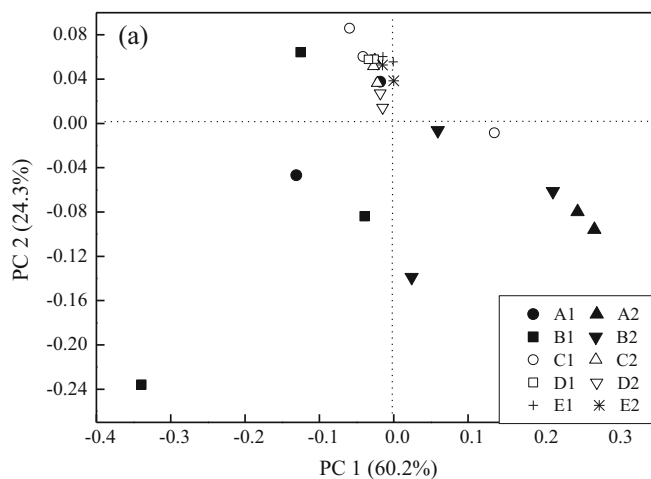
**Fig. 2** Percentages of sediment Org-N,  $\text{NH}_4\text{-N}$ , and  $\text{NO}_3\text{-N}$  contents, which account for TN in M1 and M2 areas

species were affected by the development of the vegetation community.

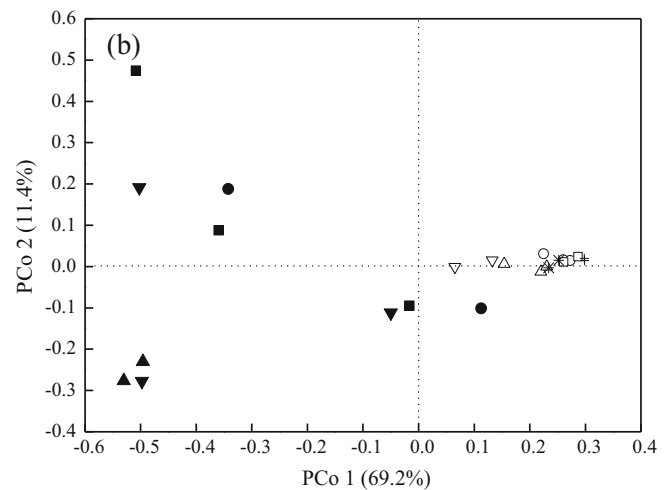
### Specific Impact of Plant Development on Microbes

Relative abundance of the top 15 phyla and top 35 genera was analyzed for their significant variations along with the development of plant community. Two-way ANOVA was for two cases based on time factor. The first case was time factor (5 levels)  $\times$  location factor (2 levels), and the second case was time factor (2 levels)  $\times$  location factor (2 levels). August 2014 and October 2014 were combined as level 1, and the other three time periods were combined as level 2 in the latter case.

Significant differences were found in 9 phyla and 22 genera based on time factor only, in 4 phyla and 10 genera based on location factor only, and in 2 phyla and 0 genus based on the interaction between time and location factors, as determined



**Fig. 3** Multivariate analysis of the sediment microbial community for different sampling locations and time periods. **a** Principal component analysis (PCA) of the microbial community at the genus level and **b** of the microbial community based on Bray-Curtis distance. Letters A to E



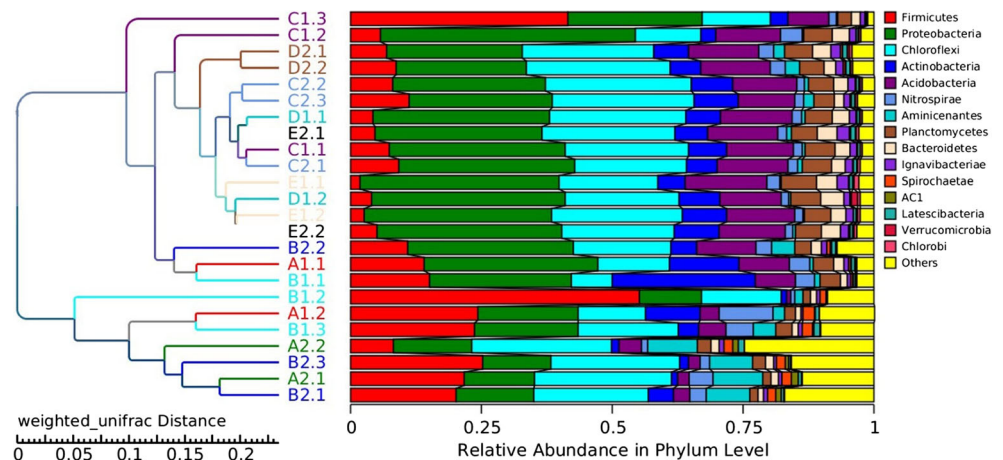
represent the five time periods (August 2014, October 2014, January 2015, April 2015, and August 2015) and the numbers 1 and 2 following each letter represent two areas

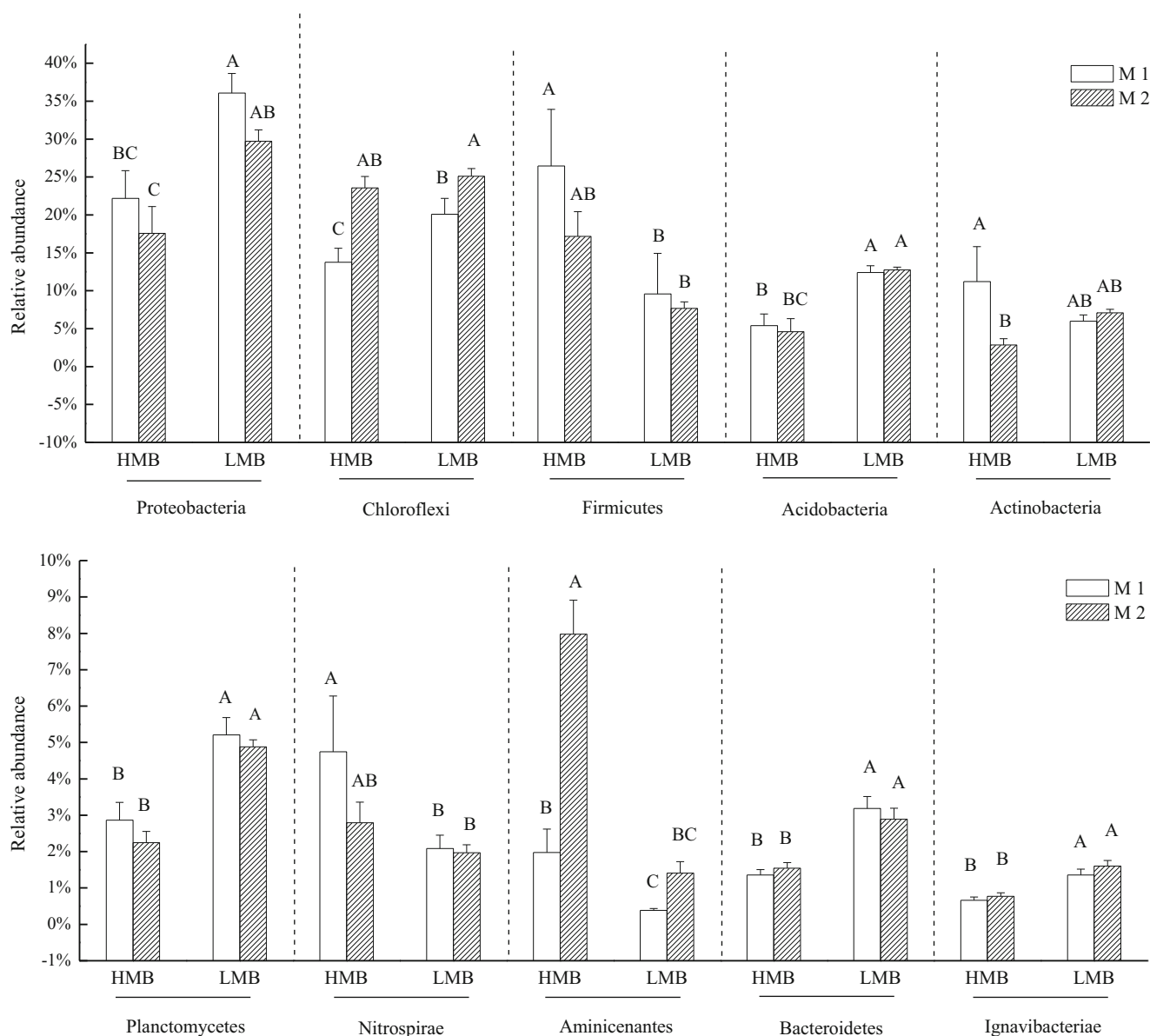
by using two-way ANOVA with 5 time levels (Table S2 and Table S3). For two-way ANOVA with 2 time levels, significant differences were observed in 12 phyla and 29 genera based on time factor only, in 4 phyla and 10 genera based on location factor only, and in 3 phyla and 2 genera based on the interaction between time and location factors (Table S4 and Table S5). More taxa presented significant differences based on time factor only and the interaction between time and location factors under 2 time levels, indicating that the microbial community was more representative when classified by the HMB and LMB periods. This was also in accordance with the results of multivariate analysis.

Specific taxa were further analyzed by one-way ANOVA. Multiple comparisons were performed among 4 treatments, which include 2 time periods and 2 locations, since microbial communities were more differentiated based on 2 time periods. The significance of the top 10 phyla and genera is presented in Fig. 5 and Fig. 6, respectively, from highest to

lowest. Relative abundance of Proteobacteria, Chloroflexi, Acidobacteria, Planctomycetes, Bacteroidetes, and Ignavibacteriae was higher during the LMB period than during the HMB period at the phylum level. Relative abundance of *Anaerolineaceae\_uncultured*, *Xanthomonadales\_Incertae\_Sedis\_uncultured*, *Alcaligenaceae\_uncultured*, *H16*, and *Vicinamibacter* was higher during the LMB period than during the HMB period at the genus level. RDA biplots of microbial taxa and nitrogen characteristics showed that  $\text{NH}_4\text{-N}$  explained the most species variation, indicating its key role in shaping the microbial communities (Fig. 7). The phyla and genera that were positively correlated with inorganic nitrogen ( $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ ) or Org-N in sediment samples were just the taxa that increased with plant development. Specifically, the increased phyla were closely correlated with the contents of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and Org-N, while the increased genera were more closely correlated with the content and percentage of  $\text{NH}_4\text{-N}$ .

**Fig. 4** Cluster tree based on unweighted pair-group method with arithmetic mean (UPGMA) with weighted UniFrac distance at the phylum level. Letters A to E represent the five time periods (August 2014, October 2014, January 2015, April 2015, and August 2015) and the numbers 1 and 2 following each letter represent two areas. The additional number after the following decimal point represent replicate cores of each sampling area





**Fig. 5** The top 10 phyla that are significantly different based on time factor through two-way ANOVA (time factor with two levels). Each bar represents the mean  $\pm$  standard deviation. Significant differences among

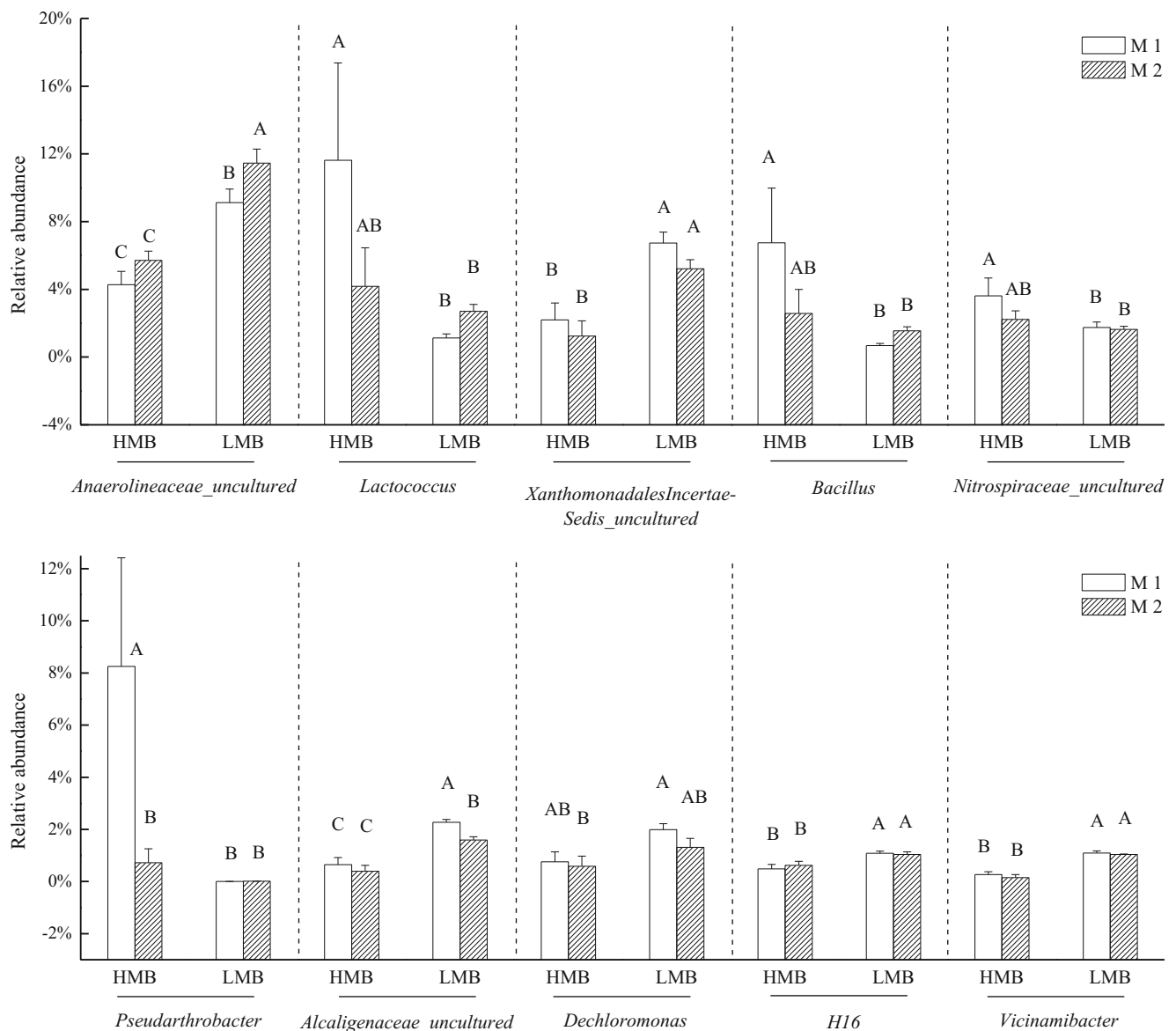
columns are indicated by uppercase letters above the bars, based on one-way ANOVA with LSD post hoc test

## Predicted Functions of Bacterial Communities in Biogeochemical Cycles

Key genes involved in N cycling were analyzed for their relative changes (Fig. 8). The abundance of *nasA* and *nir* associated with assimilatory nitrate reduction and *ureC* associated with organic N degradation was significantly enriched during the LMB period, while the genes associated with denitrification (*narG* and *nosZ*), anammox (*hzs*), and nitrogen fixation (*nifH*) were significantly less enriched during the LMB period. The genes associated with ammonia oxidation (*amoA* and *hao*) were less enriched and not significantly different. From the perspective of ammonia production and consumption,

accumulation of sediment ammonia could be attributed to the enhanced production by assimilatory nitrate reduction, organic N degradation, and/or the decreased consumption by nitrification.

Organic matter from decayed plant tissues resulted in an increase of organic N in the sediment samples, the degradation of which is usually coupled with C degradation. KEGG orthology (KO) categories involved in C degradation were analyzed for their fold changes during the LMB period compared to the HMB period. Genes associated with the moderate degradable C were enriched (Table 3). Relative abundance of special genes responsible for hemicellulose, cellulose, and chitin degradation significantly increased ranging from



**Fig. 6** The top 10 genera that are significantly different based on time factor through two-way ANOVA (time factor with two levels). Each bar represents the mean  $\pm$  standard deviation. Significant differences among

columns are indicated by uppercase letters above the bars, based on one-way ANOVA with LSD post hoc test

10.57 to 179.81%. Non-uniform changes were found in the genes associated with the most labile C starch and the most recalcitrant C aromatics listed in Table 3.

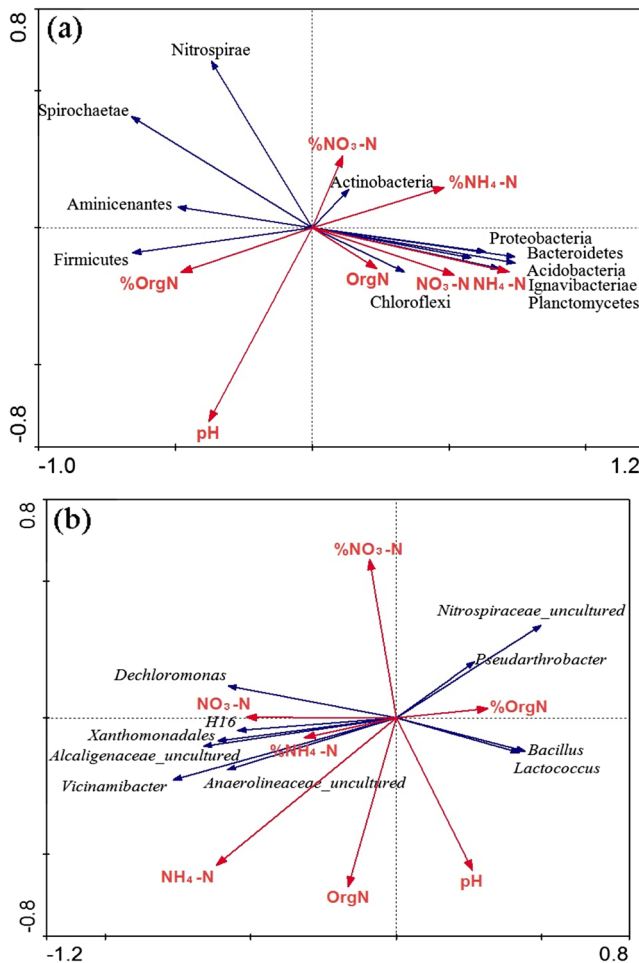
## Discussion

### Characteristics of the Unstable Preliminary Stage for the Submerged Vegetation Restoration

Pronounced fluctuations in biomass and dominant species usually occur during the early phase of submerged vegetation re-establishment [27]. A final recovery or total disappearance of the re-established species were both reported after the

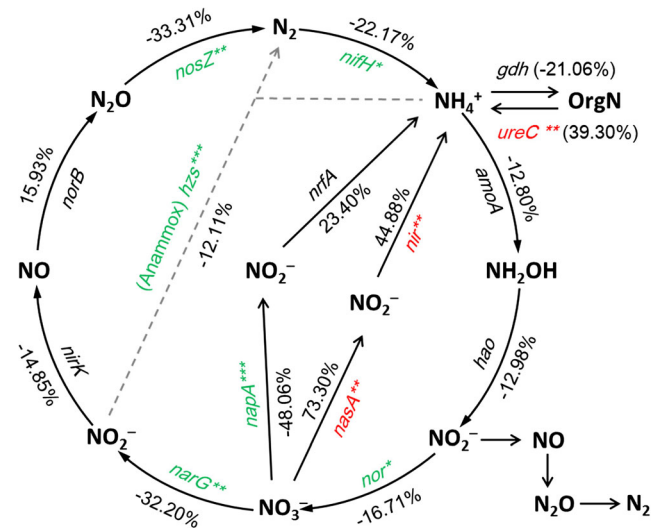
unstable early phase [7, 19]. It took about 10 years of unstable alternative states to achieve a final macrophyte-dominant clear state in Lake Veluwe (the Netherlands), in which P-loading had been strongly reduced [7]. In Lake Christina (USA), a temporary (5–10 years) unstable clear-water state induced by top-down manipulations was finally returned to a turbid state [28]. The submerged macrophyte in Lake Væng (Denmark) experienced two rounds of flourishing-decline over 10 years and finally disappeared completely or appeared only in low densities [4]. Short-term recovery of several years were more common in restoration projects worldwide. Field studies in north-western Europe indicated that 2 or 3 years after the restoration measures stopped, turbid conditions returned, and macrophyte vegetation virtually disappeared [29]. Successful





**Fig. 7** Biplot of species-environmental variables based on redundancy analysis (RDA) analysis of microbial taxa and nitrogen characteristics in sediment samples (**a** top 10 phyla—nitrogen characteristics; **b** top 10 genera—nitrogen characteristics)

short-term restoration of about 1 year for a subtropical lake using biomanipulation was achieved in the Huizhou West Lake (China). However, if the recovery effects are considered over a decade, there are probably more cases of failures than success [29]. Reasons of these fluctuations could be attributed to undesirable sediment conditions, herbivory effects by fish and waterfowls, and less competition to benthic filamentous algae [4, 19]. In general, more energy is needed to achieve a macrophyte-dominant stable state in such a system with relatively low resilience from the view of ecological resilience [30]. In this study, the submerged macrophyte community showed a flourishing-decline trend during the first year after restoration measures were applied, and it turned out that vegetation biomass was maintained only at a low level. This fluctuation could be deemed as a typical unstable preliminary stage for submerged vegetation restoration. Due to plant growth and decay, significant sediment nitrogen characteristics were observed along with plant development.



**Fig. 8** Relative changes of N-cycling genes during the lower macrophyte biomass (LMB) period. The percentage for each gene is calculated by dividing the total number of each KEGG orthology that equivalent to a N-cycling gene by the total number of all KEGG orthology categories, and then weighted by the fold change (LMB-HMB)/LMB of each gene. Red-colored genes had a significantly higher relative abundance during the LMB period than during the HMB period; green-colored genes showed the opposite (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ )

### Increased N Loading in the Sediment Has Potential Negative Impact on Plant Growth

The contents of sediment TN for the vegetated sites were significantly lower than those for the non-vegetated sites, when the submerged macrophytes were in their growing season. However, the contents increased gradually with the decline of vegetation and finally became higher than those of the non-vegetated sites. The increasing content of sediment TN for M1 reached  $3425.76 \text{ mg kg}^{-1}$  ( $p < 0.05$ ) when the vegetation biomass decreased from its maximum of  $1434.4 \text{ g m}^{-2}$  to its minimum of  $697.5 \text{ g m}^{-2}$  [20], whereas the content of sediment TN for M2 was only  $1202.57 \text{ mg kg}^{-1}$  ( $p > 0.05$ ) when the vegetation biomass decreased from its maximum of  $781.8 \text{ g m}^{-2}$  to its minimum of  $212.5 \text{ g m}^{-2}$  [20]. It should be noted that vegetation decline after flourishing may aggravate sediment nitrogen loading, but not when maximum vegetation biomass is less than  $800 \text{ g m}^{-2}$ . The sediment  $\text{NH}_4\text{-N}$  concentrations in M1 and M2 areas were  $156.46$  and  $174.96 \text{ mg kg}^{-1}$ , respectively, which would be beneficial for the growth of the artificially restored macrophytes. However, the maximum  $\text{NH}_4\text{-N}$  concentration reached  $508.60$  and  $335.56 \text{ mg kg}^{-1}$  in M1 and M2 area, respectively. A previous study showed that inorganic nitrogen in sediments was assimilated and improved the growth of *M. spicatum* when external  $\text{NH}_4\text{-N}$  concentration in sediments was less than  $200 \text{ mg kg}^{-1}$  [12]. Vegetation growth might have been restricted, as the relative growth rate of *M. spicatum* decreased markedly and the malondialdehyde

**Table 3** *T* test showing significant differences between LMB and HMB periods based on the relative abundance levels of KEGG orthology (KO) categories involved in carbon degradation. The complexity of carbon (C) is presented in order from labile to recalcitrant C. The percentage of fold change of each gene is calculated by dividing the total number of each KEGG orthology that equivalent to a C degradation gene by the total number of all KEGG orthology categories, and then weighted by the fold change (LMB-HMB)/LMB of each gene

Carbon	Gene	Fold change (%)	Significance
Starch	Alpha-amylase	-52.25	**
	pulA	-86.12	
	Glucoamylase	117.35	***
	npIT	-66.96	
	cda	1.62	
Hemicellulose	ara_fungi	43.73	**
	Mannanase	83.72	***
	Xylanase	19.13	
Cellulose	CDH	-41.23	
	Cellobiase	-16.51	*
	Endoglucanase	10.57	*
	Exoglucanase	167.14	*
Chitin	Acetylglucosaminidase	179.81	**
	Endochitinase	45.87	**
Pectin	Pectinase	3.967	
Aromatics	AceA	-91.24	
	AceB	-8.40	
	AssA	-10.41	***
	limEH	1.91	
	vanA	88.23	**
	vdh	-100	*

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

(MDA) content clearly increased, when external  $\text{NH}_4\text{-N}$  concentration was more than  $400 \text{ mg kg}^{-1}$  [12]. The autofragment production of *M. spicatum* was also inhibited when sediment  $\text{NH}_4\text{-N}$  concentration reached  $550 \text{ mg kg}^{-1}$  [31]. Meanwhile, the highly organic character of sediments may hamper the establishment of submerged species because of weak root anchorage [32] and insufficient oxygen supply to the apical root meristems [33]. Both diversity and biomass of the submerged vegetation decreased in the next growing season. Therefore, the revival of macrophyte propagule might have been impeded by excess sediment  $\text{NH}_4\text{-N}$  and Org-N that accumulated in the decline season.

### Bacterial Communities and Their Predicted Function in Biogeochemical Cycles

The bacterial community structure in the sediment was significantly influenced by the development of submerged vegetation. The HMB and LMB periods harbored distinctive microflora. Different halophyte species played a pivotal role in shaping the microbial community structure [34]. Differences in species combinations of the standing vegetation induced a distinguished soil denitrifying community pattern [35]. Specific phyla and genera in the bacterial community that changed with vegetation development were in accordance with the variation of sediment nitrogen characteristics. The

increasing phyla and genera during the LMB period compared to the HMB period were just the taxa that positively correlated with sediment  $\text{NH}_4\text{-N}$  and Org-N. The predicted functional genes involved in nitrogen cycling indicated low denitrification during the LMB period, which was in accordance with the decrease of the dominant microbes, such as genera *Lactococcus* and *Bacillus* that had been reported as denitrifiers [36, 37]. The current evidence of  $\text{NH}_4\text{-N}$  accumulation during the LMB period might be due to the higher abundance of ureC gene that transform organic nitrogen to  $\text{NH}_4\text{-N}$ , and nir and nasA genes that are responsible for assimilatory nitrogen reduction. Similarly, abundance of ureC gene is consistent with ammonium contents, as reported in both soil and groundwater [38, 39].

The phyla Chloroflexi and Acidobacteria, which are involved in the degradation of plant-derived compounds such as cellulose [40, 41], were significantly more abundant during the LMB period. The genus *Anaerolineaceae*, which is responsible for anaerobic decomposition of carbohydrates via fermentation [42], was also more abundant during the LMB period. The sediment Org-N increase during the LMB period was coupled with vegetation biomass decline. Thus, microbial degradation of plant-derived organic matter might be enhanced by specific phyla or genera that are involved in carbon cycling. This could be evidenced by the increased abundance of functional genes that are responsible for hemicellulose and

cellulose degradation. The sediment Org-N increased significantly regardless of the enhanced organic matter degradation, indicating that the plant-derived organic matter input exceeded the microbial degradation capacity. Vegetation decline leads to an increase of sediment Org-N and  $\text{NH}_4\text{-N}$ , which in turn negatively impacts the vegetation propagules and the time for achieving a stable vegetation community. Therefore, extra management measures such as plant harvesting should be applied to avoid access sediment N loading from the perspective of vegetation restoration ecology and sediment microbial ecology.

## Conclusions

Vegetation flourish-decline during the preliminary stage of the submerged vegetation restoration leads to significant variations of sediment N loading, which has potential negative impacts on the further establishment of the plant community. Bacterial assemblage in the sediment was associated with different biomass and diversity of the vegetation community. Excessive level of  $\text{NH}_4\text{-N}$  caused by vegetation fluctuation possibly impeded the growth of the submerged vegetation itself. Accumulation of sediment  $\text{NH}_4\text{-N}$  is attributed to enhanced ammonia generation through microbial metabolism. Enhanced hemicellulose and cellulose degradation potential was still not enough to deplete the plant-derived organic matter inputs. These results suggest that management measures should be considered in order to control the sediment N loading and accelerate the process for achieving a stable macrophyte-dominant clear state.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

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