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Original Research

Performance and Microbial Community Analysis of a Constructed Rapid Infiltration System at Different Depths

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Abstract

To study the removal performance of a constructed rapid infiltration (CRI) system and its microbial community characteristics, we took the demonstration project of a CRI system that has successfully operated for 15 years as an example, aiming to analyze the CRI system's removal performances for COD, NH_4^+-N, TN and TP. Meanwhile, high-throughput sequencing technology was used for the first time to study the microbial community diversity and structure in the CRI system. The results showed that the average removal efficiencies for COD and NH_4^+-N were 75.52% and 92.94%, and the average removal efficiencies for TN and TP were respectively 39.74% and 42.78%. High-throughput sequencing technology indicated that a variety of bacterial phyla were found in CRI's bacterial communities, including Bacteroidetes, Actinobacteria and Acidobacteria, among which Proteobacteria dominated. At the genus level, a spatial variation was illustrated for the diversity and structure of bacterial communities. The dominant genera on the surface layer (0 cm) of CRI were mainly *Nocardioides*, *Sphingomonas*, *Bryobacter* and other microorganisms that can degrade organic matter, and the dominant genera in the inside (30-120 cm) were mainly microorganisms that play an important role in removing nitrogen. This study provided a theoretical basis for the long-term operation of a CRI system.

Keywords: constructed rapid infiltration (CRI), wastewater treatment, high-throughput sequencing, microbial community

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Introduction

A constructed rapid infiltration (CRI) system is a new sewage treatment technology based on a traditional rapid infiltration (RI) system [1-2]. In this system, natural sand with excellent permeability and a certain amount of special filler replaced traditional natural soil layers of sewage rapid infiltration [3]. Therefore, CRI solved the problem of traditional land's low hydraulic loading of treatment and large area coverage; meanwhile, dry-wet alternate operation greatly improved the reoxygenation capability and the removal effect on pollutants [2].

The CRI system has the advantages of low cost, low energy consumption, high operation efficiency, simple and convenient management and protection [4]. It can be applied to the polluted river and rural domestic sewage treatment [4], and it has been widely applied to more than 10 provinces, cities and regions, including Beijing, Guangdong Province, Sichuan Province, Shangxi Province, Hongkong, etc. [6]. In Yunnan Province, Chao et al. [6] has studied the operational effect of utilizing CRI to treat pollutants entering Lake Dianchi through Daqing River, and the result showed that CRI has a strong removal effect and shock resistance capability for organisms. Wu et al. [8] carried out seasonal operational effect analysis on a CRI and biological wetland integration system and got the result that CRI can remove each pollutant in rural domestic sewage strongly, and the removal efficiency for COD, TN, and TP reached the highest in summer, at respectively 92.2%, 87.0% and 85.9%. In addition, Li et al. [9] and Gao et al. [10] all got the result that the CRI system is capable of reducing the concentration of COD, NH_4^+-N, TN, and TP so as to adjust and recover domestic sewage effectively. However, some research has also indicated that CRI technology exposed some problems in the concrete operation process, such as easy blockage of fillers in the CRI tank [11], unstable nitrogen and phosphorus removal effect of the system [12]. Chen et al. [13] examined the domestic sewage treatment effect of a CRI system of Chongqing in winter and found that the effluent COD, NH_4^+-N and TP cannot reach the standard. This research took the CRI system with the largest scale and longest operational period (more than 15 years) in Sichuan Province as the object and tried to analyze its practical operational performance.

Moreover, the CRI system research was mostly about the design parameters and the improvement of the technology [14-16], while the amount of research on micro-organisms has been inadequate and the research methods were traditional. Luo et al. [17] studied the growth and distribution of each micro-organism zone in the CRI system at different temperatures, but the traditional cultivation method could only examine culturable micro-organisms whose number was less than 10% of the total number of micro-organisms [18]. Therefore, this method cannot reflect the primitive existence state of the micro-organism group; Xin et al. [19] utilized PCR-DGGE technology to research the space distribution regularity of ammonia-oxidizing bacteria, and although the technology overcame the disadvantages of traditional pure cultivation method of extracting total DNA of the analytical sample, it was not very feasible for accurate quantitative research. Compared with the test methods for traditional micro-organism community structure research, the metagenome analysis method based on high-throughput sequencing has the advantage of high sequencing throughput, high accuracy and excellent cost performance [20]. However, the reports about utilizing high throughput sequencing to research micro-organisms at different heights of the CRI system and to research community structures have rarely been seen.

The aim of this study was to investigate the performance of COD, NH_4^+-N, TN and TP removal in the CRI system, which has been operated successfully for 15 years and was a demonstration project in Chengdu City of China, and reveal bacterial microbial community diversity and structure of this system at different depths. To our knowledge, this is the first study to present a variation in bacterial communities using high-throughput sequencing in CRI. CRI's performance of pollutant removal and spatial bacterial communities were an attempt to reveal the microbial mechanism and provide a theoretical basis for the long-term operation of the CRI system.

Experimental

CRI System Introduction

Phoenix River Ergou Branch Wastewater Treatment Plant (WWTP) is located at the end of the Phoenix River Ergou Branch, and it has been in operation since 2004. The main roles of the WWTP were treating rural domestic sewage, rural irrigation sewage and initial rainwater in the suburban area upriver of the Phoenix.

The project utilized the mode of the pretreatment system + CRI system + constructed wetland technology, and the designed treatment capacity was 20,000 $m^3\ d^{-1}$. The CRI system was the main part of the project, and its designed hydraulic load was 1.5 $m^3/m^2 \cdot d$. There were 9 CRI tanks in the system. Fluvial shock sand, together with 5% of marble sand as infiltration media, was filled in each CRI tank, and the thickness was 120 cm. Meanwhile, the dry-wet alternative operation plan was utilized. Water was distributed 4 times each day, and batch was operated every 7 hours. Water distribution took 1.5 h each time, and drying took 4.5 h. When the sewage water flew through the filler, wastewater was delivered. Physical, chemical and biological reactions were generated to remove the pollutants. The site of the CRI system is shown in Fig. 1.

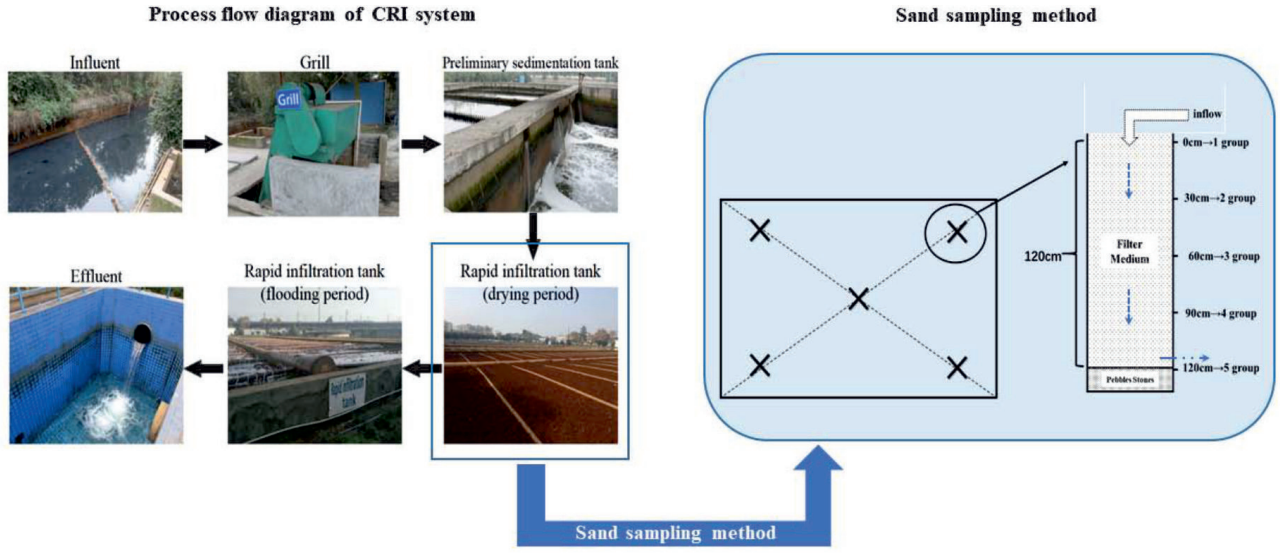

Fig. 1. Demonstration project of the Phoniex River CRI system in Chengdu, China; sand sampling locations and instructions for sampling number.

Sampling Method

Water Sampling Method

To study the pollutant removal performance of the CRI system, the influent quality and effluent quality of the CRI tank were monitored. The sampling time is from November 2017 to December 2017. During this period, the temperature changes greatly, from 0 to 22ºC, and the rainfall changes greatly. The average rainfall in November is 12.2 mm, and the average rainfall in December is 4.7 mm. Sampling frequency was once a week. When the sampling began, the water sample to be collected was used to wash the sampling apparatus and polyethylene sampling apparatus three times each. Then the samples were filled in the sampling apparatus, and the sampling apparatus was placed in the cryogenic sampling box. Next, the water sample was kept in the refrigerator in the laboratory in order to measure the concentrations of COD, NH_4^+-N,TN and TP.

Soil Sampling Method

To study the diversity variation regularity of the microorganisms in the CRI system in the whole process, and also to study the sampling feasibility and representativeness, this research utilized the quincunx method. One sampling point was set at the periphery and the center of the rapid infiltration tank. Vertical stratified sampling (the depth of the measurement was 120 cm; sampling was operated every 30 cm, and sample for five times in total) was carried out for each point from surface layer to bottom layer; then the sand samples (sand sampling locations and instructions for sampling number, shown in Fig. 1) of each sampling point were mixed evenly. After the sampling, the samples were sealed in the polyethylene sterile valve bag. Then we carried the valve bag with dry ice to the laboratory for DNA extraction and high-throughput sequencing experiment. Four parallels were carried out for all the samples. The sampling time was in December 2017.

Water Quality Analysis Method

CRI performance was monitored every week by measuring the concentrations of COD, NH_4^+-N, TN and TP in the influent and effluent, which were determined according to standard methods (APHA 2005) [21]. Before the chemical analysis, the samples were filtered through 0.45 μm filters, and each sample was conducted in quadruplicate.

Microorganism Analysis Method

(1) DNA extraction and quality control

The total DNA of samples were extracted at different depths in the CRI system with a Fast DNA SPIN kit for Soil (Mpbio, USA), and the operation followed the instructions. Then, Nanodrop one (Thermo Fisher Scientific, USA) was utilized to detect the concentration and total volume of DNA. A DYY-6C electrophoresis apparatus (Beijing Liuyi Biotechnology Co., Ltd.) was utilized for sepharose gio-gel PCR electrophoretic pre-amplification, and the completeness of the DNA samples was observed on the gel imaging system. In this way the qualification of the sample was detected.

(2) High-throughput sequencing

The extracted genome DNA was utilized to preamplify 16S rRNA V3 and V4 zones. The 20 DNA stock solution extracted from 20 sand bed samples were used as the PCR template, which was preamplified by 16S rDNA universal primer. The sequence of the universal primer was F: 341F:

(5'-CCTAYGGGRBGCASCAG-3'), R:806R(5'-GGACTACHVGGGTWTCTAAT-3'). The reaction condition of PCR included 2 min of predegeneration at 95°, 20s of denaturation at 95°, 30 s of annealing at 55°, 30s of extension at 72°, 30 circulations, 5 min of heat preservation at 72° and preservation at 4°. Quality testing was carried out for the amplified product with 1% agarose gel electrophoresis. Next, bead depuration and library construction were carried out. The constructed library was quantified with Qubit and QPCR. Computer sequencing was implemented after the library was qualified.

After the logging out of sequencing data, the quality of the sequencing data were filtered, spliced and controlled. The concrete flows include: (1) removing the barode and primer sequences at the two ends of reads; (2) spliced overlap of Read 1 and Read 2 with FLASH software, and the spliced sequence is Raw Tags. The requirement of the splicing process is that the minimum length in the overlap zone is 10 bp, and the maximum mispairing rate is 10%; (3) carried out quality control for Raw Tags with Qiime, cut off Tags with more than 5 continuous N or low-quality base group, and then filter out the Tags whose continuous high-quality base group length is less than 75% of the Tag length, and then Clean Tags was gained; (4) removing the chimera in the Clean Tags. Chimera refers to the wrong sequence generated in the process of PCR amplification. UCHIME algorithm was utilized to verify the chimera. Compared with the Gold database, Effective Tags for subsequent data analysis were gained after the removal of the chimera sequence. USEARCH method was utilized to aggregate the Effective Tags of each sample and aggregate the sequences whose sequence similarity reaches 97% in one OTU.

Results and Discussion

CRI Performance

Organic Matter Removal Performance of the CRI

The removal process of organic matter by CRI mainly involved filtration interception, filler absorption, biodegradation, etc. [22-23]. The removal effect of CRI on COD was shown in Fig. 2a), which shows that the fluctuation of influent concentration was great, ranging from 91.60 mg L^{-1} to 200.80 mg L^{-1}. The main influence factors were pollution source, initial rain, temperature, etc. The effluent concentration of COD was 26.20-42.80 mg L^{-1}, and the average removal efficiency was 75.52%. All the effluent concentration can reach the A standard of COD (<50 mg L^{-1}, GB 18918-2002, China), which showed that the CRI system had strong removal effect for organic matter, strong impulsion load resistance, and stable operation. Chen et

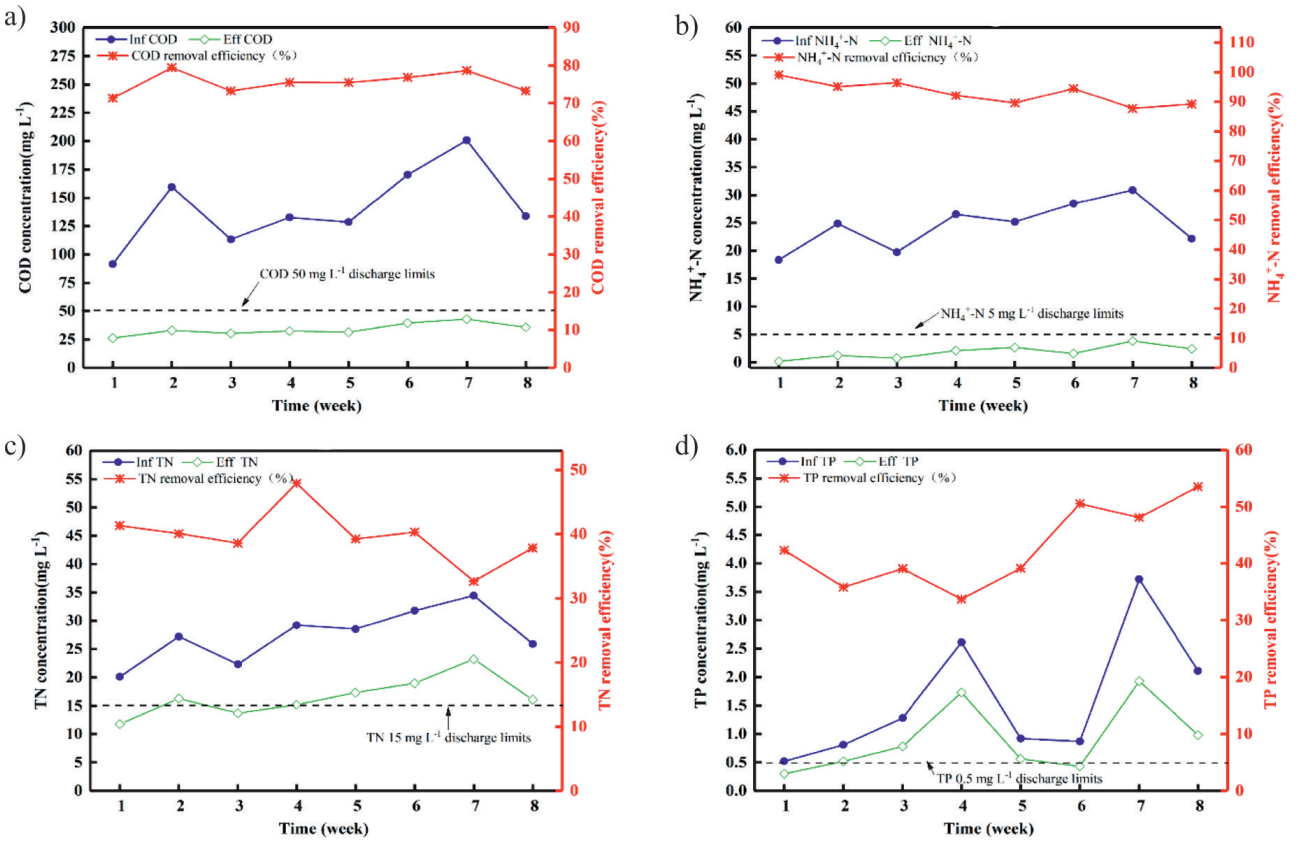

Fig. 2. Removal performance of the CRI system: a) COD, b) NH_4^+-N, c) TN, d) TP.

al. [6] investigated the removal performance in winter for a certain CRI system of Chongqing City, and found that during the whole winter, the average removal efficiency of the technology for COD was 68.58%, while the average removal rate for COD by this system was 75.52%, which showed that this system was better than the CRI system project.

Nitrogen Removal Performance of the CRI

The removal of nitrogen-type pollutants mainly involved absorption for filter materials, degradation and assimilation of micro-organisms, with microorganisms playing the main role [24]. The removal effect by the rapid infiltration tank of the CRI system on NH_4^+-N is shown in Fig. 2b). The influent concentration of NH_4^+-N was 18.35-30.90 mg L^{-1}, and the effluent concentration of NH_4^+-N was 0.16-3.81 mg L^{-1}. The average removal efficiency was 92.94%, which indicated that the removal performance of this CRI system for NH_4^+-N was strong. The effluent concentration of NH_4^+-N could reach the A standard of NH_4^+-N (<5 mg L^{-1}, GB 18918-2002, China). However, the average removal efficiency of TN was 39.74%, so the effluent concentration can seldom reach A standard of TN (<15 mg L^{-1}, GB 18918-2002, China). Liu et al. [25] and Xu et al. [14] both got the conclusion that the CRI system had strong removal effect for NH_4^+-N but weak removal effect for TN. The main possible reason was that the inadequacy of carbon source in the denitrification section in the sublayer of the CRI system led to the weak role of denitrification effect [26], and also the transference speed of nitrate nitrogen was too fast so the time of denitrification reaction period was too short [27]. Fang et al. [28] pointed out that TN removal efficiency can be improved to 75.4% after adding a postpositional waterlogged layer to coordinate the carbon nitrogen ratio.

Phosphorous Removal Performance of the CRI

Three methods remove phosphorus from CRI system: absorption, chemical precipitation and absorption of micro-organisms [5]. Different from the removal mechanisms of COD, NH_4^+-N and TN, the contribution of biological effect on phosphorus removal was limited. The absorption and precipitation of substrates were the main ways to remove phosphorus, of which the precipitation role refers to the common precipitation roles of Fe^{3+}, Al^{3+} , other metal ions and phosphate anion, which is shown in Formulas (1) and (2) [29]:

$$Fe^{3+} + H_nPO_4^{-3+n} \rightarrow FePO_4 \downarrow + nH^+ \quad (1)$$

$$Al^{3+} + H_nPO_4^{-3+n} \rightarrow AlPO_4 \downarrow + nH^+ \quad (2)$$

The removal effect of TP by the rapid infiltration tank of the CRI system is shown in Fig. 2d), where the influent concentration of TP was 0.52-3.72 mg L^{-1} and the effluent concentration of TP was 0.30-1.23 mg L^{-1}. The average removal efficiency was only 42.78%. Only few effluent concentrations can reach A standard of TP (<0.5 mg L^{-1}, GB 18918-2002, China, which showed that the CRI system had weak removal performance). Wang [30] found that a mixture of iron scrap and fly-ash can effectively remove phosphorus-containing pollutants as a phosphorus removal filler with mass ratio of 2:1. Xu et al. [31] pointed out that utilizing sponge iron as filler can improve the removal efficiency of TP to 72.5%. In conclusion, changing the filler ingredients in the rapid infiltration tank can improve TP removal efficiency. In addition, the conversion and decomposition of microorganisms on phosphorus exerted certain effects due to seasonally low temperatures.

Microbial Community Diversity Analysis of the CRI at Different Depths

OTU Analysis

To study the genus composition and diversity information of the samples, the USEARCH method was adopted to aggregate the Effective Tags of each sample. The sequences where sequence similarity reached 97% were aggregated into an OTU. Statistical analysis was carried out for the OTU generated by the 5 groups of samples (see Fig. 3), and the result showed that all the samples formed 306,222 OTU, wherein the number of samples in group 1 (surface layer) was 58,918, the number of samples in group 2 (at a depth of 30 cm) was 73,963, the number of samples in group 3 (at a depth of 60 cm) was 53,099, the number of samples in group 4 (at a depth of 90 cm) was 65,033 and the number of samples in group 5 (at a depth of 120 cm) was 55,209. As the depth of the CRI system increases, the number of OTU increased at first, decreased later, then increased again and decreased again. The number reached the

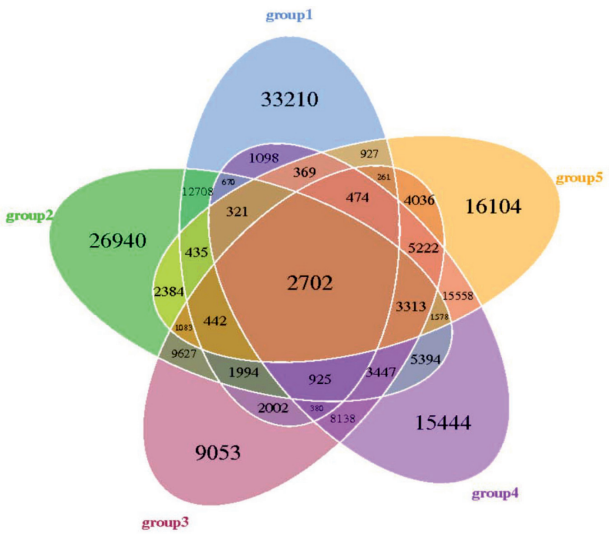

Fig. 3. Venn diagram showing distribution of OTUs.

maximum value when the depth was 30 cm. When the depth was 60 cm, the number of OTU was 53,099, which showed that the number of microorganism genus at this depth was the smallest. There were 2,702 OTU in the 5 groups of samples, which indicated that the 2702 OTU had displayed and included all the dominant bacterial communities in the CRI system. However, the number of common OTU in the 5 groups of samples only accounted for 0.88% of the total number, which indicated that the microorganism communities at different depths not only differed in number, but also in genus. That was caused by different physicochemical characteristics of the filtration tank at different depths, such as dissolved oxygen, pollutant concentration, and hydraulic condition, so the properties of the microorganisms at different depths of the filtration tank were different.

Richness and Richness of the Bacterial Community

The diversity of the bacterial communities for the CRI samples at different depths are described in Table 1.

Table 1 shows that the Chaol index indicated genus diversity, and its value was between 42,589.53 and 70,697.93. Simpson index indicated the genus uniformity whose value was between 0.997 and 0.999. Shannon index was an aggregative index of genus richness and uniformity, and its value was between 11.57 and 12.35. The larger these indexes, the more diverse the genus of the samples. The Shannon and Chaol indices indicated that the microorganism community richness and diversity on the sublayer (60-120 cm) of the CRI tank were small, and the reason was that the amount of DO and nutrient substances in the sublayer of the CRI tank was small, so the genus diversity in the sublayer was weak. The values of Simpson indexes were all close to 1, which indicated that the microorganism communities at different depths of the CRI tank had high uniformity. In addition, the Simpson index on the upper layer (0-30 cm) was higher than the Simpson index on the sublayer (60-120 cm). The Shannon and Chaol indices also took on this trend, which implies that the community structure of the microorganisms on the upper layer was more stable. Chen et al. [32] held that the more diverse the bacterial community structure, the better the removal efficiency of pollutants.

Microbial Community Structure Analysis of the CRI at Different Depths

Bacterial Community Composition at the Phylum Level

The 5 groups of CRI bacterial community structures at different depths took on high degrees of diversities at the phylum level. There were 52, 85, 51, 75 and 46 known bacterial phyla detected respectively in groups 1, 2, 3, 4 and 5. The relative proportions of bacterial phyla with comparatively higher community composition percentage (the first 10) in the 5 groups of samples are shown in Fig. 4, and the bacterial phyla mainly included Proteobacteria (24.26-29.20%), Actinobacteria (2.02-21.77%), Chloroflexi (12.14-16.66%), Acidobacteria (7.77-14.73%), Thaumarchaeota (1.23-10.86%), Bacteroidetes (0.99-8.66%), Nitrospirae (2.85-8.18%), Firmicutes (0.95-5.96%), Gemmatimonadetes (1.83-5.25%), and Saccharibacteria (0.46-3.12%).

Among the dominant bacteria phyla, Proteobacteria and Chloroflexi took up comparatively higher proportions in each sample, at respectively 24.26-29.20% and 12.14-16.66%. Proteobacteria and Chloroflexi were detected as the main dominant phyla with a high frequency for wastewater treatment [33-34]. Proteobacteria could be related to the removal of various organic matters from wastewater [35]. Meanwhile, Proteobacteria has the function of nitrogen and phosphorus removal, such as *Nitrosomonas* and *Nitrosococcus* genus in AOB, and *Nitrobacter* and *Nitrococcu* in NOB all belong to Proteobacteria phyla [36-37]. Chloroflexi is mainly detected in the sewage plant with complex treatment ingredients and large variation of water quality [38], which was consistent with the conclusion of the experiment.

According to the abundance of phyla, the abundances of Actinobacteria and Bacteroidetes in group 1 (respectively 21.77% and 8.66%) were much higher than those in groups 2-5 (respectively 3.5-6.22% and 0.99-1.75%). Namely, the surface layer contained more Actinobacteria and Bacteroidetes. Actinobacteria [35] and Bacteroidetes [39] mainly existed in the place where the organism is concentrated. The influent of the CRI was from the upper part uniformly, so the organism concentration on the surface was the highest,

Table 1. Spatial richness and diversity of bacterial community for sand samples collected from different depths in the CRI system.

Sample	Sampling number	OTU	Chao1	Shannon	Simpson
0 cm	group 1	58918	53930.11	12.16	0.999
30 cm	group 2	73963	70697.93	12.35	0.999
60 cm	group 3	53099	48313.76	11.65	0.998
90 cm	group 4	65033	52596.90	11.67	0.997
120 cm	group 5	55209	42589.53	11.57	0.998

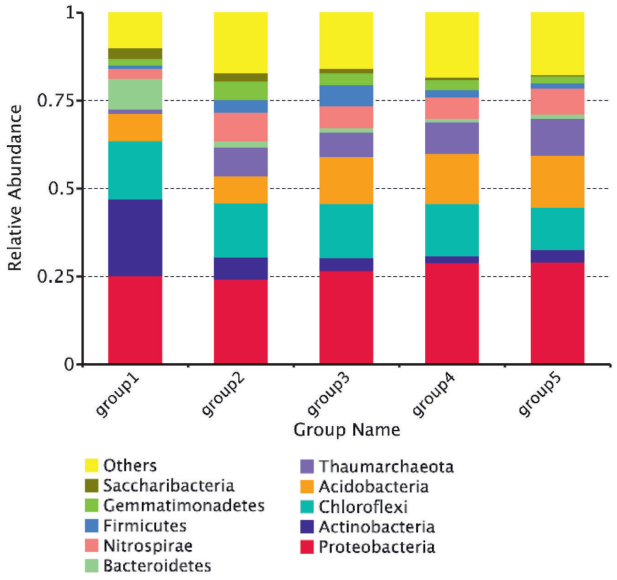

Fig. 4. Relative abundance (%) of dominant microbial phyla in all sand samples at different depths; bacterial community structure and distribution of the samples at phylum level.

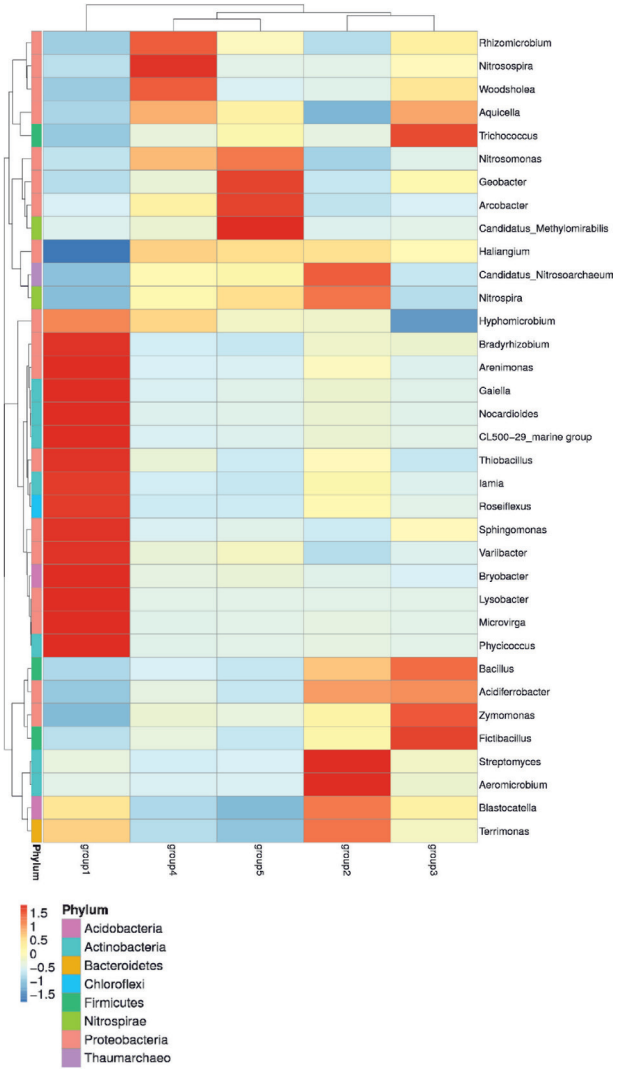

Fig. 5. Heat map of most abundant genera in bacterial communities detected from CRI samples at different depths.

which caused the abundances of Actinobacteria and Bacteroidetes to be higher. Meanwhile, it was predicted that organism degradation existed on the system surface, while the abundances of Firmicutes and Nitrospirae in group 1 (respectively 0.95 and 2.85%) were less than those in groups 2-5 (respectively 1.5-3.64% and 6.11-8.18%). That is to say the inside (30-120 cm) of CRI had more Firmicutes and Nitrospirae, which is a kind of microorganism flora for nitrogen removal. For example, Nitrospira in NOB belonged to Nitrospirae phylum; the Firmicutes bacteria had to be denitrified with nitrate [40-41]. Meanwhile, the bacillus in Phylum Firmicutes had a strong capacity for resisting exterior harmful factors [40-41], so it played a key role in the removal of nitrogen in wastewater and the maintenance of system stability. According to the distribution of Actinobacteria, Bacteroidetes, Firmicutes and Nitrospira, it can be predicted that the degradation of organisms in CRI mainly existed in the system surface, while the nitration reaction mainly happened in the inside of the system.

For a long time, researchers believed that ammonia-oxidizing bacteria were the most important carriers in the nitrogen circulation of the earth. However, since 2005 the first ammonia-oxidizing archaea (AOA) gained by separate cultivation in the seawater of Seattle Aquarium proved that archaea can also oxidize ammonia to nitrite, which broke through the cognition to nitrogen circulation. It is worth noticing that archaea Thaumarchaeota, which is initially classified as mesophilic Crenarchaeota [43], was detected in this experiment. Known from Fig. 5, the abundance values of groups 1-5 were respectively 1.23%, 8.16%, 7%, 8.92% and 10.36%. The abundance value on the sublayer of the CRI was higher, which was consistent with archaea's endurance of oxygen deficiency and the low concentration of NH_4^+-N. Research [44] indicated that Thaumarchaeota was richer than bacteria in some sand and silty clays, which was consistent with our study.

Bacterial Community Composition at the Genus Level

To get the CRI microbial community structure difference at different depths, this study was based on the genus annotation and richness information of the genus level in groups 1-5, the genus whose richness ranks were in the first 35 were drawn into heatmap, as shown in Fig. 5. The result showed that the microbial community structure varied as the change of depth. The clustering analysis on the abundance of genus-grade microorganisms showed the difference between the microbial community structure on the surface layer (group 1) of CRI and the microbial community structure in the inside (group 2-5) of CRI was large,

but the middle and upper layers (group 2) in the inside of CRI shared similarities with the middle layer (group 3), middle and lower layers (group 4) and bottom layer (group 5) in the microbial community structure. Meanwhile, the similarities of microbial community structures between the upper layer, middle layer and the similarities in the middle lower layer and bottom layer were larger than the similarities between the upper layer, middle layer and the surface layer. In addition, principal co-ordinates analysis (PCoA) was made for all the samples based on weighted unifrac distance and unweighted unifrac distance. As shown in Fig. 6, the result was consistent with the aggregation analysis of genus-grade microorganism abundance caused by the difference and similarities in the micro-environment (substrate concentration, oxygen, pH and Eh).

The great difference between the community structures of group 1 and groups 2-5 was mainly due to the big gap in dominant genus. In group 1, the abundance degree of *Bradyrhizobium, Arenimonas, Gaiella, Nocardioides, CL500−29_marine group, Thiobacillus, Iamia, Roseiflexus, Sphingomonas, Variibacter, Bryobacter, Lysobacter, Microvirga,* and *Phycicoccu* were high, ranging from 0.43% to 1.62%, while the bacteria abundance in group 2-5 were low, ranging from 0% to 0.2%, wherein *Bradyrhizobium*, *Variibacter* and *Microvirga* had the role of nitrogen fixation [45]. Thus, in the process of sampling, it was found that the surface layer of CRI had a small amount of weed, which was due to the long-time operation of this system in which the influent nitrogen concentration is high. Furthermore, most of these bacteria had the capacities of utilizing carbon source and decomposing organic matter. For example, *Bryobacter* was frequently detected in cultivated soil, and it can decompose organic matter [46]. Meanwhile, the abundance of *Bryobacter* is

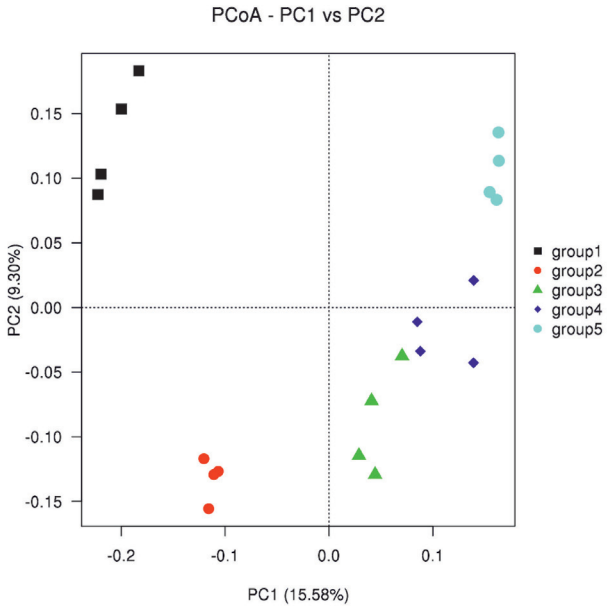

Fig. 6. PCoA plot on the OTU level.

positively correlated to TP [46]. *Arenimonas* [48], *Iamia* [49] *and Roseiflexus* [50] were frequently detected in activated sludge belonging to heterotrophic bacterium; *Thiobacillus* was both heterotrophy nitrate-reducing bacteria and also autotrophic sulfide-oxidizing [51]. Zhang et al. [52] found the predominance of *Thiobacillus* in different sulfur-based denitrification systems after treating nitrate-contaminated wastewater, and its existence was related to the sulfur cycle. Furthermore, in the dominant genus of group 1, some bacteria had the functions of transforming heavy metal and degrading toxic and harmful substances. The research showed that *Nocardioides* could not only decompose cellulose, lignin, polycyclic aromatic hydrocarbon and related molecular organic components, but also could degrade toxic compounds such as fungicide, herbicide and insecticide, and can degrade petroleum hydrocarbon compound [53-54]. *Sphingomonas* had the biodegradation capacity of an aromatic compound, but as the knowledge of *Sphingomonas* was gained relatively later, its ecological and economic value potential were great [55]. *Phycicoccus* can degrade xenobiotics, degrade ritalinic acid and related substances [56]. *Gaiella* had some relation with the transformation of heavy metal. Liu et al. [57] discovered that the abundance degree of *Gaiella* in nonferrous metal (loid) tailings sites was high.

In the system, the transformation of oxynitride was mainly concentrated in groups 2-5. The nitrogen transformation functional bacteria ranking in the TOP35 of heatmap were selected and classified into 5 types (see Table 2) according to the transformation regularity of oxynitride: the first was AOB, which oxidized NH_4^+-N into NO_2^--N; the second was NOB, which oxidized NO_2^--N into NO_3^--N; the third was AOA, which oxidized NH_4^+-N into NO_2^--N; the fourth was denitrifying bacteria, which reduced NO_3^--N into N_2; and the fifth was novel bacteria – N-DAMO and ANAMMOX. Nitrite-dependent anaerobic methane oxidation (N-DAMO) was a novel nitrogen removal method, coupling the reduction of nitrite and the anaerobic oxidation of methane of methane, and the reaction formula was shown in formula (3) [58]. Furthermore, research indicated that the N-DAMO microorganism usually coexisted with anaerobic ammonia-oxidizing microorganisms [59], so ANAMMOX bacteria were found at the depth of 90-120 cm. Meanwhile, it was positively correlated to abundance degree of N-DAMO bacteria. The equation of ANAMMOX was shown in Equation (4) [60]:

$$3CH_4 + 8NO_2^- + 8H^+ \rightarrow 3CO_2 + 4N_2 + 10H_2O \quad (3)$$

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.256N_2 + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (4)$$

Table 2 shows that the abundance of nitrobacteria in groups 1-5 was high, wherein the abundance of *Nitrospira* was the highest. Even on the CRI bottom

Table 2. Characteristics of nitrogen transformation functional bacteria in the CRI system samples.

Functional bacteria		Genus	Relative Abundance (%)				
			group 1	group 2	group 3	group 4	group 5
Nitrifying bacteria	AOB	Nitrosospira	0.03%	0.11%	0.20%	0.62%	0.11%
		Nitrosomonas	0.07%	0.04%	0.11%	0.34%	0.43%
	NOB	Rhizomicrobium	0.01%	0.11%	0.96%	1.88%	0.67%
		Nitrospira	2.36%	5.58%	2.81%	3.95%	4.55%
Archaea	AOA	Candidatus Nitrosoarchaeum	0.01%	2.83%	0.43%	1.25%	1.39%
Denitrifying bacteria		Arcobacter	0.29%	0.21%	0.28%	0.68%	1.33%
		Haliangium	0.18%	0.46%	0.39%	0.48%	0.47%
		Bacillus	0.18%	0.89%	1.21%	0.29%	0.25%
		Acidiferrobacter	0.04%	1.31%	1.36%	0.40%	0.22%
Novel bacteria	N-DAMO	Candidatus Methylomirabilis	0.00%	0.00%	0.02%	0.07%	0.63%
	Anammox	Candidatus Brocadia	0.00%	0.00%	0.01%	0.03%	0.09%

layer (120 cm) which was poor in nutrient substance and low in oxygen amount, the abundance was as high as 4.55%, which was because when the NO_2^--N was adequate, *Nitrospira* preferred the low dissolved oxygen environment [61]. What is interesting is that we found that the abundance of archaea *Candidatus Nitrosoarchaeum* was larger than that of AOB in the inside (groups 2-5) of CRI, ranging from 0.43-2.83%. The synergistic effect of nitrobacteria and archaea improved the removal efficiency of NH_4^+-N in CRI. Known from Table 2, the summation of denitrifying bacteria *Arcobacter, Haliangium, Bacillus* and *Acidiferrobacter* was the highest in group 3, and the value was 3.24%. This indicated that the denitrifying role in CRI was mainly in the middle layer of the system.

In addition, some microorganisms that can decompose molecular organic matter existed in groups 2-5, such as *Woodsholea, Woodsholea, Trichococcus* and *Hyphomicrobium*. No phosphorus-removing microorganism was found in soil remediation and in the first 35 genus, which implied that the contribution of microorganisms to phosphorus removal was limited in the CRI system.

Above all, each microorganism played its own role in CRI. Through mutual synergistic effect, the CRI system possessed strong pollutant-removal capacities.

Conclusions

1. As the influence of pollution source, initial rainwater, temperature, etc., the influent concentrations of COD, NH_4^+-N, TN and TP were high. After CRI treatment, the effluent concentrations of COD and NH_4^+-N can reach A standard (COD<50 mg L^{-1}, NH_4^+-N<5 mg L^{-1} GB 18918-2002, China).

2. Microorganisms at different depths differ in species and quantity, and that is mainly due to different physicochemical properties, such dissolved oxygen, pollutant concentration, hydraulic condition, etc. The diversity indexes (Shannon, Simpson, chao1) of the microorganisms within 0-30 cm were all higher than those in the under layer.

3. Most of the microorganisms on the surface layer (0 cm) of CRI possess the capacities to degrade organic matter, and some of the microorganisms possess the capacities of degrading toxic compounds and converting heavy metal.

4. Nitrogen transformation functional bacteria were mainly distributed in 30-120 cm. The nitrifying bacteria were mainly *Nitrospira* (2.81-5.58%), and the denitrifying bacteria were mainly *Acidiferrobacter* (0.22-1.31%). The archaea were mainly *Candidatus Nitrosoarchaeum* (0.43-2.83%). In addition, Novel nitrogen transformation functional bacteria, N-DAMO bacteria – *Candidatus Methylomirabilis* (0.02-0.63%) and Anammox bacteria – *Candidatus Brocadia* (0.01-0.09%) were found within 60-120 cm.

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Conflict of Interest

The authors declare no conflict of interest.

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