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Article

Reproducible Microbial Community Dynamics of Two Drinking Water Systems Treating Similar Source Waters

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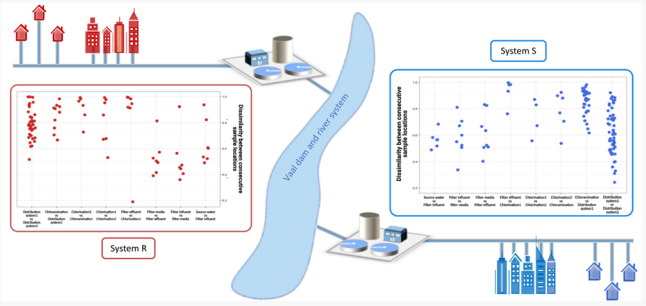
ABSTRACT: Understanding whether the spatiotemporal dynamics of the drinking water microbiome are reproducible in full-scale drinking water systems is an important step in devising engineering strategies for effectively managing and manipulating it. However, direct comparisons across full-scale drinking 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 spatiotemporal dynamics of the drinking water microbiome in two drinking water treatment plants (DWTPs) with identical sequences of treatment strategies. These DWTPs treat source waters from the same river system and treated drinking water is distributed within the same large-scale distribution system (DWDS) with similar disinfectant residual regiments. Dissimilarities in source water communities were tempered by the predisinfection treatments, resulting in highly similar postfiltration 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 degree of similarity of the microbial communities in the two systems increased during transit through the DWDS despite the 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 hygienically relevant microorganisms and limit microbial growth in drinking water distribution systems (DWDSs) ultimately reducing microbial concentrations 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 total cell concentrations in finished drinking water can vary from 10^3 to 10^5 cells/mL,[11−15] depending on the presence, absence, and concentrations of the disinfectant residual. Disinfectant residuals impact not only community size but also community structure[3,10,16−18] and functional potential.[18,19] Similarly, filtration processes impact downstream microbial communities both by filtration-mediated seeding[7] 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 DWTPs and DWDSs, it is often impossible to perform a paired comparison between drinking water systems 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 multiple DWTPs that use similar treatment

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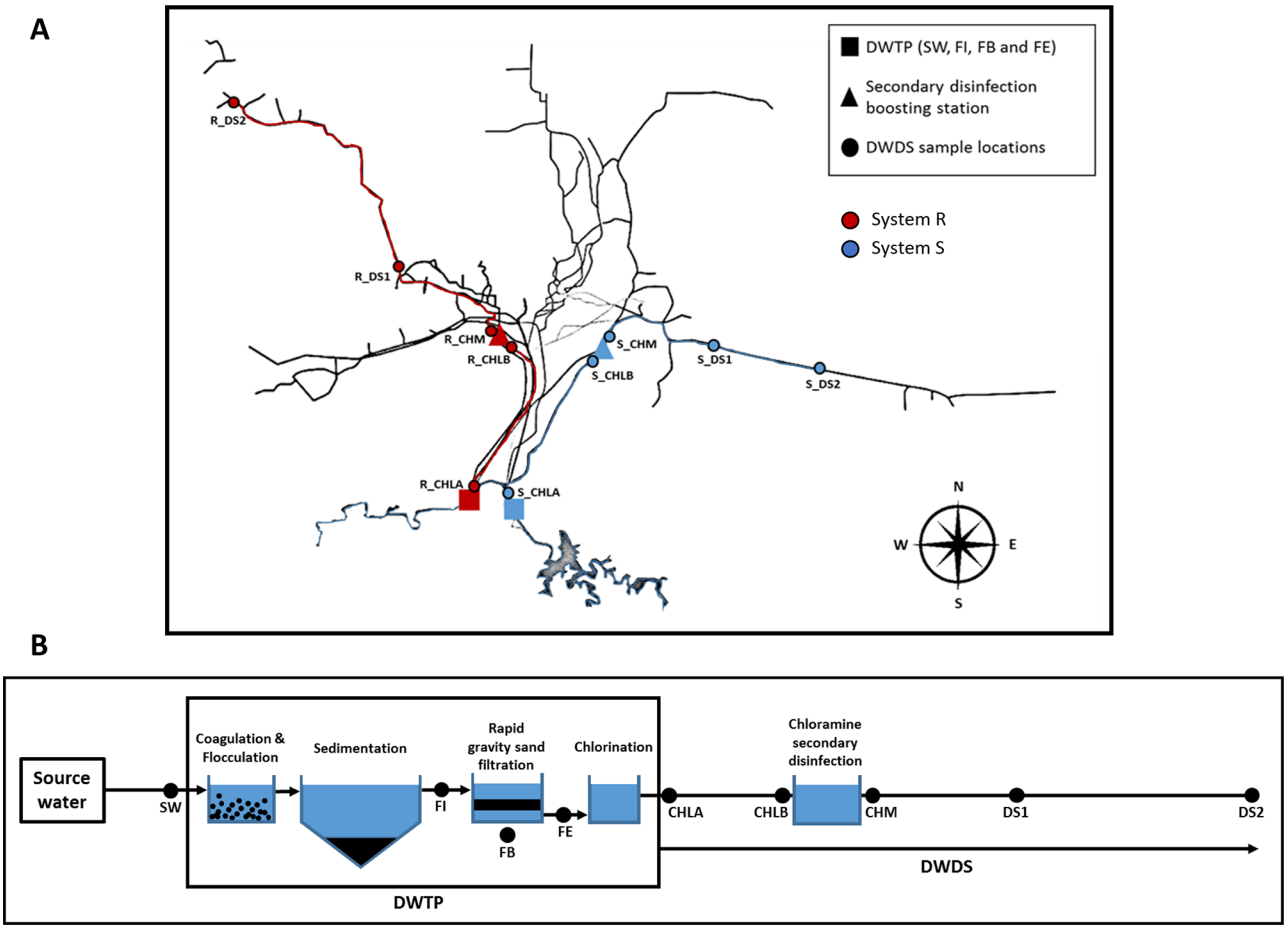

Figure 1. (A) Site map of the location of the drinking water treatment plants (R_DWTP and S_DWTP) and their corresponding distribution systems (R_DWDS and S_DWDS). System R is colored red, and system S blue. The two treatment plants are represented as squares; the two secondary disinfection boosting 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 medium (FB), and filter effluent (FE) samples were collected. All other sample locations are indicated on the figure and described in the text.

processes but utilize different source waters or utilize the same source water but different treatment approaches and/or disinfection residual free systems.[24−28] In addition, often such comparisons are limited to either the treatment system or the distribution system but rarely encompass a comprehensive source-to-tap analysis. To the best of 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 the same 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 the reproducibility of the drinking water microbiome dynamics and the impact of treatment and 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 an identical sequence of treatment technologies within two DWTPs treating similar source waters result in similar changes in the microbial community, (ii) the extent to which similarities in temporal dynamics between DWTPs are conserved (or not) between their respective distribution systems, and (iii) the extent to which the impact of physicochemical parameters on microbial community structure is conserved between the two drinking water systems. To the best of our knowledge, this mirrored study has not been previously performed and is critical for improving our understanding of the spatiotemporal dynamics of the drinking water microbiome.

2. MATERIALS AND METHODS

2.1. Site Description. This study involves two drinking water systems (systems R and S) that are operated by the same drinking water utility (Figure 1A). These two systems supply on average 3653 million L/day to approximately 12 million people within a large metropolitan region and local municipalities as well as for industrial use through a network of large diameter pipelines stretching more than 3056 km. The source water is drawn primarily from the same catchment, i.e., the Vaal river and dam system, into two drinking water treatment plants (R_DWTP and S_DWTP) approximately 20 km from each other, which abstract, treat, and distribute 98% (approximately 4320 million L/day) 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

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dam. Treatment of the source waters in both DWTPs consists of identical treatment steps (Figure 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 in both DWTPs. 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 and 2.5 mg/L after 20 min of 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 at the booster station, which supplies approximately 1100 million L of chloraminated water/day, serving predominately the northwest area of the distribution system (R_DWDS). Chlorinated water originating from the S_DWTP was also monitored at another booster station, supplying approximately 700 million L of chloraminated water/day to the eastern parts of the distribution system (S_DWDS) (Figure 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 about the range of physicochemical parameters for both systems were obtained from the utility (Tables S1 and S2).

2.2. Sample Collection and Processing. Samples were collected for 8 months (February to September 2016) on a monthly basis from the two DWTPs (R_DWTP and S_DWTP) and their associated DWDSs (R_DWDS and S_DWDS) (Figure 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 medium (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) (Figure 1A,B). Bulk water samples from the distribution system sites (DS1 and DS2) for both systems were collected from key points routinely sampled by the utility. These sample locations consisted of established sites with direct connections to large distribution system transport pipes. 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 after 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.[10] To obtain the microbial biomass from the filter bed medium (FB) samples, 10 g of filter medium was mixed with 50 mL of 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-amino-1-propanesulfonate) followed by sonication for 1 min to remove the microbial biomass attached to sand particles.[29] 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 was sent to the Department of Microbiology and Immunology, University of Michigan Medical School (Ann Arbor, MI), for 2 × 250 bp sequencing of the V4 hypervariable region of the 16S rRNA gene using the Illumina MiSeq platform.[30] 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 version 1.14[31] 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 the Illumina MiSeq 2 × 250 V4 region of the 16S rRNA gene. Specifically, reads with ambiguous bases were removed (maxN = 0), 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, whereby 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.[31] Merged reads were then used to construct an amplicon sequence variant (ASV) table;[32] 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. The resulting ASV table was imported into mothur (version 1.35.1),[33] 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. α diversity measures (i.e., richness, Shannon diversity index, Inverse Simpson diversity index, and Pielou's evenness) were calculated using the summary.single function in mothur with the parameters subsampling = 1263 (sample with the fewest sequences) and iters = 1000 (1000 subsampling of the entire data set). Due to subsampling, 10 samples were excluded from the analyses and Good's coverage estimates were calculated to assess whether a 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)[34] and post hoc Tukey honest significant difference (HSD) tests were performed in R (http://www.R-project.org) using the stats package[35] to determine the statistical significance between spatial and temporal groupings within the α diversity.

Bray−Curtis and weighted UniFrac were used to determine pairwise 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

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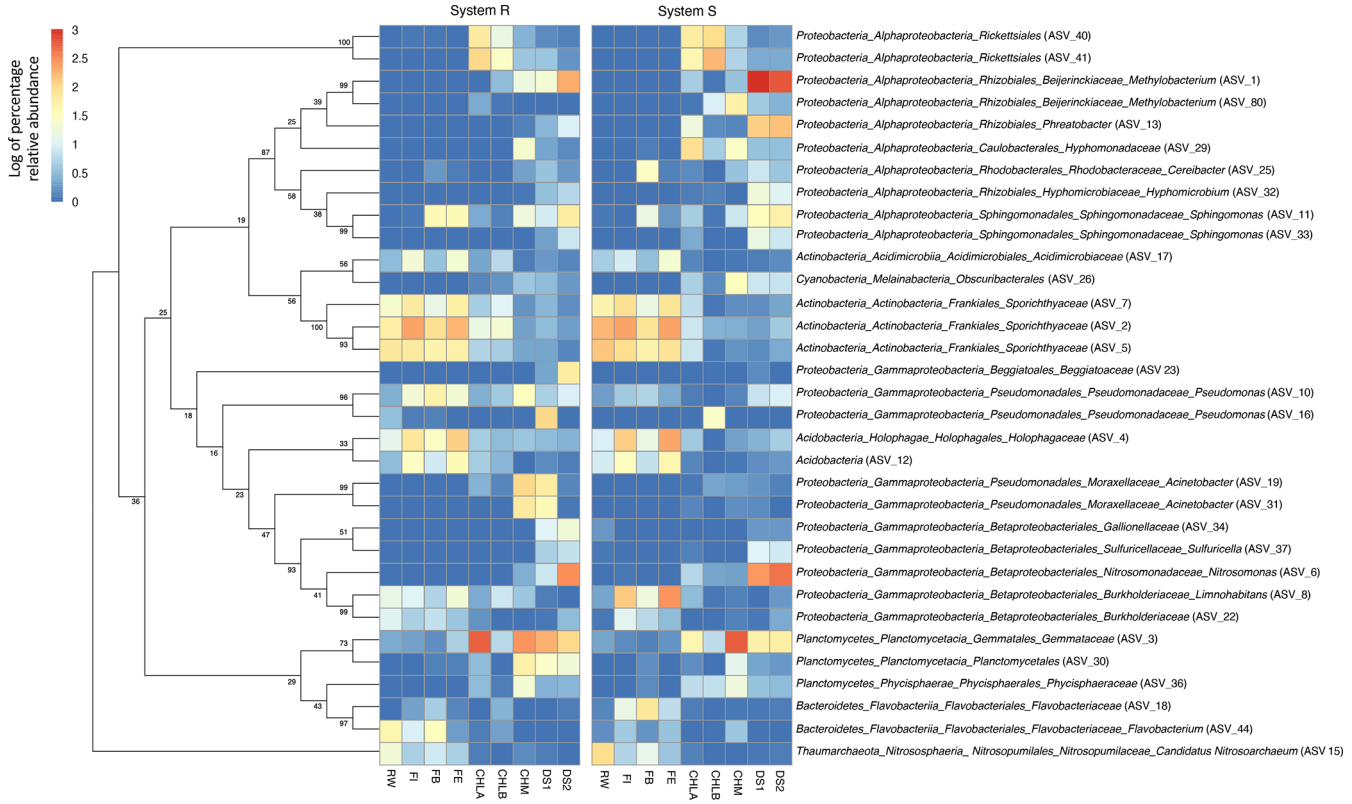

Figure 2. Maximum-likelihood phylogenetic tree (left) showing the groupings of the top ASVs with a relative abundance of >0.5 (33 ASVs). The phylogenetic tree was constructed on the basis of representative ASV DNA sequences with a bootstrap analysis of 1000 replicates (bootstrap values indicated as percentages). The log percentages of the average relative abundance of those top ASVs for each sample site in each system are shown in heat maps (center) followed by their taxonomic association (right). The average relative abundance for each sample location was averaged over the duration of the study for each system. The log percentage relative abundance of each ASV is indicated in the legend at the left of the figure. See Table S4 for mean relative abundances (MRAs) of the most dominant ASVs.

mothur also with the parameters subsampling = 1263 and iters = 1000.[36,37] Pairwise analysis of molecular variance (AMOVA) was performed using the amova function in mothur on all β diversity matrices, to determine the effect of sample groupings based on DWDS sample location, DWDS section, and season.[38,39] β 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.[40] All plots were constructed using the ggplot2 package.[41] To identify the contribution of environmental parameters and their combinations to microbial community structure, distance-based redundancy analysis (dbRDA) was used. The function dbrda() from R package "vegan"[42] 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 fraction 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 4921399 sequences post-quality control and a total of 10012 ASVs. Taxonomic classification of these ASVs revealed that bacteria dominated the microbial community [mean relative abundance (MRA) of 98.74 ± 0.02% across all samples] followed by archaea (MRA of 1.04 ± 0.01%). The bacterial community was primarily composed of Gammaproteobacteria (including Betaproteobacteriales), Actinobacteria, and Planctomycetes (Figure S1 and Table S4).

3.1. Treatment Processes Increased the Degrees of Similarity between Microbial Communities across the Two Drinking Water Systems. Despite the similar water quality (Table S5) and 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 β diversity measure) and shared only 22.5% of the detected ASVs (Figure S2). These differences could likely be a result of varying hydrological conditions at the two locations (i.e., river drawn R_SW vs from the dam S_SW), which may translate to the variable occurrence of low-abundance and transient taxa.[43] Despite harboring more ASVs over the period of the study (Figure S2), R_SW was less diverse than S_SW (ANOVA; $p < 0.05$) per time point (Figure S3 and Table S6). 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[44,45] as compared to S_SW. This is supported by the fact that R_SW exhibited higher temporal variability compared to that of S_SW based on pairwise β diversity measures (Figure S4).

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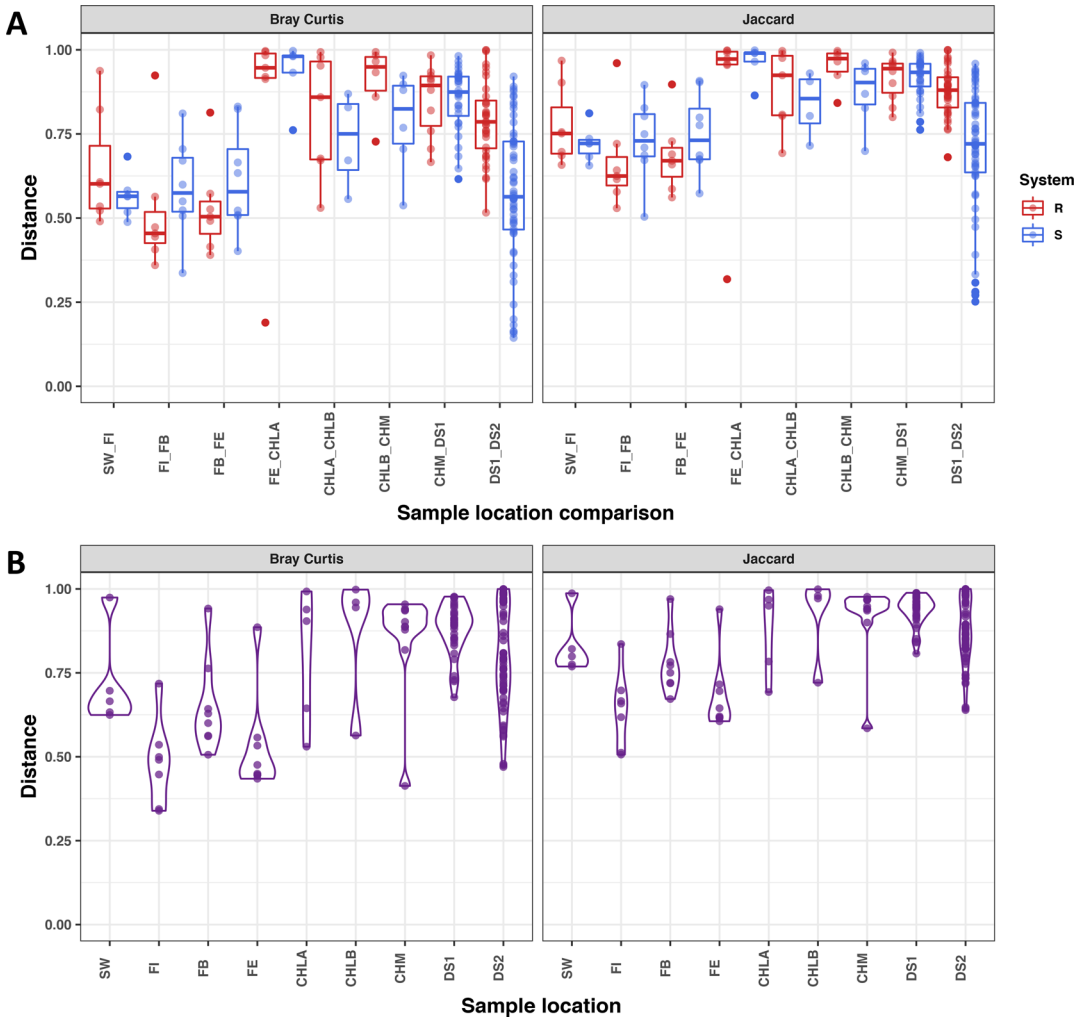

Figure 3. (A) Average pairwise β diversity comparisons of both the structure-based metric (Bray−Curtis) and the membership-based metric (Jaccard). Comparisons are between consecutive locations within each of the two systems for corresponding months. Sample abbreviations on the *x*-axis refer to comparisons 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 medium (FI_FB), filter bed medium 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 are colored red, and those for system S blue. (B) Direct pairwise β diversity comparisons (Bray−Curtis and Jaccard) between corresponding samples from the two systems. Pairwise β diversity comparisons include samples from the same month. Sample abbreviations on the *x*-axis refer to source waters (SW), filter inflows (FI), filter bed media (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). Means and standard deviations of each comparison are listed in Table S8. See Figure S5 for corresponding weighted and unweighted Unifrac plots.

The prechlorination 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 of >1%) such as Acidobacteria, Cyanobacteria, Verrucomicrobia, and Planctomycetes (Figure S1 and Table S4). The 33 most abundant ASVs, i.e., ASVs with an MRA of >0.5%, showed similar trends between the two DWTPs in terms of the increase or decrease in MRA with each sequential treatment step (Figure 2 and Table S7). These dominant ASVs within both DWTPs 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 α diversity measures in both DWTPs (Figure S3). On the basis of an ANOVA, these changes were found to be significant (for richness, F_{ST} = 19.67 and $p < 0.05$; for the Shannon diversity index, F_{ST} = 9.78 and $p < 0.05$; for the inverse Simpson diversity index, F_{ST} = 15.64 and $p < 0.05$; and for Pielou's evenness, F_{ST} = 4.79 and $p < 0.05$). Richness and diversity consistently decreased during treatment processes [excluding filter bed samples (FB)], with the most significant decrease immediately following chlorination (Figure S3 and Table S6). This shows that the decrease in microbial abundance and diversity, typical during treatment processes,[1,5,46−48] was reproducible in both systems.

β diversity analyses indicated that microbial communities became increasingly similar from source water through

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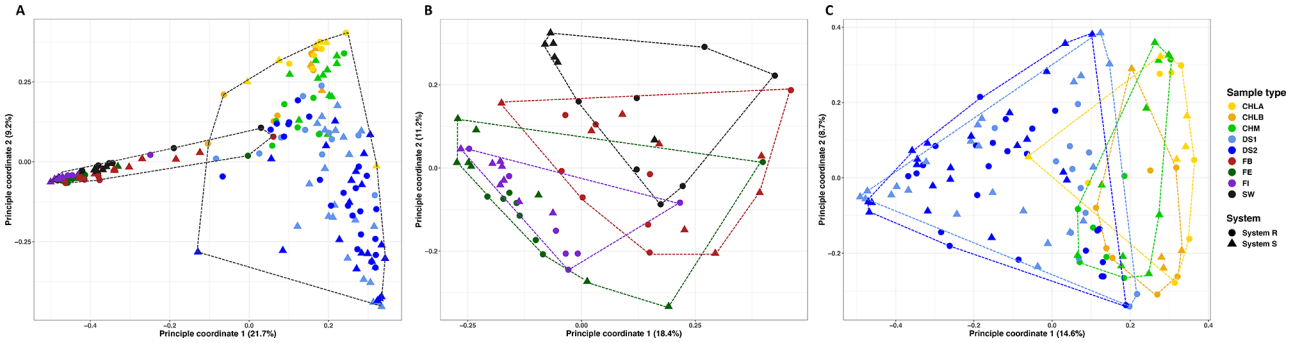

Figure 4. Principal-coordinate analysis plot (based on Bray−Curtis dissimilarity) showing the spatial and temporal variability of the bacterial community structure among (A) all samples from both systems, (B) within the two DWTPs, and (C) within the two corresponding DWDSs. Spatial groupings are shown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

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 (for the community structure, Bray−Curtis, 0.55 ± 0.17; for the community membership, Jaccard, 0.7 ± 0.13) (Figure 3A). Similar trends in community structure and membership were observed in weighted and unweighted Unifrac analyses (Figure S5). Following similar prefiltration treatments of the source water, filter influent samples between the two systems showed the greatest similarity (Figure 3B). Although filter bed microbial communities (R_FB and S_FB) showed higher degrees of dissimilarity in community structure and membership (Figure 3B and Table S8) between the two DWTPs, they were also significantly different from those of 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 degree of dissimilarity between the FB from the two DWTPs could also be attributed to the inherent high heterogeneity of attached growth microbial communities in the filter medium.[7,23] However, microbial communities were more similar between filter bed samples of the two DWTPs than the source waters that feed them. In addition, 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 the Degree of Dissimilarity across the Two Drinking Water Systems. In contrast to the DWTP (i.e., prechlorination) where treatment processes enhance similarity in the microbial communities between the two DWTPs, the rapid change in the microbial communities due to chlorination resulted in a significant increase in the degree of dissimilarity between locations within and across the two drinking water systems. Following chlorination, the microbial communities between the 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 β diversity measure) (Figure 3A). This significant decrease in the degree of similarity was also observed in weighted and unweighted Unifrac analyses (Figure S5). In addition, this significant change following disinfection was also observed as a shift in the relative abundance of dominant ASVs (Figure 2).

In addition, the degree of similarity in community structure and membership (Bray−Curtis, 0.77 ± 0.16; Jaccard, 0.86 ± 0.11) of chlorinated samples CHLA and CHLB increased slightly but they remained highly dissimilar, though not significantly different. However, following secondary disinfection, the degree of dissimilarity in microbial 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 β diversity measure) between chlorinated (CHLB) and chloraminated water (CHM) samples increased again in both systems (Figure 3A) [same trends observed for Unifrac analyses (Figure S5)]. This was consistent for both R and S systems. β diversity comparisons between paired samples from systems R and S immediately after chlorination (R_CHLA and S_CHLA, respectively) showed an increase in the degree of dissimilarity in both community structure and membership (Figure 3B and Table S8). This was also observed between R_CHLB and S_CHLB samples. Samples within the DWDS (CHM, DS1, and DS2) showed consistent temporal trends where the degree of dissimilarity of samples from both systems taken 6 months apart increased, consistent with the observed changes in temperature (Figures S4 and S6). However, temporal variability remained high within DS1 and DS2 samples where pairwise comparisons between consecutive months within each sample location were dissimilar in community membership (i.e., Jaccard, 0.80 ± 0.06; unweighted UniFrac, 0.67 ± 0.06) and structure (i.e., Bray−Curtis, 0.69 ± 0.13; weighted UniFrac, 0.52 ± 0.13) (Figure 3B).

Disinfection significantly reduces bacterial cell concentrations and has a substantial influence on community composition and structure.[3,10,24,46,49,50] While microbial communities prior to chlorination were similar between the two DWTPs, the microbial community composition and structure in both systems were highly dissimilar postchlorination. This dissimilarity was also observed on a temporal scale, where samples after disinfection showed greater temporal variability as compared to the prechlorinated 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 after disinfection and a greater degree of

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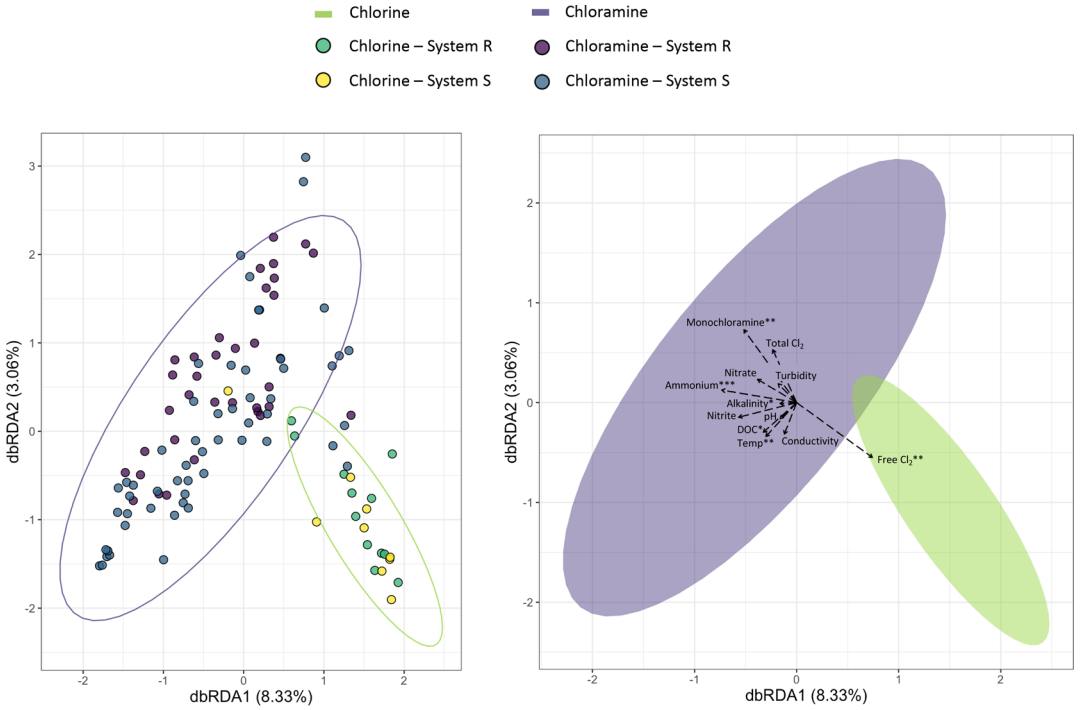

Figure 5. Relationship between measured water quality parameters and changes in the microbial community profile of locations of the distribution systems of the two systems where disinfectant residuals were applied. The plots represent the same distance-based redundancy analysis (dbRDA) based on Bray−Curtis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

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 a limited impact in preventing biofilm development and in some cases may even promote biofilm formation as a stress response.[51,52] Another explanation could be a decrease in microbial abundance and diversity due to disinfection may lead to the detection of low-abundance or rare taxa that may not have a 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 disturbs 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.[6,53,54] 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 impacts of the 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 the system (R or S) (Figure 4). For instance, the DWTP locations clustered independently from the DWDS across both systems (Figure 4A). Even within the DWTP, microbial communities did not cluster on the basis of the system from which they originated (Figure 4B). The same was consistent for the DWDS, where the chlorinated and chloraminated sections of the DWDS clustered close together (Figure 4C). In addition, the measured water quality parameters were highly similar at each location between the two DWTPs and DWDS (Figure S6, Figure S7, and Table S2). To assess the extent to which measured water quality parameters impacted the microbial community, we performed dbRDA by focusing primarily on the postchlorination samples (i.e., chlorinated and chloraminated DWDS). This was done because each section of the DWDS across the two DWDSs has multiple samples per month over the 8 month sampling period, resulting in significant sample size for both dbRDA and subsequent variance partitioning tests. Consistent with PCoAs, dbRDA revealed that samples clustered on the basis of the disinfectant residual type (chlorinated or chloraminated) and not on the basis of the system from which they originated. On the basis of dbRDA, 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, $p < 0.001$) (Figure 5 and Table S9). An increased concentration 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 water 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

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combined) identified as being most significant in the dbRDA (Figure 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 to 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 (Figure S8 and Table S10). Here, ammonium and monochloramine concentrations and temperature best explained the variability in chloraminated samples from both distribution systems as the disinfectant residual concentration and water temperatures did not differ greatly between the two lines (Figures S6 and S7). This is well supported as these variables have long been considered to be important factors in shaping the drinking water microbiome.[3,17,27] Therefore, the observed dissimilarity between the two distribution lines may be attributed 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.[55,56] 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.[51] 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 of these factors have a considerable impact on the drinking water microbiome.[3]

An increase in the degree of similarity was observed in locations toward 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 materials, 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.[57] 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 an increasing residence time in the DWDS was more pronounced in samples from summer and autumn. Here, increased 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, a seasonal increase in water demand could increase shear stress, resulting in increased detachment of similar established biofilms.[57]

In addition, where chlorine may be nonspecific in its action in reducing bacterial cell concentrations, chloramine (with the addition of ammonia) may be more selective toward microbial taxa capable of using ammonia as an energy source. This may explain the observed increase in *Nitrosomonas* sp. (ASV_6) in chloraminated DWDS samples from both systems. Here, the addition of chloramine as a secondary disinfectant has been shown to support the growth of nitrifying bacteria in a DWDS.[58] The long residence time and associated lower disinfectant residual concentrations, together with the release of ammonia through disinfection decay, result in an increased abundance of nitrifiers and therefore potential nitrification.[50] Thus, decreasing monochloramine residual concentrations associated with increased water age nearing the end of both DWDSs may also contribute to the growth of similar bacterial assemblages at these sites.

4. CONCLUSIONS

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 spatiotemporal dynamics of two independent DWTPs and their corresponding DWDSs within 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 predisinfection microbial communities. However, the differential impact of chlorination was highlighted in postdisinfection samples from the two systems, resulting in dissimilar microbial communities between the two systems. Lastly, the distribution system was observed to select for certain dominant taxa, thereby increasing the degree of 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 and low-abundance taxa. However, due to the lack of quantitative and viability analyses, the bacterial cell concentrations and the proportion of viable cells in each system are unclear. Nevertheless, this does not detract from the observed spatial and temporal changes in the microbial community composition and structure in the two systems using 16S rRNA gene sequence data. This study confirms the role of treatment and distribution in shaping the microbial community and suggests that under the same conditions, the membership and structure of the drinking water microbiome will be reproducible throughout the varying stages of the drinking water system. Here, investigating the dynamics of drinking water microbial communities is critical in revealing the impact of source water quality, environmental conditions, system infrastructure, and process operations on water quality. This provides utilities with the information they may need to adjust operational conditions and mitigate potential microbiome-associated problems. Ultimately, this will allow utilities to engineer and actively shape these communities for the production of optimal drinking water quality.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.1c00093.

Additional data regarding water quality parameters, sample collection, and the relative abundance of dominant bacterial groups (Tables S1−S5 and S7); additional α and β diversity measures and statistical data (Tables S6 and S8−S10); the taxonomic association of

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ASVs (Figure S1); ASVs shared among source waters (Figure S2); spatial and temporal changes in α and β diversity measures (Figures S3–S5); changes in water quality parameters (Figures S6 and S7); and Varpart analysis (Figure S8) (PDF)

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Notes

The authors declare no competing financial interest.

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