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ORIGINAL ARTICLE

Diversity and composition of bacterial community in the rhizosphere sediments of submerged macrophytes revealed by 454 pyrosequencing

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Abstract Freshwater lake sediments support a variety of submerged macrophytes that may host groups of bacteria exerting important ecological functions. We collected three kinds of commonly found submerged macrophyte species (*Ceratophyllum demersum*, *Vallisneria spiralis* and *Elodea nuttallii*) to investigate the bacterial community associated with their rhizosphere sediments. High-throughput 454 pyrosequencing and bioinformatics analyses were performed to examine the diversity and composition of the bacterial community. The results obtained indicated that the diversity of the bacterial community associated with the rhizosphere sediments of submerged macrophytes was significantly lower than that of the bulk sediment. Remarkable differences in the bacterial community composition between the rhizosphere and bulk sediments were also observed.

Keywords Submerged macrophytes · Rhizosphere sediment · Bacterial community · High-throughput pyrosequencing

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Introduction

The rhizosphere is the narrow zone of soil containing numerous bacteria and under the direct influence of the root exudates of plant. It is considered to be one of the most dynamic interfaces on Earth (Philippot et al. 2013). Many studies have investigated the bacterial community located in the rhizosphere of terrestrial plants, including its diversity (Teixeira et al. 2010), composition (Bulgarelli et al. 2012; DeAngelis et al. 2009; Peiffer et al. 2013; Uroz et al. 2010), activity (Chaparro et al. 2014; Ofek-Lalzar et al. 2014), variations according to plant species (Garbeva et al. 2008; Berg and Smalla 2009) and variations according to plant development (Chaparro et al. 2014). However, little is known about the bacterial communities living in close association with freshwater macrophytes, such as submerged macrophytes. Submerged plants could repair the environment by absorbing nutrient elements, such as nitrogen and phosphorus, and are a crucial component of the lake ecosystem (Chmielewski et al. 1997; Jeppesen et al. 1998; Lembi 2001). Additionally, various buffering mechanisms that keep water in its clear state, including bicarbonate utilization, nutrient uptake and allelopathy, are maintained by submerged macrophytes (Jatin et al. 2008).

Zeng et al. (2012) reported that macrophytes can influence shifts in the bacterioplankton community in Lake Taihu. Hempel et al. (2008) also demonstrated that the composition of the epiphytic bacterial community was affected by submerged macrophytes. However, there have been only a few studies which have targeted the overall bacterial community in the rhizosphere of submerged macrophytes in the freshwater ecosystem.

In the study reported here, we collected three species of submerged macrophytes (*Ceratophyllum demersum*, *Vallisneria spiralis* and *Elodea nuttallii*) from the water of Huashen Lake, Nanjing, China. Microcosms for culturing the submerged macrophytes were constructed. Sediment

samples were collected from the rhizosphere sediment, bulk sediment and surface sediment of each microcosm. Bacterial community composition was investigated using 454 high-throughput pyrosequencing of the 16S ribosomal RNA (16S rRNA) gene. The aim of our investigation was to determine whether the diversity and composition of the bacterial community associated with the rhizosphere sediment of submerged macrophytes differed from those of the bulk sediment.

Materials and methods

Microcosm construction and sample collection

In July of 2012, sediment samples were collected from Huashen Lake using a core sampler (model DM60; China Mingyu Holdings Group, Shanghai). At the same time, samples of lake water and of three species of submerged plants (*Ceratophyllum demersum*, *Vallisneria spiralis and Elodea nuttallii*) were collected. All samples were brought back to the laboratory immediately after collection and prepared for construction of the microcosm systems.

Microcosms were constructed in plexiglass tubes (internal diameter 40 cm, length 25 cm) to simulate the natural lake environment. The top 12 cm of the collected sediment cores were sectioned into 2-cm intervals, and sediment samples collected at the same depth were pooled together. The sediments were then sieved through mesh to remove the macrofauna and large particles before being fully homogenized and placed in plexiglass tubes with 2-cm intervals corresponding to their original depths. Filtered lake water was added to the plexiglass columns with intravenous needles. The height of the lake sediment in the plexiglass tubes was 12 cm and that of the overlying water was 10 cm in the constructed microcosms. The microcosms were pre-incubated for 2 days, then the above-mentioned three kinds of commonly found submerged macrophytes, *C. demersum*, *V. spiralis* and *E. nuttallii*, were added into the microcosm separately. Each kind of submerged macrophyte was cultivated in three replicate columns, and each column contained one submerged plant. Two control columns (with no submerged macrophytes) were also prepared. The microcosms were incubated at 25 °C and light was provided 12 h a day.

After incubation for 80 days, the surface sediments (top 0–1 cm) and bulk sediments (depth 5–10 cm) were collected from each microcosm with a self-made sampler. The bulk sediment was collected at least 15 cm away from the planted submerged macrophytes in order to avoid contact with the roots. The rhizosphere sediment associated with each kind of submerged macrophyte was collected from all over the root zone by shaking off sediment that was loosely adhering to the roots, as described by Herrmann et al. (2009). Replicate samples were homogenized. Sediment samples were stored at −70 °C for further analysis.

DNA extraction and PCR amplification

DNA was extracted from duplicate soil samples (0.25 g) with the PowerSoil DNA Isolation kit (MoBio Laboratories, Solana Beach, CA) according to the manufacturer's instructions. The quality of the extracted DNA was measured using a biophotometer (Eppendorf, Hamburg, Germany). The extracted DNA was stored at −70 °C for further analysis.

PCR analyses were performed to amplify the bacterial 16S rRNA gene with the primers 27 F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 533R (5′-TTACCGCGGCTGCTGGCAC−3′) (Lu et al. 2012; Zhao et al. 2014a; Zeng et al. 2016). The PCR primers were fixed with the Roche 454 pyrosequencing adapter (Roche Applied Science, Penzberg, Germany), and an individual 10-bp barcode nucleotide for each sample was attached to the reverse primer 533R. The PCR reaction mixture with a total volume of 50 μl included 10 μl of 5× PrimeSTAR Buffer (plus Mg^{2+}), 0.2 mM dNTPs, 0.4 μM each of the forward and reverse primers, 2.0 U TaKaRa PrimeSTAR HS DNA Polymerase, 10–20 ng of DNA template and ddH_2O. The thermal cycling conditions for PCR amplification consisted of a 5-min initial denaturation at 95 °C followed by 24 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. Each sample was amplified in triplicate, and the combined PCR products were verified by 2% (w/v) agarose gel electrophoresis and purified with the AxyPrep DNA gel purification kit (Axygen Biotechnology Hangzhou Ltd., Hangzhou, China).

Equal amounts of PCR products amplified from each sample were sent to the Majorbio Company in Shanghai for pyrosequencing on the Roche 454 FLX Titanium platform (Roche Applied Science). The obtained raw sequences have been deposited in the Genbank database under the accession number SRP091979.

Sequence processing and data analysis

Raw sequence reads were denoised and trimmed according to the online 454 standard operating procedure of the Mothur software package (Schloss et al. 2011). Sequences shorter than 200 nucleotides (excluding the primer and barcode), sequences containing ambiguous base calls to the primer sequences and homopolymers longer than 8 nucleotides were excluded from further analysis (Victor et al. 2010). The remaining sequences were transformed to the reverse complements and aligned using the nearest alignment space termination algorithm against a bacterial SILVA 16S rRNA gene template (Schloss et al. 2011). Putative chimeric sequences with potential pyrosequencing errors were removed with the

command 'chimera.uchime' in Mothur. The command 'pre.cluster' was further employed to prune the dataset and accelerate the distance-running procedure (Huse et al. 2010).

The subsampled sequences were used to analyze the alpha diversity, including number of operational taxonomic units (OTUs) and Chao1 indices, using the command 'summary.single' in Mothur. The phylogenetic diversity (PD) was estimated using Faith's index (Faith 1992). Nonmetric multidimensional scaling (NMDS) analysis was carried out using the vegan package in R (version 2.15.0) to investigate the similarities in bacterial community composition. The Bray–Curtis metric (Bray and Curtis 1957) was employed to calculate the dissimilarities in community composition for each pair of samples. Duncan's multiple range test was performed to test the differences in bacterial community composition among different sediment groups (e.g., the surface sediments, bulk sediments and rhizosphere sediments) using the SPSS 17.0 software package (IBM Corp., Armonk, NY). Heatmaps were implemented with HemI (Heatmap Illustrator, version 1.0) to compare the bacterial community of the most abundant genera in each sample (Deng et al. 2014).

Results and discussion

Richness and diversity of the bacterial communities in different sediment groups

After denoising, filtering out chimeras and removing the archaeal sequences, the remained number of sequences ranged from 5679 to 8037 for each sample. The obtained sequences in each sample were subsampled to the minimum number (5679) of sequences for comparing the richness and diversity in samples.

For the richness and alpha diversity analyses the sediment samples collected from the microcosms planted with the submerged macrophytes were divided into the following three groups: surface sediment (depth 0–1 cm), bulk sediment (depth 5–10 cm) and rhizosphere sediment (Table 1). The differences among the groups were compared using analysis of variance. The numbers of OTUs and Chao1 indexes of the bacterial community in the surface sediment samples were comparable with those of the rhizosphere samples ($P > 0.05$), but they were significantly different from those of the bacterial community in the bulk sediment samples. The PD was comparable among the three groups.

The diversity of the bacterial community associated with the rhizosphere sediments of submerged macrophytes was significantly lower than that of the bulk sediment ($P < 0.05$; Table 1). Peiffer et al. (2013) also reported a significant reduction in the α-diversity of the bacterial community for the maize rhizosphere microbiome under field conditions ($P < 0.05$). Although a number of studies have reported decreased bacterial community diversity in the rhizosphere soils of terrestrial plants, due to variations in these studies, such as the growth of the plants and the different sampling strategies, it is difficult to obtain a general description of the rhizosphere microbiome (for more detail, see Philippot et al. 2013).

Differences in bacterial community composition among the different sediment samples

The classifier results of the taxon assignments at the phylum level for each sample are shown in Fig. 1. Proteobacteria, which accounted for 18.97–36.09% of the total effective sequences, was the most abundant phylum in all samples except for the surface sediment of *V. spiralis* and the bulk sediment of the control group. Other phyla or subphyla, including Chloroflexi (14.9% ± 0.06), Deltaproteobacteria (11.15% ± 0.04), Bacteroidetes (9.92% ± 0.02) and Betaproteobacteria (8.51% ± 0.03), were also dominant phyla in our study (Fig. 1).

Duncan's multiple range test was performed to compare differences in the taxonomic groups of bacteria among surface sediments, bulk sediments and rhizosphere sediments (Table 2). At the phylum level, the relative abundances of Actinobacteria and Firmicutes were found to be significantly higher in the bulk sediment than in the surface and rhizosphere sediments ($P < 0.05$) (Table 2). However, the bacterial community derived from the surface and rhizosphere sediments maintained a higher relative abundance of the Proteobacteria group ($P < 0.05$). Relative abundances in the classes of Actinobacteria, KD4-96 and Clostridia were significantly higher in bulk sediments ($P < 0.05$). At the order level, the percentages of Acidimicrobidae, Rubrobacteridae, Clostridiales and Myxococcales were significantly higher in the

Table 1 Richness and diversity of the bacterial community derived from the surface, bulk and rhizosphere sediments collected for analysis

Parameters of richness and diversity of bacteria community	Surface sediment (depth 0–1 cm)	Bulk sediment (depth 5–10 cm)	Rhizosphere sediment
Number of OTUs	2731.33 ± 87.79 a	3209.67 ± 227.1 b	2419.33 ± 320.15 a
Chao1 index	5238.86 ± 137.68 a	6307.36 ± 477.75 b	5072.26 ± 346.89 a
PD	215.74 ± 3.36 a	232.43 ± 15.08 a	219.96 ± 10.9 a

Values in table are given as the mean ± standard deviation (SD) of triplicate ($n = 3$) samples. Values in each row (different sediment groups) followed by different lowercase letters are significantly different ($P < 0.05$)

OTU, Operational taxonomic unit; PD, phylogenetic diversity

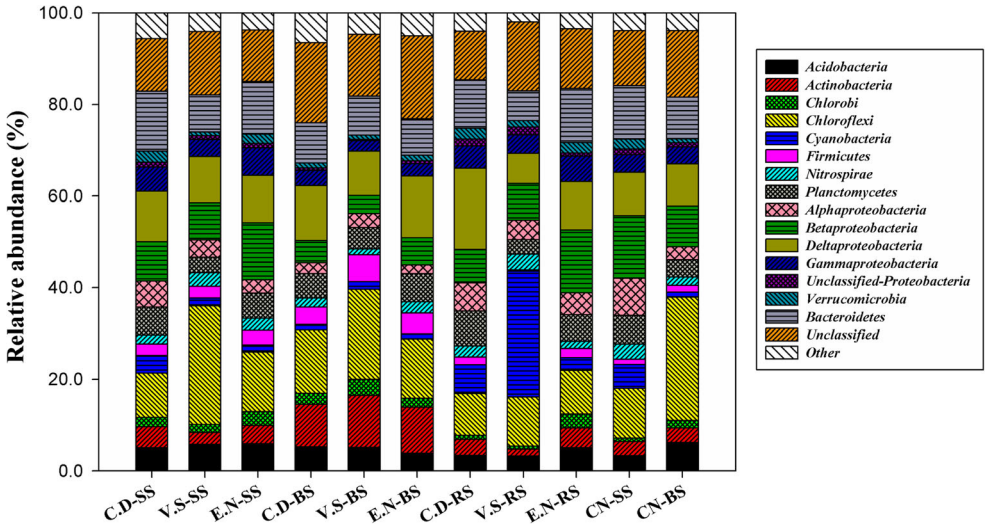

Fig. 1 Relative abundance of the dominant bacterial taxa (phyla and subphyla) in each bacterial community. *C.D Ceratophyllum demersum*, *V.S Vallisneria spiralis*, *E.N Elodea nuttallii*, *SS* surface sediment (depth 0–1 cm), *BS* bulk sediment (depth 5–10 cm), *RS* rhizosphere sediment, *CN* control

bulk sediments than in the surface and rhizosphere sediments ($P < 0.05$); however, the Xanthomonadale group was significantly less abundant in the bulk sediments ($P < 0.05$). At the family level, Acidimicrobiales, Actinomycetales, AKIW543 and Clostridiaceae were more abundant in the bulk sediments ($P < 0.05$). The relative abundances of Comamonadaceae were higher in the bacterial community derived from the surface and rhizosphere sediments.

The top ten abundant genera in each sample were selected (a total of 30 genera), and their abundancess were compared to those in other samples using the heatmap analysis (Fig. 2). The genera *Rubrivivax*, *Sulfuricurvum* and *Thiobacillus* (all affiliated with Proteobacteria) were abundant in the surface sediment samples, whereas the genera *Acidimicrobineae* (affiliated with Actinobacteria), *Clostridium* (affiliated with Firmicutes) and *Caldilinea* (affiliated with Chloroflexi) were abundant in the bulk sediment samples of both inoculated and uninoculated microcosms. Two genera *Nitrospira* (affiliated with Nitrospirae) and *Opitutus* (affiliated with Verrucomicrobia) were abundant in the rhizosphere and surface sediments of the uninoculated microcosms.

At the phylum level, the percentages of phyla Firmicutes, Actinobacteria and Proteobacteria in the rhizosphere sediments significantly differed from those in the bulk sediments ($P < 0.05$). These results are consistent with those reported by DeAngelis

Table 2 Differentially abundant taxa among bacterial communities derived from the surface, bulk and rhizosphere sediments collected for analysis

Taxonomic level	Taxon	Surface sediment (%)	Bulk sediment (%)	Rhizosphere (%)
Phylum	Actinobacteria	3.73 ± 0.85 a	10.23 ± 0.88 b	3.12 ± 1.18 a
	Firmicutes	2.76 ± 0.3 a	4.74 ± 0.85 b	1.35 ± 0.75 a
	Proteobacteria	30.68 ± 2.98 ab	22.38 ± 2.29 a	32.66 ± 5.66 b
Class	Actinobacteria	3.73 ± 0.85 a	10.23 ± 0.88 b	3.12 ± 1.18 a
	KD4-96	1.35 ± 0.37 b	1.78 ± 0.17 b	0.56 ± 0.39 a
	Clostridia	2.23 ± 0.31 b	3.79 ± 0.69 c	1.16 ± 0.63 a
Order	Actinobacteridae	1.21 ± 0.18 a	2.46 ± 0.20 b	1.17 ± 0.30 a
	Rubrobacteridae	0.65 ± 0.35 a	2.45 ± 0.34 b	0.45 ± 0.26 a
	Clostridiales	2.23 ± 0.31 b	3.78 ± 0.69 c	1.16 ± 0.63 a
	Rhodospirillales	1.24 ± 0.42 a	0.73 ± 0.18 a	1.14 ± 0.03 a
	Myxococcales	1.27 ± 0.11 a	2.43 ± 0.3 b	1.82 ± 0.36 a
	Xanthomonadales	1.86 ± 0.36 b	0.89 ± 0.11 a	2.04 ± 0.64 b
Family	Acidimicrobiales	0.98 ± 0.42 a	2.01 ± 0.25 b	0.89 ± 0.42 a
	Actinomycetales	1.21 ± 0.18 a	2.46 ± 0.2 b	1.17 ± 0.30 a
	AKIW543	0.62 ± 0.33 a	2.24 ± 0.29 b	0.41 ± 0.23 a
	vadinHA17	1.29 ± 0.25 b	1.32 ± 0.05 b	0.69 ± 0.08 a
	Clostridiaceae	0.95 ± 0.11 b	1.97 ± 0.29 c	0.26 ± 0.15 a
	Comamonadaceae	1.33 ± 0.11 b	0.6 ± 0.11 a	1.93 ± 0.19 b

Values in table are given as the mean ± SD of triplicate ($n = 3$) samples. Values in each row (different sediment groups) followed by different lowercase letters are significantly different ($P < 0.05$)

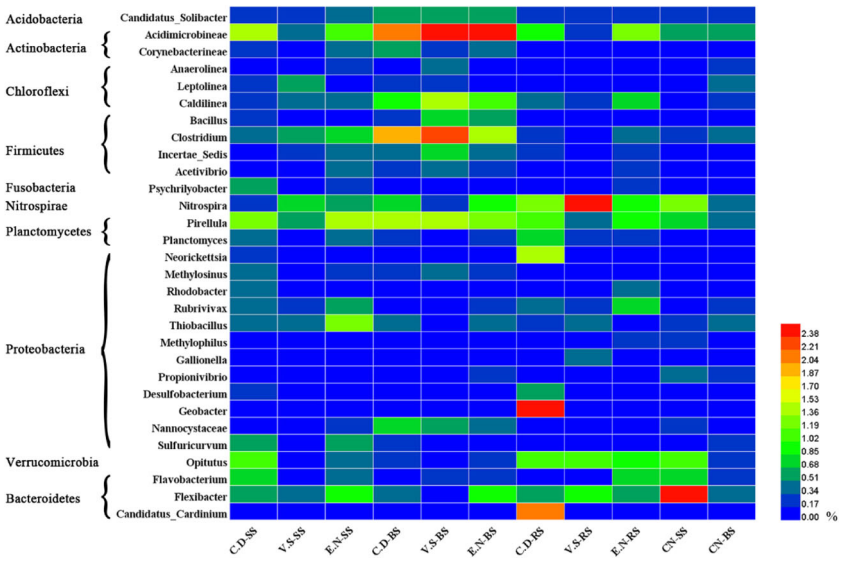

Fig. 2 Heatmap of the ten most abundant genera in each sample. The *color intensity in each box* indicates the relative percentage of a genus in each sample. For abbreviations, see caption to Fig. 1

et al. (2009) for the bacterial community associated with the wild oat root. These authors found that the relative abundances of 7% of the bacterial taxa derived from the wild oat root were significantly different from those in the bulk soil. They also reported that Firmicutes, Actinobacteria or Alphaproteobacteria was the dominant group in the bacterial communities studied, with significantly different relative abundances between the rhizosphere and bulk soils. Several previous studies have found that some genera of Proteobacteria were the dominant bacterial community members in the rhizosphere of *Avena fatua* (DeAngelis et al. 2009), maize (Gomes et al. 2001) and grain legumes (Sharma et al. 2005). One explanation may be the presence of fast-growing r-strategists of the Proteobacteria that are able to absorb a broad range of root-derived carbon substrates (Philippot et al. 2013).

The results of the heatmap analysis indicated that the relative abundance of *Nitrospira* was remarkably higher in the rhizosphere sediments than in the bulk sediments of both inoculated and uninoculated microcosms. *Nitrospira* plays an important role in the process of ammonia oxidation, which is a vital step of nitrification (Purkhold et al. 2000). Previous studies have found significantly elevated abundances of the bacterial *amoA* gene, which encodes the active site of ammonia monooxygenase, in the rhizosphere sediment of *C. demersum* and *V. spinulosa* ($P < 0.05$) (Zhao et al. 2014b). The process of ammonia oxidation requires oxygen (Kowalchuk and Stephen 2001). It is therefore possible that the higher relative abundance of *Nitrospira* found in the rhizosphere sediment may be attributable to the oxygen released from the rhizosphere of submerged macrophytes.

Non-metric multi-dimensional scaling analysis

The results of the NMDS analysis clearly indicated that the bacterial community composition was strongly related to the different sediment groups. The bacterial communities derived from the bulk sediment of the microcosms with submerged macrophytes clustered together (Fig. 3a). Bacterial communities in the surface sediments of the microcosms with submerged macrophytes were also similar. However, remarkable differences in the composition of the bacterial community were observed for the rhizosphere sediments of the three kinds of submerged macrophytes (Fig. 3a). The composition of the bacterial community derived from the bulk sediment of the control columns was similar to that of the bulk sediment planted with submerged macrophytes, whereas different bacterial community compositions were found in the surface sediments between the inoculated and uninoculated microcosms (Fig. 3a).

To further investigate the composition of the bacterial community for the general and rare bacterial groups, we further divided the overall bacterial community into the following three ecological categories: general OTUs (the OTUs which containing ≥ 11 sequences in all samples), rare OTUs (the OTUs which contained only one sequence in all samples) and other OTUs (the OTUs beyond general and rare OTUs). The NMDS analysis was conducted for the general and rare bacterial groups separately (Fig. 3b, c). The results showed that the composition of the bacterial community of the general OTUs was similar to that of the overall bacterial community (Fig. 3b). However, the bacterial community composition of rare OTUs was clearly different from that of the overall bacterial community (Fig. 3c). It was evident that the bacterial community derived from the rhizosphere sediments of different submerged macrophytes clustered together (Fig. 3c), suggesting that the rare bacterial groups associated with the rhizosphere sediments of different submerged macrophytes were similar.

The results of the NMDS analysis indicated that the composition of the bacterial community in the bulk sediment of both the uninoculated microcosm systems and those with the three kinds of submerged macrophytes

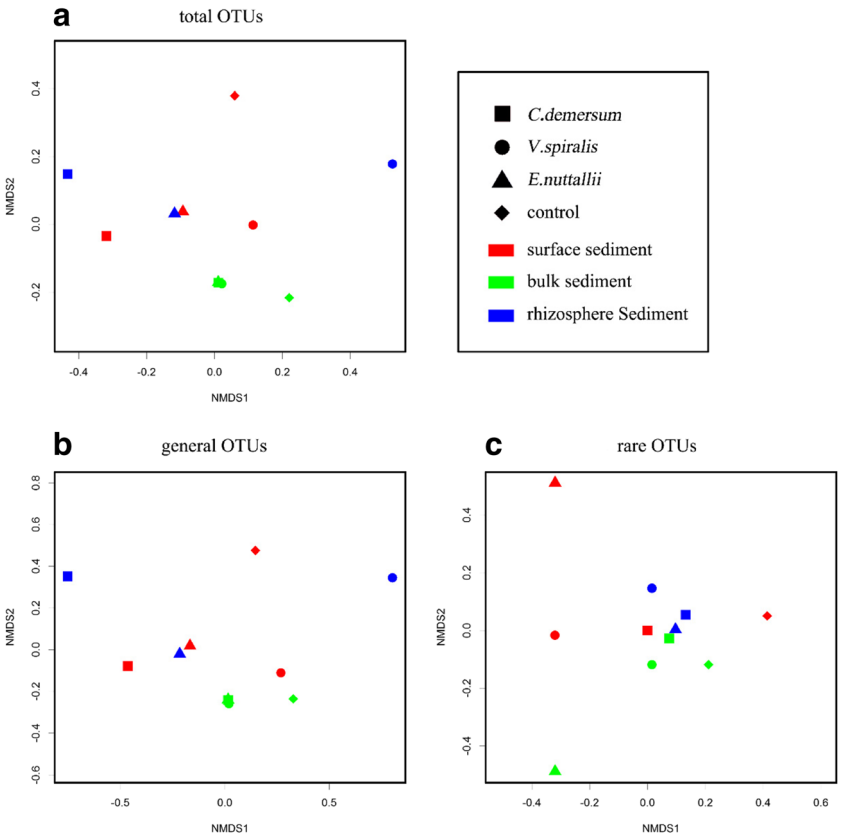

Fig. 3 Nonmetric multidimensional scaling analysis of bacterial community composition in 11 sediment samples based on total operational taxonomic units (OTUs) (**a**), based on general OTUs (**b**) and based on rare OTUs (**c**)

clustered together and was clearly different from that of the bacterial community derived from the rhizosphere sediment samples (Fig. 3a). Many previous studies have reported different relative abundances of microbial populations in the rhizosphere of crops and of cultivated and native plant species (Garbeva et al. 2008; Oh et al. 2012; Teixeira et al. 2010). Plants may influence the microorganisms associated with their rhizosphere through the release of exudates by the roots. Terrestrial plants have been shown to release secondary compounds, such as phenols and alkaloids, which could affect the bacterial communities (Berg and Smalla 2009).

Further comparison of the NMDS patterns of the abundant and rare bacterial groups revealed remarkably different patterns in the NMDS plot (Fig. 3b, c). Most bacterial communities include a great number of species. Only a few of these bacterial species are very abundant, and a great number of bacterial species contain only a few individuals (Sogin et al. 2006). In recent years, the rare biosphere of bacteria has been examined, revealing that the distribution patterns of rare and abundant taxa are seldom similar (Galand et al. 2009; Gobet et al. 2012). In our study, the bacterial community derived from the rhizosphere sediments of different submerged macrophytes clustered together for the rare bacterial groups (Fig. 3c), suggesting that the rare bacterial groups associated with the rhizosphere sediments of the different submerged macrophytes were similar.

The results of our study indicate that the diversity of the bacterial community associated with the rhizosphere sediments of the submerged macrophytes was significantly lower than that of the bulk sediment. Remarkable differences in the composition of the bacterial community between the rhizosphere and bulk sediments were also observed. Further studies are needed to investigate the functional characterization of the bacterial community colonizing in the rhizosphere sediments of submerged macrophytes. The results of such studies would improve our understanding of the ecological functions of submerged macrophytes in the freshwater ecosystem.

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References

Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68:1–13

Bray JR, Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. Ecol Monogr 27:325–349

Bulgarelli D, Rott M, Schlaeppi K, van Themaat EVL, Ahmadinejad N, Assenza F (2012) Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature 488:91–95

Chaparro JM, Badri DV, Vivanco JM (2014) Rhizosphere microbiome assemblage is affected by plant development. ISME J 8:790–803

Chmielewski TJ, Radwan S, Sielewicz B (1997) Changes in ecological relationships in a group of eight shallow lakes in the Polesie Lubelskie region (eastern Poland) over forty years. In: Kufel L, Prejs A, Rybak JI (eds) Hydrobiologia in shallow lakes '95. Springer Netherlands, Dordrecht, pp 285–295

DeAngelis KM, Brodie EL, DeSantis TZ, Andersen GL, Lindow SE, Firestone MK (2009) Selective progressive response of soil microbial community to wild oat roots. ISME J 3:168–178

Deng W, Wang Y, Liu Z, Cheng H, Xue Y (2014) HemI: a toolkit for illustrating heatmaps. PloS One 9: e111988

Faith DP (1992) Conservation evaluation and phylogenetic diversity. Biol Conserv 61:1–10

Galand PE, Casamayor EO, Kirchman DL, Lovejoy C (2009) Ecology of the rare microbial biosphere of the Arctic Ocean. Proc Natl Acad Sci USA 106:22427–22432

Garbeva P, van Elsas JD, van Veen JA (2008) Rhizosphere microbial community and its response to plant species and soil history. Plant Soil 302:19–32

Gobet A, Boer SI, Huse SM, van Beusekom JEE, Quince C, Sogin ML, Boetius A, Ramette A (2012) Diversity and dynamics of rare and of resident bacterial populations in coastal sands. ISME J 6:542–553

Gomes NCM, Heuer H, Schönfeld J, Costa R, Mendonca-Hagler L, Smalla K (2001) Bacterial diversity of the rhizosphere of maize (*Zea mays*) grown in tropical soil studied by temperature gradient gel electrophoresis. Plant Soil 232:167–180

Hempel M, Blume M, Blindow I, Elisabeth MG (2008) Epiphytic bacterial community composition on two common submerged macrophytes in brackish water and freshwater. BMC Microbiol 8:58–67

Herrmann M, Saunders AM, Schramm A (2009) Effect of lake trophic status and rooted macrophytes on community composition and abundance of ammonia-oxidizing prokaryotes in freshwater sediments. Appl Environ Microbiol 75:3127–3136

Huse SM, Welch DM, Morrison HG, Sogin ML (2010) Ironing out the wrinkles in the rare biosphere through improved OTU clustering. Environ Microbiol 12:1889–1898

Jatin S, Amit G, Harish C (2008) Managing water quality with aquatic macrophytes. Rev Environ Sci Biotechnol 7:255–266

Jeppesen E, Sondergaard M, Sondergaard M, Christofferson K (1998) The structuring role of submerged macrophytes in lakes. Springer, New York, NY, USA, pp 197–216

Kowalchuk GA, Stephen JR (2001) Ammonia-oxidizing bacteria: A model for molecular microbial ecology. Annu Rev Microbiol 55: 485–529

Lembi CA (2001) Limnology, lake and river ecosystems. J Phycol 37: 1146–1147

Lu L, Xing DF, Ren NQ (2012) Pyrosequencing reveals highly diverse microbial communities in microbial electrolysis cells involved in enhanced H_2 production from waste activated sludge. Water Res 46:2425–2434

Ofek-Lalzar M, Sela N, Goldman-Voronov M, Green SJ, Hadar Y, Minz D (2014) Niche and host-associated functional signatures of the root surface microbiome. Nat Commun 5:4950

Oh YM, Kim M, Lee-Cruz L, Lai-Hoe A, Go R, Ainuddin N, Rahim RA, Shukor N, Adams JM (2012) Distinctive bacterial communities in the rhizoplane of four tropical tree species. Microb Ecol 64:1018–1027

Peiffer J, Spor A, Koren O, Zhao J, Tringe SG, Dangl JF, Buckler ES, Ley RE (2013) Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proc Natl Acad Sci USA 110: 6548–6553

Philippot L, Raaijmakers JM, Lemanceau P, van Der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789–799

Purkhold U, Pommerening-Roser A, Juretschko S, Schmid MC, Koops HP, Wagner M (2000) Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and *amoA* sequence analysis: implications for molecular diversity surveys. Appl Environ Microbiol 66:5368–5382

Schloss PD, Gevers D, Westcott SL (2011) Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. PLoS One 6:e27310

Sharma S, Aneja MK, Mayer J, Munch JC, Schloter M (2005) Characterization of bacterial community structure in rhizosphere soil of grain legumes. Microb Ecol 49:407–415

Sogin ML, Morrison HG, Huber JA, Mark Welch D, Huse SM, Neal PR, Arrieta JM, Herndl GJ (2006) Microbial diversity in the deep sea and the underexplored 'rare biosphere'. Proc Natl Acad Sci USA 103:12115–12120

Teixeira LC, Peixoto RS, Cury JC, Sul WJ, Pellizari VH, Tiedje J, Rosado AS (2010) Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay, maritime Antarctica. ISME J 4: 989–1001

Uroz S, Buee M, Murat C, Frey-Klett P, Martin F (2010) Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. Environ Microbiol Rep 2:281–288

Victor K, Anna E, Howard O, Philip H (2010) Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates. Environ Microbiol 12:118–123

Zeng J, Bian YQ, Xing P, Wu QL (2012) Macrophyte species drive the variation of bacterioplankton community composition in a shallow freshwater lake. Appl Environ Microbiol 78:177–184

Zeng J, Zhao DY, Li HB, Huang R, Wang JJ, Wu QL (2016) A monotonically declining elevational pattern of bacterial diversity in freshwater lake sediments. Environ Microbiol 18:5175–5186

Zhao DY, Huang R, Zeng J, Yu ZB, Liu P, Cheng SP, Wu QL (2014a) Pyrosequencing analysis of bacterial community and assembly in activated sludge samples from different geographic regions in China. Appl Microbiol Biotechnol 98:9119–9128

Zhao DY, Luo J, Zeng J, Wang M, Yan WM, Huang R, Wu QL (2014b) Effects of submerged macrophytes on the abundance and community composition of ammonia-oxidizing prokaryotes in a eutrophic lake. Environ Sci Pollut Res 21:389–398