Environment International 125 (2019) 117–124

Contents lists available at ScienceDirect

Environment International

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

Organic carbon: An overlooked factor that determines the antibiotic resistome in drinking water sand filter biofilm

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ARTICLE INFO

Handling Editor: Yong-Guan Zhu

Keywords:
Antibiotic resistance genes
Organic carbon
Biofilter
Bacterial community

ABSTRACT

Biofilter, an essential water treatment process, is reported to be the harbor of bacterial antibiotic resistance genes (ARGs). Due to the oligotrophic characteristic of source water, filter biofilm is largely influenced by the concentration of organic carbon. The objective of this study was to investigate the effect of organic carbon concentration on shaping bacterial antibiotic resistome in filter biofilm. Our study was based on pilot-scale sand filters, and we investigated the antibiotic resistome using high-throughput qPCR. A total of 180 resistance genes from eight categories of antibiotics were detected in 15 biofilm samples of three sand filters. The results indicated that higher concentration of influent organic carbon led to lower diversity of bacterial community and richness of antibiotic resistance genes (ARGs) in biofilm. We discovered a negative correlation ($p \leq 0.01$) between the richness of ARGs and the corresponding TOC level. Moreover, the absolute abundance of ARGs was positively correlated ($p \leq 0.05$) with the abundance of 16S rRNA gene and was determined by the organic carbon concentration. Sand filters with gradient influent organic carbon concentration led to the formation of different antibiotic resistomes and canonical correspondence analysis (CCA) indicated that difference in bacterial community composition was likely the main reason behind this difference. We also observed a similar trend in the relative abundance of ARGs, which increased with the depth of sand filters. However, this trend was more pronounced in filters with low organic carbon concentrations. Overall, this study revealed that the organic carbon concentration determined the absolute abundance of ARGs and also shaped the diversity and relative abundance of ARGs in drinking water sand filters. These results may provide new insights into the mechanism of persistent bacterial antibiotic resistance in drinking water treatment.

1. Introduction

Biofilters are widely used in drinking water treatment since they exhibit the potential to remove pollutants such as biodegradable organics, ammonia and nitrate. Bio-oxidation of organic matter and ammonia can also decrease the amount of precursors available for disinfection by-product formation. However, biofilters have limitations when faced with emerging contaminants such as antibiotic resistance genes (ARGs). Recent studies suggest that ARGs, as well as antibiotic-resistant bacteria (ARB), persist in biofilter-treated water (Zhang et al., 2009; Khan et al., 2016; Schwartz et al., 2003).

Bacterial antibiotic resistance is often associated with reduced bacterial fitness. Specifically, studies speculated that expression of ARGs consumes extra energy, and it has been proposed that a reduction in antibiotic use would likely benefit the susceptible bacteria, allowing them to outcompete their resistant counterparts over time. In full-scale drinking water biological treatment processes, selective pressures such as antibiotics and heavy metals are usually present at extremely low (ppb) levels (Zhang et al., 2018a, 2018b) and frequently are not even detectable (Simazaki et al., 2015). Given such low selective pressures, ARB screening is rarely possible. However, ARB and ARGs are widely detected in either drinking water treatment plants (DWTP) or distribution systems (Schwartz et al., 2003; Xi et al., 2009). Moreover, recent studies found that water treatment processes such as biofiltration and water supply pipelines might increase the diversity and relative abundance of ARGs and may serve as an important reservoir for the spread of antibiotic resistance to opportunistic pathogens (Jia et al., 2015; Xu et al., 2016; Guo et al., 2014). Although residual chlorine has been reported to have a co-selective property and to screen for bacteria that are resistant to both chlorine and antibiotics in the water supply

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https://doi.org/10.1016/j.envint.2019.01.054
Received 8 October 2018; Received in revised form 17 January 2019; Accepted 17 January 2019
Available online 01 February 2019

pipelines (Jia et al., 2015; Shi et al., 2013), the notion of inconsistent effects that ARB and ARGs exert on the environment with extremely low selective pressure such as sand filter and carbon filter warrants additional investigation.

In principle, the attenuation, persistence and enrichment of ARGs or ARB in the environment are ecological processes that are affected by various factors (Andersson and Hughes, 2011). In addition to the known selective pressures such as antibiotics and heavy metals, other environmental factors play a major role in this process. One of such typical factors is organic carbon. Convincing evidence suggests that limiting the organic carbon could improve bacterial phenotypic tolerance to antibiotics (Tuomanen et al., 1986), and recent studies found that ARB were less costly in fitness and grew faster compared to susceptible strains under oligotrophic conditions (Paulander et al., 2009; Lin et al., 2018). These studies indicate that low concentration of carbon source is likely to promote both bacterial tolerance and resistance to antibiotics at the population level. However, at the community level, whether the oligotrophic condition improves the formation of bacterial antibiotic resistome remains unknown.

In this study, three pilot sand filters were established in parallel. Same feeding water was used for all filters but the total organic carbon (TOC) level was modified in each filter via addition of glucose to the influent. Filter biofilm was harvested after 52 days of operation to evaluate bacterial community composition and profile the antibiotic resistome with different organic carbon concentrations. A high-throughput q-PCR-based approach (HT-qPCR) was used to characterize profiles of ARGs in filter biofilms. Additionally, Illumina sequencing coupled with bioinformatic analysis was used to investigate the bacterial community structure and its relationship with the antibiotic resistome. The results of this study are likely to increase the understanding behind the inconsistency of ARGs persistence with the low selective pressures at the community level.

2. Material and methods

2.1. Setup and operation of sand filters

The experimental setup is depicted in Fig. S1. Three sand filters were operated in parallel. The sand filters were fed with dechlorinated tap water since the residual chlorine has the potential to select for ARB (Li et al., 2012). Dechlorination of tap water was achieved by downflow filtration with a granular-activated carbon filter. Before passing through the sand bed, a nutrient solution composed of organic carbon, nitrogen and phosphorus was injected into the dechlorinated tap water at the top of each filter column to stimulate the growth of indigenous bacteria in the sand filter column (Zhang et al., 2011). The sand filter parameters and the nutrient solution ingredient list are shown in Tables S1 and S2, respectively.

The experiment ran in two phases. In phase 1, glucose was added to the nutrient solution at a final concentration of 10 mg/L to ensure that all filter columns had the same TOC level in the influent (Table S2). The phase 1 lasted for 7 days which allowed for the formation of the initial biofilm on the three sand filters. In phase 2, the glucose concentration was increased in the nutrient solution of filters B and C to 100 and 1000 mg/l (Table S2), respectively, to create a gradient of organic carbon concentration among the three filters. Studies report that the TOC levels in source water range from 2 to 17 mg/l in typical drinking water treatments. Moreover, such gradients are designed to simulate the different levels of TOC in source water mimicking full-scale drinking water treatment processes (Volk et al., 2000). The phase 2 took 45 days, wherein backwash was performed every 12 h to minimize microorganism overgrowth in filter columns.

2.2. Water quality analysis

During the experiment, tap water and dechlorinated water were monitored for the residual chlorine by the chlorine Pocket ColorimeterTM II (HACH). Influent and effluent for each sand filter were monitored for total organic carbon (TOC), dissolved oxygen (DO) and turbidity using the total organic carbon analyzer (TOC-V, SHIMADZU), multi-parameter meter (Multi 34,210 SET D, WTW) and turbidity meter kit (Qrion AQ4500, Thermo), respectively. On day 52, the water samples were collected at different depths of the column (0, 10, 20, 35 and 50 cm) for the analyze of TOC, DO and total nitrogen (TN) and total phosphorus (TP). TN and TP were determined according to the APHA (2005) guidelines and three-dimensional excitation-emission matrix (EEM) fluorescent spectroscopy was used to determine the fluorescence of organic component ingredients in all water samples.

2.3. Biofilm collection, pretreatment and DNA extraction

Quartz sand at different depths of the column (Fig. S1B) was sampled to collect the associated biofilm. Sand sampling was performed before each backwash. To separate bacterial cells from sand particles, 3 g of quartz sand was treated in 50 ml pyrophosphate solution using an ultrasonic bath (600 W, 35 kHz) performed three times with 10 min exposure and 5 min intervals. Next, 1 ml of suspension was taken for heterotrophic plate count (HPC) determination and the remaining suspension was concentrated by a vacuum filtration apparatus onto 0.22 μm filters. The filters were stored at −80 °C until further use. Specifically, total DNA extraction was performed on samples collected on day 52, using the FastDNA Soil Kit (MP Biomedicals, CA, USA). The DNA concentration and purity were measured by micro-spectrophotometry (NanoDrop-100, Thermo). Finally, the DNA was diluted to 50 ng/μl with sterile water and stored at −20 °C until further analysis.

2.4. Plate cultivation for total culturable bacteria

HPC was utilized to determine total culturable bacteria after sample taking. The collected suspension was serial diluted with 0.9% sterile saline and used for plate inoculation. An aliquot of the 100 μl diluted samples was plated on media and then incubated for 5 days at 28 °C for colony counting. R2A agar was used as media for culturable bacteria detection. Triplicate plate counts with the available counts in the range of 30–300 per plate were used in this study.

2.5. HT-qPCR

A total of 299 primer sets (Table S6) were used to amplify the DNA. These primer sets targeted resistance genes for 8 categories of antibiotics (286 primer sets), transposase genes (9 primer sets), integrase genes (3 primer sets) and the 16S rRNA gene (Zhu et al., 2012; Corrêa et al., 2014; Ekkapobyotin et al., 2008). HT-qPCR was performed using the Wafergen SmartChip Real-time PCR system. For each primer set, a non-template negative control was included. The thermal cycle consisted of 10 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 30 s and annealing at 60 °C for 30 s (Wang et al., 2014). Melting curve analyses were automatically generated by Wafergen software.

All quantitative PCRs were performed in technical triplicates. Amplifications efficiency beyond the range (90%–110%) were discarded. Only data for samples with three technical replicates and at least three repeated sampling replicates that generated amplification products were regarded as positive and used in further data analysis. Relative copies were calculated following methods (Looft et al., 2012):

$$\text{Gene copies} = 10^{(31-CT)/(10/3)}$$

where CT refers to quantitative PCR results and 31 refers to the detection limit. The relative ARG copies generated by the HT-qPCR were transformed into absolute copies by normalization to the absolute 16S rRNA gene copies.

Absolute 16S rRNA copies were determined by the standard curve

method of quantification using the Roche 480 system. Each 20 µl qPCR consisted of 10 µl 2× LightCycle 480 SYBR Green I Master (Roche Applied Sciences), 1 µg/µl bovine serum albumin, 1 µM each primer, 1 ng/µl DNA as template and 6 µl nuclease-free PCR-grade water. The thermal cycle consisted of a 10-min initial enzyme activation at 95 °C, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s and extension 72 °C for 15 s. A plasmid control containing a cloned and sequenced 16S rRNA gene fragment (1.39×10^{10} copies/l) was used to generate eight-point calibration curves from tenfold dilutions for standard calculation. All qPCRs were performed in technical triplicates with negative controls.

2.6. Bacterial community structure analysis

To investigate the bacterial community structure of filter biofilm, V3–V4 region of 16S rRNA was selected for amplification with primers 341F: CCTAYGGGRBGCASCAG and 806R: GGACTACNNGGGTATCT AAT (Yu et al., 2005). PCR amplification was performed using the Phusion® High-Fidelity DNA Polymerase (New England Biolabs) with the following cycle conditions: 98 °C for 30 s, followed by 35 cycles of 98 °C for 5 s, 56 °C for 20 s and 72 °C for 20 s and a final extension of 72 °C for 5 min. Then, the amplification products were purified and submitted to Illumina HiSeq2500 platform (Novogene, Beijing, China) for sequencing. Raw pair-end reads were assembled after the filtering adaptor, low-quality reads, ambiguous N, and barcode to generate clean joined reads capturing the complete V3-V4 region of the 16S rRNA gene by FLASH (V1.2.7, http://ccb.jhu.edu/software/FLASH/) (Magoč and Salzberg, 2011). The generated high quality sequences were processed and analyzed using Quantitative Insights Into Microbial Ecology (QIIME, V1.7.0, http://qiime.org/index.html) (Caporaso et al., 2010). The open-reference operational taxonomic unit (OTU) picking was performed following the online instruction of QIIME. OTU was defined at the 97% similarity level using Uparse software (Uparse v7.0.1001) (Edgar, 2013). Representative sequence for each OTU was screened based on RDP classifier (http://sourceforge.net/projects/rdp-classifier/) for annotate taxonomic information. All sequences have been deposited in the National Center for Biotechnology Information Sequence Read Archive under the accession number SRP150492.

2.7. Statistical analysis

In this study, the richness of ARGs was defined as the number of the detected ARGs. The absolute abundance of ARG was defined as the ARG copies per gram sand, representing the copies of ARGs in biofilm that attached to unit mass of quartz sand. The relative abundance of ARG was defined as the normalized ARG copies to the 16S rRNA copies. Averages and standard deviations of all data were determined using Microsoft Excel 2016. One-way analysis of variance (ANOVA) as well as paired-*t*-test was carried out to assess the variation (https://www.graphpad.com) and $p < 0.05$ was considered statistically significant. Pearson correlation analysis was conducted by using GraphPad Prism 7 either to reveal the correlation between the richness of ARG and the corresponding TOC level as well as the number of detected species, and the correlation between the absolute abundance (copies/g sand) of 8 categories of ARGs and the corresponding abundance of 16S rRNA, tranposases and integrons. Canonical correspondence analysis (CCA) was performed to find the relationship between the percentages of the genera and the abundance of detected ARGs. All figures were generated using GraphPad Prism 7. Shannon diversity and CCA were conducted using R3.3.3 (https://www.r-project.org/) with the vegan package (Jia et al., 2015).

3. Results

3.1. Filter performance

During the course of the pilot experiment, the GAC filter effectively removed the residual chlorine in the tap water (Table S3), providing a chlorine-free environment for the growth of the biofilm in the sand filter columns. In phase 1, the same glucose concentration assured the same TOC level (0.74 ± 0.16 mg/l) in influent of three sand filters (ANOVA, $p \geq 0.05$). After increasing the glucose concentration of filter B and filter C in phase 2, the influent TOC of the B and C reached 2.49 ± 0.30 mg/l and 12.24 ± 1.28 mg/l, respectively, which provided a gradient difference in the influent organic carbon concentration of the three filter columns (Fig. S2). After three weeks of operation, plate cultivation revealed that the amount of cultured bacteria in three filters was as follows: C > B > A (Fig. S3). Water quality analysis revealed that after two weeks of operation, the effluent TOC of three sand filters was rather stable and the top layer of sand bed (0–10 cm) provided the maximum TOC removal (Table S4) in all filters. It is worth noting that although the deep layer of sand bed (10–50 cm) had limited effect on TOC removal, the results of EEM spectra indicated that both soluble microbial by-products-like (SMPs, Ex = 250–340 nm, Em < 380 nm) and humic acid-like substances (HA, Ex > 280 nm, Em > 380 nm) increased in concentration and diversity when the water flowed (Fig. S4).

3.2. Bacterial communities in filter biofilm

After assembly and quality filtering, a total of 862,394 high quality sequences were obtained from 15 biofilm samples collected at different depths from three sand filters. The sequences ranged from 48,155 to 64,319 per sample and were clustered into 4901 operational taxonomic units (OTUs) at the 3% dissimilarity level (Table S5). At phyla level, Proteobacteria was dominant in samples from the three filters (Fig. S5), while *Massilia* was the dominant genus (Fig. 1a). Filter A presented higher abundance of genera *Phreatobacter*, *Hyphomicrobium*, *Mycobacterium* and *Methylophilus* (8.61% ± 5.47%, 5.87% ± 0.65%, 1.98% ± 2.29%, 1.80% ± 0.54%, respectively) compared with filter B (1.60% ± 0.97%, 0.73% ± 0.24%, 0.07% ± 0.02%, 0.26% ± 0.02%, respectively) and filter C (0.17% ± 0.07%, 0.22% ± 0.05%, 0.02% ± 0.01%, 0.08% ± 0.04%, respectively). Moreover, filter B was more abundant in genus *Pseudomonas* (11.59% ± 4.47%) compared with filter A (1.08% ± 0.18%) and with filter C (0.21% ± 0.08%). Shannon index identified that a higher influent organic carbon concentration likely leads to lower bacterial diversity in the sand filter (Fig. S6a) and results in higher structural similarity of bacterial community among different filter sampling sites as evidenced by principal coordinates analysis (PCoA, Fig. 1b). Along the column, bacterial diversity and the abundance of *Sphingomonas* increased with depth in all sand filters (Fig. S6b).

3.3. Richness and diversity of ARGs

Results of HT-qPCR indicated that a total of 80,124 and 105 ARGs were detected in filters A, B and C, respectively. Importantly, the richness of detected ARGs was basically identical in the top layer of three filters (Fig. 2) but presented a different trend with the increasing depth (10–50 cm). Specifically, the richness and Shannon diversity of ARGs remained stable in filter A and increased with depth in filter C, while the richness in filter B increased with the column depth but the Shannon diversity remained stable, suggesting that all ARG categories increased in richness along the filter. Furthermore, a negative and significant correlation ($p \leq 0.01$, Fig. S7) was found between the richness of ARGs and the corresponding TOC level, while the correlation between the richness of ARGs and the diversity of detected species was positive albeit not significant. We discuss the correlation between

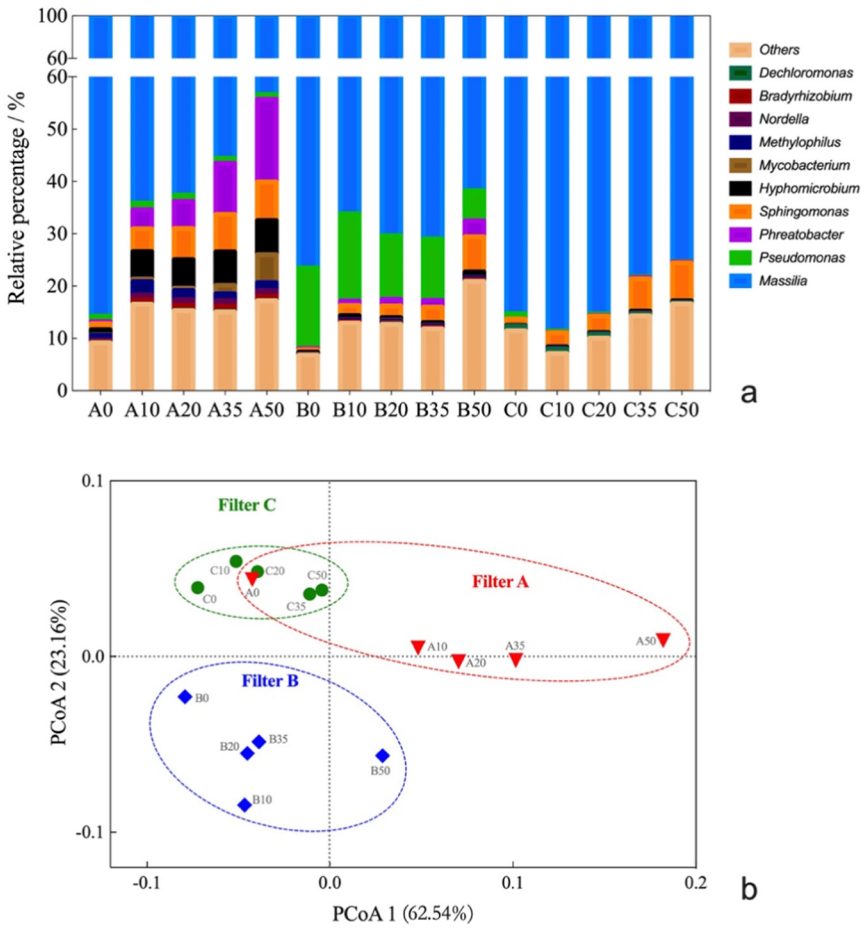

Fig. 1. (a) Bacterial community composition based on the average percentage of the top 10 largest taxonomic genera and (b) principal coordinates analysis (PCoA) based on the weighted Unifrac distance showing the overall distribution of bacterial communities in three sand filters. Samples A0 to C50 refer to biofilm samples collected at different sampling sites.

the ARG richness and the TOC level in detail later in the manuscript.

3.4. Absolute and relative abundance of ARGs

Based on the results of HT-qPCR, we calculated the absolute abundance of 16S rRNA gene, ARGs, tranposases and integrons in biofilm of three sand filters (Fig. S8). The 16S rRNA gene and ARGs presented highest absolute abundance in filter C, followed by filters B and A, which was also supported by HPC results (Fig. S3). The absolute abundance of the 16S rRNA gene and ARGs decreased with depth in filter B, and filter C and upper portion of filter A (0–20 cm), but remained stable in lower portion of filter A (20–50 cm). Among all detected samples, positive correlations ($p < 0.0001$) in absolute abundance were found between the ARGs and 16S rRNA gene and the integrons (Fig. 3). Data in Fig. 4 show the relative abundance of ARG category of each sample. Specifically, filter B presented higher abundance of aminoglycoside and MLSB resistance genes but was less abundant in FCA resistance genes compared with filter A and filter C (ANOVA, $p \leq 0.05$). Along the column the relative abundance of all detected ARG category increased with increasing depth in all filters. For each detected ARG, the fold change in relative abundance from surface (0 cm) to deep layers (10–50 cm) was calculated as depicted in Fig. S9. In deeper layers (10–50 cm) of filter A 73.8 ± 14.8% of ARGs increased and 12 ± 3% decreased in abundance compared with the surface (0 cm). This value of 56.8 ± 8.4% increased, while 8 ± 4.4% decreased in filter B and 35.9 ± 15.2% increased and 47.6 ± 10.1% decreased in filter C.

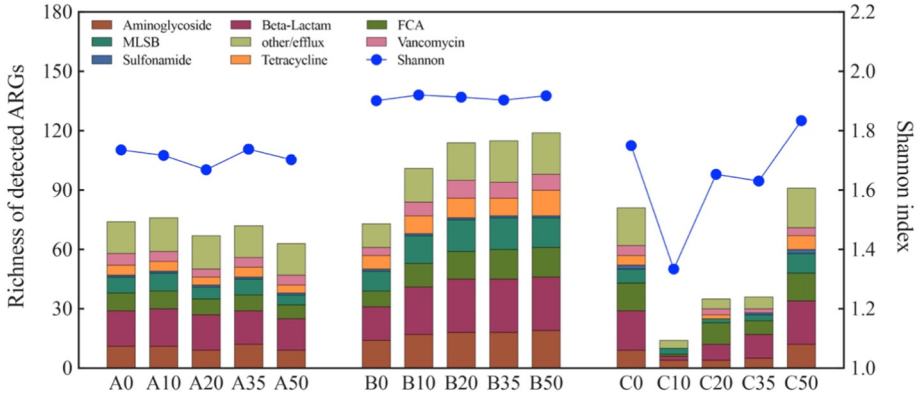

Fig. 2. Richness of detected ARGs and Shannon index indicating diversity of detected ARGs. Samples A0-C50 refer to biofilm samples collected at different sampling sites. MLSB = Macrolide-Lincosamide-Streptogramin B resistance genes, FCA = fluoroquinolone, quinolone, florfenicol, chloramphenicol and amphenicol resistance genes.

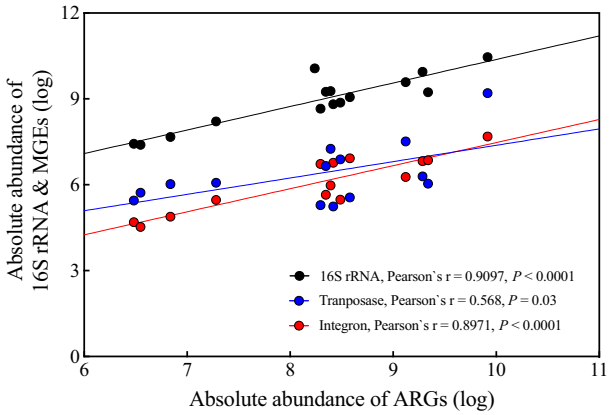

Fig. 3. The correlations between the absolute abundance (copies/g sand) of ARGs and the corresponding absolute abundance of 16S rRNA, tranposases and integrons in 15 biofilm samples. The data were log transformed.

3.5. Correlation between bacterial community and antibiotic resistome

The relationships among bacterial communities and ARGs were evaluated using canonical correspondence analysis (CCA, Fig. 5a). A total of 65.69% difference in antibiotic resistome among filters could be explained by bacterial community variables. Specifically, 9/10 most abundant genera were significantly correlated with the relative abundance of ARG ($p \leq 0.05$). Genera *Methylophilus*, *Mycobacterium*, *Hyphomicrobium*, *Nordella*, *Phreatobacter* and *Bradyrhizobium* statistically contributed to the relative abundance of ARGs in filter A, genus *Pseudomonas* was significantly correlated with the ARGs abundance in filter B, while *Massilia* and *Dechloromonas* were significantly correlated with the abundance of ARGs in filter C. Moreover, partial CCA was conducted to separate the effects of bacterial communities and mobile genetic elements (MGEs), including tranposases and integrons, on the variation of overall ARGs patterns among the filters (Fig. 5b). Bacterial communities and MGEs altogether explained 85.87% difference in antibiotic resistome, of which the bacterial communities explained 49.91%, which was much higher compared to MGEs alterations (4.65%). A substantially larger variation (31.31%) could be attributed to the interactions between bacterial communities and MGEs.

4. Discussion

In this study, we analyzed the bacterial antibiotic resistome and community structure in sand filter biofilm shaped by different organic carbon concentrations. We found that organic carbon can influence the bacterial antibiotic resistome in three ways: the richness, the absolute abundance and the relative abundance of ARGs. The results indicated that the reduction in concentration of organic carbon might lead to the decrease in absolute abundance of ARGs in filter biofilm; however, such reduction might also increase or maintain the richness and relative abundance of ARGs.

4.1. Influent organic carbon level shapes bacterial community structure in sand filter

We evaluated different bacterial communities that were shaped in sand filter with gradient organic carbon concentration. This result is in agreement with previous studies showing that addition of assimilable organic carbon altered bacterial community structure by reducing biodiversity in soil (Drenovsky et al., 2004) and aquatic environments (Judd et al., 2006), and a previous study showed that the shift in bacterial community composition along the TOC gradient is similar to the patterns of growth efficiency and growth rate (Eiler et al., 2003). Specifically, we found that higher influent organic carbon concentration leads to lower bacterial diversity and results in higher similarity in the structure of bacterial community among different filter sampling sites. An increase in nutrients allows for an increase in the complexity of the food web, whereas at high levels of eutrophication, more nutrients may be channeled to a few dominating species, further decreasing the evenness of species distribution. In extreme cases, an accumulation of toxic metabolites or other detrimental effects can occur, and this is likely to further reduce the diversity (Torsvik et al., 2002).

4.2. Concentration of organic carbon determines the absolute abundance of ARGs in filter biofilm

We found that the absolute abundance of ARG was significantly correlated with the abundance of 16S rRNA gene and integrons in filter biofilm, suggesting that the biofilm biomass drives the absolute abundance of ARG. It is well known that organic carbon is the limiting factor for bacterial growth in drinking water treatment and the TOC level determines the biomass of the filter biofilm (Van der Kooij and Hijnen, 1985; Escobar et al., 2001; Thayanukul et al., 2013). In turn, the concentration of organic carbon indirectly determines the absolute abundance of ARGs in filter biofilm.

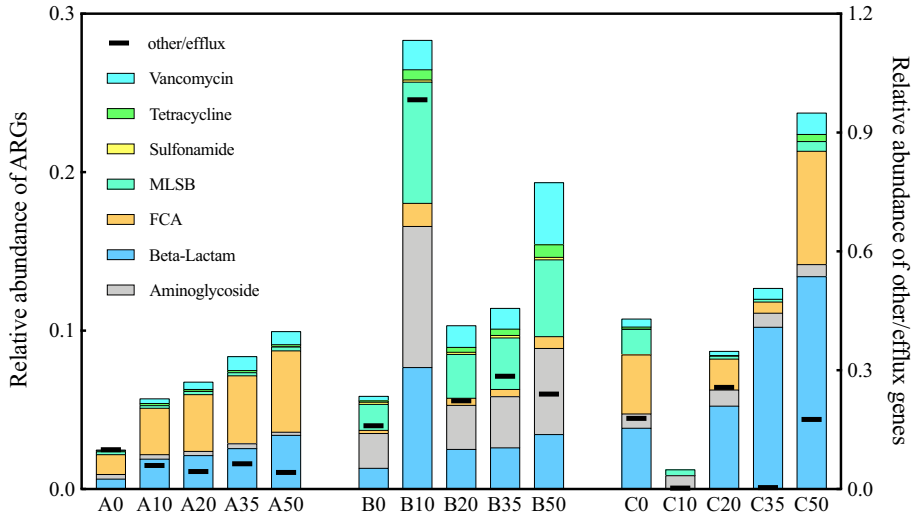

Fig. 4. Relative abundance of 8 ARG categories at each sampling site. Samples A0-C50 refer to biofilm samples collected at different sampling sites.

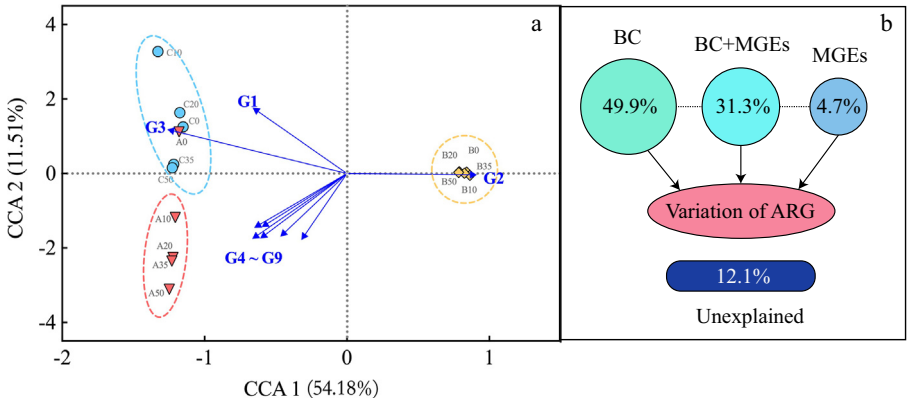

Fig. 5. (a) Canonical correspondence analysis of the quantitative correlation between the top 10 largest taxonomic genera and detected ARGs and (b) partial CCA differentiating the effects of bacterial community (BC) and MGEs on the shift of overall ARGs. Blue arrows in the CCA represent the nine genera (*Massilia* (G1), *Pseudomonas* (G2), *Dechloromonas* (G3), *Methylophilus* (G4), *Mycobacterium* (G5), *Hyphomicrobium* (G6), *Nordella* (G7), *Phreatobacter* (G8), *Bradyrhizobium* (G9)) which were positively correlated with persistent ARGs distribution ($p \leq 0.05$). The partial CCA was conducted based on the percentages of major genus and the relative abundance of MGEs, including tranposases and integrons. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.3. Influent organic carbon concentration affects the composition of antibiotic resistome of sand filter

Significant differences were found in the relative abundance of ARGs among filters with different levels of influent TOC levels. Moreover, the aminoglycoside and MLSB genes were more abundant in sand filter B that operated under 2.49 ± 0.30 mg/l influent TOC level. CCA indicated that the different bacterial community composition might be the main cause for the differences and *Pseudomonas* are likely responsible for the high abundance of aminoglycoside genes in filter B. Many studies suggest that differences in bacterial community structure are the leading cause of differences in antibiotic resistome in various environments (Jia et al., 2015; Chen et al., 2016; Su et al., 2015; Forsberg et al., 2014; Jia et al., 2017). In this study, the influent organic carbon shaped the bacterial community structure of the sand filter and indirectly affected the antibiotic resistome in biofilm. Previous study reported that *Pseudomonas*, especially *Pseudomonas aeruginosa*, has the potential to maintain aminoglycoside resistance genes such as *aadA* family (Gu et al., 2007) and MLSB resistance genes such as *ereA*, *mefA* (Lerma et al., 2014), and these genes were likely the predominant reason for the high abundance of aminoglycoside and MLSB in this study (Table S8). It is worth noting that *Pseudomonas* was reported as the core microbiota in natural biofilms in the drinking water treatments (Nguyen et al., 2014; Emtiazi et al., 2004; Stewart et al., 1990), and this study further suggested that TOC level around 2.49 ± 0.30 mg/l facilitates the growth of *Pseudomonas* spp. in aquatic biofilm.

4.4. Variation of antibiotic resistome with depth in the sand filter

In this study, the relative abundance of ARGs was increased with the depth in all sand filters and the richness of ARGs was positively correlated with the corresponding TOC level. We suspect this was likely caused by three reasons. First, the concentration of organic carbon could indirectly increase the richness of ARGs by influencing the biodiversity of bacterial community. The consumption of organic carbon in the sand filter is accompanied by an increase in organic carbon species, and may result in an increased diversity of bacteria in the biofilm. Previous study suggested that genetic diversity is positively correlated with bacterial diversity (Vellend and Geber, 2005), and such positive correlation was also found in this study (Fig. S7). Secondly, the oligotrophic environment is conducive to the survival of ARB. This conclusion was supported by the increasing abundance of *Sphingomonas* spp. in three filters. In other studies, *Sphingomonas* isolated from drinking water exhibited various antibiotic resistance patterns including β-lactams, ciprofloxacin and cotrimoxazole (Vaz-Moreira et al., 2011; Zhang et al., 2018a, 2018b). According to Monod equation, bacteria can grow at maximum rates (μ_{max}) when organic carbon is sufficient and at limited rates when organic carbon is insufficient. In sand filters, organic carbon is relatively adequate in the top layer and becomes a limiting nutrient with the increase in depth. Therefore, bacteria with higher μ_{max} will likely dominate in the top layer in the competition with resources such as space, while bacteria with lower μ_{max} will likely be eliminated, such competition will be weakened with the consumption of organic carbon along the filter column and this may improve the fitness of lower μ_{max} bacteria. Previous studies suggested that the acquisition of ARGs through either mutation or horizontal gene transfer may increase the bacterial fitness cost and may decrease the μ_{max} of bacteria (Mariam et al., 2014; Schrag and Perrot, 1996; Dahlberg and Chao, 2003; Bouma and Lenski, 1988; Lenski and Bouma, 1987; Smith and Bidochka, 1998). As a result, the slow-growing ARG-carrying bacteria show an increasing fitness with the depth in sand filtration. Third, the fitness cost of bacterial antibiotic resistance is directly affected by the carbon source (Foucault et al., 2010; Hiltunen et al., 2017; Andersson and Hughes, 2010). Specifically, a previous study found that ARB exhibit lower fitness cost in poor organic carbon environments (Paulander et al., 2009).

5. Conclusions

Overall, this study found that the decrease of TOC level leads to the increase in the diversity and relative abundance of ARGs, and the low level of TOC is conducive to the maintenance of bacterial antibiotic resistome. The results increase our understanding of shaping of the environmental bacterial antibiotic resistome by carbon source and may help explain the persistence of ARGs in drinking water treatment and distribution systems. Importantly, there is a unique difference in water quality between the dechlorinated tap water used in this experiment and the source water in the actual water treatment. Therefore, future studies are needed to evaluate the relationship between environmental organic carbon and bacterial antibiotic resistome in actual drinking water treatment.

Acknowledgements

This research was supported by the National Key Research and Development Program of China-International Collaborative Project from Ministry of Science and Technology (Grant No. 2017YFE0107300), the Natural Science Foundation of China (51678551), the National Key Research and Development Program of China (2018YFC1901000), the Natural Science Foundation of China (51478450), K.C. Wong Education Foundation and the Knowledge Innovation Program of the Chinese Academy of Sciences (IUEQN201504). We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.01.054.

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