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From source to filter: changes in bacterial community composition during potable water treatment

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Abstract

Rural communities rely on surface water reservoirs for potable water. Effective removal of chemical contaminants and bacterial pathogens from these reservoirs requires an understanding of the bacterial community diversity that is present. In this study, we carried out a 16S rRNA-based profiling approach to describe the bacterial consortia in the raw surface water entering the water treatment plants of two rural communities. Our results show that source water is dominated by the *Proteobacteria*, *Bacteroidetes*, and *Cyanobacteria* with some evidence of seasonal effects altering the predominant groups at each location. A subsequent community analysis of sections through a biological carbon filter in the water treatment plant revealed a significant increase in the proportion of *Proteobacteria*, *Acidobacteria*, *Planctomycetes*, and *Nitrospirae* relative to raw water. Also, very few enteric coliforms were identified in either the source water or within the filter, although the abundance of *Mycobacterium* was high, and was found throughout the filter along with *Aeromonas*, *Legionella*, and *Pseudomonas*. This study provides valuable insight into bacterial community composition within drinking water treatment facilities, and the importance of implementing appropriate disinfection practices to ensure safe potable water for rural communities.

Introduction

Globally, many communities rely on small surface water reservoirs as their sole water source for the production and distribution of potable water (The World Bank 2016; Bogdan and Kulshreshtha 2016; Hardie and Alasia 2009). Such reservoirs are recharged with runoff water during seasonal precipitation events as well as with spring snowmelt flow from surrounding land. The systems are open and vulnerable to environmental conditions and surrounding land use activities, making the water quality variable and sometimes unpredictable (Cessna et al. 2015; Delpla et al. 2015; Gough et al. 2016; McLeod et al. 2014). One concern is the threat posed by waterborne pathogens, such as *Campylobacter*, *Legionella* and various enteropathogenic coliforms, such as *E. coli* and *Shigella*, which can be introduced into watersheds by runoff from agricultural lands contaminated with animal wastes (Ferguson et al. 2003; Leclerc et al. 2002; World Health Organization 2011). Also of concern are chemical contaminants, including pesticides, organics, biotoxins and heavy metals, which pose a direct risk to human health (Kolpin et al. 2002). Consequently, appropriate strategies must be utilized for microbial disinfection as well as chemical contaminant remediation to ensure safe, potable water.

Ozone-assisted biofiltration systems reduce or eliminate a wide range of natural and anthropogenic and other organic pollutants in raw water by exploiting the metabolic and filtration characteristics of microorganism-derived biofilms that form on a medium of typically sand, anthracite, or granular activated carbon (GAC) (Basu et al. 2016; Gottinger et al. 2011; Liao et al. 2015; Rochex et al. 2008; Zhang et al. 2011). These filters facilitate the oxidation or biodegradation and adsorption of organics such as phenol, disinfection byproducts, pharmaceuticals, and heavy metals (Camel and Bermond 1998; Gu et al. 2016; Jang et al. 2008; Kim and Kang 2008; Niquette et al. 1998; Stackelberg et al. 2007; Ternes et al. 2002; Xiaojian et

al. 1991; Zhang et al. 2009). In some instances, more than half of the organics present in raw water, including pesticides, sterols, flame retardants, and pharmaceuticals have been reported to be removed by GAC filters (Stackelberg et al. 2007). But, biofilter efficiency depends not only on the properties of the filter medium, such as porosity, degree of compaction, and water retention capacity, but also its capacity to host and sustain a robust microbial population that can enhance contaminant removal (Basu et al. 2016; Song et al. 2015; Srivastava and Majumder 2008). Microbial communities that establish within the filter metabolize and precipitate contaminants through biodegradation and oxidation-reduction reactions (Zhu et al. 2010), and are a function of the diversity of the community from which they are drawn, as well as substrate availability (Basu et al. 2016; Curtis and Sloan 2004; Liao et al. 2015; Liao et al. 2016). The subsequent performance of the filter communities appears to be influenced by a multitude of factors that include species richness, dynamics, evenness, and functional redundancy of the bacterial community (Basu et al. 2016; Boon et al. 2011; Briones and Raskin 2003; Fish et al. 2015), making an understanding of the composition and interplay of microbial communities in source water, as well as within biofilters essential for enhancing drinking water treatment and water source management.

Recent analyses examining influent, effluent and biofilters of drinking water treatment facilities have yielded new insight into the predominant microbial communities influencing drinking water remediation. A 15-month survey of the bacterial communities in a water treatment plant (WTP) and corresponding distribution system in Michigan revealed that the *Alpha*-, *Beta*-, and *Gammaproteobacteria* predominated, along with candidate phylum OD1 (*Candidatus* Parcubacteria) (Harris et al. 2004; Pinto et al. 2014). *Betaproteobacteria* dominated during the summer months while *Alphaproteobacteria* dominated in winter, with *Acidovorax*

being a dominant Betaproteobacterial group (Pinto et al. 2014). Bacterial community structure correlated with various water quality parameters, including pH, ammonium, sulfate, phosphate, and carbon concentrations, which in turn, were correlated with season (Pinto et al. 2014). These results were consistent with another microbial survey of a WTP in Australia, which evaluated microbial diversity at different stages along the treatment train (Shaw et al. 2015). This work showed that bacterial consortia were altered after disinfection, suggesting treatment was effective, although several bacterial groups known to contain pathogenic species were identified in samples containing high concentrations of disinfectant (Shaw et al. 2015). A further analysis revealed that bacteria colonizing the biofilter were composed of predominantly *Alphaproteobacteria*, *Betaproteobacteria*, *Bacteroidetes*, and *Actinobacteria*, with *Acidovorax*, *Hydrogenophaga*, and *Denitratisoma* being found throughout the system, both pre- and post-disinfection (Shaw et al. 2015). The filter communities were shown to be strongly correlated with water pH, and to a lesser extent, carbon and phosphorus (Shaw et al. 2015). Notably, some effluent samples were shown to be more similar to source water than to post-disinfection water, suggesting that microbial repopulation of water occurred later within the distribution system (Shaw et al. 2015). This not only reinforces the importance of maintaining disinfectant residuals throughout the system, but also reinforces the importance of understanding microbial community composition given that nitrifying bacteria like *Nitrosomonas* may metabolize monochloroamine disinfectants and reduce residuals (Berry et al. 2006; Maestre et al. 2013). Thus, a fundamental understanding of bacterial community dynamics of these systems, including the links between filter communities and source water allows for enhanced oversight and optimization of filter efficiency for the production of safer, potable water.

In this study, we used 16S rRNA profiling to describe the bacterial communities of water entering the drinking water treatment plants of the village of Osage and the hamlet of Benson in rural Saskatchewan, both of which use surface water as their water source. We show that the bacterial consortia in each water source are compositionally distinct, and that the predominant bacterial groups change with seasonality. A further evaluation of the microbial communities in three transects within a biological activated carbon (BAC) filter revealed a dramatic shift in community composition from raw water, and that *Legionella*, *Pseudomonas*, *Aeromonas*, and especially *Mycobacterium* established within the filter. Our work suggests that BAC filters may provide a suitable niche favouring the accumulation of some bacteria, making disinfection post-filtration, and the maintenance of disinfectant residuals throughout the distribution system critical to ensuring safe potable water for rural communities.

Materials and Methods

Study Sites

Two small rural communities, the village of Osage (population 20) and the Hamlet of Benson (population 95), have potable water treatment plants whose ozone-assisted biofiltration systems were underperforming according to operating specifications. These communities are home to the longest running and provincially regulated ozone-assisted biofiltration plants in the province. The Osage WTP has a design flow rate of 11 m³/day and includes one 0.66 m diameter roughing filter, one 1.75 m diameter biological sand filter and one 1 m diameter BAC filter (Zanacic et al. 2016). A re-circulation system recycles non-chlorinated water at 0.6 m³/hr through the BAC filter to increase aeration. Treated water is then chlorinated and stored in a storage tanks prior to distribution. Two ozone generators (VMUS-04) provide an ozone dosage of 4 g/hr at 5 L/min airflow and 7 mg/L at average flow rate. Thus, the applied ozone dose is

approximately 17 mg/L (Zanacic et al. 2016). An air dryer was installed in April 2012 to improve the efficiency of the ozone generator.

The Benson WTP is designed to provide potable water at a rate of 45 m³/day and includes one 1.5 m diameter roughing filter, two 2.1 m diameter biological sand filters and two 2.1 m diameter BAC filters (Zanacic et al. 2016). Much like the Osage WTP design, aeration of BAC filters is enabled by a re-circulation system that recycles non-chlorinated water back into BAC filters at 0.6 m³/hr. Treated water is chlorinated and stored in tanks prior to distribution. Four ozone generators (VMUS-04) are each capable of generating up to 7 mg/L of ozone per unit at 6 L/min of oxygen resulting in a full operating capacity ozone dose of 17 mg/L (Zanacic et al. 2016).

Sample Collection and DNA extraction

Raw water samples were collected from surface water reservoirs in 1 L plastic bottles in both communities in fall (September) 2012 and spring (March/April) 2013. Approximately 0.35 L of raw water samples were vacuum filtered using 0.2 µm Millipore filters (GSWP04700). Filter discs were placed in a conical tube, 35 mL of raw water added, and the mixture vortexed to re-suspend the sediments/turbidity particles collected on the filter. The filter paper was removed from the conical tube, and the suspensions centrifuged at 186 x g for 10 minutes. The resulting pellet was recovered and processed using the MoBio PowerSoil® kit (Mo Bio, California), as per the manufacturer's instructions.

BAC filter samples were collected using a grain coring tool approximately eight (8) inches from the centre of the filter and at three depths within the BAC filter (top 1/3, middle 1/3 and bottom 1/3) at the Osage WTP in the fall. Samples were placed in sterile bottles, 50 mL of

distilled water added, and samples vortexed for 10 minutes to dislodge microbes from the BAC medium. Suspensions were then centrifuged, and DNA extracted from the sample pellet using the MoBio PowerSoil® DNA Isolation Kit (Mo Bio, California, USA) as per the manufacturer's instructions.

16S rRNA amplification

Amplification of the V4 region of the 16S rRNA was carried out in 50 µL PCR reactions containing 1 unit of Phusion DNA polymerase (2 U/ µL) (New England Biolabs, Massachusetts, USA) 10 µL of 5x HF buffer, 0.25 µL of the 515F 16S rRNA primer (100 µM) and 0.25 µL of the 806R 16S rRNA primer (100 µM) containing a unique barcode for multiplexing, 0.40 µL 10 mM dNTP and approximately 10 ng of template DNA. Three PCR amplifications were prepared for each sample, and subsequently pooled. Cycling conditions were as follows: one cycle of 98°C for 2 min, followed by 98°C/10 sec, 50°C/30 sec, 72°C/15 sec) for 20 cycles, followed by a final polymerization step of 72°C for 7 sec (Bartram et al. 2011). Samples were visualized following separation with gel electrophoresis, and amplicons purified using the E.Z.N.A. Gel Extraction Kit (Omega Bio-Tek, Georgia, USA). The concentration and purity of samples were confirmed by NanoDrop UV-Vis Spectrophotometer. Sequencing of amplicons was carried out on an Illumina GAIIx platform by McGill University and Génome Québec Innovation Centre (Montréal, Quebec, Canada). Datasets are available under MG-RAST accession/project numbers (4528546, 4528547, 4528548, 4528549, 4528550, 4528553 and 4528554). Summary statistics for these datasets are available in Table S1.

Microbial Community Analyses

Reads were processed with MG-RAST (Meyer et al. 2008) to remove eukaryotic contamination, and the resulting reads that passed quality control were subsequently analyzed, clustered, and classified using the RDPipeline using a cutoff of 80% and 16S copy number correction (Cole et al. 2014; Wang et al. 2007). The RDPipeline was also used to calculate alpha diversity (Shannon Index) at a distance of 0.03, and to generate rarefaction curves at a distance of 0.03. Analyses of significant differences between taxonomic abundances of two samples (including unclassified reads) were conducted with STAMP, using two-sided G-test (with Yates') + Fisher's and Storey-FDR multiple-test correction (Parks and Beiko 2010).

Results

Microbial Diversity

The microbial composition of surface water from the reservoirs of Osage and Benson was evaluated in both fall and spring using 16S rRNA amplicon sequencing. Approximately 5-15% of the representative reads for the four samples could not be classified to the level of domain, although 50-70% were assigned at least to the level of phylum within either the *Bacteria* or the *Archaea* (Figure 1A). The Shannon Diversity Index for all four raw water samples ranged from 7.33 to 7.96 (Table S1; Figure 2). The *Proteobacteria* dominated both sites in both seasons, accounting for 20-30% of overall diversity (Figure 1B), with the *Betaproteobacteria* making up the majority (30-55%), followed by the *Alphaproteobacteria* (20-30%), *Gammaproteobacteria* (15-20%), and *Deltaproteobacteria* (10-20%) (Figure 1C). A further evaluation of the identifiable genera within these phyla showed that one genus, *Polynucleobacter* (*Betaproteobacteria*), was present across both sites in both seasons, reaching almost 7% in Benson in the spring. In contrast, other groups correlated with season, such as the

Betaproteobacterial groups, *Polaromonas* (1.4-1.7%) and *Rhodospirillum rubrum* (0.5%), which were higher in the spring (Figure 2).

The Cyanobacteria/Chloroplast group was also well-represented across all four samples, but had higher representation in water collected from Osage in both the fall and spring (almost 20%) than in Benson (15% and 6%) (Figure 1), with the majority of the Benson cyanobacterial and/or chloroplast sequences being derived from the eukaryotic groups, *Bacillariophyta*, *Cryptomonadaceae*, and *Chlorophyta* (Figure 2). In contrast, 16-17% of the Cyanobacteria/Chloroplast group represented at Osage were derived from the above eukaryotic groups, with the remainder being composed of Group I Cyanobacteria, including *Aphanizomenon*, *Anabaena*, and *Anabaenopsis*, as well as the Group IIa genera, *Cyanobium*, *Microcystis* and *Synechococcus*. The *Bacteroidetes* accounted for 8-12% of the overall sample diversity across both sites in both seasons, with a greater abundance in Benson (12%) than at Osage (8%)(Figure 1). One of the more predominant *Bacteroidetes* genera common to both sites in both seasons was *Flavobacterium* (0.6-0.8%), while *Pedobacter* (0.5-0.7%) was more abundant in the spring at both sites. The *Actinobacteria* increased from around 3% in the fall to 8% in the spring at both Osage and Benson (Figure 1), with the predominant spring taxon being *Mycobacterium* (0.8-1.1%) (Figure 2). The *Bacteroidetes*, *Proteobacteria*, and *Cyanobacteria*, together with the *Verrucomicrobia* (4-7%), *Actinobacteria* (3-9%), *Planctomycetes* (3-7%), and *Acidobacteria* (2-3%) accounted for more than 90% of the taxonomically-defined diversity at both sites and in both seasons. A temporal (season-specific) analysis showed that the *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* were always significantly higher in the spring, independent of the site (1-6% difference in proportions, G-test, $p < 10^{-4}$) (Figure 3A). A spatial (site-specific) analysis of the relative abundance of the different phyla revealed that the

Bacterioidetes and *Proteobacteria* were higher at Benson than at Osage in both seasons (3-6% difference in proportions, G-test, $p < 10^{-4}$), while *Cyanobacteria* were significantly higher at Osage than at Benson (3-10% difference in proportions, G-test, $p < 10^{-15}$) (Figure 3B). The phyla exhibiting large spatial and temporal differentials are summarized in Figure 3C.

Given the central role of water treatment in reducing waterborne bacterial pathogens, we evaluated the presence of pathogens and toxin-producing bacteria in raw water entering both treatment plants. Of particular concern are those organisms often associated with human disease, including species of *Burkholderia*, *Campylobacter*, *Escherichia/Shigella*, *Legionella*, *Leptospira*, *Mycobacterium*, *Salmonella*, and *Vibrio* (Ferguson et al. 2003; Leclerc et al. 2002; World Health Organization 2011), although we also surveyed for the presence of several other genera that are commonly associated with water reservoirs, including *Acinetobacter*, *Aeromonas*, *Helicobacter*, *Pseudomonas*, and *Staphylococcus*, as well as the enteric coliforms, *Enterobacter*, *Klebsiella*, *Cronobacter*, *Serratia*, and *Yersinia* (World Health Organization 2011). *Acinetobacter*, *Yersinia*, and *Leptospira* were at extremely low abundance in most samples ($<0.02\%$), while *Aeromonas* and *Pseudomonas* reached up to 0.1% in some samples. In contrast, *Mycobacterium* was more abundant in both spring samples at both sites, being found at 1.1% at Osage and 0.8% at Benson (Figure 2). In the fall, it was found at only around 0.2-0.3% at both sites. We extended our search to a broader range of taxa that contain pathogenic species, and noted the presence of *Staphylococcus* and *Clostridium*, although these were also at extremely low levels ($<0.01\%$).

Microbial community composition of the BAC filter

To evaluate changes in microbial community composition through the BAC filter, a sample core was taken from the Osage BAC filter in the fall, split into three (top, middle,

bottom), and microbial diversity assessed on each fraction. Comparisons of community composition in the filter were made to the Osage Fall sample, which served as the source raw water entering the filter. Approximately 95% of sequences from the three BAC samples were assigned to the *Bacteria* or *Archaea*, with approximately 5% being unclassified (Figure 1A). Of the sequences classified to either the *Bacteria* or *Archaea*, approximately 35-40% could not be classified to phylum (Figure 1B). Of the sequences that could be classified, members of the *Proteobacteria* comprised the majority of dominant taxa throughout the BAC filter at approximately 23% (Figure 1B), decreasing in proportion with increased depth. Of the Proteobacterial classes represented, *Alpha* (23-30%), *Delta* (21-25%), and *Betaproteobacteria* (21%) were more abundant throughout the filter than the *Gammaproteobacteria* (15-17%) (Figure 1C). Some of the predominant Proteobacterial genera included *Arenimonas*, *Thiothrix*, and *Thiobacillus* (0.6-0.7%), all of which were more abundant in the top fraction, along with *Bdellivibrio*, which was found at approximately 0.4% throughout the three layers (Table 1). One member of the *Proteobacteria*, *Polynucleobacter*, which was found at 1.5% in raw water was reduced to 0.2-0.4% in the filter (Table 1).

Members of the *Planctomycetes* and *Acidobacteria* were significantly overrepresented in the filter as compared to raw water entering the filter (12-15% versus 4%, G-test, $p < e-15$) (Figure 3D), and both showed a trend of increasing abundance with increasing depth (Figure 1B). The Planctomycete, *Pirellula*, increased from 0.6% in raw water to as high as 3% in the filter, while Group 6 of the *Acidobacteria*, increased from 1% in the raw water to as high as 4% in the filter (Table 1). Many other groups of the *Acidobacteria*, including Gp4, Gp16, Gp3, Gp7, and Gp10 were all represented throughout the filter, each at an abundance of $>0.3\%$. Similarly, the *Nitrospirae* made up a significantly larger proportion of the filter community as compared to

raw water (1% versus 5-7%, G-test, $p < e-15$) (Figure 3D), with *Nitrospora*, a known nitrifier (Lucker et al. 2010) increasing to up to 7% within the filter (Table 1). Other groups represented within the filter in relatively higher proportions included *Gemmatimonas* (*Gemmatimonadetes*) (~1%), members that have been reported accumulate polyphosphate (Zhang et al. 2003), the Group 5 in the *Armatimonadetes* (0.6%) (Lee et al. 2013), and both *Candidatus* groups in the *Verrucomicrobia*, Subdivision 3 (~0.6%) and *Spartobacteria* (~0.4%) (Table 1). Among the less studied and more ambiguous taxa that increased in the filter included the genus *Litorilinea* (up to 0.4%) from the *Chloroflexi*, representatives from the two candidate phyla, WPS-1 (0.3%), and *Saccharibacteria* (0.3%), and even representatives of AR13 from the recently proposed Archaeal phylum, *Pacearchaeota* (~0.3%) (Castelle et al. 2015). In contrast, several groups were reduced significantly within the filter relative to raw water, including the Group I (*Aphanizomenon*, *Anabaena*, and *Anabaenopsis*) and Group IIa (*Cyanobium*, *Microcystis* and *Synechococcus*) *Cyanobacteria*, which were reduced from 0.7-1.5% to $< 0.4\%$ in the filter (Figure 3D). Similarly, the eukaryotic (chloroplast) groups, *Cryptomonadaceae*, *Bacillariophyta*, and *Chlorophyta*, which together comprised about 15% of the raw water diversity were reduced to approximately 1%, on average, throughout the filter (Figure 3D).

Assessment of potential pathogens in the BAC filter

Given that the raw water samples, including the Osage spring sample contained some bacterial groups with human pathogenic potential, we attempted to assess the fate of these bacteria within the filter. Many of the waterborne pathogens that are of greatest concern, including most of the enteric coliforms such as *E. coli*, *Yersinia*, *Serratia*, *Salmonella*, *Enterobacter*, and *Klebsiella*, which were largely absent from raw water were also at negligible densities within the filter. *Legionella*, which was found at approximately 0.02% in raw water

remained at approximately this concentration throughout the different filter fractions, while *Pseudomonas* and *Aeromonas*, which were each present at approximately 0.1% in raw water were reduced to approximately 0.02% (Figure 4). In contrast, *Mycobacterium*, which was at approximately 0.15% in raw water, increased slightly to 0.2-0.3% in the filter (Table 1; Figure 4).

Discussion

In this study, we used 16S rRNA profiling to assess the microbial communities found in surface water reservoirs that feed into the WTPs of two rural sites. In addition, we examined how these bacterial communities relate to microbial diversity within three transects of a BAC filter localized within one of those WTPs. Our sequencing depth of the different samples varied, despite our consistent sampling and processing methods (Table S1; Figure S1). Of the sample reads, around 25-35% of sequence reads could only be assigned to domain (Figure 1), reflecting the considerable gap in our understanding of bacterial diversity. In addition, several of our raw water samples had eukaryotic DNA contamination, and more specifically chloroplast rRNA, likely from the highly abundant diatoms and phytoplankton in our water samples.

An evaluation of bacterial community composition at the level of phylum at the two sites revealed several spatial and temporal differences in microbial diversity and distribution. The abundance of *Cyanobacteria*, *Bacteroidetes* and *Proteobacteria* appeared to correlate with location, with former being more abundant at Osage, and the latter two more abundant at Benson, irrespective of season. The higher abundance of *Cyanobacteria* at Osage is particularly noteworthy, given that strains of *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Microcystis*, *Nodularia*, *Nostoc*, *Oscillatoria*, *Planktothrix*, *Raphidiopsis*, and *Umezakia* produce

a variety of anatoxins, saxitoxins, microcystins, and cylindrospermopsins (Carmichael 2001; Lyra et al. 2001; Neilan et al. 1999; World Health Organization 2011). *Aphanizomenon*, *Anabaena*, *Anabaenopsis*, and *Microcystis* were identified in raw water samples, some species of which produce hepatotoxins and neurotoxins such as cylindrospermopsin, saxitoxin and anatoxin-A (Carmichael 2001; Lyra et al. 2001). Because these *Cyanobacteria* were not as abundant at Benson, it could suggest differences in the availability of limiting micro- and macronutrients (Elser et al. 2007), although other factors, such as turbidity, can also reduce solar light penetration through the water column, thereby limiting growth of these autotrophs. The Osage reservoir is shallower and contained lower dissolved organic carbon at sampling (~12 mg/L) than did the Benson reservoir (~30 mg/L) (Zanacic et al. 2016), possibly favouring more light penetration. Paradoxically, throughout the year, nitrogen levels at Benson ranged from 2.4-3.9 mg/L, about two times those of Osage (1.0-1.8 mg/L) while phosphorus levels at Benson were around 0.2-1.3 mg/L, also slightly higher than those found at Osage (0.4-0.8 mg/L) (Zanacic et al. 2016). This could suggest alternative environmental factors impacting cyanobacterial abundance, such as the presence of bacteriophage that may have caused the prevailing cyanobacterial populations to collapse. This was noted in one freshwater environment, where not only did cyanobacterial population collapse due to bacteriophage, but this caused the increased the population of *Actinomyces*, as well as some members of the *Bacteroidetes* (van Hatten et al. 1999). The greater abundance of the *Bacteroidetes* at Benson is consistent with this finding, and could reflect an increase in the availability of nutrition due to lysis of predominant cyanobacterial populations, which is also supported by the slight increase in measured DOC from the fall (30.4 mg/L) to the spring (35.6 mg/L) (Zanacic et al. 2016).

The abundance of several phyla changed temporally (with season), including the *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*, all three of which were higher in the spring at both Osage and Benson. The *Proteobacteria*, whose abundance we found also correlated with site (higher in Benson samples), has been previously suggested to remain prevalent throughout the season (60-70% of the community) (Pinto et al. 2014), with some studies demonstrating that the *Alphaproteobacteria* class is more abundant in the winter, and the *Betaproteobacteria* class in the summer (McCoy and VanBriesen 2012). Partially consistent with this, our results show that the *Betaproteobacteria* are higher in the spring at both sites, whereas the *Gammaproteobacteria* are higher in the fall at both sites. In addition, our results were also consistent with reports that the Proteobacterial communities, as well as the *Actinobacteria* may occupy specific temporal niches within aquatic environments, oscillating between seasons depending on the availability of various nutrients (Glockner et al. 2000). Because many pathogenic bacterial groups fall within the *Proteobacteria*, identifying any seasonal effects on the prevalence of these groups has important implications for disinfection practices and management within WTPs.

The BAC filter plays a central role in the biodegradation and adsorption of dissolved organic carbon and disinfection byproducts, as well as the nitrification of ammonia (Kim and Kang 2008; Niquette et al. 1998; Xiaojian et al. 1991). We noted very similar proportions of unclassified bacteria in the Osage filter (37-39%), which were slightly higher than those in the raw water samples (Figure 1A), suggesting an as-yet unknown community diversity that may drive biofilter activity and efficiency. This may also be a reflection of niche-specific colonization of the BAC by specific unclassified groups, which is supported by the increase in the relative proportion of the unclassified *Proteobacteria* in the filter (Figure 1B), as well as the

overall dramatic shift in microbial community composition as compared to the raw water samples. For example, the *Alphaproteobacteria* and *Deltaproteobacteria* increased in the filter relative to raw water, while the *Betaproteobacteria* decreased. Although the *Betaproteobacteria* decreased, they still comprised 20% of the diversity, which has been suggested to be due to the diverse metabolic capabilities of this group (Kaarela et al. 2015; Niemi et al. 2009). We also found that the higher abundance of *Deltaproteobacteria*, which made up a higher proportion in the filter, and which decreased with increasing depth, was consistent with the diverse metabolic capabilities of its many anaerobic members, such as the metal-reducing *Anaeromyxobacter* and *Geobacter*, as well as the sulfur- and sulfate-reducing anaerobic members within the *Desulfobacterales* (Barton and Fauque 2009; Castro et al. 2000). We noted the greater proportion of *Bdellovibrio* within the filter, a predatory group within the *Deltaproteobacteria* (Rendulic et al. 2004; Stolp and Starr 1963), which may be serving to alter community composition.

One of the other obvious shifts in filter composition was the striking increase in the *Planctomycetes*, *Acidobacteria*, and *Nitrospirae* in the filter, relative to raw water. The most abundant taxa within these phyla were *Nitrospora* (*Nitrospirae*), Gp6 (*Acidobacteria*), and *Pirellula* (*Planctomycetes*), all three of which were consistent throughout the filter layers. Many of these groups have been suggested to play important roles in nutrient cycling in a variety of environments (Fierer et al. 2007; Lucker et al. 2010; Strous et al. 2006). *Nitrospira*, for example, which are nitrifying bacteria that transform ammonia to nitrate (Lucker et al. 2010), shifted from 1.1% in raw water to 7% in the bottom of the BAC filter, indicating a possible prominent ecological role within the filter. *Nitrospora* spp. were also found to be abundant in several other studies evaluating microbial diversity across various stages of drinking water treatment (Kaarela

et al. 2015; Pinto et al. 2012; Shaw et al. 2015; Yapsakli et al. 2010), but it is important to note that the metabolic activities of nitrifying bacteria, including *Nitrosomonas* that was also identified within the Osage BAC filter, have been shown to decrease disinfectant residuals of chloroamines (Berry et al. 2006; Maestre et al. 2013, 2016). It is therefore important to actively monitor disinfectant residuals to ensure they are not being rapidly degraded by these microbes.

The Planctomycete, *Pirellula*, and Gp6 (subdivision 6) of the *Acidobacteria*, increased from less than one percent in raw water to around 3% in the filter, and therefore make up a decent proportion of this BAC bacterial community. Representative strains of *Pirellula* use a wide variety of carbon sources and can produce H₂S from thiosulfate (Clum et al. 2009). They have been identified as members of communities involved in alcohol manufacturing wastewater treatment plants (Yang et al. 2007), further reinforcing their role in nutrient cycling. A recent genomic analysis of one representative of Gp6 (subdivision 6) of the *Acidobacteria* revealed a diversity of predicted metabolic functions, including nitrate and sulfite reduction, as well as genetic determinants for arsenate, arsenite, antimonite, cobalt, zinc, lead, cadmium, and mercury detoxification (Huang et al. 2016). Other subdivisions of the *Acidobacteria*, including Gp4, Gp16, Gp3, Gp7, and Gp10, also increased within the filter, indicating that the biology of these groups may be important for understanding biofilter function.

Because BAC filters are readily colonized by a diversity of microbes, we were interested in the fate of pathogens and cyanobacteria within the filter. We found that *Cyanobacteria* in the Osage raw water, which accounted for approximately 2% of the diversity, were barely detectable in any transect of the Osage BAC filter, suggesting they are being reduced to negligible numbers. This was also true of several groups within the *Flavobacteria* (*Bacteroidetes*) (Table 1). Furthermore, we noted that many of the waterborne pathogens of greatest concern, including

E.coli/Shigella, *Salmonella*, *Campylobacter*, and *Vibrio* were not as abundant in the source water at either site, and were also not identified in the filter at any notable concentration. This was somewhat surprising given that fecal coliforms, as well as bacteria normally found in gut of animals, such as *Campylobacter*, are often expected to be more prevalent in areas like Osage and Benson where animals pasture in fields and surrounding plots (Huang et al. 2015; Wilkes et al. 2011). Other genera that include potentially pathogenic species, like *Pseudomonas* and *Aeromonas*, were found in raw water, but were reduced to < 0.05% in the filter. The particular species representing these two genera could not be identified with any confidence, although previous work has suggested that some pathogenic species that carry virulence factors, such as those of *Aeromonas*, can persist in municipally treated water (Sen and Rodgers 2004). Likewise, *Legionella*, which was identified in raw water, persisted within the filter, albeit at a relatively low abundance (0.02%). *Legionella* is an intracellular pathogen, which has been reported to be a natural colonizer of GAC (Wang et al. 2013), and which can persist through water treatment systems within amoebae, such as *Acanthamoeba* (Thomas et al. 2008). This has also been demonstrated for species of *Mycobacterium*, which in our study increased from about 0.15% in raw water to around 0.3% in the filter (Table 1). Like *Legionella*, species of *Mycobacterium* are natural colonizers of BAC (Wang et al. 2013), can survive within amoebae (Thomas et al. 2008), and have been identified as a prevalent microbe in both experimental and treatment plant filters and distribution systems (Dailloux et al. 2003; Revetta et al. 2013; Revetta et al. 2016; Stanish et al. 2016; Thomas et al. 2008; Wang et al. 2013). Species of *Mycobacterium* are of particular concern for drinking water safety, given that they are the causal agent of human and animal tuberculosis, as well as a variety of other respiratory infections in both healthy and immunocompromised individuals (Wagner and Young 2004). Given that backwashing is

important for management of bacterial biomass and proper filter performance (Urfer et al. 1997), the recommended backwash frequency for the Osage BAC filter is every 4 to 5 months (Zanacic et al. 2016), which may also help to purge pathogen populations.

Although Benson and Osage have similar water sources (surface water reservoirs) recharged by runoff from agricultural fields where similar cropping and cultivation systems are applied in the same climatic region, the microbial diversity and composition are different. The proportion of *Cyanobacteria* was higher in Osage samples, while *Proteobacteria* and *Bacteroidetes* were greater in Benson samples. The *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia* showed a temporal effect, increasing in the spring at both sites. We also found that community composition of the BAC filter shifted relative to raw water, with a distinctive increase in the proportion of *Proteobacteria*, *Acidobacteria*, *Planctomycetes*, and *Nitrospirae*, and a decrease in the *Bacteroidetes* and *Cyanobacteria*/Chloroplast groups. The BAC filter contained populations of *Legionella*, *Aeromonas*, *Pseudomonas* and *Mycobacterium*, with proportions of the latter remaining consistent throughout the filter media. This has implications for ensuring low titres of pathogens in the distribution system using disinfection residuals, with the caveat that nitrifiers identified within the BAC filter may be contributing to the degradation of monochloroamine compounds used for disinfection. The concentration of potentially pathogenic groups within the filter reinforces the vital importance of post-filter disinfection practices and active monitoring to ensure disinfection residuals are maintained throughout the treatment process.

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Figure Legends

Figure 1. A. Proportion of classified *Bacteria* and *Archaea* in four surface water (raw) samples at Osage and Benson in the fall of 2012 and the spring of 2013, and three biological activated carbon (BAC) samples collected at Osage in the fall of 2012. B. Relative proportion of Bacterial and Archaeal phyla in four surface water samples at Osage and Benson, and three biological activated carbon (BAC) samples collected at Osage. “Others” includes 27 phyla and candidate phyla, most of which had $< 1\%$ relative abundance. C. Proportion of six different classes of *Proteobacteria* across samples. “Unclassified” sequences could not be assigned to a taxonomic group at an 80% confidence threshold with the Ribosome Database Project Classifier (Cole et al. 2014; Wang et al. 2007).

Figure 2. Proportion of predominant genera ($> 0.3\%$) in seasonal samples taken at Osage and Benson. Reads classified to genus level, but which comprised $< 0.3\%$ of diversity are represented in the “other classified” slice. Sequences not classified to genus level at an 80% confidence threshold are represented by the “unclassified” slice. Asterisks indicate *candidatus* or *incertae sedis* status.

Figure 3. A. Comparison of the proportions of the *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia* between seasons within each site. B. Comparison of the proportions of the *Proteobacteria*, *Bacteroidetes*, and *Cyanobacteria* between Osage and Benson for each season. C. Summary of the phyla that show temporal (season-specific) and spatial (site-specific) increases in their relative proportions at Osage and Benson. D. Phyla that exhibit significant changes in relative proportion between the Osage raw water and the Osage BAC filter (top

fraction). Two-sample comparisons were carried out using two-sided G-test (with Yates') + Fisher's and Storey-FDR multiple-test correction. All unclassified sequences were retained for these analyses. * $p < 0.02$; ** $10^{-3} > p > 10^{-12}$; *** $p < 10^{-12}$.

Figure 4. Relative abundance of *Legionella*, *Pseudomonas*, *Aeromonas* and *Mycobacterium* from Osage raw water (Raw), through the different biological activated carbon (BAC) filter layers (top, middle, bottom).

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Table 1. Relative abundance of predominant genera in Osage raw water relative to the different layers of the biological activated carbon (BAC) filter.

Phylum	#Genus	BAC FILTER			
		Raw	Top	Middle	Bottom
<i>Nitrospirae</i>	<i>Nitrospira</i>	1.1%	5.5%	5.0%	6.7%
<i>Acidobacteria</i>	Group 6	0.9%	2.4%	3.1%	4.2%
<i>Planctomycetes</i>	<i>Pirellula</i>	0.6%	2.4%	2.8%	3.2%
<i>Gemmatimonadetes</i>	<i>Gemmatimonas</i>	1.3%	1.1%	0.8%	1.1%
<i>Verrucomicrobia</i>	Subdivision3*	0.4%	0.6%	0.7%	0.5%
<i>Armatimonadetes</i>	Group 5	0.3%	0.6%	0.6%	0.6%
<i>Proteobacteria</i>	<i>Arenimonas</i>	0.6%	0.6%	0.4%	0.3%
<i>Candidatus Saccharibacteria</i>	<i>Saccharibacteria</i> *	0.3%	0.3%	0.4%	0.4%
<i>Acidobacteria</i>	Group 16	0.2%	0.5%	0.8%	0.6%
<i>Proteobacteria</i>	<i>Thiothrix</i>	0.0%	0.7%	0.4%	0.6%
<i>Proteobacteria</i>	<i>Thiobacillus</i>	0.2%	0.6%	0.4%	0.4%
<i>Verrucomicrobia</i>	<i>Spartobacteria</i> *	0.1%	0.4%	0.5%	0.4%
<i>Proteobacteria</i>	<i>Bdellovibrio</i>	0.2%	0.4%	0.4%	0.4%
<i>Acidobacteria</i>	Group 10	0.1%	0.3%	0.4%	0.4%
<i>Acidobacteria</i>	Group 4	0.1%	0.3%	0.3%	0.4%
<i>Proteobacteria</i>	<i>Aquicella</i>	0.0%	0.4%	0.3%	0.3%
<i>Acidobacteria</i>	Group 3	0.1%	0.3%	0.4%	0.3%
candidate division WPS-1	WPS-1*	0.0%	0.3%	0.3%	0.3%
<i>Chloroflexi</i>	<i>Litorilinea</i>	0.1%	0.2%	0.3%	0.4%
<i>Proteobacteria</i>	<i>Nitrosomonas</i>	0.0%	0.2%	0.3%	0.3%
<i>Actinobacteria</i>	<i>Gaiella</i>	0.0%	0.4%	0.2%	0.5%
<i>Acidobacteria</i>	Group 7	0.2%	0.4%	0.2%	0.3%
<i>Pacearchaeota</i>	<i>Pacearchaeota</i> (AR13)*	0.1%	0.4%	0.2%	0.3%
<i>Acidobacteria</i>	<i>Aridibacter</i>	0.1%	0.3%	0.3%	0.2%
<i>Actinobacteria</i>	<i>Mycobacterium</i>	0.2%	0.2%	0.3%	0.2%
<i>Armatimonadetes</i>	<i>Armatimonas</i> (Group I)	0.3%	0.0%	0.0%	0.0%
<i>Verrucomicrobia</i>	<i>Luteolibacter</i>	0.3%	0.1%	0.1%	0.1%
<i>Proteobacteria</i>	<i>Rheinheimera</i>	0.4%	0.1%	0.2%	0.1%
<i>Bacteroidetes</i>	<i>Fluviicola</i>	0.5%	0.1%	0.1%	0.1%
<i>Bacteroidetes</i>	<i>Flavobacterium</i>	0.6%	0.1%	0.1%	0.1%
<i>Verrucomicrobia</i>	<i>Opitutus</i>	0.7%	0.3%	0.3%	0.3%
<i>Cyanobacteria</i>	Group IIa	0.7%	0.0%	0.0%	0.1%
(Chloroplast)	<i>Chlorophyta</i>	1.0%	0.1%	0.1%	0.1%
<i>Cyanobacteria</i>	Group I	1.4%	0.2%	0.2%	0.1%
<i>Proteobacteria</i>	<i>Polynucleobacter</i>	1.5%	0.4%	0.4%	0.2%
(Chloroplast)	<i>Cryptomonadaceae</i>	3.8%	0.2%	0.3%	0.2%
(Chloroplast)	<i>Bacillariophyta</i>	9.0%	0.6%	0.7%	0.5%

* Asterisk indicates *incertae sedis*

Figure 1

251x296mm (300 x 300 DPI)

Figure 2

209x208mm (300 x 300 DPI)

Figure 3

122x75mm (300 x 300 DPI)

Figure 4

52x21mm (300 x 300 DPI)

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Figure S1. Rarefaction curves of 16S rRNA sequences for raw water and filter samples at the 97% sequence similarity cut-off.

Supplemental Table

Table S1. Statistical overview of datasets.

Location	Season	Sample	*Sequences	**OTUs	***Corrected Sequence #	**Shannon Index (H)	MG-RAST Accession
Osage	fall	raw (intake)	10557	4803	4802	7.96236	4528546
	spring	raw (intake)	25099	6042	11226	7.84606	4528553
	fall	filter, biological activated carbon, top 1/3	12436	4411	5806	7.88273	4528547
	fall	filter, biological activated carbon, mid 1/3	8016	3065	3720	7.57846	4528548
	fall	filter, biological activated carbon, bottom 1/3	13516	4294	6456	7.79999	4528549
Benson	fall	raw (intake)	6941	2680	3002	7.32593	4528550
	spring	raw (intake)	16396	5717	7515	7.87802	4528554

*Sequence number after quality control and dereplication . **OTUs and Shannon Index calculated at a distance of 0.03.

***Sequence number corrected for 16S rRNA copy number, as implemented by RDP.