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Bacterial community assembly and beta-lactamase (*bla*) genes regulation in a full-scale chloraminated drinking water supply system

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ABSTRACT

Beta-lactamases (*bla*) are the largest class of antibiotic resistance genes (ARGs) and can survive drinking water treatment processes. However, the key influencing factors of *bla* genes and the control measures in drinking water supply systems (DWSSs) remain unclear. Quantitative PCR, metagenomic, and 16S rRNA gene sequencing assays were performed to investigate the *bla* genes in the DWSS. The main carriers of *bla* genes are genera *Caenimonas* and *Sphingomonas*, which were the dominant bacterial genera in the DWSS. The abundance of *bla* genes was associated with bacterial community assembly events. When the heterotrophic plate count (HPC) in water was below 500 CFU/mL, stochastic assembly was the major contributor to bacterial community assembly and the bacterial community was less affected by the environment. When the HPC in water was above 500 CFU/mL, deterministic assembly played an important role and decreasing the organic matter improved the efficiency of *bla* genes control. Organic matter can affect *bla* genes by shifting the diversity of the microbial community, and less organic matter appeared to be beneficial to reducing the bacterial niche width in the DWSS. Nanofiltration (NF) can decrease the effluent organic matter in waterworks and slow the dissemination of bacteria carrying *bla* genes in the DWSS.

1. Introduction

Antibiotic resistance has been recognized as a global public health challenge in the 21st century [7]. Over 30 kilotons of beta-lactam antibiotics are sold globally per year [23]. Beta-lactamases (*bla*) are the largest class of antibiotic resistance genes (ARGs) and contribute to almost 50%– 70% of all known antibiotic resistance incidences [32]. *bla* genes are the dominant ARGs in water supply systems, and the *ampC* gene, which encodes resistance to beta-lactam, have detection frequencies of 79.8% [16,23,53]. One group of extended-spectrum beta--lactamases (ESBLs), which include expanded-spectrum cephalosporins, penicillins, and monobactams, can hydrolyze and cause resistance to various types of newer beta-lactam antibiotics [36]. The most active beta-lactams are carbapenems, which are used in the treatment of infections caused by ESBL-producing Enterobacteriaceae but can be hydrolyzed by New Delhi Metallo-beta-lactamase (NDM) [11]. However, both ESBLs and bla_{NDM-1}-producing bacteria have occurred in drinking water supply systems (DWSSs) [2,20].

Water utilities should provide quality tap water for consumers and not only quality effluent water (freshly treated water). In drinking water treatment processes, chlorine disinfection can promote the exchange of ARGs across bacterial genera by natural transformation [19]. Disinfection may increase the transfer of ARGs to pathogenic bacteria through horizontal gene transfer, thus, increasing the human health risk [39]. When entering drinking water distribution systems (DWDSs), the complex piping environment, such as material release, biofilm formation and detachment, and loose deposit accumulation and resuspension, leads to irregular changes in the bacterial ARGs [28]. The amount of ARGs may increase due to biofilm detachment [21,56]. Environmental stressors, such as antibiotics, temperature and heavy metal, can accelerate ARG replication in the microbial population [14,30]. Additionally, researchers compared three pilot-scale sand filters, and ARGs presented highest abundance in the filter biofilm with the highest glucose concentration, which showed the effects of organic matters on ARGs [48]. However, an effective method to remove the dominant ARGs in full-scale DWSSs has not been developed, which represents a great challenge to water utilities.

Potential indicators controlling microbial ARGs can be analyzed according to the influence levels of environmental factors on microbes and ARGs [58]. Physicochemical index of water quality showed

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significant correlations with ARG categories rather than total concentration of all ARGs [15]. Recent studies have suggested that the assembly of microbial species in a local community is influenced by two types of ecological processes, namely, deterministic and stochastic processes, and bacterial communities in aquatic ecosystems are often assembled to a large extent by stochastic processes [43,61]. Deterministic processes involve selection by environmental factors, with environmental variables determining the species niche width (the range of environmental conditions that allow species to survive) and further shaping the biodiversity across environmental gradients by influencing species growth and propagation [12,43]. Stochastic processes include unpredictable disturbance, probabilistic dispersal, and random birth-death events, thereby forming communities that are indistinguishable from those produced by random chance [54]. Microbial communities catalyze the turnover of carbon and other nutrients; therefore, the microbial community composition and ecological processes underlying the assembly of these communities can have important effects on the drinking water quality and functions of the ecosystem [13]. In DWDSs, the total community assembly is largely governed by the combination of deterministic and stochastic processes, and city water supplies contribute largely to the water community assembly in proximal pipes closer to the city [22,27]. However, the factors that affect the stochastic and deterministic processes that govern the assembly of microbial communities in DWSSs are poorly understood.

Shanghai is the core city of the Yangtze Delta Estuary, which harbors the most populous metropolises in the world and the most antibiotic-contaminated river basin in China [50]. Few studies have reported the control measures of *bla* genes in full-scale DWSSs. This meta-analysis examined a total of 106 temporal drinking water samples to address the following questions. (1) What are the major environmental factors that affect *bla* genes in full-scale DWSSs, and how do these factors correlate with *bla* genes? (2) What is the extent of the contributions made by deterministic and stochastic ecological events to bacterial community assemblies in DWSSs? (3) How can *bla* gene levels be effectively reduced in the full-scale DWSS? The insights derived from these questions will be crucial for the establishment of desirable ecological niches for bacterial communities in DWSSs to improve drinking water quality.

2. Material and methods

2.1. Sample collection and water quality analysis

This research was conducted in a full-scale drinking water treatment plant and its corresponding water distribution system in Shanghai, China. The plant treats surface water from the Jinze reservoir coming from Lake Taihu. A total of 28 sampling sites were selected, and water samples were collected every month from May 2018 to Oct 2020 (Fig. S1). Water samples were collected from Yuanjiang waterworks (i.e., raw water, after sedimentation, after sand filtration, after ozone oxidation, after biological activated carbon (BAC) filtration, and after chloramine disinfection), the primary DWDS (PrimDWDS) and secondary DWDS (SecDWDS). Specifically, PrimDWDS refers to the distribution main and SecDWDS refers to the infrastructures and pipes that are used to store, pressurize and transport water from the distribution main to taps in the community [24]. A nanofiltration (NF) treatment system (1 nm rated pores and 85% recovery rate) was added after sand filtration in the waterworks and began to operate on July 1, 2020. After five minutes of flushing, each water sample was collected for 20 L of water by using two 10 L bottles which had been disinfected by high-pressure steam sterilization. All samples were transported to the laboratory on ice and processed within 12 h.

The total chlorine and temperature measurements were performed in situ. The conductivity, pH, resistivity, turbidity, alkalinity, hardness, total dissolved solids, total organic carbon (TOC), chlorides, sulfates, nitrite nitrogen, bicarbonate, total iron, Ryznar saturation index (RSI), Larson ratio (LR), assimilable organic carbon (AOC), standard plate counts, heterotrophic plate counts (HPC), total cell concentration, and intact cell concentration measurements were performed in the laboratory. The detection methods for the abovementioned water quality parameters are shown in Tab. S1.

2.2. High-throughput sequencing and shotgun metagenomics sequencing

The enrichment of biological cells in water was performed by using the membrane filtration method. Twenty liters of water was filtered through a sterile 0.22-μm mixed cellulose membrane (Bandao, China) with a magnetic filter funnel (Pall Life Science, USA) and a vacuum pressure pump (Pall Life Science, USA). Genomic DNA was extracted from the membrane by using an optimized phenol:chloroform-based method [31]. The concentration of DNA was detected using a One-Drop OD-1000 spectrophotometer. DNA quality was checked by 2% agarose gel electrophoresis. Moreover, qualified DNA was adjusted to 50 ng/μL and stored at − 80 °C.

A total of 106 water samples were used for high-throughput sequencing, and they are shown in Tab. S2 and included 37 samples in the waterworks, 41 samples in the PrimDWDS, and 28 samples in the SecDWDS. The V3-V4 hypervariable regions of the bacterial 16 S rRNA gene were amplified with primers 338 F and 806 R [33]. Purified amplicons were sequenced on the Illumina MiSeq platform (Illumina, San Diego, USA) [51]. Raw fastq files were demultiplexed, quality-filtered by Trimmomatic, and merged by FLASH [5]. Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff for 16S-generated sequences using UPARSE. Furthermore, chimeric sequences were identified and removed using UCHIME [9,10]. Taxonomy was analyzed by the QIIME and Ribosomal Database Project (RDP) Classifier algorithm using a confidence threshold of 70% [49]. The 16 S taxonomic alignment was performed against the Silva Seed v138 database [37], and putative microbial functions were predicted by Tax4Fun and FAPROTAX [54].

To quantify the microbial community assembly processes, we implemented the null modeling methods developed by Stegen et al. Stegen et al., ($year$) [43]. In short, the beta-mean nearest taxon distance (βMNTD) among communities was calculated to represent the pairwise phylogenetic turnover. Then, the standard deviation of the observed βMNTD was calculated based on the null model. This standard deviation is the beta-nearest taxon index (βNTI), which represents the strength of environmental impacts in the community assembly. To further evaluate the potential drivers of community assembly, the pairwise βNTI was regressed against Euclidean distance matrices of environmental factors [46]. The statistical significance of the resulting relationships was evaluated with Mantel tests (999 permutations) in R (version 3.5.1) using the mantel function in the ecodist package.

Six representative samples were selected for shotgun metagenomic sequencing, and they included raw water, effluent water, PrimDWDS (i.e., initial and terminal water in the PrimDWDS), and SecDWDS (i.e., roof tank water and underground tank water). Sequencing was performed using Illumina Hiseq 2000. Libraries were prepared with a fragment length of approximately 400 bp using Covaris M220 and NEXTFLEX™ Rapid DNA-Seq Kit. Raw reads were quality controlled using fastq [6]. To reduce gene misestimation, transcripts per million reads (TPMs) were used to express the gene abundance of each sample. CD-HIT (http://www.bioinformatics.org/cd-hit/), SOAPaligner, and BLASTP were used for abundance calculations of nonredundant gene sets and annotation of the KEGG orthologies and modules [25]. The phenotypic traits of the bacterial community were predicted with BugBase (http://bugbase.cs.umn.edu).

2.3. Quantitative PCR

Quantification of the dominant *bla* genes in DWSSs, including *ampC*, bla_{TEM}, bla_{OXA}, bla_{NDM-1}, bla_{IMP}, and bla_{CTX-M}, was performed by

quantitative PCR (qPCR) with a CFX Connect™ Real-Time System (Bio-Rad, USA) [16,52]. Details of the qPCR primers are described in the supplementary information (Tab. S3). The DNA template (1 μL, 50 ng/μL) and appropriate primers (1 μL, 10 μM) were combined with HieffTM qPCR SYBR® Green Master Mix (YEASEN) to create 20 μL reaction volumes. The thermal cycling conditions were the same as those published in previous studies [52]. The absolute abundance of each gene was calculated by the formula mentioned earlier [60]. Briefly, the gene copy numbers were calculated by using the following equation: Gene Copy Number $= 10^{((31-C_t)/(\frac{10}{3}))}$, where C_t (cycle threshold) refers to the qPCR result with a detection limit set to 31 [29]. The relative copy number of each gene was calculated by normalizing each gene's copy number and bringing its ratio to the 16 S rRNA's normalized copy number. The absolute abundance of each gene was recorded as the product of the relative copy number and the 16 S rRNA's absolute copy number. For all samples, amplification was confirmed with more than two positive replicates.

2.4. Effect of organic matter changes on bla genes levels

The evaluation of the effect of organic matter changes on *bla* genes was performed by using the environmental water samples and the experimental water samples. Specifically, the absolute abundance of *bla* genes (i.e., *ampC*, bla_{TEM}, bla_{OXA}, bla_{NDM-1}, bla_{IMP} and bla_{CTX-M}) in the DWDS were compared before and after NF treatment in the waterworks. The experimental water was made of effluent water from a household water purifier and mixed with tryptone and yeast extract to get the desired organic carbon concentration and was left for 40 h at room temperature. The water purifier consists of polypropylene cotton filter, ultrafilter and activated carbon filter. Absolute abundance of *bla* genes in the above mixed water was detected by using qPCR.

2.5. Statistical analysis

All data analyzed in this study were considered statistically significant at $p < 0.05$ unless indicated otherwise. All environmental variables were evaluated using one-way univariate analysis of variance (ANOVA) to select reasonable measurements. Bacterial alpha-diversity, including richness indices (i.e., Sobs, Ace and Chao index) and diversity indices (i.e., Shannon and Simpson index) were calculated using the vegan packages [8]. Bacterial beta-diversity was calculated through principal component analysis (PCA) based on Bray-Curtis dissimilarity [8]. The first and second representative principal component (PC1 and PC2, respectively) of Bray-Curtis dissimilarity were adopted to represent the beta-diversity. Fast expectation-maximization microbial source tracking (FEAST) was used to identify the microbial percentage contribution of each potential source of the bulking water in different month [41]. Variance partitioning canonical correspondence analysis (VPA) was used to quantify the explanatory degree of the influence of different water quality parameters on the structural change of the microbial community [38]. Structural equation modeling (SEM) was used to evaluate the relative importance of ARGs based on environmental or biological factors [12]. The models should meet multiple goodness-of-fit criteria, i.e., nonsignificant χ^2 test ($p > 0.05$), goodness-of-fit index (GFI)> 0.90 and root mean square error of approximation (RMSEA)$<$ 0.08 [12]. To evaluate the effects of the environmental factor-driven metabolic theory on the microorganisms in the DWSS, we applied a niche width analysis framework and constructed ecological niche models to assess the niche width of taxa along environmental gradients [12]. A higher niche width value means that the species was more evenly presented and distributed on a large scale. The difference between groups was performed by using Welch's T-test (two groups) and Scheffe's significant difference test (multiple groups).

3. Results

3.1. Bacterial community composition in the DWSS

The water quality parameters showed strong seasonal changes over the two years of investigation (Fig. 1a). Spatial variations in the relative abundance of different bacterial families and genera are shown in Fig. 1b. The treated effluent water was divided based on the total chlorine concentrations into three groups: high (>0.90 mg/L), middle (0.50 mg/L<chlorine≤0.90 mg/L) and low (≤0.50 mg/L). The common dominant bacteria in the high and low total chlorine environments were Comamonadaceae, Caenarcaniphilales, *Oligoflexales*, and *Bdellovibrio*. *Sphingomonas* (family: Comamonadaceae) increased with the decay of chloramine. A total of 77.10% of bacteria were heterotrophic according to FAPROTAX (Tab. S4). An average of 72.96% of bacteria co-interacted to survive under chloramine oxidative stress, and this type of bacteria decreased from 78.42% to 67.24% with the decay of chloramine (Fig. S2, Tab. S5). In addition, according to the phenotype prediction of BugBase, 95.07% of the bacteria in the DWSS were gram-negative bacteria (Tab. S6).

3.2. Succession of bla genes in the DWSS

A total of 1.14% ± 0.05% of the genes were predicted as ARGs according to the functional prediction of PICRUSt2 by using high-throughput sequencing (Tab. S7). No significant difference (Scheffe's test $p > 0.05$) was observed after chloramine decay in the DWDS (Fig. 2a, Tab. S8). In addition to *bla* genes, the ARGs predicted by PICRUSt2 also included cationic antimicrobial peptide (CAMP) resistance genes (0.25%), platinum drug resistance genes (0.21%), vancomycin resistance genes (0.19%), and antifolate resistance genes (0.15%) (Tab. S7).

Shotgun sequencing indicated the contribution of bacteria to *bla* genes (Fig. 2b, Tab. S9). The dominant bacterial genera that carried *bla* genes were *Caenimonas*, *Sphingomonas,* and *Bdellovibrio* in the DWDS. Additionally, the contribution of genera *Sphingomonas* and *Caenimonas* to *bla* genes increased with the decay of chloramine, which could be due to the detachment of the biofilm.

3.3. Organic matter influences bla gene levels by shifting microbial community diversity

SEM was performed to quantify the contribution of interactive drivers, including the TOC concentration and microbial community diversity (i.e., Bray-Curtis dissimilarity PC1 and PC2), to the relative abundance variation of *bla* detected by high-throughput sequencing (Fig. 3a). All of the variables explained 61% of the relative abundance variation of *bla* genes (χ^2 =0.20, $p = 0.65$, GFI=0.998 and RMSEA<0.001). Although the direct effect of TOC on *bla* genes was small, organic matter exerted an indirect influence based on changes in the bacterial community diversity.

A shotgun sequencing analysis based on the KEGG database showed that 6564 genes were related to the beta-lactam resistance pathway (ko01501), and an analysis based on the CARD database identified 460 genes as *bla* genes in the DWSS (Tab. S10). The significantly positive correlations among the TOC, the composition of *bla* genes and the relative abundance of the beta-lactam resistance pathway suggested that organic matter also determines the composition of *bla* genes (Table 1).

To identify the dominant environmental factor affecting the bacterial communities, Spearman correlations between 22 water parameters and bacterial richness (i.e., Sobs, Ace and Chao indices) and diversity (i.e., Shannon and Simpson indices) were analyzed. The results showed that chlorides, conductivity, hardness, nitrite, temperature, and TOC were all significantly correlated with the bacterial richness and diversity (Tab. S11). More specifically, the correlation coefficient between the TOC and alpha diversity was the highest, which suggested the importance of

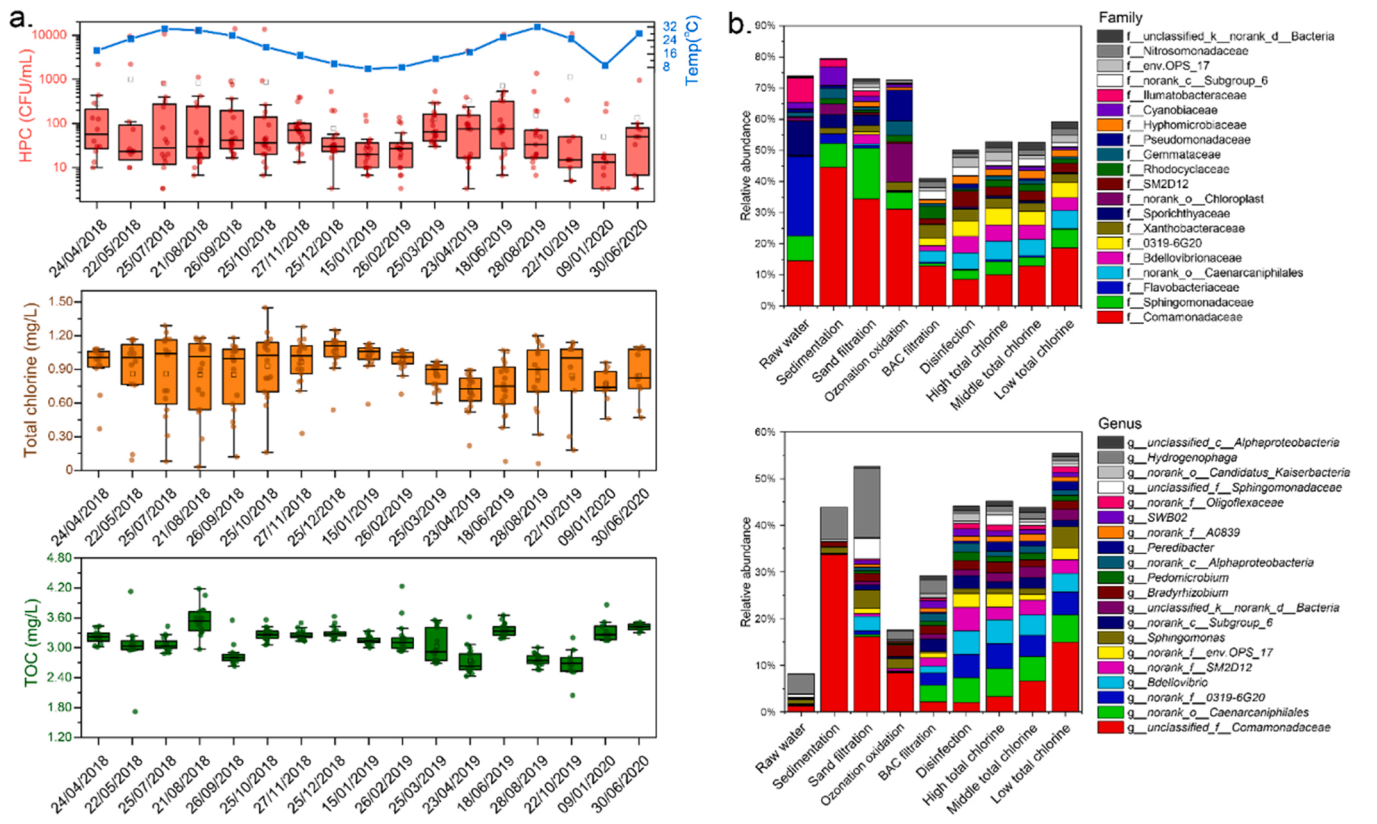

Fig. 1. Water parameters and bacterial composition in all samples during the study. (a), Temporal change in water parameters (i.e., temperature, HPC, total chlorine and TOC) in the DWDS. Boxes were used to indicate the positions of the upper and lower quartiles; the interior of box indicated the innerquartile range, which was the area between the upper and lower quartiles and consists of 50% of the distribution. Lines were extended to the extrema of the distribution, either minimum and maximum values in the dataset. Spots not fall between the inner and outer fences were used to indicate the outliners. **(b)**, Spatial change of bacterial composition at family and genera level (top 20).

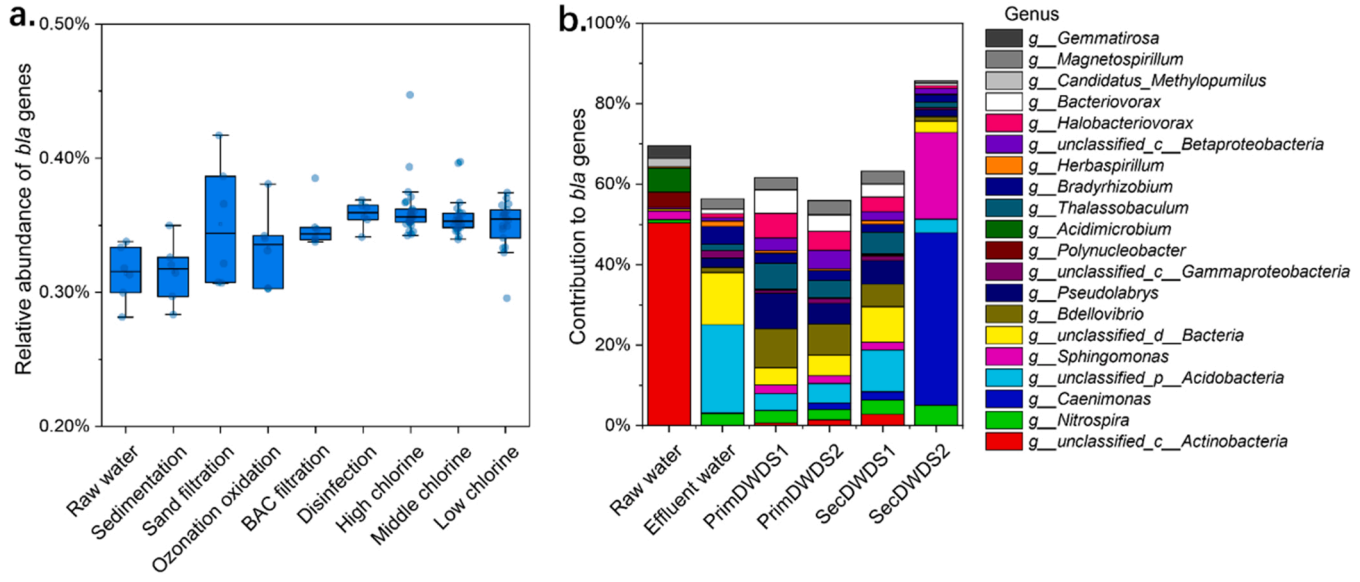

Fig. 2. Succession, contributor and influence factors of *bla* genes in the DWSS in all samples during the study. (a), Relative abundance of *bla* genes in the DWSS by high-throughput sequencing analysis. (b), Contribution of bacterial genera (top 15) to *bla* genes by shotgun sequencing. **(c)**, SEM quantification of the indirect and direct effects of TOC concentration and microbial community diversity on bacterial *bla* gene relative abundance variation. Solid and dotted arrows indicate positive and negative effects, respectively. The width of the arrows is proportional to the strength of the path coefficients (numbers adjacent to arrows). * ** indicate $p < 0.001$; * * indicate $p < 0.01$.

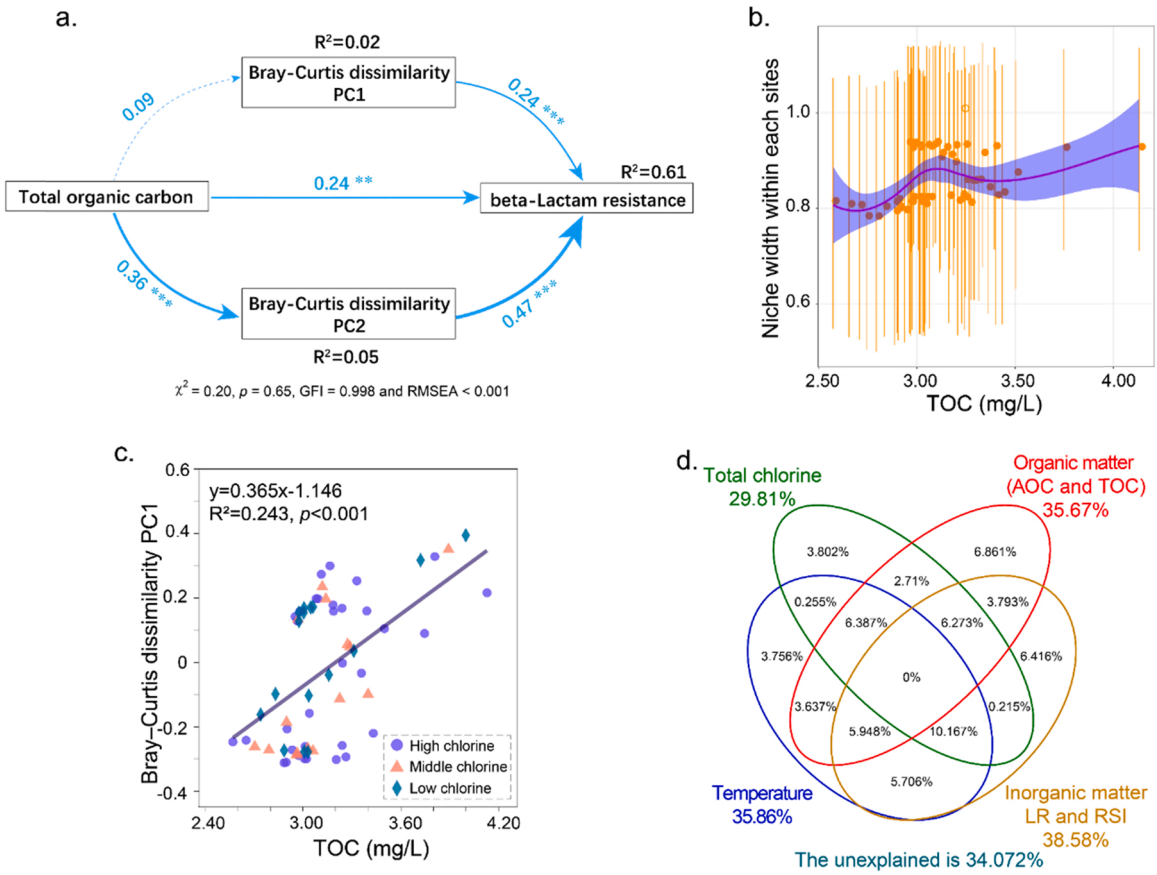

Fig. 3. Quantification of influence factors on bacterial *bla* genes and effect of water parameters on the bacterial community. (a), SEM quantification of the indirect and direct effects of TOC concentration and microbial community diversity on bacterial *bla* gene relative abundance variation. Solid and dotted arrows indicate positive and negative effects, respectively. The width of the arrows is proportional to the strength of the path coefficients (numbers adjacent to arrows). * ** indicate $p < 0.001$; * * indicate $p < 0.01$. (b), Bacterial niche widths across the TOC niche position. (c), Linear regression of bacterial Bray–Curtis dissimilarity and TOC. The shaded area indicates the 95% confidence interval. (d), VPA of the effects of water temperature, total chlorine, organic matter, and inorganic salt on bacterial community structure. Percentages indicate the variation of the bacterial community structure explained by the four sets of environmental factors.

Table 1
Correlation between TOC levels and beta-lactam resistance based on KEGG and CARD databases by shotgun sequencing analysis.

	KEGG pathway description: beta-lactam resistance (ko01501)	CARD antibiotic resistance ontology description:*bla* gene
Mantel statistic R	0.54	0.53
Significance	0.03	0.04

organic matter to bacterial diversity.

Organic matter is important for bacterial growth in DWSSs. We evaluated the TOC-based niche width for bacteria over their respective gradient ranges in the water supply system. The first two PCA components (PC1 and PC2 explained 24.86% and 18.35% of the variance, respectively) were adopted to represent the environmental stress after exclusion of TOC (Tab. S12). The TOC-based niche width for the observed bacterial species became significantly wider with increasing TOC (Fig. 3b). Based on the results of the niche width model, less organic matter was beneficial to reducing the niche width of bacteria within the DWDS. In addition, the Bray–Curtis dissimilarity of the bacterial community significantly decreased with increasing TOC (Fig. 3c). Therefore, less organic matter was beneficial to reducing the dissimilarity of the bacterial community structure between effluent and terminal water.

Organic matter was not the only factor underlying changes in the bacterial community in the DWDS. The VPA results revealed that water temperature, total chlorine, organic matter, and inorganic salt (i.e., conductivity and LR ratio) explained 35.86%, 29.81%, 35.67%, and 38.58% of the bacterial community variations, respectively (Fig. 3d). However, there were still 34.07% of the bacterial community changes unexplained by the environment.

3.4. Effect of organic matter changes on bla gene dissemination

To evaluate the effect of organic matter on the *bla* genes, we investigated the effect of NF on the organic matter and the *bla* genes in the DWDS. TOC in the DWDS decreased from 3.13 mg/L (± 0.33 mg/L) to 2.48 mg/L (± 0.50 mg/L) after the NF treatment in the waterworks (Fig. 4a). Simultaneously, the relative abundance of *bla* carriers decreased from 21.32% (± 5.86%) to 14.75% (± 4.20%) (Fig. 4b). After the NF treatment, the copy numbers of *bla* genes, including *ampC*, bla_{TEM}, bla_{OXA}, bla_{NDM-1}, bla_{IMP}, and bla_{CTX-M}, were reduced by three orders of magnitude (Fig. 4c, d). In addition, the copy numbers of bla_{TEM} gene decreased with TOC decrease in water in a lab-scale experiment (Fig. 4e). Other *bla* genes, including *ampC*, bla_{OXA}, bla_{NDM-1}, bla_{IMP}, and bla_{CTX-M}, were not detected in such water. All these results suggested that reducing organic matter in the effluent of the waterworks can effectively slow the dissemination of *bla* genes.

3.5. Bacterial community assembly processes affect bla genes

A significant Pearson correlation was observed between βNTI and the differences in beta-lactam resistance levels (Fig. 5a), which suggests that

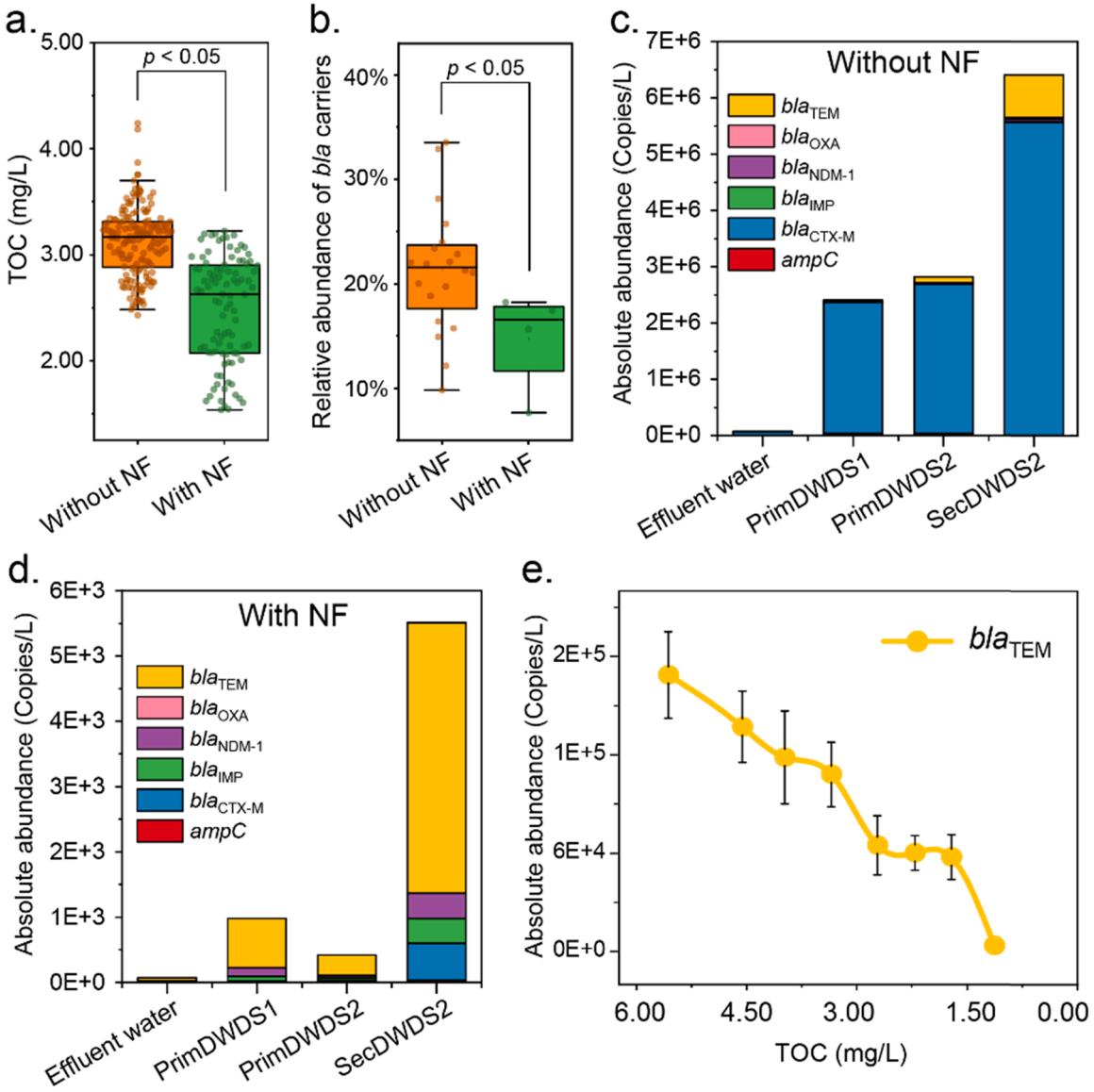

Fig. 4. Effect on *bla* genes after adding NF in the waterworks. (a), Comparison of the TOC in the DWDS before and after NF treatment in the waterworks. *p* values lower than 0.05 were considered as significant differences. (b), Comparison of the relative abundance of *bla* carriers in the DWDS before and after NF treatment in the waterworks. *p* values lower than 0.05 were considered as significant differences. (c), Absolute abundance of *bla* genes before NF treatment in the waterworks. (d), Absolute abundance of *bla* genes after NF treatment in the waterworks. **(e), Decrease of a**bsolute abundance of *bla* genes with the decrease of TOC in a lab-scale experiment. Error bar indicated the standard deviation of triplicate positive results.

bla gene levels were associated with bacterial community assembly events. To determine the relative influence of deterministic and stochastic assembly processes along the water distribution system, we examined the spatiotemporal changes in βNTI (Fig. S3). Deterministic assembly processes increased with the decay of chloramine in the DWDS (Scheffe's test, $p < 0.05$). A large proportion of bacterial stochastic processes ($-2 <$βNTI<2) corresponded to part of the bacterial community changes not determined by environmental factors by VPA (Fig. 3d), and a large fraction (45.26% ± 16.71%) of bacteria were traced back to unknown sources by FEAST (Tab. S13).

The national primary drinking water regulations (NPDWRs) recommend that HPC levels in municipal drinking water should be less than 500 CFU/mL [47]. A total of 12.50% of the water samples in the SecDWDS were found to exceed the prescribed limit in our study. The bacteria in the water with HPC greater than 500 CFU/mL showed significantly lower βNTI than the bacteria in other water (Scheffe's test; $p < 0.01$) (Fig. 5b), which suggested that the bacterial assembly mechanism of the water with HPC levels greater and less than 500 CFU/mL was significantly different.

Our results also showed that microbial community assembly turnover was quantitatively governed by different proportions of deterministic processes (homogeneous and heterogeneous selection) and stochastic processes (dispersal limitation and homogenizing dispersal) and a small proportion of undominated processes (ecological drift). When the HPC in water was below 500 CFU/mL, stochastic assembly, in particular dispersal limitation events (54.87% ± 2.28%), was the major contributor to bacterial assembly (Fig. 5c). In contrast, when the HPC was above 500 CFU/mL, the influence of dispersal limitation events decreased (36.11%) and homogeneous selection events (55.56%) gained importance (Fig. 5c). The above results suggested that the occurrence of microbial excess in the DWSS led to an increase in deterministic assembly processes (i.e., homogeneous selection).

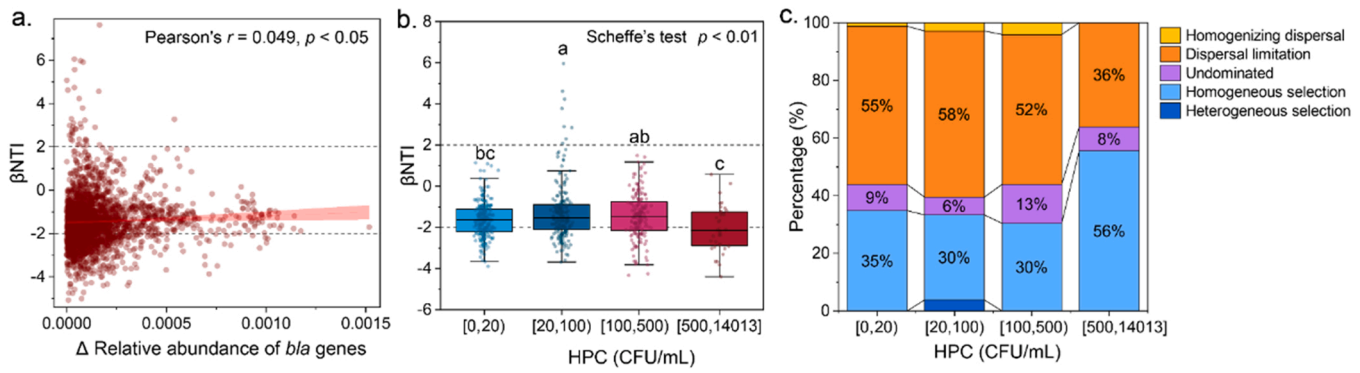

Fig. 5. Ecological processes in the assembly of the bacterial community. (a), Relationship between βNTI and differences in the relative abundance of *bla* genes in the DWDS. The shaded area indicates the 95% confidence interval. (b), Patterns of βNTI across successional bacterial numbers. Different lowercase letters indicate significant differences between months for that parameter. Data that do not share a letter are significantly different between months ($p < 0.05$; multiple comparisons with Scheffe's tests). Squared parenthesis indicated closed intervals and round parentheses denote semi-open intervals. (c), Quantification of ecological processes governing the bacterial community assembly across successional bacterial numbers. The percentages are relative contributions of each process to one group of the community. Squared parenthesis indicated closed intervals and round parentheses denote semi-open intervals.

4. Discussion

4.1. Main bacterial carriers of bla genes in the DWSS

This study focused on the control of bacterial *bla* genes in a chloraminated DWSS. Some plasmid-mediated genes, such as $bla_{\text{NDM-1}}$, can readily migrate among bacteria and result in extreme drug-resistant phenotypes [3]. Gram-negative bacteria are more readily to acquire antimicrobial resistance than gram-positive organisms, and the newest and gravest challenge among gram-negative resistant bacteria is posed by $bla_{\text{NDM-1}}$ [34]. To evade the bactericidal effects of beta-lactam antibiotics, gram-negative bacteria have evolved multiple strategies, such as production of *bla* (predominant strategy), production of novel penicillin-binding proteins with reduced affinity to beta-lactam antibiotics, mutations in porins, and multi-drug efflux pumps [55]. Most of the bacteria detected in this study were gram-negative. Therefore, beta-lactam antibiotic resistance was the potential risk in DWSSs and effective control measures of bacterial beta-lactam resistance are particularly important and need further exploration.

Antibiotic resistance can be intrinsic, conferred by horizontal gene transfer, or acquired through spontaneous mutations within chromosomal genes [40]. In this study, the dominant bacterial genera *Caenimonas* and *Sphingomonas* were the main carriers of *bla* genes. The highly chlorine-resistant genera *Sphingomonas* has been shown to secrete exopolysaccharides for biofilm formation [45]. Parts of biofilms can be torn apart by external physical forces such as friction, pressure and rapid water flow in DWDSs [1]. More importantly, horizontal gene transfer related genes included transposase, integrase, and recombinase coding genes are prevalent in *Sphingomonas* genomes [59]. As a result, horizontal gene transfer may occur in biofilms and suspended bacteria in water, which increases the risk of *bla* gene transmission.

4.2. Effect of organic matter on bla genes levels

Chlorination and some environmental stressors significantly alters microbial antibiotic resistance in drinking water, as confirmed in previous studies [14,30,42]. More specifically, pH, temperature, and Zn^{2+}, Cu^{2+}, Co^{2+}, Mn^{2+}, and Fe^{3+} ions can affect the hydrolysis of beta-lactam antibiotics [23,32]. However, the diversity of the microbial community may have a larger effect on microbial antibiotic resistance than environmental factors. Similarly, reports have shown that the bacterial community promotes antibiotic resistance during drinking water chlorination [17].

Our analysis of 69 water samples from the DWDS in different months showed that the abundance of bacterial *bla* genes were mainly driven by the concentration of organic matter. Why does organic matter affect *bla* genes? First, organic matter is the limiting factor for bacterial growth in DWSSs [48]. In our water supply system, most bacteria were classified as heterotrophic bacteria (Tab. S4), and the competition of these bacteria increased with the decay of chloramine (Tab. S5), which clarified the important role of organic matter in bacterial growth. Additionally, the main carriers of *bla* genes were the dominant bacteria in the DWDS. Therefore, the concentration of organic matter determines the absolute bacterial abundance and *bla* genes. Second, organic compounds, i.e., o-xylene, ethylbenzene, trioxymethylene, styrene, 2,4-dichloroaniline, and malachite green, can stimulate the horizontal transfer of ARGs [18]. Thus, organic matter may promote the horizontal transfer of *bla* genes, and the *bla* gene levels may increase with the growth of more *bla* bacterial carriers. Third, dissolved organic matter tends to form complexes (e.g., copper-dissolved organic matter ligands) with metal ions, which may catalyze the degradation of beta-lactam antibiotics, which are considered to be poorly stable due to the susceptibility of the beta-lactam ring to hydrolysis [23,57]. Accordingly, reducing organic matter may contribute to slowing the dissemination of bacteria carrying *bla* genes.

Furthermore, organic matter can also affect bacterial phenotypic tolerance to antibiotics, and recent studies found that antibiotic-resistant bacteria (ARB) were less costly in fitness and grew faster than susceptible strains under poor nutrient conditions (TOC<5.00 mg/L) caused by chromosomal mutations [26,35]. The TOC level in all water samples in this DWDS was less than 5.00 mg/L; therefore, the low fitness cost of ARB may promote bacterial growth, as indicated by the increase in the TPM and *bla* genes copy number by 51.33% and more than twice from the terminal PrimDWDS to SecDWDS (Fig. 4c, Tab. S14).

4.3. Control of bla genes in the DWSS

In principle, the persistence and enrichment of ARGs in the environment are ecological processes affected by various factors [4]. However, we found that the *bla* gene level was associated with bacterial community assembly events. An understanding of the assembly mechanism of the microbial community in water systems, especially the specific assembly events in the respective deterministic and stochastic processes, can provide important information for the diagnosis of system operation and performance [54].

NF treatment was found effective for reducing organic matters in drinking water. As a result, after the NF treatment in the waterworks, both *bla* gene levels and the dominance of *bla* genes carriers, which were composed of a large number of heterotrophic bacteria, decreased significantly. The main carriers of *bla* genes were the dominant bacterial

genera in the DWDS, and their relative abundance increased with the decay of chloramine. The dominant biofilm-forming *Sphingomonas* can facilitate the reappearance of these genes in SecDWDSs, even if the water treatment process is improved. This finding is supported by the twofold increase in *bla* gene levels from PrimDWDS to SecDWDS after NF treatment. When the HPC in water was below 500 CFU/mL, stochastic assembly was the major contributor to bacterial assembly. Bacteria are less affected by the environment. When the HPC in water was above 500 CFU/mL, deterministic assembly gained importance. It is more efficient to regulate the bacterial community by decreasing organic matter.

5. Conclusions

The main carriers of *bla* genes, *Caenimonas* and *Sphingomonas*, were the dominant bacterial genera in the DWDS. Organic matter influences *bla* genes by shifting the microbial community diversity. Less organic matter was beneficial to reducing the bacterial niche width in the DWDS and the change of the bacterial community from the waterworks to terminal water. Reducing organic matter may slow the dissemination of bacteria carrying *bla* genes. After the NF treatment process, both TOC and *bla* genes levels were reduced in the DWDS. Simultaneously, the abundance of *bla* genes was associated with bacterial community assembly events. When the HPC in the water was below 500 CFU/mL, stochastic assembly was the major contributor to bacterial assembly and bacteria were less affected by the environment. When the HPC in the water was above 500 CFU/mL, deterministic assembly gained importance and controlling *bla* genes by decreasing the organic matter content was more efficient.

This research brings new insight regarding the relationship between bacterial community assembly and *bla* genes control in DWSSs. It will be crucial for water utilities to create a desirable ecological niche for bacterial communities in DWSSs to improve drinking water quality.

CRediT authorship contribution statement

Xiaocao Miao: Data curation, Methodology, Visualization, Investigation, Writing – original draft. **Lingling Zhu:** Data curation. **Xiaohui Bai:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2022.107677.

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