Research Paper

Assessment of bacterial diversity in western Accra, Ghana, drinking water samples

Gertrude Ecklu-Mensah, Sammy T. Sackey, Hilary G. Morrison, Mitchell L. Sogin, Leslie G. Murphy and William S. Reznikoff

ABSTRACT

The design and performance characteristics of municipal drinking water systems can profoundly influence public health. To assess the operational attributes of an Accra, Ghana drinking water distribution system, high-throughput 454 pyrosequencing was employed to characterize its bacterial community composition. Samples from the waterworks and four household sources (one household tap and three polytank storage units) were analyzed within one of the Accra's distribution networks over a 4-month period. Samples provided between 9,059 and 20,076 reads (average = 13,056) that represented a broad range of bacterial diversity, including rare genera. Minimum Entropy Decomposition (MED) analysis showed that the sequences described four major assemblages. Assemblages 1 and 2 dominated the waterworks and household tap samples while polytank storage unit samples, with one exception, contained assemblages 3 or 4. The considerable bacterial taxonomic difference between different sources suggests that contamination and/or selective growth shapes bacterial community structures after treatment at the waterworks. Of particular interest are the major differences between the polytank samples following storage and the tap/waterworks samples, suggesting that water storage (stagnation) can select for unique microbial populations.

Key words | 454 pyrotag sequencing, bacterial diversity, drinking water, polytanks, water storage

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INTRODUCTION

Human health and development in every society depend upon access to safe drinking water. Despite the widespread use of vigorous sand filtration and disinfection of water supplies, contamination frequently leads to the transmission of microbial infections that cause serious illnesses and associated mortality worldwide (Craun *et al.* 2010). In Ghana, contaminated water accounts for about 70% of reported diseases (International Fact-Finding Mission 2002; WaterAid 2005). In many parts of the world, inconsistent supplies coupled with increased demand forces local storage of water (International Fact-Finding Mission 2002) that increases the potential to introduce other sources of water contamination. Thus, water quality depends upon the initial water treatment, the water transmission from the water plant to the point of use, and on-site water storage systems.

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In Ghana, specifically Accra, residents face acute water supply shortages. An estimated one quarter of its residents receives a continuous municipal water supply, while approximately 35% have access only 2 days each week (WaterAid 2005). To insure access to sufficient amounts during the non-supply periods, most Ghana citizens must store water. Inhabitants of Accra employ various forms of water storage tanks, but black polyethylene tanks referred to as 'polytanks' have gained wide acceptance. The storage of water for hours or days encourages stagnancy that can result in microbial contamination of otherwise good quality drinking water (Ling *et al.* 2018).

Microbiological monitoring of water quality generally relies upon conventional culturing of indicator organisms. However, these culture-dependent approaches underestimate bacterial diversity and do not provide assessments of pathogenic species (Amann *et al.* 1995). Several reports describe the presence of microbes associated with waterborne disease outbreaks in treated drinking water that meet coliform regulations (Payment *et al.* 1991), and in some cases, bacterial biofilms in drinking water distribution systems (DWDS) persist despite heavy chlorine treatment (LeChevallier *et al.* 1996). As an alternative, sequence analyses of polymerase chain reaction (PCR) amplicons for 16S ribosomal ribonucleic acid (rRNA) genes can document the presence of viable and non-viable microbes in drinking water networks that conventional microbiological approaches would fail to detect (Williams *et al.* 2004; Eichler *et al.* 2006).

High-throughput sequencing of PCR amplicons from hypervariable regions in 16S rRNA genes provides an efficient means to profile bacterial communities including the detection of sequence reads from very low abundance taxa that constitute less than 0.1% of a microbial community (Tan *et al.* 2015). Taking advantage of this technology, this investigation characterized the bacterial diversity in treated water samples collected from the Weija waterworks in Accra, Ghana and its distribution system over a 4-month time frame (September 2009 to December 2009; rainy season to the beginning of the dry season). Analysis of thousands of amplicon sequences for the 16S rRNA gene hypervariable regions V5–V6 (such regions are bordered by constant sequences convenient for PCR amplification and have been used in multiple DNA sequence studies for microbial identification) provided information about the occurrence, relative abundance and diversity of bacterial microflora in this Ghanaian drinking water supply system and how the microbial communities differed based upon the source (waterworks versus direct domestic tap versus polytank storage units) and seasonal collection time of the water samples.

MATERIALS AND METHODS

Sampling sites

The Ghana Water Company Limited provides drinking water for people in western Accra through its operation of the Weija waterworks located 15 km west of Accra, Ghana (see Supplementary Figure 1). The warm and humid climate of the region varies from 24 °C in August to 32 °C in March with a mean temperature of 27 °C. A dry period extends from December through March, followed by a rainy period with two peaks in June and September (Karikari & Ansa-Asare 2006). The waterworks (GPS coordinates 5.575493 N 0.343162 W) is supplied by a dam impoundment on the river Densu that forms a reservoir 14 km long, 2.2 km wide, with a total surface area of 38 km^2 and a mean depth of 5 m (Vanden Bossche & Bernacsek 1990). Treatment at the waterworks involves the abstraction of water from the intake into the treatment train: aeration, flocculation and coagulation using alum, sedimentation, rapid sand filtration and, finally, pH adjustment and chlorination with hydrated lime and chlorine gas (Addico *et al.* 2006). The distribution pipes supply about 64 million m^3 of treated water annually to consumers (Van Rooijen *et al.* 2008) by gravity feed.

This study sampled treated water over a 4-month period in 2009 (30 September, 28 October, 25 November and 15 December) from the waterworks and four randomly selected households located in Dansoman, a suburb of western Accra, which is approximately 12 km from the waterworks (see Supplementary Figure 1). Water was sampled from households with either a direct tap supply or an emergency polyethylene storage tank (polytank), all connected to the waterworks supply network. The three polytanks that were

analyzed in this work were labeled as A, B and C (as shown in Figure 1 and located in Supplementary Figure 1).

Sample collection and filtration

Prior to sampling, water was allowed to flow from each tap for about 3 min, followed by flame sterilization of the faucet. We collected 2.5 L of water samples over a 3-min time interval into pre-sterilized bottles. The samples were maintained at 4 °C and processed within 4 h of collection. Temperature and pH were determined at the time of sampling. Microorganisms were harvested by filtering 1.9 L of each water sample through a 0.2 μm Sterivex™ GP filter unit (Millipore, Billerica, MA) and stored at −18 °C until DNA isolation.

Nucleic acid extraction and purification

Nucleic acids were extracted from the Sterivex™ GP filter units using the Puregene DNA extraction kit (Gentra Systems, Minneapolis, MN) following the manufacturer's protocol with modifications (Sinigalliano *et al.* 2007). The reservoir of each filter cartridge was flooded with 2 mL of Puregene lysis buffer and 10 μL of the lytic enzyme (Qiagen, Valencia, CA) and incubated at 37 °C for 30 min with occasional shaking. Ten microlitres of Proteinase K (Qiagen, Valencia, CA) was added, and the sample was mixed thoroughly and incubated at 60 °C for 70 min with occasional agitation. The lysed cell suspensions were removed from the reservoir surrounding the filters using sterile 3 mL syringes and transferred into 2 mL sterile screw-cap tubes. Two hundred and thirty-five microlitres of protein precipitation solution (Qiagen, Valencia, CA) was then added, and the suspensions were incubated on ice for 15 min followed by centrifugation at 12,000 × *g* for 5 min. The supernatants were transferred into tubes containing 930 μL of 100% isopropanol. DNAs were pelleted by centrifugation at 12,000 × *g* for 5 min, washed with 700 μL of ice cold 70% ethanol, and the suspensions were centrifuged again at 12,000 × *g* for 5 min. The DNA pellets were air-dried at room temperature and resuspended in 30 μL of DNA hydration buffer (Qiagen, Valencia, CA). The quality of the extracted DNAs was assessed by agarose gel electrophoresis, and concentrations were determined on a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Polymerase chain reaction

PCR amplification of the V4 through V6 hypervariable regions of bacterial 16S rRNA genes employed fusion primers as described previously (Newton *et al.* 2013). We generated PCR amplicons in triplicate in 30 μL reaction volumes and included a separate no-template negative control. Reactions were performed in a GeneAmp PCR System 2720 cycler (Applied Biosystems, Foster City, CA) with amplification conditions described previously (Newton *et al.* 2013).

The resulting PCR products were purified using AMPure size exclusion beads (Agencourt Bioscience Corporation, Beverly, MA) to eliminate fragments below 400 bp and eluted in 24 μL of Buffer EB. The quality and size distribution of the amplicons were assessed on a Bioanalyzer 2100 (Agilent, Palo Alto, CA) using a DNA 1000 Lab Chip™ while the concentrations were determined using the Invitrogen Quant-IT™ Picogreen assay (Molecular Probes, Carlsbad, CA) with fluorescence measured on a TBS 380 Minifluorometer (Turner Biosystems, Promega, Madison, WI). PCR products with unique barcode sequences were pooled together at equimolar concentrations (25 ng each) for emulsion PCR.

Sequencing

We amplified the pooled DNA products in PCR mixture-in-oil emulsion and sequenced from the V6 end of the amplicon using Roche GS-FLX Titanium (Roche, Switzerland) 454 protocols.

Sequence analysis

Our bioinformatics process removed chimeras and low-quality reads as previously described (Huse *et al.* 2007). Reads were mapped to a bacterial reference alignment, degapped and re-trimmed to an average length of 261 nt (V5–V6) using PyNAST (Caporaso *et al.* 2010) and oligotyping tools (Eren *et al.* 2013). The final aligned dataset served as input to the Minimum Entropy Decomposition (MED) pipeline,

an information theory-based clustering algorithm for sensitive partitioning of high-throughput marker gene sequences followed by the determination of their co-occurrence in various environments (Eren *et al.* 2015). MED identifies 'amplicon sequence variants' (ASVs), previously called MED nodes or oligotypes (Callahan *et al.* 2017). ASVs were assigned rank-based taxonomy using vsearch v1.9.5 (Rognes *et al.* 2016) and the silva v119 reference database (Quast *et al.* 2013).

MED pipeline-linked analyses

First, each sample (location and month) was analyzed with regard to the detected ASVs and their relative abundances. Second, the samples were arranged according to whether they shared ASVs, and the degree of similarity is plotted on the horizontal distribution (see Figure 1). Third, the detailed analysis of the abundance of all 154 ASVs with the presence of at least 1% in at least one sample month is presented in Table 1 and in the vertical distribution of Figure 1. Note that additional 178 ASVs were detected whose abundance was below the 1% cut-off. The entire list of detected ASVs is presented in Supplementary Table 1. Of note, some different ASVs with the same family name are located at different positions in the taxonomic list. This is because the different individual members are represented by different, but related 16S RNA sequences and presumably have different genome constituents, and thus different and perhaps unique physiological capabilities.

RESULTS AND DISCUSSION

Results

General water quality metrics

pH ranged between 6.6 and 7.6, within WHO standards for drinking water (6.5–8.5). Temperature variation ranged between 27 and 33.5 °C. There were no significant differences in either pH or temperature between sites on the same sampling date, but there were significant differences in both metrics between different months (e.g. November pH = 7.00 vs. August pH = 7.48).

Bacterial diversity

We recovered an average of 11,839 and 14,159 sequences from the September 2009 through December 2009 waterworks and tap samples. Water samples from polytanks A, B and C yielded averages of 14,466, 12,572 and 12,247 sequences, respectively.

Figure 1 presents a heat map that displays the relative abundance of 154 ASVs versus their occurrence in different sample sources (each sample site is color-coded in both Figure 1 and Supplementary Figure 1). Table 1 lists these ASVs in the same order as Figure 1 along with their percentage relative abundance and taxonomic affinity. Inclusion of an ASV in Table 1 and Figure 1 required their relative abundance to be equal or exceeded 1.0% of the reads in one of the collection sites for at least 1 month. The relative abundance of any given ASV in any of the 20 samples is indicated by the color of the sample bar [red indicates high abundance (>16% representation in the sample), blue for low abundance (<1% or not observed)]. This beta diversity analysis identified four MED assemblages. With the exception of the September sample from polytank A, assemblages 1 and 2 dominated the waterworks and tap samples, and assemblages 3 or 4 dominated polytank samples. The enumeration of major taxa for each site also identified the same difference patterns between sampling sites and months (Table 1). We observed distinctive community composition for the different sites despite their having a common source, the Weija waterworks. All samples included representatives of most phyla, but each sample contained multiple ASVs that resolved to taxa that did not appear in other samples. Several distinct MED nodes resolved to the same taxonomic assignments. For all of the sampling sites (except for the polytank A September sample), the identified taxa fell into the same assemblages from month to month, although there were considerable differences in relative abundances of taxa in different months.

Analysis of the abundant microbial taxa (genus-level classification) at the waterworks showed the presence of fairly constant qualitative genus distributions with considerable quantitative changes each month. Household sample populations also showed constant qualitative genus distributions with considerable quantitative changes each

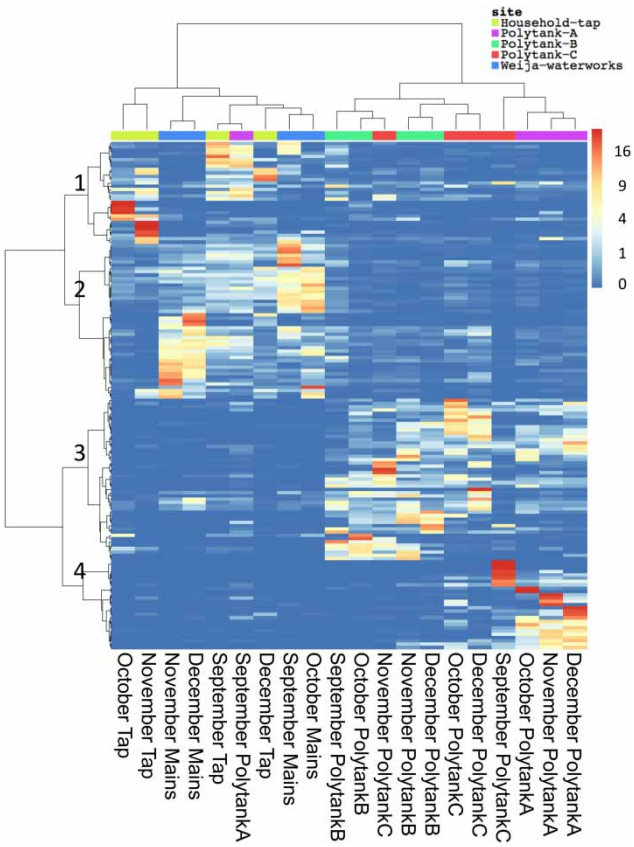

Figure 1 | Sequence variant relative abundance versus the sample sources and the month of collection using MED analysis. The cluster heatmap was produced as part of the MED analysis (Eren *et al.* 2015), using the R packages *vegan* (Oksanen *et al.* 2016) and *pheatmap* (Kolde 2015). The underlying matrix was calculated using the Bray–Curtis dissimilarity metric (Bray & Curtis 1957). Clustering indicates similarity among samples (top dendrogram) and among ASV abundances (left dendrogram). The source [Weija waterworks/Mains (blue); Household tap (green), etc.] and month of the collection (September 2009; October 2009, etc.) of different samples are presented horizontally. One hundred and fifty-four (154) different ASVs were identified by the 16s sequence in the samples (listed in the same vertical order in Table 1). The % abundance of any given ASV in any of the 20 samples is indicated by the color of the sample bar (the color and number 0–16 refer to the sample abundance). These ASVs fell into four different MED microbial assemblages (1, 2, 3 and 4 on the left). The four assemblages were analyzed versus their distribution in regard to sources and sampling months (Figure 1); waterworks and tap samples are dominated by assemblages 1 and 2 and, with one exception (polytank A September sample), all polytank samples are dominated by assemblage 3 or 4.

month. However, comparative analysis revealed that, with the exception of September, the dominant microbial community at the waterworks and tap varied drastically from the dominant bacterial populations observed in all but one of the polytank samples (Figure 1). These results demonstrate the presence of distinctive bacterial populations at the various sampling points.

Discussion

Our analysis described the diversity and relative taxon abundance for bacterial communities in a metropolitan DWDS in western Accra, Ghana. Sequence comparisons with existing databases revealed wide bacterial diversity with differing membership at each sampling point. There were members unique to each of the samples, and in general, the different sources manifested distinct populations with month-to-month changes in their relative abundance. For instance, the waterworks samples for all months were dominated by taxa from cluster 1 (Figure 1), but with different subpopulations in different months, while the household tap samples were dominated by taxa from cluster 2. This suggests that although the waterworks and household tap samples are related, there is likely some contamination and/or selection for different water taxa subsets during the delivery of water to the household taps. It would be of interest to study the relationship of microbial populations in other tap sources. The differences between most polytank samples (all but one, September polytank A) and the waterworks and household tap samples were dramatic for all sampling periods. Most polytank samples were dominated by taxa from clusters 3 or 4.

The origins of dominant taxa in the tap samples and polytank samples are not known definitively by the data in Table 1. However, we can look at whether the dominant taxa in the tap samples are present at detectable levels in the relevant waterworks samples (and thus could be potentially seeded by waterworks water), and we could do a similar analysis of the dominant polytank taxa (Table 1). For instance, the tap sample from December was dominated (21.26%) by ASV #979 that was found at low abundance in the October (0.83%) and November (0.08%) waterworks samples (Table 1). Thus, the ASV #979 could have been seeded by waterworks water. Conversely, the dominant taxon in polytank A samples (ASV #1386 for October (20.34%), November (10.55%) and December (12.66%)) was not detectable in either the tap or the waterworks samples (Table 1), suggesting that contamination after the waterworks treatment contributed to the presence of this taxon in the polytanks. Regardless of the original source of this and other taxa in the tap and polytank sources, it is likely that the high abundance of various taxa reflects

Table 1 | Microbes found in a Ghanaian drinking water system. The 154 ASVs presents are indicated, with reference to Figure 1; that is, the listing is in the same order as presented in Figure 1

ASV id	ASV size	September mains	October mains	November mains	December mains	September tap	October tap	November tap	December tap	September polytankA	October polytankA	November polytankA	December polytankA	September polytankB	October polytankB	November polytankB	December polytankB	September polytankC	October polytankC	November polytankC	December polytankC	Taxonomic classification
2636	602	0.32	0.34	1.90	2.13	0.51	0.04	0.00	0.09	1.24	0.12	0.30	0.09	1.55	0.20	0.30	0.04	0.05	0.28	0.21	0.43	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Alcaligenaceae
46	789	0.85	0.41	2.29	3.06	2.10	0.03	0.00	0.23	1.57	0.02	0.27	0.23	0.68	0.10	0.25	0.28	0.02	0.01	0.21	0.78	Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales
2940	602	0.41	0.58	1.59	1.90	2.04	0.17	0.00	0.16	1.38	0.14	0.06	0.18	0.26	0.34	0.15	0.31	0.00	0.07	0.16	0.33	Bacteria; Proteobacteria; Deltaproteobacteria; Gammaproteobacteria; Xanthomonadales; Myxococcales
2540	291	0.05	0.32	2.05	0.43	0.92	0.06	0.00	0.09	0.56	0.00	0.00	0.07	0.21	0.03	0.05	0.00	0.00	0.01	0.05	0.09	Bacteria; Planctomycetes; Phycisphaerae; Phycisphaerales; Phycisphaeraceae
78	387	0.88	0.24	0.73	0.93	1.32	0.06	0.00	0.10	0.94	0.19	0.09	0.05	0.53	0.08	0.04	0.06	0.00	0.10	0.09	0.16	Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales
2643	273	0.23	0.17	1.15	1.08	0.17	0.00	0.00	0.13	0.17	0.17	0.11	0.00	0.43	0.03	0.23	0.09	0.00	0.15	0.18	0.13	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Alcaligenaceae
838	1,615	0.55	0.34	0.64	1.81	0.55	0.48	0.07	0.07	0.57	1.48	0.75	0.85	3.32	1.60	3.85	0.84	0.07	2.44	1.16	4.02	Bacteria; Actinobacteria; Actinobacteria; Frankiales; Sporichthyaceae
155	847	0.93	0.21	0.44	0.63	0.38	0.06	0.02	0.03	0.42	0.14	0.38	0.11	0.85	2.29	1.40	0.16	0.05	0.44	1.83	2.73	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Burkholderiaceae; *Polynucleobacter*
842	734	0.20	0.30	0.78	1.53	0.13	0.23	0.02	0.07	0.32	0.46	0.43	0.45	1.85	0.61	1.17	0.37	0.11	0.81	0.67	1.44	Bacteria; Actinobacteria; Actinobacteria; Frankiales; Sporichthyaceae
232	1,204	0.85	1.09	7.07	2.24	0.29	0.48	0.26	0.44	0.74	0.32	0.47	0.16	0.94	0.52	0.78	0.22	0.04	1.13	0.65	1.28	Bacteria; Verrucomicrobia
3042	727	2.26	0.51	0.75	2.35	0.74	0.35	0.05	0.07	0.53	0.22	0.09	0.18	0.66	0.13	0.30	0.19	0.00	1.05	0.47	1.12	Bacteria; Actinobacteria; Acidimicrobiia; Acidimicrobiales; Acidimicrobiaceae
3041	576	1.03	0.94	0.86	2.11	0.42	0.46	0.00	0.19	0.28	0.22	0.11	0.14	0.17	0.25	0.11	0.22	0.00	0.58	0.30	1.21	Bacteria; Actinobacteria; Acidimicrobiia; Acidimicrobiales; Acidimicrobiaceae
171	239	0.28	0.09	0.08	0.22	0.53	0.01	0.00	0.01	0.45	0.24	0.12	0.09	0.81	0.25	0.14	0.04	0.05	0.43	0.05	0.06	Bacteria; Verrucomicrobia; Spartobacteria; empty_order; empty_family; *Chthoniobacter*
153	438	1.76	0.51	0.42	0.60	0.51	0.30	0.00	0.06	0.57	0.17	0.08	0.11	0.58	0.08	0.14	0.04	0.04	0.63	0.21	0.40	Bacteria; Actinobacteria; Acidimicrobiia; Acidimicrobiales; Acidimicrobiaceae
69	313	2.26	0.06	0.08	0.15	0.23	0.04	0.02	0.04	0.39	0.27	0.08	0.11	0.45	0.02	0.08	0.01	0.00	0.54	0.07	0.17	Bacteria; Verrucomicrobia; Spartobacteria; empty_order; empty_family; *Chthoniobacter*
840	208	0.07	0.00	0.10	0.24	0.06	0.06	0.00	0.03	0.07	0.20	0.14	0.20	0.40	0.13	0.40	0.13	0.00	0.17	0.23	0.67	Bacteria; Actinobacteria; Actinobacteria; Frankiales; Sporichthyaceae
2471	351	0.00	0.11	0.25	1.12	0.31	0.01	0.05	0.04	0.32	0.09	0.17	0.07	0.47	0.29	0.10	0.10	0.00	0.29	0.07	1.78	Bacteria; Proteobacteria; Betaproteobacteria; Methylophilales; Methylophilaceae

(*continued*)

Table 1 | continued

ASV id	ASV size	September mains	October mains	November mains	December mains	September tap	October tap	November tap	December tap	September polytankA	October polytankA	November polytankA	December polytankA	September polytankB	October polytankB	November polytankB	December polytankB	September polytankC	October polytankC	November polytankC	December polytankC	Taxonomic classification
168	288	0.32	3.06	0.51	0.86	0.08	0.09	0.12	0.29	0.08	0.00	0.00	0.00	0.06	0.00	0.00	0.01	0.00	0.04	0.00	0.03	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
2286	340	0.30	1.03	1.83	1.36	0.17	0.03	0.00	0.47	0.13	0.02	0.17	0.20	0.02	0.00	0.10	0.01	0.00	0.01	0.05	0.07	Bacteria; Proteobacteria; Gammaproteobacteria; Legionellales; Legionellaceae; *Legionella*
278	322	0.51	1.18	1.00	1.21	0.34	0.15	0.00	0.25	0.36	0.03	0.05	0.20	0.02	0.00	0.01	0.00	0.00	0.14	0.09	0.11	Bacteria; Proteobacteria; Gammaproteobacteria; Legionellales; Legionellaceae; *Legionella*
3100	384	0.45	0.71	1.63	0.71	0.44	0.04	1.06	0.91	0.18	0.00	0.11	0.07	0.04	0.00	0.14	0.04	0.02	0.03	0.14	0.10	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
3166	310	0.03	1.82	1.83	0.45	0.06	0.01	0.90	0.35	0.15	0.00	0.00	0.00	0.02	0.00	0.01	0.03	0.00	0.03	0.04	0.09	Bacteria; Cyanobacteria
3167	214	0.00	0.98	1.39	0.56	0.04	0.03	0.47	0.37	0.00	0.00	0.00	0.04	0.02	0.00	0.03	0.01	0.00	0.01	0.00	0.00	Bacteria; Cyanobacteria
3023	434	0.10	0.71	2.75	1.94	0.04	0.00	0.00	0.38	0.01	0.02	0.08	0.09	0.04	0.07	0.14	0.06	0.00	0.01	0.21	0.80	Bacteria; Proteobacteria; Alphaproteobacteria
2539	407	0.00	0.43	1.85	3.32	0.27	0.01	0.00	0.51	0.38	0.02	0.02	0.07	0.11	0.00	0.04	0.04	0.00	0.03	0.02	0.03	Bacteria; Planctomycetes; Phycisphaerae; Phycisphaerales; Phycisphaeraceae
2542	386	0.02	0.34	2.37	1.98	0.27	0.00	0.00	0.42	0.27	0.43	0.08	0.05	0.15	0.05	0.03	0.01	0.00	0.00	0.05	0.16	Bacteria; Planctomycetes; Phycisphaerae; Phycisphaerales; Phycisphaeraceae
133	357	0.05	0.17	2.10	2.09	0.57	0.01	0.00	0.23	0.53	0.00	0.02	0.02	0.09	0.02	0.10	0.01	0.04	0.00	0.04	0.07	Bacteria; Planctomycetes; Phycisphaerae; Phycisphaerales; Phycisphaeraceae
2283	303	0.05	0.19	0.05	2.69	0.00	0.03	0.00	1.86	0.00	0.00	0.02	0.05	0.00	0.03	0.01	0.06	0.00	0.01	0.02	0.03	Bacteria; Proteobacteria; Gammaproteobacteria; Legionellales; Legionellaceae; *Legionella*
189	232	0.00	0.04	0.92	2.48	0.00	0.00	0.00	0.53	0.01	0.02	0.00	0.02	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.03	Bacteria; Proteobacteria; Gammaproteobacteria; Methylococcales; Methylococcaceae; *Methylocaldum*
681	267	0.00	0.11	1.29	2.43	0.02	0.03	0.00	0.64	0.01	0.00	0.02	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.07	Bacteria; Proteobacteria; Gammaproteobacteria; Methylococcales; Methylococcaceae; *Methylocaldum*
1349	3,463	0.00	0.02	0.03	0.00	0.04	46.95	0.05	0.00	0.01	0.00	0.03	0.00	0.00	2.88	0.01	0.00	0.00	0.10	0.65	0.00	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; *Vogesella*
108	455	0.23	0.73	0.03	0.00	0.46	1.97	0.00	0.10	0.43	0.00	0.00	0.11	0.02	3.25	0.00	0.01	0.04	0.03	0.04	0.00	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; *Aquabacterium*
2946	400	0.02	0.00	0.12	0.07	0.23	4.18	0.09	0.00	0.01	0.05	0.99	0.00	0.06	0.07	0.00	0.07	0.00	0.00	0.02	0.03	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; *Pelomonas*
1348	350	0.00	0.00	0.00	0.00	0.00	4.40	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.71	0.00	0.00	0.00	0.00	0.02	0.00	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; *Vogesella*

(*continued*)

Table 1 | continued

ASV id	ASV size	September mains	October mains	November mains	December mains	September tap	October tap	November tap	December tap	September polytankA	October polytankA	November polytankA	December polytankA	September polytankB	October polytankB	November polytankB	December polytankB	September polytankC	October polytankC	November polytankC	December polytankC	Taxonomic classification
57	841	0.00	0.00	0.25	0.00	0.00	9.28	2.44	0.07	0.00	0.00	0.08	0.00	0.30	0.42	0.23	0.03	0.02	0.04	0.14	0.01	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; *Acinetobacter*
64	630	0.07	0.06	0.63	0.09	0.00	4.24	4.47	0.10	0.03	0.02	0.27	0.02	0.02	0.51	0.40	0.00	0.04	0.00	0.14	0.01	Bacteria; Proteobacteria; Gammaproteobacteria; Aeromonadales; Aeromonadaceae; *Aeromonas*; *caviae*
2232	1,456	0.00	0.00	0.02	0.02	2.96	0.87	21.31	2.53	1.03	0.07	0.05	0.05	0.02	0.10	0.25	0.45	0.05	0.04	0.23	0.10	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae
1963	1,019	0.38	4.86	0.05	0.00	0.23	0.09	12.75	2.06	0.07	0.05	0.29	0.16	0.00	0.03	0.05	0.10	0.00	0.00	0.28	0.04	Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Hyphomonadaceae
2942	472	0.00	0.00	0.00	0.00	0.00	0.81	9.20	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.15	0.04	0.00	Bacteria; Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; *Dechloromonas*; CL
162	245	0.00	0.00	0.00	0.00	0.19	0.07	4.52	0.20	0.18	0.00	0.00	0.07	0.09	0.00	0.00	0.03	0.02	0.00	0.00	0.00	Bacteria; Proteobacteria; Deltaproteobacteria
996	466	0.95	0.09	0.02	0.00	0.63	0.84	4.68	0.34	0.45	0.10	0.06	0.07	0.11	0.19	0.16	0.18	0.05	0.00	0.04	0.00	Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; *Sandarakinorhabdus*
42	417	0.48	0.00	0.00	0.00	0.67	0.28	1.68	1.95	0.85	0.05	0.11	0.05	0.11	0.02	0.10	0.36	0.02	0.07	0.19	0.01	Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales
980	439	0.81	0.02	0.03	0.00	0.32	0.33	2.10	2.21	0.36	0.07	0.21	0.11	0.06	0.10	0.07	0.27	0.00	0.03	0.37	0.03	Bacteria; Proteobacteria; Alphaproteobacteria; *Rhizobiales*
160	434	0.88	0.49	0.44	0.45	0.38	0.30	1.68	0.20	0.17	0.03	1.17	0.34	0.11	0.17	0.19	0.13	0.00	0.21	0.12	0.16	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Rickettsiaceae; *Rickettsia*
986	380	2.17	0.58	0.03	0.00	0.42	0.23	2.32	0.29	0.32	0.03	0.02	0.04	0.13	0.00	0.07	0.18	0.00	0.03	0.00	0.14	Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; *Sphingomonas*; *alpina*
1731	348	0.07	0.00	0.10	0.21	0.59	0.44	0.66	0.12	1.33	0.09	0.05	0.04	0.21	0.00	0.15	0.15	0.05	0.29	0.84	0.30	Bacteria; Proteobacteria; Alphaproteobacteria; *Rhizobiales*
3195	378	0.08	0.00	0.00	0.00	1.03	0.45	0.59	0.31	0.85	0.12	0.02	0.04	1.15	0.00	0.14	0.28	0.04	0.30	0.90	0.09	Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; *Sphingomonas*; BAC151
102	264	0.00	0.00	0.02	0.11	0.21	0.15	0.54	0.25	0.38	0.20	0.53	0.07	0.15	0.03	0.14	0.31	0.90	0.06	0.18	0.18	Bacteria; Proteobacteria; Deltaproteobacteria
2945	384	0.00	0.09	0.17	0.02	0.11	1.87	0.76	0.31	0.34	0.58	0.33	0.13	0.04	0.19	0.73	0.01	0.16	0.00	0.25	0.06	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; Roseateles; *Mitsuaria*

(*continued*)

Table 1 | continued

ASV id	ASV size	September mains	October mains	November mains	December mains	September tap	October tap	November tap	December tap	September polytankA	October polytankA	November polytankA	December polytankA	September polytankB	October polytankB	November polytankB	December polytankB	September polytankC	October polytankC	November polytankC	December polytankC	Taxonomic classification
2787	226	0.00	0.00	0.00	0.00	0.11	0.74	0.73	0.39	1.01	0.09	0.06	0.09	0.08	0.05	0.00	0.19	0.00	0.00	0.07	0.01	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales
249	1,013	0.22	2.57	3.90	4.20	2.38	0.03	0.00	0.78	2.00	0.07	0.11	0.13	0.49	0.08	0.05	0.09	0.04	0.12	0.25	0.26	Bacteria; Planctomycetes; Phycisphaerae; Phycisphaerales; Phycisphaeraceae
3144	1,121	2.34	3.45	3.22	2.69	1.56	0.07	0.19	0.47	1.78	0.26	0.05	0.11	1.06	0.13	0.00	0.07	0.07	1.10	0.35	0.48	Bacteria; Cyanobacteria; empty_class; Chroococcales; empty_family; *Microcystis*; *aeruginosa*
3054	1,578	5.59	4.56	2.58	3.43	3.34	0.70	0.02	2.66	3.27	0.05	0.06	0.05	0.25	0.02	0.00	0.09	0.11	0.15	0.02	0.06	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
3072	1,678	3.67	5.95	4.19	1.36	1.93	1.26	2.81	2.87	2.06	0.31	0.29	0.36	0.28	0.08	0.25	0.25	0.20	0.47	0.46	0.37	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
1495	1,312	3.83	4.11	1.27	1.38	1.81	0.91	1.23	3.64	1.58	0.38	0.12	0.13	0.55	0.29	0.08	0.12	0.09	0.50	0.16	0.30	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
3131	1,088	7.80	1.01	0.54	0.78	2.86	0.22	0.38	1.07	2.59	0.05	0.03	0.04	0.34	0.07	0.04	0.01	0.07	0.14	0.07	0.10	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
5	926	4.13	1.58	0.71	1.18	2.90	0.13	0.05	1.56	2.46	0.07	0.06	0.04	0.25	0.07	0.05	0.06	0.07	0.08	0.04	0.07	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
3143	608	2.17	3.83	0.39	1.25	0.95	0.09	0.05	0.23	1.23	0.09	0.02	0.04	0.36	0.08	0.04	0.01	0.00	0.06	0.07	0.03	Bacteria; Cyanobacteria; empty_class; Chroococcales; empty_family; *Microcystis*; *aeruginosa*
3145	569	1.43	2.80	0.71	1.51	0.61	0.07	0.07	0.22	1.37	0.07	0.02	0.09	0.43	0.24	0.00	0.01	0.02	0.11	0.11	0.18	Bacteria; Cyanobacteria; empty_class; Chroococcales; empty_family; *Microcystis*
1457	907	4.41	2.29	1.02	0.76	1.72	0.45	0.61	0.98	1.61	0.19	0.09	0.07	0.66	0.17	0.08	0.07	0.07	0.12	0.11	0.11	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
1456	721	2.46	3.08	0.59	0.69	1.07	0.57	0.17	1.05	1.20	0.20	0.12	0.05	0.38	0.10	0.04	0.07	0.05	0.21	0.16	0.18	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
3112	604	1.82	1.95	1.07	0.80	0.92	0.30	0.35	1.26	1.09	0.10	0.05	0.07	0.06	0.07	0.04	0.07	0.02	0.17	0.11	0.03	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
979	3,169	1.33	0.83	0.08	0.00	9.27	2.52	2.10	21.26	8.61	0.22	0.08	0.22	0.43	0.32	0.14	1.50	0.09	0.21	0.21	0.17	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales
50	2,679	1.09	3.32	0.05	0.00	4.21	3.48	6.08	18.06	3.30	0.34	0.14	0.22	1.19	0.25	0.29	0.87	0.33	0.25	0.47	0.07	Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Hyphomonadaceae
1192	3,486	5.21	12.82	4.68	7.91	2.27	1.36	3.74	12.53	2.67	1.13	0.41	0.47	0.75	0.20	0.37	0.75	0.11	0.80	0.81	1.36	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
3022	3,703	1.74	6.08	21.43	14.16	2.78	0.44	0.00	2.27	2.68	0.15	0.33	0.72	2.15	0.49	1.20	0.40	0.20	0.28	0.93	5.06	Bacteria; Proteobacteria; Alphaproteobacteria

(*continued*)

Table 1 | continued

ASV id	ASV size	September mains	October mains	November mains	December mains	September tap	October tap	November tap	December tap	September polytankA	October polytankA	November polytankA	December polytankA	September polytankB	October polytankB	November polytankB	December polytankB	September polytankC	October polytankC	November polytankC	December polytankC	Taxonomic classification
721	3,239	8.08	2.55	8.26	8.51	4.37	0.33	0.00	0.92	5.05	0.44	0.65	1.01	3.83	1.03	1.83	0.94	0.11	0.70	1.42	4.14	Bacteria; Proteobacteria; Alphaproteobacteria
3084	444	1.89	1.54	0.31	0.26	0.88	0.23	0.12	0.80	0.87	0.03	0.03	0.02	0.23	0.00	0.00	0.00	0.02	0.25	0.00	0.09	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
3113	381	2.41	1.05	0.31	0.32	0.48	0.10	0.17	0.72	0.49	0.07	0.02	0.02	0.15	0.03	0.01	0.06	0.00	0.07	0.02	0.03	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
1649	420	2.06	2.29	0.36	0.54	0.38	0.10	0.07	0.04	0.50	0.14	0.00	0.00	0.30	0.13	0.08	0.04	0.04	0.19	0.09	0.11	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Acetobacteraceae; *Roseomonas*; NA; SK_65; NA
1455	350	0.93	1.84	0.03	0.34	0.61	0.30	0.07	0.78	0.38	0.10	0.03	0.02	0.09	0.07	0.01	0.10	0.02	0.26	0.00	0.09	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
3098	343	1.54	1.84	0.15	0.19	0.61	0.07	0.02	0.42	0.46	0.15	0.00	0.00	0.28	0.00	0.01	0.00	0.02	0.12	0.09	0.07	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
3132	266	1.39	0.45	0.20	0.35	0.61	0.12	0.07	0.23	0.70	0.03	0.02	0.02	0.11	0.03	0.01	0.03	0.04	0.01	0.04	0.01	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
3056	228	0.66	0.92	0.12	0.11	0.46	0.16	0.07	0.35	0.70	0.02	0.00	0.04	0.19	0.05	0.00	0.01	0.00	0.03	0.02	0.00	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
3086	225	0.80	0.71	0.07	0.24	0.38	0.16	0.12	0.42	0.38	0.07	0.00	0.02	0.11	0.02	0.01	0.06	0.02	0.18	0.00	0.06	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
984	564	0.30	0.15	0.00	0.00	2.76	0.61	0.45	1.43	2.56	0.10	0.03	0.02	0.26	0.12	0.11	0.06	0.00	0.08	0.04	0.03	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales
2338	913	0.35	0.30	0.02	0.02	6.23	0.29	0.45	0.04	5.76	0.12	0.03	0.04	0.87	0.00	0.41	0.00	0.04	0.03	0.07	0.00	Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; *Novosphingobium*; *aromaticivorans*; NA; NA
983	880	0.10	0.00	0.00	0.00	3.79	0.55	4.26	0.25	3.75	0.15	0.15	0.13	1.45	0.07	0.32	0.04	0.04	0.25	0.33	0.00	Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; *Sphingomonas*
139	533	2.12	0.00	0.02	0.02	2.67	0.01	0.00	0.00	2.46	0.00	0.00	0.00	1.26	0.10	0.00	0.00	0.04	0.14	0.00	0.01	Bacteria; Chlamydiae; Chlamydiae; Chlamydiales; Chlamydiaceae
74	624	2.24	0.41	0.20	0.11	3.15	0.03	0.02	0.01	2.46	0.14	0.00	0.02	1.06	0.08	0.03	0.03	0.09	0.18	0.02	0.20	Bacteria; Chlamydiae; Chlamydiae; Chlamydiales
2943	462	1.56	0.04	0.03	0.09	2.36	0.32	0.14	0.18	1.66	0.29	0.02	0.02	0.53	0.03	0.08	0.06	0.13	0.03	0.09	0.04	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; *Pelomonas*
2944	348	1.92	0.00	0.00	0.00	2.48	0.17	0.07	0.16	0.60	0.02	0.00	0.00	0.17	0.02	0.01	0.03	0.04	0.07	0.21	0.00	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; *Inhella*

(*continued*)

Table 1 | continued

ASV id	ASV size	September mains	October mains	November mains	December mains	September tap	October tap	November tap	December tap	September polytankA	October polytankA	November polytankA	December polytankA	September polytankB	October polytankB	November polytankB	December polytankB	September polytankC	October polytankC	November polytankC	December polytankC	Taxonomic classification
340	221	0.43	0.00	0.00	0.00	0.95	0.06	0.02	0.04	1.29	0.02	0.00	0.00	0.60	0.02	0.00	0.03	0.11	0.01	0.02	0.01	Bacteria; Proteobacteria; Deltaproteobacteria; Bdellovibrionales; Bacteriovoracaceae; *Peredibacter*
1013	245	0.03	0.69	0.00	0.00	2.31	0.01	0.05	0.00	0.98	0.00	0.02	0.00	0.23	0.00	0.01	0.01	0.00	0.00	0.00	0.03	Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; *Sphingomonas*
3239	2,214	0.00	0.11	0.05	0.04	0.10	0.04	0.09	0.03	0.06	0.00	0.02	0.11	19.51	9.20	0.49	0.04	0.94	0.12	8.71	0.04	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; *Pseudorhodoferax*
3242	1,398	0.00	0.04	0.07	0.15	0.00	0.03	0.07	0.00	0.00	0.00	0.20	0.00	0.49	14.95	0.43	0.03	0.00	0.28	6.71	0.26	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae
1689	5,588	0.00	0.11	0.00	0.00	0.00	0.06	0.21	0.03	0.08	9.26	1.82	1.25	3.77	6.89	15.90	29.28	0.74	3.51	4.44	8.09	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; Chitinophagaceae; Lacibacter
1389	3,245	0.07	0.13	0.00	0.00	0.10	0.04	0.05	0.04	0.34	3.10	3.45	2.94	8.53	4.68	0.78	3.00	0.60	8.39	8.19	7.55	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; Chitinophagaceae; *Sediminibacterium*
45	2,210	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.32	2.40	3.12	1.95	3.74	4.90	2.45	2.50	2.80	3.54	7.34	0.97	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Oxalobacteraceae; Paucimonas
3206	3,202	0.48	0.11	0.02	0.00	0.06	0.03	0.05	0.07	0.52	7.59	2.55	2.29	10.24	5.76	4.92	4.84	0.85	2.80	7.32	2.06	Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; *Novosphingobium*; FW – 6
2570	1,585	0.05	0.00	0.02	0.02	0.11	0.00	0.02	0.00	0.08	0.05	0.17	0.00	0.19	3.35	8.56	7.57	0.00	0.14	2.81	0.65	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; *Pseudorhodoferax*
128	1,137	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.04	0.12	0.05	0.04	0.98	1.69	4.92	3.61	0.05	3.79	1.46	0.10	Bacteria; Proteobacteria; Deltaproteobacteria
1852	1,066	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	1.94	0.14	0.29	0.00	0.15	2.02	5.22	3.48	0.00	0.70	1.84	0.03	Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; *Caulobacter*; *Brevundimonas*
269	956	0.00	0.00	0.02	0.04	0.02	0.00	0.02	0.00	0.04	0.03	0.00	0.00	3.58	4.50	2.35	0.84	0.20	0.22	3.86	0.21	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; *Polaromonas*
123	597	0.00	0.00	0.03	0.00	0.13	0.59	0.09	0.00	0.07	0.00	0.00	0.02	1.09	2.65	2.80	1.15	0.02	0.06	0.58	0.04	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; NA; *Piscinibacter*
924	668	0.05	0.21	0.00	0.00	0.00	0.01	0.09	0.00	0.10	0.03	0.11	0.02	0.45	1.58	2.75	1.20	0.02	0.55	1.55	1.51	Bacteria; Proteobacteria; Betaproteobacteria

(*continued*)

Table 1 | continued

ASV id	ASV size	September mains	October mains	November mains	December mains	September tap	October tap	November tap	December tap	September polytankA	October polytankA	November polytankA	December polytankA	September polytankB	October polytankB	November polytankB	December polytankB	September polytankC	October polytankC	November polytankC	December polytankC	Taxonomic classification
1650	434	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	6.15	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Acetobacteraceae; *Roseomonas*; *gilardii*; *NA*; *NA*; *subsp._rosea*
146	998	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.07	0.68	0.64	0.42	0.02	1.77	0.16	0.82	0.11	0.12	7.03	4.22	Bacteria; Verrucomicrobia
145	643	0.12	0.00	0.07	0.00	0.06	0.09	0.09	0.06	0.13	0.03	0.24	0.18	0.06	0.13	0.99	1.75	0.23	0.18	3.58	2.10	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae; *Planctomyces*
2148	351	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.07	0.99	0.28	0.00	0.32	3.90	0.13	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae; *Planctomyces*
2573	344	0.00	0.02	0.02	0.04	0.11	0.62	0.26	0.00	0.10	0.02	0.23	0.02	0.98	1.40	0.82	0.01	0.16	0.06	0.79	0.03	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; *Acidovorax*
1994	428	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.06	1.47	0.14	0.40	0.26	0.86	1.51	0.31	0.00	0.29	1.26	0.23	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Acetobacteraceae; *Gluconacetobacter*; *diazotrophicus*; ICB557; NA
1151	296	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.66	1.11	1.53	0.00	0.02	0.00	1.12	0.00	Bacteria; Cyanobacteria; Chloroplast
1849	270	0.02	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	1.28	0.71	1.55	0.21	0.00	0.00	0.54	0.00	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; *Rhodobacter*
1734	337	0.00	0.00	0.00	0.00	0.02	0.07	0.00	0.03	0.11	0.46	0.20	0.09	1.15	0.37	0.40	1.83	0.13	0.14	0.46	0.03	Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; *Brevundimonas*
680	328	0.00	0.04	0.02	0.00	0.00	0.00	0.02	0.00	0.22	0.14	0.03	0.00	1.81	0.15	0.25	1.33	0.54	0.19	0.12	0.50	Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; *Thermomonas*; R039N
2685	228	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.03	0.44	0.11	0.00	0.54	0.12	0.27	0.02	0.14	1.98	0.06	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales
1265	206	0.00	0.00	0.00	0.06	0.06	0.03	0.00	0.01	0.17	0.09	0.05	0.00	0.15	0.44	0.43	0.25	0.00	0.61	0.76	0.11	Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Hyphomonadaceae; *Hirschia*
1956	195	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.06	0.27	0.09	0.20	1.04	0.24	0.00	0.26	0.07	0.16	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Hyphomicrobiaceae; *Hyphomicrobium*
3223	194	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.26	0.05	0.08	0.22	0.43	0.00	0.02	1.35	0.00	0.11	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales
2470	262	0.00	0.00	0.00	0.00	0.06	0.00	0.14	0.04	0.39	0.03	0.08	1.01	0.04	0.00	0.27	0.07	0.54	1.24	0.21	0.00	Bacteria; Proteobacteria; Betaproteobacteria; Nitrosomonadales; Nitrosomonadaceae

(*continued*)

Table 1 | continued

ASV id	ASV size	September mains	October mains	November mains	December mains	September tap	October tap	November tap	December tap	September polytankA	October polytankA	November polytankA	December polytankA	September polytankB	October polytankB	November polytankB	December polytankB	September polytankC	October polytankC	November polytankC	December polytankC	Taxonomic classification
14	347	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.39	0.43	0.99	0.13	0.13	0.60	0.34	0.07	0.61	0.07	1.51	Bacteria; Acidobacteria
621	499	0.00	0.00	0.03	0.07	0.00	0.00	0.02	0.00	0.03	0.73	1.38	1.08	0.00	0.39	0.65	0.60	0.23	0.48	0.11	1.88	Bacteria; Proteobacteria; Betaproteobacteria; Nitrosomonadales; Nitrosomonadaceae
76	536	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.01	0.36	0.70	0.73	0.87	0.13	0.37	0.48	0.49	0.51	1.50	1.33	0.85	Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales
182	426	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.48	0.90	2.17	0.02	0.32	0.26	0.85	0.67	0.21	0.37	0.70	Bacteria; Verrucomicrobia
2778	291	0.00	0.00	0.00	0.00	0.10	0.00	0.07	0.04	0.11	0.14	0.55	0.88	0.02	0.19	0.37	0.45	0.49	0.40	0.18	0.63	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae
1961	955	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	2.24	0.98	0.00	2.14	0.55	0.16	0.13	4.51	0.14	3.20	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; *Pseudorhodobacter*; *Rhodobacter*; NA; 16 – 62; NA
2683	904	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	1.32	0.11	0.59	0.80	0.70	0.00	3.76	0.00	5.80	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales
285	538	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.29	0.00	0.78	0.07	0.49	0.00	3.38	0.44	2.33	Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; *Zymomonas*
15	678	0.00	0.00	0.15	0.09	0.04	0.00	0.00	0.04	0.00	0.00	0.05	0.20	0.00	0.22	1.54	2.64	0.00	2.98	0.04	1.79	Bacteria; Proteobacteria; Betaproteobacteria; Methylophilales; Methylophilaceae
1957	583	0.03	0.04	0.00	0.02	0.00	0.00	0.00	0.04	0.01	0.92	0.47	0.47	0.06	0.39	1.13	1.23	0.20	1.89	0.12	1.68	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; empty_family; *Reyranella*
2334	772	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.01	1.26	0.67	1.54	0.17	0.54	1.98	0.55	0.00	2.76	0.07	2.00	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales
3219	714	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	1.09	0.64	1.44	0.08	0.35	1.03	0.78	0.02	3.03	0.11	2.10	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales
110	1,531	0.00	0.00	0.02	0.02	0.17	0.00	0.05	0.04	0.01	0.97	1.89	4.55	0.02	0.08	0.04	0.19	18.16	0.47	0.04	0.26	Bacteria; Verrucomicrobia; Opitutae; Opitutales; Opitutaceae; *Opitutus*
1242	1,440	0.00	0.00	0.00	0.00	0.04	0.01	0.00	0.00	0.06	0.00	0.00	0.00	0.06	0.00	0.00	0.01	25.73	0.00	0.09	0.00	Bacteria; Chlamydiae; Chlamydiae; Chlamydiales; Simkaniaceae; *Candidatus*_Rhabdochlamydia
94	612	0.00	0.00	0.00	0.00	0.02	0.01	0.09	0.01	0.24	0.44	0.99	1.08	0.02	0.13	0.45	0.33	3.96	1.46	0.07	0.63	Bacteria; Gemmatimonadetes; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae
2145	603	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.44	1.28	0.56	0.00	0.00	0.00	0.16	5.62	1.34	0.04	0.57	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales
16	305	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.56	1.05	1.14	0.00	0.00	0.00	0.00	2.48	0.01	0.00	0.00	Bacteria; Nitrospirae; *Nitrospira*

(*continued*)

Table 1 | continued

ASV id	ASV size	September mains	October mains	November mains	December mains	September tap	October tap	November tap	December tap	September polytankA	October polytankA	November polytankA	December polytankA	September polytankB	October polytankB	November polytankB	December polytankB	September polytankC	October polytankC	November polytankC	December polytankC	Taxonomic classification
180	250	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.01	0.01	0.12	1.05	0.00	0.00	0.02	0.03	0.01	2.98	0.00	0.02	0.00	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae; Planctomyces
2895	716	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.03	0.21	0.00	0.00	0.02	0.02	0.94	0.22	5.76	3.99	0.21	0.00	0.06	Bacteria; Verrucomicrobia
32	435	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.86	0.00	0.00	0.00	Bacteria; Verrucomicrobia
82	304	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.00	0.00	0.00	0.00	5.42	0.00	0.00	0.00	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae; *Gemmata*
2896	505	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.02	0.11	6.39	0.04	0.00	0.19	0.12	0.22	0.04	0.14	1.25	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae; Planctomyces
124	370	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.36	1.14	3.67	0.02	0.00	0.00	0.00	0.47	0.54	0.00	0.04	Bacteria; Proteobacteria; Betaproteobacteria; Nitrosomonadales; Nitrosomonadaceae
1733	291	0.00	0.06	0.00	0.00	0.04	0.01	0.17	0.57	0.06	0.19	0.88	2.67	0.04	0.00	0.01	0.04	0.14	0.01	0.02	0.03	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae
126	224	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.56	0.82	1.14	0.00	0.00	0.15	0.04	0.38	0.50	0.00	0.04	Bacteria; Acidobacteria
65	360	0.03	0.02	0.02	0.06	0.00	0.00	0.05	0.01	0.06	0.87	1.58	2.02	0.02	0.00	0.15	0.04	0.23	0.08	0.07	0.58	Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae
113	216	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	1.49	1.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Bacteria
97	255	0.00	0.00	0.00	0.02	0.04	0.03	0.02	0.04	0.01	0.77	1.51	1.32	0.00	0.00	0.03	0.01	0.36	0.04	0.04	0.00	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales
620	410	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.77	2.27	1.79	0.00	0.02	0.01	0.01	0.04	0.73	0.00	0.00	Bacteria; Proteobacteria; Betaproteobacteria; Nitrosomonadales; Nitrosomonadaceae
3196	572	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	2.05	3.22	2.33	0.02	0.07	0.10	0.00	1.66	0.03	0.00	0.04	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales
1735	418	0.00	0.00	0.00	0.00	0.00	0.01	0.05	0.00	0.03	2.17	1.40	2.62	0.00	0.00	0.00	0.00	0.47	0.23	0.04	0.06	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; empty_family; *Reyranella*
3186	521	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.20	1.82	0.70	1.34	0.15	0.12	1.61	0.16	0.23	1.52	0.04	0.16	Bacteria; Planctomycetes; Phycisphaerae; Phycisphaerales; Phycisphaeraceae
1149	287	0.00	0.00	0.02	0.07	0.00	0.01	0.02	0.06	0.41	1.31	0.21	2.15	0.00	0.05	0.01	0.16	0.29	0.00	0.05	0.04	Bacteria
2146	522	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.04	0.22	6.39	0.49	0.27	0.21	0.00	0.01	0.03	0.45	0.47	0.02	0.00	Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; *Caulobacter*; Es3; MDB1–11; NA
24	315	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.34	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	Bacteria; Verrucomicrobia
261	290	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.07	0.04	0.02	0.00	0.00	0.00	0.00	1.09	0.00	0.00	Bacteria

(*continued*)

Table 1 | continued

ASV id	ASV size	September mains	October mains	November mains	December mains	September tap	October tap	November tap	December tap	September polytankA	October polytankA	November polytankA	December polytankA	September polytankB	October polytankB	November polytankB	December polytankB	September polytankC	October polytankC	November polytankC	December polytankC	Taxonomic classification
311	470	0.02	0.00	0.00	0.00	0.11	0.04	0.33	0.07	0.80	0.26	4.23	1.28	0.02	0.02	0.01	0.04	0.07	0.03	0.09	0.04	Bacteria; Proteobacteria; Deltaproteobacteria
163	755	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	5.81	2.82	0.00	0.00	0.00	0.00	0.00	2.95	0.00	0.03	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales
2231	845	0.02	0.00	0.00	0.19	0.02	0.03	0.00	0.01	0.01	0.80	9.35	2.00	0.00	0.00	0.00	0.22	0.05	0.45	0.02	0.06	Bacteria; Proteobacteria
1386	2,909	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.34	10.55	12.66	0.00	0.03	0.04	0.01	0.07	4.05	0.00	0.28	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; Chitinophagaceae; *Ferruginibacter*
925	1,808	0.00	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.06	3.26	7.01	5.24	0.02	0.07	2.35	3.33	2.42	2.98	0.09	1.52	Bacteria; Proteobacteria; Betaproteobacteria
2143	1,324	0.10	0.02	0.12	0.28	0.21	0.04	0.07	0.07	0.45	1.36	2.54	2.35	0.11	1.31	1.84	1.36	0.78	5.64	0.97	0.70	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales
3184	1,131	0.00	0.06	0.03	0.02	0.02	0.00	0.00	0.03	0.55	2.63	1.72	3.88	0.43	0.25	2.76	1.29	0.98	2.49	0.07	0.53	Bacteria; Planctomycetes; Phycisphaerae; Phycisphaerales; Phycisphaeraceae

To be included in Figure 1 and this table, a given ASV must have at least a 1.0% presence in one of the collection sites for at least one sampled month. Each ASV is assigned to microbial assemblage 1 or 2 or 3 or 4 based upon where they were found as determined by the MED analysis. Each sample was found to contain multiple ASVs drawn from different bacterial phyla, and most phyla are represented in all samples. However, the particular ASV identified is quite different from the different assemblages found in the different sampling sites and months.

selection pressures. It should be noted that the polytanks offered distinct habitats compared to the waterworks and taps.

The September polytank A taxa types were unique in that they primarily fell into clusters 1 and 2 similar to the September tap and waterworks samples (Figure 1). A possible explanation for this result is that polytank A was cleaned (emptied, cleaned with household detergent and rinsed) a few days before the September sampling occurred, so it may not have had time to undergo the contamination/selective community change experienced by polytanks B and C. By October, this distinction had disappeared. It should be noted that these observations are consistent with the observation that water stagnation can give rise to distinct microbial assemblages in water taps (Ling *et al.* 2018).

It is of interest to determine whether any of the taxa found in the water system were likely to be human pathogens. It is not possible to definitively determine this without complete genome sequences and/or experimental tests. Nonetheless, the MED analysis identified taxa from some orders worthy of further investigation. Of particular concern were taxa from the Burkholderiales that were represented by several ASVs found in several sites including samples from the waterworks, tap source and various polytanks. Pathogenic strains resistant to antibiotics and disinfectants have been identified within the Burkholderiales. Waterborne disease outbreaks associated with this order have been reported previously (Inglis *et al.* 1999). Another order of particular concern is the Rickettsiales that were represented by several taxa mostly restricted to tap water and polytank sources. Rickettsiales are typically obligate intracellular pathogens of free-living amoeba in drinking water ecosystems (Fritsche *et al.* 1999).

The results from these studies indicate that while the water quality of the treated waterworks samples may be similar to that of other water plants (Eichler *et al.* 2006), the water quality for the ultimate consumers also depends upon the delivery system and storage systems. Storage in polytanks results in unique microbial contamination problems that should be considered in their design and/or maintenance regardless of efforts to improve the quality of water at the waterworks.

CONCLUSIONS

1. Water treatment plants can provide water contaminated with complex microbial assemblages that are similar month to month over a 4-month period.
2. Water taps provide water with microbial assemblages similar to that found in the water plant water.
3. Water storage tanks newly cleaned and filled with water have a microbial assemblage similar to that observed in the tap water.
4. Water stored for a month or more contains microbial assemblages distinct from those found in the water plant, in the tap and in the newly filled water tank.
5. The existence of distinct microbial assemblages in stored water suggests that the provision of adequate dependable quantities of water (thus bypassing the need for domestic water storage) should be a priority in water system improvements or alternatively that water storage tanks should be cleaned at frequent intervals.

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SUPPLEMENTARY MATERIAL

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