Journal of Water & Health

Journal of Water and Health Vol 20 No 12, 1733 doi: 10.2166/wh.2022.177

Diversity and dynamics of bacterial communities in the drinking water distribution network of a mid-sized city in Brazil

Ana Maria Moreira Batista [a,b], Juliano Curi de Siqueira [c], Paola Meynet [d], David Werner [d], Graziella Patricio Pereira Garcia [b], Russell J. Davenport [d], Alyne Duarte Pereira [b], Luciene Alves Batista Siniscalchi [c], Juliana Calábria de Araújo [b] and Cesar Rossas Mota Filho [b,*]

[a] Department of Natural Resources, Environmental Sciences and Technologies, State University of Minas Gerais (João Monlevade Unit), Brasília Avenue, 1304 – Bau, 35930-314 João Monlevade, Minas Gerais, Brazil
[b] Department of Sanitary and Environmental Engineering, Federal University of Minas Gerais, Av. Antônio Carlos, 6627, 31270-010 Belo Horizonte, Minas Gerais, Brazil
[c] Department of Environmental Engineering, Federal University of Lavras, Aquenta Sol, 37200-900, Lavras, Minas Gerais, Brazil
[d] School of Civil Engineering and Geosciences, Newcastle University, NE1 7RU Newcastle upon Tyne, UK
*Corresponding author. E-mail: cesar@desa.ufmg.br

AMMB, 0000-0002-9836-267X; JCdS, 0000-0002-6346-8917; PM, 0000-0002-2816-5902; DW, 0000-0002-6741-1256; GPPG, 0000-0002-7442-6683; RJD, 0000-0003-3272-4778; ADP, 0000-0001-9364-0573; LABS, 0000-0001-7892-0112; JCdA, 0000-0002-5497-8675; CRMF, 0000-0002-8020-3232

ABSTRACT

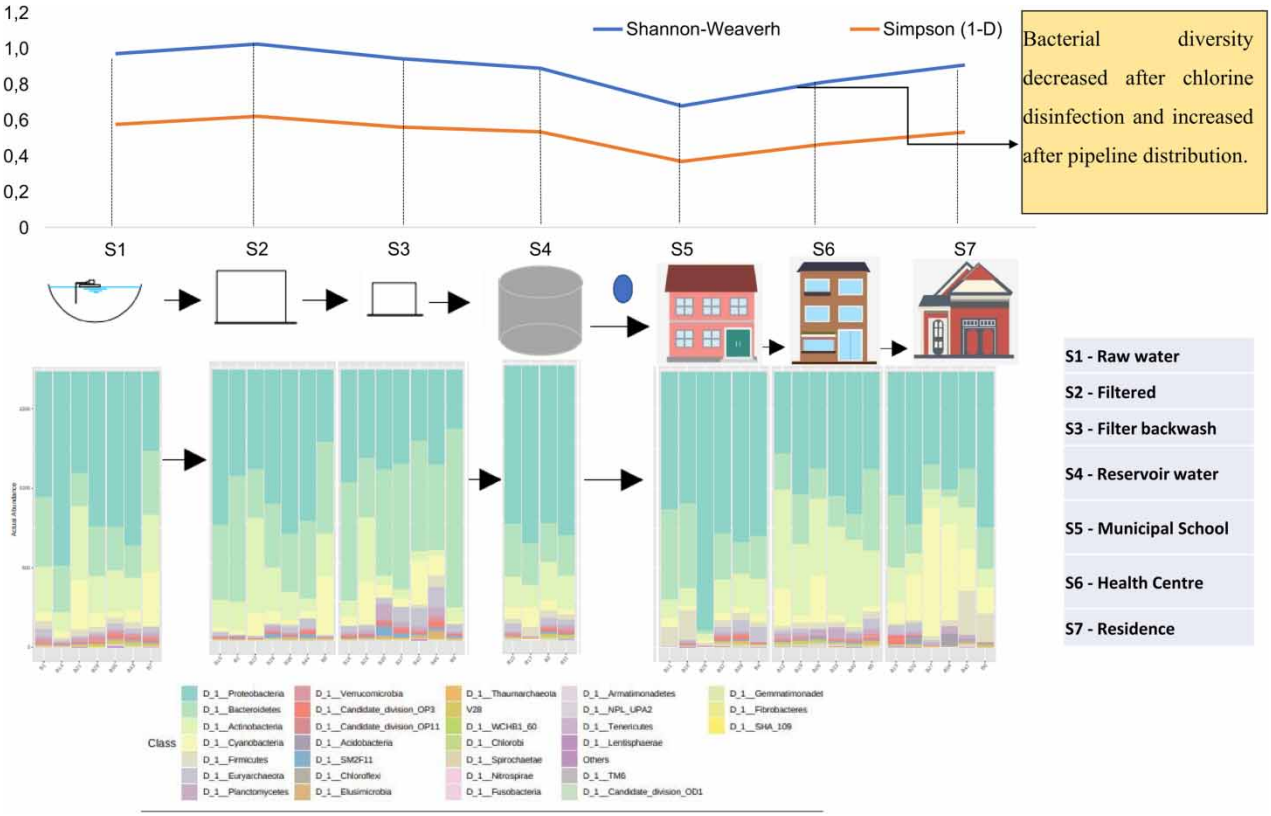
This study assessed the bacterial community composition of a drinking water system (DWS) serving a mid-sized city (120,000 inhabitants) in Brazil. Water samples, including raw and treated water, were collected at seven points throughout the DWS. DNA was extracted and analysed using high-throughput sequencing (Ion Torrent). Free chlorine and turbidity were measured *in situ*. Results showed that the highest relative abundance of 16S rRNA genes was from phyla Proteobacteria, followed by Bacteroidetes and Actinobacteria. The next most abundant phylum was Cyanobacteria, represented by Arthronema, Calothrix, and Synechococcus. An interesting observation was that the DNA-based analysis suggested a bacterial community change in the distribution network, with treated reservoir water being very different from the network samples. This suggests active microbiology within the distribution network and a tendency for bacterial diversity to decrease after chlorine disinfection but increase after pipeline distribution. In raw water, a predominance of Proteobacteria was observed with reduced Cyanobacteria, showing a negative correlation. In treated water, Proteobacteria were negatively correlated with Bacteroidetes. Finally, 16S rRNA genes from Firmicutes (especially *Staphylococcus*) had a high abundance in the chlorinated water, which may indicate the phylum's resistance to chlorine residuals. Opportunistic pathogens, e.g., *Mycobacteria*, *Legionella*, and *Staphylococcus*, were also observed.

Key words: bacterial composition, chlorination, DNA-based method, high-throughput sequencing, microbiota, water treatment

HIGHLIGHTS

- Next-generation sequencing (NGS) revealed substantial changes in microbiota from a small drinking water system (DWS) after disinfection.
- Proteobacteria, Bacteroidetes, Actinobacteria, and Cyanobacteria had high abundance.
- Firmicutes in the chlorinated water may indicate resistance to chlorine residuals.
- The chlorinated water sample presented opportunistic pathogens.
- Environmental degradation may have led to bioremediation species' growth.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Despite the Sanitation Law (11.445/2007), which obliges all Brazilian municipalities to provide water supply, sanitary sewage, urban cleaning, and solid waste management in ways appropriate to protect public health and the environment, recent data from the Institute Brazil and National Sanitation Information System (SNIS) show that, although sanitation has improved, half of Brazilians still have no access to sewerage (50.3%) and the coverage for water supply is only 83.3% (SNIS 2019). When proposing solutions that aim to comply with the Sanitation Law, managers and service providers often encounter factors that will limit the size of the system, which therefore will not include the entire population and generate social inequality in relation to the universal right to safe water and sanitation (Heller & Pádua 2016). Overcoming both non-structural and structural measures is important to realize the human right to safe water, especially among rural communities (Aleixo *et al.* 2019).

Another challenge is that the reduction in water availability in rivers and lakes has forced several water companies to use water from multiple sources to meet demand. This was the case for the city of Itabira, in the state of Minas Gerais (southeastern Brazil), which is the subject of the current study. Since 2014, the water providers for the city had to source approximately 25% of their abstraction from a river that suffers from unregulated urban development (ANA 2015; Batista *et al.* 2018).

In this context, academic research is often the only reliable source of information on the actual water quality, waterborne infectious diseases, and parasitic diseases in regions with poor enforcement of regulations (Marinho *et al.* 2016; Castro *et al.* 2020). Reports of microbial water quality during treatment and distribution are very few in Brazil (Batista *et al.* 2018), and most studies only focus on raw water quality (Köchling *et al.* 2017; Tessler *et al.* 2017). Bacterial diversity monitoring provides an additional tool in water safety assessment, especially in places where water availability is inadequate, either due to quality or quantity issues, such as is the case in most Brazilian cities (WHO 2017). Without proper maintenance, drinking water systems (DWSs) can compromise water safety after treatment and harbour risks for some waterborne diseases caused by opportunistic pathogens (Liu *et al.* 2019).

With the advent of modern molecular microbiology, more research on microbial communities in DWS has been done (Pinto *et al.* 2012, 2014; Wang *et al.* 2015; Han *et al.* 2020). Determining bacterial diversity and abundance in DWS has become an important topic for water resource management and public health. Knowing which bacterial communities are present in DWS and their response to major operational parameters can enable better water treatment, disinfection, and distribution strategies (Gomez-Alvarez *et al.* 2012; Chao *et al.* 2013; Huang *et al.* 2014; Pinto *et al.* 2014; Li *et al.* 2017; Liu *et al.* 2017a, 2017b, 2019; Han *et al.* 2020). The literature agrees that it is essential to better understand the dynamics of the DWS microbiome with the help of molecular microbiology methods, which also help identify the presence of microorganisms that pose a risk to consumers (Liu *et al.* 2017b).

In this study, water samples were repeatedly collected at different points for 6 months in a mid-sized DWS (serving a population of 120,000 inhabitants), and next-generation sequencing (NGS) was used to investigate the bacterial community composition in these water samples. The objectives of this study were to (a) assess the main bacterial taxa composition based on 16S rRNA gene sequencing; (b) investigate the relationships between the abundance of different types of bacteria and water quality parameters; and (c) determine spatiotemporal variation in bacterial diversity in the assessed DWS.

2. METHODS

2.1. Sampling

The study area covered sampling points along a DWS for the mid-sized city of Itabira, Minas Gerais, Southeast Brazil. Over the study period, the Cardiopolis and Peixe rivers supplied 75 and 25% of the water to the city, respectively. The DWS has four single layers (1.8 m thickness) – rapid, upflow filters that are backwashed every 12 h with treated water and operate with an empty bed contact time of 7 min. The pipeline material is polyvinyl chloride (PVC). Free chlorine and fluoride are added to filtered water at concentrations of 1.5 mg Cl_2 L^{-1} and 1.0 mg F L^{-1}, respectively, with a contact time of approximately 30 min. Sampling points along the DWS included the inlet to the water treatment plant or (S1) raw water; (S2) filtered water; (S3) filter backwash; (S4) reservoir water (final treated and chlorinated water); and water from three points along the distribution network: one located on the lowest point in the DWS (near a municipal school, S5), one in an intermediate location (near a health centre, S6), and one far away from the water treatment plant (near a residence, S7; Figure 1). Sampling from all points was performed monthly from July to December 2015.

Samples of treated water (S4, S5, S6, and S7) were kept in sterile glass bottles containing one tablet of 10% sodium thiosulphate to remove the free chlorine. The other samples (without chlorination – S1, S2, and S3) were kept in sterile glasses without sodium thiosulphate. All samples were filtered using cellulose acetate membrane filters (GF1 filter, 0.2 μm, Macherey and Nagel), and the final volume of filtrate ranged from 1 to 4 L. The filters were preserved at −20 °C for DNA extraction. Free chlorine concentrations were measured *in situ* using a 21055-colorimeter pocket Hach kit (Hach Lange, UK). Turbidity

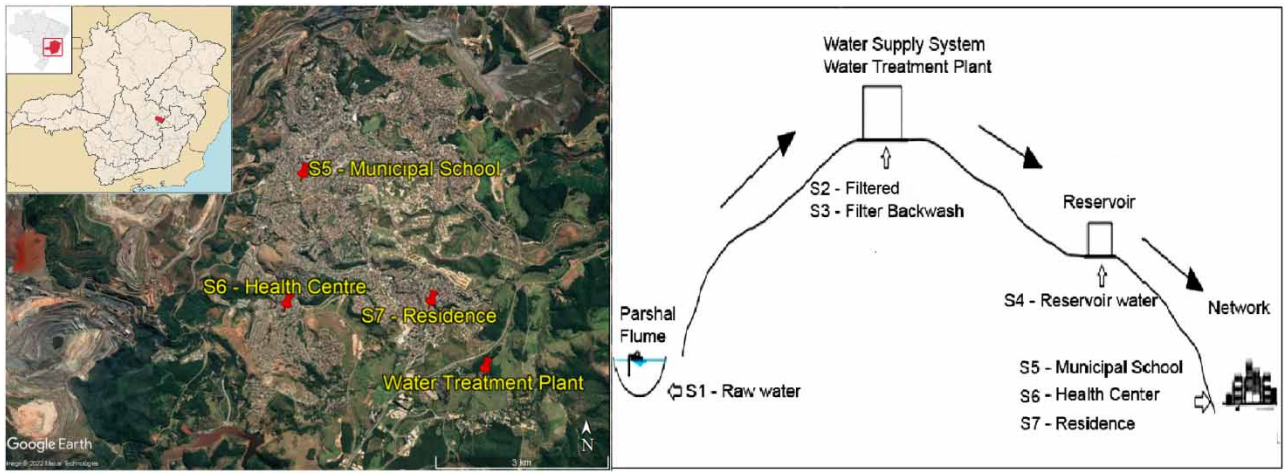


Figure 1 | Illustration of the sampling points (S1–S7) on the DWS; raw water (S1), filtered (S2), filter backwash (S3), reservoir water (S4), municipal school (S5 – 19°38′2.83″S 43°13′50.16″W), health centre (S6 – 19°39′15.61″S 43°13′59.86″W), and residence (S7 – 19°39′14.12″S 43°12′34.11″W). Modified from information provided by the National Mining Agency.

was measured using a 2100Q portable turbidity meter. In addition, 100 mL of each sample was analysed for coliforms and *Escherichia coli* using the ONPG-MUG (Colilert®) method.

2.2. DNA extraction and polymerase chain reaction amplification

DNA was extracted from membrane filters using a FastDNA Spin Kit for Soil (Qbiogene) according to the manufacturer's instructions. The concentration and purity of the DNA extracts were determined using a Nanodrop 1000 spectrophotometer (Thermo Scientific). Amplicon libraries were generated from each DNA sample by direct polymerase chain reaction (PCR) amplification, using the universal primers set (515F and 926R), targeting the V4 and most of the V5 region of the 16S rRNA gene, containing the Ion adapters A (50-CCATCTCATCCCTGCGTGTCTCCGACTCAG-30) and trP1 (50-CCTCTCTATGGG-CAGTCGGTGAT-30) for forward and reverse primers, respectively (Caporaso *et al.* 2011; Quince *et al.* 2011; Parada *et al.* 2016). Samples were differentiated by adding unique 12 base pair barcodes to the forward primer through a 'GAT' space.

The PCR was carried out using the FastStart High Fidelity PCR System and the PCR Nucleotide Mix (Roche Diagnostics GmbH, Mannheim, Germany). This kit contains NHþ 4 buffer, $MgCl_2$, dNTPs, and DNA polymerase. For the PCR, 1 μL of the 1:10 dilution for each of the DNA extract (a least 10 ng mL^{-1} of DNA template) was added to a mixture containing 5 μL FastStart High Fidelity Reaction Buffer, 1 μL dNTP, 1 μL of each primer (final primer concentration in 1× reaction: 0.2 μM), 0.5 μL of enzyme blend, and 40.5 μL of nuclease-free water to obtain a final reaction volume of 50 μL. The following PCR programme was used: initial denaturation at 95 °C for 4 min, followed by 25 cycles of 95 °C for 1 min, 55 °C (annealing) for 45 s, 72 °C (extension) for 1 min, and final elongation of 72 °C for 7 min. PCR products were checked by electrophoresis running at 100 V for 45 min using 5 μL of product/extract plus loading buffer on 2% agarose gels containing Nancy-520 DNA Gel Stain (Sigma-Aldrich) and in 1× Tris-acetate–EDTA buffer. Gels were visualized by UV illumination using a Bio-Rad Fluor-S Multi Imager (Bio-Rad, UK). Purification of PCR products was performed using the Agencourt AMPure XP PCR Purification system, which employs Agencourt's solid-phase paramagnetic bead technology for high-throughput purification of PCR amplicons. The resulting purified PCR product was used directly in the NGS workflow.

2.3. DNA library prepared for Ion Torrent sequencing

The individual amplicon libraries were quantified using a Qubit 2.0 Fluorometer (Invitrogen), a Qubit dsDNA HS reagent Assay kit, and Qubit™ assay tubes. A Qubit working solution was prepared for all samples by diluting the Qubit dsDNA HS reagent 1:200 in Qubit dsDNA HS buffer. Each sample tube required 199 μL of Qubit working solution and a 1 μL sample. The amplicon libraries were pooled in equimolar quantities into a unique solution for the downstream template preparation procedure for clonal amplification on Ion Spheres. The diluted library was freshly prepared before being used on the Ion One-Touch2 System.

2.4. Analysis of the bacterial communities by 16S rRNA gene amplicon sequencing (Ion Torrent platform)

Raw sequences generated by the Ion Torrent PGM were analysed using the QIIME (v 1.7.0) bioinformatics pipeline (Caporaso *et al.* 2010), and sequencing data were analysed using the SILVA ribosomal RNA gene database project (Quast *et al.* 2013). The final results were expressed as relative abundance (%) and were grouped into operational taxonomic units (OTUs) with 97% similarity.

Alpha- and beta-diversity indices were calculated using the R platform (R Development Core Team 2011) with the Phyloseq (McMurdie & Holmes 2013) and Vegan packages (Oksanen *et al.* 2019). A heatmap was constructed with the most abundant taxa identified from the sequencing data (≥1% in at least one of the samples). Resampling of the sequences was performed to calculate the diversity indices, so that all samples had the same number of sequences as the one with the lowest number of recovered sequences (October sample, S5 – 1,735 sequences).

A phylum-level heatmap was constructed to identify the most abundant phyla and cluster analysis was performed based on Ward's method and Euclidean distance measure using the software Microbiome Analyst (Dhariwal *et al.* 2017; Chong *et al.* 2020). The community profile was also analysed through core microbial composition (core microbiome analysis) to identify the main taxa present in the different sample groups based on their prevalence and relative abundance. Graphs with the relative abundances of the main phyla and genera were constructed using the same software.

2.5. Statistical methods

The similarities of the relative abundance of the OTUs detected from different sampling points were calculated by cluster analysis using Euclidean Distance based on the diversity of the more abundant orders (Proteobacteria, Actinobacteria, and Bacteroidetes) considering relative abundance values above 0.01%.

For the sampled variables from S1 to S7 (chlorine, turbidity, number of OTUs, and taxonomic phyla), Pearson's and Spearman's correlation coefficients were calculated for all sampled points (total correlation), and between points from S1 to S3 (raw water, excluding chlorine data) and S4 to S7 (treated water, excluding turbidity data). The correlation was calculated within the R programming environment (R Development Core Team 2011) from the algorithms of the 'Openair' (Carlaw & Ropkins 2012) package. The value of 70% was considered representative in the correlation matrices if the statistical significance was established ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Dynamics and diversity of bacterial communities

Sequencing on the Ion Torrent platform generated a minimum of 1,735 to a maximum of 24,367 reads per sample. Table 1 summarizes the Shannon–Weaver index, Simpson's diversity index, dominance, and the number of reads and OTUs for each sample for the monitoring period. The rarefaction curves tended towards a plateau, indicating sufficient sample coverage (Supplementary Fig. A.1).

Increasing bacterial diversity was observed at the final points of water distribution (S5–S7) and in treated water (S3–S5), as shown in Table 1 and Supplementary Table A.1.

In general, the four most abundant phyla in all samples were Proteobacteria, Bacteroidetes, Actinobacteria, and Cyanobacteria (Supplementary Table A.2). Other bacteria are the species with very low total abundance (<0.01%) in the samples for each OTU (Supplementary Table A.3). When we compared the prevalence of phyla for each sample, the same phyla were detected (Figure 2).

The presence of Cyanobacteria among the most abundant phyla (as shown in Figure 2) in the DWS after coagulation and flocculation steps could imply a risk to water quality, since Cyanobacteria continued to produce metabolites that may impact water quality, such as microcystin and geosmin, for approximately 10 days in sludge (Pestana *et al.* 2016). In the case study area, the supernatant from the sludge treatment facility is returned to the stream, and from there to the inlet of the plant. Cyanobacterial 16S rRNA genes affiliated with *Synechococcus*, *Arthronema*, and *Calothrix* were detected (Figure 3). This is of concern because the water treatment train does not have a final treatment for the removal of cyanotoxins such as activated carbon.

Proteobacteria, Actinobacteria, and Bacteroidetes are commonly detected in freshwater environments (Berg *et al.* 2009; Goecke *et al.* 2013); however, inside a DWS, which is an oligotrophic environment, these microorganisms have less nutrition

Table 1 | Sample description, Ion Torrent sequencing data, and diversity indices

Samples	Sequences[a]	OTUs[b]	Dominance[c]	Shannon–Weaver[d]	Simpson[e]	Evenness[f]
S1 – Raw water	58,725	30,021	0.4259	0.9689	0.5741	0.8784
S2 – Filtered	122,970	65,697	0.3807	1.023	0.6193	0.9271
S3 – Filter backwash	119,933	57,925	0.4402	0.9427	0.5598	0.8556
S4 – Reservoir water	53,997	33,761	0.4669	0.8874	0.5331	0.8096
S5 – Municipal school	78,323	41,042	0.6328	0.6776	0.3672	0.6564
S6 – Health centre	78,557	38,340	0.5362	0.8103	0.4638	0.7495
S7 – Residence	100,443	52,559	0.4693	0.9067	0.5307	0.8254

[a]Number of short-read sequences.
[b]Number of OTUs.
[c]Simpson's dominance (*D*);
[d]Shannon–Wiener's diversity (*H'*).
[e]Simpson's diversity (1 – *D*).
[f]Pielou's evennesss (*J*).

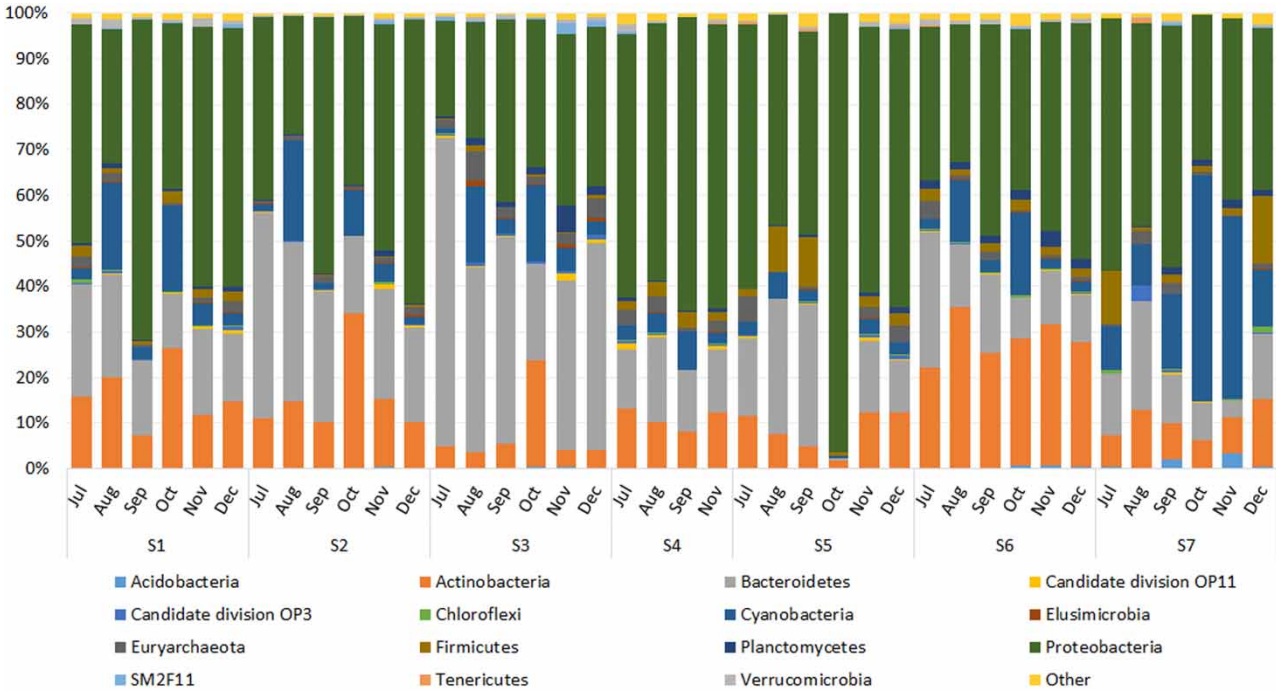


Figure 2 | Relative abundance (%) of the main phyla for each sampling point in the assessed DWS.

and are expected to be attached to the surfaces of the pipe and other particles forming a biofilm (Henne *et al.* 2012). Although the samples investigated in this study are not from biofilms, they may have contained bacteria detached from biofilms.

At all times, α-Proteobacteria followed by the β-, γ-, and δ-subdivisions represented the majority of sequences in raw water (S1) and distribution network samples from the municipal school (S5) and health centre (S6). In contrast, Bacteroidetes were more abundant in the filtered (S3) and reservoir water samples (S4). Actinobacteria were most abundant in the filter

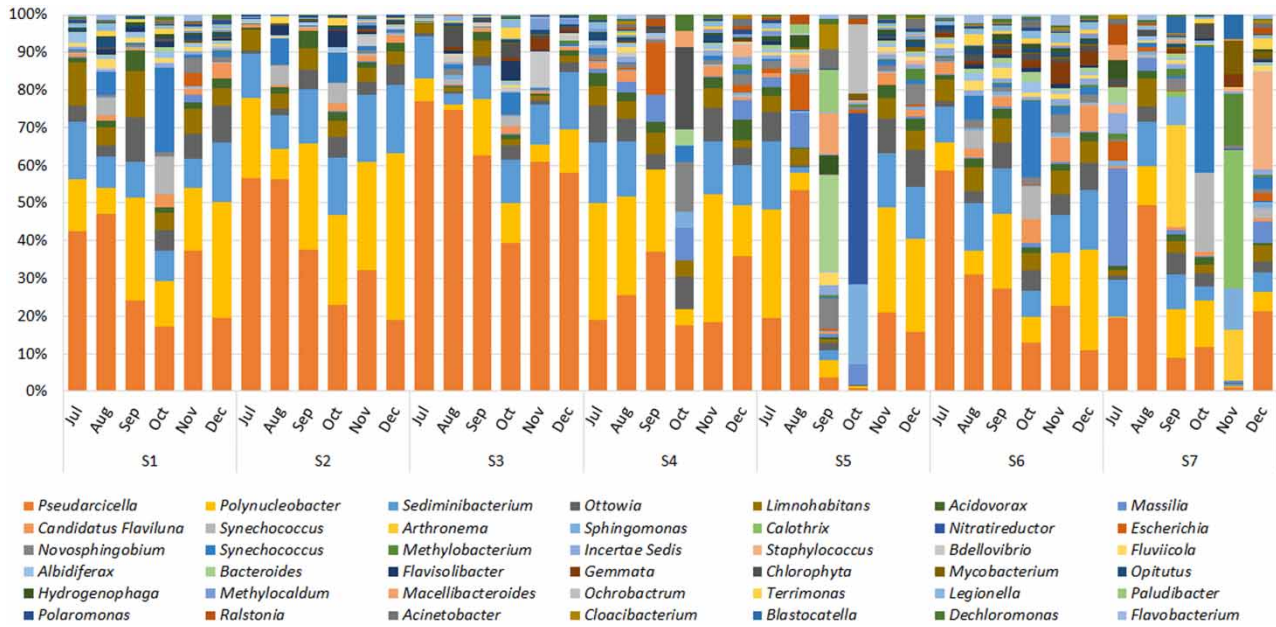


Figure 3 | Relative abundance (%) of the main genus for each sampling point in the assessed DWS.

backwash (S3) and filtered water samples (S2). Cyanobacteria and Proteobacteria were at all times the most abundant phyla at the residence sampling point (S7) situated at the end of the distribution network.

Studies of water distribution systems reveal microbial communities dominated by the phyla Proteobacteria (i.e., α-, β -, and γ-Proteobacteria), Firmicutes, Nitrospirae, and Actinobacteria (Gomez-Alvarez *et al.* 2012; Chao *et al.* 2013; Huang *et al.* 2014; Pinto *et al.* 2014; Wang *et al.* 2014). Proteobacteria were also found to be dominant in raw water in the study of Zhu *et al.* (2020). Moreover, the authors observed that Actinobacteria was the most abundant class in treated water (after chlorination), suggesting the potential chlorine resistance of this bacterial group (Zhu *et al.* 2020). Hou *et al.* (2018) detected the groups Actinobacteria, Proteobacteria, and Firmicutes during the water treatment process. On the contrary, in treated water, the proportion of Actinobacteria decreased, whereas that of Proteobacteria and Firmicutes increased and predominated.

At the class level, β-Proteobacteria (*Albidiferax*, *Dechloromonas*, *Polynucleobacter*, *Ottowia*, *Ralstonia*, *Acidovorax*, *Polaromonas*, and *Hydrogenophaga*), which include ammonia-oxidizing and arsenic-resistant soil bacteria such as *Polynucleobacter* (Heckmann & Schmidt 1987), are frequently an abundant fraction of freshwater bacterioplankton. *Polynucleobacter* has some species that are living in association with ciliates as an obligate symbiont, namely *Polynucleobacter necessarius* (Newton *et al.* 2011).

The clade α-Proteobacteria contains the genera *Nitratireductor*, *Novosphingobium*, *Ochrobactrum*, and *Sphingomonas* and was found in the DWS (Figure 4).

There was an increase in the relative abundance of versatile degraders of complex organic substrates such as autotrophic perchlorate-degrading bacteria (*Dechloromonas*) – Figure 3 (Nov-15 sample) – in S5 and S7. The anthropogenic impacts such as fishing, lack of riparian forests, loss of habitats, and agricultural activities all have contributed in recent years to the environmental degradation in this river (Carneiro *et al.* 2019).

Sha *et al.* (2020) also observed an increase in *taxa* from the Betaproteobacteria as well as Bacteroidetes, Actinobacteria, and Saccharibacteria in microbial communities exposed to a high concentration of perchlorate. In contrast, there was a decrease in the relative abundance of *taxa* such as *Chloroflexi*, *Verrucomicrobia*, and Gammaproteobacteria (Sha *et al.* 2020). The regrowth of this genus *Dechloromonas* in DWS points to a possible selection of species more resistant to chlorination. Wang *et al.* (2013) warn that breakdown of bacterial cells by chlorination releases precursors for the formation of disinfection by-products (trihalomethanes, haloacetonitriles, chloral hydrate, chloropicrin, and 1,1,1-trichloro-2-propanone).

Pinto *et al.* (2012) observed that Alphaproteobacteria increased from approximately 6% in the source water to 38% in the filter and 23% in the DWS. Conversely, the relative abundance of Betaproteobacteria ranged from 34% in the source water to 43% in the DWS.

The predominant OTUs (>0.01% relative abundance) at the genus level were *Pseudarcicella*, *Polynucleobacter*, *Sediminibacterium*, and *Candidatus-Panktophila* in S1–S4, corresponding to the raw water until the end of the treatment. From the beginning of the water distribution network (S5), there was a change in the composition of the bacterial communities in relation to the OTUs observed between the months; except for S6, where the same OTUs were always present, i.e., *Pseudarcicella*, *Polynocleobacter*, *Sediminibacterium*, and *Candidatus* (Figure 3). These *taxa* with tolerance for a broad pH spectrum further emphasize differences in habitat-specific adaptations. For example, *Sediminibacterium* (family Chitinophagaceae, phylum Bacteroidetes) was first described by Qu & Yuan (2008). This genus has only two species that were isolated from reservoir sediment samples, *Sediminibacterium salmoneum*, and from soil, *Sediminibacterium ginsengisoli* (Kim *et al.* 2013).

At S5, there was a significant change in the composition of the community with a greater presence of the genera *Novosphingobium*, *Synechococcus*, *Staphylococcus* (October sample, S5) and *Burkholderia* followed by *Candidatus-Flaviluna*, *Sphingomonas*, and *Calothrix* (October sample, S5). The same phenomenon was observed in S7, with notable occurrence in September and November (S7) for the OTUs *Arthronema*, *Sphingomonas*, and *Calothrix*, and from the phylum Firmicutes the genera *Chlorobi*, *Spirochaetae*, *Chloroflexi*, and *Nitrospire* (Figure 4).

3.2. Bacterial community composition

Bacterial diversity decreased after disinfection with chlorine (S4) but increased along the distribution networks (S5–S7, Supplementary Fig. A.1), where bacteria might include the microbial community of the filter (Pinto *et al.* 2012), loose deposits, and detachment of pipeline biofilms (Liu *et al.* 2017a, 2017b).

Active microbiology within the distribution network has been linked to the dispersal of biofilm-associated microbes during passage through the water distribution pipes (Huang *et al.* 2014), in which the material of the pipes may have influenced. The

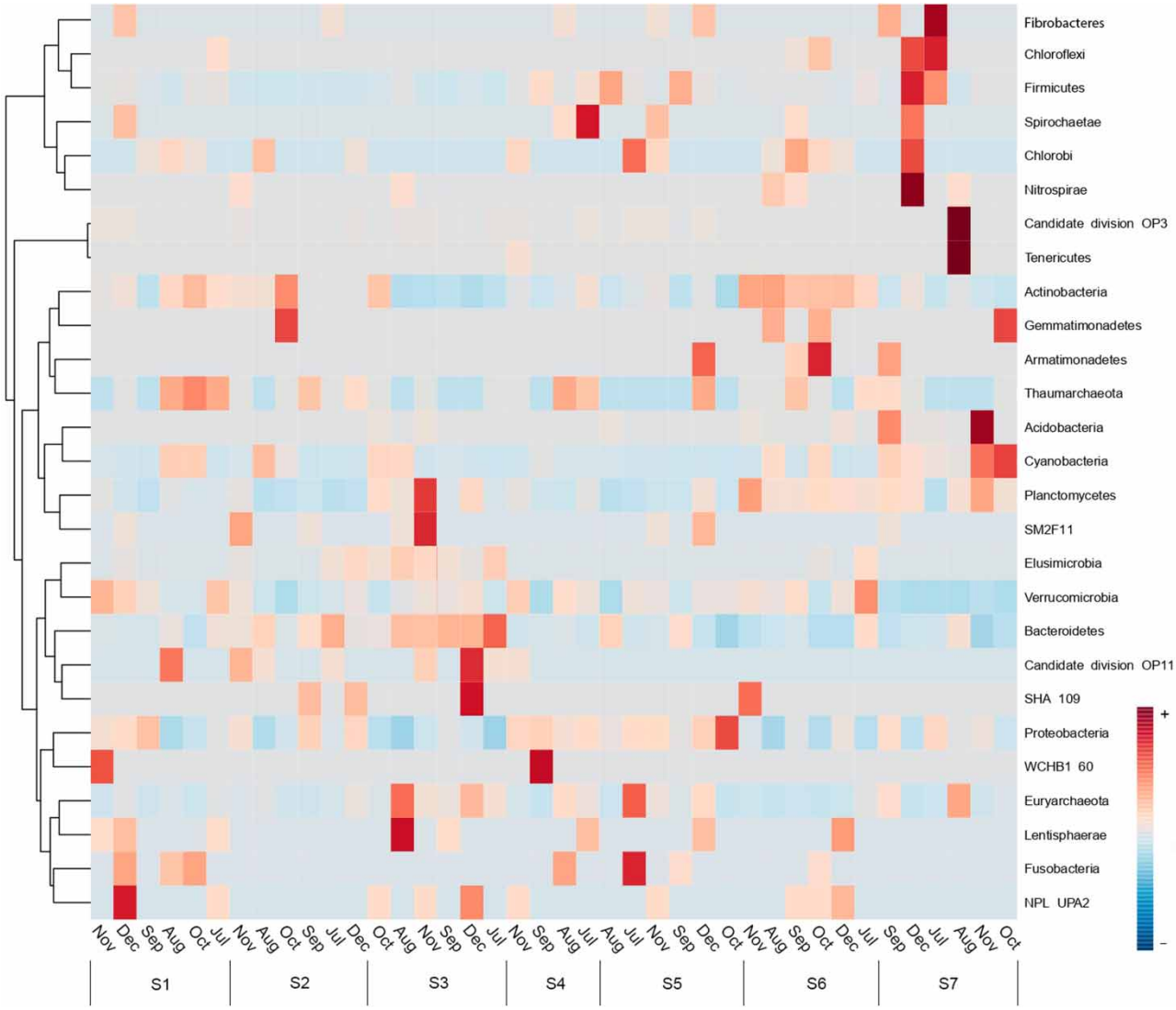


Figure 4 | Heatmap with major clades found for all sampling points in the assessed DWS.

PVC material of pipes can serve as a support for microorganisms, in addition to serving as a carbon source. This condition is aggravated by the age and maintenance of the pipes, which is quite deficient in developing countries, increasing the possibility of pipe fractures and adverse pressure events in the DWS (Liu *et al.* 2017b).

Deterioration of water quality along the network can be caused by long retention times or 'water age', as observed worldwide (Liu *et al.* 2017b). Long retention times have been associated with microbial communities dominated by the phyla Proteobacteria, Actinobacteria, and Bacteroidetes (Gomez-Alvarez *et al.* 2012; Chao *et al.* 2013; Huang *et al.* 2014), regardless of the water treatment plant and disinfection type (Table 2). In addition, because removing all microorganisms from source water is impossible, microbial regrowth may occur in DWSs (Reynolds *et al.* 2008), even after the advanced treatment processes (Cui *et al.* 2020).

Disinfection could select resistant bacterial populations. Differential resistance of drinking water bacterial communities was observed when *Legionella*, *Escherichia*, *Geobacter* (in a lab-scale system), and *Mycobacterium*, *Sphingomonas,* and *Coxiella* (in a full-scale system) increased in their relative abundance during monochloramine treatment (Chao *et al.* 2013). Furthermore, the detachment of biofilms from pipes promoted by disinfectants can affect general bacterial resistance to antibiotics in tap water (Zhang *et al.* 2018).

Table 2 | Community patterns observed in different studies of water distribution systems

Treatment processes	Disinfection	Main phyla	Platform	Reference
Coagulation, flocculation with pH adjustment followed by sand filtration	Free chlorine	Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria	Ion Torrent	Present study
Lime-softening compounds, sedimentation, ozonation, and dual medium filtration	Free chlorine and ammonia to produce chloramine prior to distribution	Proteobacteria (Betaproteobacteria and Alphaproteobacteria)	454 GS-FLX	Pinto *et al.* (2014)
Flocculation and settling with pH adjustment followed by sand filtration and granular activated carbon	Free chlorine	Proteobacteria, Firmicutes, Nitrospirae, and Actinobacteria	GS-FLX Titanium	Gomez-Alvarez *et al.* (2012)
Flocculation, sedimentation, and sand filtration	Free chlorine	Proteobacteria (Beta, Gamma, and Alpha)	HTS	Chao *et al.* (2013)
Sand filtration	Free chlorine	Proteobacteria, Firmicutes, Nitrospirae, and Actinobacteria	Illumina MiSeq	Huang *et al.* (2014)
Biofiltration	Free chlorine	Acidobacteria and Gemmatimonadetes	454 FLX Titanium	El-Chakhtoura *et al.* (2015)
Preozonation, flocculation, sedimentation, sand filtration, post-ozonation and biological activated carbon filtration	Free chlorine	Proteobacteria and Bacteroidetes	Illumina MiSeq	Li *et al.* (2017)
Aeration, rapid sand filtration, softening, and activated carbon filtration	UV	Proteobacteria (Beta) and Bacteroidetes	Illumina MiSeq	Liu *et al.* (2017a)
Coagulation, sedimentation, dual-media filtration	Free chlorine	Actinobacteria, Proteobacteria, and Firmicutes	Illumina MiSeq	Hou *et al.* (2018)
Annular reactors (ARs, Model 1320LJ, BioSurface Technologies Co., USA), inoculated with groundwater, simulating the DWS	UV and free chlorine	Opportunistic pathogenic bacteria from Proteobacteria and Bacteroidetes	Illumina MiSeq	Liu *et al.* (2019)
Flocculation, coagulation, sedimentation, and filtration	Chlorine	Proteobacteria	Illumina MiSeq	Han *et al.* (2020)
Annular reactors (ARs, Model 1320LJ, BioSurface Technologies Co., USA), Cl_2 disinfection	No. of combinations of UV, free chlorine, and NaClO	Proteobacteria, Planctomycitecia, and Sphingobacteria	GS-FLX Titanium	Zhu *et al.* (2020)
Pre-ozone, coagulation, sedimentation, and sand filtration	Ozone-biological activated carbon (O_3-BAC)	Proteobacteria (*Pseudomonas* spp.)	Ion S5™ XL	Bian *et al.* (2021)
The facility operates two trains of water treatment processes in parallel (Train I uses horizontal flow sedimentation and a V-filter, while Train II uses Multiflo-sedimentation and a TGV filter). Both trains contain coagulation–sedimentation, filtration, and O_3-BAC	Chlorine disinfection	Proteobacteria, Actinobacteria, Cyanobacteria, Firmicute, Bacteroides, Acidobacteria, Planctomycete, and Patescibacteria	Illumina MiSeq	Tang *et al.* (2022)

Enterobacteriales (*Escherichia*) were detected via NGS analyses in smaller quantities at several points inside the DWS, e.g., at the health centre (S6, August), municipal school (S5, September and December), residence (S7, July and November), and in raw water (S1, November) – Figure 3 – which could indicate some faecal contamination entering the DWS. However,

the routine method ONPG-MUG (Colilert®) detected *E. coli* only in the non-treated samples (S1–S3) (Supplementary Table A.4). The regrowth of these microorganisms due to inadequate operational practices can affect the microbiological safety of the DWS. We also observed that some bacterial genera containing opportunistic pathogens, such as *Legionella*, *Klebsiella*, and *Shigella* showed a higher abundance in treated water than in raw water (Figure 3, and more details in Batista *et al.* 2018). Liu *et al.* (2017a) observed that loose deposits in PVC pipes constitute a hotspot for adenosine triphosphate (ATP) and bacteria from the genera *Aeromonas*, *Mycobacterium*, and *Legionella*. Opportunistic pathogens can be very resistant to the disinfection residuals in drinking water when adhered to biofilm, corrosion products, and loose deposits, which may render them tolerant to disinfection with UV and chlorine (Liu *et al.* 2019).

Of every 100 L of water collected and treated in Brazil, only 63 L are consumed. In addition, 37% of water in the country is lost because of leaks, theft or clandestine connections, lack of measurement, or incorrect measurements of water

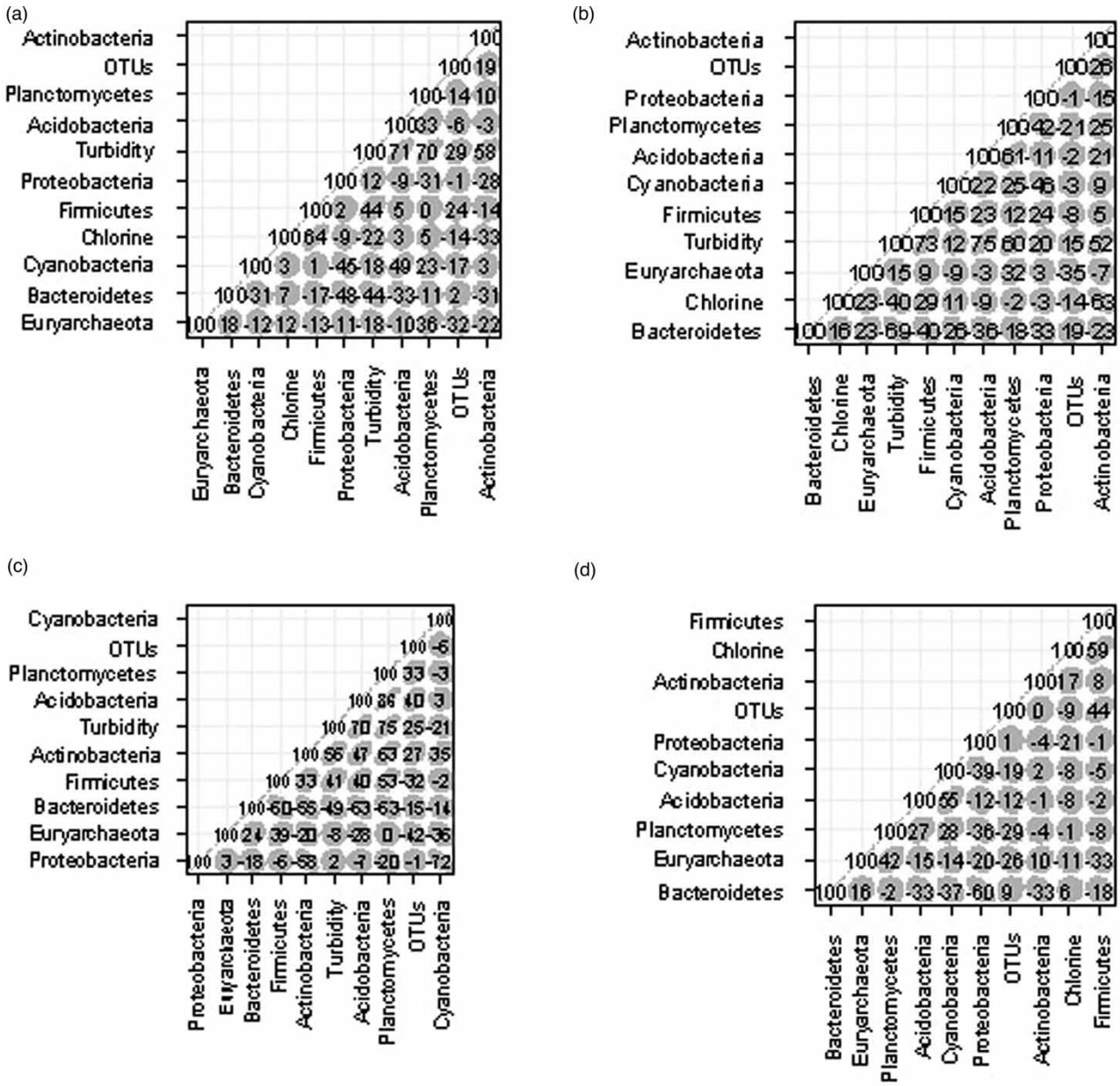


Figure 5 | (a) Pearson's correlation for all sampling points (S1–S7); (b) Spearman's correlation for all sampling points (S1–S7); (c) Pearson's correlation for all sampling points (S1–S3); and (d) Pearson's correlation for all sampling points (S4–S7).

consumption. In the state of Minas Gerais, the loss is 36% (SNIS 2020). This situation favours the introduction of contaminants external to the distribution system, such as faecal contaminants (LeChevallier *et al.* 2003).

3.3. Association between chorine and turbidity versus OTUs detected

Pearson's correlation matrices for the raw (S1–S3) and treated water sample points (S4–S7) are presented in Figure 5. For total correlation (S1–S7), it was observed that turbidity showed a high correlation with Acidobacteria (70%, p-value = 0.000591) and with Planctomycetes (71%, p-value = 0.0007769). Acidobacteria is prevalent in soils and has been linked to resistance to pollutants such as petroleum derivatives and nitrophenols. This resistance, in general, has been attributed to their exopolysaccharide (EPS) production capacity (Kielak *et al.* 2016). Thus, it is suggested that suspended particles that contribute to turbidity may carry microorganisms from this phylum and Planctomycetes since EPS facilitates the formation of aggregates. Li *et al.* (2017) evaluated a water treatment plant in China and observed that Planctomycetes outcompete Actinobacteria and Verrucomicrobia when turbidity is quite high.

When only samples S1–S3 (raw water) were considered, in addition to the high correlation of turbidity with the phyla Planctomycetes (75%, p-value = 0.0008502) and Acidobacteria (70%, p-value = 0.002742), Acidobacteria and Planctomycetes correlated positively (86%, p-value = 0.0007602). It should also be noted that the increase in Proteobacteria implied a reduction in Cyanobacteria, with a negative correlation of 72% (p-value = 0.0007655). Li *et al.* (2019) also showed a strong negative correlation between Cyanobacteria and Proteobacteria.

High correlations were not observed in the samples from S4 to S7 (treated water); therefore, the correlation matrix of 50% was considered. Thus, it was observed that the increase in Cyanobacteria implies an increase in Acidobacteria ($r = 55$, p-value = 0.005745). The rise in Proteobacteria was negatively correlated by 60% (p-value = 0.001924) with Bacteroidetes. Finally, Firmicutes seem to better tolerate the increase in chlorine concentrations since the increase in chlorine implied a rise in this phylum ($r = 59\%$, p-value = 0.002281). A significant increase of the relative abundance after chlorination in the DWS, mainly in the residence point (S7) (Figures 2 and 5) at the end of the DWS, was observed for Firmicutes (*Staphylococcus*), which tend to predominate in chlorinated and unchlorinated biofilms (Mi *et al.* 2015), and together with Proteobacteria can predominate in treated water (Hou *et al.* 2018). Several studies on DWS observed that disinfection with chlorine promoted the predominance of Firmicutes (Hou *et al.* 2018; Zhang *et al.* 2019), and disinfection strategy could have a strong influence on the abundance of Firmicutes in the bacterial community present within these systems (Mi *et al.* 2015).

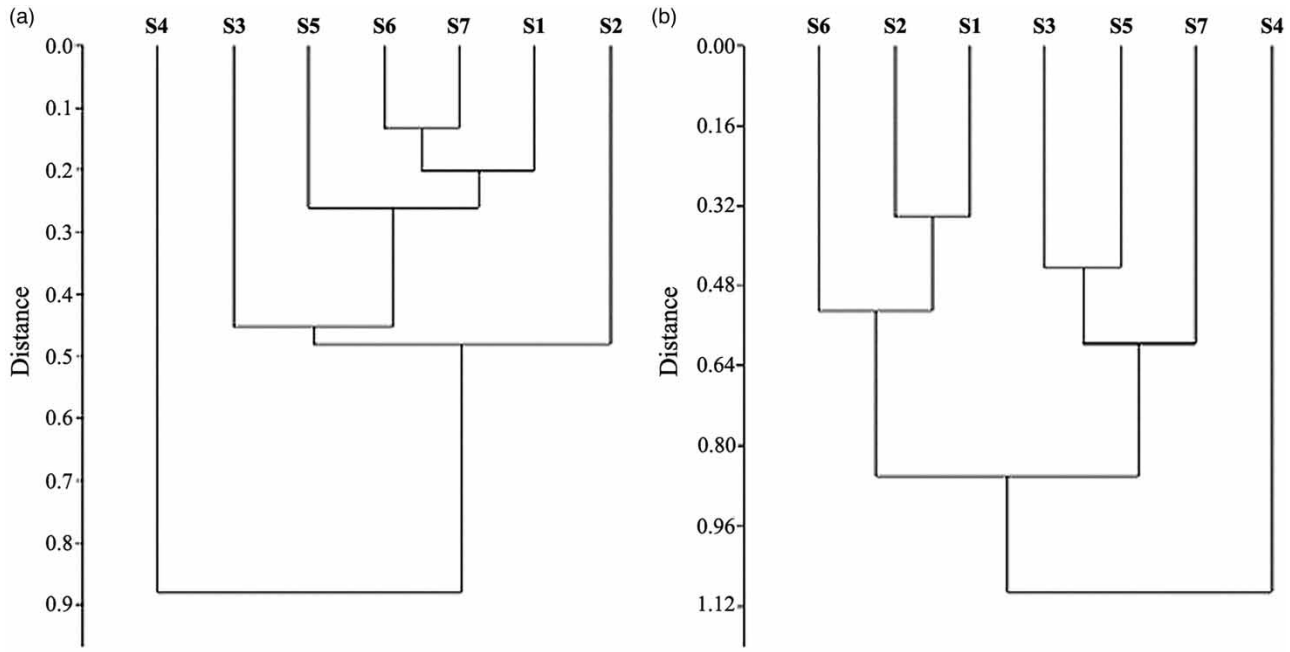


Figure 6 | Cluster analysis from (a) dry and (b) wet seasons to the monitoring points (distance represents similarity between the samples).

3.4. Seasonal distribution of the microbiota

Cluster analyses showed in the dry season (Figure 6(a)) that the relative abundance of some samples from network distribution (S6 and S7) was similar to the relative abundance detected in raw water (S1). However, a more significant similarity was observed in the wet season (Figure 6(b)) between the samples of the backwash filter (S3) and some points of the distribution network (S5 and S7).

After the rains began (in mid-October 2015), there was an apparent change in microbial structure dynamics, which was determined more by backwash (S3) than by raw water (S1). With precipitation, it is expected to increase the suspended solid concentrations. So, there is a natural increase in the load of microorganisms adhered to these particles that tend to be carried to the filters and consequently colonize the sludge filters, since this could be an ecological survival strategy for bacterial communities in DWSs (Pinto *et al.* 2012). As a result, the recirculation of this water with a higher load of particles and microorganisms will influence the quality of the water treated and be distributed to the population. Water sampling at the tap water of the treatment outlet (S4) presented the greatest dissimilarity in relation to the relative abundance of the orders observed in the other points, both in dry and wet seasons, therefore indicating that there is a spatial variation of the community dynamics after the treatment.

4. CONCLUSIONS

This study investigated the bacterial community of water samples collected in the DWS for 6 months in Itabira, Brazil, based on the Ion Torrent sequence analysis of 16S rRNA gene amplicons. Predominant phyla and changes in microbial dynamics over space and time were identified from raw water to supplied water samples.

The most abundant phyla detected were Proteobacteria, Bacteroidetes, and Actinobacteria, which are usually reported in water samples, especially in samples of freshwater environments. However, we observe a change in the microbial community structure in the assessed DWS after disinfection, with a dominance of resistance taxa (Firmicutes), versatile degraders of complex organic matter, and some opportunistic pathogens. Moreover, Cyanobacteria have also been detected and pose a potential health risk, since no cyanotoxin removal technology is used in the DWS.

ACKNOWLEDGEMENTS

The authors acknowledge the support from FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), Brazil, and the Project Global Innovation Partnership to Investigate, Restore, and Protect the Urban Water Environment, funded by the British Council.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

Aleixo, B., Pena, J. L., Heller, L. & Rezende, S. 2019 Infrastructure is a necessary but insufficient condition to eliminate inequalities in access to water: research of a rural community intervention in Northeast Brazil. *Science of the Total Environment* **652**, 1445–1455. doi:10.1016/j.scitotenv.2018.10.202.

ANA – Agência Nacional de Águas e Saneamento Básico (Brazil). 2015 *Conjuntura dos recursos hídricos. No Brasil. 2015 Report for Situation of Water Resources, Brasília*, p. 163. Available from: https://arquivos.ana.gov.br/institucional/sge/CEDOC/Catalogo/2015/ConjunturaDosRecursosHidricosNoBrasil2015.pdf (accessed 5 January 2022).

Batista, A. M. M., Meynet, P., Garcia, G. P. P., Costa, S. A. V., Araujo, J. C., Davenport, R. J. & Mota Filho, C. R. 2018 Microbiological safety of a small water distribution system: evaluating potentially pathogenic bacteria using advanced sequencing techniques. *Water Supply* **18** (2), 391–398. doi:10.2166/ws.2017.091.

Berg, K. A., Lyra, C., Sivonen, K., Paulin, L., Suomalainen, S., Tuomi, P. & Rapala, J. 2009 High diversity of cultivable heterotrophic bacteria in association with cyanobacterial water blooms. *The ISME Journal* **3** (3), 314–325. doi:10.1038/ismej.2008.110.

Bian, K., Wang, C., Jia, S., Shi, P., Zhang, H., Ye, L., Zhou, Q. & Li, A. 2021 Spatial dynamics of bacterial community in chlorinated drinking water distribution systems supplied with two treatment plants: an integral study of free-living and particle-associated bacteria. *Environment International* **154**, 106552. doi:10.1016/j.envint.2021.106552.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K. & Knight, R. 2010 QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7** (5), 335–336. doi:10.1038%2Fnmeth.f.303.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N. & Knight, R. 2011 Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *PNAS* **108** (S1), 4516–4522. doi:10.1073/pnas.1000080107.

Carlaw, D. C. & Ropkins, K. 2012 Openair: an R package for air quality data analysis. *Environmental Modelling & Software* **27–28**, 52–61. doi:10.1016/j.envsoft.2011.09.008.

Carneiro, C. S., Pena, E. H. B., Cordeiro, J., dos Santos, C. I. F., Bernardes, C. D. & Cordeiro, J. L. 2019 Diagnóstico da degradação ambiental de um trecho do Rio do Peixe em Itabira (MG) e proposição de medidas para a sua restauração. *Research, Society and Development* **8** (3), 20. doi:10.33448/rsd-v8i3.843.

Castro, R. S. D., Cruvinel, V. R. N. & Oliveira, J. L. D. M. 2020 Correlation between drinking water quality and occurrence of diarrhea and hepatitis A in the Federal District/Brazil. *Saúde em Debate* **43** (3), 8–19. doi:10.1590/0103-11042019s301.

Chao, Y., Ma, L., Yang, Y., Ju, F., Zhang, X., Wu, W. & Zhang, T. 2013 Metagenomic analysis reveals significant changes of microbial compositions and protective functions during drinking water treatment. *Scientific Reports* **3**, 3550. doi:10.1038/srep03550.

Chong, J., Liu, P., Zhou, G. & Xia, J. 2020 Using MicrobiomeAnalyst for comprehensive statistical, functional, and meta-analysis of microbiome data. *Nature Protocols* **15**, 799–821. doi:10.1038/s41596-019-0264-1.

Cui, Q., Liu, H., Yang, H. W., Lu, Y., Chen, Z. & Hu, H. Y. 2020 Bacterial removal performance and community changes during advanced treatment process: a case study at a full-scale water reclamation plant. *Science of the Total Environment* **705**, 135811. doi:10.1016/j.scitotenv.2019.135811.

Dhariwal, A., Chong, J., Habib, S., King, I., Agellon, L. B. & Xia, J. 2017 Microbiomeanalyst – a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. *Nucleic Acids Research* **45** (W1), W180–W188. doi:10.1093/nar/gkx295.

El-Chakhtoura, J., Prest, E., Saikaly, P., van Loosdrecht, M., Hammes, F. & Vrouwenvelder, H. 2015 Dynamics of bacterial communities before and after distribution in a full-scale drinking water network. *Water Research* **74**, 180–190. doi:10.1016/j.watres.2015.02.015.

Goecke, F., Thiel, V., Wiese, J., Labes, A. & Imhoff, J. F. 2013 Algae as an important environment for bacteria – phylogenetic relationships among new bacterial species isolated from algae. *Phycologia* **52** (1), 14–24. doi:10.2216/12-24.1.

Gomez-Alvarez, V., Revetta, R. P. & Santo Domingo, J. W. 2012 Metagenomic analyses of drinking water receiving different disinfection treatments. *Applied and Environmental Microbiology* **78** (17), 6095–6102. doi:10.1128/AEM.01018-12.

Han, Z., An, W., Yang, M. & Zhang, Y. 2020 Assessing the impact of source water on tap water bacterial communities in 46 drinking water supply systems in China. *Water Research* **172**, 115469. doi:10.1016/j.watres.2020.115469.

Heckmann, K. & Schmidt, H. J. 1987 Polynucleobacter necessarius gen. nov., sp. nov., an Obligately Endosymbiotic Bacterium Living in the Cytoplasm of Euplotes aediculatus. *International Journal of Systematic and Evolutionary Microbiology* **37** (4), 456–457. doi:10.1099/00207713-37-4-456.

Heller, L. & Pádua, V. L. 2016 *Abastecimento de água para consumo humano*, 3rd edn. UFMG, Belo Horizonte, p. 870 (in Brazilian Portuguese).

Henne, K., Kahlisch, L., Brettar, I. & Hofle, M. G. 2012 Analysis of structure and composition of bacterial core communities in mature drinking water biofilms and bulk water of a citywide network in Germany. *Applied and Environmental Microbiology* **78**, 3530–3538. doi:10.1128%2FAEM.06373-11.

Hou, L., Zhou, Q., Wu, Q., Gu, Q., Sun, M. & Zhang, J. 2018 Spatiotemporal changes in bacterial community and microbial activity in a full-scale drinking water treatment plant. *Science of the Total Environment* **625**, 449–459. doi:10.1016/j.scitotenv.2017.12.301.

Huang, K., Zhang, X.-X., Shi, P., Wu, B. & Ren, H. 2014 A comprehensive insight into bacterial virulence in drinking water using 454 pyrosequencing and Illumina high-throughput sequencing. *Ecotoxicology Environmental Safety* **109**, 15–21. doi:10.1016/j.ecoenv.2014.07.029.

Kielak, A. M., Barreto, C. C., Kowalchuk, G. A., van Veen, J. A. & Kuramae, E. E. 2016 The ecology of *Acidobacteria*: moving beyond genes and genomes. *Frontiers in Microbiology* **7**, 744. doi:10.3389/fmicb.2016.00744.

Kim, Y. J., Nguyen, N. L., Weon, H. Y. & Yang, D. C. 2013 *Sediminibacterium ginsengisoli* sp. nov., isolated from soil of a ginseng field, and emended descriptions of the genus *Sediminibacterium* and of *Sediminibacterium salmoneum*. *International Journal of Systematic and Evolutionary Microbiology* **63** (3), 905–912. doi:10.1099/ijs.0.038554-0.

Köchling, T., Sanz, J. L., Galdino, L., Florencio, L. & Kato, M. T. 2017 Impact of pollution on the microbial diversity of a tropical river in an urbanized region of northeastern Brazil. *International Microbiology* **20** (1), 11–24. doi:10.2436/20.1501.01.281.

LeChevallier, M. W., Gullick, R. W., Karim, M. R., Friedman, M. & Funk, J. E. 2003 The potential for health risks from intrusion of contaminants into the distribution system from pressure transients. *Journal of Water and Health* **1** (1), 3–14. PMID: 15384268.

Li, H., Alsanea, A., Barber, M. & Goel, R. 2019 High-throughput DNA sequencing reveals the dominance of pico- and other filamentous cyanobacteria in an urban freshwater Lake. *Science of The Total Environment* **661**, 465–480. doi:10.1016/j.scitotenv.2019.01.141.

Li, Q., Yu, S., Li, L., Liu, G., Gu, Z., Liu, M. & Ren, L. 2017 Microbial communities shaped by treatment processes in a drinking water treatment plant and their contribution and threat to drinking water safety. *Frontiers in Microbiology* **8**, 2465. doi:10.3389/fmicb.2017.02465.

Liu, G., Tao, Y., Zhang, Y., Lut, M., Knibbe, W. J., van der Wielen, P. & van der Meer, W. 2017a Hotspots for selected metal elements and microbes accumulation and the corresponding water quality deterioration potential in an unchlorinated drinking water distribution system. *Water Research* **124**, 435–445. doi:10.1016/j.watres.2017.08.002.

Liu, G., Zhang, Y., Knibbe, W. J., Feng, C., Liu, W., Medema, G. & van der Meer, W. 2017b Potential impacts of changing supply-water quality on drinking water distribution: a review. *Water Research* **116**, 135–148. doi:10.1016/j.watres.2017.03.031.

Liu, L., Xing, X., Hu, C., Wang, H. & Lyu, L. 2019 Effect of sequential UV/free chlorine disinfection on opportunistic pathogens and microbial community structure in simulated drinking water distribution systems. *Chemosphere* **219**, 971–980. doi:10.1016/j.chemosphere.2018.12.067.

Marinho, J. S., Jesus, I. M. D., Asmus, C. I. R. F., Lima, M. D. O. & Oliveira, D. C. 2016 Waterborne infectious and parasitic diseases and respiratory diseases in an industrial area, Northern Brazil. *Cadernos Saúde Coletiva* **24** (4), 443–451. doi:10.1590/1414-462X201600040120.

McMurdie, P. J. & Holmes, S. 2013 Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **8** (4), e61217. doi:10.1371/journal.pone.0061217.

Mi, Z., Dai, Y., Xie, S., Chen, C. & Zhang, X. 2015 Impact of disinfection on drinking water biofilm bacterial community. *Journal of Environmental Sciences* **37**, 200–205. doi:10.1016/j.jes.2015.04.008.

Newton, R. J., Jones, S. E., Eiler, A., McMahon, K. D. & Bertilsson, S. 2011 A guide to the natural history of freshwater lake bacteria. *Microbiology and Molecular Biology Reviews* **75**, 14–49. doi:10.1128/MMBR.00028-10.

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H. B. A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M. O., Lahti, L., McGlinn, D., Ouellette, M.-H., Cunha, E. R., Smith, T., Stier, A., Ter Braak, C. J. F. & Weedon, J. 2019 vegan: Community Ecology Package. R package version 2.5-6. Available from: https://CRAN.R-project.org/package=vegan (accessed 2 January 2022).

Parada, A. E., Needham, D. M. & Fuhrman, J. A. 2016 Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology* **18** (5), 1403–1414. doi:10.1111/1462-2920.13023.

Pestana, C. J., Reeve, P. J., Sawade, E., Voldoire, C. F., Newton, K., Praptiwi, R., Collingnon, L., Dreyfus, J., Hobson, P., Gaget, V. & Newcomb, G. 2016 Fate of cyanobacteria in drinking water treatment plant lagoon supernatant and sludge. *Science of the Total Environment* **565**, 1192–1200. doi:10.1016/j.scitotenv.2016.05.173.

Pinto, A. J., Xi, C. & Raskin, L. 2012 Bacterial community structure in the drinking water microbiome is governed by filtration processes. *Environmental Science & Technology* **46** (16), 8851–8859. doi:10.1021/es302042t.

Pinto, A. J., Schroeder, J., Lunn, M., Sloan, W. & Raskin, L. 2014 Spatial-temporal survey and occupancy abundance modeling to predict bacterial community dynamics in the drinking water microbiome. *mBio* **5** (3), 1135–1114. doi:10.1128/mBio.01135-14.

Qu, J. H. & Yuan, H. L. 2008 *Sediminibacterium salmoneum* gen. nov., sp. nov., a member of the phylum Bacteroidetes isolated from sediment of a eutrophic reservoir. *International Journal of Systematic and Evolutionary Microbiology* **58** (9), 2191–2194. doi:10.1099/ijs.0.65514-0.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. & Glöckner, F. O. 2013 The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* **41** (D1), D590–D596. doi:10.1093/nar/gks1219.

Quince, C., Lanzen, A., Davenport, R. J. & Turnbaugh, P. J. 2011 Removing noise from pyrosequenced amplicons. *BMC Bioinformatics* **12** (38). doi:10.1186/1471-2105-12-38.

R Development Core Team 2011 *A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. Available from: https://cran.r-project.org/bin/windows/base/old/ (accessed 25 November 2021).

Reynolds, K. A., Mena, K. D. & Gerba, C. P., 2008 Risk of waterborne illness via drinking water in the United States. In: *Reviews of Environmental Contamination and Toxicology*, Vol. 192, pp. 117–158 (Whitacre, D. M., ed.). Springer-Verlag, Berlin. doi:10.1007/978-0-387-71724-1_4.

Sha, J., Wu, J., Bi, C., Chen, C., Su, Q., Wang, S., Wang, C. & Zhou, Y. 2020 Responses of microbial community to different concentration of perchlorate in the Qingyi River. *3 Biotech* **10**, 21. doi:10.1007/s13205-019-2012-1.

SNIS – Sistema Nacional de Informações sobre Saneamento 2019 *24° Diagnóstico dos Serviços de Água E Esgotos*. Brasília. Available from: http://www.snis.gov.br/downloads/diagnosticos/ae/2018/Diagnostico_AE2018.pdf (accessed 25 November 2021).

SNIS – Sistema Nacional de Informações sobre Saneamento 2020 *Perdas de água 2020 (SNIS 2018): Desafios para disponibilidade hídrica e avanço da eficiência do saneamento básico*. Available from: https://tratabrasil.org.br/images/estudos/Relat%C3%B3rio_Final_-_Estudo_de_Perdas_2020_-_JUNHO_2020.pdf (in Portuguese).

Tang, W., Liu, Y., Li, Q., Chen, L., Li, Q., Li, P. & Xia, S. 2022 The impact of extreme weather events on bacterial communities and opportunistic pathogens in a drinking water treatment plant. *Water* **14** (1), 54. doi:10.3390/w14010054.

Tessler, M., Neumann, J. S., Afshinnekoo, E., Pineda, M., Hersch, R., Velho, L. F. M. & Mason, C. E. 2017 Large-scale differences in microbial biodiversity discovery between 16S amplicon and shotgun sequencing. *Scientific Reports* **7**, 6589. doi:10.1038/s41598-017-06665-3.

Wang, J.-J., Liu, X., Ng, T. W., Xiao, J. W., Chow, A. T. & Wong, P. K. 2013 Disinfection by product formation from chlorination of pure bacterial cells and pipeline biofilms. *Water Research* **47** (8), 2701–2709. doi:10.1016/j.watres.2013.02.038.

Wang, H., Masters, S., Edwards, M., Falkinham, J. III & Pruden, A. 2014 Effect of Disinfectant, Water Age, and Pipe Materials on Bacterial and Eukaryotic Community Structure in Drinking Water Biofilm. *Environmental Science & Technology* **48** (3), 1426–1435. doi:10.1021/es402636u.

Wang, H., Masters, S., Falkinham, J. I. I. I., Edwards, M. & Pruden, A. 2015 Distribution system water quality affects responses of opportunistic pathogen gene markers in household water heaters. *Environmental Science & Technology* **49** (14), 8416–8424. doi:10.1021%2Facs.est.5b01538.

WHO – World Health Organization 2017 *Guidelines for Drinking-Water Quality*. WHO Press, Switzerland. Available from: https://www.who.int/publications/i/item/9789241549950 (accessed 2 February 2022).

Zhang, J., Li, W., Chen, J., Qi, W., Wang, F. & Zhou, Y. 2018 Impact of biofilm formation and detachment on the transmission of bacterial antibiotic resistance in drinking water distribution systems. *Chemosphere* **203**, 368–380. doi:10.1016/j.chemosphere.2018.03.143.

Zhang, H. Y., Tian, Y. M., Kang, M. X., Chen, C., Song, Y. R. & Li, H. 2019 Effects of chlorination/chlorine dioxide disinfection on biofilm bacterial community and corrosion process in a reclaimed water distribution system. *Chemosphere* **215**, 62–73. doi:10.1016/j.chemosphere.2018.09.181.

Zhu, Y., Chen, L., Xiao, H., Shen, F., Deng, S., Zhang, S. & Gong, L. 2020 Effects of disinfection efficiency on microbial communities and corrosion processes in drinking water distribution systems simulated with actual running conditions. *Journal of Environmental Sciences* **88**, 273–282. doi:10.1016/j.jes.2019.09.009.

First received 12 July 2022; accepted in revised form 8 November 2022. Available online 17 November 2022