Microbial community compositions in different functional zones of Carrousel oxidation ditch system for domestic wastewater treatment

Dong Xu1,2, Sitong Liu*, Qian Chen1,2 and Jinren Ni1,2,3*

Abstract

The microbial community diversity in anaerobic-, anoxic- and oxic-biological zones of a conventional Carrousel oxidation ditch system for domestic wastewater treatment was systematically investigated. The monitored results of the activated sludge sampled from six full-scale WWTPs indicated that Proteobacteria, Chloroflexi, Bacteroidetes, Actinobacteria, Verrucomicrobia, Acidobacteria and Nitrospirae were dominant phyla, and Nitrospira was the most abundant and ubiquitous genus across the three biological zones. The anaerobic-, anoxic- and oxic-zones shared approximately similar percentages across the 50 most abundant genera, and three genera (i.e. uncultured bacterium PeM15, Methanosaeta and Bellilinea) presented statistically significantly differential abundance in the anoxic-zone. Illumina high-throughput sequences related to ammonium oxidizer organisms and denitrifiers with top50 abundance in all samples were Nitrospira, uncultured Nitrosomonadaceae, Dechloromonas, Thauera, Denitratisoma, Rhodocyclaceae (norank) and Comamonadaceae (norank). Moreover, environmental variables such as water temperature, water volume, influent ammonium nitrogen, influent chemical oxygen demand (COD) and effluent COD exhibited significant correlation to the microbial community according to the Monte Carlo permutation test analysis (p < 0.05). The abundance of Nitrospira, uncultured Nitrosomonadaceae and Denitratisoma presented strong positive correlations with the influent/effluent concentration of COD and ammonium nitrogen, while Dechloromonas, Thauera, Rhodocyclaceae (norank) and Comamonadaceae (norank) showed positive correlations with water volume and temperature. The established relationship between microbial community and environmental variables in different biologically functional zones of the six representative WWTPs at different geographical locations made the present work of potential use for evaluation of practical wastewater treatment processes.

Keywords: Carrousel oxidation ditch, Biologically functional zone, Wastewater treatment, Microbial community structure, Illumina high-throughput sequencing

Introduction

Oxidation ditches are widely applied in wastewater treatment, especially in the small-scale and medium-scale wastewater treatment plants (WWTPs) in China (Zhang et al. 2016). As a modified activated sludge process, oxidation ditches have distinct advantages in simple construction, flexible operating mode, low sludge production, and special capability of nitrification and denitrification within the same tank. An oxidation ditch is often used to achieve simultaneous nitrification and denitrification because of the alternation of aerobic and anoxic zones within a channel, which can be formed by regulating the oxygen supply with surface aeration equipment or surface aeration combined with microporous aeration (Ammary and Radaideh 2005; Liu et al. 2010; Jin et al. 2015; Zhou et al. 2015).
The Carrousel oxidation ditch system, usually with an external pre-anaerobic zone and an internal pre-anoxic zone, is one of the most commonly used type of oxidation ditches (Peng et al. 2008; Jin et al. 2014). Carrousel oxidation ditch is designed to achieve an excellent condition for denitrifying and nitrifying bacteria in alternating anoxic–oxic zones in the looped channel system. In recent decades, operational conditions (e.g. temperature, hydraulic retention time, dissolved oxygen and operational mode), ditch geometry, aerator design and mode of aeration have been extensively investigated to optimize the treatment process and enhance nitrogen removal (Liu et al. 2010; Saida et al. 2010; Jin et al. 2015). Meanwhile, mathematical models have also been developed for simulating hydrodynamics, oxygen mass transfer, carbon oxidation, nitrification and denitrification processes, and optimizing the complicated alternating aerobic and anoxic conditions in oxidation ditches (Saida et al. 2010; Xie et al. 2011; Yang et al. 2013; Lei and Ni 2014).

As an important microbially mediated process for wastewater treatment and nitrogen removal, the efficiency and stability of Carrousel oxidation ditch system is entirely dependent upon the concerted and syntrophic activity of microorganisms belonging to different functional nitrifying, anammox and denitrification (Vanwonterghem et al. 2014; Rodríguez et al. 2015). Recently, several molecular technologies based on microbial 16S rRNA have been used to investigate the microbial community structures in oxidation ditch system (Zheng et al. 2015; Xia et al. 2016). For instance, Guo et al. (2013) studied the community structure of the nitrifying bacteria using fluorescence in situ hybridization (FISH). Zhou et al. (2015) identified the simultaneous nitrification and denitrification in an oxidation ditch by observing coexistence of nitrifying and denitrifying bacteria as well as some microaerophilic microorganisms using FISH and polymerase chain reaction–denaturing gradient gel electrophoresis methods. Jin et al. (2015) investigated the effect of different aeration modes on microbial communities of a Carrousel oxidation ditch by high-throughput 454 Pyrosequencing, of which activated sludge samples were sampled from anoxic and oxic zones and mixed with same proportion.

In this study, Illumina high-throughput sequencing was employed to reveal the microbial community diversity and structure of the activated sludge sampled from six full-scale WWTPs with Carrousel oxidation ditch systems. The core microbial populations and distribution of ammonium oxidizer organisms and denitrifiers in different functional zones in Carrousel oxidation ditch systems were studied. Differentially abundant features of the core microorganisms between the three biological functional zones and between the six different geographically located WWTPs were evaluated. More importantly, relationship between microbial community and environmental variables were established, which is of significance to diagnosis of practical wastewater treatment processes.

**Materials and methods**

**Sample collection and determination**

The investigated six full-scale Carrousel oxidation ditch systems (all equipped with an external pre-anaerobic zone and an internal pre-anoxic zone) respectively belong to six WWTPs at geographically different location in China, represented by XJYQ, ZZLQ, HBLY, BJYF, GDHZ and MYYX. The descriptions of these WWTPs were shown in Additional file 1: Table S1. The anaerobic-(A1), anoxic-(A2) and oxic-(O) activated sludge samples were collected respectively from the corresponding biologically functional zones in Carrousel oxidation ditch systems. Each activated sludge sample was fixed on site by mixing with 50% ethanol (v/v) on site, kept in an ice box for transport and stored at $-20\,^\circ\mathrm{C}$ in laboratory before DNA extraction. Concentrations of chemical oxygen demand (COD) and ammonium nitrogen in the influent/effluent of the WWTPs were measured according to standard analytical procedures (Clesceri et al. 1998). The level of pH and DO was determined on site by a pH sensor (pHS-25) and a DO sensor (WTW Oxi 3401), respectively.

**DNA extraction**

Genomic DNA was extracted from each activated sludge sample using the PowerWater DNA Isolation Kit (MO BIO, CA, USA) according to manufacturer’s protocols. The extracted DNA samples were stored at $-20\,^\circ\mathrm{C}$ for subsequent assays. The intact DNA was confirmed on 1.5% agarose gel electrophoresis. The concentration and quality of the extracted DNA were assessed with a Nanodrop2000 microspectrophotometry (Thermo Scientific, DE, USA).

**Polymerase chain reaction (PCR) amplification and high-throughput sequencing**

The hypervariable V3–V4 region of 16S rRNA genes were amplified from all DNA extracts with barcoded primers 340F (CCTACGGGNGGCAGCAG) and 805R (GACTACNVGGGTATCCTAATCC) under following conditions: initial denaturation at 95 °C for 3 min, followed by 30 cycles at 95 °C for 30 s, 50 °C for 30 s and 72 °C for 60 s and final extension at 72 °C for 7 min by the LabCycler PCR (Sensoquest, Germany). The 50 µL PCR mixture contained 5 µL of 10× buffer, 38.8 µL of 5 dH2O, 1 µL of dNTP (10 mM), 2 µL of each primer, 0.2 µL of KAPA Taq polymerase (KAPA Biosystems, USA) and 1 µL of genomic DNA. After confirmed by 1.5% agarose
gel electrophoresis, the PCR products were mixed to get equal concentration of DNA fragment for each sample and purified using MinElute PCR Purification Kit (QIAGEN, Germany), and then were sequenced using the Illumina Hiseq2500 PE250 platform.

High-throughput sequencing data analysis
All the obtained paired-end reads of 16S rRNA gene PCR amplicons were quality filtered and denoised to remove low quality or ambiguous reads. Then the treated forward and reverse reads were merged with PANDAseq (Masella et al. 2012). The putative chimeric sequences were identified and excluded with USEARCH61 pipeline (Edgar et al. 2011) in QIIME (Caporaso et al. 2010). The remaining set of high quality sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity threshold using UCLUST methods (Edgar 2010) embedded in QIIME. The taxonomic identities of the representative sequences from each OTU were classified via the Ribosomal Database Project (RDP) classifier with the SILVA databases (Wang et al. 2007). The Illumina sequencing raw data obtained from this study were deposited in the NCBI Sequence Read Archive with accession No. SRP093686 (PRJNA354474).

Statistical analyses
Heatmap of the top 50 genera in each sample was conducted using R packages. The open source software Cytoscape v3.3.0 (Shannon et al. 2003) was employed for clustering network analysis to visualize the most abundant OTUs and to compare their abundance among the different samples. The linear discriminant analysis (LDA) effect size (LEfSe) pipeline (http://huttenhower.sph.harvard.edu/galaxy/) (Segata et al. 2011) was used to identify differentially abundant features among the different biological functional zones of Carrousel system and different sampling WWTPs. The differential features were identified on the OTU level (relative abundance >1%). The non-parametric factorial Kruskal–Wallis (KW) rank sum test was used to detect taxa with significant differential abundances. LDA was used to evaluate the effect size of each differentially abundant trait. The LEfSe analysis was performed under the alpha value for the Kruskal–Wallis test is <0.05, and the threshold on the logarithmic LDA score for discriminative features is >2.0 (Zhang et al. 2013). Principal component analysis (PCA) was conducted using Canoco 4.5 (Microcomputer Power, USA). Redundancy analysis (RDA), a form of constrained ordination, was employed to analyze the relationships between the abundance of core genera and environmental variables using R software with the vegan and ggplot2 packages.

Results
Biodiversity and microbial community profiles in Carrousel system
Using Illumina Sequencing of 16S rRNA Gene Amplicons, 8017-10681 OTUs obtained from the individual activated sludge sample. The OTUs, Good’s coverage, Chao1, ACE, Shannon and Simpson of each sample could be seen in Additional file 1: Table S2. Forty-eight bacterial and 7 archaeal samples were retrieved from 164,591 Illumina effective sequences obtained from the 18 activated sludge samples, while the reads for archaeal phyla only accounted 1.22% of the total phyla.

The major phyla in each sample (the sequence percentage is above 2% in at least one WWTP) were Proteobacteria, Chloroflexi, Bacteroidetes, Actinobacteria, Acidobacteria, Verrucomicrobia, Gemmatimonadetes, Planctomycetes, Parcubacteria, TA06, Chlorobi, Woesearchaeota (DHVEG-6) and Bacteria (norank) (Fig. 1). Proteobacteria was the most dominant phylum and accounted 33.9–50.9% of all the phyla, followed by Chloroflexi (12.1–18.8%), Bacteroidetes (6.6–20.8%), Actinobacteria (5.1–11.8%), Verrucomicrobia (1.4–9.4%), Acidobacteria (1.4–6.3%) and Nitrospirae (0.8–6.1%). At the class level for Proteobacteria, β-proteobacteria represented 13.1–25.1% of total sequences, followed by δ-proteobacteria (5.2–9.0%), α-Proteobacteria (5.4–8.4%) and γ-proteobacteria (4.1–9.4%).

At the genus level, a total of 1594 genera were identified in the 18 activated sludge samples. Relative abundance of the top 50 genera from the anaerobic-(A1), anoxic-(A2) and oxic-(O) zones in the six WWTPs is shown in Fig. 2. These top 50 genera belong to 11 bacterial phyla (including Nitrospirae, Proteobacteria (α-, β-, γ-, δ- and norank), Bacteroidetes, Gemmatimonadetes, Actinobacteria, Chloroflexi, Verrucomicrobia, Parcubacteria, Latecibacteria, TA06 and Acidobacteria) and an archaeal phylum of Woesearchaeota (DHVEG-6).

The most abundant genus is Nitrospira with a relative abundance varying from 0.8 to 6.1% in individual samples, while the second is Gemmatimonadaceae (belonging to Gemmatimonadetes phylum) with a relative abundance varying from 1.1 to 3.6% in individual samples. The other top 10 abundant genera detected in all samples were Comamonadaceae (no rank), Thauera, uncultured Nitrosomonadaceae, Dechloromonas and Rhodocyclaceae (no rank) belonging to β-proteobacteria, Candidatus Microthrix belonging to Actinobacteria phylum, OPB35 soil group (no rank) belonging to Verrucomicrobia phylum, and Chloroflexi (no rank and uncultured) belonging to Chloroflexi phylum.
Distribution of ammonium oxidizer organisms and denitrifiers

Nitrification and denitrification occur in Carrousel oxidation ditch wastewater treatment system for the catalysis of physiologically distinct clades of involved ammonium oxidizing bacteria (AOB) (oxidation of ammonium to nitrite), nitrite-oxidizing bacteria (NOB) (oxidation of nitrite to nitrate), and/or complete ammonium oxidizer (Comammox) (complete oxidation of ammonium to nitrate), and denitrifiers (reduction of nitrate via nitrite and intermediate gaseous nitrogen oxide products to dinitrogen). Illumina sequences associated with ammonium oxidizer organisms and denitrifiers of anaerobic-(A1), anoxic-(A2) and oxic-(O) activated sludge samples of the six WWTPs were shown in Fig. 3. *Nitrospira* (including detected *Candidatus Nitrospira defluvii* spp. and other two uncultured *Nitrospira* organisms) can conduct oxidation of nitrite to nitrate, with relative abundance over 3% of the activated sludge samples of the investigated WWTPs except for XJYQ. *Nitrosomonadaceae* (uncultured) as a group of the main contributors for oxidation of ammonium to nitrite occupied 1.5 to 3.8% of each sample from the WWTPs of BJYF, GDHZ and MYXY. The ratio of NOB to AOB [i.e. *Nitrospira/Nitrosomonadaceae* (uncultured)] ranged from 119 to 687% of all samples, and HBLY and ZZLQ might highlight the excellent nitrification process for the high ratios (406–687%).

Five denitrifying groups, *Dechloromonas*, *Thauera*, *Denitratisoma*, *Rhodocyclaceae* (norank) and *Comamonadaceae* (norank) with relative abundance ranged from 0.2 to 4.8% were detected in all the samples. *Dechloromonas*, *Thauera*, *Denitratisoma* and *Rhodocyclaceae* (norank) belongs to *Rhodocyclaceae* family.

PAC revealed that samples of anaerobic-(A1), anoxic-(A2) and oxic-(O) zone from each WWTP were grouping together, but the relative abundance of the ammonium oxidizers and denitrifiers differed apparently with different WWTPs (Fig. 3b). Among the six WWTPs, the most relative abundant genera involved in nitrogen transformation in BJYF were *Dechloromonas* (3.1–3.8%), *Nitrosomonadaceae* (uncultured) (2.7–6.1%) and *Denitratisoma* (1.8%), and in XJYQ were *Thauera* (2.9–4.8%) and *Comamonadaceae* (norank) (3.7–4.3%), while that in ZZLQ and GDHZ exhibited *Nitrospira* (4.8–6.1%) and *Rhodocyclaceae* (norank) (2.5–3.4%), respectively. XJYQ presented the highest relative abundance of the sum of the five denitrifiers among the six WWTPs, while MYXY was the lowest. The differences in wastewater quality and environmental factors may result in the bacterial abundant discrepancies among the WWTPs.

Abundance differences between the 3 biological functional zones

Clustering network analysis by Cytoscape was applied to gain a better insight into the differences of the anaerobic-, anoxic- and oxic-zones in carrousel oxidation ditch system. Figure 4 showed the most abundant 50 OTUs and presented the relative distribution and abundances between the 3 biological functional zones. *Nitrospira* (including *Candidatus Nitrospira defluvii* spp.
and other two uncultured Nitrospira organisms) was the most abundant and ubiquitous bacterial genus with dominant occurrence among the 3 zones. The organisms involved in ammonium-oxidizing and denitrification as Nitrosomonadaceae, Denitratisoma, Thauera, Dechloromonas were also shared by the anaerobic-, anoxic- and oxic-zones with nearly the same percentage of the weighted-degree of the nod. Furthermore, the anaerobic-, anoxic- and oxic-zones shared approximately similar percentages across the 50 most abundant OTUs.

The differentially abundant features among the anaerobic-, anoxic- and oxic-zones in carrousel oxidation ditch system were identified by LEfSe analysis (Fig. 5).

The results showed that the taxa with significantly differential abundances were only detected from the anoxic-zone (A2). Figure 5a presented that 13 microbial clades showed statistically significant and biologically consistent differences in the anoxic-zone. The significantly differential abundant taxa in the anoxic-zone belonged to an archaeal phylum of Euryarchaeota, two bacterial classes of Actinobacteria and γ-Proteobacteria and an archaeal class of Methanomicrobia, and 3 genera of uncultured bacterium PeM15, Methanoseta and Bellilinea (Fig. 5b). γ-proteobacteria had the highest LDA score, followed by Actinobacteria class and Bellilinea genus (within the phylum of Chloroflexi, class of Anaerolineae, order of Anaerolineales and family of Anaerolineaceae).
Fig. 3 Distribution of ammonium oxidizers and denitrifiers of the 18 activated sludge samples. a Percentages of ammonium oxidizers and denitrifiers, b PCA based on the abundance

Fig. 4 Profile clustering Cytoscape network visualize the top 50 abundant OTUs across the anaerobic-zone (A1) (red), anoxic-zone (A2) (green) and oxic-zone (O) (blue) in carrousel oxidization ditch system. Node sizes pie chart presented the relative abundances of the OTU over the respective samples.
Abundance differences between the geographically distributed Carrousel oxidation ditches

Six full-scale Carrousel oxidation ditch WWTPs represented by XJYQ, ZZLQ, HBLY, BJYF, GDHZ and MYYX, which geographically locate at Northwest, Central South region, Central region, Southern and Southwest respectively, of China were investigated. The most abundant 50 OTUs and the relative distribution and abundances between the 6 WWTPs were analyzed by Cytoscape network analysis (Fig. 6). The dominant and ubiquitous bacterial genera among all the 6 WWTPs were *Nitrosospira* (including *Candidatus Nitrospira defluvii* spp. and uncultured organism *Nitrospira*), *Candidatus Microthrix*, *Anaerolineaceae*, uncultured Chloroflexi, uncultured Nitroso monadaceae, *Acidimicrobiales*, *Thaurea*, *Dechloromonas* and *Denitratisoma*; however, the abundance proportion of each WWTP presented obvious discrepancy.

To gain insight into the differences of the six Carrousel oxidation ditches, LEfSe analysis was employed (Fig. 7). The results showed that the significant differential abundances occurred in three WWTPs i.e. GDZH, MYYX and ZZLQ, of which 36 differentially abundant taxonomic clades were found. Taxa of *Nitrospiraceae*, *Nitrospira*, *Nitrospirales* and *Acidobacteria* in ZZLQ had the highest LDA score among all OTUs. *Chloroflexi* and *Haliangium* presented the highest LDA score in the OTUs of MYYX and GDZH, respectively.

The most differentially abundant taxa in ZZLQ belonged to two bacterial phyla of *Nitrospirae* and *Actinobacteria*, while that in MYYX belonged to two bacterial phyla of *Chloroflexi* and *Gemmatimonadaceae*, and that in MYYX belonged to a bacterial phylum of *Planctomycetes* (Fig. 7b). At family level, 7 differentially abundant taxonomic clades were detected in ZZLQ (including *Hyphomicrobiaceae*, *Planctomycetaceae*, *Nitrospiraceae*, uncultured soil bacterium *JG30-KF-CM45*, uncultured bacterium *KD4-96, DS-100* and *GR-WP33-58*), while that of 2 in MYYX (*Gemmatimonadaceae* and I-10) and one in GDZH (*Haliangiaceae*). At genus level, also 7 differentially abundant taxonomic clades were detected in ZZLQ (including *Hyphomicrobiun*, *Planctomycetes*, *Nitrospira*, uncultured soil bacterium *JG30-KF-CM45*, uncultured bacterium *KD4-96, DS-100* and uncultured *XanthomonadalesIncertaeSedis*), while 2 differentially abundant taxonomic clades were detected in MYYX (*uncultured Gemmatimonadaceae* and uncultured bacterium *I-10*) and one in GDZH (*Haliangium*). Except the genera within the above phyla, 4 differentially abundant genera of *Hyphomicrobiun* (within *Hyphomicrobiaceae* family and *Rhizobiiales* order and *α-proteobacteria*), uncultured bacterium *I-10* (within *I-10* family and *Rhodospirillales* order and *α-proteobacteria*), *Haliangiun* (within *Haliangiaceae* family and *δ-Proteobacteria* calss) and uncultured *XanthomonadalesIncertaeSedis* (within *Xanthomonadales* order, *γ-proteobacteria*) were also detected.

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**Fig. 5** LEfSe analysis results of the anaerobic-, anoxic- and oxic-zones in carrousel oxidization ditch system. **a** LDA scores to feature differentially abundant taxa (yellow represented non-significant, and red indicated the anoxic-zone). Each circle’s diameter was proportional to the taxonomic abundance.
The relationships between environmental variables and microbial community

The relationship between environmental variables and the abundance of microbial community referred to the major genera (top50) relative abundances were identified by RDA (Fig. 8). Nine environmental variables including geographical location parameters (i.e. eastern longitude and northern latitude of the WWTP), operating parameters of each WWTP (i.e. water volume, influent/effluent concentration of COD and ammonium nitrogen), and physicochemical parameters of each sample (i.e. water temperature and dissolved oxygen) (Additional file 1: Table S1) were investigated. Arrows indicate the direction and magnitude of environmental variables associated with bacterial community structures. The results showed that the eastern longitude and dissolved oxygen were not as strongly correlated to microbial community composition as the other environmental variables. Six environmental variables, i.e. water temperature, northern latitude, water volume, influent ammonium nitrogen, influent COD and effluent COD exhibited significant correlation to microbial community according to the Monte Carlo permutation test analysis ($p < 0.05$). Also, water temperature and northern latitude were the crucial factors shaped the variations in microbial community composition. Among the genera of ammonium oxidizer organisms and denitrifiers, the abundance of *Nitrospira*, *Nitrosomonadaceae* (uncultured) and *Denitratisoma* presented strong positive correlations with the northern latitude and influent/effluent concentration of COD and ammonium nitrogen, respectively, while *Dechloromonas*, *Thauera*, *Rhodocyclaceae* (norank) and *Comamonadaceae* (norank) presented positive correlations with the water volume and temperature.

**Discussion**

Proteobacteria, Chloroflexi, Bacteroidetes, Actinobacteria, Verrucomicrobia, Acidobacteria and Nitrospirae were dominating phyla across the anaerobic-, anoxic- and oxic-zones in Carrousel oxidation ditch system, and
accounted 75.2–85.8% of all sequences of the six investigated full-scale WWTPs. **Proteobacteria** (mainly including α-, β-, γ-, δ-proteobacteria) was the most dominant phylum and accounted 33.9–50.9% of all the monitored phyla. Previous reports on activated sludge from municipal WWTPs have showed that the above phyla are common and **Proteobacteria**, **Actinobacteria**, **Bacteroidetes** and **Verrucomicrobia** dominated in both DNA and cDNA sets (Yu and Zhang 2012; Cydzik-Kwiatkowska and Zielińska 2016); also **Proteobacteria** commonly dominated 21–65% of all phyla and the **β**-proteobacteria is the most abundant class which is mainly responsible for organic and nutrient removal (Nielsen et al. 2010; Hu et al. 2012; Wang et al. 2014b; Cydzik-Kwiatkowska and Zielińska 2016). Compared to common wastewater treatment activated sludge system, the phylum **Nitrospirae**, of which members are usually found as predominant NOB in WWTPs (Nielsen et al. 2010; Rodríguez et al. 2015), presented in the subdominant phyla in the Carrousel oxidation ditch system for nitrogen removal function.

The genus **Nitrospira** was the most abundant and ubiquitous bacterial genus across the anaerobic-, anoxic- and oxic-zones in the Carrousel oxidation ditch systems. The second most abundant genus is **Gemmatusmonadaeceae**, which family members had also been detected in activated sludge sewage treatment systems, and could grow by both aerobic and anaerobic respiration (Zhang et al. 2003; Shu et al. 2015). The other subdominant genera were **Comamonadaeceae** (no rank), **Thauera**, uncultured **Nitrosomonadaeceae**, **Dechloromonas**, **Rhodocyclaceae** (no rank) **Candidatus Microthrix parvicella** is been considered as a specialized lipid consumer with a physiological potential analogous to polyphosphate-accumulating organisms (PAOs) and glycogen accumulating organisms.
(GAOs) (Nielsen et al. 2002). The Verrucomicrobia OPB35 soil group, the most abundant prokaryotic taxa (Lanzén et al. 2015), were also detected in the 18 activated samples with the abundance ranged from 0.5 to 3.4%. The relative abundance of Chloroflexi (no rank and uncultured) ranged from 0.2 to 4.9% of the samples. Members of phylum Chloroflexi are commonly detected from sediment and involve carbon cycling, organohalide respiration, fermentation, CO2 fixation, and acetogenesis with ATP formation by substrate-level phosphorylation (Hug et al. 2013).

Illumina sequences associated with ammonium oxidizer organisms and denitrifiers included Nitrospira (including Candidatus Nitrospira defluviit, and other two uncultured Nitrospira organisms), uncultured Nitrosomonadaceae, Dechloromonas, Thauera, Denitratisoma, Rhodocyclaceae (norank) and Comamonadaceae (norank), which presented top50 abundant genera in all samples. Nitrospira as the most diverse known group of NOB in classical two-step nitrification theory (oxidation of ammonium via nitrite to nitrate), in which of Candidatus Nitrospira inopinata is recently discovered to be complete ammonium oxidizer (Comammox) organisms i.e., novel one step nitrification theory which completely oxidize ammonium to nitrate (Daims et al. 2015; van Kessel et al. 2015). Candidatus Nitrospira defluviit spp., a predominant nitrite oxidizer in WWTP, possesses a periplasmically oriented enzyme nitrite oxidoreductase (NXR), which differs from other known nitrite oxidizers as Nitrobacter and Nitrococcus (Lucker et al. 2010; Nielsen et al. 2010; Rodríguez et al. 2015). The periplasmic forms of NXR are considered to be more efficient because of more proton-motive force produced by per oxidized nitrite and no nitrite/nitrate transport across the cytoplasmic membrane (Lucker et al. 2010; Rodríguez et al. 2015). Nitrosomonadaceae (uncultured) presented a relative abundance varying from 0.27 to 3.8% in individual samples. Also, genus Nitrosomonas was detected in all the samples with relative low abundance of 0.03–0.35% (out of the top 50 genera). The genera within family Nitrosomonadaceae as Nitrosomonas and Nitrosospira are the most detected AOB in WWTPs (Li et al. 2016). The other dominant genera of Comamonadaceae (no rank), Thauera, Dechloromonas and Rhodocyclaceae (no rank) are capable of denitrification (Hwang et al. 2006; Lu et al. 2014). Rhodocyclaceae and Comamonadaceae (both within β-proteobacteria) were reported as the core families
with responsibility for denitrifying and aromatic degrad-
ing processes in wastewater treatment activated sludge
systems (Loy et al. 2005; Ma et al. 2015), of which *Thauera*
1 is one of the most often detected genera and functionally
important denitrifier in activated sludge systems (Lu et al.
2014; Ma et al. 2015). Furthermore, *Dechloromonas*-related
bacteria have been demonstrated with the capability of
nitrate/nitrite reduction, acetate uptake and polyphos-
phate and polyhydroxycacids storage (Cydzik-Kwiatkowska
and Zielińska 2016). Among the anaerobic-(A1), anoxic-
(A2) and oxic-(O) function zones, the abundances of AOB,
NOB and denitrifiers clustered together within each Car-
roussel oxidation ditch system (Fig. 3b), because Carroussel
oxidation ditch is a looped channel system, and the most
of the activated sludge separated from the final clarifier is
returned to the anaerobic-zone to maintain the proper bio-
mass level in the system.

Furthermore, the phylogeny-based UniFrac analysis
(Fig. 1), clustering network analysis (Fig. 4), and LEfSe
analysis (Fig. 5) demonstrated that the activated sludge
samples were certainly similar between those taken from
the same type of plant (i.e. the different biological zones).
The clustering network analysis showed that anaerobic-,
anoxic- and oxic-zones shared approximately similar per-
centages across the 50 most abundant OTUs. The stan-
dard deviation (SD, calculated by weighted-degree of the
three zones at a certain OTU) of the top 50 OTUs ranged
from 0.2 to 14.3, and Coefficient of variation (CV, calcu-
lated as the ratio of the SD to the mean and multiplied
by 100), evaluating dispersion of a probability distribu-
tion among the three zones, ranged from 3.0 to 19. Only
6 OTUs (referred to the genera of *Terrimonas*, *Caldini-
eaceae* (uncultured), *β-proteobacteria* (no rank), uncul-
tured *B1-7BS*, *Denitratissoma*, *Caldithrix* and uncultured
*Hydrogenophilaceae*) presented the CV value higher than
15, which indicated that the probability distributions of
the 6 OTUs exhibited relative higher dispersion degree
among the 3 biological functional zones. The LEfSe
analysis result indicated only13 microbial clades within
an archaean phylum of *Euryarchaeota* and two bacterial
classes of *γ-proteobacteria* (with the highest LDA score)
and *Actinobacteria*, showed statistically significant dif-
fferences in the anoxic-zone among the 3 biological zones
(Fig. 5). At genus level, there were 3 differential abundant
taxa (i.e. uncultured bacterium *PeM15*, *Methanoseta*
and *Bellilinea*) in the anoxic-zone among the 3 biologi-
cal zones. The members of *Actinobacteria* phylum have
been verified as important PAOs in enhanced biological
phosphorus removal systems, and also some may contain
nitrite reductase genes involved in denitrification (Rod-
íguez et al. 2015). *Methanoseta* within *Methanosarcina-
les* is an obligate aceticlastic methanogen (Rodríguez
et al. 2015). *Methanosarcinales* showed statistically
significant differences in the anoxic-zone among the 3
biological zones might be caused by the high acetate con-
centrations (Anderson et al. 2009), which were added into
the anoxic-zone as external carbon source to enhance the
denitrification process.

The 6 studied biological WWTPs with Carrousel oxi-
dization ditches process shared the major phyla (Fig. 1)
and the most abundant genera (top50) (Fig. 2), but were
dissimilar in the microbial abundance distribution. These
results were complying with previous reported microb-
ial community structure distribution in geographi-
cally distributed biological WWTPs (Xia et al. 2010; Shu
et al. 2015). The distribution of detected AOB, NOB and
denitrifiers also exhibited apparently differences among
the 6 WWTPs (Fig. 3). The significant differential abun-
dances occurred in three WWTPs i.e. GDZH, MYYX and
ZZLQ by the LEfSe analysis (Fig. 7), which indicated
that 36 microbial clades within 7 bacterial phyla of *Nitro-
spira*, *Planctomycetes*, *Gemmatimonadales*, *Chloro-
flexi*, *Acidobacteria* and *Proteobacteria* presented statisti-
cally significant differences. At genus level, there were 10 sig-
nificantly differential abundant taxa, of which genera
*Nitrosospirochaetes*, uncultured *Gemmatimonadales* and *Hal-
angium* showed the highest LDA score in the activated
sludge samples from the ZZLQ, MYYX and GDHZ,
respectively. Such discrepancies in microbial community
structures among the geographically distributed WWTPs
harbored the same biological treatment process might be
correlated with the different environmental conditions and
operating parameters among the different geographi-
cally located WWTPs (Hu et al. 2012; Xia et al. 2016).

The RDA analytical results (Fig. 8) showed that the abundance of *Nitrosospirochaetes*, uncultured *Nitrosomonadaceae*
and *Denitratisoma* presented strong positive correlations
with the northern latitude of the WWTP, and influent/
effluent concentration of COD and ammonium nitrogen.
*Dechloromonas*, *Thauera*, *Rhodocyclaceae* (norank) and
*Comamonadaceae* (norank) presented positive correla-
tions with the water volume and temperature. Among
the tested environmental variables, the temperature
and water quality play important parts in the microbial
metabolisms, nitrifying process and denitrification (Ju
et al. 2014; Lu et al. 2014; Wang et al. 2014a). The temper-
ature and influent ammonium nitrogen can impact the
abundance of AOB and the balance between AOB and
NOB in activated sludge systems (Cydzik-Kwiatkowska
and Zielińska 2016). Also, *Nitrosospirochaetes* and *Nitrosomonas*
have been verified more susceptible to seasonal variations
and displayed higher abundances in relative higher tem-
perature (Wan et al. 2011; Ju et al. 2014). In the present
study, the abundance of *Nitrosospirochaetes*, *Nitrosomonadaceae*
(uncultured) and *Denitratisoma* were greatly influenced
by not only the water quality, but also the northern
latitude of the WWTP. Shanks et al. (2013) reported that city latitude could result in the variation of the sewage infrastructure community composition among cities with a wide range of geographic locations. Zhang et al. (2012) found that although some detected microbial genera existed in all WWTPs, the microbial composition of biomass differed with different geographically location. Thus, city latitude might impact the microbial community structures of untreated wastewaters, which further influence the microbial community of biological treated system of WWTP. Also, the effects of city latitude on microbial composition might due to the fact that the temperatures, technological system and water quality of wastewater treatment vary in different locations (Cydzik-Kwiatkowska and Zielinska 2016). The northern latitude of the WWTP presented significant correlation with the water quality (i.e. influent/effluent concentration of COD and ammonium nitrogen) in this study (Fig. 8). However, further study on spatial and temporal dynamics of ammonium oxidizer organisms and denitrifiers in activated sludge system is needed. In general, the established relationship between microbial community and environmental variables in different biologically functional zones of the six representative WWTPs at different geographical locations made the present work of potential use for evaluation of practical wastewater treatment processes.

Additional file

Additional file 1: Table S1. Characteristics of the six representative full-scale WWTPs. Table S2: Raw and trimmed reads, Good’s coverage, Chao1, ACE, Shannon, Simpson, and plus numbers of OTUs of the activated sludge samples.

Abbreviations

WWTPs: wastewater treatment plants; FISH: fluorescence in situ hybridization; COD: chemical oxygen demand; PCR: polymerase chain reaction; OTUs: operational taxonomic units; LDA: linear discriminant analysis; LEfSe: linear discriminant analysis effect size pipeline; PCA: principal component analysis; RDA: redundancy analysis; AOB: ammonium oxidizing bacteria; NOB: nitrite-oxidizing bacteria.

Authors’ contributions

DX performed the research, analyzed data and wrote this manuscript. QC helped to draft the manuscript. SL and JN designed the research and polished the manuscript. All authors read and approved the final manuscript.

Author details

1. Department of Environmental Engineering, Peking University, Beijing 100871, China. 2. The Key Laboratory of Water and Sediment Sciences, Ministry of Education, Beijing 100871, China. 3. State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining 810016, Qinghai, China.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The data are included within the manuscript, and all data are fully available without restriction.

Consent for publication

This manuscript does not contain any individual person’s data.

Ethics approval and consent to participate

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

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