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RESEARCH ARTICLE

Bio-cord plays a similar role as submerged macrophytes in harboring bacterial assemblages in an eco-ditch

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Abstract

Artificial carriers are widely used to enhance the formation of biofilm and improve pollutants' removal efficiency in agricultural wastewater treatment ditches (eco-ditches), yet comprehensive insight into their bacterial community is scarce. In this study, bacterial diversities in four different habitats—the water column, surface sediments, submerged macrophytes (*Myriophyllum verticillatum* L.), and the artificial carriers (bio-cord)—were compared in a Chinese eco-ditch. Comparable richness and evenness of bacterial communities were observed on *M. verticillatum* and bio-cord, both being higher than for free-living bacteria in the water column but lower than for bacteria in the surface sediment. The highest similarity of bacterial community composition and structure also occurred between *M. verticillatum* and the bio-cord, dominated by α- and γ-*proteobacteria*, *Verrucomicrobia*, and *Bacteroidetes*. *Firmicutes* and *Planctomycetes*, respectively, were the exclusive abundant phyla in *M. verticillatum* and the bio-cord, probably indicating the unique interaction between *M. verticillatum* and their epiphytic bacteria. Some abundant genera, such as *Roseomonas*, *Pseudomonas*, and *Rhodopirellula*, which were exclusively observed in *M. verticillatum* or the bio-cord, have been reported to have the same capacity to remove nitrogen and organic matter in wastewater treatment systems. In conclusion, in the studied eco-ditch, the bio-cord was found to play a similar role as submerged macrophytes in harboring bacterial assemblages, and we therefore propose that bio-cord may be a good alternative or supplement to enhance wastewater treatment in agricultural ditches.

Keywords Ecological ditch system · Artificial carrier · *Myriophyllum verticillatum* L. · Biofilm bacterial community

Introduction

The increasing discharge of domestic, industrial, and agricultural wastewater has led to serious river and lake pollution, especially in developing and rural areas (Feng et al. 2017), to an extent that it thus poses a threat to the health of local populations and to the economic development. Many environmentally friendly measures have been applied to reduce the nutrient loading and amount of organic compounds in rural areas (Chen et al. 2015; Mi et al. 2015; Wu et al. 2017), one of these being the eco-ditch technology, which has proved to effectively intercept and filter agricultural wastewater before it exits into downstream receiving waterbodies (Xiong et al. 2015). Eco-ditches are generally composed of drainage channels and include different kinds of aquatic plants, artificial substrates, and microorganisms in diverse combinations adapted to the local environment (Chen et al. 2015).

Bacterial assemblages attached to different submerged substrates are highly metabolically active and have a complex

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composition and structure (Guan et al. 2015). Therefore, bacteria, which are widely distributed in water and sediment, and on natural and artificial substrates, play a crucial role in eco-ditches established for decomposition, transformation, and uptake of water pollutants (He et al. 2015; Lopez-Garcia et al. 2011; Ma et al. 2015; Ye and Zhang 2013). Furthermore, bacterial community assemblages formed in different niches can be used to predict particular functions (Burke et al. 2011a; Salles and Roux 2009; Yang et al. 2005) and they are sensitive to the ambient environment; hence, the composition and structure of bacterial community assemblages can reflect the status and function of the eco-ditch ecosystem (Guan et al. 2015). In addition, identification of bacterial community structure can improve our knowledge of biological processes in eco-ditch systems, which may be of benefit to the rational design and maintenance of eco-ditches (He et al. 2015). So far, most previous studies have concentrated on the nutrient removal and purification capacity of the eco-ditches (Chen et al. 2015; Wu et al. 2014b; Wu et al. 2011; Xiong et al. 2015) or on the different efficiencies of macrophyte species (Dhote and Dixit 2009; Kumwimba and Zhu 2017), while insight into the composition and structure of the bacterial communities responsible for the purification in the eco-ditches is fragmented.

Macrophytes, such as *Lemna gibba*, *Hydrilla verticillata*, and *Myriophyllum verticillatum*, play a key role in eco-ditches and are efficient nutrient and heavy metal removers (Dhote and Dixit 2009). Moreover, macrophytes are essential for providing substrates and habitats for bacterial colonization (Kumwimba and Zhu 2017). Several studies have documented that strong interactions between macrophytes and epiphytic bacterial biofilm communities improve the removal capacity, especially in the rhizosphere (Hempel et al. 2008; Jiang et al. 2013). Additional artificial carriers such as non-woven fabrics, polyurethane sponges, bio-cord, and AquaMats (Yuan et al. 2012) have been used to enhance the formation of bacterial assemblages and thus improve the removal efficiency (Feng et al. 2017; Peng et al. 2017). These artificial carriers are characterized by high porosity and large superficial areas, and they can be adapted to the river flow conditions and, as a result, help provide suitable conditions and support media for bacterial growth (Ateia 2016). To date, few studies have compared the composition and structure of bacterial community assemblages on artificial carriers and submerged macrophytes using traditional methods like denaturing gradient gel electrophoresis (DGGE) and fluorescence in situ hybridization (FISH) (Hempel et al. 2009). Whether the bio-cord can play a similar role as submerged macrophytes, sediment, or water in eco-ditch ecosystems is still unknown. The recently developed high-throughput sequencing technology has been successfully applied to analyze microbial communities (Gulay et al. 2016; Tang et al. 2017; Zhang et al. 2012) and may provide us with new information allowing a more efficient identification of the entire profile of microbial communities than traditional methods (Ye and Zhang 2013).

The objective of this study was to examine the composition and structure of bacterial communities and indicator bacterial groups in water, surface sediment, submerged macrophytes, and artificial carrier-attached habitats simultaneously in an eco-ditch system. We hypothesized that bio-cord would play a similar role as submerged macrophytes in harboring bacterial assemblages due to habitat preference in the eco-ditches.

Materials and methods

Study site and sample collection

The eco-ditch (~ 42°01′N, 86°49′E) used in our study is situated in a remote arid area in Xinjiang, northwestern China (Fig. S1). The length, width, and depth of the ditch are 150, 4, and 1.5 m, respectively. It receives drainage effluent from agriculture and has been planted with the submerged macrophytes *Myriophyllum verticillatum* L. In addition, bio-cords have been submerged in order to enhance purification of the water. Bio-cord is a cord contact filtration material in which fine polypropylene fibers and vinylon are woven into a three-dimensional form (outer diameter 45 mm, specific surface area 53.12 m^2/kg, porosity > 99%). A total of 12 samples were collected at three parallel sites placed within 20 m on October 11, 2016, of water, surface sediment, *M. verticillatum*, and bio-cord (Table S1), when the bio-cord submerged on August 2 was sufficiently stable for nutrient removal according to an earlier study in this ditch (Cai et al. 2017). Water was collected into a pre-sterilized plastic bucket by a conventional hydrophore. The samples were subsequently filtered through 0.2 μm polycarbonate membranes (47 mm diameter; Millipore) in the laboratory for further treatment. Surface sediment (to 1 cm depth) was collected with a 60-cm-long gravity corer (UWITEC, Austria). Bio-cord and *M. verticillatum* samples were cut by sterile scissors and mixed with sterile Milli-Q water, respectively. Ultrasound was sequentially applied to separate attached microbes from the bio-cords and submerged macrophytes using the method detailed in Cai et al. (2014).

DNA extraction, PCR amplification, and 16S rRNA gene sequencing

Total DNA were extracted from the 12 samples using the FastDNA Spin Kit for Soil (MP Biomedicals, USA) according to the manufacturer's protocol. The quality and quantity of extracted DNA were checked by electrophoresis in 1.5% (wt/vol) agarose gel with 0.5% (vol/vol) Gold View I (Salarbio, USA) and by using a NanoDrop ND-1000 UV/Vis spectral photometer (Thermofisher, USA).

After DNA extraction, the V4 regions of bacterial 16S rRNA genes were amplified using the primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al. 2012). The PCR reaction mixtures contain 1× PCR buffer, 0.4 μM of each primer, 0.2 mM dNTPs, 0.5 U of Takara DNA Polymerase (Takara, Japan), 20 ng diluted DNA template, and sterile Milli-Q water in a final volume of 25 μl. PCR amplifications were performed in triplicate for each sample using a 25-thermal-cycle scheme: after initial denaturation at 94°C for 5 min, each cycle consisted of denaturation at 94°C for 30 s, primer annealing at 58°C for 30 s, and primer extension at 72°C for 30 s, followed by a final extension at 72°C for 7 min. PCR products were run on 2% agarose gels and purified using the QIAquick® Gel Extraction kit (Qiagen, USA) according to the manufacturer's instructions. Purified amplicons were pooled and quantified with Quant-iT PicoGreen dsDNA Assay Kit (Thermofisher, USA). Finally, clean amplicon pools for each sample in equal concentrations were pair-end sequenced (2 × 250) on an Illumina MiSeq platform at Beijing Genomics Institute (BGI), China. All the sequences used in this study are available from the NCBI Sequence Read Archive (SRA) under accession number SRP125423.

Sequences processing and taxonomy classification

The raw data was pre-processed to get clean data by filtering out the reads with sequencing adapters, N base, poly base, low quality, etc. with default parameters (Fadrosh et al. 2014). The high-quality paired-end reads were combined to tags based on overlaps by Flash v.1.2.11 software (Fast Length Adjustment of Short reads) with minimal overlapping length of 15 base pairs (Magoč and Salzberg 2011). Tags were renamed and filtered according to the MiSeq standard operation procedure, i.e., MiSeq SOP (http://www.mothur.org/wiki/MiSeq_SOP), using Mothur v.1.39.5 software (Schloss et al. 2009). Precluster commands with permitted max mismatches less than 1/100 (http://www.mothur.org/wiki/Pre.cluster) were used to screen and remove noise to obtain unique sequences. UCHIME in Mothur software (http://www.mothur.org/) was performed to remove chimera sequences (Edgar et al. 2011). The OTU-representative reads were classified using Mothur's version of the Ribosomal Database Project (RDP) Bayesian classifier through a normalized RDP training dataset (Cole et al. 2009). Sequences from chloroplasts, mitochondria, archaea, and eukaryotes were removed because our study focus was on the composition of bacterial communities. Operational taxonomic units (OTUs) were defined by clustering of sequences at the 97% similarity threshold (Edgar 2010). In addition, low confidence singletons (OTUs) with a read count smaller than 2 were removed from the downstream analysis.

Data and statistical analysis

The subsample data set was constructed in Mothur on 24,401 sequences per sample based on the sample with the smallest sequencing effort. α-Diversities were assessed using observed species (i.e., OTUs), invsimpson (the inverse Simpson index), Chao1 (a non-parametric species richness estimator), Shannon index (a combination of richness and evenness), and phylogenetic diversity (PD whole tree) (Faith 2006) calculated in Mothur from the subsample data set. β-Diversities (similarity of samples) were estimated using Jclass (the Jaccard similarity coefficient based on OTUs), Bray–Curtis distance (a non-phylogenetic metric), Unweighted UniFrac (a qualitative phylogenetic metric), and Weighted UniFrac (a quantitative phylogenetic metric) (Chao 1984; Lozupone and Knight 2005; Real and Vargas 1996; Schloss 2008) and visualized by non-metric multidimensional scaling (NMDS) of the subsample data set. In addition, samples were clustered using Bray–Curtis distance and Weighted UniFrac. Analysis of similarity (ANOSIM) based on Bray–Curtis distance and Weighted UniFrac, which is a non-parametric test of differences between two or more groups (Clarke and Ainsworth 1993), was used to statistically test the variation of bacterial community composition across different habitats. In addition to α- and β-diversities, it is important to identify the significantly enriched bacterial groups (biomarkers) in each habitat. Linear discriminate analysis (LDA) effect size (LEfSe) (Segata et al. 2011), which is an algorithm for high dimensional biomarker discovery between two or more biological conditions or classes, was applied to distinguish biomarker at genus level in different habitats.

Most data were plotted with the packages "ggplot2" (Wickham 2009) in R v.3.4.1 or base graphics. Kruskal–Wallis rank tests were used with Bonferroni correction to compare the distribution of phyla and genera among different habitats. Venn diagram was generated by Mothur at OTU level. A heat map of the most abundant bacterial genera (average relative abundance in each sample type > 1%) was made using the "pheatmap" package in the R v.3.4.1.

Results

Sequencing and quality control

A total of 12 samples of water (W), surface sediment (S), *M. verticillatum* (M), and bio-cord (B) were taken from four habitats in the eco-ditch. We generated 490,178 tags with an average length of 253 bp using Illumina Miseq. After quality filtering, denoising, and removal of chimeras, archaeal and eukaryotic sequences, and singleton OTUs, a total of 357,851 reads and 8002 OTUs (97% similarity cut-off) were obtained. Plots of OTU number versus read number, i.e., the

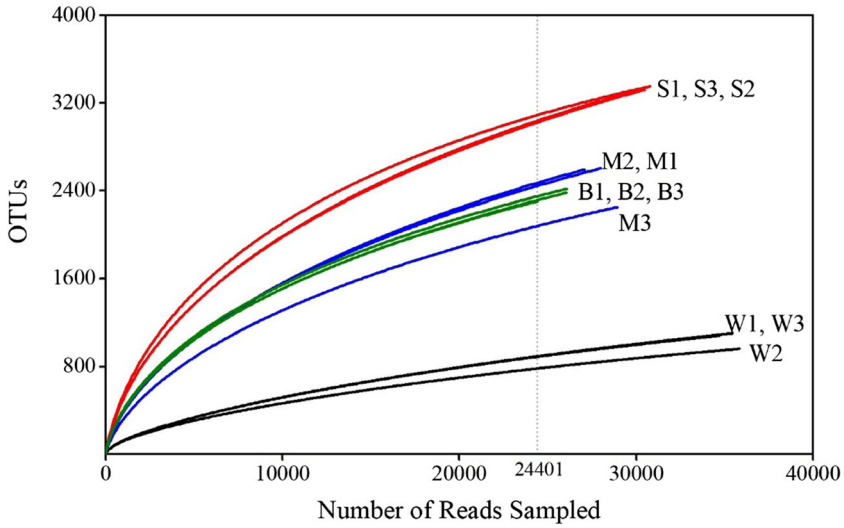

Fig. 1 Rarefaction curves for OTUs number at 97% similarity. W, S, M, and B represent water, surface sediment, *M. verticillatum*, and bio-cord samples, respectively. The vertical dashed line represents the smallest reads among the 12 samples

rarefaction curves, revealed that the sequencing depth was sufficient to cover most of the bacterial diversity in each sample, as documented by the modest slope at the end of the rarefaction curves and the sampling coverage (Fig. 1; Table 1).

Diversity of the bacterial community

To compare α- and β-diversities among the four habitats, we normalized the sequencing number of each sample to 24,401 reads (the smallest sample among the 12 samples). All α-diversity indices, including observed species (i.e., OTUs), invsimpson (the inverse Simpson index), Chao1, Shannon index, and phylogenetic diversity (PD whole tree), were highest in the sediment samples (S1–S3), followed by the *M. verticillatum* (M1–M3), and the bio-cord samples (B1–B3), and the water samples (W1–W3) had by far the lowest α-diversity. These results were also consistent with the rarefaction curves (Fig. 1). Average OTUs, Chao1, and phylogenetic diversity were slightly higher in the *M. verticillatum* than on the bio-cord, whereas average invsimpson and Shannon indexes were slightly higher on the bio-cord. This reflected a relatively higher evenness but lower richness in the bio-cord.

NMDS was performed to compare β-diversity among the different sample types using non-phylogenetic Jclass (the Jaccard similarity coefficient based on OTUs) and Bray–Curtis distances and phylogenetic (Unweighted UniFrac and Weighted UniFrac)-based ordination (Fig. 2). We found that the triplicate samples of each type were grouped together, reflecting a similar bacterial community membership and structure within each sample type. The similarity of bacterial community membership was essentially the same whether non-phylogenetic (Jclass) or phylogenetic (Unweighted UniFrac)-based ordination was used. However, the similarity in bacterial community structure was much higher using phylogenetic (Weighted UniFrac)-based ordination than non-phylogenetic (Bray–Curtis distances)-based ordination. The lowest similarity between bacterial community assemblage and structure appeared between the water samples and the other three samples. The surface sediment, *M. verticillatum*, and bio-cord samples showed relatively closer resemblance, and the strongest similarity was observed between the *M.*

Table 1 Comparison of α-diversity indices among the bacterial communities of water (W), sediment (S), *M. verticillatum* (M), and bio-cord samples (B)

Samples	Reads	Coverage (%)	OTUs	Invsimpson	Chao1	Shannon	PD whole tree
W1	34,677	98	1082	13.4	1832	3.50	72.0
W2	35,824	99	963	12.3	1634	3.43	63.7
W3	35,426	98	1104	10.6	1987	3.29	74.0
S1	30,759	96	3352	264	4300	6.74	194
S2	30,468	96	3322	186	4875	6.54	189
S3	30,163	96	3319	210	4752	6.56	187
M1	27,984	96	2604	74.1	4200	5.78	146
M2	27,076	96	2593	90.0	3919	5.82	148
M3	28,919	96	2252	16.4	3583	4.92	129
B1	26,061	96	2382	81.4	3662	5.85	136
B2	26,093	96	2418	88.5	3693	5.91	138
B3	24,401	96	2304	95.0	3470	5.91	131

Diversity indices were calculated using a subset of 24,401 reads per sample selected randomly based on the sample with the smallest sequencing effort

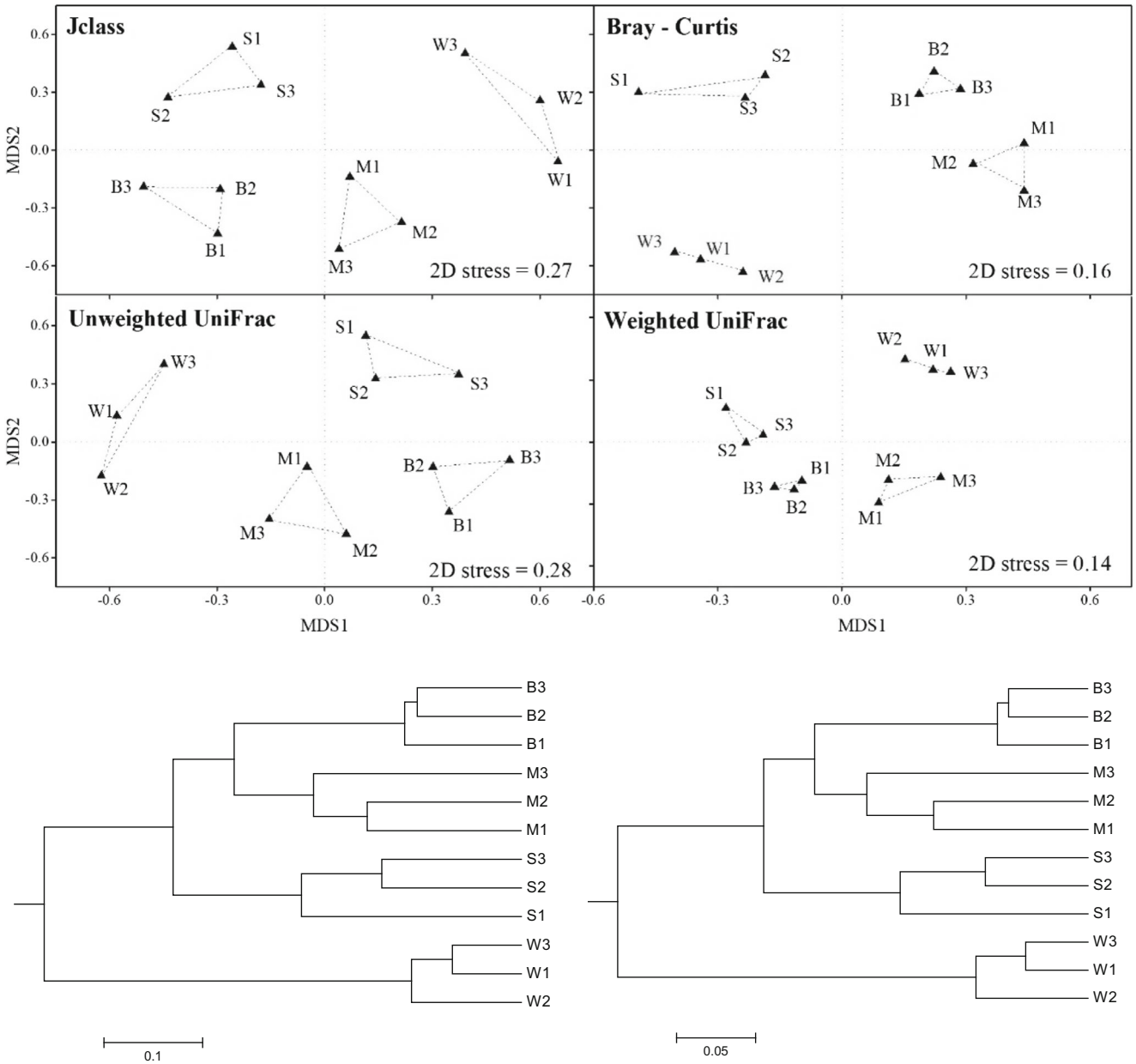

Fig. 2 NMDS based on Jaccard similarity coefficient, Bray–Curtis distances, unweighted UniFrac and Weighted UniFrac (upper), and cluster-based on Bray–Curtis distances and Weighted UniFrac (lower). W, S, M, and B represent water, surface sediment, *M. verticillatum*, and bio-cord samples, respectively

verticillatum samples and the bio-cord samples using both Bray–Curtis distances and Weighted UniFrac-based ordination (Fig. 2). Accordingly, among 7939 OTUs, after normalizing the sequencing number, an average of 1329 OTUs was shared between the *M. verticillatum* sample and the bio-cord sample, which was by far the highest average among any other comparisons. The shared OTUs accounted for 51.4 and 52.8% in the *M. verticillatum* sample and the bio-cord sample, respectively. Moreover, the number of OTUs shared between the water sample and the other samples was low (Fig. 3). ANOSIM showed a significant difference among the four sample types using both Bray–Curtis distances and Weighted UniFrac ($p < 0.001$).

Bacterial community composition and structure

Overall, all reads were classified into 26 phylum-level taxonomic groups, and nine of them accounted for 98.7% of all reads (Fig. 4). The bacterial community composition and structure of the water samples differed notably from the other three sample types. The water samples (W1–W3) were dominated by *Actinobacteria*, which accounted for 37.9 ± 5.4%

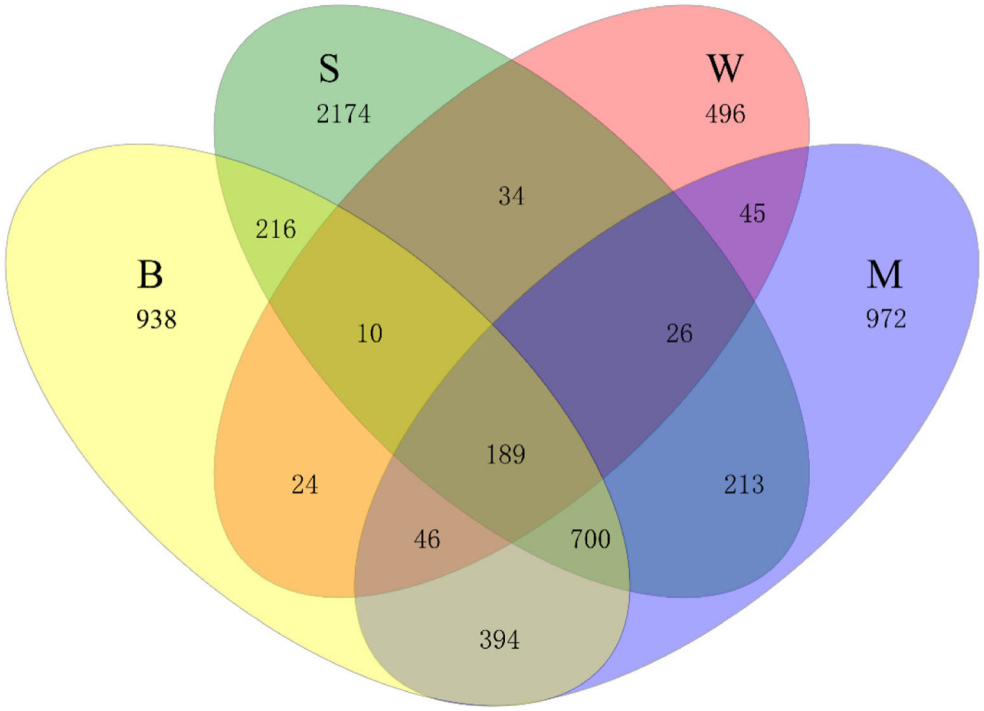

Fig. 3 Venn diagram showing operation taxonomic units (OTUs) shared between samples. W, S, M, and B represent water, surface sediment, *M. verticillatum*, and bio-cord samples, respectively

(mean ± standard deviation), followed by *Proteobacteria* (29.5 ± 3.5%) and *Bacteroidetes* (21.3 ± 7.9%). The most abundant phyla in the surface sediment samples (S1–S3) were *Proteobacteria* (41.5 ± 0.2%), *Bacteroidetes* (11.4 ± 1.1%), *Verrucomicrobia* (9.6 ± 3.0%), and *Acidobacteria* (7.7 ± 0.6%). The *M. verticillatum* samples (M1–M3) were dominated by *Proteobacteria* (54.6 ± 7.3%), *Verrucomicrobia* (11.9 ± 2.2%), and *Bacteroidetes* (7.6 ± 1.6%). The abundant phyla in the bio-cord samples (B1–B3) were *Proteobacteria* (45 ± 2.4%), *Verrucomicrobia* (17.8 ± 1.2%), *Bacteroidetes* (11.8 ± 1.62%), and *Planctomycetes* (5.25 ± 0.49%). Within *Proteobacteria*, the water samples were dominated by β-

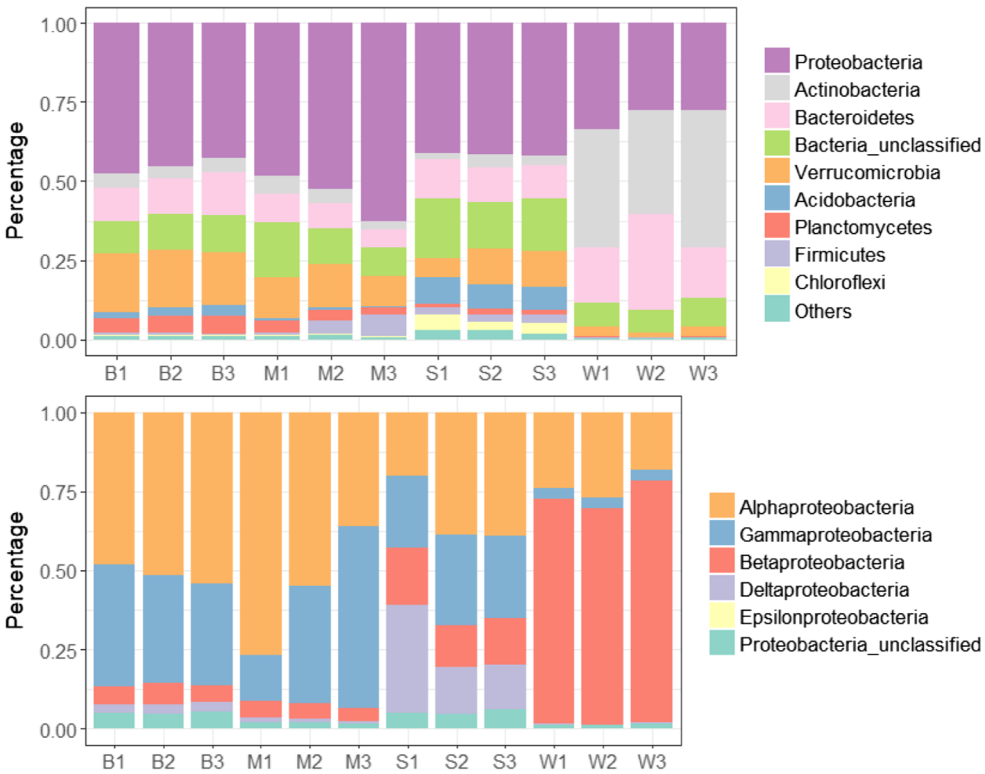

Fig. 4 Percentage of the dominant bacterial phyla (average relative abundance > 1%, upper) and classes in *Proteobacteria* (lower) in all samples. Phyla with relative abundance < 1% were grouped as others. W, S, M, and B represent water, surface sediment, *M. verticillatum*, and bio-cord samples, respectively

subdivisions, followed by α-subdivisions, whereas the *M. verticillatum* and bio-cord samples were dominated by α- and γ-subdivisions. α-, β-, γ-, and δ-subdivisions were distributed evenly in the sediment samples, and only few ε-subdivisions were observed. Overall, the highest percentages of *Actinobacteria* and *Bacteroidetes* appeared in the water sample, the highest percentages of *Acidobacteria* and *Chloroflexi* in the sediment sample, the highest percentages of *Firmicutes* and *Proteobacteria* in the *M. verticillatum* sample, and the highest percentages of *Verrucomicrobia* and *Planctomycetes* in the bio-cord sample. In addition, in each sample, a relatively large percentage (7.9–16.7%) of effective bacterial reads could not be assigned to any taxa at phylum level, and unclassified read percentages in the total community gradually increased from the phylum to the genus level, indicating the extent of novel reads captured in this study.

The abundant genera (average relative abundance > 1% in each sample) were selected (a total of 47 genera for all 12 samples) and compared with their abundances in other samples (Figs. 5 and 6). More than half were only classified at family or order level. There were 22 (46.8%) *Proteobacteria* genera among the abundant genera, including α- (nine genera), β- (four genera), γ- (five genera), and δ-subdivisions (three genera), followed by seven genera of *Bacteroidetes* and six genera of *Verrucomicrobia*. Only an unclassified genus belonging to the family *Chitinophagaceae* was shared by all four habitats. *Rhodobacteraceae*, *Rhizobiales*, *Luteolibacter*, and *Ilumatobacter* were shared by the sediment, *M. verticillatum*, and bio-cord samples; however, significantly higher proportions of *Rhodobacteraceae* and *Rhizobiales* were observed in the *M. verticillatum* samples (Kruskal–Wallis rank test, $p < 0.05$). Three genera, *Spartobacteria_genera_incertae_sedis*, *Rhodobacter*, and an unclassified genus belonging to the family *Microbacteriaceae*, were only shared by the water and the *M. verticillatum* samples. *Sphingobacteriales* was the only order shared by the sediment and the *M. verticillatum* samples. *Gp4* and *Haliea* were shared by the sediment and the bio-cord samples. *Planctomycetaceae* and *Porphyrobacter* were shared by the *M. verticillatum* and the bio-cord samples, while a significantly higher proportion of *Porphyrobacter* was observed in the *M. verticillatum* samples (Kruskal–Wallis rank test, $p < 0.01$). The abundant genera or families only appearing in the water samples were *Comamonadaceae_unclassified* (*β-proteobacteria*), *Cytophagaceae_unclassified* (*Bacteroidetes*), *Flavobacterium* (*Bacteroidetes*), and *Polynucleobacter* (*β-proteobacteria*). The abundant genera only appearing in the sediment samples were *Desulfuromonas* (*δ-proteobacteria*), *Desulfobacteraceae_unclassified* (*δ-proteobacteria*), and *Anaerolineaceae_unclassified* (*Chloroflexi*). The abundant genera only appearing in the *M. verticillatum* samples were *Psychrobacter* (*γ-proteobacteria*), *Roseomonas* (*α-proteobacteria*), *Pseudomonas* (*γ-proteobacteria*), and *Carnobacterium* (*Firmicutes*). The abundant genera only appearing in the bio-cord samples were *Aquicella* (*γ-proteobacteria*), *Sandaracinobacter* (*α-proteobacteria*), and *Rhodopirellula* (*Planctomycetes*).

Bacterial groups with statistical differences

LEfSe results (Fig. 6) showed that three groups of bacteria, namely, *Flavobacteria* (from class to genus), *Comamonadaceae* (a family of *β-proteobacteria*), and SR1 (from phylum to genus), were enriched in the water sample. Two groups of bacteria, *Chloroflexi* (from phylum to genus) and *Desulfobacterales* (from order to genus), were enriched in the sediment sample, while two groups of bacteria, *α-proteobacteria* and *Erythrobacteraceae* (the order and its family), were enriched in the *M. verticillatum* sample. *Planctomycetales* (from phylum to genus) were enriched in the bi-cord sample. All these eight lineages had an LDA value > 3.5, and five lineages had an LDA value > 4, namely, *Flavobacteria*, *Comamonadaceae*, *Desulfobacterales*, *α-proteobacteria*, and *Erythrobacteraceae*.

Discussion

Our study is, to the best of our knowledge, the first to demonstrate that bio-cord plays a similar role as *M. verticillatum* in harboring bacterial assemblages and that its role differs substantially from the surface sediment and not least the water in ditches. We found similar richness and evenness on bio-cords as on *M. verticillatum*, and the levels recorded were substantially higher than in the free-living bacterial assemblages in the water column (Table 1). A higher physiologic activity has also been reported for bacterial populations in biofilms on attached substrate than for planktonic bacteria in rivers (Araya et al. 2003). The higher richness might be explained by a higher habitat heterogeneity on *M. verticillatum* and the bio-cords (Acinas et al. 1999; Hempel et al. 2009; Yuan et al. 2012). The higher biofilm diversity in the sediment environment than in the water column and substrate materials recorded in our study (Table 1) is in consistence with previous studies showing that bacteria can exploit sediment niches with high structural and chemical complexity (Jiang et al. 2006; Torsvik et al. 2002; Ye et al. 2009).

Furthermore, according to the NMDS ordinations and the cluster results (Fig. 2), we found a similar bacterial community composition and structure on bio-cords and *M. verticillatum* and a notably different bacterial community structure than in the sediment and not least in the water. *Actinobacteria* was the most abundant phylum in the water column, followed by *β-proteobacteria* and *Bacteroidetes* (Fig. 4), as found in other freshwater studies (Holmfeldt et

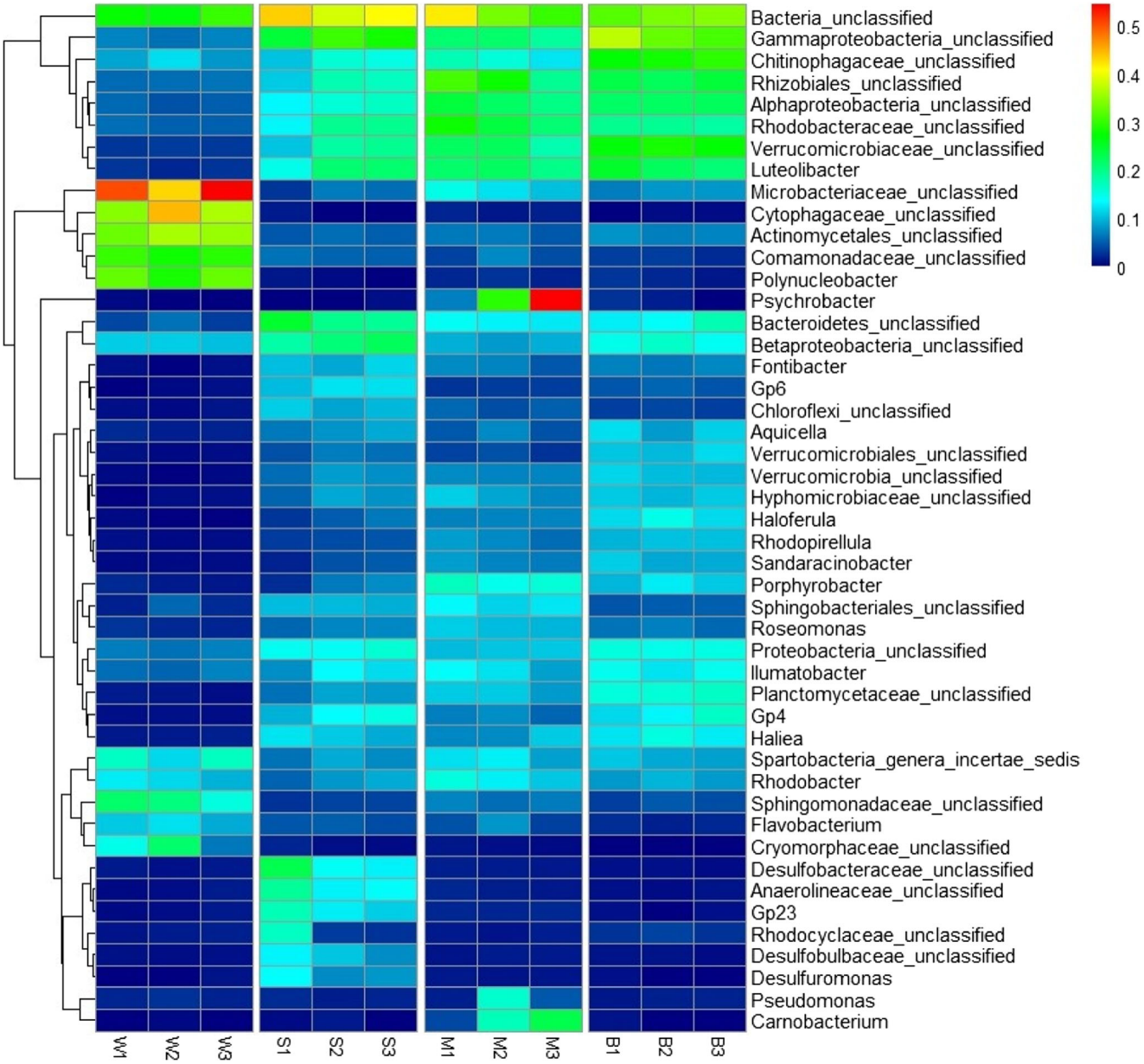

Fig. 5 Heat map of the dominant bacterial genus (average relative abundance > 1% in each sample). The color intensity shows the relative abundance (sqrt transformed) of the genera. W, S, M, and B represent water, surface sediment, *M. verticillatum*, and bio-cord samples, respectively

al. 2009; Parfenova et al. 2013). *Polynucleobacter*, *Cytophagaceae*, *Flavobacterium*, and *Comamonadaceae* were the exclusively abundant genera or biomarkers in the water environment and are all members of the most typical freshwater clusters (Andersson et al. 2008; Araya et al. 2003; Cottrell et al. 2005). In contrast, *Proteobacteria* were the dominant phylum in the bio-cord and *M. verticillatum* environments, as well as in the sediment environment (Fig. 4). *Proteobacteria* is also the most abundant phylum in the bacterial communities in active sludge, biofilm reactors, and constructed wetlands (Guan et al. 2015; Singh et al. 2006; Wagner and Loy 2002; Wu et al. 2011; Zhang et al. 2012). Thus, *Proteobacteria* plays a central role in the biological processes involved in the removal of organic matter and nitrogen in the eco-ditch systems, but the distributions of subdivision may differ depending on the ambient environment, its salinity, and whether they are facing aerobic or anaerobic conditions (Ye and Zhang 2013). Bio-cord and *M. verticillatum* were both dominated by α-proteobacteria and γ-proteobacteria, whereas α-, β-, γ-, and δ-subdivisions of *Proteobacteria* were distributed evenly in the sediment environment, which is indicative of higher habitat complexity and heterogeneity in the sediment. Moreover, a few ε-subdivisions typically occurring in high abundances at the oxic–anoxic interfaces (Campbell et

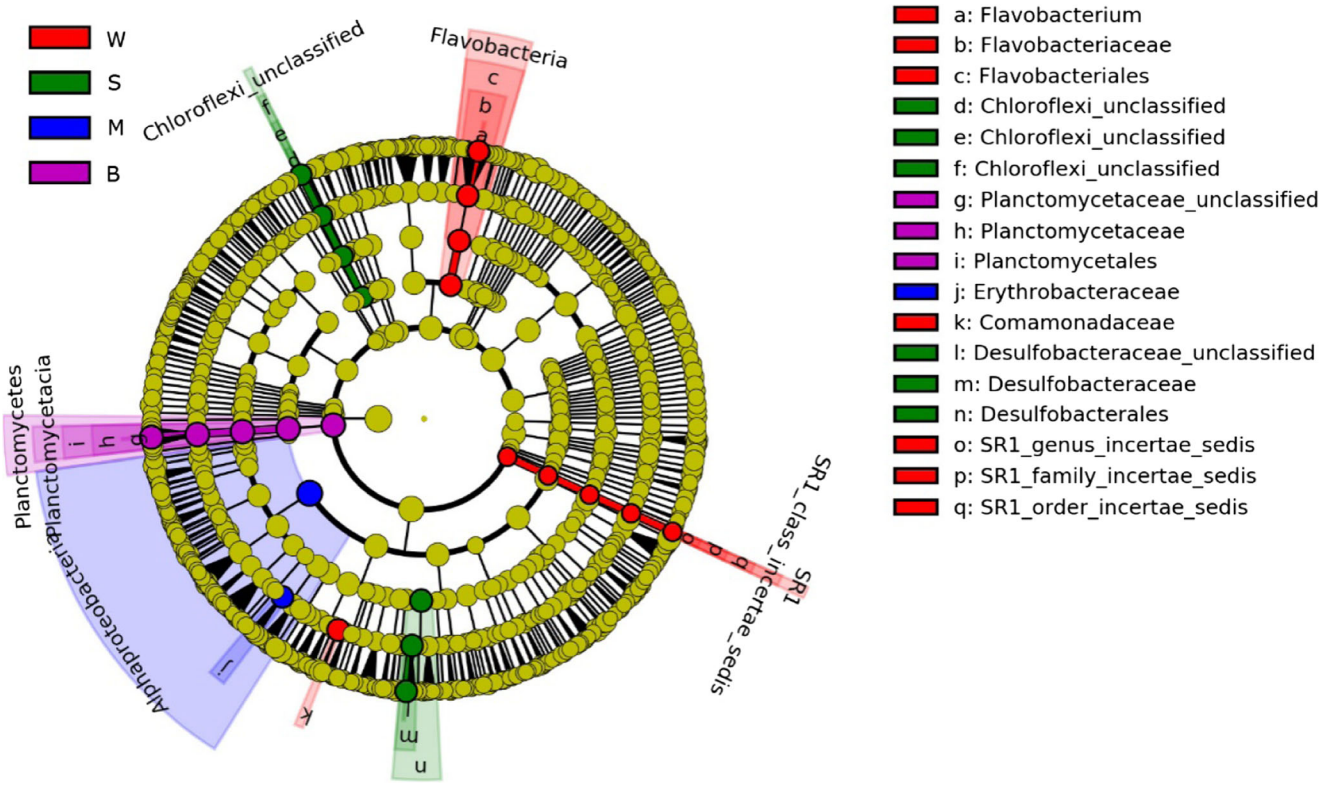

Fig. 6 Cladogram depicting the phylogenetic distribution of bacterial lineages associated with the four types of samples; lineages with an LDA value higher than 3.5 determined by LEfSe are exhibited. Biomarkers of each sample type are represented by different colors (red, green, blue, and purple, indicating the water, surface sediment, *M. verticillatum*, and bio-cord sample, respectively; yellow circles indicate no significant difference). Circles show phylogenetic levels from domain outside to genus inside

al. 2006; Wang et al. 2012) were exclusively observed in the sediment environment (Fig. 4). In addition to *Proteobacteria*, other abundant phyla shared by the bio-cord, *M. verticillatum*, and the sediment were *Bacteroidetes*, *Verrucomicrobia*, *Actinobacteria*, and *Planctomycetes*, which are also widespread and of great importance for pollutant degradation in wastewater treatment systems (Andersson et al. 2008; Ma et al. 2015; Wagner and Loy 2002; Ye and Zhang 2013). The bacterial compositional differences between bio-cords and *M. verticillatum* and the sediment environment reflect that the sediment harbored more of the most abundant phyla or biomarkers, *Acidobacteria* and *Chloroflexi*, and the unique and abundant genera *Desulfuromonas*, *Desulfobacteraceae*, and *Anaerolineaceae*, suggesting the occurrence of more anoxic microniches involving sulfur reduction than in the bio-cord and *M. verticillatum*.

The similar bacterial composition of the bio-cord and *M. verticillatum*, i.e., α- and γ-*proteobacteria*, *Verrucomicrobia*, and *Bacteroidetes*, are mostly reported to be highly related to substrate-attached properties and biofilm formation (Andersson et al. 2008; Feng et al. 2017; Jones et al. 2007; Lee et al. 2008; Singh et al. 2006). A majority of the shared bacteria can produce extracellular polymeric substances and form an organic matter layer that traps nutrients from the surrounding aquatic environments (Chen et al. 2013; Feng et al. 2017; Jones et al. 2007). Biofilm bacteria attached to the bio-cord and the submerged macrophytes are beneficial for the degradation of recalcitrant compounds due to their relatively high microbial biomass and capability of immobilizing compounds (Singh et al. 2006; Wetzel and Søndergaard 1998). Previous studies have confirmed the nutrient removal function of artificial carriers and submerged macrophytes; for instance, 46–56% and 13–19% reductions of total nitrogen (TN) and total phosphorus (TP) by bio-cord were observed in a polluted river treatment system (Yuan et al. 2012) and 64 and 60% reductions of TN and TP were recorded after ditch plant domestic sewage treatment (Kumwimba and Zhu 2017). The average removal rate of TN and TP were 26 and 33% in the studied eco-ditch according to Cai et al. (2017). It should be noted that the bacteria composition and removal efficiency of the biofilm may differ depending on types of carriers and plants (Guan et al. 2015; Wu et al. 2014a) and the growth status of plants (Cai et al. 2013) as well as environmental conditions and variation in, e.g., temperature, conductivity, water level, and hydrodynamic stability (Cattaneo and Kalff 1978; Chen et al. 2015; Hempel et al. 2009; Lyautey et al. 2005).

Submerged macrophytes interact with bacteria in various ways (Burke et al. 2011b; Haichar et al. 2008; Hempel et al. 2008; Wetzel and Søndergaard 1998), and this probably explains why the bacterial composition of submerged

macrophytes is more unique than that of bio-cords. For example, epiphytic biofilms offer the macrophytes organic compounds and carbon dioxide and boost nutrient recycling (Hempel et al. 2008; Zhang et al. 2016). Further evidence of uniqueness is the fact that the *α-Proteobacteria* in *M. verticillatum* include much more abundant lineages such as *Porphyrobacter* (*Erythrobacteraceae*), *Rhodobacteraceae*, *Rhizobiales*, *Roseomonas*, and *Pseudomonas* than in the bio-cord. These lineages are widely reported as epiphytic microorganisms which have close relationship with plants (Burke et al. 2011b; Tujula et al. 2010; Zhang et al. 2016). *Firmicutes* are exclusively abundant in the *M. verticillatum* and include the genus *Carnobacterium* that comprises pathogenic organisms or probiotic cultures (Leisner et al. 2007; Voget et al. 2011). This is further substantiated by the close interactions between the submerged macrophytes and the biofilm bacteria. *Planctomycetes*, the biomarker of the bio-cord, include the uniquely abundant genus *Rhodopirellula*, and this is probably related to the root-like structure (holdfast) of *Planctomycetes* that anchors the substrate. A different genus composition of periphyton between artificial carriers and submerged macrophytes were also found in previous studies under both field and experiment conditions (Hao et al. 2017; Hempel et al. 2009; Townsend and Gell 2005). This emphasizes that different kinds of plants and carriers with distinctive physical and chemical complexities can influence the periphyton community and the nutrient uptake capacity. Despite the fact that the genera recorded differed between the bio-cord and the submerged macrophytes, a few were reported to have a similar capacity to remove nitrogen and organic matter, for instance *Roseomonas*, *Pseudomonas*, and *Rhodopirellula* (Feng et al. 2016; Zhao et al. 2017). Moreover, previous studies have confirmed that the nutrient removal efficiency may be enhanced by using two or more types of substrate instead of only one (Zou et al. 2013), likely due to a higher diversity in the microenvironment when using different types of substrates. Artificial carriers may therefore be used as a supplement to submerged macrophytes to augment nutrient removal in wastewater treatment systems.

Conclusions

Our eco-ditch study demonstrates that the bio-cord acts relatively similar to submerged macrophyte by harboring bacterial assemblages, consisting of *α-* and *γ-proteobacteria*, *Verrucomicrobia*, and *Bacteroidetes*. This was evidenced by detailed comparisons between the bacterial communities sampled in four habitats in the eco-ditch. Our results have important implications for ecosystem remediation in that it suggests that the bio-cord might be an alternative suitable substitute for or a supplement to submerged macrophytes in wastewater treatment systems (eco-ditches) or natural aquatic ecosystems.

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