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Community shift of biofilms developed in a full-scale drinking water distribution system switching from different water sources

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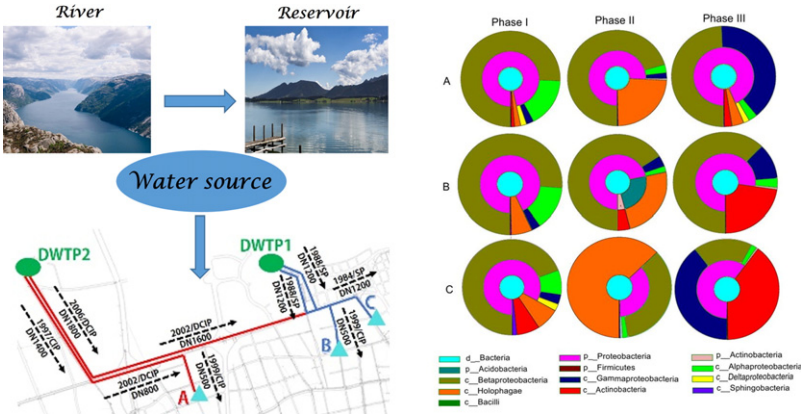
HIGHLIGHTS

- Biofilms sampled from a full-scale drinking water distribution system.
- Drinking water distribution system successively suffered two kinds of water sources with different grades.
- High-throughput sequencing of biofilm community
- Water source switching produced substantial impact on the biofilm community.

GRAPHICAL ABSTRACT



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ABSTRACT

The bacterial community of biofilms in drinking water distribution systems (DWDS) with various water sources has been rarely reported. In this research, biofilms were sampled at three points (A, B, and C) during the river water source phase (phase I), the interim period (phase II) and the reservoir water source phase (phase III), and the biofilm community was determined using the 454-pyrosequencing method. Results showed that microbial diversity declined in phase II but increased in phase III. The primary phylum was Proteobacteria during three phases, while the dominant class at points A and B was Betaproteobacteria (>49%) during all phases, but that changed to Holophagae in phase II (62.7%) and Actinobacteria in phase III (35.6%) for point C, which was closely related to its water quality. More remarkable community shift was found at the genus level. In addition, analysis results showed that water quality could significantly affect microbial diversity together, while the nutrient composition (e.g. C/N ration) of the water environment might determine the microbial community. Furthermore, *Mycobacterium* spp. and *Pseudomonas* spp. were detected in the biofilm, which should give rise to attention. This study revealed that water source switching produced substantial impact on the biofilm community.

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1. Introduction

Human health is closely related to drinking water quality, and access to safe, reliable drinking water in sufficient quantities is fundamental for good health and well-being (Bain et al., 2014). In China, there were 271 drinking water contamination incidents except Tibet autonomous region, of which 63% were classified as biological during 1996–2006 (Zeng et al., 2015). According to the World Health Organization, diarrheal diseases (including cholera) kill 1.8 million people every year, 90% of whom are children under five years of age, and 88% of the cases are attributed to unsafe water supply, inadequate sanitation or hygiene (Lee, 2004). In the United States, decreasing numbers of waterborne outbreaks reported per year since 1982, but an increasing percentage was attributed to drinking water distribution system (DWDS) issues (NRC, 2006). Therefore, investigation of microbes survived in the DWDS is of great significance in terms of drinking water safety.

As one of the world's greatest technological advancements of the 20th century, DWDS play an important role on keeping drinking water safety as it not only significantly affected drinking water quality but also drastically decreased the opportunity of human contraction of infectious waterborne diseases. However, the water quality would fluctuate due to water age, temperature, hydraulic conditions, decaying of residual disinfectants and bacterial regrowth when finished water is transported through a DWDS, whereby the biofilm was gradually developed in the DWDS (Chu et al., 2005; Manuel et al., 2007). Actually, the potential threat to human health derived from biofilms (Flemming and Wingender, 2010) in the DWDS had never been completely eliminated, and microbes in the biofilm have been generally recognized as the primary source of microorganisms in DWDS (Berry et al., 2006), and the potential threat to human health derived from biofilms (Flemming and Wingender, 2010) has never been completely eliminated. It is true that many types of opportunistic pathogens, such as *Legionella pneumophila* (Thomas and Ashbolt, 2011), *Nontuberculosis mycobacteria* (NTM) (Falkinham, 2009), and *Pseudomonas aeruginosa* (von Baum et al., 2010), and *Bacteroides* (Douterelo et al., 2014) have been detected in DWDS. Therefore, gaining insight into the bacterial community structure of biofilms was of great significance.

Besides DWDS, high-quality water sources also play an important role on guaranteeing drinking-water safety. In China, many drinking water treatment plants (DWTP) are changing water sources due to the population increase in cities as well as the rapid development of economy. Water source switching, however, could cause striking effects on the water quality of the DWDS. In 2008, Beijing attempted to replace its local water source with a long-distance water source transported from a neighboring province. Nevertheless, heavy red water occurred in the pipes transporting ground water when 80% of local source water was replaced. This problem was studied subsequently and the results showed that corrosion products contributed by specific bacteria were responsible for this issue (Zhu et al., 2014). Although the reasons of heavy red water was clear, the effects of water source switching on microbial community structure of biofilms need to be further studied.

Due to the difficulty of sampling, research on biofilms in DWDS generally focused on simulating DWDS (Douterelo et al., 2013; Wang et al., 2012b; Zhu et al., 2014). However, various factors (Janjaroen et al., 2013) affecting the microbial community of biofilms in the practical DWDS were difficult to simulate comprehensively. Consequently investigation of practical DWDS was worth conducting. Both culture-based and culture-independent methods have been adopted in previous studies, but growing evidence has shown that culture-independent methods (i.e., high-throughput sequencing) can be a powerful tool for a comprehensive overview of microbial communities in various environmental samples (Huang et al., 2014; Li et al., 2015; Ye and Zhang, 2011), so culture-independent method was selected in our research.

Our objective of this study was to seek information about the microbial community shift of pipe biofilms under the condition of various types of surface source water of varying quality. The full-scale DWDS studied in this research was characterized by multiple pipe materials and a long running time. Based on the growing pattern of biofilms and a strict sampling schedule, this study was conducted over the course of three years. The microbial community of biofilms was determined by using the 454-pyrosequencing method and was compared between different phases. This research provided insights into the influence of water source switching on the microbial community of pipe biofilms.

2. Materials and methods

2.1. Description of water sources and water treatment plants

The relative positions of DWTP and sampling points were illustrated in Fig. 1. Based on water sources, water treatment processes (Table 1) and water quality (Table S1), our research covered three phases. During phase I (03/2012–02/2013), the DWDS was supplied by DWTP 1 and DWTP 2, which received water from river water of grade III-V. Moreover, DWTP1 stopped supplying B and C at the end of this phase and they were supplied by DWTP 2 simultaneously. At the beginning of phase II, the water source supplying DWTP2 was switched from river water of grades III-V to reservoir water of grade II at the end of phase I. Water quality of bulk water samples in the practical DWDS varied to the largest extent during this phase, especially for total organic carbon (TOC) and conductivity (Table S1). Therefore, phase II (03/2013–02/2014) was regarded as the interim period. During phase III (03/2014–02/2015), the DWDS only was supplied by DWTP2 receiving water from reservoir water of grade II. It should be noted that the water treatment techniques applied in DWTP1 and DWTP 2 were different. DWTP2 added ozonation and granular activated carbon filtration before the disinfection process on the base of the conventional treatment process (Coagulation + Sedimentation + Filtration + Disinfection), whereas DWTP1 employed only the conventional treatment process. Considering these conditions, the effects of water source switching on the diversity and relative abundance of the microbial community in the biofilm developed in the practical DWDS was primarily discussed in this research, whereas the water treatment process was also considered to determine the difference between phase I and phase II.

2.2. Biofilm sampling device

Three sampling points (A, B, C) were selected in the practical DWDS of city Y in south China (Fig. 1). To facilitate biofilm sampling in the full-scale DWDS, a new valve well ($L \times B \times H = 3\ m \times 2\ m \times 1.2\ m$) equipped with biofilm sampling device was built at each sampling point. The schematic diagram of biofilm sampling device was shown in Fig. 2(a). In brief, a main pipe, a bypass pipe, three valves and one tap were installed in the valve well. Two valves were situated at the inlet (V_i) and outlet (V_o) of the main pipe, and the third valve (V_m) was equipped at the bypass pipe and six coupons and a tap were installed between V_i and V_o of the main pipe. The coupon [Fig. 2(b)] surface was cement mortar material with an area of about 2.9 cm^2. Biofilms developed when V_m was closed and V_i and V_o were kept opening.

2.3. Sampling

Tap water and biofilms were collected from each sampling point. Firstly, the tap was opened about two minutes to drain the stagnant water in the pipe, then the residual chlorine of water sample was determined on the spot (PC II, HACH, USA) and the bulk water (2 L) was collected subsequently. The tap was then sterilized with lighted alcohol cotton, and bulk water (1 L) was sampled again in a sterilized glass bottle to determine the heterotrophic plate counts (HPC). Upon arrival at the lab, bulk water was used to analyze pH, conductivity (PE20, METTLER, Switzerland), turbidity (2100Q, HACH, USA), and TOC (TOC4100, Shimadzu, Japan), and ammonia, nitrate, and nitrite were

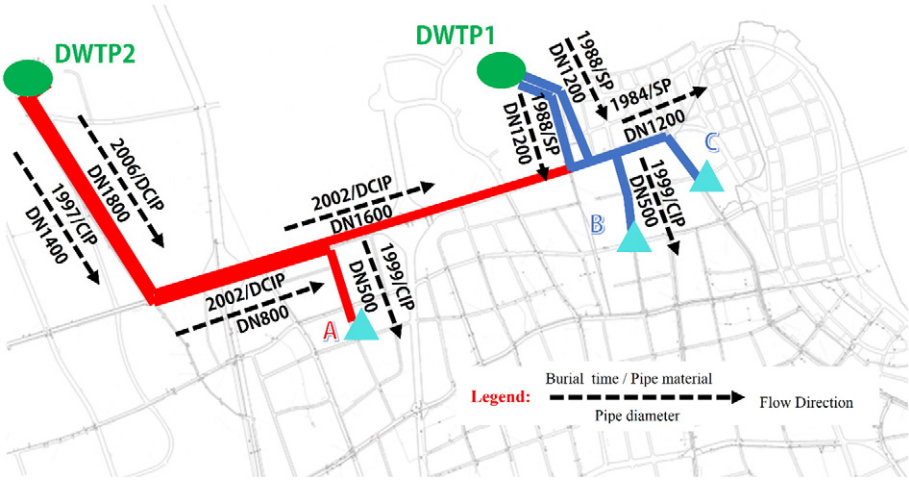

Fig. 1. Relative positions of sampling points and DWTPs in the practical DWDS of City Y. Abbreviation: CIP (Cast-Iron Pipe), SP (Steel Pipe), DCIP (Ductile Cast-Iron Pipe).

measured according to the State Standard of the People's Republic of China (GB/T5750.5-2006). As for HPC, samples were appropriately diluted and then plated on nutrient agar(peptone 10 g, beef extract 3 g, sodium chloride 5 g, agar 15–20 g, distilled water 1000 mL, pH 7.2–7.4), and the number of colonies were determined after 48 h of incubation at 37 °C according to the State Standard of the People's Republic of China (GB/T5750.12-2006). The detailed water quality of A, B, and C for three years is presented in Table S1.

When sampling biofilms, V_m was opened and V_i and V_o were closed, and the residual pressure in the bypass pipe was released by opening the tap. Two coupons with biofilm [Fig. 2(b)] were sampled with sterilized tweezers and placed in sterilized stainless steel containers filled with bulk water in the pipe respectively. New sterilized coupons were installed immediately after the old ones were removed. After sampling, V_i and V_o were opened and V_m was closed. All of the samples were stored in a car refrigerator at 4 °C until reaching the lab. Then, the bulk water in the stainless steel container was replaced with sterilized saline (50 mL) and then shaken with an amplitude of 13 for 2 min by an ultrasonic processor (S-4000, SONICATOR Company, USA). The suspended mixture was then poured into a sterilized disposable 100 mL polytetrafluoroethylene bottle for DNA extraction. In general, two coupons were sampled for one location at the same time for repeatability validation. Especially, during the initial phase of this research, the first and the second coupons were sampled one month after installation, two months elapsed for the third and the fourth coupons, and three months elapsed for the fifth and the sixth coupons, after which another sampling cycle was continued. This method and schedule of sampling were strictly executed over three years, and about 40 group samples were gathered in all. Biofilms discussed in this article were sampled at the same month of three years in a row, and all were developed for approximately 90 days. This sample selecting criteria was helpful to reduce bias induced by environmental factors.

2.4. DNA extraction and preparation for 454-pyrosequencing

Approximately 50 mL of saline solution containing turbid biofilm was obtained after the biofilm was pretreated, and DNA was extracted using a FastDNA SPIN Kit for soil (MP Biomedical, Solon, OH) according to the manufacturer's protocol. A 60-μL DNA sample of each biofilm was obtained eventually. The PCR was conducted using forward primer 27f: 5′-AGAGTTTGATCCTGGCTCAG-3′ and reverse primer 519r: 5′-GGTTACCTTGTTACGACTT-3′ to amplify the 16S rDNA gene of DNA sample, and each 50-μL reaction mixture included Premix Ex *Taq*™ Hot Start (25 μL), forward primer (1.2 μL), reverse primer (1.2 μL), DNA template (2 μL) and ddH_2O (20.6 μL). The PCR program for bacterial amplification was performed by initial denaturation at 94 °C for 3 min, followed by 32 cycles of amplification (30 s denaturation at 94 °C, 30 s annealing at 55 °C and 1 min elongation at 72 °C), and 7 min at 72 °C to finish elongation, and finally preserved at 15 °C.

The 454-pyrosequencing method was applied to characterize bacterial communities and examine their relative abundance and diversity in biofilm samples. First, the PCR products amplified with tag-encoded primers (27F and 519R) were purified with a QIAquick Gel Extraction Kit (QIAGEN, Germany), and the extracted DNA was then sent to the company for bacterial 16S rDNA gene pyrosequencing. Prior to sequencing, the quantity of purified PCR amplicons was determined using the Quant-iT™ PicoGreen® dsDNA Reagent Kit (Life Technologies™ Corporation, Grand Island, NY). The same amount (in moles) of PCR amplicons of different samples was pooled together and subjected to emulsion PCR to generate amplicon libraries, as recommended by 454 Life Sciences (Margulies et al., 2006).

2.5. Data analysis

Sequence data were analyzed using a combination of software applications UPARSE (usearch version v7.0.1090) (Edgar, 2013), QIIME (version 1.8) (Kuczynski et al., 2002) and R (version 3.0.2). The raw 16S rRNA gene sequences were analyzed with script split_libraries.py in QIIME to remove the primers, demultiplex reads and filter reads according to Phred quality scores with default parameters (a minimum sequence length of 200 bp, a maximum sequence length of 1000 bp, a minimum average quality score of 25, a maximum homopolymer length of 6 bp, no ambiguous bases or mismatches in the primer sequence, no barcode errors). The demultiplexed reads were clustered

Table 1
Different water sources and water treatment processes in three phases.

Phase no.	Date	Water source	Water grades	DWTP	Water treatment process
Phase I	03/2012–02/2013	River water	III–V	DWTP1 & DWTP2	Conventional and advanced treatment processes
Phase II	03/2013–02/2014	Reservoir water	II	DWTP2	Advanced treatment process
Phase III	03/2014–02/2015	Reservoir water	II	DWTP2	Advanced treatment process

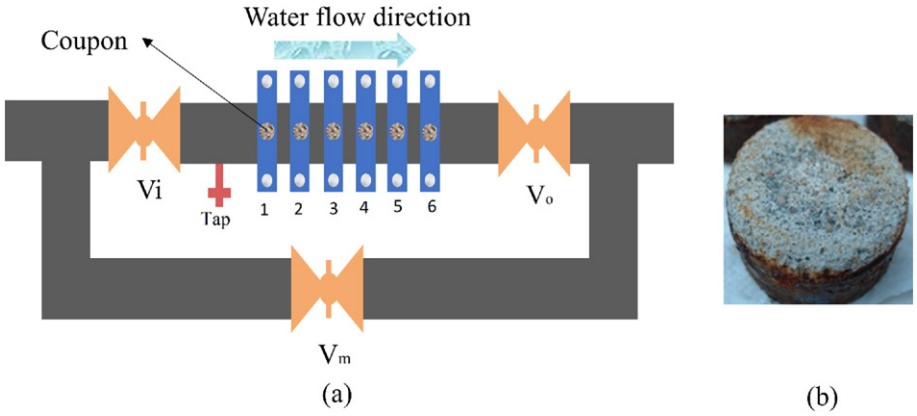

Fig. 2. Schematic diagram of biofilm sampling device (a) and the cement-mortar coupon (b).

at 97% sequence identity into operational taxonomic units (OTU) using the UPARSE pipeline (http://drive5.com/usearch/manual/uparse_cmds.html). The OTU representative sequences were aligned against the greengenes reference template set (http://greengenes.lbl.gov/Download/Sequence_Data/Fasta_data_files/core_set_aligned.fasta.imputed) based on PyNAST (version 1.2.1) (Caporaso et al., 2010). The phylogenetic tree was constructed using FastTree (version 2.1.3) (Price et al., 2010) with the filtered alignment and visualization based on GRAPHLAN (https://bitbucket.org/nsegata/graphlan/wiki/Home). The Ribosomal Database Project (RDP) Classifier (version 2.7) (Wang et al., 2007) was employed for taxonomy assignment against RDP 16S rRNA training set 9 with confidence score ≥0.8. For alpha-diversity metrics, the estimated number of species based on the Observed_species and Chao 1 were calculated. All of the figures were generated with customized R scripts.

The correlation analysis and multiple linear regression analysis were both conducted with the software of IBM SPSS statistics (version 23) in attempts to seek relations between microbial diversity and water quality. In addition, Canonical correlation analysis (CCA) was performed to search relevance between microbial species and environmental factors by using CANOCO (version 4.5).

3. Results

3.1. Properties of water samples

Table 2 shows the physicochemical data and colony counts of the samples. Water quality improved greatly from phase I to phase III. In detail, the turbidity of tap water decreased to blow 0.2 NTU in phase III. The average TOC of the three water samples decreased from 4.1 (phase I) to 1.2 mg/L (phase III). When compared among different sampling points, the difference of water quality parameters was also manifest. Turbidity, conductivity, nitrate and nitrite, their concentrations at points B and C were always higher than those at A in all three phases; Moreover, the colony counts of point C were always the highest during all phases. Therefore, the water quality of sampling point C was worse than that of the other two points. This could be attributed to the long transporting distance, long working time and complicated pipe materials of it's transporting pipes.

3.2. Microbial diversity

Overall, approximately 46,706 sequence reads of 16S rRNA genes with an average length of approximately 480 bp were obtained after trimming and chimera removal, and the effective reads of each sample were all higher than 70%. A 97% similarity cutoff was used to delineate OTUs in the downstream analyses. 68 OTUs for bacteria were acquired eventually. The results of 454-sequencing in various phases are shown in Table 3. The biodiversity of the three water samples was investigated based on Chao 1 and Observed_species analyses at cutoff levels of 3%. This research was conducted on the basis of a full-scale drinking water distribution system, which was of nature properties, such as low nutrient, unstable hydraulic conditions, buffered in temperature. Although coupons with cement mortar material surface were used in this research, the growth of microbes on the coupon surface was limited to some degree. Therefore, the Observed_species was low in this research.

Table 2
Physical and chemical properties of water and biofilm samples.

Phase no.	SP	Biofilm	Water								
		HPC (CFU/mL)	HPC (CFU/mL)	Total chlorine (mg/L)	TOC (mg/L)	pH	Turbidity (NTU)	Conductivity (us/cm)	Ammonia(mg/L)	Nitrate (mg/L, as N)	Nitrite (mg/L, as N)
Phase I	A1[a]	10	4	1.3	3.9	7.6	0.27	62	0.15	2.7	0.001
	B1	1000	15	1.4	4.1	7.5	0.3	62	0.27	2.7	0.003
	C1	4000	1	1.5	4.2	7	0.3	61	0.28	2.6	<0.001
Phase II	A2	150	0	1	1.5	8	0.28	53	0.15	1.3	0.046
	B2	510	1	0.89	1.6	8	0.33	53	0.13	1.3	0.195
	C2	2800	7	1	1.6	8	0.3	53	0.21	1.3	0.011
Phase III	A3	2800	0	1.1	1	7.9	0.11	45	0.23	/	0.001
	B3	2800	2	1.1	1.2	7.8	0.17	30	0.22	1.3	0.002
	C3	16,000	5	1.3	1.3	7.2	0.18	38	0.19	1.1	<0.001

[a] "A" represents sampling point A, "1" represents phase I, others were similar to this note.

Table 3
Results of biofilm samples by 454-sequencing and average value of diversity parameters in different phases.

Phase no.	Sample	Raw reads	Reads_qc	Effective reads ratio (%)	Reads in OTU	Chao1	Observed_species
Phase I	A1[a]	13,918	10,757	77.29	4204	41.48	37.9
	B1	8819	6525	73.99	2094	37.61	35.8
	C1	3275	2320	70.84	546	25.63	24.6
Phase II	A2	5033	4116	81.78	947	22.14	20.9
	B2	3753	2797	74.53	592	29.91	21.1
	C2	5565	4833	86.85	883	16.11	15.7
Phase III	A3	7504	5796	77.24	2155	33.48	30.7
	B3	5588	4608	82.46	2016	26.20	25.9
	C3	5833	4954	84.93	2113	31.41	29.6

[a] "A" represents sampling point A, "1" represents phase 1, others were similar to this note.

In terms of Chao 1 and Observed_species, the value in phase I was the highest, whereas it decreased in phase II, after which it increased again during phase III. This result could be attributed to the new water environment and bacterial species that accompanied the water source switching.

3.3. Microbial community

Fig. 3 shows the differences among various phases and samples regarding the relative abundance of microorganisms. The three dominating phyla in the three phases were Proteobacteria (37%–96.6%), Acidobacteria (0.05%–24.3%), and Actinobacteria (0%–39.6%). At the phylum level, Proteobacteria was dominant in almost all of the biofilm samples except for sampling point C in phase II (37%), which was replaced by Acidobacteria (62.7%). Compared to phase I, the relative abundance of Acidobacteria (2.0%–7.7%) and Actinobacteria (0.2%–7.5%) obviously increased in phases II (24.0%–62.7%) and III (2.4%–39.6%), respectively. Apparently, water source switching generated obvious influence on the community of biofilms developed in the practical DWDS.

Differences were much more evident when compared at the class level, and the relative abundance of changes in Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria were representable. Similar to previous studies (Clemmensen et al., 2013; Holinger et al., 2014; Luo et al., 2013; Shaw et al., 2014), Betaproteobacteria was the most abundant class in phases I and II (34.6%–76.2%), but it was challenged by Gammaproteobacteria in phase III (11.3%–39.9%). As for Alphaproteobacteria, it's relative abundance was the highest in phase I (8.2%–16.6%) and it was kept at almost the same level during the latter two phases (1.6%–2.9%). In addition, it was apparent that the relative abundance of Holophagae was of high level only during phase II (24.0%–62.7%), while that of Gammaproteobacteria and Actinobacteria increased in phase III (39.5% and 39.6%), and this rule was extremely typical at point C.

At the level of genus, microbial community composition changes in biofilm samples were much more evident (Table S2). *Dechloromonas* was dominant in biofilm samples of B in all three phases, and its relative abundance decreased from 57.6% in phase I to 29.2% in phase II;

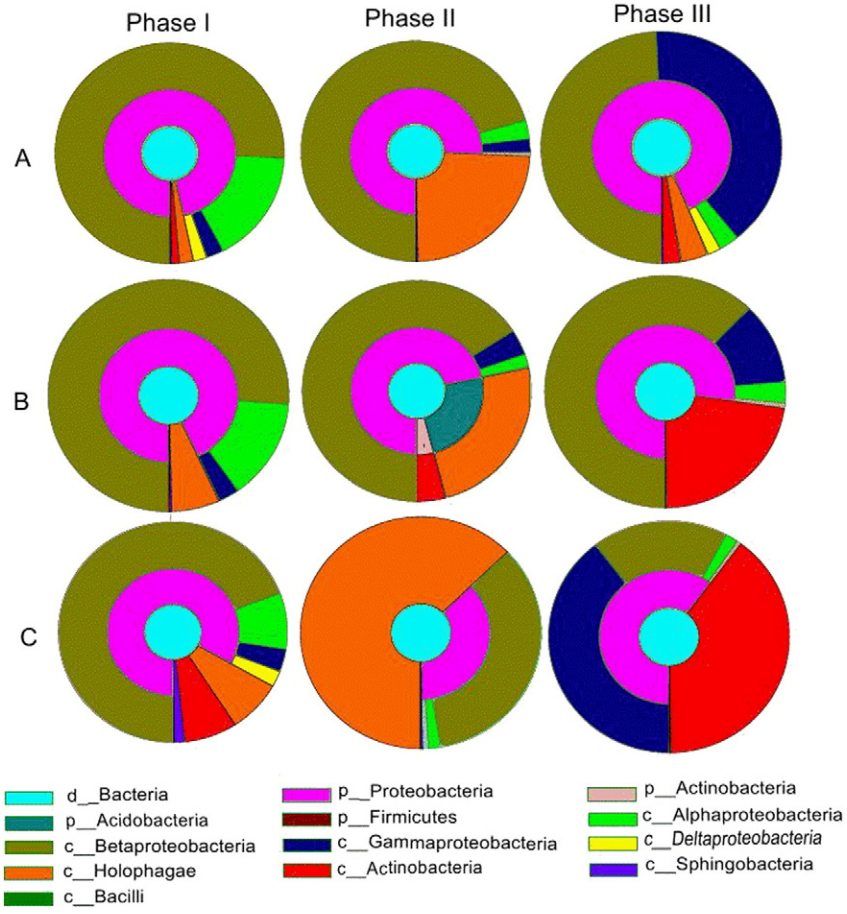

Fig. 3. Community shift of biofilm samples in various phases. The inner circle represents kingdom level (d), the middle circle represents phylum level (p), and the external circle represents class level (c).

however, it increased again to 37.9% in phase III. In sampling point A, the main genus in phase I (62.4%) and phase II (40.8%) was also *Dechloromonas*; however, it changed to *Acinetobacter* in phase III (39.6%). In general, community shift happened in our research.

4. Discussion

4.1. The effects of water quality on microbial diversity

As described in the section of microbial diversity, the microbial diversity presented irregular pattern. This pattern was closely related to water quality (Table 2). Although high total chlorine content was maintained, high TOC was retained in phase I and primarily contributed to high colony counts and sample biodiversity. In phase II, the microbial ecosystem formed in phase I was disturbed by the new water source because the water source grade changed from III–V to II (Table 1). The average TOC level drastically decreased nearly 70% from phase I to phase III, and the attacking effect induced by the new water source determined the decline of biodiversity in the biofilm. In phase III, TOC and colony counts continually decreased, but the new ecosystem gradually established along with time, and many organisms regrew and new species also developed. Therefore, the biodiversity of the biofilm increased again. However, the biodiversity in phase III was not as high as it was in phase I; this was also determined by improved water quality.

In order to get detailed information of correlations between alpha-diversity metrics and water quality parameters, correlation analysis was performed and the result was shown in Table 4. The results manifested that Chao1 and Observed_species presented positive correlation with HPC, TOC, Total Chlorine and Conductivity, and negative correlated with pH and Turbidity. However, the Pearson correlations between microbial diversity indexes and each single environmental factor were not significant ($P > 0.05$). Multiple Linear Regression (MLR) analysis were conducted by taking more than two environmental factors, and the results were shown in Table 5. Observed_species significantly correlated with TOC, Turbidity, Ammonia and HPC ($P < 0.05$), and this model was well fitted ($R^2 = 0.95$), while the fitting result of Chao1 and water quality parameters was not as well as that of Observed_species. Based on the results, Turbidity and Ammonia were both negatively correlated with microbial diversity, while TOC was helpful to increase microbial diversity. This indicated that water quality could generate different effects on microbial diversity.

4.2. The effects of water quality on microbial community

As demonstrated in the microbial community section, microbial community shifts were remarkable during the three phases of this research. The reasons for such shifts are various, and water quality is likely to make contributions to this issue. Canonical correlation analysis (CCA) was performed with the purpose of exploring relations between microbial species and water quality in this study, and the results are shown in Fig. 4. Water parameters were labeled with arrows, microbes were expressed with triangles, and circles were used to mark sampling points. The angle between microbial species and water quality indexes was on behalf of the positive (acute angle) and negative correlation (obtuse angle) between them. Based on Fig. 4(a) and the above illustration, it could be summarized that many parameters, including TOC, were negatively correlated with the relative abundance of Gammaproteobacteria, but they were positively correlated with the relative abundance of Alphaproteobacteria and Betaproteobacteria. In addition, it should be noticed that Ammonia was positively related with Gammaproteobacteria and Alphaproteobacteria, while Betaproteobacteria was negatively correlated with it. This implied that organic matter and ammonia were good for the growth of different classes of Proteobacteria phylum. As we all known, ammonia was one kind of nitrogen sources, while TOC represented carbon source. The switching of source water might alter the ratio of C/N of the water environment in the DWDS, and this change probably promoted the community shift of pipe biofilms in our research.

Chlorine was one prevalent disinfectant used in the water treatment process, and it played an important role on shaping the community composition of biofilms (Behnke et al., 2011; Gagnon et al., 2005; Wang et al., 2014). It's true that the chlorine resistant ability was different with regard to different microbial species. According to Fig. 4(a), the angle between Alphaproteobacteria and Total chlorine was less than that between Betaproteobacteria, Gammaproteobacteria and Total chlorine, this suggested that Betaproteobacteria and Gammaproteobacteria are more resistant to chlorine comparing to Alphaproteobacteria in the biofilm. The varying levels of drinking-water bacterial populations' resistance to monochloramine disinfection was investigated by Tzu-Hsin Chiao, *Dechloromonas*, *Acidovorax* and *Geothrix* were also detected in their test and they were defined as the most sensitive genera to monochloramine (Chiao et al., 2014). However, *Dechloromonas* was dominant in phase I with the highest total chlorine level in sampling point C in this study (Table 2), this might be due to the real amount of chlorine required to kill bacteria not being completely reflected by the total chlorine level.

As for sampling point C, the analytical results of correlations between community species and water environment parameters were shown in Fig. 4(b), it was obvious that pH and HPC were positively correlated with the relative abundance of Holophagae, Total chlorine and HPC were positively correlated with the relative abundance of Gammaproteobacteria and Actinobacteria. In particular, the relative high pH condition possibly promoted the increasing of Holophagae during phase II. However, the reasons for the increase of the relative abundance of Actinobacteria was still not clear, more efforts need to be spent on it in the future. In addition to the effects of water quality, competition among bacterial species can also result in the growth of specific bacterial populations that take advantage of others (Prest et al., 2014). Above all, the reasons of microbial community shift was complex, more investigations should be conducted in the future.

4.3. Opportunistic pathogens

Biofilms grown on the inner pipe wall of the DWDS have been considered as a reservoir for pathogens (Wingender and Flemming, 2011) and pose significant threats to human health (Ortolano et al., 2005). Some species of *Mycobacterium* spp. (Norton et al., 2004) and *Pseudomonas* spp. (Lee et al., 2011) were common opportunistic pathogens as well as detected in this study (Table S2). In addition to opportunistic pathogenicity, previous studies have shown that some species of

Table 5
MLR analysis between microbial diversity and water quality.

Dependent Variable	Variable Coefficient					R^2
	Constant	TOC	Turbidity	Ammonia	HPC	
Chao1	56.48*	6.76*	−93.50*	−94.47	/	0.70
Observed_species	54.78*	6.17*	−108.14*	−85.37*	0.62*	0.95

* $P < 0.05$.

Table 4
Correlation analysis between microbial diversity and water quality.

Parameter	HPC	TOC	pH	Total Chlorine	Turbidity	Conductivity	Ammonia
Chao1	0.27	0.44	−0.29	0.45	−0.18	0.23	−0.03
Observed_species	0.36	0.50	−0.41	0.62	−0.29	0.17	0.18

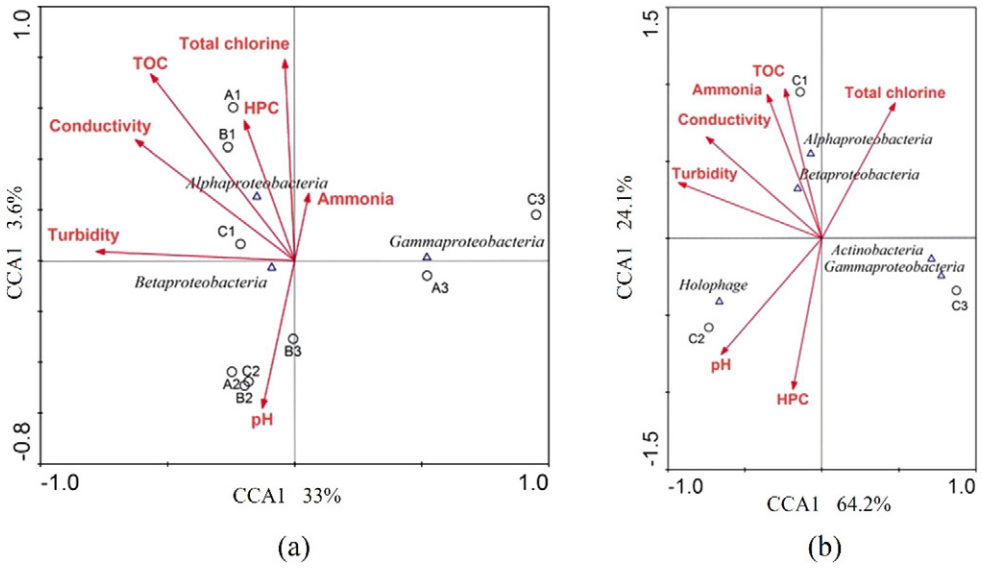

Fig. 4. The correlations among sampling points, water quality parameters and microbial species at different phases (a) and point C (b).

Mycobacterium spp. and *Pseudomonas* spp. also demonstrated chlorine resistance and should be the subject of much more attention. *Mycobacterium avium* was detected at a DWDS in Virginia (United States); that species can survive in water with 2 mg/L chloramine (Wang et al., 2012a). *Pseudomonas aeruginosa* has also been detected in water with 0.5 mg/L chlorine (Shrivastava et al., 2004). In this study, the relative abundance of *Mycobacterium* spp. increased in general, particularly at sampling points B (22.6%) and C (39.4%) in phase III (Table S2), whereas the total chlorine decreased from 1.4 mg/L (phase I) to 1.1 mg/L (phase III) at sampling point B and from 1.5 mg/L (phase I) to 1.3 mg/L (phase III) at sampling point C (Table 2). Compared to *Mycobacterium* spp., the relative abundance of *Pseudomonas* spp. was always maintained at a low level (<2.9%) during the experiment (Table S2). This result demonstrated different chlorine resistance of *Mycobacterium* spp. and *Pseudomonas* spp. At total chlorine concentration of 1.1–1.3 mg/L, *Mycobacterium* spp. and *Pseudomonas* spp. both could survive, but only the former could regrow.

5. Conclusions

- This study provides a detailed view on the community shift of biofilms developed in a full-scale drinking water distribution system involving different water sources. Results suggested that microbial community and microbial diversity varied among different phases. Proteobacteria was the main phylum during all phases, and the dominant class of it was Betaproteobacteria during the first two phases, while it was challenged by Gammaproteobacteria in phase III. Taking sampling points into consideration, the community shift of point C did representative own to relative great fluctuation of water quality. Besides, microbial diversity and microbial similarity also varied depending on different phases.
- Water quality could generate influence on microbial diversity and community structure, and different water quality parameters produced different effects. According to the results of statistical analysis, Turbidity, Ammonia, TOC and HPC could significantly affect microbial diversity together, and the former and the latter two parameters were positively and negatively correlated with microbial diversity, respectively. Moreover, Alphaproteobacteria and Betaproteobacteria were positively correlated with TOC, and Gammaproteobacteria was positively correlated with ammonia.
- Some specific opportunistic pathogens were detected at all phases, this remind us that more efforts should be spent on seeking the reasons of this problem, while new strategy must be developed to control the multiply of them at the same time.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.11.121.

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