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Combined effects of phosphate-solubilizing bacterium XMT-5 (*Rhizobium* sp.) and submerged macrophyte *Ceratophyllum demersum* on phosphorus release in eutrophic lake sediments

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Abstract

Simulation experiments were conducted using sediments collected from the Taihu Lake to determine the combined effects of submerged macrophytes *Ceratophyllum demersum* and phosphate-solubilizing bacteria (PSB) strain XMT-5 (*Rhizobium* sp.) on phosphorus (P) concentrations in overlying waters and sediments. After 30 days of experimental incubation, the total phosphorus (TP) and dissolved total phosphorus (DTP) concentrations of the overlying water subjected to AMB and AHMB treatments (both with the combined effects of PSB cells and submerged macrophytes) were generally lower than those of the AM (with individual effects of inoculated *C. demersum*) and AB (with individual effects of a smaller amount of inoculated PSB cells) control treatments but higher than that of the A (with no effects of inoculated PSB cells or *C. demersum*) and AHB (with individual effects of a larger amount of inoculated PSB) control treatments. The TP contents of the sediment in the AMB and AHMB treatments were significantly lower than those of the other control treatments. The TP contents of the *C. demersum* cocultured with the PSB strain XMT-5 cells in the AMB and AHMB treatments were all significantly higher than that of the AM treatment, indicating the enhancement of P uptake by submerged plants inoculated with PSB. The bacterial diversity structures of the rhizosphere sediment subjected to different treatments were also analyzed by the high-throughput sequencing method. According to the ACE and Chao 1 indices, the bacterial diversity in the AMB and AHMB treatments were the highest. Although many sources contributed to the decrease in the nutrient loads of the lake sediment, harvesting macrophytes inoculated with PSB cells prior to their senescence might constitute a significant in-lake measure for reducing internal P load.

Keywords Phosphate-solubilizing bacteria · Submerged macrophytes · Eutrophication · Sediment · Phosphorus release

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Introduction

Excessive nitrogen (N) and phosphorus (P) inputs lead to eutrophication, which causes severe damage. Eutrophication has become a worldwide environmental issue and has been a serious threat to surface water bodies, including lakes, reservoirs, and rivers (Qin et al. 2007; Wang et al. 2017; Song et al. 2017). In China, several areas of eutrophic lakes and other areas approaching eutrophication are about 8700 and 14,000 km^2, respectively (Wang et al. 2015).

As a major factor causing eutrophication (Schindler et al. 2008), P contents in lakes and reservoirs generally originate from discharge water and bottom sediments or from external and internal P sources (Zhang et al. 2017). Various P removal

methods (de-Bashan and Bashan 2004) were developed to control P from point and non-point source pollution. However, these methods remain insufficient in removing massive amounts of accumulated P in sediments during polluting periods. In extreme cases, internal P loading can reach 80% of the total phosphorus (TP) inputs of a lake (Penn et al. 2000). To date, eutrophication management remains a grim challenge because removal of releasing P from sediments is increasingly difficult especially in large lakes. Thus, internal P loading from lake sediments must be reduced for eutrophication control (Huang et al. 2016b; Wang et al. 2017).

Environmental factors, such as oxygen, temperature, pH, light, and organic matter, affect the P release from sediments (Christophoridis and Fytianos 2006; Jiang et al. 2008; Wang et al. 2008; Huang et al. 2016a). Some researchers considered the biological impacts on P release in river or lake sediments. Wu et al. (2008) mentioned that indigenous phosphobacteria in sediments play an important role in P release processes in river sediments. Meanwhile, Jiang et al. (2008) investigated the effects of total benthic alga and bacteria on P release from sediments, and their results showed that the organisms can stimulate the process of P release. Qian et al. (2011) found that biological metabolism strongly stimulates P release, and its intensity can reach five times higher than that from the physicochemical procedures.

In aquatic ecosystems, submerged macrophytes also play a central role because of their numerous functions in nutrient and organic matter balance and food web regulation (Declerck et al. 2011; Lauridsen et al. 2015; Barbara et al. 2016). Macrophytes also reduce sediment suspension and water turbidity (Gu et al. 2016), thereby contributing to the maintenance of a clear water state. Therefore, submerged macrophytes are widely used to control water pollutions, including eutrophication (Gao et al. 2017). The uptake of P from water and sediment by submerged macrophytes greatly contributes to P cycling in lakes and other water bodies (Granéli and Solander 1988). In particular, submerged macrophytes can uptake P from overlying water and sediment. Therefore, these macrophytes reduce the P concentrations in sediments, which are their primary sources of P (Jukka and Leena 2003; Li et al. 2014; Wang et al. 2017). Compared with *Elodea canadensis*, *Potamogeton crispus*, *Myriophyllum spicatum*, and *Vallisneria spiralis*, *Ceratophyllum demersum* has the highest TP removal rate during spring and autumn (Gao et al. 2009). Furthermore, decaying macrophytes may act as a kind of internal P source in water bodies (Granéli and Solander 1988). Thus, harvesting macrophytes prior to their senescence is a reasonable measure for reducing internal P load.

Phosphate-solubilizing bacteria (PSB) can release a soluble form of P from mineral phosphates, including $Ca_3 (PO_4)_2$, $Fe_3 (PO_4)_2$, and $AlPO_4$, into soil or a solution (Liu et al. 2015). In sediments, PSB affect the internal P cycle in water ecosystems. The amount of P released from sediments is closely related to the amount of PSB (Wu et al. 2008). The results of biogeochemical function of an efficient PSB strain on the P cycling at water-sediment interface showed that inoculated PSB cells can accelerate the release of P from sediments (Qu et al. 2013). PSB promotes plant growth and P uptake in the agricultural soils (Tao et al. 2008; Anzuay et al. 2015; Istina et al. 2015). When PSB is inoculated in the rhizosphere sediments of submerged macrophytes, changes occur during the P release processes. Meanwhile, the association between the introduction of PSB and submerged macrophytes in sediments should stimulate the transporting of P from the sediments to the submerged macrophytes. Furthermore, given that planted submerged macrophytes might affect the bacterial diversity of the rhizosphere sediment, more work is needed to understand whether the combined application of PSB and submerged macrophytes reduces the internal P loading in sediments. Considerably reducing internal P loading is an extremely reasonable method for the restoration of eutrophic water bodies, including lakes.

In the present study, laboratory assays were conducted to simulate the water-sediment conditions of eutrophic water body and determine the combined effects of inoculated PSB strain XMT-5 and submerged marophytes *C. demersum* on the P release process of eutrophic sediments. We performed several tests to determine the changes in pH, dissolved oxygen (DO), TP, and DTP concentrations in overlying water; variations in the TP concentrations in sediments; and the P uptake of *C. demersum*. The effects of submerged macrophytes on bacterial structure and composition were also analyzed by the high-throughput sequencing method. We aimed to improve the understanding of the effects of PSB activities, effects of *C. demersum* on P release in sediments, and potential application of the association between PSB strains and submerged marophytes. Our results may contribute to the discovery of methods for the reduction of internal P loading in eutrophic water bodies and may serve as a basis for the long-term management of eutrophication.

Materials and methods

Eutrophication sediment sampling

Surface sediment (0–20 cm in depth) was collected from Zhushan Bay (16[#] sample site of Taihu Lake) with a grab sampler (Chen et al. 2015). This bay is one of the most contaminated regions, and harmful cyanobacterium blooms occur nearly every summer (Chen et al. 2015). The samples were placed in sealed plastic bags, which were placed on ice and transported immediately to the laboratory. The samples were stored at 4 °C in dark conditions before further analysis. The sediments were grounded and sieved into particles to a diameter of less than 2 mm after freeze-drying. The processed

sediments were used for the simulation of the P release process.

PSB strain and submerged macrophyte species

The PSB XMT-5 identified as a bacterium belonging to the genus *Rhizobium*, which was isolated from the rhizosphere soil of wheat using a modified Menkina medium (Niewolak 1980) composed of 10.0 g of glucose, 0.5 g of $(NH_4)_2SO_4$, 0.3 g of NaCl, 0.03 g of $MgSO_4·7H_2O$, 0.03 g of $MnSO_4·4H_2O$, 10 g of $Ca_3(PO_4)_2$, 18 g of bacteriological agar, and 1 L of ddH_2O. The pH was adjusted to 7.0 with 1 mol/L of NaOH and sterilized by an autoclave for 30 min at 115 °C. The Genbank accession number of the strain is KR653316. Meanwhile, the phosphate solubilizing properties of the strain were reported previously (Li et al. 2017b).

Submerged macrophyte species *C. demersum* used in this study were collected from Lake Donghu. Samples were first rinsed to remove impurity and precultured in the bucket with sediment and ddH_2O for 10 days. Then, the sturdy plants (approximately 10 cm in length) were weighted and transplanted to different treatment beakers.

P release experimental design

P release experiments were performed in 18 Pyrex tall beakers (12.8 cm in diameter, 24.5 cm in height, and 2 L in volume). Each beaker contained 200 g of dry sediment samples (3 cm in height). As shown in Table 1, six groups were used for the experiment and contained three beakers each. Beakers A-i, A-ii, and A-iii were included in the control treatment A, which was only loaded with sediment and ddH_2O. Beakers AB-i, AB-ii, and AB-iii were included in the AB control treatment group. These beakers were loaded with sediment, ddH_2O, and PSB strain XMT-5 cells (about 1.2×10^6 CFU/g sediment). Beakers AHB-i, AHB-ii, and AHB-iii were included in the AHB control treatment group. They were loaded with sediment, ddH_2O and PSB strain XMT-5 cells (about 6.3×10^6 CFU/g sediment). Beakers AM-i, AM-ii, and AM-iii were included in the AM control treatment group. They were loaded with sediment and ddH_2O and planted with five shoots of *C. demersum*. Beakers AMB-i, AMB-ii, and AMB-iii were included in the experimental treatment AMB group. They were loaded with sediment, ddH_2O, and five similar shoots of submerged plant *C. demersum* and inoculated with PSB strain XMT-5 cells (about 1.2×10^6 CFU/g sediment). Beakers AHMB-i, AHMB-ii, and AHMB-iii were included in the AHMB experimental treatment group. They were each loaded with sediment, ddH_2O, five similar shoots of submerged plant *C. demersum*, and PSB strain XMT-5 cells (about 6.3×10^6 CFU/g sediment). All glassware and sample bottles were soaked in diluted HCl solution for more than 12 h and rinsed three times with ddH_2O.

XMT-5 cells were cultivated in liquid Luria-Bertani medium (10 g/L of tryptone, 5 g/L of yeast extract, and 10 g/L of NaCl, pH 7.2) to the exponential growth phase at 28 °C, 180 r/min conditions. The cells were harvested from the culture by centrifugation at 8000×g for 10 min and rinsed twice with sterile distilled water. The centrifuged cells were thoroughly mixed with the sediment and then planted with *C. demersum* when needed. An aliquot from 1600 mL of ddH_2O (15 cm in height) was added carefully as overlying water to cover the sediments in the beakers. Aseptic ventilated films were used to cover the beakers to prevent pollution and minimize splashing and evaporation. During the experiment, the beakers were all transferred into an illumination incubator under controlled conditions of 24 ± 1 °C, 2200 ± 100 lx, and 12:12 h light-dark cycle.

During the P release experiment of over 30 days, 20 mL of overlying water samples were collected from each beaker using a syringe for the determination of pH values and concentrations of TP and DTP at defined time points (i.e., days 1, 4, 7, 10, 15, 20, 25, and 30). Then, 20 mL of ddH_2O was then added to maintain the volume of the overlying water. After removing the overlying water from the beakers at the end of the experiment, plant samples were removed carefully from the sediment and then washed to remove any sediment particle adhered. After being dried with filter paper, 0.1 g of the plant samples was selected for TP analysis. Surface sediment samples (0–1 cm in height) were divided into two parts. One part was used for bacterial community analysis, and the other was stored at − 20 °C for TP analysis after freeze-drying.

Table 1 The P release experimental design. +: added or inoculated; −: without added or inoculated

Treatments	Sediment	ddH$_2$O	Submerged plants	Smaller number of PSB cells	Larger number of PSB cells
A	+	+	−	−	−
AB	+	+	−	+	−
AHB	+	+	−	−	+
AM	+	+	+	−	−
AMB	+	+	+	+	−
AHMB	+	+	+	−	+

Sampling and analysis

After powdering, TP in the dried sediment and plant tissue were determined by digesting the samples with HNO_3-$HClO_4$ (APHA 2005; Bao 2000), and ascorbic acid-molybdenum blue colorimetric method was performed (Murphy and Riley 1962; APHA 2005). The DTP of the overlying water was determined using the same procedure as the ascorbic acid-molybdenum blue colorimetric method after digestion with $K_2S_2O_8$ in an autoclave (120 °C) for 30 min, and TP was determined using the same method as DTP, but the water was not filtered with 0.22 μm cellulose membrane (APHA 2005). The overlying water pH was determined with a pH meter (PHS-3C, Shanghai, China). DO concentration was tested by a portable water quality meter (HQ30d, USA). The data were subjected to ANOVA by the Tukey's range test ($p < 0.05$) performed on the SPSS software (SPSS 19.0, SPSS Inc., Chicago, USA).

Amplification of 16S rRNA gene sequences in the sediment

Sediment bacterial DNA was extracted by the FastDNA® Spin Kit for Soil (Mpbio, CA, USA). The 16S rRNA V3 + V4 region was amplified using forward primer 338F: (5′-ACT CCT ACG GGA GGC AGC A-3′), and reverse primer 806R: (5′-GGA CTA CHV GGG TWT CTA AT-3′). The primers were provided by Biomarker Technologies (Beijing, China), and polymerase chain reaction (PCR) was performed using the method described by Li et al. (2017a).

Sequence analysis

Based on 97% similarity, the high-quality sequences were clustered into different operational taxonomic units (OTUs) (Caporaso et al. 2010), and the OTUs were aligned with the Silva reference gene database (http://www.arb-silva.de/). Alpha diversity (like the Chao 1 richness and Shannon diversity indices) analyzed by Mothur version v.1.30 (http://www.mothur.org/) was used to estimate the diversity of bacteria in each sample (Schloss et al. 2009). Principal component analysis (PCA) was performed to assess the bacterial composition of the samples (Jiang et al. 2013). Beta diversity measurements obtained by Unifrac were used to compare the difference among the bacterial community compositions of all the sediment samples (Lozupone and Knight 2005), and LefSe (http://huttenhower.sph.harvard.edu/lefse/) was applied to analyze the significant differences among the bacterial community compositions of the samples.

Results and discussion

The apparent characteristics of different treatments at water-sediment interface

As shown in Fig. 1, the overlying water appeared clearer in the AM treatment than those in the A, AB, and AHB treatments. The reduction in the number of phytoplankton cells in the AM treatment was also greater than those in the other negative control treatments. The results were confirmed with the direct counting method ($10^7 < 10^9$ cells/L). The combined effects of PSB cells and submerged macrophytes minimized the number of phytoplankton cells in the AMB and AHMB treatments ($10^6 < 10^7 < 10^9$ cells/L). The phytoplankton density of the oligotrophic Lugu Lake with a TP concentration of 0.01 mg/L was 4.1×10^5 cells/L (Dong et al. 2012). The phytoplankton density of the mesotrophic Erhai Lake with a TP concentration of 0.07 mg/L was 4.8×10^7 cells/L (Wei et al. 2012), and the eutrophic Taihu Lake with a TP concentration of 0.2 mg/L was 3.8×10^7 cells/L (Gu et al. 2011). The *C. demersum* can suppress algae biomass and enhance water transparency (Dai et al. 2014), and the combined effects of PSB XMT-5 cells and *C. demersum* may have more contributions to the improvement of water quality than their individual effects.

TP, DTP, pH, and DO concentrations of overlying water

As shown in Fig. 2, the lowest TP and DTP concentrations were obtained in the overlying water in the AHB systems from the fourth day, and the maximum values were obtained on the 20th day. The maximum values were 0.42 and 0.31 mg/L. Meanwhile, the highest TP and DTP concentrations in the water column were observed in the AM systems before the 10th day. The maximum values (2.69 and 2.42 mg/L) were obtained in the AB systems on the 15th day. Thus, the inoculation of a small number of PSB cells (approximately 1.2×10^6 CFU/g sediment) was expected to accelerate the release of P from the sediment compared with those of the control systems, including treatment A. When the number of PSB cells reached approximately 6.0×10^6 CFU/g sediment, the TP and DTP concentrations were usually less than those of the control systems. Similar result was also observed in the previous study (Qu et al. 2013). The P released from the sediment might have been transformed and uptaken by the inoculated growing bacteria and other planktons. Thus, the number of inoculated PSB cells may have significantly affected the P release process from sediment.

C. demersum can potentially remove TP nutrients from water (Gao et al. 2009; Dai et al. 2014). The best TP and DTP removal rates were 73.82 and 78.54%, respectively, in the AM systems on the 20th day. In the AMB systems inoculated with PSB cells, the TP and DTP concentrations in the
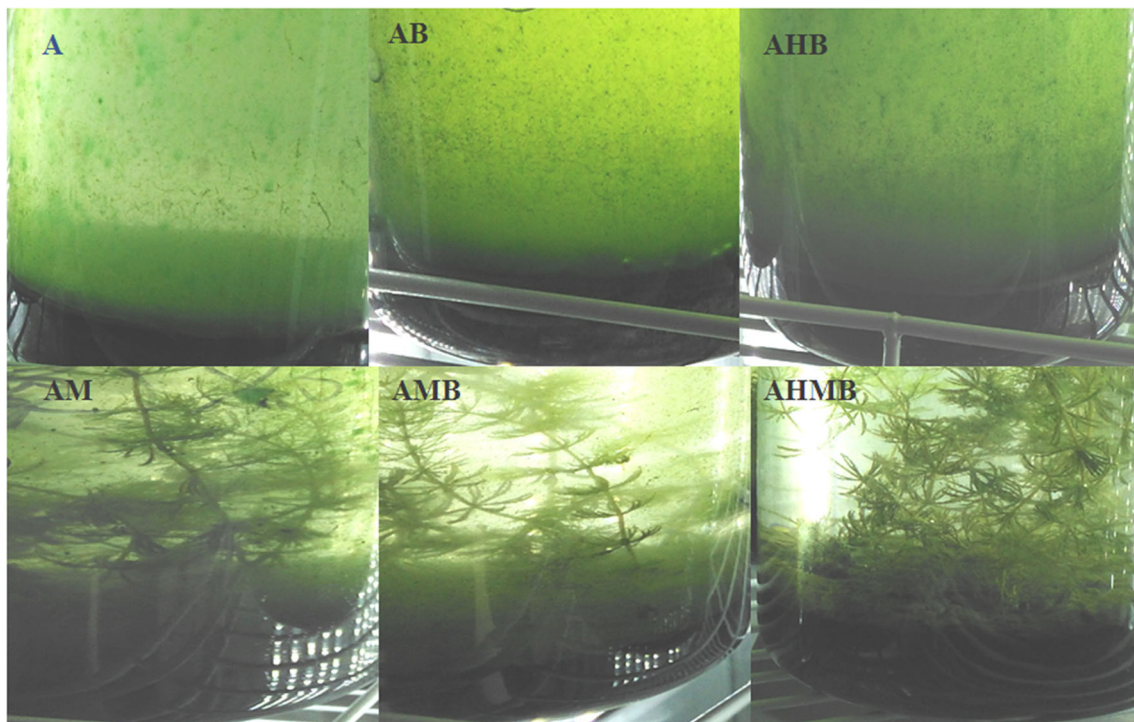
Fig. 1 Final water state of different treatments on the 30th day

water column were all lower than those of the AM systems, showing a quite similar effect on the P release process of *C. demersum* even in the AHMB systems. Thus, P release may have been increasingly affected by the submerged plant under experimental conditions. Therefore, the P release that decreased under the combined application of PSB cells and *C. demersum* to a greater extent than that affected by *C. demersum* alone.

The highest pH value of overlying water (Fig. 3a) was detected in the AHB treatment before the 20th day, reaching 9.77. The changes in pH were similar to those observed in the AHB treatment in other treatments. All the pH values slowly increased before the 10th day and then rapidly after the 15th day. In addition, the pH contents in the AM treatment were the lowest in general. Owing to the effects of the inoculated PSB cells, the pH of the overlying water usually increased (Qu et al. 2013). Meanwhile, a minimal increase in pH occurred in the sterilized systems unaffected by biological factors (Qu et al. 2013). In the present study, changes in pH might have been mainly caused by the metabolism of bacteria, algae, and other living beings in the water systems. Macrophytes also affected the chemical environment, including DO and pH, which in turn influenced P cycling in the lake systems (Granéli and Solander 1988). The photosynthesis of the submerged macrophytes can increase pH in lake water and may affect P release from sediments (Granéli and Solander 1988).

The highest DO concentrations (Fig. 3b) in the overlying water from the AHB treatments were obtained from the first day to the 20th day, during which the concentrations started to

decrease. In the AMB and AHMB treatments, the DO concentrations were nearly 0 before the seventh day, although they reached their maximum values on the 15th or 20th day before decreasing again. In the AM treatment, the DO concentration increased throughout the experiment. For analysis, the photosynthetic processes of the submerged macrophytes and phytoplankton increased the DO concentrations in the overlying water. When inoculated with PSB cells alone at relatively large numbers, the solubilizing P might promote algal growth in the overlying water. Under the combined effects of submerged macrophytes and PSB cells, the growth of algae was inhibited to some extent. Thus, DO in the overlying water was mainly generated by the submerged macrophytes at a low level. The proliferation of organisms also consumed O_2, and DO concentrations were extremely low in the first 7 days.

TP contents of sediment and submerged macrophyte *C. demersum*

For the systems planted with *C. demersum* in the same manner as AM treatments, the TP contents of the sediments were 1.59 mg/g (Fig. 4a). The lowest values were obtained in the AMB (1.55 mg/g) and AHMB (1.54 mg/g) treatments. These values were significantly lower than those of the AM treatments ($p < 0.05$). The application of PSB cells enhanced the growth and P uptake of *C. demersum* throughout the experiment, as indicated by the visual observation and chemical analysis (Fig. 4b). The fresh weight of *C. demersum* increased by 87.10 and 100.43% in AMB and AHMB treatments,
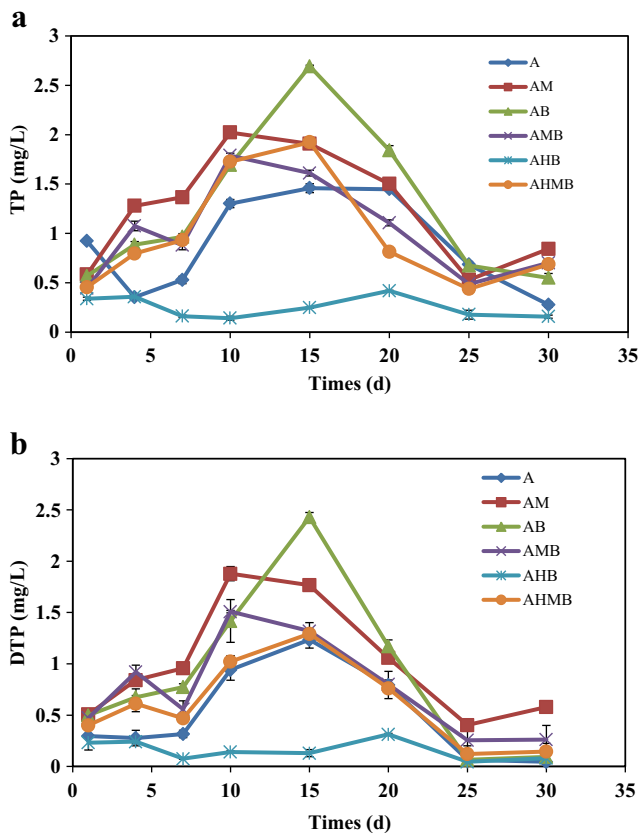
Fig. 2 Concentrations of TP (**a**) and DTP (**b**) of overlying water of different treatments

Fig. 3 Variations in pH (**a**) and DO (**b**) in overlying water of different treatments

respectively. The dry weight respectively increased by 20.79 and 28.07% (data not shown). The AB and AHB treatments had the highest TP concentrations in the sediments (1.64 and 1.66 mg/g, respectively), followed by the A control treatment (1.63 mg/g). These findings suggested that although PSB cells can accelerate the release of P from the sediments in the middle of the experiment, the extent of internal P pollution will not be affected.

The submerged macrophytes can obtain more than 70% of their TP content from sediments (Granéli and Solander 1988). Thus, the TP contents of sediments in the AM treatment were lower than those in sediments without *C. demersum*. The PSB cells effectively promote the growth and P uptake of plants (Anzuay et al. 2015; Istina et al. 2015). In the present study, PSB cells also improved the growth and P uptake of the submerged macrophytes. As shown in Fig. 4b, the TP contents of *C. demersum* in AM, AMB, and AHMB treatments were 1.45, 1.67, and 1.67 mg/g, respectively. These results indicated that P uptake significantly increased ($p < 0.05$) after the employment of PSB cells for the submerged macrophyte *C. demersum* grown in the sediments. The internal P loads can be effectively reduced in this manner. Therefore, harvesting macrophytes inoculated with PSB cells prior to their senescence is necessary because a large amount of the P released
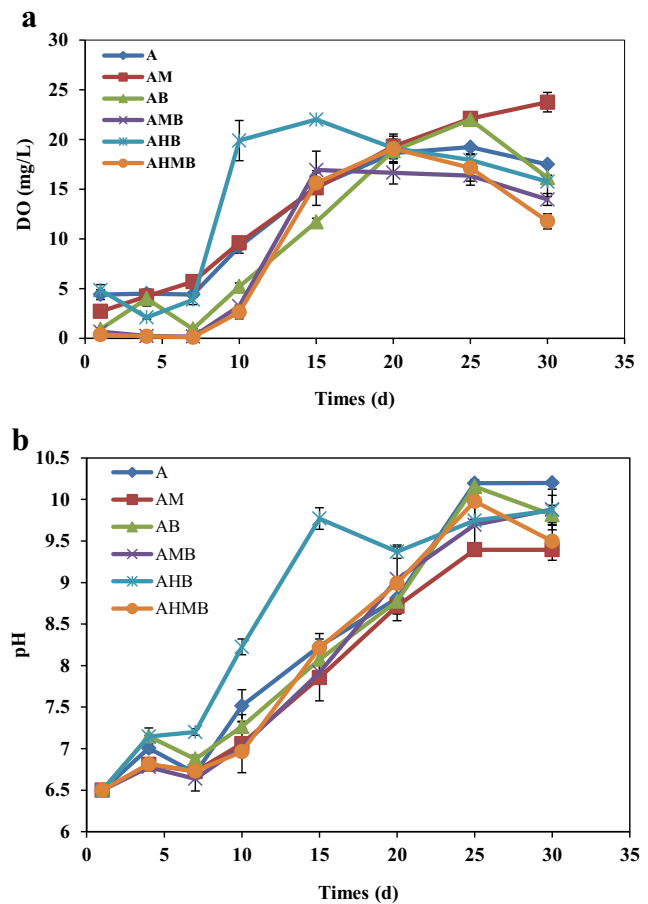
from the decaying plants may be retained by the sediments and may act as an internal P source (Granéli and Solander 1988).

Taxa diversity of sediment with different treatments

The number of OTUs, Chao 1 richness, ACE, and Shannon indices at a level of 3% are shown in Table 2. The average OTU numbers of samples A, AB, AHB, AHMB, AM, AMB, and ASMB (a plant died in one beaker in the AMB treatment and was replanted after 3 days of the experiment) were 1146, 1068, 1044, 1241, 1244, 1268, and 1264, respectively. In all the samples, the bacteria in the ASMB and AHMB treatments were more diverse than those of the AM, and A, AB, and AHB (treatments without the combined effects of PSB and submerged plants). The Chao 1 estimator indicated good sample richness throughout, and the Shannon diversity index indicated a diverse microbiota (Grice et al. 2009; Quigley et al. 2012). The AMB treatments had higher ACE richness and Chao 1 estimators. Meanwhile, lower Simpson index and higher Shannon indices indicate high bacterial biodiversity
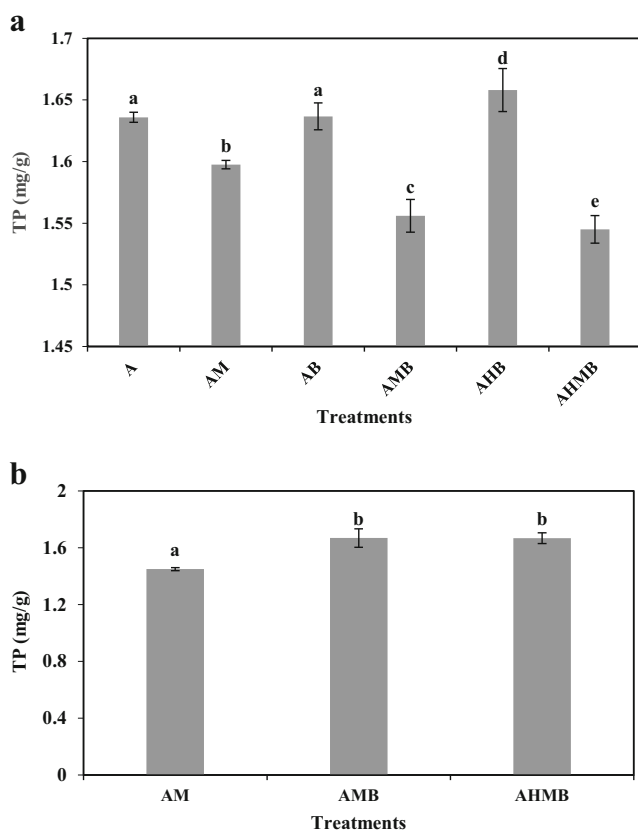
a

b

Fig. 4 Concentrations of TP in the sediments (**a**) and submerged plants (**b**) of different treatments. Letters indicate significant difference at $p < 0.05$

Table 2 Different estimator parameters for alpha diversity in each sediment samples

Sample ID	OTU	ACE	Chao 1	Shannon	Simpson
AMB	1268	1314.49	1320.53	4.80	0.050
AHMB	1241	1290.95	1302.76	5.67	0.009
ASMB	1264	1309.73	1325.17	5.73	0.009
AM	1244	1270.51	1284.51	5.45	0.018
A	1146	1182.19	1196.56	5.52	0.012
AB	1068	1169.83	1170.05	4.75	0.040
AHB	1044	1109.79	1122.07	5.31	0.016

in a sample (like the AHMB or ASMB treatments). So more bacterial diversity occur in the AMB, AHMB and ASMB treatments, indicating that inoculating of both PSB cells and submerged macrophytes can increase bacterial community diversity in rhizophere sediments.

In the Venn diagram (Supplemented Fig. 1), 772 OTUs were found to be common to all the seven tested sediment samples, which contained 9.33% of the sequences. The main bacteria of the shared OTUs belonged to Proteobacteria, Firmicutes, Chloroflexi, Acidobacteria, Bacteroidetes, Gemmatimonadetes, and Cyanobacteria in phylum level and *Geobacter* and *Bryobacter* in the genus level.

The relative abundance of bacterial community proportions in the genus level is shown in Fig. 5a. At the genus level, *Limnothrix* content was extremely high (9.67%) in the AM treatment, and *Lutispora* (4.64%), *Magnetospirillum* (3.74%), *Geobacter* (3.62%), and *Lysobacter* (3.62%) were the main genera. The dominant genera were *Limnothrix* (4.74–16.72%), *Geobacter* (4.52–5.11%), *Methylomonas* (4.74–5.03%), *Chlorobaculum* (3.57%) and *Magnetospirillum* (3.21%) in the AMB, ASMB, and AHMB treatment samples, in which the proportions of *Rhizobium* were from 0.02 to 0.04%. *Geobacter* (5.79%), *Phenylobacterium* (4.94%), *Thiobacillus* (3.57%), *Magnetospirillum* (3.21%), and

Rhizobium (0.05%) were in the A control treatment. In the AB and AHB treatment samples, the predominant bacteria were *Thiobacillus* (3.87–19.18%), *Caulobacter* (8.09%), *Phenylobacterium* (6.07%) *Gemmatimonas* (5.77%), *Geobacter* (4.16%), and *Bryobacter* (3.28%), and proportions of *Rhizobium* were from 0.02 and 0.03%. According to these results, the inoculation of PSB cells did not cause an apparent increase of the proportions of the genus *Rhizobium* in AB, AHB, AMB, and AHMB treatments. So except for the inoculated PSB, the interactions between other bacteria and the submerged macrophyte in the rhizosphere might play important roles in the growth and P uptake of *C. demersum*. Meanwhile, the predominant bacterial communities showed differences.

A total of 11 identified main phyla were found, and Proteobacteria (41.59–65.22%) was the dominant phylum in all the samples (Supplemented Fig. 2). The second dominant phylum was markedly different, including Firmicutes in the A (11.26%), AM (15.86%), and AHMB (11.81%) treatments; Gemmatimonadetes (6.05%) in AB treatment; Acidobacteria (13.51%) in the AHB treatment; and Cyanobacteria (20.01%) in the AMB treatment.

As shown in Fig. 5b, no dominant community whose LDA score log10 was more than 4 in group c (A1 referred to the surface or rhizospheric sediment of 0–1 cm in height and A2 referred to the remained bottom sediment) was found. Members in the phyla of Cyanobacteria and Spirochaetes and the genus of *Limnothrix* and *Methylomonas* formed the dominant bacteria in group d (AM1 and AM2). Bacteria belonged to the family of Hydrogenophilaceae, and the genus of *Thiobacillus* was dominant in group e (AB1 and AB2). For group f (AHB1 and AHB2), the main bacteria were affiliated to Acidobateria, Holophagae, and an unknown family. Meanwhile, the phylum of Cyanobacteria was the dominant member in group i (AMB1, AMB2, AHMB1, AHMB2, ASMB1, and ASMB2). This finding indicates that the employment of PSB cells or submerged plant or the combined effects of the two kinds of organisms, the bacterial diversity in the sediment varied greatly in different treatments.
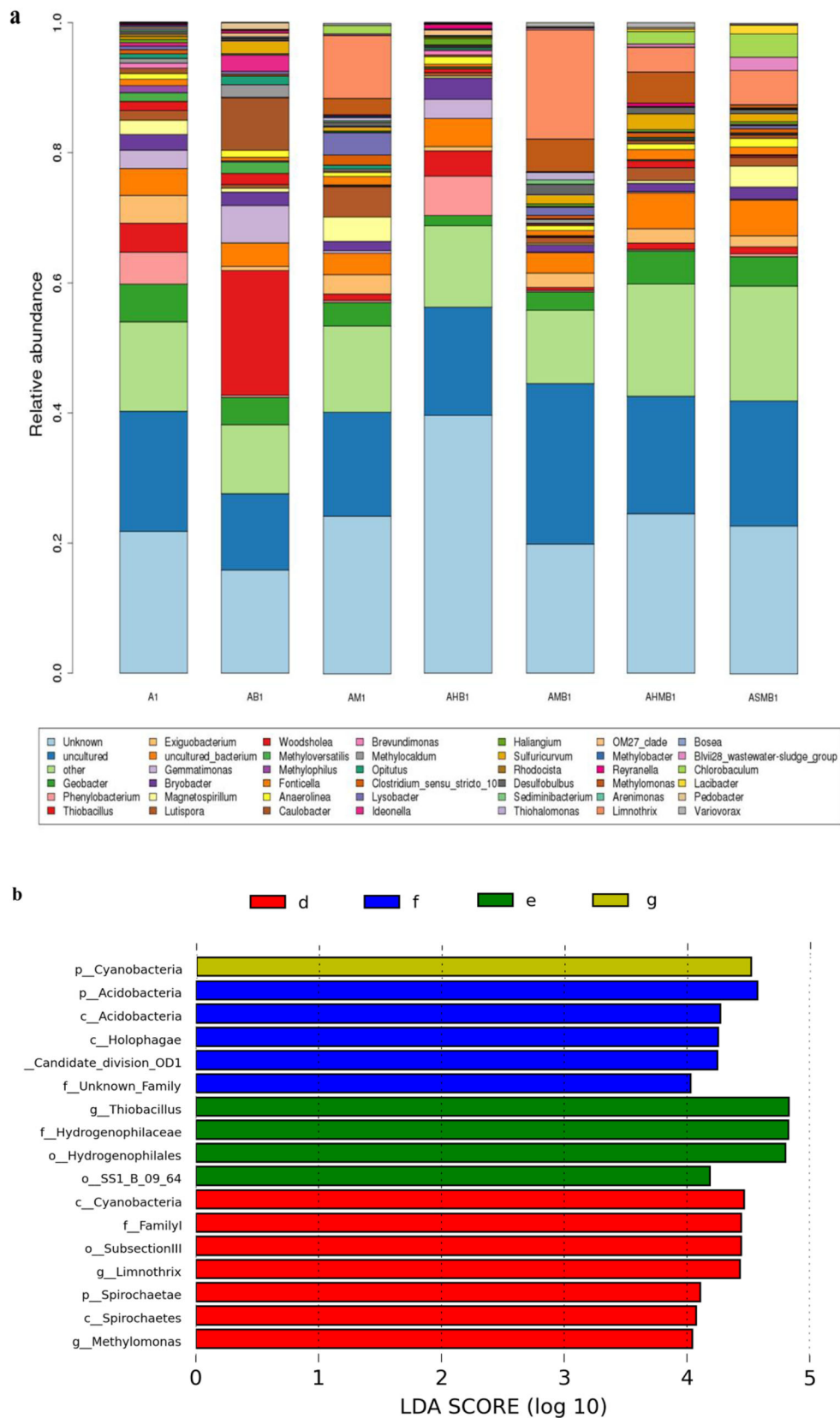
Fig. 5 Differences in the bacterial diversity at the genus level (**a**) and dominant populations (**b**) in sediment of different treatments. Represented taxa occurred at > 1% abundance in at least one sample

Comparison of bacterial community of different treatments

The multiple samples' similarity tree and PCA analysis results are shown in Fig. 6. As shown in Fig. 6a, a distinct separation of the bacterial communities was found in the samples from group g, including both the PSB strain XMT-5 cells and *C. demersum*, and group d with the individual effects of submerged plant *C. demersum*. The groups of c, e, and f, which were affected by the bacteria in sediment and the inoculated PSB strain XMT-5 cells, clustered together.

For the PCA analysis in Fig. 6b, the first and second axes showed that the values of the cumulative percentage variance of the species were 33.36 and 15.12%, respectively. In total, 48.48% of the variances of the species were explained by the two axes. The results suggested that the bacterial communities were similar between groups g and d, indicating that the individual effects of PSB cells or combined inoculation of submerged macrophytes and PSB cells caused a clear shift in the assemblage of bacterial communities. The use of PSB cells led

to a slight change in groups c, e, and f. The bacterial communities in the sediment might have been mainly affected by the submerged macrophytes.

Conclusions

The combined effects of PSB XMT-5 cells and submerged macrophytes enhanced the water transparency in the AMB and AHMB treatments, and the contents of TP and DTP in overlying water were lower in the two experimental treatments than those of the control treatments AM and AB. The lowest TP and DTP contents in overlying water and the highest TP content in the sediment were found in the AHB treatment. At the end of the experiment, the TP concentrations of the sediment in the AMB and AHMB treatments were significantly lower and the TP contents of the *C. demersum* were all significantly higher than those of the AM treatment ($p < 0.05$), indicating that the inoculation of PSB cells can promote the P uptake by *C. demersum*. The composition of bacterial communities in experimental treatments (AMB and AHMB) was greatly differed from those of the control treatments (A, AB, AHB, and AM). The inoculation of sole or combined PSB cells or *C. demersum* influenced the bacterial communities in the sediments. Meanwhile, the number of the inoculated PSB cells also affected the P release process and composition of the bacterial community.

Fig. 6 Multiple samples' similarity tree (**a**) and PCA analysis (**b**) of bacterial community in sediment of different treatments. c: A1, A2; d: AM1, AM2; e: AB1, AB2; f: AHB1, AHB2; g: AMB1, AMB2, ASMB1, ASMB2, AHMB1, AHMB2. (1 referred to the surface sediment (0–1 cm in height), and 2 refers to the remained bottom sediment)

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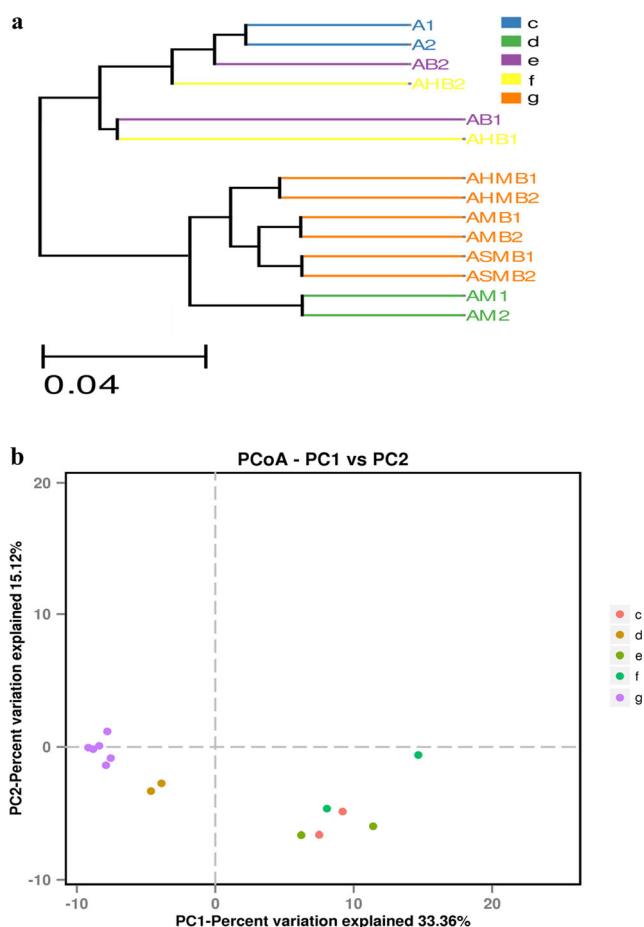
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