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Bacterial Community Structure in the Drinking Water Microbiome Is Governed by Filtration Processes

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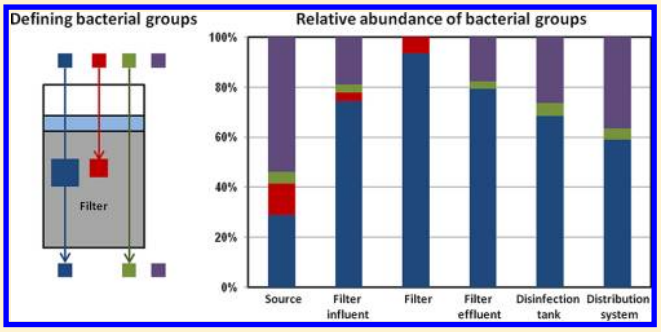
Supporting Information

ABSTRACT: The bacterial community structure of a drinking water microbiome was characterized over three seasons using 16S rRNA gene based pyrosequencing of samples obtained from source water (a mix of a groundwater and a surface water), different points in a drinking water plant operated to treat this source water, and in the associated drinking water distribution system. Even though the source water was shown to seed the drinking water microbiome, treatment process operations limit the source water's influence on the distribution system bacterial community. Rather, in this plant, filtration by dual media rapid sand filters played a primary role in shaping the distribution system bacterial community over seasonal time scales as the filters harbored a stable bacterial community that seeded the water treatment processes past filtration. Bacterial taxa that colonized the filter and sloughed off in the filter effluent were able to persist in the distribution system despite disinfection of finished water by chloramination and filter backwashing with chloraminated backwash water. Thus, filter colonization presents a possible ecological survival strategy for bacterial communities in drinking water systems, which presents an opportunity to control the drinking water microbiome by manipulating the filter microbial community. Grouping bacterial taxa based on their association with the filter helped to elucidate relationships between the abundance of bacterial groups and water quality parameters and showed that pH was the strongest regulator of the bacterial community in the sampled drinking water system.



INTRODUCTION

Bacterial communities in drinking water systems (DWSs) can play a positive role through biologically mediated chemical contaminant removal; most commonly implemented in filtration systems,[1] but can also have a negative impact if DWSs harbor potential pathogens[2,3] and by contributing to infrastructure deterioration due to biologically induced corrosion.[4] In an effort to minimize these negative aspects, most drinking water utilities try to limit microbial growth in the drinking water treatment plant (DWTP) and the drinking water distribution system (DWDS). Specifically, most filters are designed to remove turbidity and bacterial growth substrates to limit bacterial growth downstream from the filter and disinfection (e.g., ozonation, chlorination, chloramination, or UV treatment) is used to inactivate bacteria. Further, a disinfection residual is maintained in most DWDSs to prevent bacterial regrowth.[5] Despite these efforts, all DWSs harbor a vast diversity of bacteria[6,7] and bacterial concentrations in drinking water are estimated to be around 10^6–10^8 cells per liter.[6,8] As it is not possible to eliminate bacteria from drinking water with current treatment technologies, it is critical to identify the different types of bacteria and their relative abundance in DWSs, and to determine which water quality parameters and/or treatment processes shape the bacterial community structure in DWSs.

Previous studies have determined bacterial abundance at different points in a DWS,[6] elucidated seasonal and diurnal changes in bacterial community membership at multiple sampling locations,[8,9] and tried to identify sources of bacteria in the DWDS.[10] These studies have sought to answer questions such as "who is present?" and "how does composition change between sites or over time?". However, they generally did not evaluate the role of water quality or process operation in shaping community structure. Characterizing the drinking water microbiome without identifying the forces that influence it is not only challenging, but also represents a lost opportunity. While factors shaping bacterial community structure are undeniably site specific due to differences in plant configurations, operational practices, and water quality, developing an approach that can identify the forces that determine the bacterial community structure provides a framework that can be

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applied to most DWSs. To our knowledge, such an approach has not yet been developed.

To this end, we conducted a sampling campaign for the Ann Arbor, Michigan DWS, including multiple sample locations at the DWTP and DWDS. We used β-diversity analyses based on 16S rRNA based pyrosequencing to compare bacterial communities across sampling locations and seasons to determine (i) which source(s) and/or process(es) seed and shape the bacterial community in the DWTP and DWDS, and (ii) how water quality contributes to the structure of bacterial communities.

MATERIALS AND METHODS

Drinking Water Treatment Plant and Distribution System. The Ann Arbor DWTP supplies water to the City of Ann Arbor, Michigan. The two source waters treated at this plant consist of surface water from the Huron River and groundwater from local wells. The surface water to groundwater ratio varies through the year ranging from approximately 2:1 in the winter to 8:1 in the summer. Treatment includes lime softening, coagulation, flocculation, sedimentation, ozonation, dual media filtration, and addition of free chlorine and ammonia before distribution (Supporting Information (SI) Figure S1). The DWTP has 26 dual media filters consisting of a layer of granular activated carbon (GAC) on sand (bed height ratios range from 2:1 to 3:1) supported by gravel, garnet, or Leopold integral media support (IMS) caps (Zeilenpole, PA) at the bottom of the filters. At any given time, approximately 7−10 filters are operated with an empty bed contact time of less than 10 min. The filters are backwashed every 70−90 h (or after filtering 75−100 million gallons of water) with finished water containing chloramine at a concentration of approximately 3 mg Cl_2/L. Free chlorine is added to the filter effluents at a concentration of 5 mg Cl_2/L and, after a contact time of approximately 1 min, ammonia is added (∼1 mg/L of ammonia) to generate chloramine in the clear wells, which is used as the residual disinfectant in the DWDS. The pH of the finished water is maintained between 9.1 and 9.3, to ensure the formation of primarily monochloramine, as compared to di/trichloramines, as well as to ensure the stability of monochloramine in the DWDS.

Sampling, Sample Processing, And Chemical Analyses. The sampling campaign was conducted over six months in 2010, specifically April (26−29), June (8−10), July (5−8), August (16−19), September (14−16), and October (27−30). Samples were collected at seven points in the DWTP (SI Figure S1) and 13 points in the DWDS. All samples were bulk water samples, except for the dual media filter samples, for which GAC medium was collected directly from the top of three filter beds that were in use. Equal amounts of GAC media from each filter were combined to generate a single filter sample for each sampling time point. Bulk water samples were collected in 4 L sterile Nalgene polycarbonate bottles, which were transported on ice to the laboratory.

Upon arrival in the laboratory, samples for biomass analyses were filtered through sterile (by autoclaving) 0.22 μm polycarbonate membrane filters (Millipore catalog no: GTTP04700, Billerica, MA). Specifically, 250 mL of surface water, 1000 mL of groundwater, 1000 mL of filter influent, and 2000 mL each for the remaining samples were filtered, and the filter membranes with collected biomass were transferred to sterile microcentrifuge tubes and stored at −80 °C. All filtration equipment (Millipore), that is, filter manifold, funnels, and filter supports, were sterilized by autoclaving immediately prior to filtration. Water samples were analyzed immediately for total chlorine, pH, conductivity, and turbidity using standard methods.[11] Upon arrival in the laboratory, samples for additional chemical analyses were filtered through 0.2 μm nylon filters (Fisher catalog no: 09-719C, Chicago, IL) and stored at 4 °C for a maximum of 10−14 days. Samples were analyzed for total ammonia (NH_3 + NH_4^+-N), nitrite (NO_2^--N), nitrate (NO_3^--N), total organic carbon (TOC), phosphate-P, chloride, and sulfate according to standard protocols.[11]

DNA Extraction. The nucleic acid extraction protocol was optimized for reliable and reproducible DNA recovery from the postfiltration samples including the DWDS samples for which the bacterial abundance was expected to be very low. The volume of sample filtered for postfiltration samples, as listed above, was determined after an extensive DNA extraction protocol optimization (SI Figure S2). Briefly, the filters with collected biomass were incubated with 1 mL of 25:24:1 (v:v:v) phenol:chloroform:isoamyl alcohol (Sigma-Aldrich, St. Louis, MO) in a 70 °C water bath for 5 min to allow for breakup of the polycarbonate filter material. Following this, a previously described DNA extraction protocol was used,[12] with the addition of two more 2-min bead beating steps[13] such that the second and third beating steps were preceded by replacement of the aqueous phase with fresh lysis buffer to minimize shearing of the DNA already recovered in the aqueous phase. The extracted DNA was purified, precipitated,[12] and dissolved in 50 μL of sterile nuclease free water and stored at −80 °C.

PCR Amplification and 454-Sequencing. PCR amplification was conducted in triplicate using Roche 454 titanium-compatible primers targeting bacterial 16S rRNA genes as described earlier.[14] Triplicate PCR products were pooled and purified using a QiaQuick PCR purification kit (Qiagen Inc., Valencia, CA). The amount of PCR product from each sample was quantified in triplicate using a Quant-iT dsDNA assay kit (Invitrogen, Carlsbad, CA) on a Nanodrop 3300 (Thermo Scientific, Wilmington, DE).

The PCR products of each sample were further combined to generate 18 final PCR pools for sequencing (summarized in SI Table S1). Specifically, two monthly samples from each sampling location were combined to generate seasonal samples, that is, April−June, July−August, and September−October were pooled to form Spring, Summer, and Fall samples, respectively. Some of the pooled seasonal samples were further combined to limit the number of samples to be sequenced. Specifically, surface and groundwater samples were combined to form one source water sample, clear well and reservoir samples were combined to generate a disinfection tank sample, and all 13 DWDS samples were combined to generate a DWDS pool. This resulted in six PCR pools for each of the three seasons (source water, filter influent, filter, filter effluent, disinfection tank, and DWDS). These 18 samples were sequenced at the Michigan State University Research Technology Support Facility (East Lansing, Michigan) on $^1/_8$ pico-titer plate (60 samples unrelated to the current study were also included).

Data Processing. All data processing was conducted using Mothur[15] and focused primarily on β-diversity based analyses. A total of 5717 sequences were obtained for the 18 samples sequenced for this study. The sequences were trimmed to remove primers and barcodes, quality filtered, and chimera checked as defined previously,[14] resulting in a total of 5623 sequences in the final data library. The numbers of quality

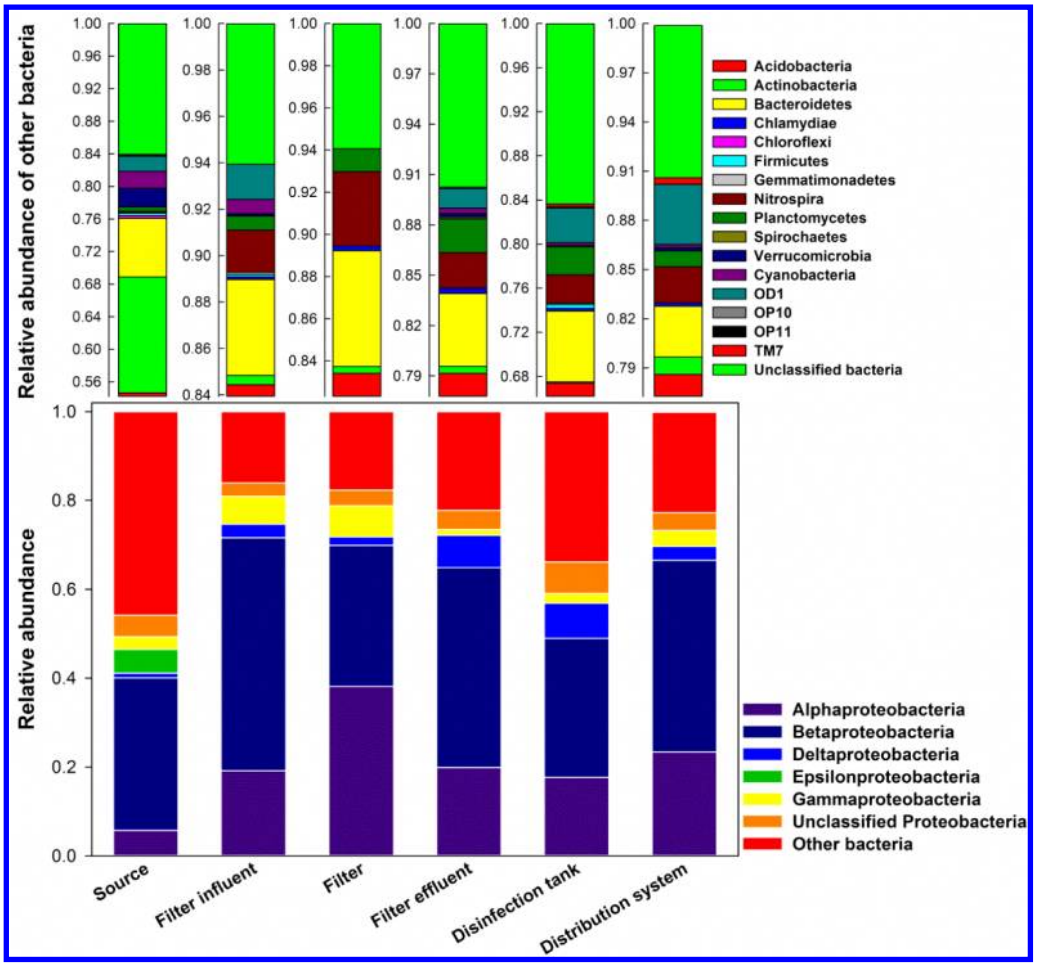

Figure 1. Relative abundance of bacterial phyla at the six sampling locations averaged over three seasons. The dominant phylum, *Proteobacteria,* is not represented in the upper panel, but is divided into its five classes in the lower panel. The upper panel shows the distribution of all bacteria other than *Proteobacteria*, the sum of which are shown in red in the lower panel. Classification was conducted by using the RDP training set provided through Mothur[15] using a confidence level cutoff of 75%.

filtered and chimera-free reads in each sample are provided in SI Table S2. While the sequencing effort in this study was not very deep, it has been shown that β-diversity based analyses can be effectively performed without the requirement for deep sequencing.[14,16] Specifically, deeper sequencing does not improve the accuracy of β-diversity estimates, but only improves precision.[14]

β-Diversity and Statistical Analyses. The sequences were clustered using the average neighbor approach[17] to form operational taxonomic units (OTUs) at 97% sequence similarity cutoff (3% sequence divergence). All samples were normalized to ensure equal number of sequences in each sample, prior to further analyses.[18] Phylogenetic trees were constructed by using the Clearcut program[19] and the parsimony test[20] was performed to determine significance of structural similarity among communities across sampling locations and seasons. The Fast UniFrac online tool[21] was used to estimate weighted (WUnF) and unweighted (UWUnF) UniFrac metrics,[22] to perform Jackknife clustering (1000 iterations), and to carry out principal coordinate analyses (PcoA). Classification of reads was performed using an RDP training set[23] using a confidence level cutoff of 75%. Linear regression analyses to determine correlation between water quality parameters and the relative abundance of relevant OTUs were determined using PAST.[24]

RESULTS AND DISCUSSION

Drinking Water Systems Host Diverse Bacterial Communities. Figure 1 shows the phylum level classification for all sequences detected in the six sampling locations averaged over the three seasons for which samples were obtained from the Ann Arbor DWS. The DWS harbored a large diversity of bacterial phyla with 14, 14, 9, 14, 16, and 13 different bacterial phyla detected in the source water, filter influent, filter, filter effluent, disinfection tank, and DWDS, respectively. The seven dominant phyla for all sampling locations combined, in descending order of their relative abundance, were *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Nitrospira*, OD1, *Planctomycetes*, and *Acidobacteria*. *Proteobacteria* constituted approximately 74% of the total number of OTUs for all samples combined, whereas its subclasses were represented in the following order of decreasing abundance: *Betaproteobacteria* (40%), *Alphaproteobacteria* (21%), *Deltaproteobacteria* (4%), *Gammaproteobacteria* (4%), and *Epsilonproteobacteria* (1%), with an additional 4% of unclassified *Proteobacteria*. The relative abundance of the remaining six dominant phyla averaged 2.3 ± 1.5%, whereas ten other phyla represented from 0.02% to 0.6% of the total number of OTUs. Despite the dominance of *Betaproteobacteria* and *Alphaproteobacteria,* which is consistent with previous observations,[9,10] the DWS contained a vast diversity of bacterial groups. When moving from the head of

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the DWTP to the DWDS, the relative abundance of the *Alphaproteobacteria* increased from approximately 6% in the source water to 38% in the filter and 23% in the DWDS. In contrast, the relative abundance of *Betaproteobacteria* did not change as dramatically and ranged from 34% in the source water to 43% in the DWDS. Even though *Epsilonproteobacteria* constituted approximately 5% of the bacterial community in the source water, none of the sequences in the DWTP and DWDS samples fell in this group. The greatest decrease in relative abundance when moving through the DWTP was seen for the phylum *Actinobacteria*, specifically the *Actinomycetales* order. Members of this order constituted approximately 14% of the source water samples and represented only about 0.5% in the samples in the DWTP and DWDS for all three seasons.

It is important to note that the detection of bacterial OTUs using DNA based analyses does not ascertain that the corresponding bacteria are viable or active.[25,26] Numerous approaches have been recommended for discrimination of "live" and "dead" bacteria,[26−30] but no single method or a combination of methods can guarantee the selective detection of viable bacteria. Alternatively, some studies have suggested that targeting RNA instead of DNA would limit the detection to active bacteria,[31,32] under the assumption that RNA is only synthesized in actively growing cells. However, lack of activity (dormancy) by bacteria within the DWS does not necessarily mean that they cannot become active outside the DWS, for example, inside the human host. As a result, the selective detection and characterization of viable bacteria is a challenging task and an important research question by itself. Previous research has shown that increasing the size of the target amplicon selectively discriminates between intact and damaged DNA and that larger amplicons tend to correlate with viability.[29,30] Hence, we have chosen DNA damage as the primary mechanism for discrimination between live and dead cells by selecting pyrosequencing PCR primers that generate relatively large amplicons (~600 bp).

The Source Water Seeds the Drinking Water System. To determine the ability of the source water to seed the DWS, we estimated the similarities in community membership across the six sampling locations. Specifically, we binned the detected OTUs into three categories, namely core, variable, and unique OTUs. An OTU was categorized as a core OTU,[33] if it was found in all sampling locations at even one seasonal time point. An OTU was classified as a variable OTU if it was not detected at all locations, but was found in more than one location at any time point. Unique OTUs were defined as OTUs that were detected only in one sampling location. Of the 606 detected OTUs, 4%, 36%, and 60% were classified as core, variable, and unique, respectively. Deeper sequencing (more sequences per sample) would likely increase the number of detected OTUs, but would only improve the representation of rare taxa and would not have a significant impact on the structure of the drinking water microbiome[14] (SI Figure S3). SI Figure S4 shows the phylogenetic relationships among all core OTUs, their relative abundance across all sampling points, and a heatmap showing the variation in relative abundance from source water to DWDS. These core OTUs likely exhibit a variety of functional traits that allow their survival in a range of environments, from eutrophic conditions in one of the source waters (surface water) to substrate limited conditions accompanied by chronic disinfectant stress in the DWDS. This is highlighted by the fact that some OTUs with high similarity (>97%) to the core microbiome have been recovered from disparate environmental samples, ranging from *Beijerinckiaceae* in soils[34] to skin associated *Rhodospirillaceae*[35] and *Nitrospira* in drinking water[36] to *Rhodobacteraceae* in wastewater systems.[37]

The Filter Shapes the DWDS Bacterial Community. The source water and filter bacterial communities clustered independently from the postfiltration samples for two out of the three seasons (SI Figure S5A) and this was supported by a high Jackknife fraction (>90% support). When data from all three seasons were combined, the postfiltration samples clustered together, as compared to the prefiltration samples which were separated on distinct nodes (SI Figure S5B). Additionally, a pairwise comparison across sampling locations shows that the bacterial community structure for the source water sample was distinct from all of the DWS bacterial community structures (parsimony $p < 0.001$) within each season (data not shown) and also when sequences from all three seasons were combined (SI Figure S5B). These results clearly indicate that even though the source water seeds the DWS, it does not shape the bacterial community structure of the drinking water microbiome. Further, parsimony analysis indicate only three pairwise comparisons with significantly similar community structure (filter−filter effluent, filter effluent-disinfection tank, and disinfection tank-DWDS) (SI Figure S5B), indicating conservation of community structure as the water moves from the filter into the DWDS in contrast to the prefiltration samples.

Evidence of clear separation of dynamics between the pre- and postfiltration samples can be seen by comparing the stability of community membership and structure at each sampling location over the three seasons (Figure 2). Higher UwUnF and WUnF similarities indicate greater stability of the bacterial community membership and structure, respectively. The source water exhibited the lowest stability in terms of shared membership and structure, consistent with the observation that seasonal changes in water quality were greatest for the source water (SI Table S3). In contrast, the filter bacterial community exhibited the greatest amount of shared membership across seasons, that is, ~37% shared membership and greater than 75% shared structure. This clearly shows that the filter maintained a more stable bacterial community as compared to the other sampling locations, despite the seasonal changes in source water and intermittent exposure to chloramines present in the backwash water. Furthermore, all postfiltration samples exhibited a higher stability than the prefiltration samples. It is likely that the stable filter bacterial community continuously seeds the postfiltration samples through sloughing of biofilms attached to the filter media, which stabilizes the postfiltration bacterial communities.[38]

We constructed PcoA plots using pairwise WUnF distances between all sampling locations and seasonal time points (Figure 3). Three key features were noted for the Ann Arbor DWS: (1) the strikingly similar spatial dynamics in community structure irrespective of the season, (2) the spatial dynamics of the pre- and postfiltration samples are distinctly different (changes in prefiltration samples are primarily along principal coordinate 1, whereas changes in postfiltration samples are less substantial, but take place along principal coordinate 2), and (3) the stability of the filter bacterial community compared to the other sampling locations. This further reaffirms the notion that the filter exerts a stabilizing force on the postfiltration communities and seeding by the filter drastically changes the dynamics of the

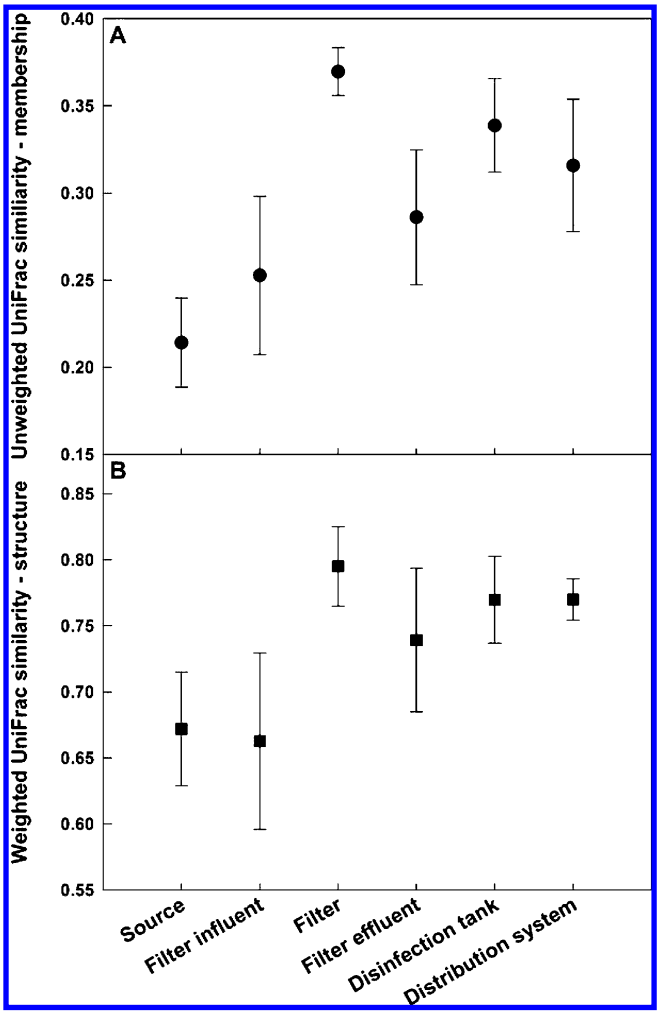

Figure 2. Mean UwUnF (A) and WUnF (B) similarities at each sampling location averaged across the three seasons. Error bars indicate standard deviations. Lower similarities in UwUnF and WUnF means lower stability of the bacterial community at each sampling location, whereas higher similarities indicate stable membership and structure across the seasons.

postfiltration samples such that they are largely decoupled from the seasonal effects seen in the prefiltration samples.

To further understand the influence of the filter on the postfiltration communities and to provide a framework to analyze filter colonization as a potential mechanism by which bacteria dominate the drinking water microbiome, all OTUs were grouped based on their association (or lack thereof) with the filter into four different categories as follows. The group of leaky colonizers (LC) consisted of OTUs detected in the filter and all postfiltration samples. The LC group presents the greatest potential to seed the postfiltration samples as they are derived from a stable reservoir of bacterial biomass on the filter that can be sloughed off into the filter effluent and eventually into the DWDS. Their abundance on the filter can be replenished by growth on the filter, through seeding from the source water over the long-term, or through reseeding with backwash water over the short-term. The group of strict colonizers (SC) includes OTUs that are detected in the filter, and possibly in prefiltration samples, but never in postfiltration samples. These taxa are not important from the perspective of the DWDS bacterial community, but rather they may be direct competitors with the LC group from a filter colonizing perspective. Since these OTUs are absent from postfiltration samples, they may primarily represent biofilm forming bacteria that are not competitive in a planktonic state. The third category was the pass-through (PT) group defined as OTUs that were found in all pre- and postfiltration samples, but were never detected in the filter samples. In contrast to the SC group, the PT group represents bacteria that are either not able to form biofilms, or are weak competitors with LC and SC groups in biofilm mode and thus are unable to colonize the filter. The final category of OTUs was the filter-independent variable (FIV) group. These OTUs were never detected on the filter media and not at all of the other five sampling locations. The FIV group were primarily composed of OTUs that were unique to each sampling location (>80%) and some OTUs that were sporadically detected in more than one sampling location.

Figure 4A and B show the relative abundance of each of the four groups and their contribution toward community membership at the six sampling locations, respectively. The LC group constitutes approximately 75% of the filter influent, 93% of the filter media, 80% of the filter effluent, 68% of the disinfection tank, and approximately 59% of the DWDS bacterial communities (Figure 4A). These high values translate into the LC group comprising approximately 67% of the drinking water microbiome, but only 16% of the total membership (Figure 4B). The LC group accounts for approximately 25% and 20% more of the membership and relative abundance, respectively, of the DWS as compared to the core microbiome. This is because an analysis based on the core microbiome approach is restricted by the time frame of sampling, that is, it is unable to address historical seeding of the DWS by the source water. Rather, by binning the taxa based on their association with the filter, which acts as a stable reservoir, we are able to evaluate taxa that may have been introduced into the filter from the source water before the commencement of the sampling campaign. Approximately 42% of the LC group were composed of *Alphaproteobacteria*, with the majority (>70%) belonging to three bacterial orders (*Rhizobiales* > *Rhodospirillales* > *Sphingomonadales*). Another 16% of the LC group was composed of *Betaproteobacteria* with the majority belonging to the order *Burkholderiales*. The SC group was primarily composed of *Proteobacteria* (*Betaproteobacteria* > *Alphaproteobacteria* > *Deltaproteobacteria* > *Gammaproteobacteria*), *Bacteroidetes*, and *Actinobacteria* phyla. The PT group was composed of *Proteobacteria* (*Alphaproteobacteria* = *Betaproteobacteria*) > OD1 > *Bacteroidetes*, and all sequences in the *Bacteroidetes* phylum consisted of *Sphingobacteria*. Though this broad categorization helps to determine the contribution of the filter in shaping the DWDS bacterial community, physiological differences between different OTUs are also equally important. The three most dominant OTUs in the complete data set were successfully classified to the genus level. These OTUs were *Acidovorax* and *Hydrogenophaga*, both belonging to the family *Comamonadaceae* in the *Burkholderiales* order, and *Denitratisoma*, a member of the *Rhodocyclaceae* family in the *Rhodocyclales* order (confidence thresholds for genus level assignments were: 92 ± 3%, 85 ± 11%, and 78 ± 4.5%, respectively). Figures 4C–E show the relative abundance of each of these genera at the different sampling locations. *Hydrogenophaga* dominated the filter microbial community suggesting this genus has a competitive advantage in biofilms. In contrast, *Acidovorax* was the dominant genus in all bulk water sample locations. In contrast to *Acidovorax* and *Hydrogenophaga*, whose relative abundance was significantly ($p < 0.05$) lower in the disinfection

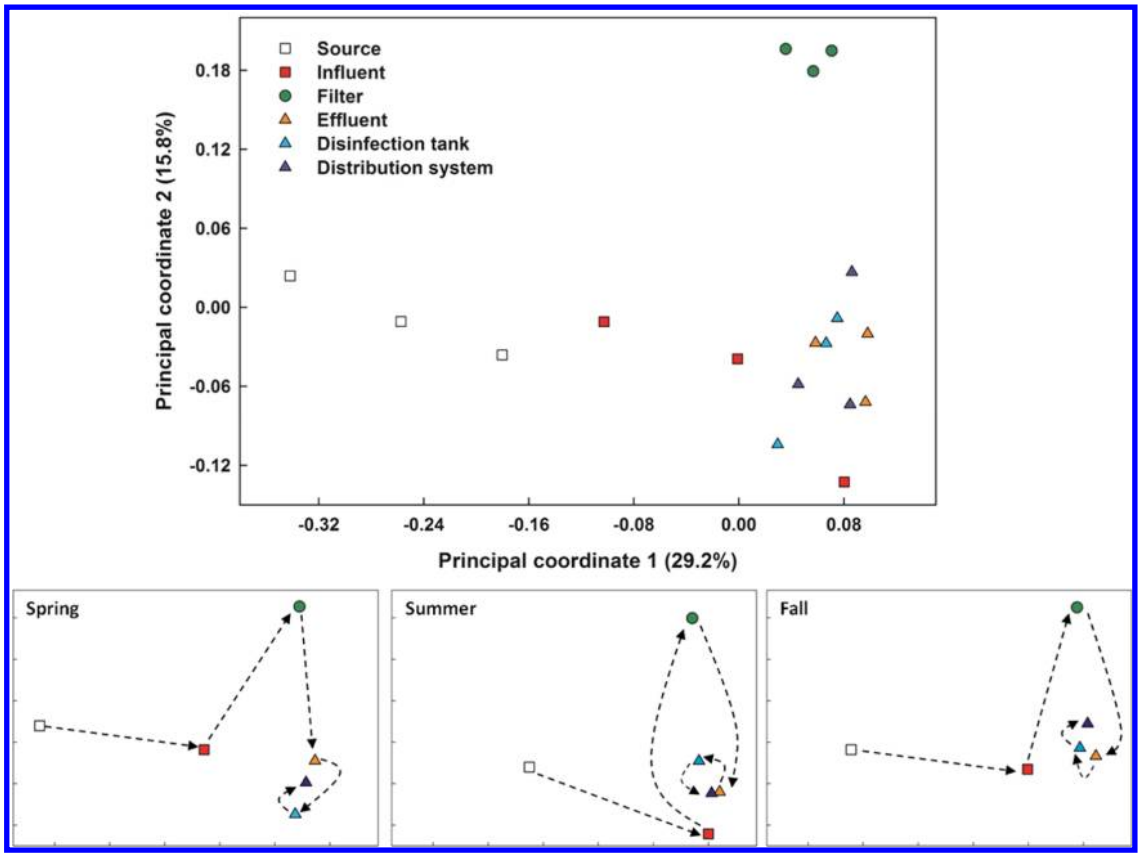

Figure 3. PcoA plot generated using WUnF metrics for all sampling locations for the three seasons is shown in the upper panel. The lower panels show the three seasons separated to allow for better visualization of the movement of water from source to the DWDS.

tank as compared to the filter effluent, *Denitratisoma* levels were less impacted by disinfection and remained relatively consistent in all postfiltration samples including the DWDS. Sequences associated with *Denitratisoma* have been retrieved from sites contaminated with chlorinated compounds,[39] causing us to speculate that its increase in relative abundance could be attributed to greater chlorine resistance or the greater ability to utilize chlorinated organic compounds generated by the reaction of chlorine with residual organic compounds.

Grouping Bacterial OTUs Based on Their Association with Filter Media Highlights the Influence of Water Quality Parameters. To further determine if the binning of OTUs based on their association with the filter allows for deciphering of physiological traits, we performed linear regression of the relative abundance of each group at each sampling time point and location against water quality parameters. Table 1 shows the correlation coefficients and their significance values. Among the water quality parameters determined, pH exhibited the strongest effect on the relative abundance of different groups. Higher pH values were more favorable for the LC group, whereas the SC group exhibited a negative correlation with pH. Hence, the nondetection of the SC group in the postfiltration samples may be related to the high pH of finished water (9.1−9.3), making the SC group weak competitors in the bulk water phase in postfiltration samples. In contrast, their ability to survive and grow on the filter media could be due to occurrence of localized pH gradients in the filter media. The SC and LC groups also show opposite correlations with TOC and phosphate-P. Specifically, the LC group was present at a higher relative abundance in low TOC and high phosphate-P environments, whereas the SC group was able to compete better in high TOC and low phosphate-P environments. These observations confirm the ability of the SC group to survive on the filter where the TOC concentrations may be higher due to adsorbed particulates and the bioavailable phosphate-P concentrations may be lower as the hexametaphosphate (added immediately before filtration primarily as a corrosion inhibitor in the DWDS) may not have been broken down yet. In the postfiltration samples, the TOC concentrations are lower and the bioavailable phosphate-P concentrations are higher due to breakdown of the hexametaphosphate to orthophosphate, thus favoring the LC group. In contrast to the LC and SC group, the PT group shows no significant correlation to any measured environmental parameter. This could indicate that the bacteria in the PT group may exhibit highly flexible or diverse physiological traits, so as to survive at low relative abundance in both eutrophic conditions in one of the source waters (surface water) and oligotrophic conditions in the DWDS. It is possible that the only reason they are not able to dominate the system, despite their potential metabolic flexibility, is the inability to colonize the filter through biofilm formation. The correlation coefficients and significance values for the FIV group are similar to those of the SC group. The variable and sporadic occurrence of the FIV group in conjunction with its similarities with the SC group, may indicate that these OTUs are remnants of the SC group from past filter colonization events. This analysis shows that water quality parameters play an important role in shaping the bacterial communities not only in specific processes,[10,38,40] but also within the entire drinking water microbiome. Nonetheless, the presence of large numbers of OTUs can complicate the ability to decipher relationships between water quality

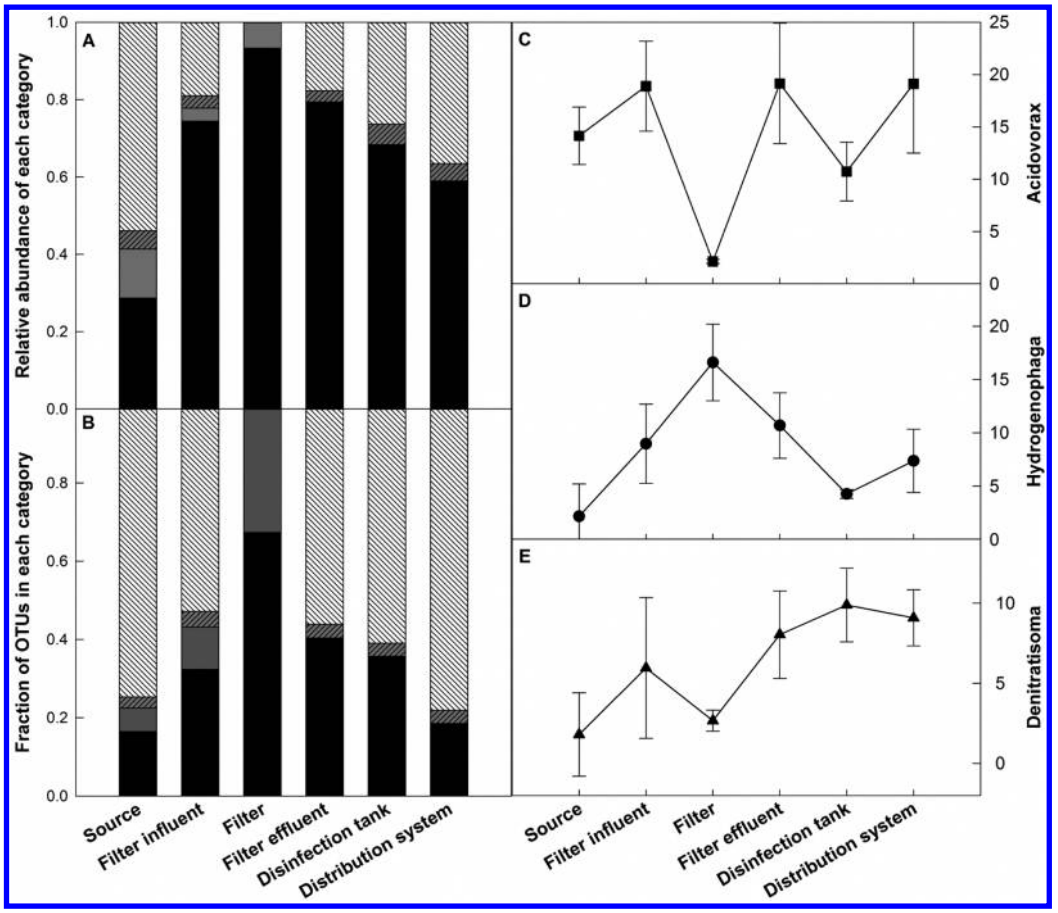

Figure 4. The contribution of the leaky colonizers (LC) (black), strict colonizers (SC) (gray), pass through (PT) (gray hashed), and filter independent variable (FIV) (white hashed) groups toward the relative abundance (A) and membership (B) of bacterial communities at each sampling location. Panels C, D, and E show the mean relative abundance for the three seasons at each sampling location of the three dominant OTUs, *Acidovorax*, *Hydrogenophaga*, and *Denitratisoma*, respectively, belonging to the leaky colonizer group. Error bars indicate standard deviations of the relative abundances for the three seasons.

Table 1. Linear Regression Analysis Showing Coefficients and Statistical Significance Values for the Correlation between Water Quality Parameters and the Relative Abundance of Each of the Four Bacterial Groups Defined in Terms of Their Association with the Filter

parameter	leaky colonizers (LC)	strict colonizers (SC)	pass-through (PT)	filter-independent variable (FIV)
total organic carbon	−0.74[b]	0.76[a]	0.34	0.61[a]
total ammonia-N	0.23	0.59	0.38	−0.20
temperature	0.36	−0.47	0.09	−0.35
pH	0.96[d]	−0.90[d]	0.44	−0.91[d]
chloride	−0.21	0.02	0.33	0.23
sulfate	−0.58[a]	0.36	−0.09	0.70[b]
phosphate-P	0.69[b]	−0.79[a]	−0.06	−0.68[b]

[a]Significance < 0.05. [b]Significance < 0.01. [c]Significance < 0.001. [d]Significance < 0.00001.

parameters and relative abundance of OTUs. This is primarily due to the fact that DWSs are complex systems with a diverse range of physical and chemical environments containing complex microbial communities for which a specialized function is often not discernible. Hence, it is inherently difficult to correlate a particular water quality parameter with the relative abundance of a specific OTU, since many OTUs may exhibit similar responses to changes in water quality. Rather, the effect of environmental characteristics can be effectively determined if the OTUs are lumped together into groups based on an ecologically relevant survival strategy, such as filter colonization as presented in this study. This approach allowed us to show the significance of water quality parameters based on their correlation with the relative abundance of the four groups defined with respect to their association with the filter was in the following order: pH > TOC > phosphate-*P* > sulfate > total ammonia-N, temperature, chloride.

In conclusion, the ability of the filter microbial community to shape the Ann Arbor drinking water microbiome and the capacity of the filter colonizing LC group to dominate it presents a possible opportunity to assist in controlling and managing the microbial quality of the DWDS. The correlations of the water quality parameters with the relative abundance of different bacterial groups clearly indicate the operational possibility to control the bacterial community structure by altering water quality parameters. For example, it may be possible to manipulate the filter colonizing LC group through various operational strategies to ensure that it is populated by (1) innocuous bacteria, (2) bacteria that can effectively outcompete risky bacteria (e.g., pathogens, corrosion, or odor causing bacteria) either on the filter or in the DWDS, or (3) bacteria that are beneficial to human health. Additionally, the

potential to control the DWDS by focusing on the filter would centralize risk management in DWSs, thus providing the potential for reducing operational costs and uncertainties.

■ ASSOCIATED CONTENT

Supporting Information

Additional data, figures, and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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