Science of the Total Environment 803 (2022) 149868

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Functional microbial community structures and chemical properties indicated mechanisms and potential risks of urban river eco-remediation

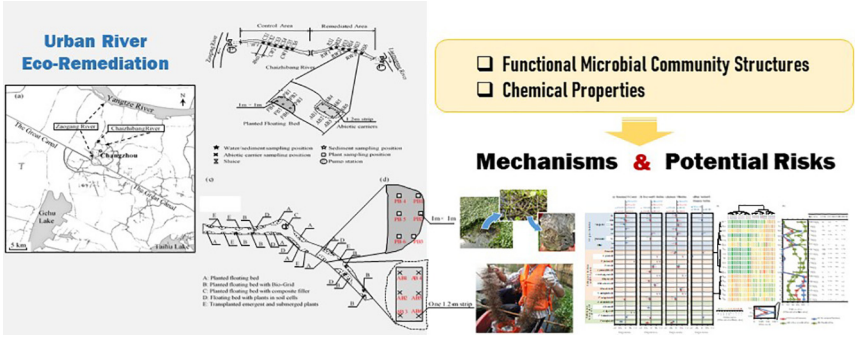
Jiao Sun [a], Ziyu Lin [a], Daliang Ning [a,b,*], Hui Wang [a,**], Zuotao Zhang [a], Zhili He [b], Jizhong Zhou [a,b,c]

[a] State Key Joint Laboratory on Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing, China
[b] Institute for Environmental Genomics, Department of Microbiology and Plant Biology, School of Civil Engineering and Environmental Science, University of Oklahoma, Norman, OK, USA
[c] Earth and Environmental Sciences, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

HIGHLIGHTS

- The eco-remediation significantly changed the microbial community structures.
- Labile-organics-degrading and ammonia-oxidizing gene families were increased.
- The eco-remediation facilities showed absorption of N, P and heavy metal.
- Transparency and sedimentation of some heavy metals were increased.
- Most detected pathogens were not significantly affected by eco-remediation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 July 2021
Received in revised form 17 August 2021
Accepted 19 August 2021
Available online 27 August 2021

Editor: Warish Ahmed

Keywords:
Eco-remediation
Urban river
Functional gene
Community structure
Heavy metal
Antibiotic resistance
Pathogen

ABSTRACT

To investigate the mechanisms and potential risks of river eco-remediation, river water, sediment, and biofilms in remediation facilities were sampled from a 2-year full scale eco-remediation site in an urban river in southeastern China. The samples from both remediated and adjacent control areas were analyzed for chemical properties and functional microbial community structures. The eco-remediation significantly changed the community structures in the river and introduced much more diverse functional microorganisms in facility biofilms. Corresponding to effective reduction of organics and ammonium in river water, some labile-organics-degrading and ammonia-oxidizing gene families showed higher abundances in river water of remediated area than control area, and were obviously more abundant in facility biofilms than in river water and sediment. The eco-remediation facilities showed obvious absorption of N, P, and heavy metals (Mn, Cr^{VI}, Fe, Al, As, Co), contributing to nutrients and metals removal from river water. The eco-remediation also increased transparency and sedimentation of some heavy metals (Cu, Pb, Zn), which probably associated with colloids breakdown. Various metal-resistance microorganisms showed different abundances between facility biofilms and sediment, in accordance with relative metals. Most detected pathogens were not significantly affected by eco-remediation. However, our measurements in sediment and facilities showed heavy metals accumulation and development of some pathogens and several antibiotic-resistance pathogens, alerting us to investigate and control these potential risks to ecosystem and human health.

* Correspodence to: D. Ning, State Key Joint Laboratory on Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing, China.
** Corresponding author.
E-mail addresses: ningdaliang@ou.edu (D. Ning), wanghui@tsinghua.edu.cn (H. Wang).

https://doi.org/10.1016/j.scitotenv.2021.149868
0048-9697/

1. Introduction

Water pollution is an acute problem along with urbanization in the past and nowadays developing countries. Various pollutants rapidly disperse through urban rivers to risk the health of human and natural ecosystems, which make urban rivers a key node for water pollution investigation and control. The huge population, continuous rapid economic development, speedy urbanization and complicated environmental problems in the past decades in China are rarely seen in human history. To date, up to 80% of urban rivers were contaminated to varying degrees and accumulated various problems, including dissolved oxygen depletion, excessive nutrients, heavy metals, recalcitrant organic pollutants, pathogens, and antibiotic-resistance microorganisms (Hao et al., 2015; Qu and Fan, 2010).

To solve the problems, besides pollution source control and channel reconstruction, various eco-remediation technologies have been increasingly researched and widely applied in urban rivers. These technologies primarily included planted floating bed systems, phytoremediation, constructed wetlands, bio-manipulation, and several combined techniques (Qu and Fan, 2010). For instance, the planted floating bed system is an aquaponic technology which consists of aquatic or terrestrial plants growing in a hydroponic manner with buoyant frames floating on the surface of waters (Bout well, 1995; Hu et al., 2010). It was regarded as a low-cost, solar-energy-based and eco-friendly technology for in situ purification of surface water and has been applied in Europe (Garbett, 2005; Hoeger, 1988), the United States (Burton, 2012; Chang et al., 2012; Stewart et al., 2008), Canada, Japan (Nakai et al., 2010; Nakamura and Mueller, 2008), Korea (Seo et al., 2013), India (Kamble and Patil, 2012), and China (Li et al., 2010; Qu and Fan, 2010; Zhou et al., 2012).

Although designed to remove pollutants and restore health ecosystem, river eco-remediation is still artificial intervention to the ecosystem. Therefore, it is crucial to monitor and evaluate its effect on both physic-chemical properties and the diverse communities when applied to a river. Many studies demonstrated the positive effects of river eco-remediation on water quality, such as effective removal of organics (Berglund et al., 2014), nutrients (Billore et al., 2009; Huang et al., 2010; Masters, 2012) and heavy metals (Agunbiade et al., 2009; Rai, 2009; Zhao et al., 2012) from river water. Our previous study further revealed the impact of eco-remediation on sediment properties, including the increase of nutrients and some heavy metals in sediment (Ning et al., 2014). Several studies including our previous work found significant change of phytoplankton diversity and structures in river after eco-remediation (Ning et al., 2011). In contrast, we know little about the effect of eco-remediation on river microbial communities.

Microbial communities mediate many biogeochemical transformations underpinning ecosystem functioning (Winter et al., 2007) and include various hazardous microorganisms which are of great concern. Microbial studies in urban rivers mostly engaged on pathogenic microorganisms (Duris et al., 2013; Jang et al., 2011; Peeler et al., 2006; Reeves et al., 2004; Surbeck et al., 2010) and also revealed the significant impact of urban effluents on water quality and microbial communities (Cebron et al., 2004; Perryman et al., 2011a; Tiquia, 2011; Wang et al., 2011). Some researches in urban rivers improved our knowledge on river functioning and potential risks by investigating functional genes or species involved in N-cycling (Cebron et al., 2004; Perryman et al., 2011b; Song et al., 2012; Wang et al., 2011), metal resistance (Lear et al., 2012) and antibiotic resistance (Cummings et al., 2011; Lu et al., 2010; Rodriguez-Mozaz et al., 2015; Storteboom et al., 2010; Q. Zhang et al., 2014). Furthermore, various microorganisms and/or their functional genes were proposed to be bio-indicators of complicated pollutants and potential risks in urban rivers (Ancion et al., 2010; Fechner et al., 2012; Lear et al., 2012; Sole et al., 2008; Tiquia, 2011) which can never be entirely covered by chemical analyses. In addition, microbial communities in different biofilms should be essential for many eco-remediation techniques, which have been demonstrated by widely studied analogous techniques (e.g. constructed wetland, microbial biofilm reactor, and water distribution systems, etc.) applying to wastewater treatment (Adrados et al., 2014; Correa-Galeote et al., 2013; Desta et al., 2014; Jasper et al., 2014a; Jasper et al., 2014b; Wang et al., 2014; Zhao et al., 2015). Therefore, the study on microbial communities in urban rivers under eco-remediation is compelling and informative to understand the mechanisms and evaluate the impact of eco-remediation.

We carried out a field study in an urban river eco-remediation site in southeastern China. The river water comes from the Yangtze River, received urban pollutants and contributed to the pollution of the Tai Lake and the Grand Canal. Our previous results indicated effective improvement of water quality in remediated area of this river after 2-year remediation (Ning et al., 2014). In this study, our objective is to further reveal the microbial mechanisms and potential risks of the river eco-remediation by dissecting functional microbial community structures and chemical properties in the river and the two major biofilms in eco-remediation facilities. We aim (1) to explore the effect of the functional microbial community structures in the river water and sediment by eco-remediation; (2) to indicate whether that the microbial communities in eco-remediation facilities were significantly different from those in river water and sediment, and positively contributed to water quality improvement by enhancing absorption and biotransformation of various pollutants; (3) to detect whether the biofilms on plants were significantly different from biofilms on abiotic carriers (abio-carriers), in both functional microbial communities and chemical properties. The results in this study gave these information, but also alert us the potential risks regarding to accumulation of some heavy metals and development of several antibiotic-resistance pathogens in sediment and remediation facilities.

2. Materials and methods

2.1. Site and eco-remediation

The study was carried out in a river called Chaizhibang (31.83°N, 119.98°E) in the city of Changzhou in southeastern China as we described previously (Ning et al., 2014). Changzhou city is located in Jiangsu province, south of the Yangtze River and northwest of Lake Taihu. The Chaizhibang River originates from a main river named Zaogang River which connected to Yangtze River, and ends by sluices (Fig. S1a). The width and depth of Chaizhibang are 15– 25 m and 1.5– 2 m. Most time, the water is nearly still and only slightly flows with tide. Water in Chaizhibang is periodically pumped downstream and replaced by water pumped from Zaogang River, usually once in 4 days. Before the eco-remediation, the river water, with good quality when pumped in, easily deteriorated after few days and appeared with floating oil, low transparency, black and malodorous, even in the year after sediment dredging was completed.

Eco-remediation was carried out in an area of 10,000 m^2 water surface with a length of 450 m, and completed in March 2009. The primary planted floating beds with *Hydrocotyle verticillata* Thunb and *Myriophyllum spicatum* Linn carried by nylon net were 5– 15 m in length, 3– 5 m in width, and discretely arranged beside the river bank, occupying 786 m^2 in total when they were engineered. A kind of abiotic carrier named Bio-grid, which was made of special polymer fiber, was hanged beneath a half of primary floating beds and had an entire volume of 300 m^3 (Fig. S1c). Other constructions were the same as described previously (Ning et al., 2014). The plants, mainly *Hydrocotyle verticillata* Thunb and *Myriophyllum spicatum* Linn, were harvested once a season to remove the parts exceeding original sizes. A small part of plants withered in winter were replaced in spring.

2.2. Sampling and chemical analysis

To reveal the effect of remediation on water and sediment properties, an upstream control area was set with a length of 450 m and

adjacent to the remediation area (Fig. 1). Sampling was performed on Oct. 26, 2010, 4 days after last water transfer. From the middle of each area, water at 50 cm depth was sampled at 3 positions with 80 m intervals along the river, and 5 cm surface sediment was taken from 6 positions with 40 m intervals (Fig. 1). The submerge part of plants were sampled from 6 positions of a large floating bed with an area of 1 m × 1 m at each position after the part above water surface was cropped (Fig. 1). A 1.2-m strip of abiotic carrier was sampled from each of 6 positions in a large Bio-grid bed. Samples were sealed in sterile sampling bags or tubes and transported to the lab on ice within 10 h. A set of river water and sediment subsamples were transport to lab within 2 h and analyzed for the most probable number (MPN) and fluorescein diacetate hydrolysis assays (FDA). For DNA extraction, the biofilms on the subsamples of plants and abiotic carriers were washed down by sterile buffer containing 100 mM phosphate and 100 mM TE (pH = 8.0), and then centrifuged at 15,000*g* for 15 min to get pellets. River water were filtered by 0.2-μm-pore-size membranes. Each sediment sample was mixed well before a subsample for DNA extraction was preserved. Then the samples for DNA extraction were stored at −80 °C before use. For chemical analyses, the biofilms of other subsamples were scraped off, air dried in shade, and then analyzed for total organic carbon (TOC), ammonium nitrogen (NH_4^+), nitrate nitrogen (NO_3^-), total nitrogen (TN), total phosphorus (TP), sulfate (SO_4^{2-}), Al, As, Cd, Cr, Cr^{VI}, Cu, Fe, Mn, Ni, Pb, and Zn as described previously for sediment chemical analyses (Ning et al., 2014).

2.3. DNA extraction

Microbial genomic DNA was extracted by a freeze-grinding method as described previously (Zhou et al., 1996) and purified by agarose gel electrophoresis. DNA was analyzed by NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). Purified DNA had A_{260}/A_{280} of 1.75– 1.90 and A_{260}/A_{230} of >1.7. The DNA samples were stored at −80 °C until usage.

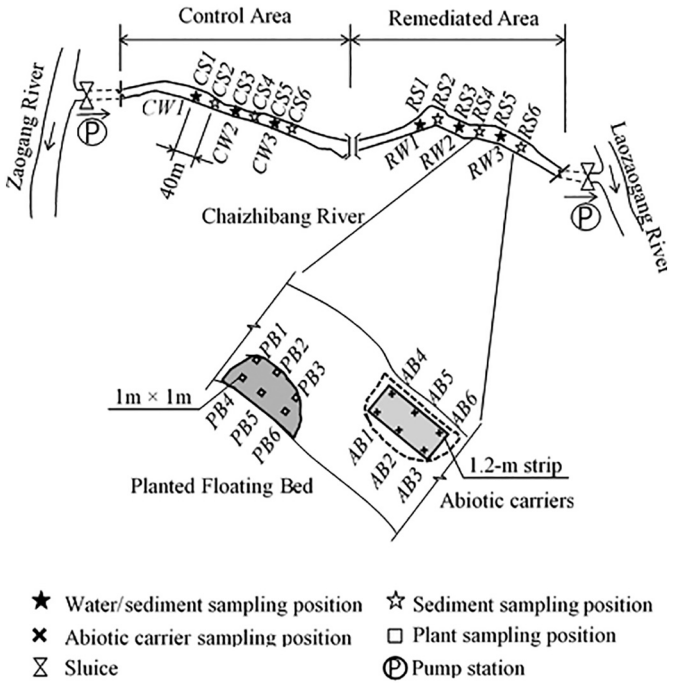


Fig. 1. Sampling positions of river water, sediment, plants, and abiotic carriers in the remediated and control areas in Chaizhibang River. Italic labels indicate sample numbering. See Fig. S1 for the location of the river, detailed maps and sample photos.

2.4. GeoChip hybridization and data pre-processing

The microbial communities were analyzed by a new version of GeoChip 4 (Tu et al., 2014) with 107,950 probes covering about 155,000 protein-coding sequences. For each sample, 1 μg of DNA was labeled with the fluorescent dye Cy3 and hybridized on GeoChip 4 as described previously (Tu et al., 2014). The arrays were scanned with a NimbleGen MS 200 Scanner (Roche NimbleGen, Madison, WI, USA). The raw data were preprocessed using a data analysis pipeline (http://ieg.ou.edu/microarray/). Across all samples, spot signals were normalized by the average signal intensity of control spots and then by the sum of all sample spot signals. Then, the spots with (i) a signal-to-noise ratio (SNR) less than 2.0, (ii) or a coefficient of variation (CV) larger than 0.8, (iii) or a normalized signal less than 1000 were removed. The microarray data presented are available at http://ieg.ou.edu/4download/.

2.5. Statistical analysis

Pre-processed microarray data and chemical properties were further analyzed: (i) hierarchical clustering for chemical properties and community structures; (ii) microbial diversity indices, the two-tailed *t*-tests and response ratio (RR) (Liang et al., 2009); (iii) detrended correspondence analysis (DCA) for community structures (He et al., 2010); (iv) dissimilarity test of microbial communities by ANOSIM, Adonis, and MRPP analysis (He et al., 2010; Lu et al., 2012); (v) canonical correspondence analysis (CCA) (Ter Braak, 1986; Zhou et al., 2008) for linking microbial communities to physic-chemical properties; (vi) Mantel test (Mantel, 1967) and Pearson correlation test for correlation analysis between some chemical properties and functional gene families or genes. All statistical analyses were performed using R v.3.1.2 (R Core Team, 2014) with the vegan v.2.2–0 package (Oksanen et al., 2014).

3. Results and discussion

3.1. Effect of eco-remediation on overall diversity and structures of functional microbial communities

The effect of eco-remediation on river microbial communities were investigated in few studies on 16S amplicons and appeared not significant (C.B. Zhang et al., 2014). In our study, Geochip 4.2 was applied to analyze the diversity and structures of functional microbial communities in river water, sediment and facility biofilms. The alpha diversity index in river water had no significant difference between remediated and control areas, while that in sediment was a bit higher in remediated area than control area with marginal significance (Fig. S3a). The eco-remediation facility biofilms showed significantly higher diversity. The plant biofilms had the highest number of detected genes and alpha diversity, showed much more unique genes than other samples and covered more than 90% genes detected in all the other media (Table S1). Interestingly, detected gene number (Fig. S4) and alpha diversity index in river water were significantly higher than the sediment in both remediated and control area. The rank of microbial alpha diversity index is plant biofilms > abio-carrier biofilms ≈ river water > sediment in remediated area > sediment in control area.

DCA, dissimilarity test and hierarchical clustering were performed based on all detected function genes to reveal the turnover of community structures. The samples from different media and different areas were completely separated on the biplot of DCA (Fig. 2a), generally in different branches in the tree of cluster (Fig. S5), and showed significant differences ($P < 0.02$) in dissimilarity test except marginally significant ($P < 0.12$) difference of Bray-Curtis dissimilarity between water samples from remediated and control areas (Table S2). Comparing to control area, the river water and sediment in remediated area had community structures obviously closer to facility biofilms. These results

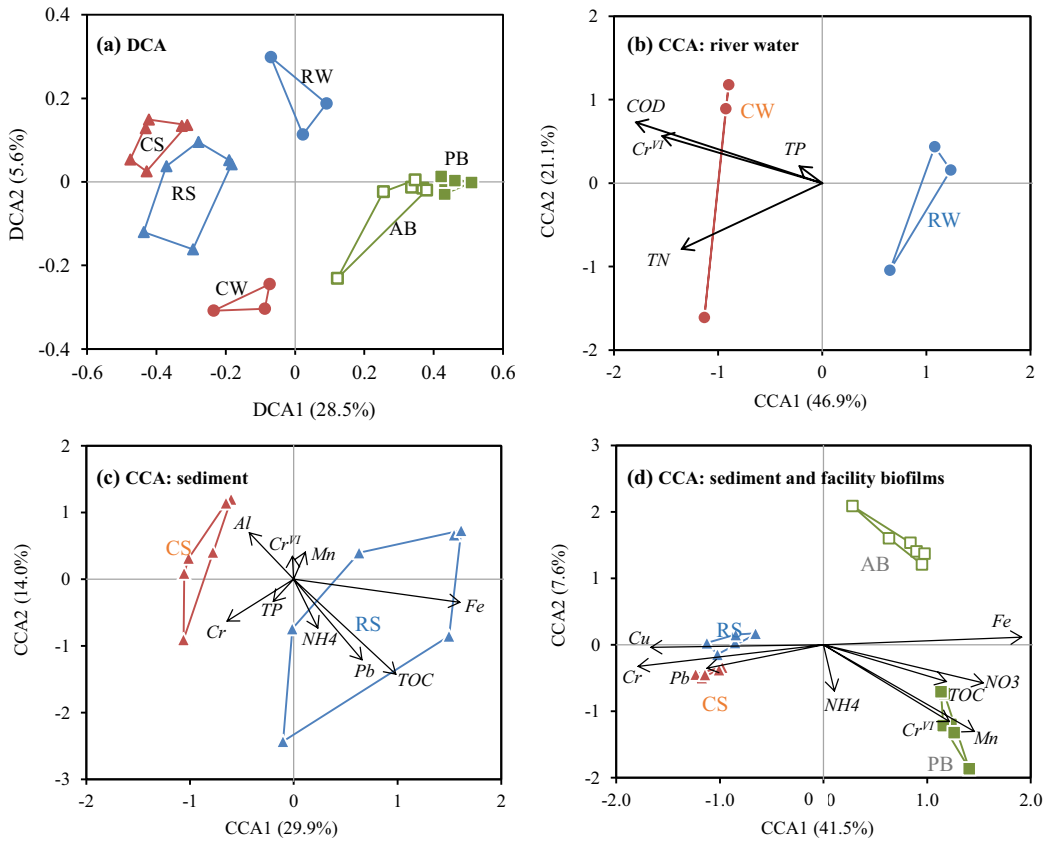


Fig. 2. (a) Detrended correspondence analysis (DCA) of functional microbial community structures and canonical correspondence analysis (CCA) of functional microbial community structures and chemical properties in (b) river water, (c) sediment, and (d) adherent-growth communities (sediment and facility biofilms). CS (red triangle), sediment samples from control area; RS (blue triangle), sediment samples from remediated area; CW (red circle), river water samples from control area; RW (blue circle), river water samples from remediated area; AB (open green square), biofilm samples from abiotic carriers; PB (closed green square), biofilm samples from plants. NO3, nitrate; NH4, ammonium; TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

revealed obvious effect of eco-remediation on microbial diversity and community structures in this river.

The physic-chemical properties in river water and sediment had been analyzed in our previous study (Ning et al., 2014). In this study, CCA was performed to analyze the relationship between these properties and microbial community structures in river water and sediment, respectively. A combination of physic-chemical properties was qualified if the CCA model was significant ($P < 0.05$) and variance inflation factors (VIFs) were less than 10. According to qualified CCA results, the combination of COD, TN, TP and Cr^{VI} explained 91.9% of observed variation of microbial community in river water ($P = 0.013$, VIFs < 7.8, Fig. 2b), while the combination of TOC, NH_4^+, TP, Fe, Al, Mn, Cr^{VI}, Cr and Pb explained 89.8% of observed microbial variation in sediment ($P = 0.017$, VIFs < 9.8, Fig. 2c). According to CCA and Mantel test with each property, the community structures in river water were correlated with COD (CCA proportion explained $E = 41.3\%$, $P = 0.064$), Mn ($E = 32.8\%$, $P = 0.053$), TN ($E = 31.5\%$, $P = 0.057$; Mantel $r = 0.662$, $P = 0.035$) and Al ($E = 30.6\%$, $P = 0.082$), while the community structures in sediment were correlated with Fe ($E = 21.6\%$, $P = 0.010$), SO_4^{2-} ($E = 17.8\%$, $P = 0.026$), NO_3^- ($E = 17.1\%$, $P = 0.024$), Cu ($E = 16.7\%$, $P = 0.033$) and TOC ($E = 16.5\%$, $P = 0.032$; Mantel $r = 0.345$, $P = 0.032$). These results suggested the relationship between microbial community turnover and the variations of organics, nutrients and heavy metals in the river.

To better understand the contributions of facility biofilms in eco-remediation, the biofilm samples were also analyzed for various chemical properties. Comparing to the sediment, the facility biofilms showed significantly higher concentrations of TN, TP, Fe, Al, NO_3^-, and SO_4^{2-}, and lower concentrations of Cd, Ni, Cr, Cu, Zn, and Pb. Particularly, the plant biofilms held obviously higher concentrations of TOC, Mn, and Cr^{VI} (Fig. 3). According to CCA, the combination of TOC, NO_3^-, NH_4^+, Mn, Cr^{VI}, Fe, Cr, Cu, and Zn explained 67.6% of observed variation of microbial communities in adherent-growth communities (sediment and facility biofilms. VIFs < 8.9, $P = 0.005$, Fig. 2d). To reveal underlying mechanisms and potential risks, the physic-chemical properties and functional microbial communities in different areas and media were further analyzed in detail by categories as follows.

3.2. Role of microbial communities in organics degradation by eco-remediation

Organic metabolism genes targeted by Geochip can be divided into two categories, so called carbon cycling and organic remediation. The category of carbon cycling includes genes of carbon fixation, acetogenesis, methane metabolism and degradation of major carbon sources, most of which are relatively labile organics (e.g. glucose, lactose, sucrose, starch, protein, lipids, etc.) except lignin, cellulose and terpenes. The category of organic remediation contains genes involved in biotransformation of hazardous organic pollutants, such as BTEXs, halo-aromatics, pesticides, etc. In our study, the 65 gene families of carbon cycling were all detected in all kinds of samples, while 97.8%–99.4% gene families of organic remediation were detected in different samples.

The labile organic pollutants are the major oxygen consuming substances in wastewater and surface water receiving urban effluent (Sawyer et al., 2003). Many studies demonstrated effective removal of labile organic pollutants from river water by eco-remediation (Billore et al., 2009; Bu and Xu, 2013; Li et al., 2010). Our previous study in

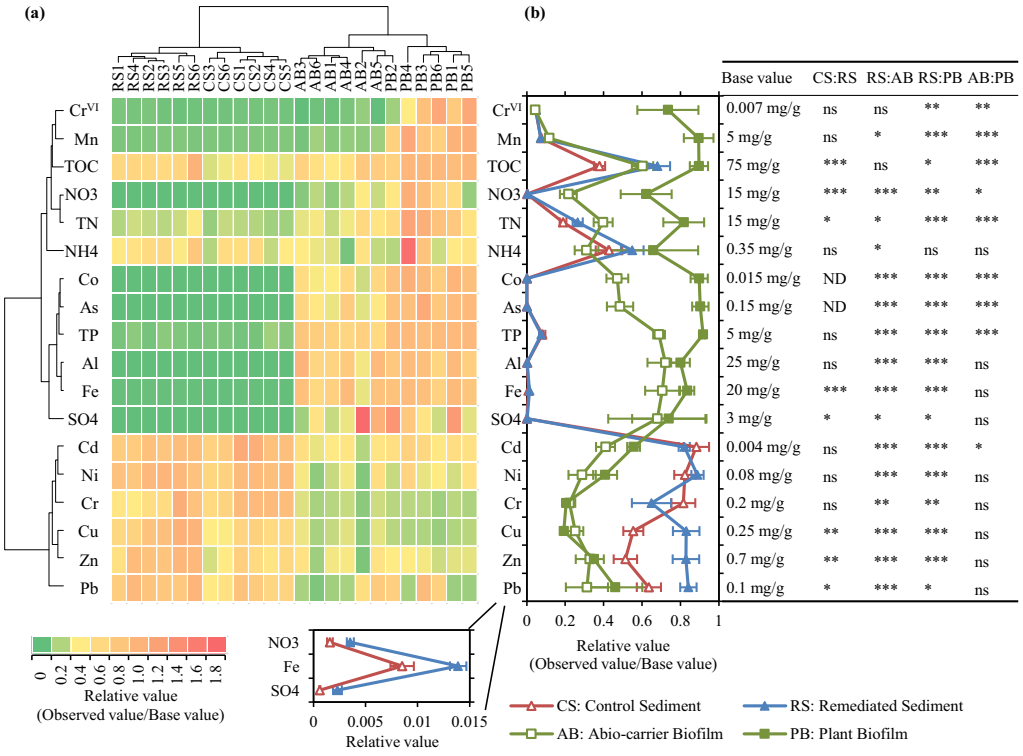


Fig. 3. Chemical properties of sediments in remediated and control areas and biofilms in eco-remediation facilities. The hierarchical clustering (left) is based on standardized data (set mean of zero and variance of 1 for each property). CS1 ~ CS6, sediment samples from control area; RS1 ~ RS6, sediment samples from remediated area; AB1 ~ AB6, biofilm samples from abiotic carriers; PB1 ~ PB6, biofilm samples from plants. The line chart (right) shows the mean (point) and standard deviation (error bar) of each property in sediment of remediated (blue) and control (red) areas, biofilms on abiotic carriers (open green) and plants (closed green). The relative values of each property were observed values divided by a designated base value in order to make different properties easier to read in one chart. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$; ns, $P > 0.05$, according to two-tail *t*-tests. As and Co were not detectable (ND) in sediment. Detection limit: As 0.007 mg/kg, Co 0.005 mg/kg. NO3, nitrate; NH4, ammonium; TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; SO4, sulfate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

this river revealed the significant decrease of COD in water of remediated area comparing to reference site and control area, as well as significantly higher sediment TOC in remediated area than in control area (Ning et al., 2014). The present study showed even higher TOC in facility biofilms than in sediment (Fig. 3). Accordingly, the increased microbial biomass and improved settlement of solid organics were regarded as the major causes of organics removal by eco-remediation as proposed previously, but the functional microorganisms involved were rarely specified in previous publications.

In this study, 25 gene families of carbon degradation showed significant (at 95% confidence interval of response ratio, i.e. 95% CI) differences between remediated and control areas in river water, and most (18 families) had higher relative abundances in remediated area (Fig. 4a and Fig. S6a). The gene families degrading cellulose, hemicellulose and lignin mostly showed much less differences between the two areas than those degrading starch, protein and lipid which are typical organics in urban wastewater. In the two types of facility biofilms, many gene families degrading labile organics showed significantly (95% CI) higher relative abundances than in river water (10 and 19 families, Fig. 4b) and sediment (20 and 24 families, Fig. 4c), while obviously fewer were less abundant than in river water (7 families) and sediment (7 and 10 families). These results revealed the internal reason and some major contributors of effective organics removal by eco-remediation facilities in this study from river water.

On the other hand, due to higher density of labile organics degrading microorganisms, the DO consumption within the eco-remediation facilities could be faster than in river water. Obviously lower DO concentrations were observed in the center (DO = 0.2– 1.2 mg/L) than in the edge (DO = 1.0– 3.5 mg/L) of several large floating beds in this river. Thus, it is worth considering to enhance oxygenation, e.g. by aerator or hydraulic construction.

In sediment, only 10 gene families of carbon cycling showed significant (95% CI) differences between the two areas, without gene families involved in degradation of typical organics in urban wastewater (e.g. glucose, starch, protein, lipid etc.) other than *spr*. This should be a reason for (rather than a result of) significantly higher TOC in sediment of remediated area than control area.

Removal of recalcitrant organic pollutants were rarely studied in river eco-remediation, although many constructed wetlands showed effective removal of aromatics (Wang et al., 2014; Zhou et al., 2013), pesticides (Budd et al., 2009; Vymazal and Bfezinova, 2015), pharmaceuticals and personal care products (Jasper et al., 2014a; Matamoros et al., 2007) from wastewater and/or agricultural runoff. In the river remediation facilities, we detected functional gene families degrading various recalcitrant organics, such as aromatics, chlorinated solvents, herbicides and pesticides. These functional microorganisms are good for recalcitrant organic pollutants removal. However, most of them showed few differences between remediated and control areas. In river water and sediment, only a few families (7% in water and 6% in sediment) showed higher relative abundance in remediated area, while more families (19% in water and 12% in sediment) had significantly higher relative abundance in control area (Fig. S6b). Meanwhile, only a few (8%–12%) families had significantly higher, but much more (27%–45%) had lower relative abundances in facility biofilms than in river water and sediment (Fig. S6b). The results suggest the current eco-remediation had much less effect on recalcitrant organic pollutants than labile organics. The remediation of rivers with recalcitrant organic pollution need enhanced or new techniques other than those applied in this study.

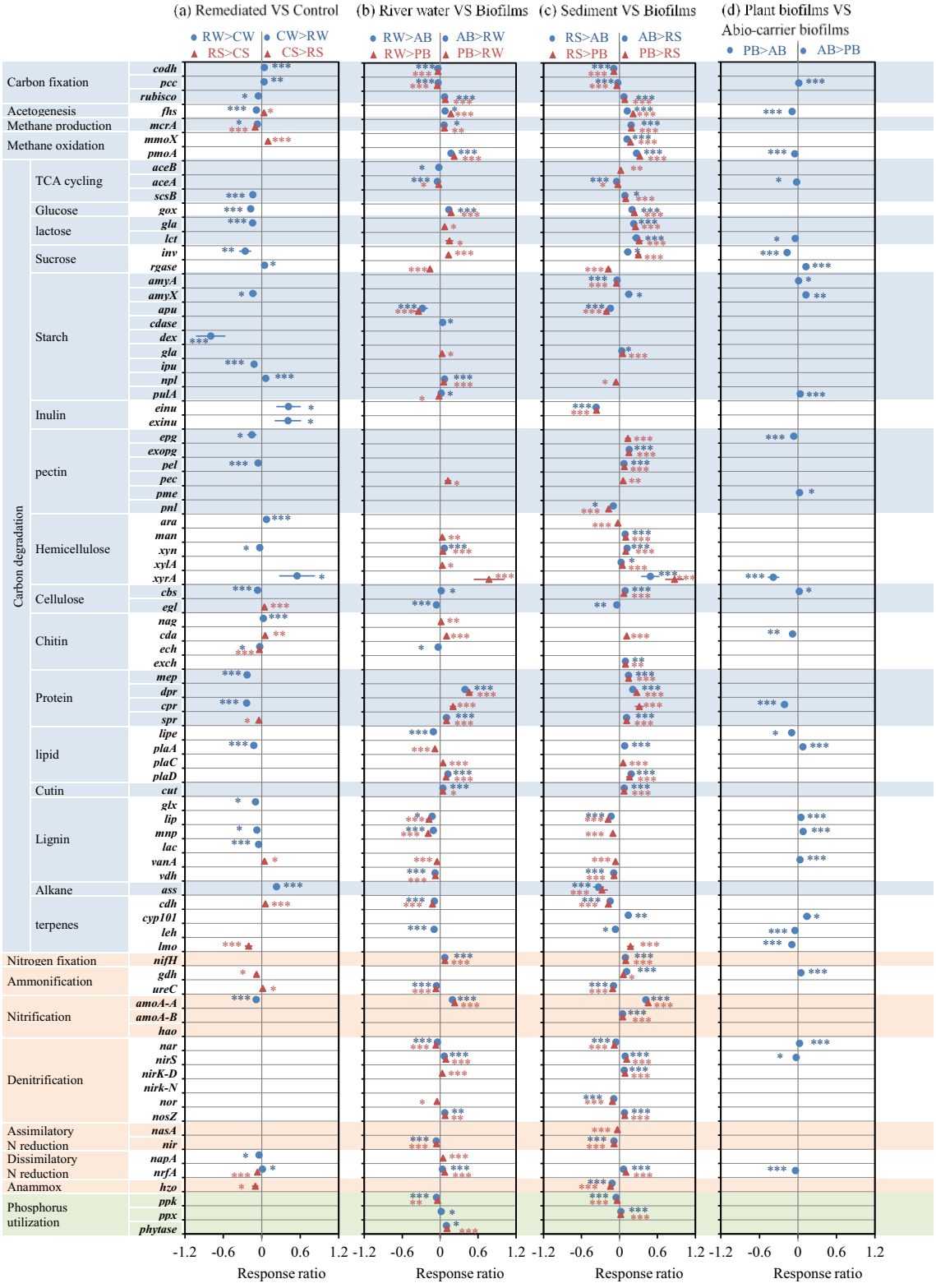


Fig. 4. Abundance response ratios of gene families (a) in river water (blue) and sediment (red) of control area to those of remediated area, (b) in biofilms on abiotic carriers (blue) and plants (red) to river water in remediated area, (c) in biofilms on abiotic carriers (blue) and plants (red) to sediment in remediated area, and (d) in biofilms on abiotic carriers to those on plants. CS, sediment samples from control area; RS, sediment samples from remediated area; CW, river water samples from control area; RW, river water samples from remediated area; AB, biofilm samples from abiotic carriers; PB, biofilm samples from plants. *, significant at 95% confidence interval (CI); **, significant at 99% CI; ***, significant at 99.5% CI. Error bars represent standard errors. Only significant (CI > 95%) data are presented. *amoA-A* and *amoA-B* are archaeal and bacterial *amoA*, respectively. See Table S3 for the description of genes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Role of microbial communities in nutrients removal or transformation by eco-remediation

Eutrophication remains the most critical problem of lakes in China today (Qu and Fan, 2010), and most of the urban lakes in China are facing serious eutrophication (Jin et al., 2005). The excessive nutrients (N and P) from urban effluents transported via urban rivers are one of the major causes. Many studies including our previous work have showed N and P removal by plants in eco-remediation facilities (Masters, 2012; Ning et al., 2014; C.B. Zhang et al., 2014). In our current study, TN and TP in facility biofilms were significantly higher (2.7 and 10.3 folds, respectively) than in sediment, reflecting the effective absorption of N and P. Nevertheless, TN and TP in river water of remediated and control areas did not show significant differences. Pearson correlation test showed TN in river water significantly correlated with denitrifier *nirK* gene ($r = -0.924$, $P < 0.01$) rather than other N cycling gene families, while TP in river water negatively correlated with *ppk* gene ($r = -0.93$, $P < 0.01$), suggesting the contribution of denitrifiers and P-accumulation organisms to TN and TP variations in river water. However, denitrification and P utilization gene families did not show any significant differences between remediated and control areas, in either river water or sediment (Fig. 4). The facility biofilms had even slightly less abundances of *nar* and *ppk* genes than river water and sediment. In contrast, TP in sediment and biofilms were clustered very closely to Fe, Al, As, and Co rather than other properties (Fig. 3), suggesting the importance of chemical precipitation rather than bioaccumulation in TP absorption by biofilms. These results suggest a worthwhile but challenging innovation direction, to increase nutrients removal by enhancing denitrifiers and P-accumulation organisms in eco-remediation facilities. For reference, denitrification can be improved by taking advantage of some constructed wetlands (Jasper et al., 2014b), vegetation design and management (Chen et al., 2014b; Tanaka et al., 2015).

On the contrary to TN, NH_4^+ was significantly lower in remediated area than control area. Correspondingly, archaeal *amoA* genes showed significantly (99.9% CI, *t*-test $P = 0.03$) higher abundances in remediated area than control area, and the facility biofilms had obviously higher abundances of both archaeal and bacterial *amoA* genes than river water (Fig. 4b) and sediment (Fig. 4c). In addition, the concentrations of NO_3^- and SO_4^{2-} in facility biofilms was 164 and 486 folds (on average) higher than in sediment (Fig. 3b), also suggesting dramatically higher oxidation activity in facility biofilms. These indicated the eco-remediation facilities definitely increased the nitrification capability of the river. The obvious increase of labile organics degrading and ammonia oxidizing microorganisms in river water could effectively reduce oxygen consuming pollutants and increase the carrying capacity of river water, which is also benefit for downstream water quality improvement. However, these functional microorganisms in sediment did not show obvious difference between remediated and control areas, and were significantly less abundant in sediment than in facility biofilms, suggesting the necessity to keep the running of remediation facilities for water quality improvement and maintenance.

The different ammonia oxidizers showed interesting variation patterns in our results. In aerobic ammonia oxidizers, ammonia oxidizing archaea (AOA) was reported to be numerically and/or metabolically dominant in some rivers (Liu et al., 2011; Sonthiphand et al., 2013; Sun et al., 2014a), preferring to aerobic and relatively low ammonia concentrations (Liu et al., 2011) or the area outside WWTP effluent plume (Sonthiphand et al., 2013). Ammonia oxidizing bacteria (AOB) was found dominant in some rivers (Sun et al., 2014a) including the areas within WWTP effluent plume (Sonthiphand et al., 2013). In our study, AOA showed much more obvious differences along with NH_4^+ variation than AOB did (Fig. 4a, b and c), and was significantly more abundant in remediated river water where NH_4^+ concentrations were much lower than control area. AOA were often demonstrated to be dominant ammonia oxidizers in acidic soils, while AOB were often more abundant under neutral pH conditions. This is probably because AOA has high ammonia affinity when the ammonia substrate availability was below the threshold of AOB under acidic condition (He et al., 2012). Accordingly, we propose a hypothesis that in both soil and water, no matter pH is high or low, AOA can get advantage or even become dominant ammonia oxidizers when the substrate (ammonia) is substantially low. These results also suggest the potential of AOA abundance or the ratio of AOA to AOB as bio-indicator in river water quality assessment.

As the least known ammonia oxidizer which could be underestimated, anaerobic ammonium oxidizing (anammox) bacteria are of increased concerns in recent years. Anammox bacteria were often detected (Sun et al., 2014c; Wang et al., 2012) or even dominant ammonia oxidizer (Sonthiphand et al., 2013) in the sediment of freshwater systems, but were much less abundant in water columns (Sonthiphand et al., 2013; Sun et al., 2014b). Our results demonstrated anammox bacteria were detectable in river water, plant biofilms, floating abio-carrier biofilms and sediment, and also showed higher relative abundances in sediment, especially in remediated area. The abundances of anammox bacteria in river sediment were reported to be correlated with nitrite (Sun et al., 2014c) or total inorganic nitrogen (Hu et al., 2012). In our results, *hzo* gene in sediment correlated with NO_3^- (Pearson correlation test using total abundance of *hzo* gene, $r = 0.533$, $P = 0.074$) and the ratio of NO_3^- to TN ($r = 0.570$, $P = 0.053$) rather than NH_4^+ ($P = 0.75$) or TN ($P = 0.47$). Anammox can also be stimulated by Fe in some studies (Chen et al., 2014a; Liu and Horn, 2012). In our study, *hzo* gene significantly correlated with Fe in sediment (Pearson correlation test using total abundance of *hzo* gene, $r = 0.673$, $P = 0.016$; Mantel test between Fe and the composition of *hzo* gene, $r = 0.240$, $P = 0.049$). We suppose that the electron accepters (such as NO_3^-, Fe^{III}) rather than donors could be the limiting factor of anammox bacteria in river sediment. On the contrary to *nirK*, *hzo* genes family did not showed any significant correlation with TN in any media ($P > 0.3$), suggesting its minor role in denitrification, in accordance with previous studies on constructed wetlands (Jasper et al., 2014b) and river riparian sediments (Wang et al., 2012).

3.4. Fates of heavy metals and metal-resistance microorganisms

Heavy metal pollution has been recognized as one of key water pollution problems in Chinese rivers since the 1970s (Qu and Fan, 2010). Our previous work in this river has revealed effective removal of some heavy metals from river water by eco-remediation and their accumulation in sediment (Ning et al., 2014). Our current study further revealed different fates of various heavy metals after eco-remediation by comparing heavy metal concentrations in different media. Firstly, Cu, Pb, Zn, Cd, Ni and Cr showed significantly higher concentrations in sediment than in facility biofilms (47%–330% higher, $P < 0.05$, Fig. 3), in accordance with more abundant Cu-, Pb-, Zn-, Cd-, and Ni-resistance gene families in sediment (Fig. S7c), indicating the tendency of these metals to settle rather than suspend in remediated area. Consequently, Cu, Pb and Zn, the common metals in urban runoff (Ancion et al., 2010), showed significantly higher concentrations in the sediment of remediated area than in control area (Fig. 3). In contrast, some metals appeared more difficult to settle. The concentrations of Fe, Al, As, and Co were significantly higher in facility biofilms than in sediment (Fig. 3), so were the relative abundances of Al-, As-, and Co-resistance gene families (Fig. S7c). The concentrations of these four metals significantly correlated with each other ($r > 0.92$ in sediment and biofilms; $r > 0.49$ in facility biofilms), which was probably attributed to chemical precipitation and adsorption of As and Co by Fe and Al. Furthermore, Mn and Cr^{VI} in plant biofilms had 12- and 16-fold higher concentrations ($P < 0.0001$) than in sediment, respectively, and more than 5 times higher concentrations than in abio-carrier biofilms (Fig. 3), suggesting the important role of plant in absorbing these heavy metals.

The fates of different metals in surface water were often found associated with their size distribution (dissolved, colloidal, and particulate) (Gaillardet et al., 2003; Pokrovsky and Schott, 2002; Sigg et al., 2000),

and heavy metals transported along rivers were often found to be associated with colloids (Taylor et al., 2012). In remediated area of this river, the transparency was significantly improved (Ning et al., 2014), but the suspended solid (>0.45 μm) concentrations were still similar to control area (Fig. S2), indicating many colloids in river water should be removed or destructed. The remediation facilities can break down colloids by adsorbing and entrapping the suspended particles (Li et al., 2010), and degrading dissolved organics which were reported to stabilize some colloids (Stumm, 1987). This definitely facilitated and could be an important cause of heavy metals removal from river water. Accordingly, we suppose that the three different fates of metals in our results could be associated with their size distribution which was changed by eco-remediation. Further size fractionation of different metals will help to elucidate the mechanism.

Metal-resistance gene families were expected to serve as bio-indicators of heavy metal pollution. Lear et al. reported higher abundances of metal-resistance genes correlated with elevation of heavy metals in a restored urban stream (Lear et al., 2012). In our results, some metals were not detectable (e.g. As and Hg) in river water or sediment, but the gene families resistant to them were detected and showed significant differences between remediated and control areas (Fig. S7a), indicating metal-resistance gene probs can have high sensitivity and lower detection limitation of the metals. However, many resistance genes may not have enough specificity to certain metals. In either river water or sediment, most metal-resistance gene families did not show significant correlation (Mantel test $P > 0.14$) with relative heavy metals (Al, Cu, Pb, Ni, and Zn), except *cueO* gene which correlated with Cu variations in sediment (Mantel test $r = 0.37$, $P = 0.016$). However, each gene families still had a few genes significantly (Pearson correlation test $P < 0.05$) correlated with relative metals in either river water or sediment, for instance, the relative abundances of 11 and 54 Cu-resistance genes correlated with Cu in water and sediment, and the relative abundances of 15 and 14 Zn-resistance genes correlated with Zn in water and sediment, respectively.

3.5. Pathogenic and/or antibiotic-resistance microorganisms

The pathogens and antibiotic-resistance microorganisms in urban river must have our attention, as they are so close to dense populations in cities and have been found as health threat in many urban rivers (Rodriguez-Mozaz et al., 2015; Sales-Ortells and Medema, 2014; Q. Zhang et al., 2014). Eco-remediation can provide new habitat for more diverse microorganisms (as we found in this study) which might benefit growth of pathogens or antibiotic-resistance microorganisms, however, little was documented about this issue in previous studies.

In our study, the function genes of 61 pathogenetic species were detected in the river by Geochip, including 38 bacterial species (223 subspecies) and 23 fungal species (34 subspecies). Most pathogenic subspecies showed insignificant differences or lower abundances (RR 95% CI) in remediated area than in control area (Fig. S8). However, the remediated area still had higher abundances (RR 95% CI) of 16% pathogenic bacterial subspecies (30 subspecies in water and 28 in sediment) and 39% pathogenic fungal subspecies (13 subspecies in water and 1 in sediment) than control area. Comparing to river water and sediment, the facility biofilms had higher abundances of 62– 84 pathogenic bacterial subspecies (30%–39%) and 12– 19 pathogenic fungal subspecies (36%–57%). See Table S4 for the list of detected pathogens with significant differences between different areas or media.

The 8 antibiotic-resistance gene families covered by Geochip were all detectable in this river. The river water of remediated area had lower relative abundances of *mfs*, *bla*, and *tet* gene families, but higher relative abundances of *mex* and *mate* gene families than control area. The sediment of remediated area had lower abundances of *mate* and *abc* gene families than control area (Fig. 5a). The facility biofilms, especially abio-carrier biofilms, had more abundant *smr* gene family than river water (Fig. 5b) and sediment (Fig. 5c), but did not cause any significant difference of this gene family between remediated and control areas in either river water or sediment. All the other detected antibiotic-resistance gene families showed similar or lower abundances in facility biofilms than in river water (Fig. 5b). Thus, the higher abundances of *mex* and *mate* gene families in remediated river water could not simply be due to dispersal from remediation facilities.

The pathogens with antibiotic-resistance genes are of special concern (Berglund et al., 2015; Hao et al., 2015; Novovic et al., 2015). The *smr* gene family in *Enterococcus faecium* detected in river water of control area became undetectable in river water of remediated area, and the *mfs* gene family in *Aspergillus niger* detected in control area became undetectable in any media in remediated area. Moreover, the *tet* gene family in *Paracoccidioides brasiliensis* Pb03 in river water was less abundant in remediated area than in control area (Fig. 5a). These results suggested these antibiotic-resistance pathogens were suppressed in remediated area. In contrast, the antibiotic-resistance genes in *Pseudomonas aeruginosa* showed higher relative abundances (RR 97.5% CI, *t*-test $P = 0.04$) in sediment of remediated area than control area, and *abc* transporter in *Penicillium marneffei* was more abundant (RR 99% CI, *t*-test $P = 0.07$) in river water of remediated area than control area. All the other detected antibiotic-resistance pathogens had no significant difference between remediated and control areas in either river water or sediment. However, the *smr* gene families in *Listeria monocytogenes* and *Neisseria meningitidis* were detected in facility biofilms rather than river water and sediment, while the *smr* gene family in *Campylobacter jejuni* was only detected in plant biofilms, suggesting potential risks emerged in remediation facilities (Fig. 5b and c). Altogether, quite a few pathogens and some antibiotic-resistance microorganisms, including several antibiotic-resistance pathogens, can develop after eco-remediation, of which the variation should be monitored and the impacts on human health and ecosystem safety ought to be investigated.

3.6. Difference between the two major biofilms in eco-remediation facilities

Many studies revealed the diverse microbial communities in biofilms on different carriers in constructed wetlands that treated wastewater (Adrados et al., 2014; Correa-Galeote et al., 2013; Desta et al., 2014; Jasper et al., 2014b; Wang et al., 2014; Zhao et al., 2015), however, the comparison between plant-associated biofilms and abiotic carriers is rarely reported, and even less in eco-remediation facilities. In our study, microbial communities in the two types of facility biofilms were much closer to each other than to other types of samples (Fig. 2 and Fig. S3b). They showed very similar compositions of labile organics degrading and ammonia oxidizing gene families (Fig. 4d). Therefore, their capability of removing oxygen consuming pollutants should mostly depend on the biomass density. This support a certain advantage of abiotic carriers which are easier to hold higher density of microorganisms. However, our results also showed some features of floating plants that are not replaceable by abiotic carriers. The plant biofilms had significantly higher concentrations of TOC, TN, TP, Mn, Cr^{VI} and Cd than abio-carrier biofilms (Fig. 3), suggesting more efficient absorption of these pollutants. Moreover, the plant biofilms exhibited higher microbial richness and diversity (Figs. S3a and S4). The plants provided more habitats for aquatic organisms, ornamented the urban rivers, and after harvested, were used as fertilizer for floriculture plantation near the city. Therefore, the eco-remediation technique can be improved by either efficient combination of both plants and abio-carriers or application of plants with more developed submerged part to hold more biofilms.

4. Conclusions

The eco-remediation facilities did not only increase the biomass, but also changed the community structures and introduced much more diverse functional microorganisms in the river, which positively contributed to water quality improvement. Microbial communities in river water showed significantly higher abundances of labile-organics-

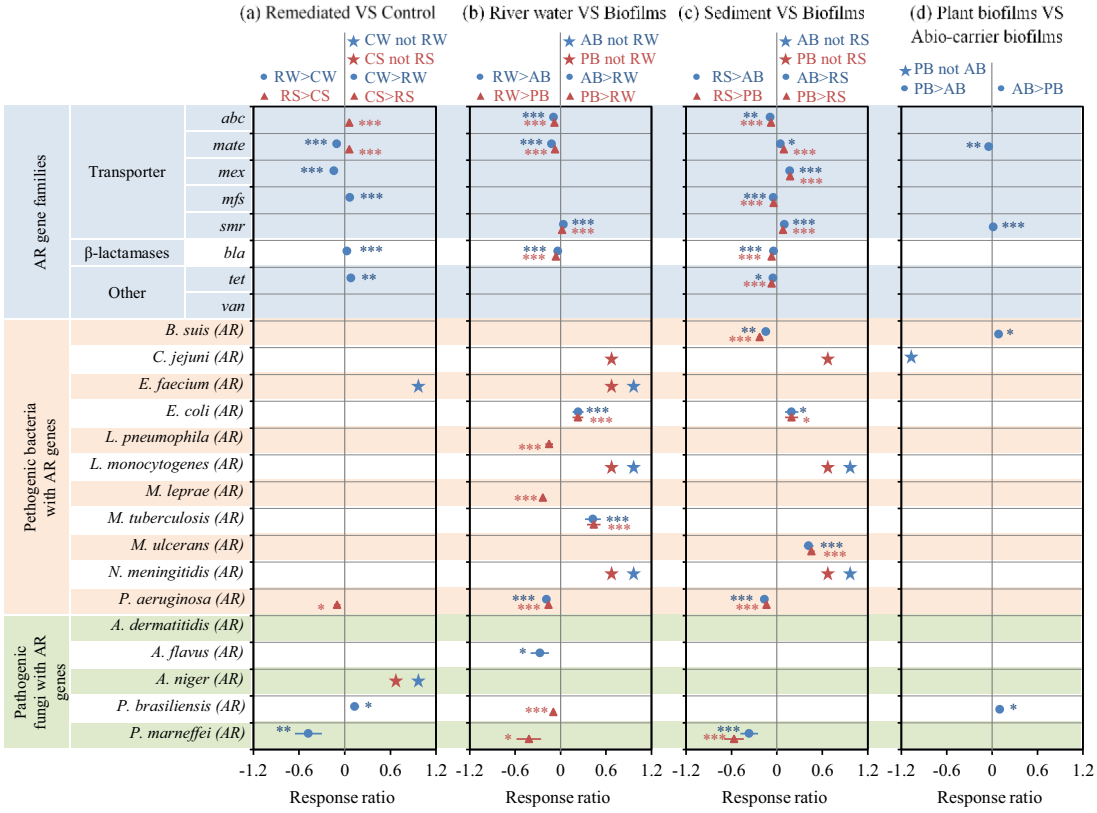


Fig. 5. Abundance response ratios of antibiotic-resistance (AR) genes families and antibiotic-resistance pathogens (a) in river water (blue) and sediment (red) of control area to those of remediated area, (b) in biofilms on abiotic carriers (blue) and plants (red) to river water in remediated area, (c) in biofilms on abiotic carriers (blue) and plants (red) to sediment in remediated area, and (d) in biofilms on abiotic carriers to those on plants. CS, sediment samples from control area; RS, sediment samples from remediated area; CW, river water samples from control area; RW, river water samples from remediated area; AB, biofilm samples from abiotic carriers; PB, biofilm samples from plants. *, significant at 95% confidence interval (CI); **, significant at 99% CI; ***, significant at 99.5% CI. Error bars represent standard errors. Only significant (CI > 95%) data are presented. The detected pathogenetic bacteria with antibiotic-resistance genes include *Brucella suis* 1330 with *smr*, *Campylobacter jejuni* subsp. jejuni CG8486 with *smr*, *Enterococcus faecium* with *mfs* or *smr*, *Escherichia coli* with *blaA*, *blaC*, *abc* and *smr*, *Legionella pneumophila Philadelphia* with *blaC*, *Listeria monocytogenes* str. 4b H7858 with *smr*, *Mycobacterium leprae* with *smr*, *Mycobacterium tuberculosis* with *smr*, *Mycobacterium ulcerans* Agy99 with *smr*, *Neisseria meningitidis* Z2491 with *smr*, and *Pseudomonas aeruginosa* with *smr*, *mfs*, *mex* and *bla*. The detected pathogenetic fungi with antibiotic-resistance genes include *Ajellomyces dermatitidis* with *tet* and *abc*, *Aspergillus flavus* with *mfs*, *mate* and *abc*, *Aspergillus niger* with *mfs*, *Paracoccidioides brasiliensis* with *tet*, and *Penicillium marneffei* with *abc*. See Table S3 for the description of the genes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

degrading and ammonia-oxidizing gene families in remediated area than in the adjacent control area. Correspondingly, the facility biofilms had significantly more abundances of these functional gene families than river water and sediment. The plants and biofilms in the facilities showed obvious absorption of N, P and various metals (especially Mn, Cr^{VI}, Fe, Al, As, and Co), contributing to removal of nutrients and heavy metals from river water. The eco-remediation also resulted in breakdown of some colloids, and thus increased transparency and settlement of some heavy metals (e.g. Cu, Pb, and Zn). The eco-remediation techniques can be furtherly improved by enhanced oxygen supply, more effective recalcitrant organics degradation, denitrification, and phosphorus accumulation, and optimized combination of abiotic carries and plants holding more biofilms. The facilities did not significantly change the abundances of most pathogens and antibiotic-resistance gene families detected in river water and sediment, and appeared to inhibit some of them. However, our results also alerted us of the potential risks regarding to the accumulation of heavy metals and the development of some pathogenic and/or antibiotic-resistance microorganisms in remediated area, which should be monitored and under control for human health and ecosystem safety.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This study was supported by the Site Soil Pollution Treatment Technology Major Project of China (Grant number 2020YFC1808801), the National Natural Science Foundation Projects of China (Grant numbers 41573065 and 41773082), and the National Water Pollution Control and Treatment Science and Technology Major Project of China (Grant number 2017ZX07202002). We would also like to thank Dr. Yuting Liang, Dr. Haiyan Zhou and Dr. Jiuxiao Hao for the constructive comments on our manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.149868.

References

Adrados, B., Sanchez, O., Arias, C.A., Becares, E., Garrido, L., Mas, J., et al., 2014. Microbial communities from different types of natural wastewater treatment systems: vertical and horizontal flow constructed wetlands and biofilters. Water Res. 55, 304–312.

Agunbiade, F.O., Olu-Owolabi, B.I., Adebowale, K.O., 2009. Phytoremediation potential of Eichornia crassipes in metal-contaminated coastal water. Bioresour. Technol. 100, 4521–4526.

Ancion, P.-Y., Lear, G., Lewis, G.D., 2010. Three common metal contaminants of urban runoff (Zn, Cu & Pb) accumulate in freshwater biofilm and modify embedded bacterial communities. Environ. Pollut. 158, 2738–2745.

Berglund, B., Khan, G.A., Weisner, S.E.B., Ehde, P.M., Fick, J., Lindgren, P.-E., 2014. Efficient removal of antibiotics in surface-flow constructed wetlands, with no observed impact on antibiotic resistance genes. Sci. Total Environ. 476, 29–37.
Berglund, B., Fick, J., Lindgren, P.E., 2015. Urban wastewater effluent increases antibiotic resistance gene concentrations in a receiving northern European river. Environ. Toxicol. Chem. 34, 192–196.
Billore, S.K., Prashant, Sharma, J.K., 2009. Treatment performance of artificial floating reed beds in an experimental mesocosm to improve the water quality of river Kshipra. Water Sci. Technol. 60, 2851–2859.
Bout well, J.E., 1995. Preliminary field studies using vegetated floating platforms. Technical Memorandum, No. 8220-95-2. U.S. Department of the Interior Bureau of Reclamation.
Bu, F.P., Xu, X.Y., 2013. Planted floating bed performance in treatment of eutrophic river water. Environ. Monit. Assess. 185, 9651–9662.
Budd, R., O'Geen, A., Goh, K.S., Bondarenko, S., Gan, J., 2009. Efficacy of constructed wetlands in pesticide removal from tailwaters in the Central Valley, California. Environ. Sci. Technol. 43, 2925–2930.
Burton, A., 2012. Floating islands to restore Rift Valley lake. Front. Ecol. Environ. 10, 459.
Cebron, A., Coci, M., Garnier, J., Laanbroek, H.J., 2004. Denaturing gradient gel electrophoretic analysis of ammonia-oxidizing bacterial community structure in the lower Seine River: Impact of Paris wastewater effluents. Appl. Environ. Microbiol. 70, 6726–6737.
Chang, N.-B., Islam, K., Marimon, Z., Wanielista, M.P., 2012. Assessing biological and chemical signatures related to nutrient removal by floating islands in stormwater mesocosms. Chemosphere 88, 736–743.
Chen, H., Yu, J.J., Jia, X.Y., Jin, R.C., 2014a. Enhancement of anammox performance by Cu (II), Ni(II) and Fe(III) supplementation. Chemosphere 117, 610–616.
Chen, Y., Wen, Y., Zhou, Q., Vymazal, J., 2014b. Effects of plant biomass on denitrifying genes in subsurface-flow constructed wetlands. Bioresour. Technol. 157, 341–345.
Correa-Galeote, D., Marco, D.E., Tortosa, G., Bru, D., Philippot, L., Bedmar, E.J., 2013. Spatial distribution of N-cycling microbial communities showed complex patterns in constructed wetland sediments. FEMS Microbiol. Ecol. 83, 340–351.
Cummings, D.E., Archer, K.F., Arriola, D.J., Baker, P.A., Faucett, K.G., Laroya, J.B., et al., 2011. Broad Dissemination of Plasmid-Mediated Quinolone Resistance Genes in Sediments of Two Urban Coastal Wetlands. Environmental Science & Technology 45, 447–454.
Desta, A.F., Assefa, F., Leta, S., Stomeo, F., Wamalwa, M., Njahira, M., et al., 2014. Microbial community structure and diversity in an integrated system of anaerobic-aerobic reactors and a constructed wetland for the treatment of tannery wastewater in Modjo, Ethiopia. PLoS One 9.
Duris, J.W., Reif, A.G., Krouse, D.A., Isaacs, N.M., 2013. Factors related to occurrence and distribution of selected bacterial and protozoan pathogens in Pennsylvania streams. Water Res. 47, 300–314.
Fechner, L.C., Versace, F., Gourlay-France, C., Tusseau-Vuillemin, M.-H., 2012. Adaptation of copper community tolerance levels after biofilm transplantation in an urban river. Aquat. Toxicol. 106, 32–41.
Gaillardet, J., Viers, J., Dupré, B., 2003. 5.09-Trace elements in river waters. In: Drever, J.I., Holland, H.D., Turekian, K.K. (Eds.), Surface and Ground Water, Weathering, and Soils. Pergamon, Oxford, pp. 225–272 Treatise on Geochemistry.
Garbett, R., 2005. An investigation into the application of floating reed bed and barley straw techniques for the remediation of eutrophic waters. Water Environ. J. 19, 174–180.
Hao, R.Z., Zhao, R.T., Qiu, S.F., Wang, L.G., Song, H.B., 2015. Antibiotics crisis in China. Science 348, 1100.
He, Z., Xu, M., Deng, Y., Kang, S., Kellogg, L., Wu, L., et al., 2010. Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO2. Ecol. Lett. 13, 564–575.
He, J., Hu, H., Zhang, L., 2012. Current insights into the autotrophic thaumarchaeal ammonia oxidation in acidic soils. Soil Biol. Biochem. 55, 146–154.
Hoeger, S., 1988. Schwimmkampen: Germany's artificial floating islands. J. Soil Water Conserv. 43, 304–306.
Hu, G.J., Zhou, M., Hou, H.B., Zhu, X., Zhang, W.H., 2010. An ecological floating-bed made from dredged lake sludge for purification of eutrophic water. Ecol. Eng. 36, 1448–1458.
Hu, B.L., Shen, L.D., Zheng, P., Hu, A.H., Chen, T.T., Cai, C., et al., 2012. Distribution and diversity of anaerobic ammonium-oxidizing bacteria in the sediments of the Qiantang River. Environ. Microbiol. Rep. 4, 540–547.
Huang, Y., Jiang, M., Zhang, Y., Du, J., 2010. Building Artificial Floating-island for Treating Bailianjing River in Shanghai. Environ. Sci. Technol. 33, 108–113.
Jang, J., Unno, T., Lee, S.W., Cho, K.H., Sadowsky, M.J., Ko, G., et al., 2011. Prevalence of season-specific Escherichia coli strains in the Yeongsan River Basin of South Korea. Environ. Microbiol. 13, 3103–3113.
Jasper, J.T., Jones, Z.L., Sharp, J.O., Sedlak, D.L., 2014a. Biotransformation of trace organic contaminants in open-water unit process treatment wetlands. Environ. Sci. Technol. 48, 5136–5144.
Jasper, J.T., Jones, Z.L., Sharp, J.O., Sedlak, D.L., 2014b. Nitrate Removal in Shallow, Open-Water Treatment Wetlands. Environ. Sci. Technol. 48, 11512–11520.
Jin, X.C., Xu, Q.J., Huang, C.Z., 2005. Current status and future tendency of lake eutrophication in China. Sci. China Ser. C Life Sci. 48, 948–954.
Kamble, R., Patil, D., 2012. Artificial floating island: solution to river water pollution in India. Case study: rivers in Pune city. Int. Proc. Chem. Biol. Environ. 41, 136–140.
Lear, G., Ancion, P.Y., Harding, J., Lewis, G.D., 2012. Use of bacterial communities to assess the ecological health of a recently restored stream. N. Z. J. Mar. Freshw. Res. 46, 291–301.
Li, X.N., Song, H.L., Li, W., Lu, X.W., Nishimura, O., 2010. An integrated ecological floating-bed employing plant, freshwater clam and biofilm carrier for purification of eutrophic water. Ecol. Eng. 36, 382–390.
Liang, Y., Li, G., Van Nostrand, J.D., He, Z., Wu, L., Den, Y., et al., 2009. Microarray-based analysis of microbial functional diversity along an oil contamination gradient in oil field. FEMS Microbiol. Ecol. 70, 324–333.
Liu, S.T., Horn, H., 2012. Effects of Fe(II) and Fe(III) on the single-stage deammonification process treating high-strength reject water from sludge dewatering. Bioresour. Technol. 114, 12–19.
Liu, Z.H., Huang, S.B., Sun, G.P., Xu, Z.C., Xu, M.Y., 2011. Diversity and abundance of ammonia-oxidizing archaea in the Dongjiang River, China. Microbiol. Res. 166, 337–345.
Lu, S.-Y., Zhang, Y.-L., Geng, S.-N., Li, T.-Y., Ye, Z.-M., Zhang, D.-S., et al., 2010. High diversity of extended-spectrum beta-lactamase-producing bacteria in an urban river sediment habitat. Appl. Environ. Microbiol. 76, 5972–5976.
Lu, Z., Deng, Y., Van Nostrand, J.D., He, Z., Voordeckers, J., Zhou, A., et al., 2012. Microbial gene functions enriched in the Deepwater Horizon deep-sea oil plume. ISME J 6, 451–460.
Mantel, N., 1967. Detection of disease clustering and a generalized regression approach. Cancer Res. 27, 209–220.
Masters, B., 2012. The ability of vegetated floating Islands to improve water quality in natural and constructed wetlands: a review. Water Pract. Technol. 7.
Matamoros, V., Arias, C., Brix, H., Bayona, J.M., 2007. Removal of pharmaceuticals and personal care products (PPCPs) from urban wastewater in a pilot vertical flow constructed wetland and a sand filter. Environ. Sci. Technol. 41, 8171–8177.
Nakai, S., Zou, G., Okuda, T., Tsai, T.Y., Song, X., Nishijima, W., et al., 2010. Anticyanobacterial allelopathic effects of plants used for artificial floating islands. Allelopath. J. 26, 113–121.
Nakamura, K., Mueller, G., 2008. Review of the Performance of the Artificial Floating Island as a Restoration Tool for Aquatic Environments. World Environmental and Water Resources Congress 2008; May 12–16. U. S. Environmental and Water Resources Institute of ASCE, Hawaii.
Ning, D., Huang, Y., Wang, H., 2011. The phytoplankton community succession during the restoration of an urban river in the Yangtze Plain. International Conference on Environment Simulation and Pollution Control. State Key Joint Laboratory of Environment Simulation and Pollution Control in China, Beijing, China.
Ning, D., Huang, Y., Pan, R., Wang, F., Wang, H., 2014. Effect of eco-remediation using planted floating bed system on nutrients and heavy metals in urban river water and sediment: a field study in China. Sci. Total Environ. 485–486, 596–603.
Novovic, K., Filipic, B., Veljovic, K., Begovic, J., Mirkovic, N., Jovcic, B., 2015. Environmental waters and bla(NDm-1) in Belgrade, Serbia: endemicity questioned. Sci. Total Environ. 511, 393–398.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., et al., 2014. Vegan: Community Ecology Package. R package version 2.2-0. http://CRAN.R-project.org/package=vegan.
Peeler, K.A., Opsahl, S.P., Chanton, J.P., 2006. Tracking anthropogenic inputs using caffeine, indicator bacteria, and nutrients in rural freshwater and urban marine systems. Environ. Sci. Technol. 40, 7616–7622.
Perryman, S.E., Rees, G.N., Grace, M.R., 2011a. Sediment bacterial community structure and function in response to C and Zn amendments: urban and nonurban streams. J. N. Am. Benthol. Soc. 30, 951–962.
Perryman, S.E., Rees, G.N., Walsh, C.J., Grace, M.R., 2011b. Urban stormwater runoff drives denitrifying community composition through changes in sediment texture and carbon content. Microb. Ecol. 61, 932–940.
Pokrovsky, O.S., Schott, J., 2002. Iron colloids/organic matter associated transport of major and trace elements in small boreal rivers and their estuaries (NW Russia). Chem. Geol. 190, 141–179.
Qu, J., Fan, M., 2010. The current state of water quality and technology development for water pollution control in China. Crit. Rev. Environ. Sci. Technol. 40, 519–560.
R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria http://www.R-project.org/.
Rai, P.K., 2009. Heavy metal phytoremediation from aquatic ecosystems with special reference to macrophytes. Crit. Rev. Environ. Sci. Technol. 39, 697–753.
Reeves, R.L., Grant, S.B., Mrse, R.D., Oancea, C.M.C., Sanders, B.F., Boehm, A.B., 2004. Scaling and management of fecal indicator bacteria in runoff from a coastal urban watershed in southern California. Environ. Sci. Technol. 38, 2637–2648.
Rodriguez-Mozaz, S., Chamorro, S., Marti, E., Huerta, B., Gros, M., Sanchez-Melsio, A., et al., 2015. Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water Res. 69, 234–242.
Sales-Ortells, H., Medema, G., 2014. Screening-level microbial risk assessment of urban water locations: a tool for prioritization. Environ. Sci. Technol. 48, 9780–9789.
Sawyer, C.N., McCarty, P.L., Parkin, G.F., 2003. Chemistry for Environmental Engineering and Science. 5th edn. New York, McGraw-Hill.
Seo, E.-Y., Kwon, O.-B., Choi, S.-I., Kim, J.-H., Ahn, T.-S., 2013. Installation of an artificial vegetating island in oligomesotrophic Lake Paro, Korea. Sci. World J. 2013, 857670.
Sigg, L., Xue, H.B., Kistler, D., Sshonenberger, R., 2000. Size fractionation (dissolved, colloidal and particulate) of trace metals in the Thur River, Switzerland. Aquat. Geochem. 6, 413–434.
Sole, M., Fetzer, I., Wennrich, R., Sridhar, K.R., Harms, H., Krauss, G., 2008. Aquatic hyphomycete communities as potential bioindicators for assessing anthropogenic stress. Sci. Total Environ. 389, 557–565.
Song, K., Kang, H., Zhang, L., Mitsch, W.J., 2012. Seasonal and spatial variations of denitrification and denitrifying bacterial community structure in created riverine wetlands. Ecol. Eng. 38, 130–134.
Sonthiphand, P., Cejudo, E., Schiff, S.L., Neufeld, J.D., 2013. Wastewatereffluent impacts ammonia-oxidizing prokaryotes of the Grand River, Canada. Appl. Environ. Microbiol. 79, 7454–7465.
Stewart, F.M., Mulholland, T., Cunningham, A.B., Kania, B.G., Osterlund, M.T., 2008. Floating islands as an alternative to constructed wetlands for treatment of excess nutrients

from agricultural and municipal wastes - Results of laboratory-scale tests. Land Contam. Reclam. 16, 25–33.
Storteboom, H., Arabi, M., Davis, J.G., Crimi, B., Pruden, A., 2010. Identification of antibiotic-resistance-gene molecular signatures suitable as tracers of pristine river, urban, and agricultural sources. Environ. Sci. Technol. 44, 1947–1953.
Stumm, W., 1987. Aquatic Surface Chemistry: Chemical Processes at the Particle-Water Interface. John Wiley, New York.
Sun, W., Xia, C.Y., Xu, M.Y., Guo, J., Sun, G.P., Wang, A.J., 2014a. Community structure and distribution of planktonic ammonia-oxidizing archaea and bacteria in the Dongjiang River, China. Res. Microbiol. 165, 657–670.
Sun, W., Xia, C.Y., Xu, M.Y., Guo, J., Wang, A.J., Sun, G.P., 2014b. Diversity and distribution of planktonic anaerobic ammonium-oxidizing bacteria in the Dongjiang River, China. Microbiol. Res. 169, 897–906.
Sun, W., Xu, M.Y., Wu, W.M., Guo, J., Xia, C.Y., Sun, G.P., et al., 2014c. Molecular diversity and distribution of anammox community in sediments of the Dongjiang River, a drinking water source of Hong Kong. J. Appl. Microbiol. 116, 464–476.
Surbeck, C.Q., Jiang, S.C., Grant, S.B., 2010. Ecological control of fecal indicator bacteria in an urban stream. Environ. Sci. Technol. 44, 631–637.
Tanaka, T.S.T., Irbis, C., Wang, P.Y., Inamura, T., 2015. Impact of plant harvest management on function and community structure of nitrifiers and denitrifiers in a constructed wetland. FEMS Microbiol. Ecol. 91.
Taylor, H.E., Antweiler, R.C., Roth, D.A., Alpers, C.N., Dileanis, P., 2012. Selected trace elements in the sacramento river, california: occurrence and distribution. Arch. Environ. Contam. Toxicol. 62, 557–569.
Ter Braak, C.J.F., 1986. Canonical correspondence-analysis - a new eigenvector technique for multivariate direct gradient analysis. Ecology 67, 1167–1179.
Tiquia, S.M., 2011. Extracellular hydrolytic enzyme activities of the heterotrophic microbial communities of the rouge river: an approach to evaluate ecosystem response to urbanization. Microb. Ecol. 62, 679–689.
Tu, Q., Yu, H., He, Z., Deng, Y., Wu, L., Van Nostrand, J.D., et al., 2014. GeoChip 4: a functional gene-array-based high-throughput environmental technology for microbial community analysis. Mol. Ecol. Resour. 14, 914–928.
Vymazal, J., Bfezinova, T., 2015. The use of constructed wetlands for removal of pesticides from agricultural runoff and drainage: a review. Environ. Int. 75, 11–20.
Wang, S.-Y., Sudduth, E.B., Wallenstein, M.D., Wright, J.P., Bernhardt, E.S., 2011. Watershed urbanization alters the composition and function of stream bacterial communities. PLoS One 6.
Wang, S.Y., Zhu, G.B., Peng, Y.Z., Jetten, M.S.M., Yin, C.Q., 2012. Anammox bacterial abundance, activity, and contribution in riparian sediments of the Pearl River Estuary. Environ. Sci. Technol. 46, 8834–8842.
Wang, Y.-f., Wu, Y., Pi, N., NF-y, Tam, 2014. Investigation of microbial community structure in constructed mangrove microcosms receiving wastewater-borne polycyclic aromatic hydrocarbons (PAHs) and polybrominated diphenyl ethers (PBDEs). Environ. Pollut. 187, 136–144.
Winter, C., Hein, T., Kavka, G., Mach, R.L., Farnleitner, A.H., 2007. Longitudinal changes in the bacterial community composition of the Danube River: a whole-river approach. Appl. Environ. Microbiol. 73, 421–431.
Zhang, C.B., Liu, W.L., Pan, X.C., Guan, M., Liu, S.Y., Ge, Y., et al., 2014a. Comparison of effects of plant and biofilm bacterial community parameters on removal performances of pollutants in floating island systems. Ecol. Eng. 73, 58–63.
Zhang, Q., Jia, A., Wan, Y., Liu, H., Wang, K., Peng, H., et al., 2014b. Occurrences of Three Classes of Antibiotics in a Natural River Basin: Association with Antibiotic-Resistant Escherichia coli. Environ. Sci. Technol. 48, 14317–14325.
Zhao, F., Xi, S., Yang, X., Yang, W., Li, J., Gu, B., et al., 2012. Purifying eutrophic river waters with integrated floating island systems. Ecol. Eng. 40, 53–60.
Zhao, C., Xie, H., Xu, J., Xu, X., Zhang, J., Hu, Z., et al., 2015. Bacterial community variation and microbial mechanism of triclosan (TCS) removal by constructed wetlands with different types of plants. Sci. Total Environ. 505, 633–639.
Zhou, J., Bruns, M.A., Tiedje, J.M., 1996. DNA recovery from soils of diverse composition. Appl. Environ. Microbiol. 62, 316–322.
Zhou, J., Kang, S., Schadt, C.W., Garten, C.T., 2008. Spatial scaling of functional gene diversity across various microbial taxa. Proc. Natl. Acad. Sci. U. S. A. 105, 7768–7773.
Zhou, X.H., Wang, G.X., Yang, F., 2012. Nitrogen removal from eutrophic river waters by using Rumex acetosa cultivated in ecological floating beds. Fresenius Environ. Bull. 21, 1920–1928.
Zhou, Y.Q., Tigane, T., Li, X.Z., Truu, M., Truu, J., Mander, U., 2013. Hexachlorobenzene dechlorination in constructed wetland mesocosms. Water Res. 47, 102–110.