Influence of microbial community diversity and function on pollutant removal in ecological wastewater treatment

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Abstract Traditional wastewater treatments based on activated sludge often encounter the problems of bulking and foaming, as well as malodor. To solve these problems, new treatment technologies have emerged in recent decades, including the ecological wastewater treatment process, which introduces selected local plants into the treatment system. With a focus on the underlying mechanisms of the ecological treatment process, we explored the microbial community biomass, composition, and function in the treatment system to understand the microbial growth in this system and its role in pollutant removal. Flow cytometry analysis revealed that ecological treatment significantly decreased influent bacterial quantity, with around 80% removal. 16S rRNA gene sequencing showed that the ecological treatment also altered the bacterial community structure of the wastewater, leading to a significant change in *Comamonadaceae* in the effluent. In the internal ecological system, because most of microbes aggregate in the plant rhizosphere and the sludge under plant roots, we selected two plant species (*Nerium oleander* and *Arundo donax*) to study the characteristics of rhizosphere and sludge microbes. Metagenomic results showed that the microbial community composition and function differed between the two species, and the microbial communities of

A. donax were more sensitive to seasonal effects. Combined with their greater biomass and abundance of metabolic genes, microbes associated with *N. oleander* showed a greater contribution to pollutant removal. Further, the biodegradation pathways of some micropollutants, e.g., atrazine, were estimated.

Keywords Ecological treatment · Microbial community · Metagenomics · *Nerium oleander* · *Arundo donax*

Introduction

In highly populated areas, centralized wastewater treatment plants (WWTPs) are essential systems for treating domestic wastewater. Activated sludge (AS) treatment is a crucial method in traditional WWTPs as it contains a highly complex microbial population (Saunders et al. 2016). However, bulking and foaming due to overgrowth of filamentous microorganisms as well as malodor problems frequently occur in AS treatment plants (Nielsen et al. 2009). To improve treatment effectiveness, decrease odor, and reduce the area covered, new treatment technologies have emerged in recent decades, including ecological wastewater treatment systems. Ecological wastewater treatment has been applied in a number of countries, including the USA (Balcom et al. 2016), and was first introduced to China in 2013. The unique feature of this process is transplanting emergent aquatic plants into the activated sludge treatment unit and utilizing ecological interactions among plants, animals, and microbes. Long-term operation has shown desirable effects on pollutant removal. However, the treatment mechanism remains largely unknown. A recent research study reported that the microbial biofilm communities growing on the surfaces of plant roots might play an important role in pollutant removal (Balcom et al.

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2016). During operation of an ecological treatment process in China, we also found a microbial film formed on plant roots, probably because the plant root secretes exudates, which are beneficial for microbial growth. Based on previous research and our observations, we hypothesize that the rhizosphere microbial community would have high diversity and strong degradation functions, thus resulting in the effective pollutant treatment.

The methodology used is a key issue to verify the above hypothesis and explore the microbial role in pollutant removal in the ecological treatment process. Recent high-throughput sequencing techniques (e.g., metagenomics) have greatly improved their specificity, thus providing a better description of biological mechanisms (Mason et al. 2014; Thomas et al. 2008; Ye et al. 2012). Prior studies have used this technique to address the fundamental issue of AS, e.g., how external factors affect the diversity and function of AS communities, as well as the impacts of the diversity of functionally important microorganisms on process stability (Fang et al. 2013; Gruber-Dominguer et al. 2015; Ibarbalz et al. 2016; Ju et al. 2014; Ye et al. 2012; Yu and Zhang 2012). The general consensus was that microbial diversity is usually very large in wastewater bio-treatment facilities (Ayache et al. 2013; Bouali et al. 2013; Madoni et al. 1996; Manz et al. 1994; Satoh et al. 2006; Wells et al. 2011). However, there exists a small core functional microbial community which may account for most pollutant removal (Saunders et al. 2016; Zhang et al. 2012).

Here, we combined flow cytometry, MiSeq 16S rRNA gene sequencing, and metagenomic analysis to unravel the underlying mechanism of ecological treatment. We aimed to investigate (i) what is the role of microbial community in pollutant removal and (ii) how the cultivated plants are beneficial for the microbial metabolism of nutrients and micropollutants. This research will improve our understanding of the ecological treatment mechanism.

Materials and methods

Sampling site and strategy

The studied wastewater treatment plant is located in Shenzhen, Guangzhou Province, China (Fig. S1a, Supporting Information), and has a designed treatment capacity of 30,000 m³/d. The actual daily treatment capacity of this treatment plant is 20,000 ~ 24,000 m³. In 2013, the traditional anoxic-oxic process was upgraded to an ecological process to meet the new strict Chinese wastewater discharge standards. This new technological process includes fine grids, ecological treatment, microfiltration, and UV disinfection (Fig. 1). Compared with the previous anoxic-oxic process in this plant, the area occupied by the new process was greatly decreased. For ecological treatment (Fig.

S1b), 25 local emergent aquatic plant species were transplanted into the WWTP, with biofilters (plastic material) placed under their roots to intensify treatment. Because wastewater was treated underground, the deodorization role of the transplanted plants markedly improved the odor compared with that of the traditional AS process (per field odor detection). From the monitoring results during 2015, more than 60 and 80% respectively of the chemical oxygen demand (COD) and NH₃-N were removed in most cases in this process. After treatment, the effluent met the Category III Chinese standard of surface water (influent and effluent water quality details in Table S1) and was discharged into the Dongjiang River (dilution factor ~ 20% annually, 2–3 m wide and 80 cm deep). During operation under the new process, we observed that the transplanted plants grew weakly in winter and autumn and their rhizospheres had fewer attached microbes, compared with spring and summer. According to our experience, it was too difficult to extract sufficient DNA for metagenomic analysis.

Based on our field observations, sampling was conducted on three separate occasions (July 2015, March 2016, and June 2016, Fig. 1). For each sampling period, we collected water samples three times (morning, noon, and afternoon, respectively) from the influent and effluent of the different treatment processes as well as upstream and downstream (surface water, 2–5 cm) of the receiving river. The three collected water samples were mixed together at equal volumes (2 L). We used 1 L of water for chemical analysis and 4 L of water (only from July 2015 and March 2016) for DNA extraction and MiSeq 16S rRNA gene sequencing. For flow cytometry analysis, 1 mL of water was loaded into 1.5-mL brown bottles and glutaraldehyde was added for microbe fixation.

We sampled the rhizosphere and sludge (under the roots and surrounding the plastic biofilter) of two dominant plant species, *Nerium oleander* and *Arundo donax*, in July 2015 and March 2016, in order to investigate the biomass, as well as the diversity and function of the microbial communities. The root (dissected into 3-cm segments from an individual plant each time) and sludge samples of the two plant species were sampled. Rhizosphere biofilm samples were obtained by washing the plant roots with 5 mL of phosphate-buffered saline (PBS) in a 15-ml sterile Falcon tube (Bulgarrelli et al. 2012) which was then subjected to centrifugation (3000×g, 10 min). In addition, sludge samples (5 mL) from under the plant roots were directly added to 50-mL sterile tubes. Lifeguard™ Soil Preservation Solution (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) was added to the biofilm and sludge samples to prevent degradation of nucleic acids. All samples were stored on ice during transport to the laboratory, then placed in a freezer at –80 °C for DNA extraction.

Chemical analysis and MiSeq 16S rRNA gene sequencing were performed for water samples. The rhizosphere biomass of two plants was roughly evaluated by washing root segments three times with 20 mL of sterile deionized water and

Fig. 1 Schematic diagram of the wastewater treatment plant and sampling sites in the plant and receiving river. The wastewater treatment includes the following processes (inside the box). Water samples were obtained from ECI (influent of ecological treatment unit), ECE (effluent of ecological treatment unit), MFE (effluent of microfiltration unit), effluent of treatment plant, upstream sites 1 and 2 (U1 and U2) of treated wastewater

outlet in receiving river, and downstream sites 1 and 2 (D1 and D2). Rhizosphere biofilm of *Arundo donax* (RA) and *Nerium oleander* (RN) and sludge of *Arundo donax* (SA) and *Nerium oleander* (SN) were obtained in the ecological treatment unit. Water samples were used for chemical analysis and MiSeq 16S rRNA gene sequencing, and biofilm and sludge samples were used for metagenomic analysis

then drying at 105° C in an oven before weighing. Sludge biomass was evaluated by directly drying 20 mL sludge at 105° C. Illumina metagenomic sequencing was adopted for rhizosphere biofilm and sludge samples.

Water quality measurements

Temperature, conductivity, pH, and total dissolved solids (TDS) were measured using a Multi-Function Water Quality Monitor (MYRON L Company, USA), and dissolved oxygen (DO) was measured using a portable DO analyzer (INESA JPB-607A, China) in the field. Collected water samples were filtered through cellulose nitrate membranes (0.45 µm pore size) for photometric analyses of $\text{NH}_3\text{-N}$, $\text{NO}_2^-\text{-N}$, and total nitrogen (TN) (State Environmental Protection Administration of China 1989). For dissolved organic carbon (DOC) determination, water samples were filtered with cellulose glass membranes and then analyzed with a TOC-VCPH analyzer (Shimadzu, Japan). Sixteen US EPA priority polycyclic aromatic hydrocarbons (PAHs) were analyzed using gas chromatography-mass spectrometry (GC/MS, Agilent 7890A/5975C) (Qiao et al. 2013), and the sum of all aromatic compounds was calculated. Twenty-one pesticides were screened and detected with solid-phase extraction and GC/MS, as described previously (Ruiz-Gil et al. 2008).

Bacterial density determination by flow cytometry

The quantity of bacteria in water was measured using flow cytometry (Brussaard et al. 2000; Brussaard et al. 2010).

Briefly, TE buffer (10 mM Tris-HCl, 1 mM EDTA (pH 8.0), filtered by 30 KD ultrafiltration membrane) was used for dilution of the water samples (1:10–1:200 for different samples). The diluted samples were stained by SYBR green I at room temperature for 15 min in the dark, then loaded into a flow cytometer (FACSCalibur™, BD Company, USA) for bacterial density determination.

DNA extraction

For DNA extraction of water samples, 200–1000 ml (depending on turbidity) of water was filtered by membranes (0.22 µm pore size, 47 mm diameter, Millipore, USA) using a vacuum pump. Total water DNA was extracted from each membrane using a PowerWater DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). For rhizosphere and sludge samples of *N. oleander* and *A. donax*, 0.5–1.0 g of centrifuged rhizosphere biofilm or sludge was used for DNA extraction using a PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). DNA extraction was performed in duplicate for each sample.

MiSeq sequencing for bacterial community composition in different treatment processes and the receiving river

The V4 region of the 16S rRNA gene was amplified using barcoded primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR products were purified and the DNA concentration was determined. Prior to sequencing, the amplicons from

duplicate samples were pooled in equal amounts based on concentration. High-throughput sequencing was performed using the Illumina MiSeq sequencing platform (Illumina, Inc., San Diego, CA, USA). The 1,101,422 raw sequence reads in total were initially filtered to remove low-quality reads and barcode primers and the filtered paired-end reads were connected to tags based on overlaps. The connected tags were then analyzed using the QIIME standard pipeline (Caporaso et al. 2010). Alpha diversity statistics, including nonparametric richness Chao1 (Chao 1987), observed operational taxonomic units (OTUs, 97% similarity), phylogenetic diversity whole tree metric, and beta diversity (principal coordinates analysis (PCoA) of weighted unifrac distance), were calculated based on 16,990 filtered sequences of each sample.

Metagenomic analysis of microbial community composition and function

Metagenomic analysis was used to characterize the composition and function of the microbial community in the rhizosphere and sludge of *N. oleander* and *A. donax*. Eight metagenomic samples were prepared, including the rhizosphere of *N. oleander* (RN), rhizosphere of *A. donax* (RA), sludge of *N. oleander* (SN), and sludge of *A. donax* (SA) from July 2015 to March 2016 samplings. The duplicate DNA samples were pooled in equal amounts based on concentration. The same amount of each sample was used to construct eight paired-end libraries (350 bp insert size) using an Illumina TruSeq DNA Sample Preparation Kit (Illumina, Inc., San Diego, CA, USA). Cluster generation was performed on an Illumina cBot automated cluster generation system per the manufacturer's instructions. The prepared templates were loaded on the Illumina HiSeq 2500 system for sequencing (150 bp paired-end reads). The 280,662,176 raw sequence reads were initially filtered to remove adapters, low-quality reads, and reads that belonged to the host. The filtered reads were directly uploaded to the MG-RAST (Meta Genome Rapid Annotation using Subsystem Technology, v3.2.2) website (<http://metagenomics.anl.gov/>) for taxonomic classification and functional annotation using the MG-RAST pipeline (Meyer et al. 2008). Microbial taxonomy was based on protein sequences determined using the RefSeq database, which can identify both prokaryotes and eukaryotes.

For community functional annotation, MG-RAST was performed with the recommended parameters (Gregoracci et al. 2012) to assign reads to different functional groups using the SEED subsystems database or metabolic pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. In this study, we focused on the metabolism of C and N pollutants. Metabolic genes present in the biofilm or sludge were identified, and the abundances of those gene sequences were calculated. PCoA of microbial composition and function was performed with PAST 3.14 software (Hammer and

Harper 2006). Statistical analysis by the metagenomic profile (STAMP) bioinformatics software (Parks and Beiko 2010) was used to compare community composition and function between the *N. oleander* and *A. donax* samples. Statistical significance was calculated using two-sided Fisher's exact tests, and the differences between proportions were analyzed using the Newcombe-Wilson method with 95% confidence interval. Heatmaps were plotted with "pheatmap" in R (RCORETeam 2015).

All 16S rRNA gene sequencing data and metagenomic datasets were archived in the NCBI Sequence Read Archive (SRA) database (Bioproject, PRJNA376229).

Results

To understand the microbial role in wastewater treatment in the ecological treatment process, we first investigated the treatment effect of the ecological process and then linked the treatment effect with the microbial biomass, diversity, and function in influent and effluent wastewater and the interior biosystem of the ecological process.

Effects of different treatment processes

In accordance with long-term monitoring of COD and NH₃-N removal, our sampling results (Table S2) also demonstrated that the ecological treatment unit made a substantial contribution to C and N removal, with 27.2–80.7% removal of DOC and 59.0–76.1% removal of TN. After ecological treatment, NH₃-N decreased sharply (85.7–99.7% removal) and NO₃-N increased markedly (1.1-fold to 37.4-fold) in the effluent, suggesting considerable ammonia oxidation during treatment. Additionally, 47.6% of polycyclic aromatic compounds (one time sampling) were also removed. In conclusion, the ecological process showed considerable contribution to pollutant removal.

Bacterial biomass, diversity, and composition in wastewater under different treatment processes

Variations in the bacterial counts in the wastewater of different processes and the receiving river are shown in Fig. 2. Results revealed that the ecological process significantly decreased the amounts of bacteria (80% removal on average) in the influent (i.e., ECi), suggesting most microbes in the influent were stored or degraded in the ecological unit. In addition, after a series of treatments, the effluent bacteria did not significantly affect the bacterial concentration in the receiving river water (Fig. 2).

To understand the variation in bacterial diversity under different treatment processes and in river discharge, MiSeq 16S rRNA gene sequencing was employed for July 2015 and

Fig. 2 Variation of bacterial amounts in the treated wastewater and receiving river. ECi: influent of ecological treatment unit; ECe: effluent of ecological treatment unit; MFe: effluent of microfilter unit; Up: upstream of receiving river; Down: downstream of receiving river. Box plot was constructed based on data from three sampling periods ($n = 9$). ** denotes $p < 0.01$ (Wilcoxon signed-rank test)

March 2016 water samples, which produced 516,685 qualified sequences (more than 17,099 sequences per sample). Chao1 richness and phylogenetic diversity (PD) whole tree analysis (Fig. S2) showed that the variation trends of bacterial richness and diversity across the sequential processes were distinct between the two sampling times, suggesting that the role of different treatment processes in microbe removal was associated with seasonal effects. In addition, PCoA (Fig. 3, left) revealed that the ecological process was most efficient in altering the bacterial community composition in wastewater, as the community dissimilarity between the influent and effluent was more apparent than that of the other processes. Although the treated wastewater was discharged into the receiving river with around 20% dilution, no obvious differences between upstream and downstream bacterial composition were observed. This strongly suggested that wastewater discharge had only a minor role in the river microbial ecosystem. To determine which community groups changed during treatment and migrated to the river, we investigated bacterial taxonomy at the phylum (Fig. S3) and family levels (Fig. 3, right). At the phylum level, *Proteobacteria* were dominant in all water samples, but the abundance varied significantly with treatment. At the family level, the abundance of *Comamonadaceae* affiliated with *Betaproteobacteria*—a specialist for degradation of a number of organic xenobiotics (Boon et al. 2001; Khan et al. 2002; Tancsics et al. 2010), varied markedly. In the July sampling, *Comamonadaceae* increased (from 3.4 to 25.2%) after ecological treatment and was still abundant after microfiltration and UV disinfection. However, in the March sampling, *Comamonadaceae* decreased from 20 to 9% after ecological treatment. When the effluent containing *Comamonadaceae* was discharged to the river, the original abundance of *Comamonadaceae* in the river remained unchanged, suggesting that the other bacteria in the river might outcompete the introduced *Comamonadaceae*.

Microbial community traits in ecological treatment

The biomass of rhizosphere and sludge microbes under the roots of both plant species (July 2015 and March 2016 sampling) were roughly evaluated, and results (Fig. S4) revealed that the rhizosphere biomass of *N. oleander* was significantly higher than (2.17-fold on average) that of *A. donax*, but the sludge biomass of the two plants was similar.

Further, we investigated the microbial community composition and function using metagenomic sequencing. After sequence quality checking in MG-RAST, 6,886,782–57,296,771 filtered sequences for each sample were obtained, with an average length of 150 bp. Among the sequences, 39.98–47.09% of filtered sequences contained predicted proteins with known function. Comparing protein sequences against the RefSeq database, we obtained the taxonomic composition of the microbial community. This revealed that bacteria dominated (approximately 98% abundance) among the three domains and *Proteobacteria* was the most abundant phylum; eukaryotes accounted for ~1% of microbes in the samples. Remarkably, 39 eukaryotic phyla were identified in the ecological unit, and most were invertebrates (e.g., *Arthropoda* and *Nematoda*), suggesting that plants, animals, and microbes had formed a complicated ecosystem in the ecological treatment unit.

We compared the community diversities in the rhizosphere and sludge of roots of *N. oleander* and *A. donax*. According to alpha diversity analysis (Table S3), the community diversity indices of the rhizosphere and sludge of both plants were similar; however, the microbial community in *A. donax* was more sensitive to seasonal effects than that in *N. oleander*. Beta diversity analysis (Fig. 4a, b) showed that plant type was the primary driver for community composition variation, with rhizosphere and sludge samples from both plants grouped into different clusters. The data also clearly indicated that the microbial community in the rhizosphere and sludge of *A. donax* was more susceptible to seasonal alternation, as March and July data were distinctly separate.

Functional annotation provided a snapshot assessment of the potential microbial biodegradation occurring in the rhizosphere biofilm and sludge. To compare pollutant degradation between the two plants, we focused on aromatic compound degradation and inorganic N transformation (nitrification and denitrification), as they are most important in wastewater treatment. Against the SEED database, 248 aromatic compound metabolism genes were identified, and key nitrification (*amo*) and denitrification (*nar*, *nir*, *nor*, *nos*) genes were detected in all metagenomic datasets. We compared three typical categories of gene sequences reflecting aromatic compound degradation, e.g., benzoate degradation gene sequences, as benzoate is a key intermediary in the microbial metabolism of various aromatic compounds (Silva et al. 2012). As shown in Fig. 4c, compared with *A. donax*, the representative aromatic degradation genes in *N. oleander* samples (including

Fig. 3 Shift in bacterial community composition in **a** treated water and **b** receiving river. Left figure is PCoA and right is heatmap of bacterial composition (dominant family, > 1%). Influent: influent of treatment plant, ECi: influent of ecological treatment unit, ECe: effluent of

ecological treatment unit, MFe: effluent of microfiltration unit, Effluent: effluent of treatment plant, up1: site 1 upstream, Up2: site 2 upstream, Down1: site 1 downstream, Down2: site 2 downstream. Samples were collected in July 2015 and March 2016

rhizosphere biofilm and sludge) were more abundant ($p < 0.05$, paired-sample sign test) in July, but were similar in March. In contrast, the abundances of nitrification and denitrification genes in both plant species were not significantly different in either July or March samples (Fig. 4c). These results also suggest that the function of the microbial community associated with *N. oleander* was more stable than that of *A. donax*.

Discussion

Ecological treatment is an emerging process for wastewater management. It is similar to constructed wetlands as they both rely on an established plant-microbe-animal ecological system. In contrast with constructed wetlands, which use specialized soil microorganisms for treating wastewater (Bouali et al. 2013), the ecological process mainly uses bacteria from the influent wastewater and initial sludge.

Treatment effect and underlying mechanism of ecological treatment

In addition to water quality comparison, we also compared the bacterial community variation in the influent/effluent of different treatment units. Our results demonstrated that ecological treatment decreased the quantity of bacteria in the influent (Fig. 2). Some of bacteria from the influent were stored in the plant roots or in sludge under the roots and, therefore, performed nutrient utilization and micropollutant degradation. A great number of N transformation genes and aromatic compound biodegradation genes were identified, solidifying the above conclusion. Unlike other treatment processes (grids, microfiltration, and UV disinfection), the ecological unit distinctly altered the composition of the wastewater microbial community.

Due to the significant decrease in the microbial amount and diversity in wastewater following ecological treatment, together with the subsequent processes, the discharged wastewater diluted the concentrations of pollutants and bacteria in the receiving river. Moreover, the inflow of treated wastewater

Fig. 4 Composition and functional traits of microbial communities in rhizosphere and sludge of *Nerium oleander* and *Arundo donax*. **a** PCoA of community composition. **b** PCoA of community function. **c** Aromatic compound metabolism-related and nitrification- and denitrification-related gene sequences retrieved in eight metagenomic datasets. Values

were normalized by randomly sampling 1,000,000 filter reads per sample. RN: rhizosphere of *Nerium oleander*, SN: sludge of *Nerium oleander*, RA: rhizosphere of *Arundo donax*, SA: sludge of *Arundo donax*. Suffix letter J denotes samples from July 2015 and M denotes samples from March 2016

did not obviously change the microbial community structure in the receiving river water and, thus, maintained the river's original microbial function.

Selection of cultivated plants for ecological treatment

The cultivated plants in current ecological treatment are emergent aquatic plants and most of them are affiliated with *Angiospermae*. In the rhizosphere of emergent aquatic plant, plant roots provide a favorable habitat and exudate for the growth of microbes, which is of benefit for pollutant removal (Shaw et al. 2006; Zhang et al. 2013; Zou et al. 2013). However, different plants have different rhizosphere structures, possibly leading to different effects on pollutant removal in wastewater. The plants selected for the ecological treatment process should exhibit (i) the ability to utilize the nutrients from wastewater, (ii) adaptation to the local climatic conditions, and (iii) a strong rhizosphere and ease of microbial attachment (Loan et al. 2014; Nhapi et al. 2003). *N. oleander* is a common plant for dry ecosystems, whereas *A. donax* is a halophyte-like plant that can provide its rhizosphere with root exudates (such as carbohydrates, amino acids/amines, and organic acids) and oxygen (Idris et al. 2012; Nackley and Kim 2015). The

treatment performance of these two plants could probably be partially attributed to the high abundance of two degradation specialists of organic xenobiotics present in their rhizospheres, *Comamonadaceae* and *Burkholderiaceae* (10.0–15.7% abundance, Table S4) (Lunsmann et al. 2016), as well as the high presence of catabolic and N-oxidizing genes (Fig. 4c).

Considering that (i) the biomass of *N. oleander* was higher than that of *A. donax*; (ii) the community diversity of *A. donax* was also more sensitive to seasonal effects; and (iii) the aromatic degradation gene abundances in the *N. oleander* samples were higher than those in *A. donax*, we conclude that microbes surrounding *A. donax* were more easily aggregated in the rhizosphere and may make a greater contribution to the pollutant removal. This might provide a valuable reference for the selection of cultivated plants in the ecological treatment process.

Biodegradation pathway identification of micropollutants based on enzyme information

Based on the metagenomic analysis, we estimated the dominant pathways of some micropollutants. Atrazine is a frequently detected herbicide in surface water, groundwater, and wastewater and is typically measured at very low (ng/l

to $\mu\text{g/l}$, in our study) levels (Hua et al. 2006). Previously, at least three microbial metabolic pathways for atrazine that funnel into cyanuric acid metabolism were documented in pure culture degradation experiments (BoundyMills et al. 1997; Desouza et al. 1995; Nagy et al. 1995; Sadowsky et al. 1998). In this research, up to 0.05 $\mu\text{g/l}$ of atrazine was detected in the wastewater (Table S2). It was difficult to determine which biodegradation pathway it undergoes during the treatment by chemical measurement, due to the fact that the concentrations of intermediates would be too low to detect. By metagenomics, we identified the presence of key enzymes (Table S5), hydroxyatrazine ethylaminohydrolase (*atzB*), N-isopropylammelide isopropylaminohydrolase (*atzC*), and cyanuric acid amidohydrolase (*atzD*), in the rhizosphere and sludge samples. This indicated that the dominant atrazine biodegradation pathway in the ecological treatment process was atrazine to hydroxyatrazine to N-isopropylammelide to cyanuric acid (Bai et al. 2014; Gao et al. 2010). For PAH degradation, several dioxygenases, e.g., PAH dioxygenase large subunit (*nidA*) and small subunit (*nidB*), phthalate 4,5-dioxygenase, and extradiol dioxygenase, were found, indicating that deoxygenation frequently occurred during PAH metabolism (DeBruyn et al. 2007; Peng et al. 2008). Thus, the metagenomic information obtained here provides a clue to the micropollutant degradation pathway.

In summary, three main outcomes were yielded in this study, i.e., (i) ecological treatment removed around 80% of microbes in the influent and obviously altered the bacterial community composition of wastewater, with a great change in *Comamonadaceae*, a specialist for organic xenobiotics degradation; (ii) biomass and metagenomic analysis of the rhizosphere biofilm and sludge under the roots of two typical plant species suggested that the microbes in the *N. oleander* root habitat provided a greater contribution to pollutant removal than the rhizospheric microbes of *A. donax*; and (iii) some micropollutant biodegradation pathways were estimated by metagenomics, e.g., the dominant atrazine biodegradation pathway: from atrazine to hydroxyatrazine to N-isopropylammelide to cyanuric acid.

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Compliance with ethical standards

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

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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