Title: Reproducible microbial community dynamics of two drinking water systems treating similar source waters.

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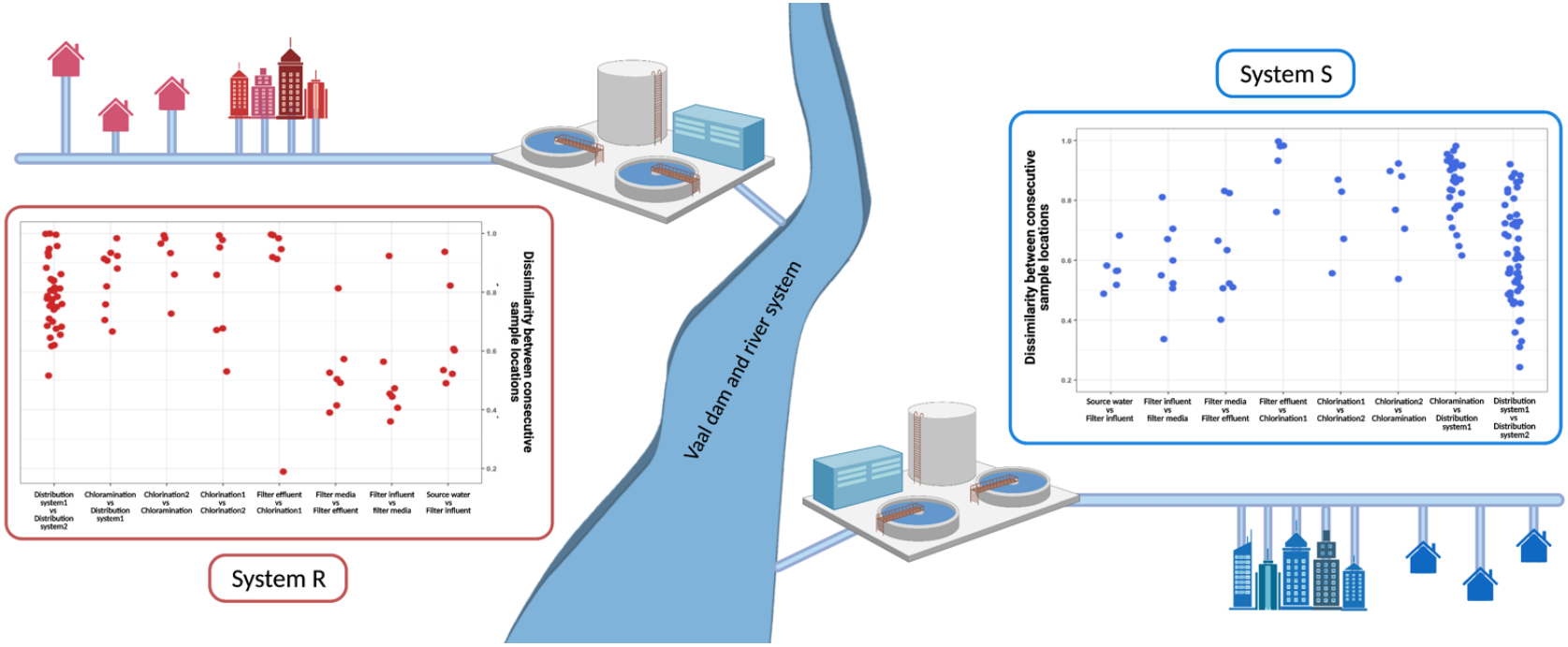
Graphical abstract



Abstract

Understanding whether the spatial-temporal dynamics of the drinking water microbiome are reproducible in full-scale drinking water systems is an important step towards devising engineering strategies to manipulate it. Yet, direct comparisons across full-scale drinking water systems are challenging because multiple factors, from source water to treatment process choice and configuration, can be unique to each system. This study compared the spatial-temporal dynamics of the drinking water microbiome in two drinking water treatment plants (DWTPs) with identical sequence of treatment strategies treating source waters from the same river system and with treated drinking water distributed in same large-scale (but independent) distribution system (DWDS) with similar disinfectant residual regiment. Dissimilarities in source water communities were tempered by the pre-disinfection treatments, resulting in highly similar post-filtration microbial communities between the two systems. However, high community turnover due to disinfection resulted in highly dissimilar microbial communities in the finished water between the two systems. Interestingly however, the microbial communities in the two systems increased in similarity during transit through the DWDS despite presence of a disinfectant residual. Overall our study finds that the drinking water microbiome demonstrated reproducible spatial and temporal dynamics within both independent but nearly identical DWTPs and their corresponding DWDSs.

Keywords: drinking water treatment; drinking water distribution; disinfection; microbial community dynamics; amplicon sequencing

1. Introduction

Drinking water treatment operations are designed to reduce microbial concentrations and limit microbial growth in drinking water distribution systems (DWDS) in order to deliver safe water to the consumer[1-3]. The choice of treatment strategies implemented to achieve this is highly site specific and is based on a range of considerations including source water type, local regulations, etc[3,4]. Drinking water treatment itself can be considered as a series of ecological disturbances implemented sequentially on the microbial community as it transits from the source water to the consumers taps[5]. These disturbances shape the microbial community composition (i.e., who is there)[6], as well as the population and community size (i.e., cell concentrations). Previous studies have shown that the drinking water microbiome is shaped by the choice of treatment strategy and conditions in the DWDS[3,5,7-10]. For instance, the microbial concentrations in finished drinking water can vary from 10^3 and 10^5 cells/mL[11-15], depending on the presence, absence, and concentrations of the disinfectant residual. Disinfectant residuals not only impact community size, but also impact community structure[3,10,16-18] and functional potential[18,19]. Similarly, filtration processes impact downstream microbial communities both by filtration-mediated seeding (Pinto *et al*., 2012) and through the biologically mediated removal of nutrients making them unavailable for microbial growth downstream[11,12,20,21].

Factors influencing the drinking water microbiome are site specific due to the unique type and combination of treatment technologies across DWTPs and DWDS configurations, source water types, and operational practices[21-23]. Thus, while the impact of individual treatment process or environmental conditions on the drinking water microbiome may be compared across DWTP's and DWDSs, it is often impossible to perform paired comparison between DWSs due to the inability to control for the type and sequential combination of treatment and distribution practices. For instance, studies have investigated drinking water

microbiome dynamics in two DWTPs that use similar treatment processes but utilize different source waters or utilize the same source water but different treatment approaches[24-27]. Further, often such comparisons are limited to either the treatment system or the distribution system, but rarely encompass a comprehensive source-to-tap analysis. To our knowledge, no study has yet investigated the impact of identical drinking water treatment regimens and similar distribution practices on the drinking water microbiome in full-scale systems utilizing similar source waters. This is a significant knowledge gap in the field, because it has direct implications for our understanding of reproducibility of the drinking water microbiome dynamics and the impact of engineering interventions.

This study presents unique insights from systematic comparisons between two DWTPs, using the same treatment strategies to treat similar source waters originating from the same river system and their corresponding distribution systems. The specific objectives of this study were to investigate: (i) whether identical sequence of treatment technologies within two DWTP's treating similar source waters results in similar changes in the microbial community, (ii) the extent to which similarities in temporal dynamics between DWTP's are conserved (or not) between their respective distribution systems, and (iii) and to what extent the impact of physicochemical parameters on microbial community structure is conserved between the two DWSs. To our knowledge this mirrored study has not been previously performed and is critical towards improving our understanding of the spatial-temporal dynamics of the drinking water microbiome.

2. Materials and methods

2.1. Site description. This is study involves two drinking water systems (System R and S) that are operated by same drinking water utility (Fig. 1A). These two systems supply on an average 3653 million liters per day to approximately 12 million people within large metropolitan

region and local municipalities as well as for industrial use through a network of large diameter pipelines stretching over 3056 km. The source water is drawn primarily from the Vaal river and dam system into two drinking water treatment plants (R_DWTP and S_DWTP), which abstract, treat, and distribute 98% (approximately 4320 ML/d) of the total water supplied by the utility. The R_DWTP (river intake pumping site) treats source water from the river downstream of the dam and the S_DWTP treats source water from a canal directly from the dam. Treatment of the source waters in both DWTPs consists of the identical treatment steps (Fig. 1B). Briefly, source water in both DWTPs is dosed with polyelectrolyte coagulants with low lime for coagulation and flocculation, with no need for pH correction after sedimentation. Although in some months in System R, a combination of polyelectrolyte and silica lime are used for coagulation and flocculation (Table S1). In these instances, following sedimentation, the water pH is adjusted to near neutral by bubbling CO_2 gas followed by filtration through rapid gravity sand filters. Finally, the filter effluent is dosed with chlorine gas via bubbling as the primary disinfection step. The total chlorine at sites following chlorination varies between 1.0 mg/L and 2.5 mg/L after 20 min contact time. Chlorinated water leaving both DWTPs is again dosed with chloramine (approximately 2 mg/L) at secondary disinfection boosting stations. For the purpose of this study chlorinated water originating from the R_DWTP was monitored to the booster station, which supplies approximately 1,100 ML/d of chloraminated water, serving predominately the northwest area of the distribution system (R_DWDS). Chlorinated water originating from the S_DWTP was also monitored to another booster station, supplying approximately 700 ML/d of chloraminated water to the eastern parts of the distribution system (S_DWDS) (Fig. 1A). Samples were also collected after the booster stations in the chloraminated sections of each DWDS, with monochloramine residuals varying on average between 0.8 mg/L in the autumn and 1.5 mg/L in the spring. Further details on

range of physical-chemical parameters for both systems were obtained from the utility (Table S1 and S2).

2.2. Sample collection and processing. Samples were collected for 8 months (February 2016 – September 2016) on a monthly basis from the two DWTPs (R_DWTP and S_DWTP) and their associated DWDS (R_DWDS and S_DWDS) (Fig. 1A). At each DWTP, samples collected included source water (SW), filter influent (FI, i.e. water entering the rapid sand filter following coagulation, flocculation, sedimentation and carbonation), filter bed media (FB), filter effluent (FE) and chlorinated water leaving the treatment plant (CHLA). In both DWDSs, chlorinated water samples were collected immediately before the booster station (CHLB), chloraminated water leaving the booster station (CHM) and chloraminated bulk water samples at two points with the DWDSs (DS1 and DS2, respectively) (Fig. 1A and 1B). Within the two DWTPs, 1 L of source water, 4 L of filter influent, and 8 L of filter effluent were collected. Typically, 8 – 16 L of bulk water was collected for samples collected post disinfection and in the DWDS. Collected water samples were filtered to harvest microbial cells followed by phenol:chloroform DNA extraction as described by Potgieter *et al*., 2018. To obtain microbial biomass from the filter bed media samples, 10 g of filter media was mixed with 50 ml extraction buffer (i.e., 0.4 g/L EDTA, 1.2 g/L TRIS, 1 g/L peptone and 0.4 g/L N-dodecyl-N, N dimethyl-3-amminio-1-propanesulfonate) followed by sonication for 1 min to remove the microbial biomass attached to sand particles[28]. After sonication, the aqueous phase was filtered through a Sterivex™-GP 0.22 µm polycarbonate membrane filter unit (Merck Milipore, South Africa) followed by phenol:chloroform DNA extraction, as with bulk water samples.

2.3. Sequencing and data processing. Extracted DNA from samples were sent to the Department of Microbiology and Immunology, University of Michigan Medical School (Ann

Arbor, USA) for the 2x250 bp sequencing of the V4 hypervariable region of 16S rRNA gene using the Illumina MiSeq platform[29]. All raw sequence data have been deposited with links to BioProject accession number PRJNA529765 on NCBI. The resultant 16S rRNA gene amplicon sequences were processed using the Divisive Amplicon Denoising Algorithm, DADA2 v1.14[30] workflow including sequence filtering, dereplication, inferring sample composition, chimera identification and removal, merging of paired-end reads and construction on a sequence table. Initial trimming and filtering of reads followed standard filtering parameters described for Illumina MiSeq 2x250 V4 region of the 16S rRNA gene. Specifically, reads with ambiguous bases were removed (maxN=0) were removed, the maximum number of "expected errors" was defined (maxEE=2) and reads were truncated at the first instance of a quality score less than or equal to truncQ (truncQ=2). Dereplication was performed where identical sequences are combined into "unique sequences" while maintaining the corresponding abundance of the number of reads for that unique sequence. The core sample inference algorithm was applied to dereplicated data and forward and reverse reads were merged together to obtain fully denoised sequences[30]. Merged reads were then used to construct an amplicon sequence variant (ASV) table[31], chimeras were identified and removed and taxonomic assignments were called using the SILVA reference database (https://www.arb-silva.de) through the DADA2 chimera removal and taxonomy assignment script.

2.4. Microbial community analysis. Resulting ASV table was imported into the mothur (v 1.35.1)[32] and the shared sequences between sample locations from the two DWTPs and corresponding DWDSs as well as the unique sequences within each sample location were calculated using the venn function in mothur. Alpha diversity measures (i.e., richness, Shannon Diversity Index and Pielou's evenness) were calculated using the summary.single

function in mothur with the parameters, subsampling=1263 (sample with the least amount of sequences) and iters=1000 (1000 subsampling of the entire dataset). Due to subsampling, 10 samples were excluded from the analyses and Good's coverage estimates were calculated to assess whether sufficient number of sequences were retained for each sample after subsampling. This indicated that subsampling at a library size of 1263 retained the majority of the richness for all samples (i.e., average Good's coverage = 95.84 ± 0.02%). One-way analysis of variance (ANOVA)[33] and post-hoc Tukey Honest Significant Differences (HSD) test were performed in R (http://www.R-project.org) using the stats package[34] to determine the statistical significance between spatial and temporal groupings within the alpha diversity.

Bray-Curtis and weighted UniFrac were used to determine pair-wise dissimilarity in community structure between samples, whereas Jaccard and unweighted UniFrac were used to infer dissimilarity in community membership. Bray-Curtis and Jaccard distances were calculated using the dist.shared function in mothur with the parameters, subsampling=1263 and iters=1000. Weighted and unweighted UniFrac distances were calculated through the construction of a phylogenetic tree with representative sequences using the clearcut command in mothur also with the parameters subsampling=1263 and iters=1000[35,36]. Pairwise Analysis of Molecular Variance (AMOVA) was performed using the amova function in mothur on all beta diversity matrices, to determine the effect of sample groupings based on DWDS sample location, DWDS section, and season[37,38]. Beta diversity metrics and metadata files containing sample location, sample type, disinfection type and season were imported into R (http://www.R-project.org) for statistical analysis and visualization. Principal-coordinate analyses (PCoA) using Bray-Curtis and Jaccard distances was performed using the phyloseq package[39]. All plots were constructed using the ggplot2 package[40]. To identify the contribution of environmental parameters and their combinations towards microbial community structure, distance-based redundancy analysis (dbRDA) was used. The function dbrda() from R package

"vegan"[41] was applied on Bray-Curtis distances estimated between samples using ASV counts to investigate relationships between the scaled environmental parameters and microbial community structure. In addition, the faction of variation explained by the environmental parameters identified as significantly associated with ASV count-based Bray-Curtis distance matrices was determined using the function varpart() in the "vegan" package.

3. Results and discussion

A total of 172 samples were sequenced for this study (Table S3) which resulted in 4,921,399 sequences post quality control and a total of 10,012 ASVs. Taxonomic classification of these ASV's revealed that bacteria dominated the microbial community (mean relative abundance, MRA: 98.74 ± 0.02% across all samples) followed by archaea (MRA: 1.04 ± 0.01%). The bacterial community was primarily composed of *Gammaproteobacteria* (including *Betaproteobacteriales*), *Actinobacteria*, and *Planctomycetes* (Fig. S1 and Table S4).

3.1. Treatment processes increased similarities between microbial communities across the two drinking water systems. Despite being drawn from the same surface water system, the two-source water microbial communities were significantly different (AMOVA, $F_{ST} \leq 3.04$, $p < 0.001$, depending on the beta diversity measure) and only shared 22.5% of the detected ASV's (Fig. S2). These differences could likely be a result of varying hydrological conditions at the two locations (i.e., river drawn R_SW vs S_SW from the dam), which may translate to the variable occurrence of low abundance and transient taxa[42]. Despite harboring more ASV's over the period of the study (Fig. S2), R_SW was less diverse than S_SW (ANOVA; $p < 0.05$) per timepoint (Fig. S3; Table S5). This was because R_SW was likely much more influenced by strong hydrological conditions such as runoff and increased flow rates during heavy rainfall events[43,44] as compared to S_SW. This is supported by the fact that

R_SW exhibited higher temporal variability as compared to S_SW based on pairwise beta diversity measures (Fig. S4).

The pre-chlorination treatment processes played an important role in tempering the differences in microbial communities across the two DWTPs. The microbial community composition of both DWTPs was highly diverse and in addition to *Proteobacteria* included other dominant phyla (i.e. MRA greater than 1%) such as *Acidobacteria, Cyanobacteria, Verrucomicrobia* and *Planctomycetes* (Fig. S1; Table S4). The 33 most abundant ASV's i.e., ASV's with an MRA of > 0.5%, showed similar trends between the two DWTPs in terms of increase/decrease in MRA with each sequential treatment step (Fig. 2; Table S6). These dominant ASV's within both DWTP included members of *Actinobacteria, Acidobacteria, Gammaproteobacteria, Bacteroidetes* and *Thaumarcheota.* This reproducible effect of sequential treatment steps was also evident at the community level. Specifically, the treatment process resulted in similar changes in alpha diversity measures in both DWTPs (Fig. S3). Based on ANOVA these changes were found to be significant (richness: $F_{ST} = 19.67$, $p < 0.05$, Shannon Diversity Index: $F_{ST} = 9.78$, $p < 0.05$, Inverse Simpson Diversity Index: $F_{ST} = 15.64$, $p < 0.05$ and Pielou's evenness: $F_{ST} = 4.79$, $p < 0.05$). Richness and diversity consistently decreased along treatment processes (excluding filter bed samples (FB)), with the most significant decrease immediately following chlorination (Fig. S3; Table S5). This shows that the decrease in microbial abundance and diversity, typical during treatment processes[1,5,45-47], was reproducible in both systems.

Beta-diversity analyses indicated that microbial communities became increasingly similar from source water through treatment and filtration where the microbial communities between the filter bed and the filter effluent were approximately 40% similar in community structure (Bray-Curtis: 0.57 ± 0.15) and 30% similar in community membership (Jaccard: 0.71 ± 0.11); this was comparable to the similarity of the filter bed and filter influent communities

(community structure: Bray-Curtis: 0.55 ± 0.17, community membership: Jaccard: 0.7 ± 0.13) (Fig. 3A). Similar trends in community structure and membership were observed in weighted and unweighted Unifrac analyses (Fig. S5). Following similar prefiltration treatments of the source water, filter influent samples between the two systems showed the greatest similarity (Fig. 3B). Although, filter bed microbial communities (R_FB and S_FB) showed higher dissimilarity in community structure and membership (Fig. 3B; Table S7) between the two DWTPs, they were also significantly different from both the filter inflow (AMOVA: $F_{ST} \leq 5.07$, $p < 0.001$) and filter effluent (AMOVA: $F_{ST} \leq 6.51$, $p < 0.001$). The greater dissimilarity between the FB from the two DWTP's could also be attributed to inherent high heterogeneity of attached growth microbial communities in the filter media[7,23]. Yet, microbial communities were more similar between filter bed samples of the two DWTPs, than the source waters that feed them. Further, an increase in the number of shared ASV's between the two filter effluents (36.04%), indicates that conditions in the filter beds were sufficiently similar to have the same effect on the resulting effluent and the selection of dominant taxa in both systems.

3.2. Rapid microbial community turnover due to disinfection increases dissimilarity across the two drinking water systems. In contrast to the DWTP (i.e., pre-chlorination) where treatment processes enhance similarity in microbial community between the two DWTPs, the rapid change in microbial community due to chlorination resulted in significant increase in dissimilarity between locations within the DWTP/DWDS and across the two systems. Following chlorination, the microbial communities between filter effluent (FE) and bulk water (CHLA) were only 8 – 11% similar in community structure (Bray-Curtis: 0.89 ± 0.2) and membership (Jaccard: 0.92 ± 0.15) (AMOVA: $F_{ST} \leq 18.22$, $p < 0.001$ depending on the beta diversity measure) (Fig. 3A). This significant decrease in similarity was also observed in weighted and unweighted Unifrac analyses (Fig. S5). In addition, this significant change

following disinfection was also observed as shift in the relative abundance of dominant ASVs (Fig. 2).

Further, chlorinated samples CHLA and CHLB increased slightly in similarity in community structure and membership (Bray-Curtis: 0.77 ± 0.16 and Jaccard: 0.86 ± 0.11) but remained highly dissimilar, though not significantly different. However, following secondary disinfection, the microbial communities between chlorinated (CHLB) and chloraminated water (CHM) again increased in dissimilarity in community structure (Bray-Curtis: 0.84 ± 0.12) and membership (Jaccard: 0.91 ± 0.08) (AMOVA: $F_{ST} \leq 4.09$, $p < 0.001$ depending on the beta diversity measure) in both systems (Fig. 3A) (same trends observed for Unifrac analyses, Fig. S5). This was consistent for both, the R and S systems. Beta diversity comparisons between paired samples from System R and S immediately after chlorination (R_CHLA and S_CHLA) showed an increase in dissimilarity in both community structure and membership (Fig. 3B; Table S7). This was also observed between R_CHLB and S_CHLB samples. Samples within the DWDS (CHM, DS1 and DS2) showed consistent temporal trends where samples from both systems increased in dissimilarity 6 months apart, consistent with the observed changes in temperature (Fig. S4; Fig. S6). However, temporal variability remained high within DS1 and DS2 samples where pair-wise comparisons between consecutive months within each sample location were dissimilar in community membership (i.e., Jaccard: 0.80 ± 0.06 and unweighted UniFrac: 0.67 ± 0.06) and structure (i.e., Bray-Curtis: 0.69 ± 0.13 and weighted UniFrac: 0.52 ± 0.13) (Fig. 3B).

Disinfection significantly reduces bacterial cell concentrations and has a substantial influence on community composition and structure [3,10,24,45,48,49]. While microbial communities prior to chlorination were similar between the two DWTPs, the microbial community composition and structure in both systems were highly dissimilar post-chlorination. This dissimilarity was also observed on a temporal scale, where samples post disinfection showed

greater temporal variability as compared to the pre-chlorinated samples. This variability could potentially arise from a few different factors. For instance, chlorine and chloramine are both strong oxidants and are likely to inactivate microorganisms indiscriminately; this could be one potential reason for higher temporal variability post-disinfection and greater dissimilarity between paired samples between the two drinking water systems. Alternatively, those surviving disinfection may be associated with biofilms. The presence of disinfectant residual has been reported to have limited impact in preventing biofilm development and in some cases may even promote biofilm formation as a stress response[50,51]. Another explanation could be reduction in microbial abundance and diversity due to disinfection may lead to the detection of low abundance or rare taxa that may not have well defined niche within the drinking water community and thus are variably present and/or detected.

Ultimately, the application of disinfection can be viewed as an ecological disturbance, where the sequential and controlled addition of disinfectants, disturb the drinking water microbial continuum[5]. The diversity of microbial communities generally decreases in response to environmental stress and disturbances, ultimately shifting the ecological balance of microbial populations within the community [6,52,53]. Following a disturbance, the surviving populations are considered to have specific properties (such as biofilm formation or oxidative stress response mechanisms), allowing them to persist in the disrupted environment.

3.3. Chloramine residuals and distribution conditions promote microbial community stability. Considering the aforementioned similar impacts of treatment process and distribution system on the microbial communities between the two systems, it is not surprising that microbial communities from both systems clustered together depending on sampling location (i.e., DWTP or DWDS), rather than system (R or S) (Fig. 4). For instance, the DWTP locations clustered independently from the DWDS across both systems (Fig. 4A). Even within

the DWTP, microbial communities clustered depending on sampling location despite the temporal variability (Fig. 4B). The same was consistent for the DWDS, where the chlorinated and chloraminated sections of the DWDS clustered together (Fig. 4C). Further, the measured water quality parameters were highly similar at each location between the two DWTPs and DWDS (Fig. S7; Table S2). To assess the extent to which measured water quality parameters impacted the microbial community, we performed dbRDA analysis by focusing primarily on the post-chlorination samples (i.e., chlorinated and chloraminated DWDS). This was done because each section of the DWDS across the two DWS's has multiple samples per month over the eight-month sampling period resulting in significant sample size for both dbRDA and subsequent variance partitioning tests. Consistent with PCoA analyses, dbRDA revealed that samples clustered based on disinfectant residual type (chlorinated or chloraminated) and not based on the system they originated from. Based on dbRDA analysis, ammonium, water temperature, monochloramine and free Cl_2 emerged as the best indicators among water quality parameters that explained the variability in microbial community structure in both systems (ANOVA: $p < 0.001$) (Fig. 5; Table S8). Increased concentrations of free chlorine (1.66 ± 0.55 mg/L) was identified as a significant variable in chlorinated samples ($p = 0.0021$).

Alternatively, among other water quality parameters, ammonium (0.36 ± 0.13 mg/L as N) and monochloramine concentrations (1.60 ± 0.38 mg/L) as well as temperature (18.28 ± 4.47 °C) were identified as significant variables in chloraminated samples ($p < 0.001$). Variance partitioning analyses were used to determine the proportion of variance that could be attributed to variables (individual or combined) identified as most significant in the dbRDA analysis (Fig. 5). This resulted in ammonium, free Cl_2, monochloramine and temperature each explaining 2.5%, 1.9%, 1.6% and 1.3% of the variance, respectively. While the contribution of these parameters towards explaining the variance in community structure was significant, it was small. In fact, 90% of the variance could not be explained by these four parameters (Fig.

S8; Table S9). Here, ammonium and monochloramine concentrations and temperature best explained the variability in chloraminated samples from both distribution systems as disinfectant residual concentration and water temperatures did not differ greatly between the two lines. This is well supported as these variables are long considered to be important factors in shaping the drinking water microbiome[3]. Therefore, the observed dissimilarity between the two distribution lines may be accredited to the initial differential response of the microbial community to chlorine residuals.

Interestingly, the changes in the microbial community in the chlorinated sections were driven predominately by chlorine residuals (specifically free Cl_2) as an initial significant ecological disturbance. However, chlorine residuals are typically short-lived and therefore chloramine residuals are added as a more stable alternative to ensure residuals are maintained over longer distances[54,55]. Nevertheless, the transport of bulk water over longer distances leads to increased water age and decreased disinfectant residual due to disinfectant decay, ultimately contributing to microbial regrowth[50]. In addition to disinfectant residuals, the microbial community is also exposed to various other distribution conditions and water quality parameters including (but not limited to) pipe material, hydraulic conditions, residence time, water temperature, concentrations of nitrogen species, organic carbon, etc. All these factors have a considerable impact on the drinking water microbiome[3].

An increase in similarity was observed in locations towards the end of the DWDS (DS2 samples). Through distribution, water is continually seeded by similar microbial communities over time thereby selecting for the same dominant taxa through similarities in pipe material, residence times, hydraulic conditions and operation practices contributing to site specific taxa and biofilms. Biofilms have also been shown to seed bulk water communities through biofilm erosion, detachment and sloughing[56]. In this study, the established (historical) biofilms in both systems have been long exposed to similar distribution conditions thereby

potentially contributing to the development of similar biofilm microbial communities. This increase in similarity in community membership and structure with increasing residence time in the DWDS was more pronounced in samples from summer and autumn. Here, elevated water temperatures in summer months may affect the bacterial community composition and structure by positively influencing the growth kinetics and competition processes of specific bacterial species in each section of the DWDS[3]. Alternatively, seasonal increase in water demand could increase shear stress resulting in increased detachment of similar established biofilms[56].

In addition, where chlorine may be non-specific in its action in reducing bacterial cell concentrations, chloramine (with the addition of ammonia) may be more selective towards microbial taxa capable of using ammonia as an energy source. This may explain the observed increase in *Nitrosomonas* sp. (ASV_6) in chloraminated distribution system samples from both systems. Here, the addition of chloramine as a secondary disinfectant has been shown to support the growth of nitrifying bacteria in DWDS[57]. The long residence time and associated lower disinfectant residual concentrations, together with the release of ammonia through disinfection decay results in increased abundance of nitrifiers and therefore potential nitrification[49]. Thus, decreasing monochloramine residual concentrations associated with increased water age at nearing the end of both DWDSs may also contribute to the growth of similar bacterial assemblages at these sites.

This study provided a unique opportunity to compare the effects of the same treatment strategies (disturbances) on similar source waters as well as the distribution of treated water on the drinking water microbiome in a large-scale system. The spatial-temporal dynamics of two independent DWTP and their corresponding DWDSs with in the same large-scale distribution system were shown to be reproducible. Similarities in design and operational parameters of the two DWTPs resulted in the development of similar pre-disinfection

microbial communities. However, the differential impact of chlorination was highlighted in post-disinfection samples from the two systems, resulting in dissimilar microbial communities between the two systems. Lastly, distribution was observed to select for certain dominant taxa, thereby increasing similarity between microbial communities due to the impact of inherent biofilms seeding the distribution system. However, dissimilarities in microbial community throughout distribution may arise from initial differences in the source waters and the differential response to chlorination ultimately leading to the presence of site specific rare/low abundant taxa. This study confirms the role of treatment and distribution in shaping the microbial community and may provide utilities with the reassurance that under that same conditions, drinking water quality will be reproducible throughout the varying stages of the drinking water system.

Supporting Information

Table S1: Water quality parameters of the source water from both systems

Table S2: Water quality parameters for DWDS sites

Table S3: The number of samples collected and processed

Table S4: Mean relative abundance of dominant bacterial phyla

Table S5: Mean and standard deviations of alpha diversity indexes

Table S6: Mean percentage relative abundance of the top 33 abundant sequence variants

Table S7: Pair-wise beta diversity comparisons

Table S8: Permutation test for distance-based redundancy analysis (dbRDA)

Table S9: Variance Partition analyses

Fig. S1: Phylum-level and proteobacterial classes of bacterial sequences detected

Fig. S2: Venn diagram of shared amplicon sequence variants (ASVs) between source waters.

Fig. S3: Spatial changes in alpha diversity indexes

Fig S4: Temporal variation in beta diversity measures

Fig S5: Pairwise beta diversity comparisons of weighted and unweighted UniFrac analyses

Fig S6: Variation in temperature over the course of the study (8 months).

Fig S7: Variation in water quality parameters

Fig S8: Venn diagram based on Varpart analysis

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FIGURES

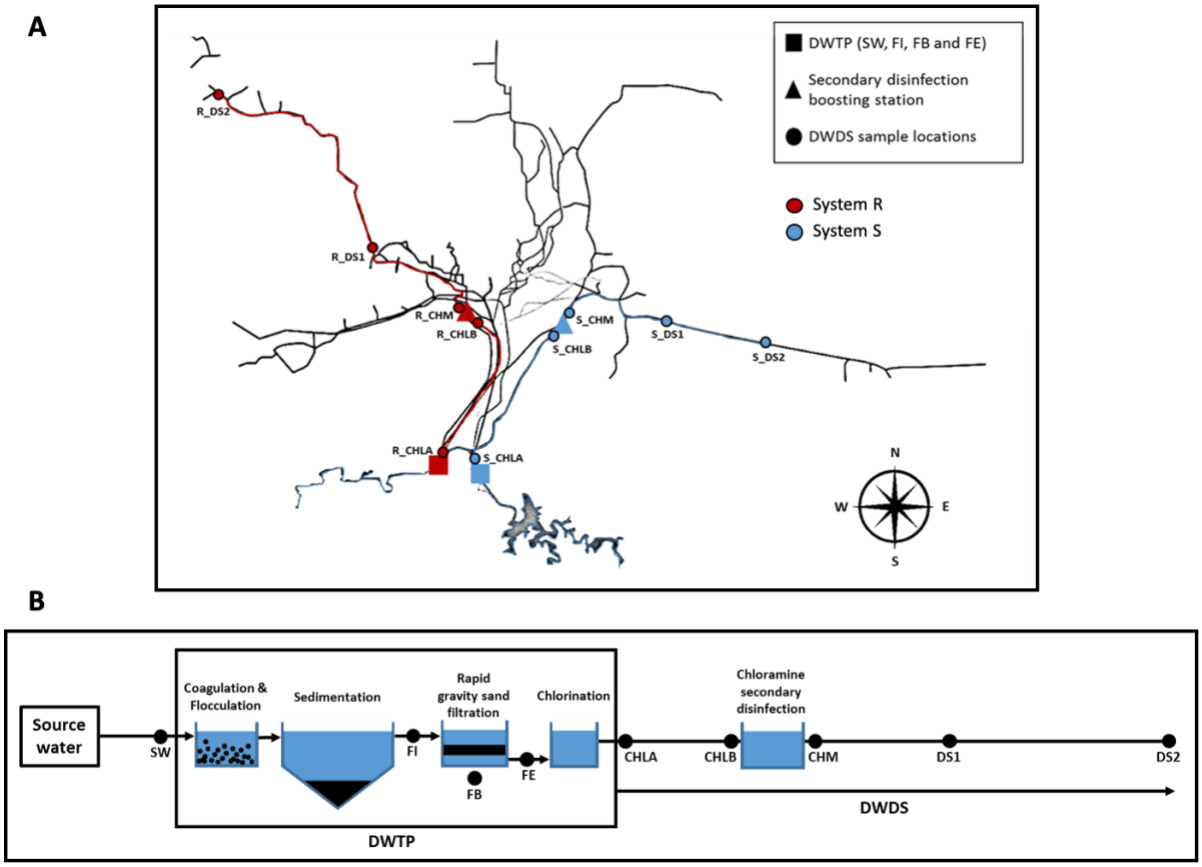


Fig. 1: (A) Site map of the location of the drinking water treatment plants (R_DWTP and S_DWTP) and their corresponding distribution systems (R_DWDS and S_DWDS). System R is indicated in red and System S in blue. The two treatment plants are represented as squares, the two-secondary disinfection boosting stations, where chloramine is added, are represented as triangles and all sample locations are represented as circles. (B) Schematic of the layout of the DWTP and DWDS showing all sample locations sampled monthly for the duration of the study. Within the two DWTPs source water (SW), filter inflow (FI), filter bed media (FB) and filter effluent (FE) samples were collected. All other sample locations are indicated on the figure and described in the text.

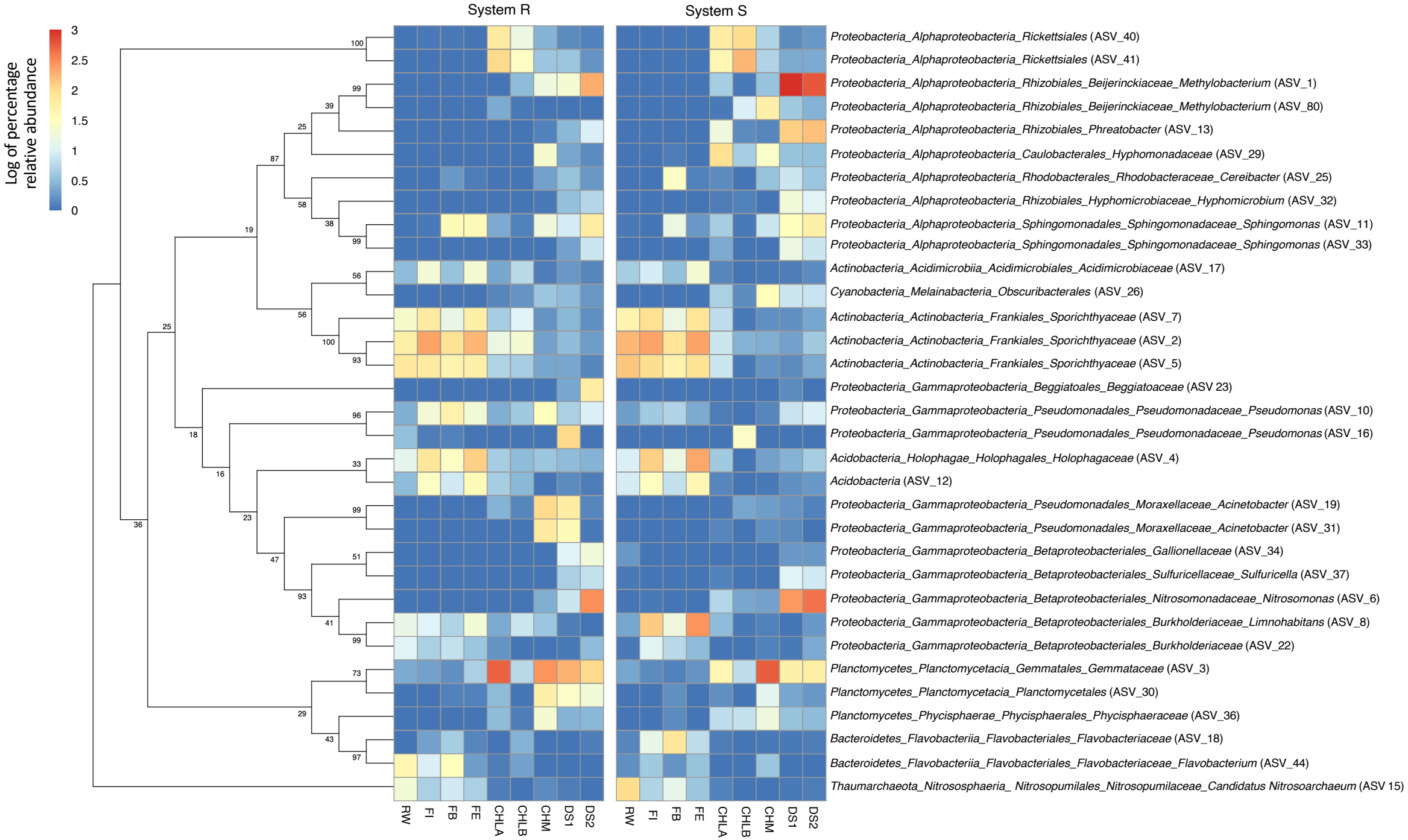
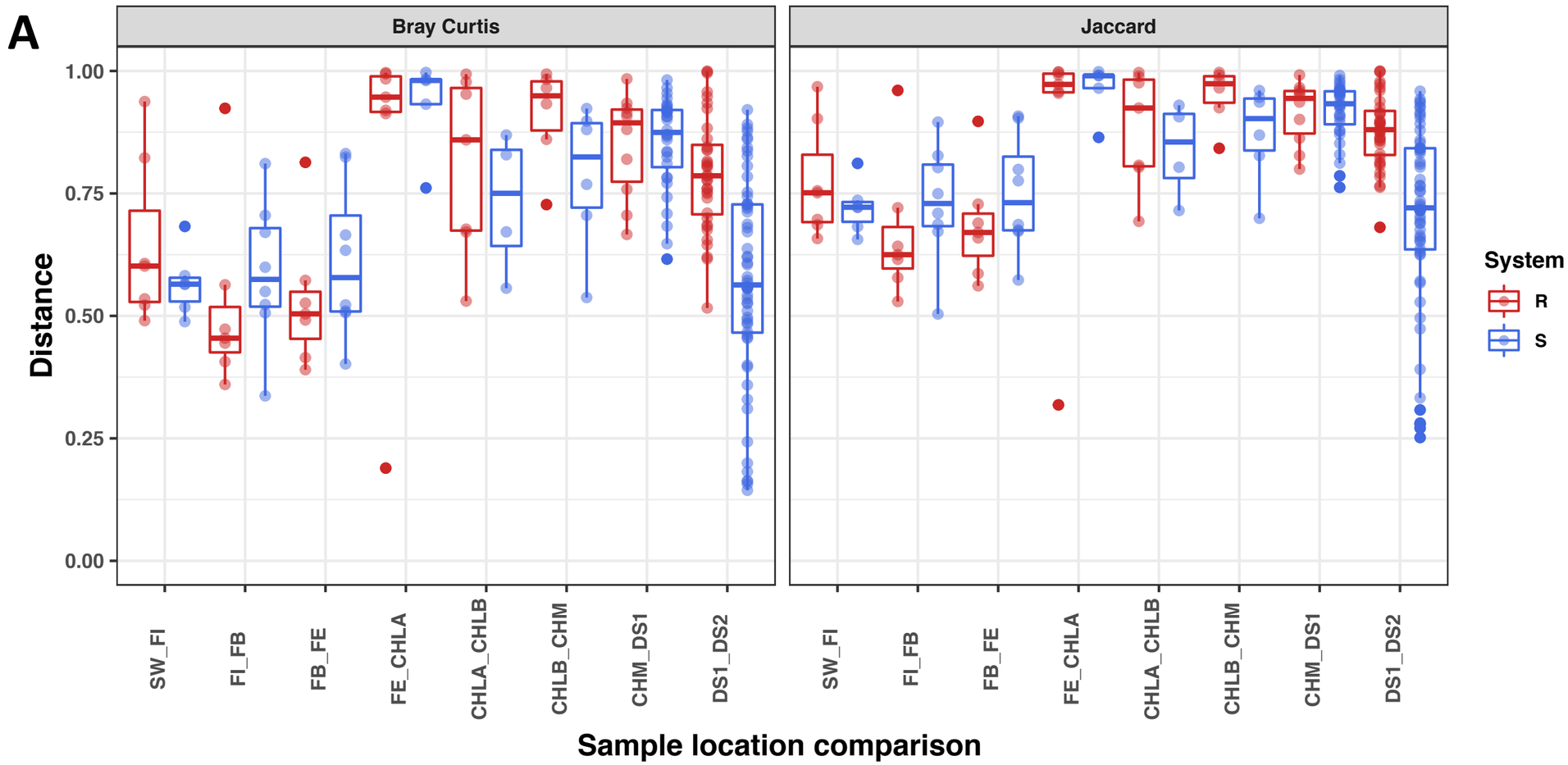

System R
System S
Log of percentage relative abundance
3
2.5
2
1.5
1
0.5
0
Proteobacteria_Alphaproteobacteria_Rickettsiales (ASV_40)
Proteobacteria_Alphaproteobacteria_Rickettsiales (ASV_41)
Proteobacteria_Alphaproteobacteria_Rhizobiales_Beijerinckiaceae_Methylobacterium (ASV_1)
Proteobacteria_Alphaproteobacteria_Rhizobiales_Beijerinckiaceae_Methylobacterium (ASV_80)
Proteobacteria_Alphaproteobacteria_Rhizobiales_Phreatobacter (ASV_13)
Proteobacteria_Alphaproteobacteria_Caulobacterales_Hyphomonadaceae (ASV_29)
Proteobacteria_Alphaproteobacteria_Rhodobacterales_Rhodobacteraceae_Cereibacter (ASV_25)
Proteobacteria_Alphaproteobacteria_Rhizobiales_Hyphomicrobiaceae_Hyphomicrobium (ASV_32)
Proteobacteria_Alphaproteobacteria_Sphingomonadales_Sphingomonadaceae_Sphingomonas (ASV_11)
Proteobacteria_Alphaproteobacteria_Sphingomonadales_Sphingomonadaceae_Sphingomonas (ASV_33)
Actinobacteria_Acidimicrobiia_Acidimicrobiales_Acidimicrobiaceae (ASV_17)
Cyanobacteria_Melainabacteria_Obscuribacterales (ASV_26)
Actinobacteria_Actinobacteria_Frankiales_Sporichthyaceae (ASV_7)
Actinobacteria_Actinobacteria_Frankiales_Sporichthyaceae (ASV_2)
Actinobacteria_Actinobacteria_Frankiales_Sporichthyaceae (ASV_5)
Proteobacteria_Gammaproteobacteria_Beggiatoales_Beggiatoaceae (ASV 23)
Proteobacteria_Gammaproteobacteria_Pseudomonadales_Pseudomonadaceae_Pseudomonas (ASV_10)
Proteobacteria_Gammaproteobacteria_Pseudomonadales_Pseudomonadaceae_Pseudomonas (ASV_16)
Acidobacteria_Holophagae_Holophagales_Holophagaceae (ASV_4)
Acidobacteria (ASV_12)
Proteobacteria_Gammaproteobacteria_Pseudomonadales_Moraxellaceae_Acinetobacter (ASV_19)
Proteobacteria_Gammaproteobacteria_Pseudomonadales_Moraxellaceae_Acinetobacter (ASV_31)
Proteobacteria_Gammaproteobacteria_Betaproteobacteriales_Gallionellaceae (ASV_34)
Proteobacteria_Gammaproteobacteria_Betaproteobacteriales_Sulfuricellaceae_Sulfuricella (ASV_37)
Proteobacteria_Gammaproteobacteria_Betaproteobacteriales_Nitrosomonadaceae_Nitrosomonas (ASV_6)
Proteobacteria_Gammaproteobacteria_Betaproteobacteriales_Burkholderiaceae_Limnohabitans (ASV_8)
Proteobacteria_Gammaproteobacteria_Betaproteobacteriales_Burkholderiaceae (ASV_22)
Planctomycetes_Planctomycetacia_Gemmatales_Gemmataceae (ASV_3)
Planctomycetes_Planctomycetacia_Planctomycetales (ASV_30)
Planctomycetes_Phycisphaerae_Phycisphaerales_Phycisphaeraceae (ASV_36)
Bacteroidetes_Flavobacteriia_Flavobacteriales_Flavobacteriaceae (ASV_18)
Bacteroidetes_Flavobacteriia_Flavobacteriales_Flavobacteriaceae_Flavobacterium (ASV_44)
Thaumarchaeota_Nitrososphaeria_ Nitrosopumilales_Nitrosopumilaceae_Candidatus Nitrosoarchaeum (ASV 15)
RW
FI
FB
FE
CHLA
CHLB
CHM
DS1
DS2
RW
FI
FB
FE
CHLA
CHLB
CHM
DS1
DS2

Fig. 2: Maximum-likelihood phylogenetic tree (left) showing the groupings of the top ASV with a relative abundance > 0.5 (33 ASVs). Phylogenetic tree was constructed based on representative ASV DNA sequences with a bootstrap analysis of 1000 replicates (bootstrap values indicated as percentages). The log percentage of average relative abundance of those top ASVs for each sample site in each system are shown in heatmaps (right) followed by their taxonomic association. The average relative abundance for each sample location was averaged over duration of the study for each system. The log percentage relative abundance of each ASV is indicated in the legends on the left of the figure. See Table for mean relative abundances (MRA) of top dominant ASVs.


A
Bray Curtis
Jaccard
Distance
1.00
0.75
0.50
0.25
0.00
SW_FI
FI_FB
FB_FE
FE_CHLA
CHLA_CHLB
CHLB_CHM
CHM_DS1
DS1_DS2
Sample location comparison
System
R
S

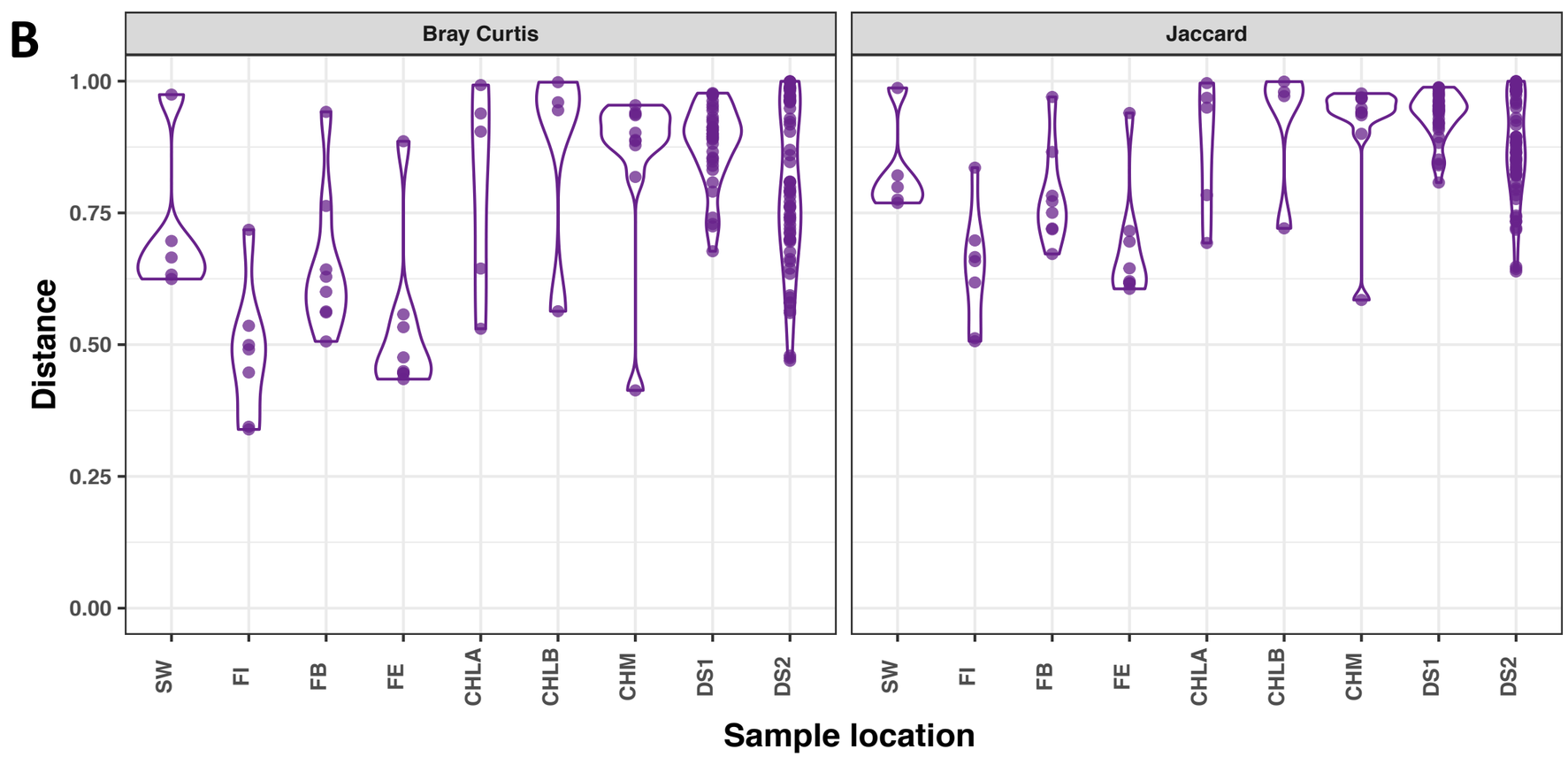

B
Bray Curtis
Jaccard
Distance
1.00
0.75
0.50
0.25
0.00
SW
FI
FB
FE
CHLA
CHLB
CHM
DS1
DS2
Sample location

Fig. 3: (A) Average pairwise beta diversity comparisons of both structure-based metric: Bray-Curtis and membership-based metric: Jaccard. Comparisons are between consecutive locations within each of the two systems for corresponding months. Sample abbreviations on the x-axis refer to comparisons of samples following the flow of bulk water through both systems, i.e. source water and filter inflow (SW_FI), filter inflow and filter bed media (FI_FB), filter bed media and filter effluent (FB_FE), filter effluent and chlorinated water leaving the DWTP (FE_CHLA), chlorinated water leaving the DWTP and chlorinated water entering the secondary disinfection boosting station (CHLA_CHLB), chlorinated water entering the secondary disinfection boosting station and chloraminated water (CHLB_CHM), chloraminated water and distribution system site 1 (CHM_DS1) and finally distribution system site 1 and distribution system site 2 (DS1_DS2). Sample comparisons from System R indicated in red and System S in blue. (B) Direct pairwise beta diversity comparisons (Bray-Curtis and Jaccard) between corresponding samples from the two systems. Pairwise beta diversity comparisons include samples from the same month. Sample abbreviations on the x-axis refer to source waters (SW), filter inflows (FI), filter bed medias (FB), filter effluents (FE), chlorinated waters leaving the DWTP (CHLA), chlorinated waters entering the secondary disinfection boosting station (CHLB), chloraminated waters (CHM), distribution system sites 1 (DS1) and distribution system sites 2 (DS2). Mean and standard deviations of each comparison is shown in Table S7. See Fig. S4 for corresponding weighted and unweighted Unifrac plots

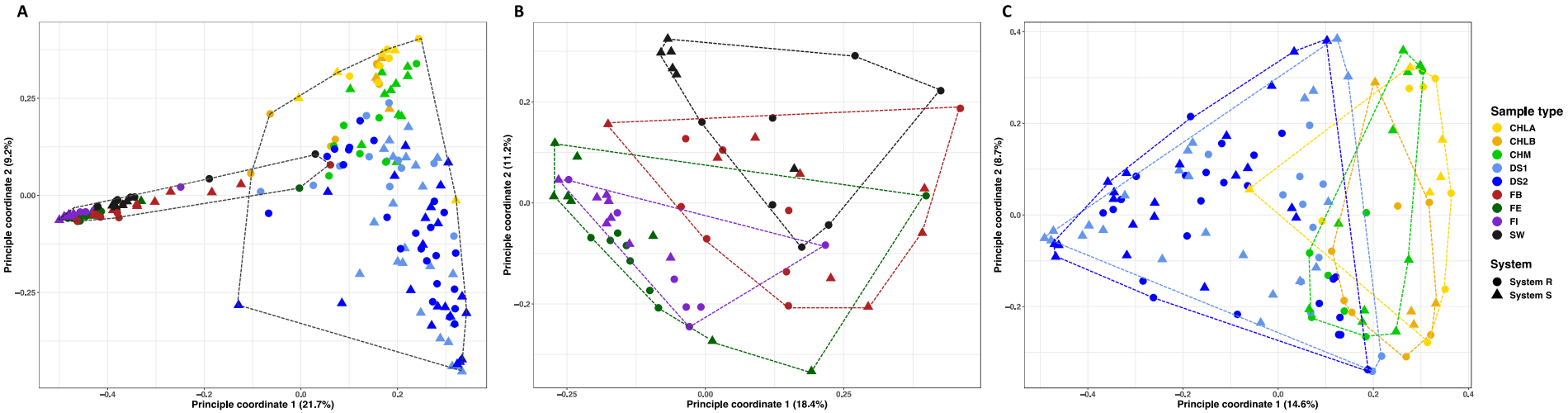


Fig. 4: Principal coordinate analysis plot (based on Bray-Curtis dissimilarity) showing the spatial and temporal variability of the bacterial community structure among all samples from both systems (A), within the two DWTPs (B) and within the two corresponding DWDSs (C). Spatial groupings are shown where data points are colored based on sample location and shaped based on the system they originate from (System R samples are indicated as circles and System S samples as triangles). Color and shapes are indicated in the legends on the right of all plots.

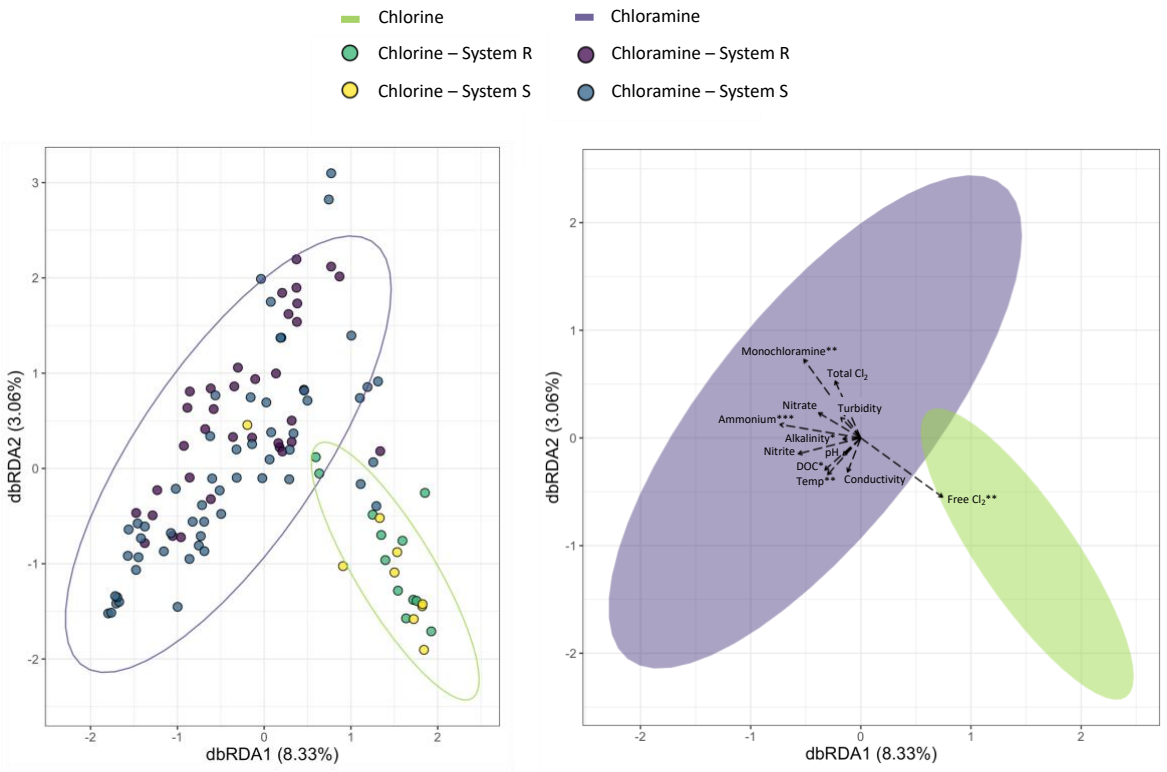


Fig. 5. Relationship between measured water quality parameters and changes in the microbial community profile of locations distribution systems of the two systems where disinfectant residuals were applied. The plots represent the same distance-based redundancy analysis (dbRDA) based on Bray-Curtis distances of 16S rRNA amplicon sequencing data showing the clustering of individual samples (left) and their impacting water quality parameters (right). Chloraminated samples are were found to cluster in the purple ellipse and chlorinated samples in the green ellipse. Significance codes based on ANOVA are indicated as follows 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.