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Capturing and tracing the spatiotemporal variations of planktonic and particle-associated bacteria in an unchlorinated drinking water distribution system

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ABSTRACT

The aperiodic changes in the quantity and community of planktonic and particle-associated bacteria have hampered the understanding and management of microbiological water quality in drinking water distribution systems. In this study, online sampling was combined with the microbial fingerprint-based SourceTracker2 to capture and trace the spatiotemporal variations in planktonic and particle-associated bacteria in an unchlorinated distribution system. The results showed that spatially, the particle load significantly increased, while in contrast, the quantity of particle-associated bacteria decreased sharply from the treatment plant to the distribution network. Similar to the trend of particle-associated bacterial diversity, the number of observed OTUs first slightly decreased from the treatment plant to the transportation network and then sharply increased from the transportation network to the distribution network. The SourceTracker2 results revealed that the contribution of particle-associated bacteria from the treatment plant decreased along the distribution distance. The spatial results indicate the dominant role of sedimentation of particles from the treatment plant, while the observed increases in particles and the associated bacteria mainly originated from the distribution network, which were confirmed directly by the increased contributions of loose deposits and biofilm. Temporally, the daily peaks of particle-associated bacterial quantity, observed OTU number, and contributions of loose deposits and biofilms were captured during water demand peaks (e.g., 18–21 h). The temporal results reveal clear linkages between the distribution system harboring bacteria (e.g., within loose deposits and biofilms) and the planktonic and particle-associated bacteria flowing through the distribution system, which are dynamically connected and interact. This study highlights that the spatiotemporal variations in planktonic and particle-associated bacteria are valuable and unneglectable for the widely on-going sampling campaigns required by water quality regulations and/or drinking water microbiological studies.

1. Introduction

There is a consensus that the microbiological quality of drinking water changes during distribution (Prest et al., 2014), which could be caused by the growth of planktonic bacteria in the water and/or the release of attached bacteria from established biofilms and loose deposits in drinking water distribution systems (DWDS) (Chen et al., 2020; Kooij, 1992; Liu et al., 2017, 2018). Studies have found that stagnation time and water demand are important factors influencing both the quantity and community of bacteria in DWDSs (Ling et al., 2018). For example, high bacterial concentrations and the growth of opportunistic pathogens in drinking water supply systems have been observed after long-term stagnation (Chan et al., 2019; Zhang et al., 2021; Zlatanovic et al., 2017). Hydraulic disturbances may cause water quality deterioration,

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such as increases in particle counts, water turbidity, and the concentrations of heavy metals and/or bacteria (Lehtola et al., 2006). All of the abovementioned water quality deteriorations pose public health concerns, especially regarding the biosafety of drinking water.

Worldwide, grab sampling is commonly used for regular assessments and statutory monitoring of drinking water quality in the DWDS. However, such low-resolution and labor-intensive sampling strategies can neither capture the aperiodic changes nor reveal the origination of physiochemical and microbiological contamination events (Banna et al., 2014). Online monitoring of particulate matter in the DWDS has been introduced since the early 2000 s (Hargesheimer et al., 2002). Studies by online particle counting/sampling and pairwise monitoring of hydraulic parameters and turbidity have clearly illustrated the daily variations in particulate profiles, but their application has been preliminarily limited to physiochemical aspects (Matsui et al., 2007; Verberk et al., 2006), such as discolorations (Mounce et al., 2015; Vreeburg et al., 2008). Recently, online flow cytometry was developed for counting total and intact bacterial cells (Hammes et al., 2012) and applied in DWDS (Prest et al., 2021). By combining online particle counts, intact cell counts (ICCs) and adenosine triphosphate (ATP) measurements, Prest et al. (2021) observed a weak correlation between ATP and flow velocity, which was attributed to the release of particle-associated bacteria from biofilms or loose deposits. However, assessing only quantitative data on water samples could neither explain the source of contamination nor offer enlightenments on effective solutions.

The understanding of the microbial ecology in DWDS has been substantially expanded by the rapid development and application of high-throughput sequencing (Zhang and Liu, 2019). This is especially true when the generated high-throughput sequencing data are combined with microbial ecology theory and mathematic modeling. For example, using an island biogeography model, Ling et al. found that pipe diameter drove the changes in the tap water bacterial community in building plumbing (Ling et al., 2018). By using the bacterial community fingerprint-based Bayesian SourceTracker, we quantified the contribution of biofilm and loose deposits to the bacteria present in tap in unchlorinated DWDS (Liu et al., 2018). However, in the study, we only collected three sets of samples in the distribution system, and capturing and tracing the aperiodic daily variations remains a critical knowledge gap. Therefore, the objective of this study is to capture and trace the aperiodic spatiotemporal variations in planktonic bacteria (PB) and particle-associated bacteria (PAB) in drinking water distribution systems. More specifically, based on the investigations of the spatial and temporal variations in the distribution system, the critical questions of what the local dominant processes are, when the peaks would occur, and why the changes may occur and where the changes may come from will be addressed. As an approach to this goal, an online sampling and monitoring system was developed, which was used and combined with the bacterial community fingerprint-based Bayesian SourceTracker. The findings obtained from this study advance the current understanding of the dynamics of aperiodic planktonic and particle-associated bacteria in drinking water distribution systems, which will be a powerful tool for water utilities to diagnose water quality problems and develop effective strategies for managing biological water quality.

2. Materials and methods

2.1. Drinking water treatment plant and sampling locations

The study was conducted in one of the drinking water supply systems of Oasen, the Netherlands. The drinking water treatment plant produces drinking water from anaerobic groundwater (340 m^3/h) through conventional treatment processes. In short, the water was treated by spray aeration, rapid sand filtration, pellet softening, carry-over submerged rapid sand filtration, granular activated carbon filtration and UV disinfection. The drinking water is distributed to customers without chlorine.

As illustrated in Fig. 1A, the sampling locations were selected at the treatment plant before the water entered the distribution system (referred to as DWTP), at the transportation network before the water was distributed into communities (referred to as TN), and at distribution network locations (referred to as DN-1 and DN-2). More specifically, DN-1 and DN-2 were located at the distal part of the secondary distribution network (DN), while TN was located at the proximal part of the secondary distribution network that is connected to the transportation network (TN). The distribution pipe material is PVC-U in the study area. In total, 147 samples were collected from the four locations, including 62 water samples (planktonic bacteria, PB), 63 suspended solids (particle-associated bacteria, PAB), 10 biofilms (BF), and 12 loose deposits (LD).

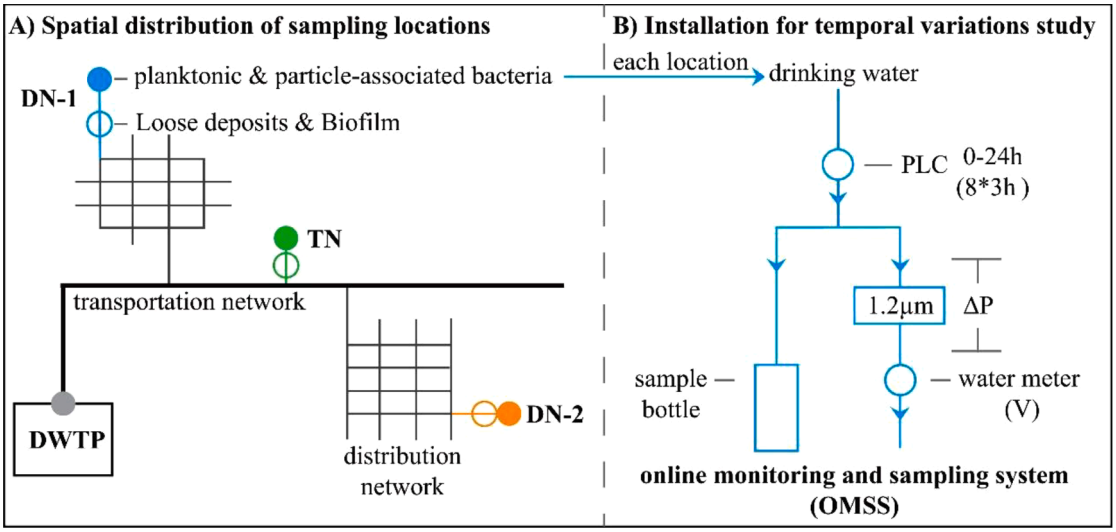

Fig. 1. (A) Spatial distribution of sampling locations. DWTP stands for drinking water treatment plant, DN stands for locations in distribution network, TN stands for location in the transportation network. At each location, water, suspended solids, biofilm and loose deposits were sampled. (B) Illustration of the installation for temporal variation study, named the online monitoring and sampling system (OMSS), which runs continuously for 24 h (8 × 3 h) to capture the daily variations. The planktonic bacteria were sampled in bottles for downstream ATP measurements and 0.22 μm filtration for DNA extraction. The particle-associated bacteria were sampled by filtrating drinking water through 1.2 μm filters online, the filters were used for ATP measurements and DNA extraction. ΔPressure (ΔP) was recorded online.

2.2. Online monitoring and sampling of water and suspended particles

A novel online monitoring and sampling system (OMSS) was developed to conduct online monitoring of water quality and continuous sampling of water and particles in DWDSs (Fig. 1B). Briefly, the system integrated water quality monitoring sensors, data loggers, water sampling bottles and particle sampling filters (Whatman, 1822–047, 1.2 μm), which were controlled and run by a preprogramed PLC for 24 h. For planktonic bacteria, the bottle sampled drinking water was used for ATP measurements and filtrated by 0.22 μm filters for DNA extraction. For particle-associated bacteria, the filter pore size of 1.2 μm was selected based on the results of our previous study (Liu et al., 2013). The online monitored physicochemical parameters included temperature, conductivity, and pH. Water and suspended particles were sampled every 3 h. Suspended particles were sampled by filtrating tap water for 3 h with particle sampling filters. Samples were collected in triplicate 8 times a day automatically ($n = 3 \times 8$, 24 samples) at sampling time periods of 0–3 h, 3–6 h, 6–9 h, 9–12 h, 12–15 h, 15–18 h, 18–21 h, and 21–24 h. The transmembrane pressure was monitored and recorded online, and the pressure differences per volume of water (ΔPressure/Volume, ΔP/V) were used as an index of particle load in water. The filters and water bottles were kept in refrigerator to guarantee the sample quality for the downstream microbiological analysis. Samples were collected and transported on ice and processed in the lab immediately after the 24 h sampling was performed. The OMSS was operated at a flow of 2 L/min, for a total of ~120 L per filter for suspended particle sampling. The flow was measured and recorded online by a digital flow meter. All data were logged every 5 min and visualized on a screen. At each location, the OMSS ran for two consecutive working days to obtain reliable and representative samples.

2.3. Biofilm and loose deposit sampling

At each location, the loose deposits and biofilm were sampled after the two days of running the OMSS as previously described (Vreeburg et al., 2008). In short, loose deposits were sampled at corresponding hydrants by flushing the pipeline with a velocity of 1.5 m/s. Afterward, sections of the flushed pipes with biofilm were cut in duplicate from the network, PVC-U, $D = 110$ mm, length= 30 cm. The pipe section was closed with sterile caps and filled with DNA-free water to keep the inner surface wet. All samples were stored in sterile plastic containers on ice and transported to the lab within 2 h. To detach the bacteria and materials from suspended solids (filters), loose deposits (suspensions) and pipe surfaces (pipe section), the samples were pretreated by ultrasonication for 3×2 min at 42 KHz in a water bath (Liu et al., 2017). The obtained suspensions were used for downstream physicochemical and microbiological analyses.

2.4. Physicochemical analysis

The concentrations of metal elements in all samples, including iron (Fe), manganese (Mn), calcium (Ca), aluminum (Al), and arsenic (As), were determined by inductively coupled plasma-mass spectrometry (ICP-MS). Quality control samples, including laboratory-fortified blanks and laboratory-fortified samples, were performed for every 10 samples.

2.5. ATP measurement

ATP content measurements were used to determine the active biomass across all samples. The ATP content was measured using the Luciferene Luciferase method (Magic-Knezev and Kooij, 2004). In brief, the ATP released from cells by nucleotide-releasing buffer (NRB, Celsis) was measured by the intensity of the emitted light in a luminometer (Celsis AdvanceTM) calibrated with solutions of free ATP (Celsis) in autoclaved tap water following the procedure given by the manufacturer.

2.6. DNA extraction and sequencing

The DNA was extracted from all samples using the FastDNA Spin Kit for Soil (Q-Biogene/MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. The extracted DNA was amplified with a primer set (341F: 5′-CCTACGGGNGGCWGCAG-3′ and 785R: 5′-GACTACHVGGGTATCTAATCC-3′) targeting the V3-V4 hypervariable regions of sequences from both bacterial and archaeal domains. The primer set was modified for the Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA) by appending the Illumina sequencing adaptors on the 5′ end. Paired-end sequencing of the amplicons (2×300 bp) was performed by BaseClear (Leiden, the Netherlands). The sequencing data have been deposited in the NCBI database (accession number PRJNA715925).

2.7. Sequence data processing

The sequences generated from the Illumina MiSeq analysis of the 16S rRNA gene amplicons were processed using the Quantitative Insights Into Microbial Ecology (QIIME2, v2020.11) pipeline with the default settings (Bolyen et al., 2019; Caporaso et al., 2010). Raw sequences were first processed using DADA2 (Callahan et al., 2016), including quality filtering, denoising, paired-end sequence merging, and chimera filtering. Unique amplicon sequence variants that were equivalent to 100% similarity operational taxonomic units (OTUs) in conventional practice were consequently generated through DADA2. Taxonomy was assigned using the q2-feature classifier (Bokulich et al., 2018), customized for the primer set used in this study with Silva SSU database release 132 (Quast et al., 2012). Multiple sequence alignment and phylogenetic tree construction were performed using the QIIME 2 plugin q2-phylogeny. Alpha and beta diversity analyses were performed using the QIIME 2 plugin q2-diversity. Principal coordinate analysis (PCoA) was conducted based on weighted UniFrac distance matrices (Liu et al., 2014). The major OTUs are defined as OTUs with a defined cutoff of relative abundance (>1%) within each sample category. The statistical analysis was performed in Past and Qiime2. Statical significant differences were identified when the p value was lower than 0.05 ($p < 0.05$).

2.8. SourceTracker analysis

The Bayesian-based SourceTracker method was performed to quantify the contribution of potential sources to the sinks (Henry et al., 2016). In the present study, the planktonic bacteria (PB) and particle associated bacteria (PAB) at each location in the network (TN, DN-1, DN-2) were identified as sinks, while the PB and PAB at DWTP and the biofilm and loose deposits at corresponding locations in the network were defined as potential sources. SourceTracker analysis was conducted using default settings with a rarefaction depth of 1000, burn-in 100, restart 10, alpha (0.001) and beta (0.01). The analysis was performed three times, and the average was calculated as previously described (Henry et al., 2016; McCarthy et al., 2017).

3. Results

In general, clear spatial and temporal variations in the physiochemical and microbiological parameters in the DWDS were captured by the online monitoring and sampling system (OMSS). Spatially, the particle load (ΔP/V) increased significantly from 1.98 ± 0.93 mbar/L in the DWTP to 3.15 ± 2.49 mbar/L in the primary transportation network (TN) and 21.36 ± 5.25 and 24.79 ± 7.24 mbar/L in the secondary distribution networks DN-1 and DN-2, respectively ($p < 0.001$, Fig. S1A). Temporally, peaks in particles and microbes were observed corresponding to the water demand peaks in the morning (06–09:00) and/or in the evening (18–21:00) (Fig. S2). In this section, the results will be presented with an emphasis on capturing and tracing the dynamics of quantity and community of the planktonic bacteria (PB) and particle-

associated bacteria (PAB).

3.1. Spatial variations in PB and PAB

Quantity. Considering the active biomass, the ATP concentrations in water were stable (8.1 ± 0.9 ng/L) among the sampling times and locations, while the particle associated ATP (P-ATP) decreased from 1.10 ± 0.40 ng/L at DWTP to 0.09 ± 0.03 ng/L at TN and then slightly increased to 0.23 ± 0.15 ng/L and 0.13 ± 0.06 ng/L at DN-1 and DN-2, respectively ($p < 0.001$, Fig. 2A). A weak negative correlation was observed between particle load and particle-associated ATP (R^2=0.17, $P = 0.001$, Fig. 2B).

Community. In total, 2655,227 sequences were obtained for 147 samples, including 62 water (PB), 63 suspended solids (PAB), 10 biofilms (BF), and 12 loose deposits (LD), which were assigned as 18,308 OTUs. The rarefication curves reached a plateau after 3000 sequences, indicating that enough sample coverage was obtained in the present study (Fig. S3). Alpha and beta diversity scores were generated after verification to an even sampling depth of 5670. For PB, the number of observed OTUs followed the same changes as P-ATP, which decreased first from DWTP (457 ± 171 OTUs) to TN (331 ± 50 OTUs) and increased in DN (636 ± 205 at DN1, 646 ± 251 OTUs at DN2) ($p <$ 0.001, Fig. 3A). Similarly, for PAB, the number of observed OTUs decreased from DWTP (456 ± 59 OTUs) to TN (381 ± 38 OTUs) and then increased in DN (803 ± 101 OTUs at DN-1, 939 ± 152 OTUs at DN-2) ($p < 0.001$, Fig. 3B). A strong positive correlation was observed between the number of observed OTUs in PAB and particle load ($R^2 = 0.69$, $p < 0.001$, Fig. 3C).

Regarding beta diversity, the PCoA plot showed that the PB community was highly similar among all locations. For the PAB community, TN clustered together with DWTP, which was different from DN-1 and DN-2 (Fig. S4). At the phylum level, both PB and PAB were dominated by Proteobacteria and Patescibacteria across all locations (Fig. S5A and B). The relative abundance of Proteobacteria decreased from the DWTP to locations in the distribution system, while the relative abundance of Patescibacteria showed the reverse trend. At the genus level, the relative abundance of OTU1525, assigned as *Polaromonas* spp., decreased dramatically from DWTP to distribution sites for both PB and PAB (Fig. S6A and B). In contrast, the relative abundances of OTU903 (assigned as the order *Candidatus Kaiserbacteria*) and OTU1540 (assigned as the family *Burkholderiaceae*) increased from DWTP to DWDS for PB, and the relative abundances of OTU1137 (assigned as *Caulobacter* spp.) and 1657 (assigned as the family *Methylomonaceae*) increased from DWTP to DWDS for PAB.

Compared to PB and PAB, higher OTU numbers were observed in loose deposits (LD, 980 ± 96) and biofilms (BF, 806 ± 302) (Table S1). According to the beta diversity shown as the PCoA plot in Fig. S4, the bacterial communities of LD and BF were highly similar regardless of phase and location. For the bacterial community composition, Proteobacteria was the dominant phylum in both LD (41.6 ± 4.0%) and BF (54.9 ± 10.4%), followed by Planctomycetes, Acidobacteria, Chloroflexi, Nitrospirae, Patescibacteria, Bacteroidetes and Gemmatimonadetes (Fig. S5C). The taxonomic profile at the OTU level is shown in Fig. S6C and Table S2.

3.2. Daily variations in PB and PAB

At the DWTP, the particle load was relatively stable (1.98 ± 0.93 mbar/L), while clear daily patterns were observed in the DWDS, with peak hours occurring at different times, e.g., 8.82 mbar/L between 6 and 9 h at TN, 25.34 mbar/L between 18 and 21 h at DN-1, and double peaks at DN-2 between 6 and 9 h (31.57 mbar/L) and between 18 and 21 h (30.64 mbar/L) (Fig. S2A). The peaks of particle load were positively correlated with the concentrations of five selected elements but not with the concentrations of PB (ATP) or PAB (P-ATP), indicating that the particles might have mainly consisted of nonbiomass (Figs. 2B and S7).

Considering the bacterial community, no clear peak in OTU number was found for PB at either DWTP or TN. Remarkably, the peak numbers were observed between 18 and 21 h at DN-1 (804 OTUs) and DN-2 (813 OTUs), which was exactly the time of peak particle loads at the location (Figs. 4A and S2A). Although it was not reflected in the beta diversity analysis results, significant daily variations in bacterial community composition were observed at the OTU level at DN-1 and DN-2. For example, the relative abundance of OTU903 reached its peak between 18 and 21 h at DN-2 (Fig. 4C).

For PAB, there were no significant daily variations in alpha and beta diversity analysis (Figs. 4B and S4), whereas differences in bacterial community composition were observed over the course of the day at each location (Fig. 4D). For example, peaks of certain OTUs were observed during the particle load peaks at TN (OTU1526, assigned as *Polynucleobacter* spp.) and DN-2 (OTU1498, assigned as *Aquabacterium* spp.) (Fig. 4D). In particular, taking location TN as an example, when comparing the peak hour 6–9 h with other sampling hours, many OTUs (i.e., OTU1526, OTU899, OTU892) were significantly enriched (Fig. S8).

3.3. Microbial source tracking of the PB and PAB variations

Spatially, the PB at the DWTP was the major contributor to the PB in water in the DWDS (47.3 ± 14.0%), the contribution of which increased slightly from 42.2 ± 10.6% at TN to 46.9 ± 15.2% at DN-1 and 52.7 ± 16.2% at DN-2 (Fig. 5A). Similarly, the PAB at DWTP was the main contributor to the PAB in the distribution system (40.2 ± 4.4%), but the

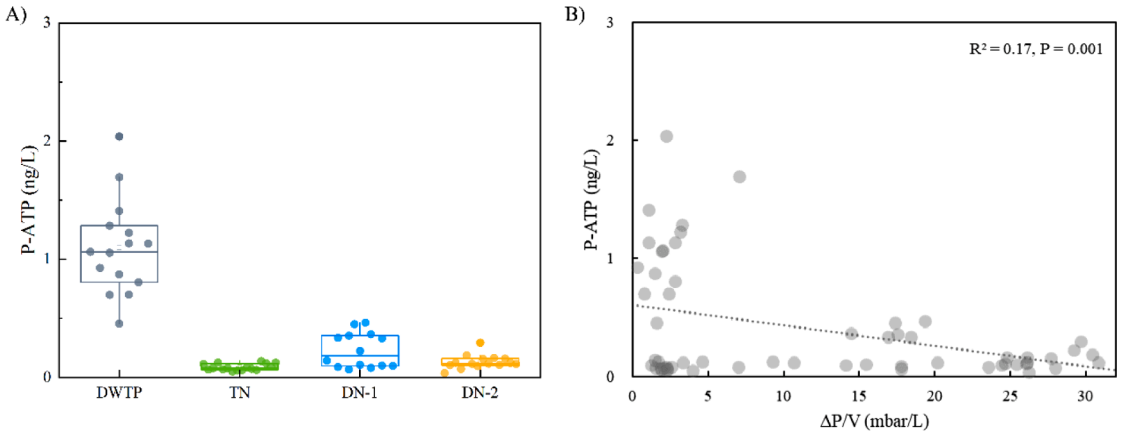

Fig. 2. Variations in particle-associated ATP (P-ATP) during distribution from DWTP to DWDS (A) and the correlation between the particle associated ATP (P-ATP) and particle load (ΔP/V) (B).

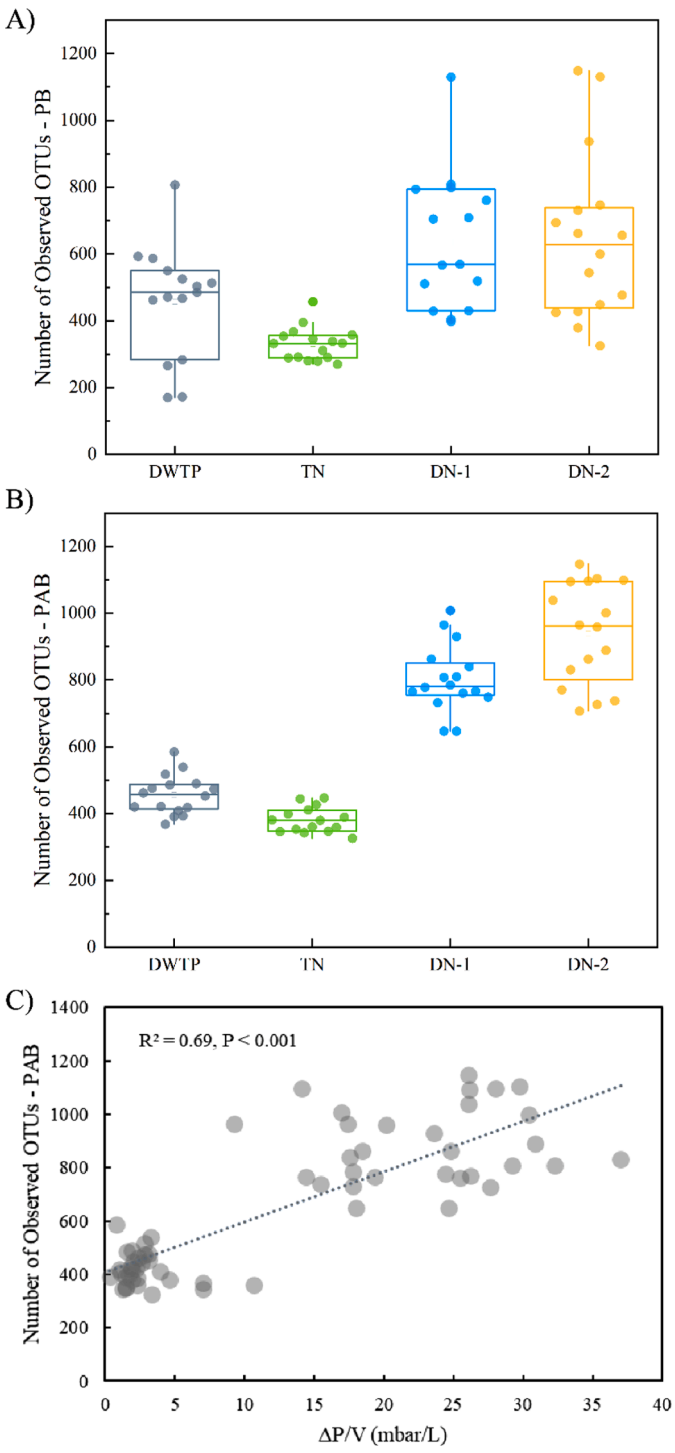

Fig. 3. The numbers of observed OTUs for (A) PB and (B) PAB at different locations; and (C) the correlation between the number of observed OTUs for PAB and particle load (ΔP/V).

exact contribution decreased from 50.7 ± 1.2% at TN to 40.0 ± 3.6% at DN-1 and 29.9 ± 8.5% at DN-2 (Fig. 5F). Noticeably, the contributions of BF and LD to the PB and PAB in DWDS increased along the distance from TN (PB,1.8 ± 0.5%; PAB 2.8 ± 3.4%) to DN-1 (PB, 2.3 ± 1.3%; PAB, 3.8 ± 1.9%) and further increased to DN-2 (PB, 5.3 ± 4.2%; PAB, 4.7 ± 1.4%) (Fig. 5C, D, G, and H). In addition, a large fraction of the contribution was from unknown sources, which may be because some possible sources were not covered in the sampling campaign.

Temporally, significant peak contributions of BF and LD to PB and PAB in DWDS were captured. The contributions from BF and LD were well correlated with the trend of the daily particle loads (Fig. S9, R2=0.22 and $p < 0.001$ for PB, R^2=0.34 and $p < 0.001$ for PAB), suggesting the potential sporadic release of BF- and LD-harbored microbes and contaminants during regular water demand fluctuations. This is especially true after the secondary distribution at DN-1 and DN-2, where multiple peaks of BF and LD contributions were observed. For example, peak contributions of BF and LD to PAB at DN-1 were captured at 6–9 h (BF, 1.8 ± 1.0%; LD, 3.1 ± 1.3%), 18–21 h (BF, 1.9 ± 0.1%; LD, 3.3 ± 1.5%), and 21–24 h (BF, 1.9 ± 0.6%; LD, 5.0 ± 0.3%) (Fig. S10), while peak contributions of BF and LD to PB at DN-2 were captured at 3–6 h (BF, 6.7 ± 1.5%; LD, 0.2 ± 0.1%), 6–9 h (BF, 4.5 ± 3.0%; LD, 0.3 ± 0.1%), and 18–21 h (BF, 5.3 ± 2.7%; LD, 0.2 ± 0.2%) (Fig. S11).

4. Discussion

An online monitoring and sampling system (OMSS) was combined with SourceTracker to assess the spatial and temporal variations in the quantity and community of planktonic bacteria (PB) and particle-associated bacteria (PAB) in a drinking water distribution system (DWDS). The spatial variations revealed a dominant process during distribution, which allowed for the understanding of general biological water quality changes. In addition, online sampling and monitoring at certain locations makes it possible to have reliable comparisons among different locations, which also uncovers the local circumstances and contributions of the DWDS to daily water quality variations.

4.1. Spatial variations in PB and PAB reveal the dominant process in the DWDS

Water quality is determined by complicated physiochemical and biological processes in DWDSs, such as the sedimentation and resuspension of particles with the associated bacteria (Vreeburg and Boxall, 2007) and the attachment and detachment of bacteria to/from pipe wall biofilms (Liu et al., 2018). There will be an improvement or deterioration of water quality from the treatment plant to the DWDS depending on the dominant processes. However, conventional sampling methods (e.g., a single collection time-point) and analysis parameters (e.g., heterotrophic plate count with insufficient sensitivity) can hardly reveal the dominant processes (Banna et al., 2014).

In the present study, the results obtained from different locations by the combination of online monitoring and sampling, integral analysis, and bacterial community fingerprint-based source tracking make it possible to explore the detailed processes that occur during water distribution. For all parameters analyzed, the newly developed online monitoring and sampling outlined the ranges of variation at each location, based on which a rational spatial comparison could be made with high resolution. In this manner, the mismatched comparison of randomly single time-point grabbed samples and any potential misunderstanding on distribution processes could be avoided, such as comparing a valley value from one location with a peak value of another location.

The newly introduced parameter 'ΔP/V' captured particle load variations (not revealed by turbidity, results not shown), which increased significantly from DWTP to DWDS, especially after the secondary network at DN-1 and DN-2. The observed increase in particle load in DWDS complied with a previous study in chlorinated (filtration, quantified offline by TSS) (Matsui et al., 2007) and unchlorinated systems (online particle counting) (Verberk et al., 2009). Considering other newly introduced measures, the decrease in active PAB (P-ATP), the decrease of PAB_{-DWTP}'s contributions to PAB_{-DWDS}, and the increase in PAB_{-DWDS}'s diversity (observed OTU numbers), it is reasonable to argue that particles supplied by DWTP were dominated by the sedimentation process, while new particles were released from loose deposits (LD) and biofilms (BF) in the secondary network. This was directly confirmed by the increases in LD and BF contributions to PB and PAB from TN to DN-1

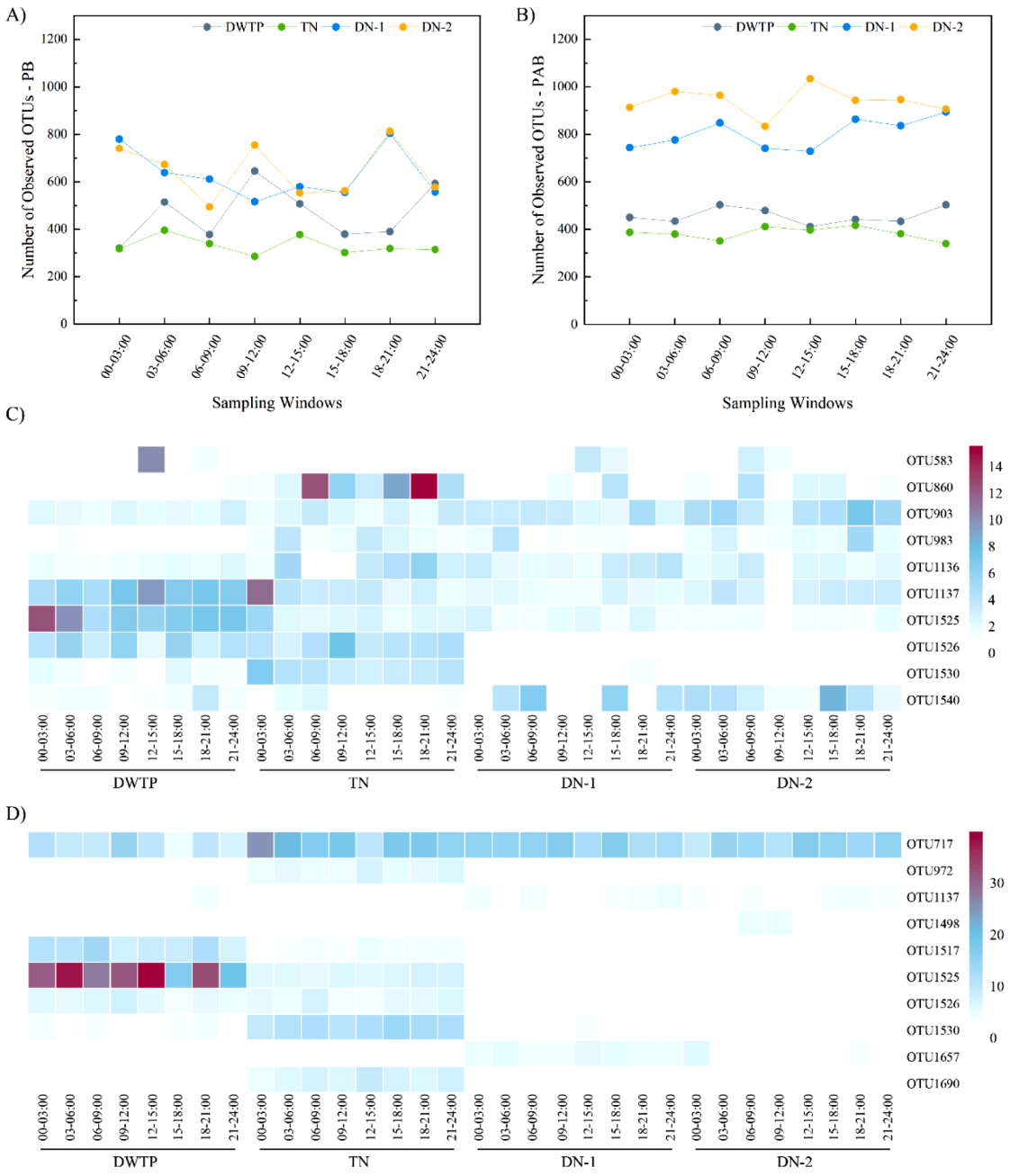

Fig. 4. The daily variations in the number of observed OTUs and the top 10 dominant OTUs over time and space in DWDS: (A) the daily variations in observed OTU numbers for PB; (B) the daily variations in observed OTU numbers for PAB; (C) the heatmap showing the top 10 dominant OTUs in PB; and (D) the heatmap showing the 10 dominant OTUs in PAB.

and DN-2, which also agree with the findings of our previous study assessing the origins of PB and PAB in unchlorinated DWDS (Liu et al., 2018).

4.2. Daily dynamics of PB and PAB uncover the local circumstances of DWDS

There were no significant daily variations for any parameters measured at the DWTP, suggesting a stable input from the treatment plant into the DWDS. In the DWDS, clear daily variations were observed, which were captured as peaks of particle loads, observed OTU numbers (PB), and certain members of the PB and PAB communities. Similar morning/evening peaks of turbidity and cell numbers in DWDSs were reported previously (Besmer and Hammes, 2016; Matsui et al., 2007). In addition, another study found daily patterns of PB community richness in chlorinated systems, but the peak periods varied among locations (e.g., 8–12 h and 0–4 h) (Bautista-de Los Santos et al., 2016). In the same study, the authors also reported significant differences in PB community structure and composition between 8 and 12 h and 16–20 h. However, no significant difference in the bacterial community was observed among the different periods for either PB or PAB in the present study. This might be because the present study was conducted in unchlorinated DWDS, where the flocculation of chlorine decay associated with water demand and usage shaping the bacterial community would not occur (Ling et al., 2018).

Considering that the feed from the DWTP is stable, it is reasonable to hypothesize that the captured daily peaks at each site were contributed by local DWDS circumstances (e.g., level of harbored contaminants and

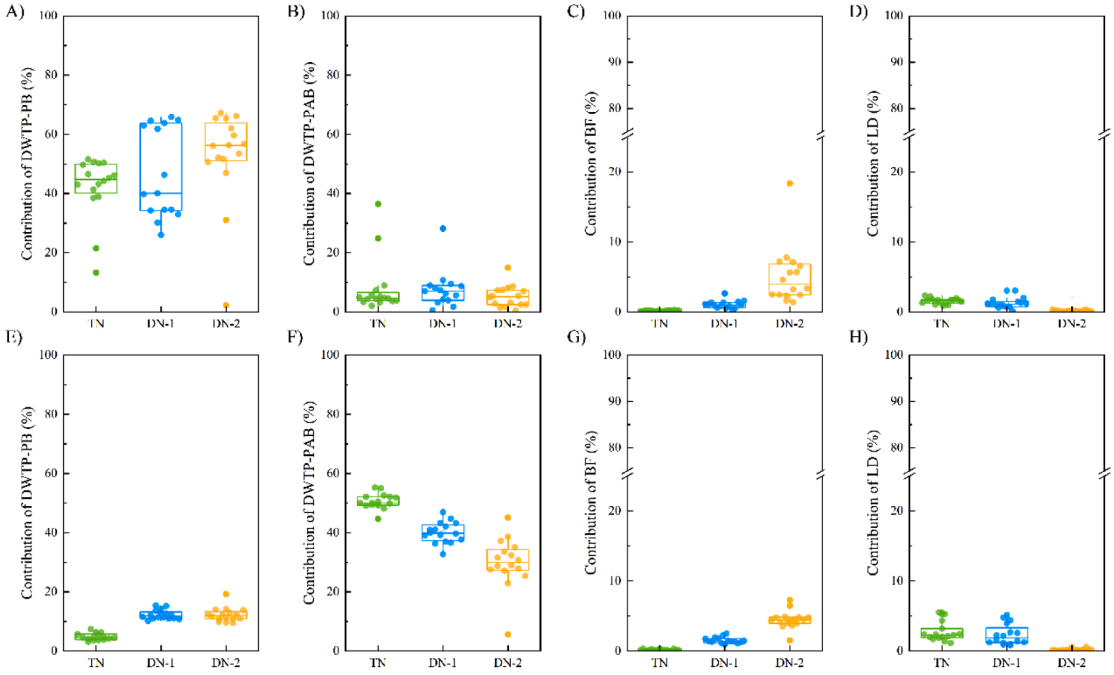

Fig. 5. The percentages of contributions from different sources (PB and PAB at DWTP, BF and LD) to PB (A–D) and PAB (E–H) in the DWDS at different locations. BF stands for biofilm, while LD stands for loose deposits.

hydraulic turbulences), which can be well illustrated by characterizing PB and PAB. This is especially true for the increase in PB OTU numbers between 18 and 21 h at DN-1 from 636 OTUs on average to 804 OTUs and at DN-2 from 646 OTUs on average to 813 OTUs. Such increases in OTU number were positively correlated with the increase in particle load, which may be because local water demand peaks lead to variable hydraulic regimes in pipes and cause different levels of BF and LD release (Carragher et al., 2012; Douterelo et al., 2013; Lucas et al., 2010). Previous studies also found that the increased flow rate accompanied by increases of shear stress and scouring forces caused the release of particles and cells into water from biofilm and loose deposits (Choi and Morgenroth, 2003; Husband et al., 2008; Paul et al., 2012). In the present study, the peaks of loose deposits and biofilm contributions to PB and PAB in the DWDS (calculated by SourceTracker) during the evening and morning hours offered direct and solid evidence to for this hypothesis. Interestingly, the increase in PB OTU numbers did not lead to significant changes in PB community structure because the contributions of LD and BF to PB were 5.3%, which were lower than our previously reported threshold (20%) in the same DWDS (Liu et al., 2017).

4.3. Practical implications

The present study sensitively captured the spatial and temporal variations in PB and PAB in a DWDS, which is important to consider for both routine sampling campaigns required by the water quality regulations and the widely conducted random collection sampling campaigns for research purposes. Ignoring such aperiodic variations would lead to mismatched comparisons of spatiotemporal series data and misunderstandings of DWDS microbial ecology. In addition, we demonstrated that the quantitative and qualitative characterizations of PB and PAB could be valuable messengers for determining local dominant processes within DWDS. However, to understand the origin of captured variations and develop an effective management strategy accordingly, high-resolution integral sampling campaigns are required to cover all potential sources. It would be even more powerful if other online microbiological analyses could be used together with OMSS, such as the commercially available automated ATP (de Vera and Wert, 2019) and the online flow cytometers (Besmer et al., 2014).

This study was conducted in an unchlorinated distribution system under regular water supply conditions. For chlorinated distribution systems subjected to supply-water changes and/or hydrological disturbances, the proposed methodology of combining OMSS with SourceTracker based on microbial community fingerprints would also be valuable for investigating water quality dynamics and transition effects. In chlorinated systems, the dynamic changes in PB and PAB because of water demand, stagnation and chlorine decay could be investigated with high resolution. For example, critical questions such as daily spatiotemporal variations of chlorine residual, its correlation with PB and PAB changes, and the local interactions among water, biofilm and loose deposits could be answered. Moreover, the methodology involves continuous online monitoring and sampling, as well as large volume preconcentration. These advantages allow it to overcome the challenges of transition effect studies, e.g., its contingency and the dilution of released bacteria when it takes place. Therefore, as we suggested previously, using OMSS will significantly increase the success rate of capturing the release events and transition effects compared to offline sampling (Chen et al., 2020). Future application of such a method is highly recommended both for studying the mechanism of water quality deterioration during water distribution and for preventing esthetic and health risks at customers' ends, especially if the distribution system is subject to supply-water quality changes (e.g., switching source water, upgrading treatments) and/or hydraulic disturbances (e.g., ultralong stagnation during the pandemic, postrepair flushing, firefighting).

5. Conclusions

In the present study, an online monitoring and sampling system (OMSS) was developed to investigate the daily variations in planktonic and particle-associated bacteria in an unchlorinated drinking water distribution system. The microbial fingerprint-based SourceTracker was used for capturing and source tracking the daily bacterial peaks, using planktonic and particle-associated bacteria in treated water and the biofilm and loose deposits in the distribution system as potential sources. The following conclusions can be drawn from this study regarding the daily variations in particle and bacterial load, changes in the

diversity and composition of bacterial communities, and the sources of increased particles and cells during hydraulic peaks:

- Spatially, the particle load slightly increased from the treatment plant to the transportation network and then sharply increased in the distribution network. In contrast, the quantity of particle-associated bacteria decreased from the treatment plant to the transportation network and the distribution network. For both planktonic and particle-associated bacteria, the number of observed OTUs first slightly decreased from the treatment plant to the transportation network and then sharply increased in the distribution network. According to the SourceTracker results, the planktonic and particle-associated bacteria in the produced water are the main contributors to bacteria in the distribution system. Along the distribution distance, the contribution of planktonic bacteria from the treatment plant increased, while the contribution of particle-associated bacteria from the treatment plant decreased.
- Temporally, clear daily patterns were observed, especially at the two locations in the distribution network (DN-1, DN-2). More specifically, the quantitative peaks of particle-associated bacteria were captured in water usage peaks during the day. According to the SourceTracker results, the contributions of biofilm and loose deposits to the planktonic and particle-associated bacteria in the drinking water distribution system (DN-1 and DN-2) spiked during the water demand peaks, accounting for 5.3 ± 2.7% and 6.7 ± 1.5%, respectively.
- Methodologically, we demonstrated that the combination of an online monitoring and sampling system (OMSS) and the microbial fingerprint-based SourceTracker is a powerful tool for studying spatiotemporal water quality variations in an unchlorinated drinking water distribution system. The particles and bacteria can be valuable messengers revealing physicochemical and microbiological processes occurring in distribution systems. To better understand and manage the microbiological quality of drinking water during distribution, future investigations are recommended to apply such a method in chlorinated systems and/or transition effect studies.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.118589.

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