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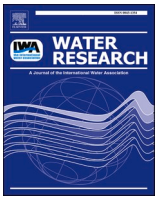

Service-lines as major contributor to water quality deterioration at customer ends

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ABSTRACT

Biofilm detachment contributes to water quality deterioration. However, the contributions of biofilm detachment from different pipes have not been quantified or compared. Following the introduction of partial reverse osmosis (RO) in drinking water production, this study analyzed particles at customers' ends and tracked their origins to water distribution mains and service lines. For doing so, filter bags were installed in front of water meters to capture upstream detached particles, while biofilm from water main and service line were sampled by cutting pipe specimens. The results showed that elemental concentrations of the biofilm in mains were higher than those of service lines (54.3–268.5 vs. 27.1–44.4 μg/cm^2), both dominated by Ca. Differently, filter bags were dominated by Fe/Mn (77.5–98.1%). After introducing RO, Ca significantly decreased in biofilms of mains but not service lines, but the released Fe/Mn rather than Ca arrived at customers' ends. The ATP concentrations of service lines were higher than mains, which decreased on mains but increased in service lines after introducing RO. For the core ASVs, 13/24 were shared by service lines (17), mains (21), and filter bags (17), which were assigned mainly to *Nitrospira* spp., *Methylomagnum* spp., *Methylocytis* spp., and *IheB2–23* spp. According to source tracking results, service lines contributed more than mains to the particulate material collected by filter bags (57.6 ± 13.2% vs. 13.0 ± 11.6%). To the best of our knowledge, the present study provides the first evidence of service lines' direct and quantitative contributions to potential water quality deterioration at customers' ends. This highlights the need for the appropriate management of long-neglected service line pipes, e.g., regarding material selection, length optimization, and proper regulation.

1. Introduction

Drinking water distribution systems (DWDSs) play a pivotal role in the delivery of safe and clean drinking water to consumers (Rosario-Ortiz et al., 2016). Moreover, microenvironments form and develop within DWDSs over decades under regular operations, e.g., biofilm, loose deposits, and pipe scaling (Liu et al., 2017b; Vreeburg and Boxall, 2007), and these microenvironments can significantly impact the water quality at customers' taps (El-Chakhtoura et al., 2015; Liu et al., 2018; Prest et al., 2016; Zhang et al., 2022). As reported, there are clear differences regarding the bacterial quantity and community between the drinking water that leaves the treatment plant and the water that arrives at downstream taps (Chan et al., 2019; Chen et al., 2020; Liu et al., 2018; Nescerecka et al., 2018; Prest et al., 2016; Thom et al., 2022; Vavourakis

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et al., 2020). For example, microbial water quality deterioration has been commonly observed as increases in the total cell concentrations and intact cell proportions (El-Chakhtoura et al., 2015; Prest et al., 2013). In extreme cases, water quality deterioration may be accompanied by esthetic and health problems resulting from the detachment/release of particles, cells, and metals in DWDSs, such as discoloration, high concentrations of heavy metals and/or the presence of (opportunistic) pathogens. This is especially true when irregular disturbances occur in distribution systems due to hydraulic or water quality changes.

In general, DWDSs are pipe networks with varying diameters and materials organized into multiloops that range in length from tens to thousands of kilometers (totaling 1.1 million kilometers in China and 19,796 kms in Beijing in 2021; 120,224 kms in the Netherlands in 2020) (Association of Dutch Water Companies (Vewin), 2022; National Bureau of Statistics of China, 2021). It is difficult to respond to water quality deterioration without knowing where the problems come from. To pinpoint the source of particle/cell/element release and develop efficient tap water quality management strategies, it is essential to explore the contributions of different pipe types to water quality deterioration (e.g., transportation pipe, typically $>$ 200 mm; distribution main pipe, typically 63–110 mm; service-line pipe that connecting main to water meter, typically 25–32 mm) (Hensley et al., 2021; Liu et al., 2017a). These pipes are different in hydraulics condition, residence time, and surface-to-volume ratio. Water quality deterioration has been widely observed in distribution water mains (Main) and premise plumbing, which has been observed as increases in cell number and/or particulate load and attributed to the long water retention time and high surface-to-volume ratio (Lautenschlager et al., 2010; Ling et al., 2018; Montagnino et al., 2022; Tsvetanova and Hoekstra, 2010a; Zlatanović et al., 2017). The service line (Service-line) is characterized as intermittent flow, prolonged stagnation and close proximity to consumers comparing to Main, yet the study on service-line is limited. Our previous study found that the quality deterioration potential of Service-line is higher than that of Main (Liu et al., 2017a). However, real release events from Main and Service-line have yet been captured, quantified, or compared in the literature.

In field distribution systems, the challenges in studying particle/cell release from pipes include the episodic occurrence of such events, the dilution effects caused by the flushing water, the limited accessibility of the biofilm matrix, and the lack of source-apportionment methods. These difficulties have been partially overcome in our previous studies, such as through the application of preconcentration tools focused on suspended particles and the introduction of microbial community fingerprint-based source tracking to investigate the contributions of loose deposits and biofilm in Main pipes (Chen et al., 2020; Liu et al., 2018) . However, the contribution from service lines remains unknown, and no long-term study has followed particle/cell release from different types of pipes. The objectives of this study are to capture and trace the particle/cell release from Main and Service-line and to compare their contributions. For this purpose, filter bags were developed and placed in front of water meters before being sampled together with paired Main and Service-line pipe specimens during the introduction of a new treatment for elemental composition, bacterial quantity and community analysis. This study provides an important opportunity to advance our understanding of episodic particle/cell release from different types of pipes in full-scale DWDSs, thereby allowing water utilities to answer the crucial question of where the major problems may come from.

2. Material and methods

2.1. Drinking water treatments

The Dutch drinking water treatment plant assessed in this study uses riverbank filtrated water as a source and produces 3 Mm^3 of drinking water per year. The source water abstracted from 2 well fields (each contributing 50%) is further treated by sprinkler aeration, rapid sand filtration, activated carbon filtration, and UV disinfection before being pumped into the distribution network. Recently, reverse osmosis was introduced to pretreat abstracted source water from one well field. Thereafter, the RO permeate is blended with abstracted source water from another well field before being subjected to subsequent aeration, rapid sand filtration, activated carbon filtration and UV disinfection treatments. The treatments applied before and after introducing RO are shown in Figure S1. In the Netherlands, chlorine is not used in the drinking water treatment and distribution for bacteriological control.

2.2. Distribution area and sampling program

The distribution system in the study area is a branched network. As illustrated in Fig. 1, four locations (L1, L2, L3 and L4) were chosen in the distribution system with gradually increasing distances (1.1 km, 4.2 km, 13.0 km, and 18.7 km) to the treatment plant (WTP). At each location, integral sample sets were taken, including pipe specimens from Main and Service-line and the filter bags installed in front of water meters at household connections. Loose deposits may have considerable contributions in releasing particles/cells, since it may be flushed out distribution network by regular hydraulic flushing, hence loose deposits were excluded from the present study. Therefore, the objectives of this study are to capture and trace the particle/cell release from biofilm in service line and water main and to compare their contributions. For this purpose, filter bags were developed and placed in front of water meters before being sampled together with paired service line and main pipe specimens during the introduction of a new treatment for elemental composition, bacterial quantity and community analysis. This study targeted on larger particles released from pipe water biofilm, which may lead to water quality deterioration at customers' taps. Therefore, a pore size of 50 μm was selected based on pre-tested particle size distribution in tap water, by particle counter HIAC Royco 9703, combined with Pharm Spec Version 2. The filter bags used in this study were made of polypropylene (PP) with a pore size of 50 μm, diameter of 10.5 cm, height of 20.3 cm, and an inner surface area of 500 cm^2 (Manufacturer: Pentair, Mexico). The vessels used for filter bag accommodation were polypropylene (PP) filter housing with a diameter of 11.4 cm and height of 25.4 cm (Manufacturer: Pentair, Mexico).

Based on our previous field study of transition effects, sampling campaigns were conducted at three stages after introducing RO in WTP for a period of 6 months (26 weeks) (Chen et al., 2020). More specifically, the 26 weeks were divided into three phases: the initial period, from week 0–4 (T_{-ini}); middle period, from week 5–21 (T_{-mid}); and final period, from week 22–26 (T_{-end}). The Main and Service-line pipe specimens were sampled as described previously (Liu et al., 2018). In short, duplicate pipe specimens (L = 20 - 40 cm) were taken after flushing out loose deposits and filled with tap water at the same site (water taken after flushing until the temperature was constant) and closed by pre-disinfected end caps to keep the inner environment humid during transportation. Considering the biofilm sampling is destructive, paired pipe specimens of main and service-line were taken from adjacent streets as close as possible to the house installed filter bags at T_{-ini} and T_{-mid}, the service-line and main connected directly to filter bags were sampled at T_{-end}. As we reported earlier (Chen et al., 2022; Liu et al., 2014) and found in the present study (Fig. 4a) reliable and reproducible biofilm results are collected. The operation of filter bags was kept as similar as possible between 38 and 40 days for T_{-mid} and T_{-end}. There may be growth during the filtration period, but it would be neglectable considering the short filtration time (comparing to biofilm formation) and high biological stability of drinking water (AOC, $\sim$ 3 μg C/L) in the studied system (Sousi et al., 2020). The collected filter bags were sealed in their original housing for transportation and evenly rinsed with 30 ml DNA-free demi water to keep filtrates from drying out (Milliq water). All samples were transported to the lab on ice, stored at 4 °C and processed within 24 h after sampling (Liu et al., 2018, 2014). In total, 23 Main samples (T_{-ini}, 7; T_{-mid}, 8; T_{-end}, 8), 19 Service-line samples (T_{-ini}, 5; T_{-mid},

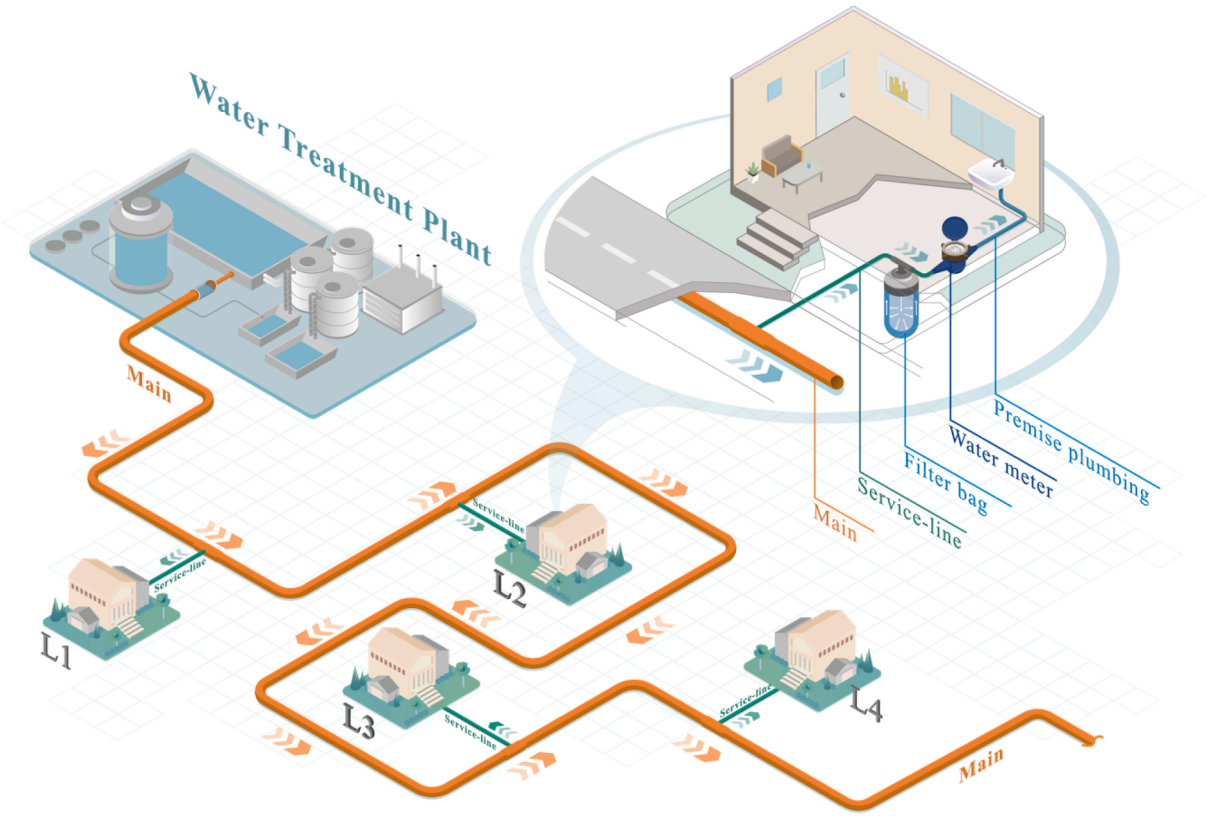

Fig. 1. Schematic illustration: the layout of sampling locations and the main pipe (Main, PVC, 110 mm (exl L1. HDPE, 50 mm)), service lines (Service-line, HDPE, 32 mm) and filter bags in front of the water meters at each location.

8; T_{-end}, 6) and 24 filter bags (T_{-mid}, 12; T_{-end}, 12) were collected throughout the campaign and were subject to chemical and microbiological analysis. General information about sampling location, pipe diameter, duration of filter bags in the system, etc. are provided in Table S1.

2.3. Sample preparation

The biofilm matrices isolated from pipes and filter bags were detached by pretreatment with low-energy ultrasonication as previously described (Liu et al., 2018; Magic-Knezev and van der Kooij, 2004). In short, 3 × 2min ultrasonication treatments at 43 kHz were applied (Branson ultrasonic water bath, 180W power output, 10L sonification chamber). The obtained suspensions were further used for the biomass quantification (ATP), DNA extraction and elemental composition analyses (Ca, Fe, Mn, Al, and As). Considering the uneven distribution of filtrates on filter bags, 3 × 1 cm^2 pieces were cut from the upper, middle, and bottom of the filter bags and treated by the same ultrasonication method. To obtain representative results, the tailer dividing of three zones (upper, middle, and bottom) were based on visually inspected particle distribution over filter bags, similar to our previous study on the radial-spatial distribution of biofilm over 360° pipe surface (Liu et al., 2020).

2.4. Inorganic element analysis by ICP–MS

The inorganic elements of calcium (Ca), iron (Fe), manganese (Mn), aluminum (Al) and arsenic (As) were quantified using inductively coupled plasma–mass spectrometry (ICP–MS) (PerkinElmer ELAN DRC-e ICP–MS), following previously description (Liu et al., 2017a). Quality control samples were performed for every ten samples assessed, including laboratory-fortified blanks and laboratory-fortified samples. For filter bag samples, the elemental concentration is the total elements amount divided by the corresponding water volume flowing through it within a sampling period.

2.5. Adenosine triphosphate (ATP)

The adenosine triphosphate (ATP) content, as an indicator of active biomass, was determined for all samples by combining BacTiter Glo reagent and a luminometer (Liu et al., 2017a; Magic-Knezev and van der Kooij, 2004). Briefly, the ATP released from bacteria is capable of luminescence after mixing with the ATP reagent at 30 °C for 2 min. Therefore, the intensity of emitted light was directly measured in a luminometer and converted to ATP using a calibration curve based on known ATP standards. For filter bag samples, the ATP concentration is the total ATP content normalized by the corresponding water volume flowing through it within a sampling period.

2.6. DNA extraction and illumina 16S rRNA gene sequencing

DNA was extracted from pretreated suspensions using the FastDNA Spin Kit for Soil in accordance with the manufacturer's instructions (Q-Biogene/MP Biomedicals, Solo, OH, USA). Amplification of the 16S rRNA gene was performed following (Kozich et al., 2013). In short, primers (341F: 5′-CCTACGGGNGGCWGCAG-3′ and 785R 5′-GACTACHVGGGTATCTAATCC-3′) targeting the V3-V4 hypervariable regions of the 16S rRNA gene were applied to amplify the extracted genomic DNA (gDNA). Paired-end sequencing of the amplicons (2 × 300 bp) was conducted on an Illumina MiSeq platform (Illumina, San Diego, USA) at a Baseclear laboratory in Leiden, the Netherlands. The sequencing data have been deposited in the NCBI database, with reference code PRJNA922967.

2.7. Sequencing data processing

Demultiplexing and adaptor trimming were performed by the sequencing center, and the retrieved data were imported into artifacts for use with the Quantitative Insights Into Microbial Ecology (QIIME2 v2020.11) platform (Bolyen et al., 2019). Plugins installed in QIIME2 were used to perform data preprocessing, 16S rRNA amplicon sequence variant (ASV) generation and phylogenetic tree construction. Primer trimming was performed by the q2-cutadapt plugin. To optimize the 16S rRNA gene-trimming parameters and increase the reproducibility of analysis, Figaro (v1.1.2) was used to determine the DADA2 trimming parameters with an overlap length of 30 bp (Sasada et al., 2020). Denoising, dereplication, paired-read merging and chimera removal were applied using q2-dada2 with flags "–p-trunc-len-f 251 –p-trunc-len-r 206 –p-max-ee-f 2 –p-max-ee-r 2″ (Callahan et al., 2016). In total, 1,231,847 16S rRNA gene sequences were obtained from 66 samples (42 pipe samples and 24 filter bag samples), with an average of 216,611 (5,399-34,780) sequences per sample. The numbers of quality-filtered and chimera-free reads in each sample are provided in Table S1, and rarefaction curves are provided in Figure S2. Taxonomy was assigned to the generated ASVs using the q2-feature-classifier, customized for the V3-V4 primer set with SILVA SSU database release 138.1 (Quast et al., 2013). Multiple sequence alignment and phylogenetic tree reconstruction processes were applied using q2-phylogeny. Alpha-diversity and beta-diversity indices were calculated based on ASV.

2.8. Microbial source tracking by SourceTracker2

To identify the contribution proportions of potential sources to the sink, the Bayesian-based SourceTracker method was applied using the ASV tables as the input files (Knights et al., 2011). In this study, the communities of bacteria associated with particles retained by/on filter bags were considered "sinks", while the communities of biofilms from upstream water main and service line were defined as "sources". The source proportioning is based on bacterial fingerprints of biofilm released particles, which would not be influenced by different flow patterns and nutrients availability between filter bags and pipe surface. When quantifying the source−sink relationships at each location, the biofilms collected from upstream water mains and service lines were assumed to be potential independent contributors. In this study, source apportionment was performed by SourceTracker2 (V 2.0.1) using the following parameter settings: source rarefaction depth of 11,547, sink rarefaction depth of 4,528, burn-in of 100, restart of 10, alpha1 of 0.001, alpha2 of 0.1 and beta of 10. The analysis was repeated in triplicate, with the average values derived as described (Liu et al., 2018; McCarthy et al., 2017).

2.9. Statistical analysis

All of the downstream analyses, including post-denoising, alpha diversity estimation, beta diversity comparisons, and further statistical analyses, as well as data visualization, were carried out in R (R Core Team, 2021; RStudio Team, 2020) by utilizing the packages phyloseq (McMurdie and Holmes, 2013), vegan (Oksanen et al., 2019), data.table (Dowle and Srinivasan, 2021) and ggplot2 (Wickham, 2016). Considering the difference in library size across samples, the read count of each ASV was normalized by the total number of reads in each sample. Alpha and beta diversity analyses were conducted to compare the samples based on normalized ASVs. While alpha diversity was calculated using the Simpson index, principal coordinate analyses (PCoA) were performed based on the Bray-Curtis distance matrix calculated for beta diversity comparison. Statistically significant differences in alpha diversity indices between different groups were evaluated using the Wilcoxon rank sum test, and permutational multivariate analysis of variance (PERMANOVA) was performed using the adonis function with dispersion in the dataset tested by the betadisp function. ASVs with a maximum abundance greater than 5% across all samples were illustrated in a heatmap using PHeatmap (Kolde, 2019).

3. Results

3.1. Elemental composition of biofilms on main and service-line and particles captured by filter bags

As shown in Fig. 2, higher elemental concentrations (sum of Ca, Fe, Al, Mn and As) were detected within the biofilm matrix in mains (54.3–268.5 μg/cm^2) than on the Service-line (27.1–44.4 μg/cm^2) at each location, both of which were dominated by Ca (97.6–99.4%, main pipes; 52.5–99.2%, service lines) (Figure S3). The other elements, in descending order, were Fe, Al, Mn, and As. In the filter bags, a total mass of 10.8–96.3 μg was detected, resulting in elemental concentrations of 3.5–31.8 μg/l. Different from the biofilm matrix, the filter bags were dominated by Fe and Mn (77.5–98.1%), while Ca accounted for only 1.4–6.5%. Taking the observed differences at different time points (T_{-ini} vs. T_{-mid}; T_{-mid} vs. T_{-end}) as indication for biofilm detachment/enrichment, it is interesting to notice that significant decreases in the measured elements (mainly in Ca) were observed on the water mains from T_{-ini} to T_{-mid} ($p < 0.05$, Figure S4) and remained stable until T_{-end}. This indicates occurrence of release from biofilm matrix on water main shortly after the introduction of the new treatment, which stopped after T_{-mid}. However, no clear variance was noticed for service lines. For the filter bags, significant decreases in Fe and Mn were observed at L3 and L4, while the content of Fe increased at L1 and L4 from T_{-mid} to T_{-end}. This observation suggested that the released Fe/Mn rather than Ca arrives at customers' locations, though the time and the exact level of release might be location dependent.

3.2. Active biomass quantification

For the biofilm matrix in main and service line, 0.01–0.7 ng ATP/cm^2 and 0.01–5.6 ng ATP/cm^2 were detected, respectively (Fig. 2). In general, the ATP concentrations were higher in the Service-line than in the Main pipes, and this was especially true at T_{-mid} and T_{-end}. After introducing new treatments, the ATP concentrations on water mains slightly decreased ($p > 0.05$), while those of Service-line slightly increased from T_{-ini} to T_{-mid} and sharply increased afterward until T_{-end} ($p < 0.05$) (Figure S5). The increased ATP within Service-line might have been caused by the deposition and accumulation of the detached biofilm from upstream main pipes. The accumulation could be favored by the longer stagnation time and intermittent water usage pattern of service-line. In addition, relatively high standard deviations were observed for service line replicates, indicating the uneven biomass distribution within service lines.

The ATP content of the filter bags ranged from 10.8 to 96.3 ng, resulting in ATP concentrations of 0.3–4.0 ng/l. The relatively high standard deviations among the top, middle and bottom of the filter bags revealed the uneven distribution of particulate matter on the filter bags. Specifically, there were clearly higher ATP at bottom of filter bags than other positions, indicating bigger particles sedimentation/accumulation driven by gravity may occur in premise plumbing. From T_{-mid} to T_{-end}, the ATP content in filter bags decreased at L1, L2 and L3 but increased at L4, indicating heavier or lighter release of biofilm matrix upstream; this process might be location- and residential water usage pattern-dependent.

3.3. Bacterial community composition

In total, 1,231,847 sequences were obtained from 66 samples (42 biofilm samples and 24 filter bag samples) and further assigned to 14,692 ASVs. For taxonomic composition, the bacterial community was assigned to 11 core phyla (relative abundance > 5%, Fig. 3, Table S7).

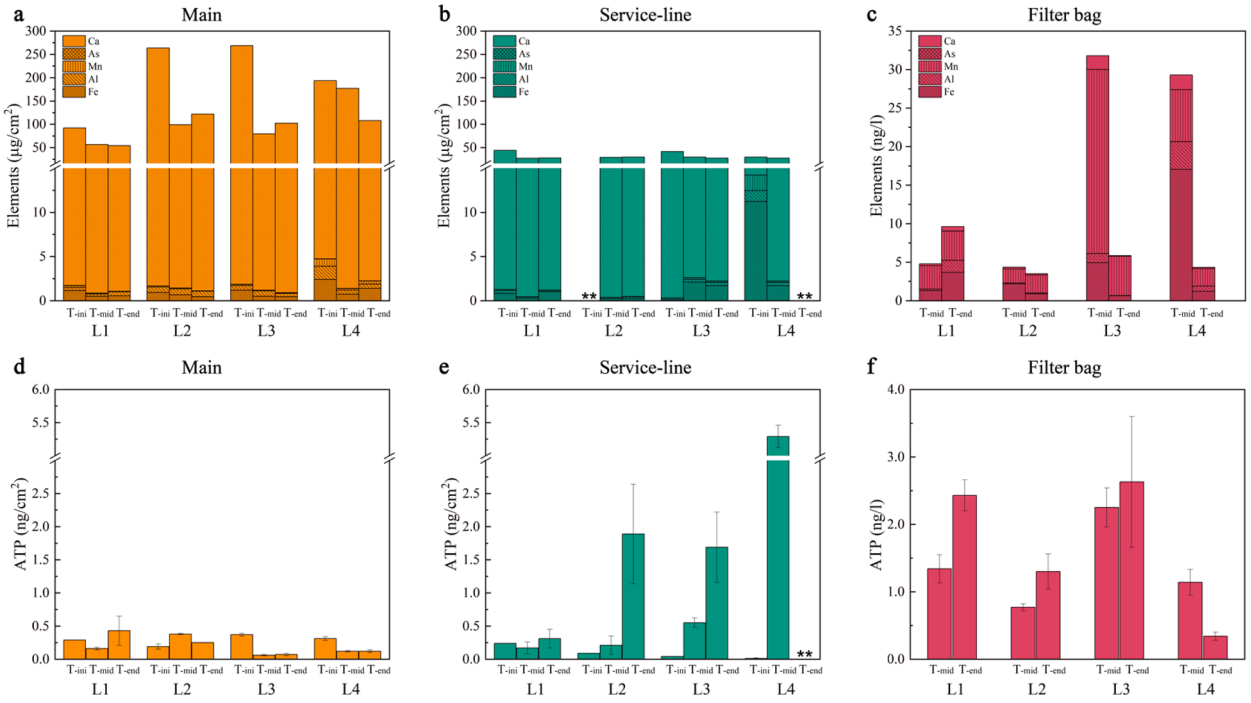

Fig. 2. Metal elements of Fe, Al, Mn, As, and Ca (a, b, c) and ATP (d, e, f) of the biofilms sampled on main and service line pipes and particles collected on the filter bags at 4 locations in a DWDS at T_{-ini}, T_{-mid} and T_{-end}. The ATP and elemental composition corresponding to each location are provided in Tables S3, S4, and S5. Elemental and ATP concentrations for filter bags was normalized by the volume of water flowing through.

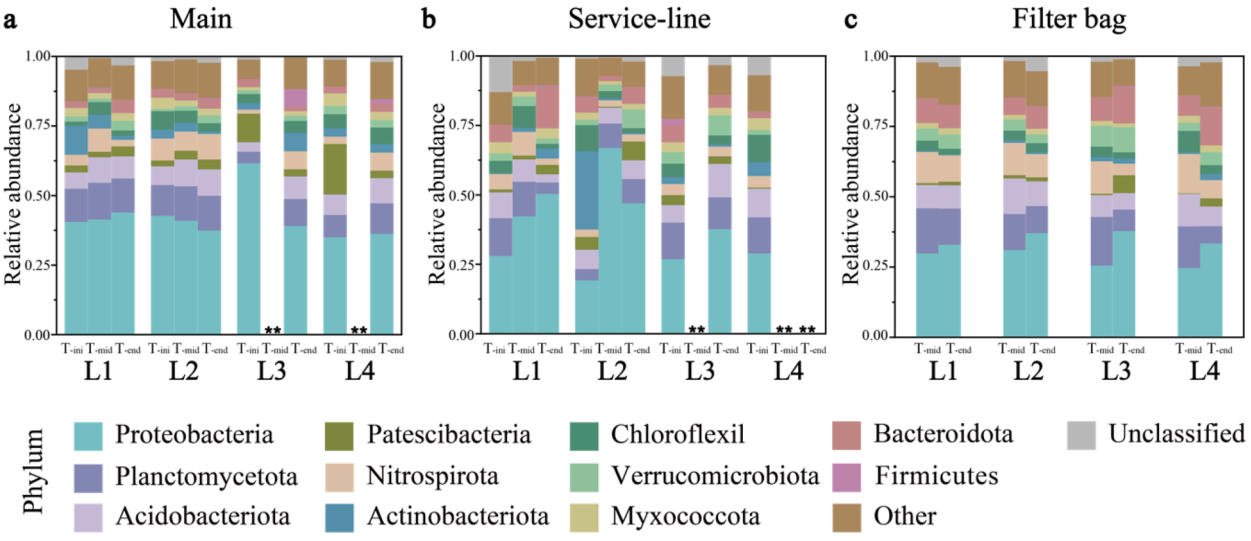

Fig. 3. Microbial community composition at the phylum level. The ASVs that could not be classified to a specific phylum were assigned as Unclassified, and the remaining ASVs were assigned together under Other.

Procteobacteria (19.4–78.6%) was the dominant phylum across all the samples regardless of the sample type. For biofilms on main pipes, the core phyla, in descending order, were Proteobacteria (25.2–78.6%), Planctomycetota (1.4–14.7%), Acidobacteriota (1.4–13.3%), Patescibacteria (0.4–33.0%), Nitrospirota (0.9–9.9%), Actinobacteriota (0.7–10.4%), Choroflexil (0.5–9.5%), Verrucomicrobiota (0.6–4. 9%), Myxococcota (0.3–8.4%), Bacteroidota (0.2–5.2%) and Firmicutes (0–10.4%). Similar to the main pipes, Proteobacteria, Planctomycetota and Acidobacteriota remained the most abundant phyla in the biofilms on Service-line. In contrast, compared to mains, the relative abundances of Bacteroidota, Verrucomicrobiota and Myxococcota were higher, while the relative abundances of Patescribacteria and Actinobacteriota were lower. The bacterial communities sampled from the filter bags contained lower Proteobacteria and Patescribacteria but higher Planctomycetota, Acidobacteriota, Nitrospirota, Bacteroidota and Verrucomicrobiota. Interestingly, Firmicutes was detected in all biofilm samples but not in the filter bag samples until T_{-end}, indicating its possible late release from the biofilm.

At the genus level, the detected ASVs mainly consisted of *Nitrospira* spp. (0.4–14.6%), *IS-44* spp. (0.1–5.0%), *Methylocystis* spp. (0–17.1%), *IheB2–2* spp. (0–9.9%), *Methyloglobulus* spp. (0–7.8%), *Crenothrix* spp. (0–23.6%), and *Pseudomonas* spp. (0–27.2%). In total, 15 core genera (maximum relative abundance > 5%) were found across all samples, among which 14/15 and 11/12 core genera in biofilms on main and

Service-line were present in the filter bags (Figure S6). ASV04 (0.3–12.8%; assigned as *Nitrospira*) was the most abundant across all samples, especially in the filter bag samples. ASV10 (assigned as *Methylocystis*) and ASV12 (genus *Unclassified*) were also abundant but were found mainly in service line biofilms (Figure S7).

3.4. Bacterial community diversity

For alpha diversity, similar trends were observed for species richness (ASV number, Figure S8a) and diversity (Simpson index, Figure S8b) for all sample groups. Specifically, the species richness and diversity of the service line community decreased from T_{-ini} to T_{-mid} and then increased from T_{-mid} to T_{-end} back to a similar level as T_{-ini}. Differently, in the filter bag community, they decreased significantly from T_{-mid} to T_{-end}; while the Main community showed no clear changes. These results indicate the relatively sharp responses of the biofilms on the Service-line on the Main pipes, which may have led to a higher contribution of the Service-line than the Main pipes to water quality deterioration. Moreover, 24 ASVs (> 5%) were identified as core ASVs (taxonomy information given in Table S6). The Venn diagram illustrated that 13/24 core ASVs were shared by the Service-line (13/17), Main (13/21) and Filter Bag (13/17) samples (Figure S9). Interestingly, there was no unique ASV contained by the Filter Bags, but all were shared with either Main (16/17) or Service-line (14/17), indicating a close relationship among the three sample types, and the materials collected by the filter bags may have all originated from upstream Service-line and main pipes. Regarding the taxonomy information, the shared core ASVs were mainly assigned to *Nitrospira* spp., *Methylomagnum* spp., *Methylocytis* spp., and *IheB2–23* spp.

The beta-diversity analysis conducted using Bray-Curtis distances exhibited clear grouping among the three sample types (PCoA plot, Fig. 4a). The biofilm samples from mains and filter bags were grouped into two separate clusters, implying their clear dissimilarity (PERMANOVA, $p < 0.001$). The cluster of biofilms on Service-line fell between the biofilms on main pipes and filter bags, indicating its similarity and connection with the other two groups. The paired distance calculation based on Bray−Curtis also confirmed that the similarity between the filter bag and Service-line was higher than that between the filter bag and main pipes (Fig. 4b). Within each group, no clear changes in microbial community composition were observed. In other words, despite biofilm sloughing, the relatively stable microbial community composition of the biofilm was maintained. This suggested that the release/detachment of the biofilm was not member-specific and did not have a significant influence on the general composition of the bacterial community. In addition, the mains were made mostly of PVC except for the samples from L1, which were made of HDPE material (shown as bordered yellow dots in the PCoA plot, Fig. 4a). The observed apparent overlap between HDPE and PVC in the main pipe group elucidated that the microbial community of biofilm was not significantly influenced by the pipe material (PERMANOVA, $p > 0.05$) but was influenced by the pipe type (Main vs. Service-line, $p < 0.001$).

3.5. Immigrant source apportionment by SourceTracker2

Bacteria in the biofilm matrices on the Main and Service-line can be potential sources of biofilm in filter-bag filtrates. The SourceTracker2 results showed that the Main and Service-line samples contributed 51% - 86% of the collected microbes on filter bags (72–86% at T_{-mid}; 51–77% at T_{-end}) (Fig. 5). Regardless of the sampling time and location, the Service-line samples outnumbered the Main samples as the major contributor of sloughing particles immigrated to end-users, hinting that these pipes would be hotspots releasing particulate matter (e.g., elements and microbes) and deteriorating water quality when the DWDS was subjected to disturbances. Interestingly, the proportion of bacteria from Service-line decreased by 3% (L1, from 67% to 64%) - 33% (L4, from 72% to 39%) from T_{-mid} to T_{-end}, suggesting that the release of cells/particles/elements was mitigated after T_{-mid}. The contribution of the Main pipes remained at the same level at L1, L3 and L4 but increased significantly at L2 from 16% to 36%, indicating that the actual release events in the DWDS are likely location- and water usage pattern-dependent.

4. Discussion

To address how the DWDS impacts water quality during water distribution (especially during disturbances) and compare the contributions of different types of pipes, we conducted a comprehensive study in an unchlorinated system after the introduction of RO in the treatment process. The sampling method of the Main and Service-line was integrated with in situ filter-bag sampling in front of water meters and microbial fingerprint-based source tracking. The successfully captured large particles in filter bags and SourceTracker2 results demonstrated that Service-line contributed more than Main pipes to the potential water quality deterioration at customers' taps.

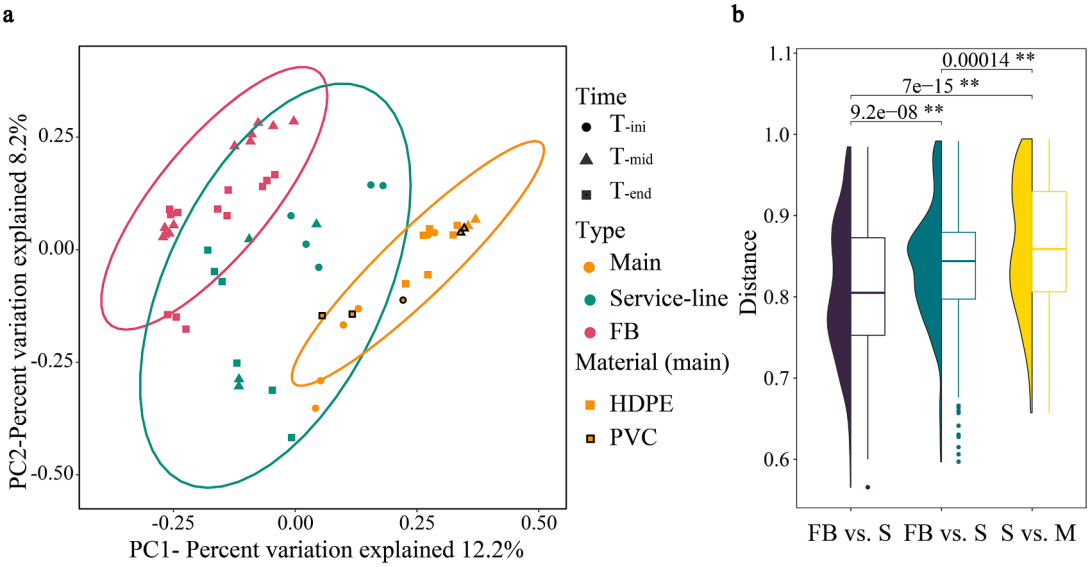

Fig. 4. Beta diversity results illustrated by a PCoA plot (a) and the dissimilarity results revealed by paired Bray−Curtis distances (b). FB stands for filter bags.

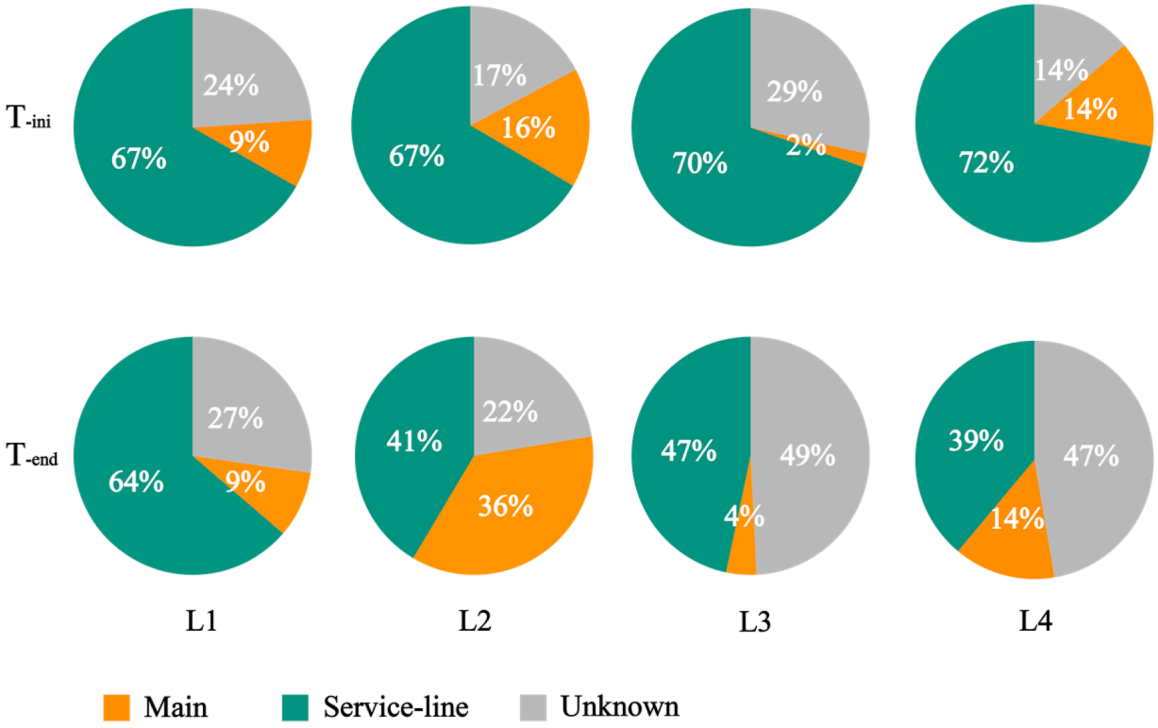

Fig. 5. Origination of filter-bag particles calculated by SourceTracker2-based microbial community fingerprints at T_{-mid} and T_{-end}. The results are presented by the filter bags collected from each location (Table S8).

4.1. Higher water quality deterioration potential lies in Service-line

According to the SourceTracker2 analysis, up to 86% (T_{-mid}, L4) and 78% (T_{-end}, L2) of microbes collected in filter bags came from the upstream Main and Service-line. There were other potential contributors not quantified, such as loose deposits in Main, particles fed by treatment plant, and the possible growth on particles in the filter bags (not likely). However, based on their absolute high contributions, it is reasonable to conclude that Main and Service-line are the major contributors to the large particles and their associated microbes released from the DWDS to customers' taps. Particulate matter reaching customers may originate from the breakthrough of particles from the source water, the release of particles from filters, and the generation of particles during distribution (e.g., the detachment of biofilm and scales from pipes). Our previous study found that Main pipes (excluding loose deposits) contributed 0.5−19.7% to particle-associated bacteria at tap, but Service-line were not considered in that study (Liu et al., 2018). The much higher contribution observed in the present study could be attributed to the inclusion of Service-line. In addition, it may also be because this study focused on much larger particles (50 μm) than previous work (1.2 μm) and/or because the sampling was conducted during disturbances induced by the introduction of RO in the treatment. Moreover, it should be noted that biofilms on main and service-line were dominated by Ca, while filter bags were dominated by Fe and Mn. This may indicate the inorganic particles collected on the filter bags could be originated differently than the bio-particles, for example, from loose deposits in main and/or service-line.

Comparing the contributions of the Main and Service-line, although there was a clear decrease in the Service-line's contribution from T_{-mid} (68.4 ± 4.6%) to T_{-end} (47.7 ± 10.4%), they contributed much more than the Main pipes regardless of the study period (57.6 ± 13.2% vs. 13.0 ± 10.6%) (Table S9). The higher contribution of Service-line could be attributed to their features of intermittent flow pattern, high surface-to-volume ratio, long stagnation and contact time, and a short distance to the customer (Liu et al., 2014; Prest et al., 2016; Tsvetanova and Hoekstra, 2010b). The decrease in the Service-line's contribution from T_{-mid} to T_{-end} (22–26 weeks) might have been due to the fading away of the disturbances induced by introducing partial RO in production. This is consistent with the observed changes of diversity and composition of biofilm bacterial communities from T_{-ini} to T_{-mid} and T_{-end}. It should be noted that the time period of (different) weeks may also influence the composition and diversity of bacterial community (Douterelo et al., 2018). However, considering the high stability of both source water and produced water (especially high biological stability of produced water) and the short time-period (weeks) comparing to biofilm age (decades), it is likely that the introduction of new treatments contributes to the observed differences in bacterial community rather than time. Moreover, Service-line has attracted very little attention compared to distribution mains and plumbing systems. Our previous study reported that Service-line possessed high-quality deterioration potential by assuming the one-time release of all harbored contaminants, e.g., Fe, Mn, As and microbes; we found that the release of 10% household biofilm significantly altered the bacterial community composition in water (Liu et al., 2017a). To the best of our knowledge, the present study provides the first evidence of Service-line's direct and quantitative contributions to potential water quality deterioration at customers' ends.

Weighting the contributions from different pipes is important from a management perspective, especially to allow water utility managers to prioritize their efforts and investments and to avoid blindness to potential risks. It should be mentioned that both Main and Service-line are important, although their contributions are different. This is especially true considering that opportunistic pathogens might be released from biofilms in both Main and Service-line, and the total length and surface area of Main pipes are much larger than those of Service-line. The results of the present study emphasize the importance of considering the contribution of Service-line to potential water quality deterioration. Proper actions should be taken on Service-line to manage the drinking water quality at taps. However, as service lines are mostly dead ends and connected directly to water meters, regular cleaning by flushing or pigging would not be feasible. Further research on service line cleaning strategies and tools is urgently needed.

4.2. Beyond DWDS monitoring: from quantitative to qualitative assessment

In the present study, 50-μm filter bags were installed in front of water meters to collect large particulate matter immigrated from the DWDS for extended periods of time without disturbing daily water consumption. This in situ and real-time sampling method continuously captured and concentrated any possible episodically released large particles from the DWDS, thereby allowing a quantitative assessment of how many particles, elements and microbes arrived at the customers and their spatio-temporal changes. However, it was not possible to determine where those particles/elements/microbes came from (treatment or distribution system), and neither the proportional contributions of different types of pipes (e.g., Main vs. Service-line). These questions can be answered by applying SourceTracker2 to paired sink (particles on filter bags) and source (Main and Service-line) samples; the results pinpointed that the highest contributions/risks came from Service-line (63–79%). This demonstrated the advantages of combining in situ preconcentration data with the community-based SourceTracker to not only quantify changes but also track and apportion the sources. Moreover, continuous sampling can be conducted in a nondestructive manner, making this approach applicable in full-scale drinking water distribution systems.

Traditionally, water utilities are commonly obligated to conduct highly labor- and capital-cost-demanding monitoring programs in DWDSs, the results of which are mostly used to ensure that the measured parameters fulfill the regulated standard. Recently, online devices have moved DWDS monitoring methods toward the emerging big-data era. The commonly installed sensors include temperature, pH, pressure, conductivity, turbidity, and free chlorine sensors. Applications of online particle counters, online flow cytometers and online ATP meters have also emerged in DWDSs. These attempts and efforts at high-frequency quantitative assessments have shown great advantages and potential for valuable leakage detection and meaningful water quality dynamics description (Buysschaert et al., 2019; Koppanen et al., 2022; Pan et al., 2021; Safford and Bischel, 2019). However, such purely quantitative measures can hardly offer insights into what is occurring, why deterioration happens, and where problems may originate. Qualitative measures, in such cases, point out an innovative route for water quality monitoring and management, moving DWDSs into the big-data era. One step beyond the current demonstration would be the possibility of combining trackable molecular results with online monitorable parameters, adopting the possible advantages of machine learning to overcome the limitation of the long processing times required for gene sequencing. Another possibility would be promoting fully automated online nanopore sequencing integrated with SourceTracking packages, though this method will not be financially feasible/ cost-feasible in the near future.

4.3. Practical implications

This study highlighted the importance of service lines for supplying the high-quality drinking water to customers' taps; the contributions of Service-line have long been neglected in previous research. According to our field experiences, these pipes connect houses to distribution water mains that are normally buried under gardens or greens, ranging in length from a few meters to a dozen of meters (mostly 5–10 m). For some special cases where the houses are located slightly further from the water mains, these lengths could be more than 20 m. To bring this issue under the spotlight, the potential risks associated with service lines could be easily mitigated by implementing stricter material selection and length optimization, which have not yet been considered. In addition, as such pipes are neither constructed under roads, like water mains are, nor in building walls, such as premise plumbing are; thus, they can be cleaned or replaced much easier than other pipes without influencing the water supply to other consumers. In short, attention should be given to these meter-long pipes close to taps, and maintenance should be performed on such pipes to avoid water quality deterioration and potential risks, especially when the DWDSs are subjected to disturbances.

5. Conclusions

Following the introduction of RO in the drinking water treatment process, in this study, we captured large particles using filter bags at customers' houses and source-tracked their origins based on bacterial community fingerprints. Based on the results of this study, the following conclusions can be drawn:

- The elemental concentrations of the main pipes were higher than those of the Service-line (54.3–268.5 μg/cm^2 vs. 27.1–44.4 μg/cm^2), both of which were dominated by Ca (97.6–99.4% in main pipes; 52.5–99.2% in Service-line). The filter bags were dominated by Fe and Mn (77.5–98.1%).
- After introducing RO, Ca significantly decreased from the water mains but not from the Service-line, while released Fe/Mn rather than Ca arrived at customers' taps.
- The ATP concentrations in Service-line were higher than those in Main pipes. After introducing RO, the ATP concentrations decreased on the water mains but increased in the Service-line.
- There were 24 core ASVs, 13/24 of which were shared by the Service-line (13/17), Main (13/21) and Filter Bag (13/17) samples. No unique core ASV was found in the Filter Bags, but all were shared with either Main (16/17) or Service-line (14/17) samples. The shared core ASVs were mainly assigned to *Nitrospira* spp., *Methylomagnum* spp., *Methylocytis* spp., and *IheB2–23* spp.
- According to SourceTracker2, service lines contributed much higher particulate material than main pipes (57.6 ± 13.2% vs. 13.0 ± 11.6%) to the filter bags, meaning that a higher water quality deterioration potential lies in service lines than in water mains. Service-lines have long been neglected. Appropriate management is necessary through means such as material selection, length optimization, and proper regulation.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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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.2023.120143.

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