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Assessing the origin of bacteria in tap water and distribution system in an unchlorinated drinking water system by SourceTracker using microbial community fingerprints

Gang Liu [a, b, *], Ya Zhang [c], Ed van der Mark [d], Aleksandra Magic-Knezev [e], Ameet Pinto [f], Bartholomeus van den Bogert [g], Wentso Liu [c], Walter van der Meer [a, h], Gertjan Medema [b, i]

[a] Oasen Water Company, P.O. Box 122, 2800AC, Gouda, The Netherlands
[b] Sanitary Engineering, Department of Water Management, Faculty of Civil Engineering and Geosciences, Delft University of Technology, P.O. Box 5048, 2600GA, Delft, The Netherlands
[c] Department of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign, 205 North Mathews Avenue, Urbana, IL, 61801, United States
[d] Dunea Water Company, P.O. Box 756, 2700 AT, Zoetermeer, The Netherlands
[e] Het Water Laboratorium, P.O. Box 734, 2003 RS, Haarlem, The Netherlands
[f] Department of Civil and Environmental Engineering, Northeastern University, Boston, United States
[g] Baseclear B.V., P.O. Box 1336, 2302BH, Leiden, The Netherlands
[h] Science and Technology, University of Twente, P.O. Box 217, 7500AE, Enschede, The Netherlands
[i] KWR Watercycle Research Institute, P.O. Box 1072, 3430 BB, Nieuwegein, The Netherlands

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ABSTRACT

The general consensus is that the abundance of tap water bacteria is greatly influenced by water purification and distribution. Those bacteria that are released from biofilm in the distribution system are especially considered as the major potential risk for drinking water bio-safety. For the first time, this full-scale study has captured and identified the proportional contribution of the source water, treated water, and distribution system in shaping the tap water bacterial community based on their microbial community fingerprints using the Bayesian "SourceTracker" method. The bacterial community profiles and diversity analyses illustrated that the water purification process shaped the community of planktonic and suspended particle-associated bacteria in treated water. The bacterial communities associated with suspended particles, loose deposits, and biofilm were similar to each other, while the community of tap water planktonic bacteria varied across different locations in distribution system. The microbial source tracking results showed that there was not a detectable contribution of source water to bacterial community in the tap water and distribution system. The planktonic bacteria in the treated water was the major contributor to planktonic bacteria in the tap water (17.7–54.1%). The particle-associated bacterial community in the treated water seeded the bacterial community associated with loose deposits (24.9–32.7%) and biofilm (37.8–43.8%) in the distribution system. In return, the loose deposits and biofilm showed a significant influence on tap water planktonic and particle-associated bacteria, which were location dependent and influenced by hydraulic changes. This was revealed by the increased contribution of loose deposits to tap water planktonic bacteria (from 2.5% to 38.0%) and an increased contribution of biofilm to tap water particle-associated bacteria (from 5.9% to 19.7%) caused by possible hydraulic disturbance from proximal to distal regions. Therefore, our findings indicate that the tap water bacteria could possibly be managed by selecting and operating the purification process properly and cleaning the distribution system effectively.

* Corresponding author. Room 4.51, Stevinweg 1, Building of CiTG, TU Delft, 2628CN, Delft, The Netherlands.
E-mail addresses: g.liu-1@tudelft.nl, gang.liu@oasen.nl, ganghow@gmail.com (G. Liu).

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1. Introduction

The presence and growth of microbes in treated drinking water and at the customers' taps is undesirable both for biosafety reasons (Wang et al., 2013) and because of process-related microbial problems during distribution (Berry et al., 2006), such as nitrification (Zhang et al., 2009), bio-corrosion (Emde et al., 1992) and persistence of pathogens (Emtiazi et al., 2004). However, the omnipresence of microbes in drinking water systems has been proven and acknowledged (Proctor and Hammes, 2015; Bautista-de los Santos et al., 2016; Liu et al., 2017b), such as their presence and multiplication in tap water (10^3-10^6 cells ml^{-1}) (Hammes et al., 2010; Liu et al., 2013c; Prest et al., 2014) and in distribution systems (biofilm: 10^6–10^8 CFU cm^{-2}; loose deposits:10^8 CFU g^{-1}) (Liu et al., 2013d; Prest et al., 2016).

To help address public health concerns associated with bacteria in tap water and to develop efficient biological water quality management strategies, it is critical to explore where these bacteria come from. Potentially, tap water bacteria may come from source water (Eichler et al., 2006; Vaz-Moreira et al., 2013), from treatment steps and treated water (Pinto et al., 2012), and from the distribution network through biofilm detachment and/or loose resuspension (Lautenschlager et al., 2010; Liu et al., 2014). However, the source of bacteria in tap water and the contribution of distribution system biofilm and loose deposits to tap water microbiological water quality still lacking systematic study.

The drinking water bacteria source apportionment can be done by microbial source tracking (MST): an approach that emerged at the end of the 20th century. This tracking is based on the assumption that by using an appropriate method and appropriate indicator bacteria sources of the microorganisms can be found and characterized (Harwood et al., 2014). However, the use of these methods can be problematic, especially when the markers are not entirely source specific, and when multiple sources within a system have similar marker concentrations (Cao et al., 2013; McCarthy et al., 2017).

The historical MST has been significantly advanced by the development and employment of high-throughput sequencing (HTS) technologies that make the characterization of entire microbial communities of environmental samples feasible and increasingly commonplace (Unno et al., 2010; Henry et al., 2016). Meanwhile, computational tools have been developed that utilise HTS data to track microbes according to source, such as SourceTracker, which applies a Bayesian framework to estimate the proportion of each source contributing to a designated sink (Knights et al., 2011). SourceTracker can estimate directly the source proportions and model the uncertainty about known and unknown source environments. In aquatic systems, it has been previously applied to identify water source contributions of microbial contamination (faecal contamination) in coastal waters, recreational beaches, urban estuaries, lakes and rivers (Cao et al., 2013; Newton et al., 2013; Neave et al., 2014; Henry et al., 2016; McCarthy et al., 2017).

In contrast to single marker MST methods, the HTS-based MST method (e.g. SourceTracker) can characterize hundreds or even thousands of markers simultaneously (Unno et al., 2010; Lee et al., 2011). Therefore, it may be used to identify any relevant source, and it is particularly useful for the sources that currently do not have and may never have a source-specific single marker gene (Knights et al., 2011; Cao et al., 2013). Recently, the HTS-based SourceTracker has been evaluated and compared with a 3-dimensional hydrodynamic model for identifying the primary sources of microorganisms (McCarthy et al., 2017) in aquatic environments. McCarthy et al. demonstrated that the HTS-based SourceTracker can identify the sources that contribute to the bacterial community in aquatic environments to understand the origin of the microorganisms and to protect environmental values and public health.

Based on HTS and SourceTracker, the objective of this study is to track the source of microbial communities at consumers' taps back to the water distribution and treatments. To track the source of bacteria in tap water and bacteria in the loose deposits and biofilm that accumulated and developed in the distribution system, this study characterized bacterial communities in bulk water (PB) and the bacteria associated with suspended particles (PAB) from source to tap, together with bacteria harboured in the loose deposits and biofilm in an operational distribution system. For the first time, the bacteria harboured in the loose deposits and biofilm in a distribution system has been incorporated into a drinking water microbial ecology study by tracking their origin from source water and purification processes (upstream tracking), and quantifying their proportional contribution to the bacteria detected in the tap water (downstream tracking). The results from this study will be valuable to water utilities in achieving a better understanding of the origin of bacteria in tap water and the development of biofilm and loose deposits in the distribution system, based on which treatments and management strategies can be developed, guaranteeing the safeguard of public health and biological water quality at the drinking water taps.

2. Material and methods

2.1. Drinking water purification processes

The study was conducted in one of the drinking water supply system of Dunea, the Netherlands. The source water, after pretreatment, is transported over 30 km to a dune area of natural lakes where it recharges the groundwater. After an average residence time of two months, the water is abstracted from the dunes. This abstracted artificial recharge and recovery water is posttreated by softening, powdered activated carbon filtration, aeration, rapid sand filtration, and slow sand filtration before being pumped into the distribution system. Chlorination and disinfectant residuals are avoided in the Netherlands.

2.2. Sample collection

As illustrated in Fig. 1, samples were taken after dune filtration (sampling point SW), after rapid sand filtration (sampling point RSF), after slow sand filtration before pumping into the distribution system (sampling point SSF/TW), and from three locations in the distribution system (sampling point DN1, DN2 and DN3).

In the treatment plant, both planktonic bacteria (bulk water samples, PB) and suspended particle-associated bacteria (filtered particle samples by 1.2 μm Whatman glass fiber filters, PAB) were taken at SW, RSW and SSF/TW. Duplicate samples from the treatment plant were taken before and after the scheduled sampling events in the distribution system.

In the distribution system, three locations were selected which were at proximal, central and distal parts of the distribution system with a distance of 6 km (DN1), 13 km (DN2) and 23 km (DN3), respectively, away from the treatment plant (illustrated in Fig. 1). At each location, an integral sampling was performed as described by (Liu et al., 2014). Briefly, bulk water samples were collected after a flushing (until the water temperature was constant) at customers' taps connected directly to the main supply pipe and close to the hydrants. The loose deposits were collected at the fire hydrants by flushing the distribution pipe with a velocity of 1.5 m/s (Vreeburg et al., 2008). Subsequently, two sections of the flushed pipe (PVC-U, D = 110 mm, length = 30 cm) were cut out to sample the biofilm in duplicate. The pipe section was closed by pre-disinfected caps

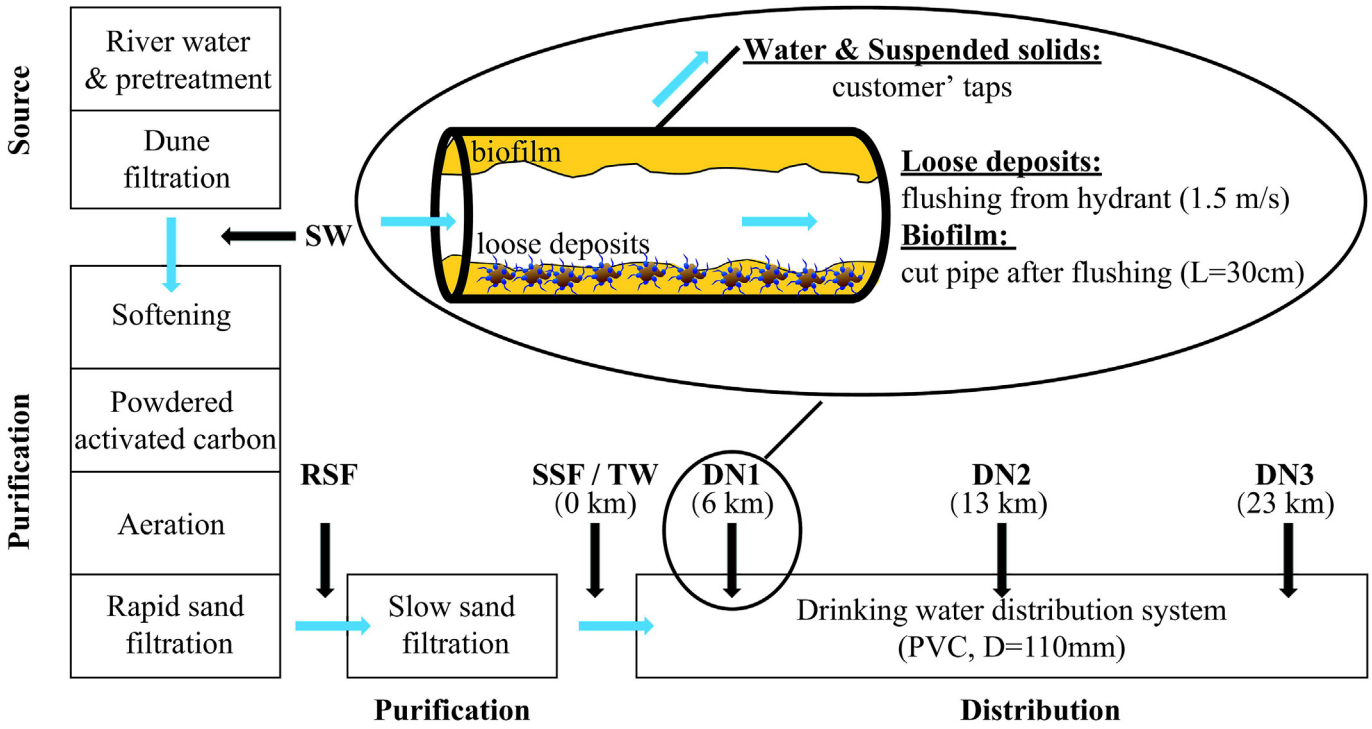

Fig. 1. Illustration of the sampling points and study design. The planktonic bacteria (PB) and suspended particle-associated bacteria (PAB) were sampled from source to tap: after dune filtration (SW), after rapid sand filtration (RSF), after slow sand filtration before pumping into the distribution system (SSF/TW) and at three locations in the distribution system at customers' taps (DN1, DN2 and DN3). At the sampling locations in the distribution system, loose deposits were sampled by flushing the distribution pipe. Biofilm was sampled in duplicates at each location by swabbing the top and bottom inner surface.

and filled with 1 L DNA-free water to keep the inner surface wet during transportation.

The order of sampling began by obtaining the water samples, then filtering suspended particles on site, flushing the distribution pipe in the street for loose deposits sampling, and finally cutting out parts of the distribution pipe. All samples were kept at 0 °C as soon as they were taken and subsequently transported at 0 °C to the lab. To detach bacteria from the associated surface of the suspended particles, loose deposits and pipe, the samples were pretreated by ultrasonification for three time periods of two minutes each at 42 KHz (Magic-Knezev and van der Kooij, 2004). The obtained suspensions were used for further microbiological analysis. All samples were processed within 24 h after they were taken.

2.3. DNA extraction and Illumina sequencing

The DNA was extracted from the water samples and other obtained suspensions after pre-treatment using the FastDNA Spin Kit for Soil (Q-Biogene/MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. 16S rRNA gene amplification was carried out as described (Kozich et al., 2013). Briefly, the extracted gDNA was amplified with a primer set (515F: 5′-GTGCCAGCMGCCGCGGTAA-3′ and 909R: 5′-CCCGTCAATTCMTTTRAGT-3′) targeting the V4-V5 hypervariable regions of sequences from both bacterial and archaeal domains. The primer set has been 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 done by BaseClear (Leiden, the Netherlands). The sequencing data have been deposited in the NCBI database, with reference code SRR5908979-5909003; the sample origin of each sequencing library is provided in Table S1.

2.4. Sequencing data processing

The sequences generated from the Illumina Miseq analysis of the 16S rRNA gene amplicons were processed (i.e., filtered, clustered, and taxonomically assigned and aligned) using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline with the default settings (Caporaso et al., 2010). The sequences were clustered using the average neighbour approach to form operational taxonomic units (OTUs) at the 97% sequence similarity cut-off.

The process consisted of quality checking, denoising, and a microbial diversity analysis. Weighted and unweighted UniFrac distance matrices were constructed from the phylogenetic tree (built by a FastTree algorithm) and used to conduct a principal coordinate analysis (PCoA) (Liu et al., 2014). The dominant OTUs are defined as the OTUs with a defined cut-off of relative abundance (>0.5%) within each phase/pipe (Ling et al., 2016). Shared and the unique core OTUs in the source water, treated water, distribution system and tap water were shown by the Venn diagram.

2.5. MST by SourceTracker

The OTU tables derived from quality filtering and OTU picking were used as input file for source tracking using "SourceTracker" as described by Knights et al. (2011). SourceTracker compares the community profiles in the 'source' to those of the 'sink', using Bayesian methods to identify the extent of contribution of each source to the sink. It was used to identify the percentage contribution of each potential source to the defined sinks, as illustrated in Fig. 2. In the present study, when identifying the sources of tap water bacteria (defined as sinks), the samples collected from the source water, the purification steps and the distribution systems were all defined as potential sources (independent contributors); while for identifying the sources of biofilm and loose deposits in

the distribution system (defined as sinks), the samples collected from the source water and purification steps were considered as potential sources (independent contributors). SourceTracker analysis was performed using default settings with a rarefaction depth of 1000, burn-in 100, restart 10, alpha (0.001) and beta (0.01) Dirichlet hyper parameter were applied. The analysis was performed three times and the average was calculated as previously described (Henry et al., 2016; McCarthy et al., 2017).

3. Results

Bacterial community profiles were generated for the 25 collected samples (10 water samples, 6 suspended solids samples, 3 loose deposits samples and 6 biofilm samples). In total 1,216,897 sequences were obtained, which were assigned to 13,489 OTUs. The rarefaction curves reached a plateau after 30,000 sequence reads were obtained, indicating that enough sample coverage was obtained in this study (Fig. S1a). Among the different sample types at the distribution site, it is clear that the observed OTUs in descending order are loose deposits, water, suspended solids and biofilm (Fig. S1b). At the Phylum level, all samples were dominated by Proteobacteria (20%–80%, Fig. 3). The unclassified group accounts for 2%–36% of the total bacterial community. The dominant OTUs in all samples (relative abundance >0.5%) are presented in the heat-map shown in Fig. 4 and the associated taxonomy information is given in Table S2.

3.1. Bacterial community composition

3.1.1. Bacterial community of planktonic and particle-associated bacteria from source to taps

For the community of planktonic bacteria, the relative abundance of Proteobacteria decreased over the treatments, from more than 70% in the source water to 30% in RSF water, and further decreased to 20% in the treated water. Its relative abundance remained at 30% (std. 8%) in the tap water at different locations. Several phyla indicated increased relative abundance during treatments and stable relative abundance during distribution, e.g. Nitrospirae (0–4%), Planctomycetes (0–3%) and Acidobacteria (0–3%). At the OTU level, in total 24 dominant OTUs were found in all water samples. There were 18, 6, 4 and 8 dominant OTUs in the source water, after rapid sand filtration, in the treated and tap water, respectively. As shown in the Venn diagram (Fig. S2a), there were 4 shared OTUs between the source water and the RSF water, which were assigned to the family of Methylococcaceae and Methylophilaceae which obtain carbon and energy from by oxidizing methane, methanol or methylamine. Between the treated water and the tap water, there were three shared dominant OTUs (3 out of 9 OTUs). There were no dominant OTUs shared by either the treated water and source water, or the treated water and RSF water. However, all of the 6 dominant OTUs in the RSF water were in the source water. Most of the dominant OTUs in the treated water (3/4) and tap water (5/8) already present in the source water at low abundance (as low as 0.006%, Fig. S2b); those OTUs are denovo168693, denovo1151294, denovo504875, denovo1286495 and denovo204270, the abundance of which increased from source to tap (taxonomy information given in Table S2).

For the community of suspended particle-associated bacteria (PAB), the relative abundance of Proteobacteria decreased over the treatments, from more than 80% in the source water to about 50% in RSF water and treated water. Their relative abundance remained 50% (std. 2%) in the tap water at different locations. As with the PB community, the following phyla showed increased relative abundance over treatments and remained stable during distribution: Planctomycetes (0–10%), Acidobacteria (0–8%), Nitrospirae (0–4%) and Chloroflexi (1%–5%). A higher number of dominant OTUs were detected for suspended particle-associated bacteria (PAB) compared to the community of PB (43 vs. 24). There were 23, 15, 14 and 13 dominant OTUs associated with PAB in the source water, RSF water, treated water and tap water, respectively (Fig. S3a). Between the community of PAB in the source water and in the RSF water, there were 9 shared OTUs, 4 of which were assigned to the genus of *Crenothix* that is related to methane and iron oxidation; 3 OTUs were the same as in the PB community which were assigned to the family of Methylococcaceae and Methylophilaceae. There was, however, only 1 shared OTU (denovo1201581, assigned to genus

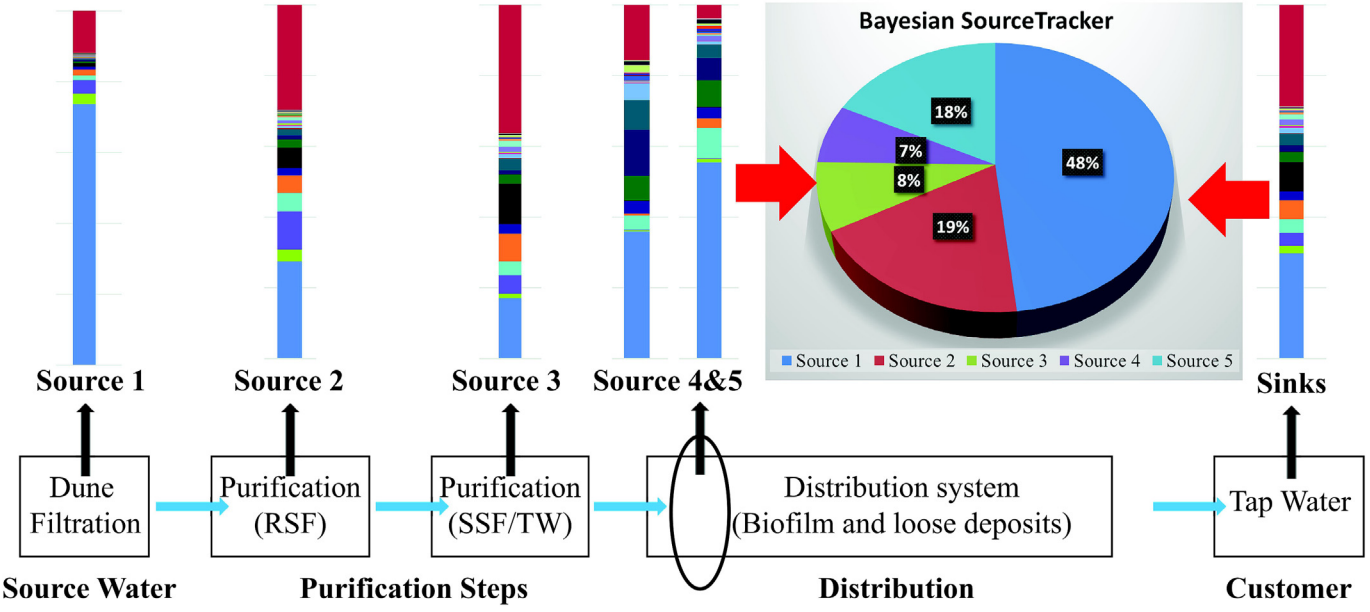

Fig. 2. Illustration of SourceTracker methodology to identify the percentage contribution of each potential source to the sinks using Bayesian model comparing the community profiles of potential sources and sinks, which compares each microbe and its relative proportion to estimate sources in the sink. In this specific illustration, the tap water bacteria (sinks) contains 48% of source 1 (source water), 19% of source 2 (RSF water), 8% of source 3 (treated water), 7% of source 4 (loose deposits) and 18% of source 5 (biofilm).

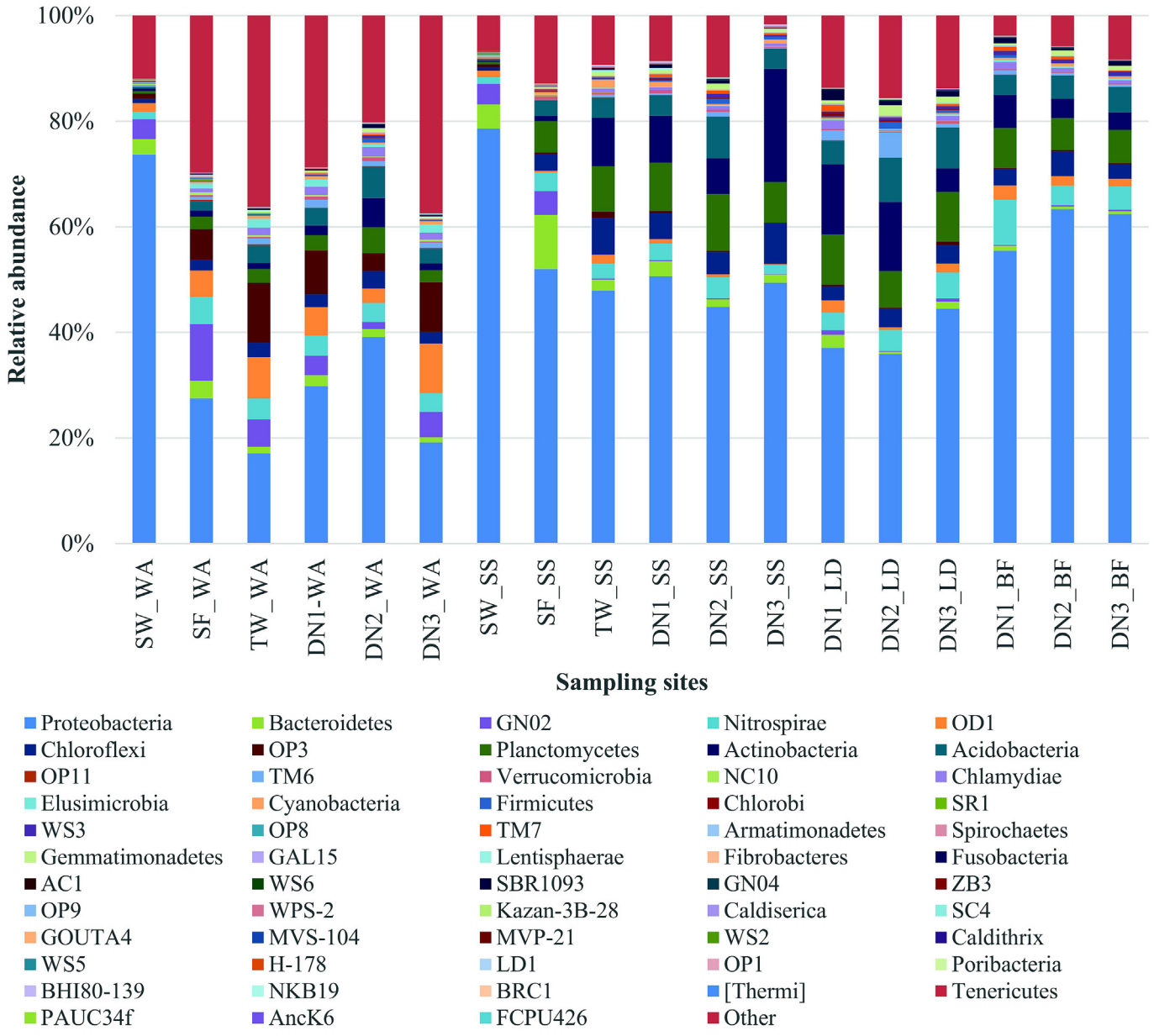

Fig. 3. Bacterial community composition at phylum level. Average of replicates was calculated for each sampling location and phase.

Pedomicrobium, related to manganese oxidation) between the PAB community in the RSF water and treated water. There were no shared dominant OTUs of the PAB in the source water, RSF water, treated water or tap water. However, 3/14 (denovo504875, denovo1382108 and denovo75529) and 3/13 (denovo1382108, denovo168693 and denovo1596590) OTUs that dominant PAB of treated and tap water were present in the PAB of the source water (Fig. S3b).

3.1.2. *Bacterial community of loose deposits and biofilm in the distribution system*

The community composition of bacteria in the loose deposits and biofilm were similar, both of which were dominated by Proteobacteria with a relative abundance of 40% (std. 3%) and 60% (std. 3%). The other dominant phyla in loose deposits and biofilm included (in descending order): Actinobacteria (10% and 4%), Planctomycetes (9% and 7%), Acidobacteria (7% and 4%), Nitrospirae (both 4%) and Chloroflexi (both 3%). At the OTU level, there were 32 dominant OTUs detected in the bacterial communities of loose deposits (15/32) and biofilm (22/32), 5 of which were shared by the two niches (denovo745756 and denovo1285130, not identified; denovo1854419 assigned to genus *Hyphomicrobium*; denovo1382108 assigned to genus *Nitrospira*, related to nitrite oxidation; denovo345586 assigned to genus *Pedomicrobium*, related to manganese oxidation).

Comparing loose deposits and biofilm with the PAB entering the distribution system, there were 6 shared dominant OTUs between the loose deposits and PAB in the treated water (6/15), and 3 shared OTUs between the biofilm and PAB in the treated water (3/22). The number of shared OTUs increased to 9 between the loose deposits and PAB in the tap water (9/15) and 5 between the biofilm and PAB in the tap water (5/22). It was revealed that 11 out of 14 dominant OTUs in the tap water PAB are shared with loose deposits and biofilm in the distribution system. A full list of these shared OTUs' IDs and taxonomy information is given in Fig. S4.

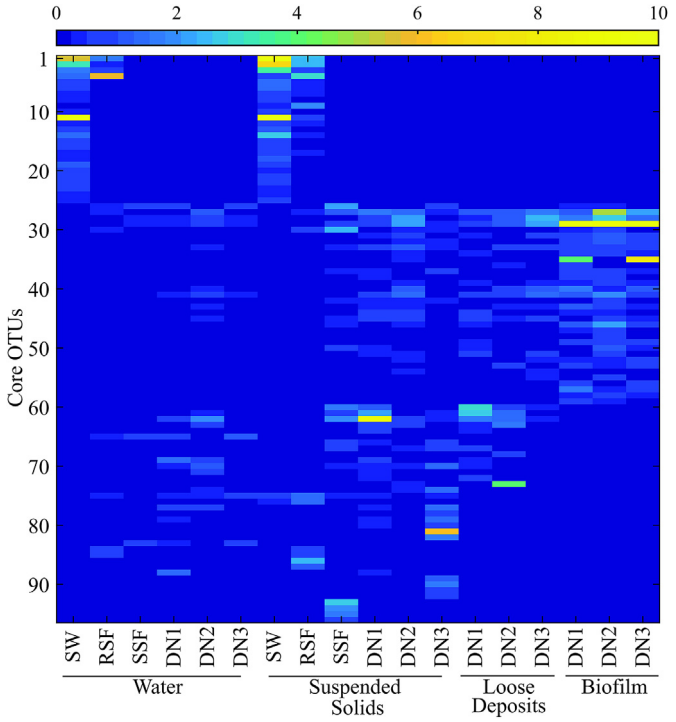

Fig. 4. Heatmap showing the dominant OTUs and their relative abundance in all samples; the average of replicates was calculated for each sampling location and phase. The full taxonomy information of the listed dominant OTUs is given in Table S2.

3.2. Beta diversity comparing bacterial community similarity

The beta diversity results are represented in a PCoA plot (Fig. 5), the high similarity of replicate samples revealed the quality of the sampling conducted in this study and the reproducibility of subsequent 16S rRNA gene sequencing and obtained results. For the community of both PB and PAB, clear differences were observed for source water, RSF water, treated water, and tap water. The community of PB and PAB were similar in the source water, while the differences became greater when going further into the purification steps.

In the distribution system, the community of suspended particle-associated bacteria, bacteria harboured in loose deposits, and biofilm bacteria clustered closely together indicate their high similarity to each other; they are also clustered closely together with the PAB in treated water entering distribution system. Whereas the community of PB in the tap waters collected at three locations in the distribution system showed clear differences, especially at DN3, the PB is more similar to the cluster of suspended solids, loose deposits, and biofilm than to the PB at DN1 and DN2. There were clear overlaps between the bacterial communities of suspended solids and loose deposits.

3.3. Source apportionment estimates using SourceTracker

3.3.1. Source tracking of planktonic and particle-associated bacteria in tap water

As illustrated in Fig. 2, bacteria in the source water, RSF water, treated water, biofilm and loose deposits in the distribution system can be potential sources of tap water bacteria. According to the source apportionments by the microbial community MST, there was a variability in the source of PB and PAB across the three selected locations in the distribution system (Table 1). For both PB and PAB in the tap water, there was no detectable contribution from source water. For the PB in tap water, the major contribution was from the PB in the treated water. When going further into the distribution system, the contribution of PB in the treated water decreased from 54.1% (DN1) to 41.0% (DN2), and further decreased to 11.7% at DN3. Correspondingly, the contributions of loose deposits (LD) increased from 2.5% at DN1 to 10.5% at DN2, and further

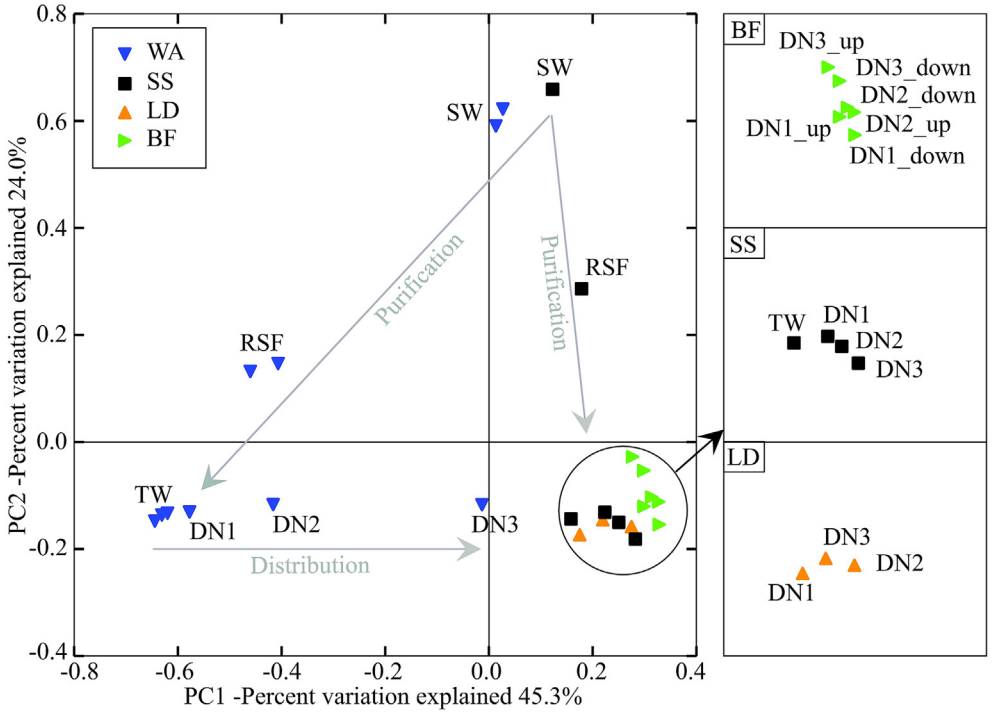

Fig. 5. PCoA plot generated using WUnF metrics for all sampling locations and phases.

Table 1
The percentage contributions and standard deviations of the potential sources for the bacteria in the tap water and the loose deposits and biofilm in the distribution system calculated by SourceTracker, presented as percentage contributions (±standard deviations).

			PAB-Purification			PB-Purification			Distribution		Unknown
			SW	RSF	SSF/TW	SW	RSF	SSF/TW	LD	BF	
Taps	PB	DN1	0	0	2.2 (±0.2)	0	6.6 (±0.2)	54.1 (±0.2)	2.5 (±0.1)	1.7 (±0.1)	32.9 (±0.1)
		DN2	0	0	9.8 (±0.2)	0	4.7 (±0.3)	41.0 (±0.2)	10.5 (±0.2)	0.5 (±0.1)	33.5 (±0.2)
		DN3	0	0	7.0 (±0.2)	0	0.8 (±0.1)	11.7 (±0.2)	38.0 (±0.3)	2.6 (±0.2)	39.7 (±0.2)
	PAB	DN1	0	0	38.0 (±0.3)	0	0	0	34.2 (±0.3)	5.9 (±0.3)	21.3 (±0.1)
		DN2	0	0	26.1 (±0.2)	0	0	0	42.7 (±0.4)	0.5 (±0.1)	29.8 (±0.2)
		DN3	0	0	6.4 (±0.2)	0	0	0	45.2 (±0.4)	19.7 (±0.3)	28.3 (±0.1)
Distribution system	Loose deposits	DN1	0	1.3 (±0.6)	32.7 (±0.7)	0	0	5.9 (±0.6)	/	/	59.9 (±0.9)
		DN2	0	0	23.0 (±0.7)	0	0	11.3 (±0.6)	/	/	65.7 (±0.7)
		DN3	0	1.9 (±0.6)	24.9 (±0.8)	0	0	13.5 (±0.9)	/	/	60.2 (±0.8)
	Biofilm	DN1	0	2.1 (±0.4)	38.1 (±1.2)	0	0	12.4 (±1.3)	/	/	47.4 (±0.6)
		DN2	0	2.8 (±0.7)	43.8 (±0.9)	0	0	10.2 (±1.2)	/	/	40.5 (±0.8)
		DN3	0	0.9 (±0.3)	37.8 (±1.2)	0	0	9.0 (±1.1)	/	/	52.3 (±0.5)

increased to 38% at DN3. There was some minor contribution from PAB treated water (2.2–9.9%), PB in RSF water (0.8–6.6%) and biofilm in the distribution system (0.5–2.6%). The unknown contributions ranged from 32.9% to 39.7%.

By contrast, the PAB in tap water only originated from the PAB in the treated water and distribution system, of which the major contributions were from PAB in treated water and loose deposits in distribution system. When going further into the distribution system, the contributions of PAB in treated water decreased from 38.0% at DN1 to 26.1% at DN2, and further decreased to 6.4% at DN3. Correspondingly, the contributions of loose deposits in the distribution system increased from 34.2% at DN1 to 42.7% at DN2, and further increased to 45.2% at DN3. At DN3, the contributions of biofilm increased sharply from 0.5% and 5.9%–19.7%. The unknown contributions ranged from 21.3% to 29.8%.

3.3.2. Source tracking of biofilm and loose deposits bacteria developed in the DWDS

The SourceTracker results showed that biofilm and loose deposits that developed in the distribution system have similar source contributions: the major known contributions were from PB and PAB in the treated water. The contributions of PB ranged from 5.9% to 13.5%, while the contributions of PAB ranged from 23.0% to 43.8%. On average, the contributions of PAB to biofilm (39.9%) are higher than their contributions to loose deposits (26.6%). There were minor contributions from the PAB in RSF water, which was less than 2.8%. The unknown contributions ranged from 40.5% to 65.7% which was higher than the unknown proportion for PB and PAB source tracking.

4. Discussion

4.1. Source of planktonic and particle-associated bacteria in tap water

4.1.1. Effects of water purification

The present study shows that the water purification processes have significant effects on the community of both planktonic bacteria (PB) and particle-associated bacteria (PAB), which is consistent with previous findings about the influences of water purification on the microbial community of treated water (Hijnen et al., 2004; Vaz-Moreira et al., 2013; Liao et al., 2015; Li et al., 2017; Ma et al., 2017). Comparing the two sand filtration processes, slow sand filtration imposed a greater alteration in the bacterial community composition than rapid sand filtration which introduced different dominant OTUs in the treated water (Fig. 4, Figs. S2 and S3). This may be because slow sand filters have higher removal efficiency of bacteria compared to rapid sand filtration, and the higher biomass in its filter bed maybe detached and released into the effluent, which can be attributed to its smaller interstices and lower filtration velocity (Huisman and Wood, 1974; Bauer et al., 2011).

In a step beyond the reported studies, this study quantified the proportional contribution of PB and PAB in source water, RSF water and treated water to tap water bacteria. The results show that the biggest contributor to tap water bacteria is treated water (35.6% for PB and 23.5% for PAB). This observation was doubly confirmed by the community profiles that show a high proportion of shared dominant OTUs for PB (33.3%) and PAB (20.8%) between treated water and tap water, while there was no shared dominant OTUs either between source water and tap water, nor between RSF water and tap water. This finding suggests the importance of filtration process (e.g. RSF and SSF) on shaping the microbial community not only in treated water, but also in tap water, which agrees with the reported governing role of the filtration process in shaping the tap water bacterial community (Pinto et al., 2012). Besides sand filtration, another recent study found changes of bacterial community structure after each drinking water treatment step of coagulation and flocculation, sedimentation, media filtration and disinfection (Ma et al., 2017).

It should be noted that although there was no detectable contribution from source water to tap water bacteria by SourceTracker, some of the dominant OTUs in treated water (3/4 PB, 3/14 PAB) and tap water (5/8 PB, 3/13 PAB) were already present in the source water, which had gone through treatment, and remained stable during distribution. The zero contribution of source water given in Table 1 may indicate that the contribution was too low to be reliably detected (e.g. <0.1%).

4.1.2. Contributions of a distribution system

It has been a wide consensus that most bacteria in a distribution system are harbored by biofilms (>95%) (LeChevallier et al., 1987; Flemming, 2002) and loose deposits (up to 98%) (Liu et al., 2014); the release of which can contribute greatly to the microbiological water quality deterioration at customers' taps (Liu et al., 2017b) and change the water bacterial community (Liu et al., 2017a). However, to the best of our knowledge, this is the first time the influence and contribution of loose deposits and biofilm on tap water bacteria has been captured and quantified.

At the proximal location (DN1), the community of PB was highly similar to PB in treated water, which was hardly influenced by loose deposits (2.5%) and biofilm (1.7%). During transit to the central location (DN2), the contribution of bacteria from loose deposits increased to 10.5%, but the contribution of biofilm was still limited. This may due to the better mobility of loose deposits compared to

biofilm makes it easier for the loose deposits to be resuspended and release the associated bacteria into water (Gauthier et al., 1999; Lehtola et al., 2004; Liu et al., 2014; Pinto et al., 2014). It is likely that detachment of microbial cells from biofilms into the bulk water required greater turbulence, due to inherit structural integrity of biofilms (Flemming and Wingender, 2010; Fish et al., 2016). Therefore, the contributions of biofilm to PB in tap water were relative low at all locations (0.5–2.6%).

This study demonstrated that with increasing distance in a distribution system, the contributions of both PB and PAB in treated water decreased considerably (by 31.6% and 42.4% at DN3), indicating a weaker contribution of treated water to tap water bacteria in the distal distribution regions. This is because bacterial community in the tap water in distal regions may also be strongly influenced by the loose deposits (re-suspension) and/or biofilm (detachment) during distribution, which is indicated by the corresponding increase of the contribution of loose deposits (35.5%, PB) and biofilm (13.8%, PAB). While approaching the distal location at DN3, the further increase in the contribution of loose deposits indicates either a positive correlation of distance (travel time, water age) and loose deposits contribution or that a bigger hydraulic disturbance occurred at DN3. The sharp increase in the biofilm's contribution by 19.7% to tap water PAB suggested the occurrence of biofilm detachment, which suggests that the increased contribution of loose deposits to tap water PB is caused by a bigger hydraulic disturbance, rather than only the distance travelled. This captured hydraulic disturbance was still a regular operational event (most likely morning peak). However, the detached biofilm with 19.7% contribution to tap water PAB was reflected by only slightly increase of biofilm's contribution to tap water PB, which was not significant. This is likely because that PAB accounted for less than 2% of the total bacteria in drinking water (Liu et al., 2014; Liu et al., 2016). The differences observed in the biofilm's contribution to PB and PAB indicates that monitoring the PAB will be a good approach to study biofilm detachment, even before they significantly influence the water bacteria (Liu et al., 2017b). This will be especially useful as an early warning methodology when biofilm based (opportunistic) pathogens pose potential risks, such as the releasing of Legionella from biofilm during the switching of the supply water (Schwake et al., 2016).

In the distribution system with chlorination, the situation may be significantly different due to the presence of disinfectant residuals. Although there is no quantifiable bacteria source tracking study can be compared to the present study, the contribution of PAB in treated water and the bacteria associated with biofilm and the loose deposits will be higher than un-chlorinated system because of the their protection effects against disinfectant residuals (Liu et al., 2013a).

4.2. Source tracking of loose deposits and biofilm in a distribution system

The bacterial communities of PAB, biofilm and loose deposits were highly similar across the proximal, central and distal locations, which concurs with previous findings in both a pilot distribution system (Martiny et al., 2003) and an operational full-scale distribution system (Liu et al., 2014), both of which contained no disinfectants. Regardless of the locations, the bacterial community of PAB, loose deposits and biofilm were similar with each other. We have discussed in previous study that it might be the process that the suspended particles is originated from biofilm detachment, and become loose deposits after settled in distribution pipes (Liu et al., 2014). In this study, the high similarity between the PAB in distribution system and in treated water revealed that the PAB in distribution system are likely originated from treated water rather than biofilm detachment, which has also been confirmed by the SourceTracker analysis results as discussed above. Moreover, it suggests that PAB in the treated water might have seeded the loose deposits and biofilm developed in the distribution system.

As revealed and proven by SourceTracker, for both loose deposits and biofilm, the major source was the PAB in the treated water (23.0–43.8%). For biofilm, it can be explained by the common characteristics of bacteria attaching to the surface of suspended particles (PAB) in treated water, and taking advantage of their capability to attach to the pipe surface and form biofilm (Liu et al., 2014; Liu et al., 2017a). The formation of loose deposits may have been started from sedimentation of suspended particles in treated water (Gauthier et al., 2001; Vreeburg and Boxall, 2007; Vreeburg et al., 2008; Liu et al., 2014), which was confirmed by the decreased contribution of PAB in treated water to the tap water PAB from proximal to distal locations in the present study.

The PB contributed around 10% to the bacteria in the loose deposits and biofilm. This contribution from PB may be caused by selection of seed bacteria from bulk water that attach and multiply on the surfaces offered by pipe wall and loose deposits (Henne et al., 2012; Fish et al., 2016). Alternatively, it may be caused by the capture of planktonic bacteria on the surfaces of suspended particles and then subsequent trapping into the biofilm matrix on the pipe surface during hydraulic peaks or settling of bio-aggregates settled in the pipe as loose deposits during stagnant periods (Liu et al., 2016).

4.3. Practical implications for tap water bacteria management

4.3.1. Managing tap water bacteria through purification

To sum up from the integral perspective of source to taps, our study showed that source water has no detectable contribution neither to tap water bacteria nor to the distribution system's harboured bacteria in loose deposits and biofilm. On the other hand, the PB and PAB in the treated water contributed 28.7–56.3% to tap water bacteria, 34.3–38.6% to loose deposits and 46.8–54.0% to biofilm. In return, as the indirect contribution from treated water, the bacteria in loose deposits and biofilm that originated from treated water also contributed to tap water bacteria (e.g. partial contribution from the 48.5% at DN3). In other words, the treated water seeded a large proportion of bacteria developed in the distribution system and were present in the tap water. Therefore, the tap water bacteria could be managed through proper water purification.

Previously, researchers proposed that, as a quantitative managing strategy, removing nutrients during purification can limit the bacterial regrowth during distribution both for planktonic bacteria and biofilm formation (Van Der Kooij, 2000; Liu et al., 2013b), and that removing particles will limit the formation of loose deposits (Vreeburg et al., 2008; Liu et al., 2013b). Beyond the quantity, the purification process is also shaping the community of tap water bacteria through changing the bacterial community of PB and PAB in the treated water (seed bacteria). Potentially, Pinto et al. (2012) have proposed that it could be possible to populate the bacterial community in treated water with desired innocuous bacteria by altering water quality and process operation parameters at the drinking water treatment plant. According to the ecological niche theory, the drinking water supply system could thus be pre-colonized via selection from source water or inoculated with microbial community which represents a desirable microbial consortia that can effectively compete in the niches of undesirable micro-organisms associated with nitrification, bio-corrosion and/or (opportunistic) pathogens and can inhibit the growth of those undesirable micro-organisms so as to take and defend their own ecological niches (Wang et al., 2013).

4.3.2. Monitoring and managing tap water bacteria during distribution

The variations across three locations (DN1, 2, 3) showed that the specific contribution of the distribution system (loose deposits and biofilm) to tap water bacteria is highly location- and hydraulics-dependent. At DN3, the distribution system's contribution (40.6% to tap water PB and 64.9% to tap water PAB) led to significant changes in the bacterial community of tap water (Fig. 5, PCoA), which concurred with a previous hypothetical evaluation that the release of 20% loose deposits or biofilm will change water bacterial community significantly (Liu et al., 2017a). Flushing the distribution system has proven to be an efficient strategy that improved drinking water quality (Lehtola et al., 2004), and the routine cleaning of the distribution system has been widely employed by water utilities as a strategy to manage tap water quality (Kjellberg et al., 2009).

The main challenge of distribution system cleaning is deciding whether, when, where and how to clean the distribution network. As such, the resuspension potential method (RPM) has been developed and is widely used in the Netherlands as a way of estimating the rate of sediment accumulation, determining the necessary cleaning frequency and monitoring the effectiveness of distribution pipe cleaning which measures the turbidity response to an induced hydraulic turbulence (Vreeburg and Boxall, 2007). However, the method is mainly based on the particle load consideration and turbidity measurements. The release of bacteria from loose deposits and biofilm into the bulk water and the associated influence on the quantity and community of tap water bacteria might be undetectable because of the dilution effects in the water column. We demonstrated that an integral sampling of PB, PAB, loose deposits and biofilm in the distribution system makes it possible to observe and quantify the water quality deterioration potential and the changes of contribution from loose deposits and biofilm to tap water bacteria (Liu et al., 2017a). Based on regular monitoring results, once the contribution of biofilm and loose deposits to tap water bacteria exceed pre-set quantitative (e.g. cell number) and qualitative (e.g. bacterial community) threshold, the decision for pipe cleaning can be made at an early stage before the distribution system harbored material can lead to water quality deterioration problems.

4.4. Unknown sources and outlook

It was noted that a considerable portion of contributions to tap water bacteria remain unknown. Firstly, this is because there were other potential sources not included in the sampling program for this study which may harbor different bacteria and make considerable contributions, for example the filter material used for water purification (Pinto et al., 2012), the distribution pipes of other materials (Broo et al., 2001; Wang et al., 2014; Ji et al., 2015; Rożej et al., 2015), and the household pipes (Liu et al., 2017a) and premise plumbing system (Proctor and Hammes, 2015; Zlatanović, van der Hoek et al., 2017).

It is highly recommended that a complete sampling and evaluation of the contributions of each component in the drinking water system from source to tap be conducted, especially including the potential influences of the premise plumbing system. The knowledge gained will be essential for distributing available labor and financial resources for the most efficient bio-safety and bio-quality management.

Secondly, the present study is only a snapshot sampling that does not include temporal variations and seasonal dynamics yet, both of which have significant contributions to particle load and the bacterial community in tap water (Matsui et al., 2007; Pinto et al., 2014). If the PB and PAB at the customer's tap can be continuously monitored online, the changes and dynamics of tap water bacteria, and the regular ranges of loose deposits and biofilm contributions in the distribution system can be investigated and quantified.

A long-term microbial observation that collects high-resolutions, uses multiple locations and yearly data sets from source to tap will enable a better assessment of the origin and development of tap water bacteria. Moreover, the SourceTracker method has the potential to be used to identify the sources of bio-contamination, such as waste water ingress, surrounding soil contamination during repairing events, and to quantify the bacteria being released during the loose deposits and biofilm destabilization during the operational parameters or when the water quality changes.

5. Conclusion

- The water purification process shaped the community of PB and PAB in treated water. The tap water bacterial community is location- and hydraulics-dependent, which varies across different locations.
- There was no detectable contribution from the source water to the bacteria developed in the loose deposits and biofilm in the distribution system and the bacteria present in the tap water.
- The PB in the treated water is the major contributor to the PB in tap water (17.7–54.1%), and the PAB in the treated water seeded the bacteria associated with loose deposits (24.9–32.7%) and biofilm (37.8–43.8%) in the distribution system.
- The loose deposits and biofilm showed significant influence on tap water PB and PAB revealed by the increased contribution of loose deposits to tap water PB (from 2.5% to 38.0%) and the increased contribution of biofilm to tap water PAB (from 5.9% to 19.7%) caused by disturbance.
- The tap water bacteria could possibly be managed by selecting and operating proper purification processes and regularly cleaning the distribution system.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.watres.2018.03.043.

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