Environmental Science
Water Research & Technology

Effects of BAC-filtration, disinfection, and temperature on water quality in simulated reclaimed water distribution systems

Journal:	*Environmental Science: Water Research & Technology*
Manuscript ID	EW-ART-06-2020-000581.R1
Article Type:	Paper

SCHOLARONE™
Manuscripts

Effects of BAC-filtration, disinfection, and temperature on water quality in simulated reclaimed water distribution systems

Ni Zhu[1], Sudeshna Ghosh[1], Laurel Strom[2], Amy Pruden[1], Marc A. Edwards[1*]

[1] Via Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, VA 24061, USA

[2] CDM Smith, 14432 SE Eastgate Way, Suite 100, Bellevue, WA, 98007

* Corresponding author. Tel.: +1 540 231 7236. Email address: edwardsm@vt.edu (M.A. Edwards).

Water Impact Statement

Achieving water sustainability goals will require improved understanding of water quality degradation during transport of reclaimed water to its point of use. This study illustrates that choice of water treatment, seasonality, water age, and levels of nutrients profoundly influence water quality. If chloramine is selected as the secondary disinfectant residual, warm temperatures can cause rapid disinfectant loss due to nitrification.

1 Effects of BAC-filtration, disinfection, and temperature on water quality in simulated reclaimed
2 water distribution systems

3 Ni Zhu[1], Sudeshna Ghosh[1], Laurel Strom[2], Amy Pruden[1], Marc A. Edwards[1*]
4 [1] Via Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, VA
5 24061, USA
6 [2] CDM Smith, 14432 SE Eastgate Way, Suite 100, Bellevue, WA, 98007
7
8 * Corresponding author. Tel.: +1 540 231 7236. Email address: edwardsm@vt.edu (M.A.
9 Edwards).
10
11 **Keywords**: Reclaimed water; Distribution system; Disinfectant; Nitrification; Water chemistry;
12 Environmental metagenomics
13

14 ## Abstract

15 The distinct characteristics of reclaimed versus potable water have important implications for
16 design and operation of reclaimed water distribution systems (RWDSs). Here we operated six
17 simulated RWDSs in parallel to determine the effects of feed water treatment (with and without
18 biologically-active carbon (BAC) filtration) and residual disinfectants (chlorine, chloramine, or
19 no residual) on the distributed water quality. Following a six-month acclimation period, the
20 experimental conditions were implemented over 2
21 years of operation, with a temperature regime of 14°C→22°C→30°C→22°C→14°C imposed to
22 simulate seasonal variation. Comprehensive water chemistry profiling and microbial sampling
23 were conducted over a range of water ages (0-d, 1-d, 2.5-d and 5-d) along the RWDSs at each

temperature phase. When ≥22°C, RWDSs became more vulnerable to deterioration of water quality due to nitrification, as evidenced by accelerated chloramine decay and increased relative abundance of nitrifier genera and functional genes. Contrary to conventional experience in potable water distribution, chlorine generally became more persistent than chloramine from the first 22°C phase onwards. Enhanced persistence of chlorine was accompanied by increased biological stability (i.e., lower cell counts and reduced dissolved oxygen demand). BAC filtration initially improved water quality (i.e., lower total organic carbon and biomass, longer persistence of disinfectants), but benefits diminished over long term of distribution system operation. Taxonomic and functional metagenomic profiles revealed that chlorine had the strongest selective effect compared to both chloramine and no residual, particularly for the unfiltered condition. The long-term operation strategy employed here enabled evaluation of distribution system management practices and their interactive effects as the RWDSs experienced temperature shifts and aged.

1. Introduction

Climate change, extreme weather and urbanization are spurring use of alternative and more sustainable water resources, including reclamation of treated wastewater effluent.[1] Reclaimed water can have distinct physiochemical and microbial water quality properties compared to conventional drinking water, including higher levels of nutrients, disinfectant demand, and elevated levels of biomass and potential pathogens.[2,3] Meeting current drinking water regulations requires a multi-barrier treatment process in order to achieve high levels of pathogen and microbial inactivation and to eliminate aesthetic concerns. On the other hand, there is a lack of well-established treatment targets and goals for reclaimed water. Instead, an overall "fit-for-purpose" guideline is intended to match treatment level to the end reuse application, resulting in a wide range of reclaimed water qualities.[1,2] In addition to the treatment process, reclaimed water distribution systems (RWDSs), i.e., systems designed to convey non-potable water, experience distinct operational conditions compared to drinking water distribution systems (DWDSs), including more intermittent demand and associated prolonged stagnation events, making RWDSs more susceptible to various biological stability-related water quality degradation issues as the water flows through the systems, including disinfectant decay , microbial regrowth, sediment deposition, and biofilm growth.[2,3] Despite these known distinctions, there is a dearth of long-term studies that systematically examine the effects of typical treatments on water quality in RWDSs.

Non-potable reuse of reclaimed water, including irrigation, toilet flushing, landscaping and industrial cooling, accounts for more than 50% of total reuse in the United States.[4] The California State Water Resources Control Board requires reclaimed water to be "oxidized" and "disinfected" for most agricultural reuse purposes, with an additional filtration step

recommended for broader surface reuse applications.[5] Granular/biological activated carbon (GAC/BAC) filtration and disinfection are amongst the most commonly applied final treatments for non-potable reuse.[5] GAC removes some organic compounds via adsorption [6] while BAC is able to remove recalcitrant organic compounds via biodegradation.[7,8]

RWDSs face many of the same issues as DWDSs, including deterioration of water quality during transport in pipes due to depletion of disinfectant residuals and biological regrowth.[9-11] The loss of biological stability during distribution; such as increase in organic matter due to leaching of organic carbon from pipes, biofilm sloughing, and excessive growth of bacteria, pathogens, and nitrifying microorganisms,[12-14] has been well-noted to result in undesirable water quality at the point of use in DWDSs. Complex interactions between nutrients, disinfectants, and the microbial community in RWDSs can further exacerbate problems associated with loss of biological stability. In DWDSs the two most common disinfectants, chlorine and chloramine, each has its strengths and weaknesses as a disinfectant dependent on temperature, source water quality, disinfection by-products, distribution system materials, water use patterns and other factors.[12,16-19] While chlorine is a stronger oxidant than chloramine,[20-21] the residual is usually lost more quickly and forms more regulated disinfection-byproducts.[22,23] In contrast, chloramine is less reactive, but forms fewer regulated disinfection-byproducts and tends to be more persistent during distribution, especially in the presence of unlined iron pipes.[18,19,23] However, in circumstances with rapid nitrification in potable water systems, chloramine decay rates can sometimes equal or exceed those of free chlorine.[19,24,25] It is critical that the relative advantages and disadvantages of these two common disinfectants are rigorously evaluated in terms of their application in

RWDSs, especially in hot climate regions that tend to be early adopters of sustainable water strategies where higher temperatures tended to exacerbate disinfectant decay.[2]

Only a few short-term (< 12 months) studies have examined the effects of different reclaimed water treatment options on finished water quality,[26,27] and relatively little is known about how water quality changes in the RWDSs and at the point of use over the course of prolonged operation and seasonal varaitions.[2] Recent field-scale studies have revealed several concerns with respect to water quality delivered by RWDSs;[3,26,28,29] The use of metagenomics-based analysis further enabled rapid detection of a broad diversity of taxonomic and functional genes to elucidate possible mechanisms about interested metabolic pathways.[2,9,11] However, it is not possible to isolate the key controlling variables in field-scale studies.

The objective of this study was to advance understanding of the treatment processes and operational factors that shape the chemical and biological water quality delivered by RWDSs over long-term operation. Conventional DWDS research over recent decades has benefited greatly from combining field surveillance of full-scale systems and lab-scale simulated reactors focused on understanding the underlying mechanisms driving the observed water chemistry and microbial trends.[27-29] Depending on the chemical and biological process of interest, a wide range of lab-scale reactor designs have been employed to illuminate important aspects of the water distribution systems. These include, batch-scale reactors, aged pipe coupons in batch reactors, and an array of scaled-down continuous flow reactors. Building upon prior field work and lab-scale studies on DWDSs, we identified the formation of redox zones, disinfectants types, BAC-filtration, flow regime, water age, and temperature to be the key parameters shaping water quality of water at the point of use, and

correspondingly designed a lab-scale RWDS suitable for their integrated evaluation. Employing these lab-scale continuous-flow RWDSs, here we conducted a controlled study of comparing effects of BAC-filtration and disinfectant residual conditions (chlorine, chloramines, and no residual) as well as seasonal temperature variation over a ~2-year period. In order to achieve a realistic water age with pipe dimensions that are comparable to field RWDSs, a high water age of 5 days was targeted while maintaining the flow velocity low using 4-in. PVC pipes. Comprehensive water chemistry analysis followed by targeted metagenomic examination of select targets in bulk water and biofilm under various conditions and at different water ages provided insight into the interplay of different factors controlling microbial and physicochemical aspects of reclaimed water quality at the point of use.

2. Methods

2.1 Design and operation of simulated RWDSs

Final effluent before UV-disinfection, collected from a local municipal wastewater treatment plant (Virginia, USA), was used as the source water to simulate reclaimed water before pretreatment. The source water was collected twice per week and stored at 4 °C for a maximum of 3 days before treatment to minimize changes in the source water quality. Two levels of treatment were sequentially performed on the source water: BAC-filtration and chlorine-based disinfection. BAC-filtration was conducted by re-circulating the source water through two filters (Culligan D-20A Drinking Water Filter) connected in series for 60 hours.

A standardized filter replacement protocol was applied throughout the course of the study to maintain a relatively consistent filtration performance, wherein the second aged filter was moved into the first position in the flow sequence every three months, and a new filter was added to the second position where it could be quickly inoculated. Three disinfectant conditions: free chlorine, chloramine and breakpoint chlorination (no residual), were subsequently applied to both the filtered and unfiltered source water to achieve a stable disinfectant residual target of 4 mg/L free chlorine for the free chlorine conditions, 4 mg/L total chlorine for the chloramine conditions (4:1 weight ratio of final chlorine to ammonia was used to create monochloramine), and <0.2 mg/L free chlorine residuals for the breakpoint chlorination conditions. To achieve a stable 4 mg/L free chlorine residual in the unfiltered water, the source water was dosed to 8 mg/L free chlorine and allowed to sit for 30 hours at 4°C to destroy the high chlorine demand over time. The pre-chlorinated water was readjusted to 4 mg/L after 30 hours. The six pretreated feed water reservoirs; including chlorine, chloramine and no residual (3 levels of disinfection), with and without BAC-filtration (2 levels of filtration) for each, were maintained at 4°C before the water was fed to the simulated RWDSs. Influent feed reservoirs were changed every 30 hours. A detailed characterization of the influent water quality including impacts of BAC-filtration are reported in a prior study.[30]

The RWDSs were constructed from three consecutive 4-in. diameter PVC pipe segments connected with 3/8-in. diameter PVC tubing. PVC was selected because it is the most widely-used pipe material in sewer systems[31] and provides a relatively chemically and biologically inert surface relative to other materials, such as iron.[32] The pipe diameter was selected to achieve realistic pipe surface area to volume ratio and the pipe length was

selected to achieve the targeted hydraulic residence times. The smaller diameter tubing connecting each larger pipe segment created zones with slightly higher flow velocity (8.66×10^{-6} m/s in the main pipe sections and 9.85×10^{-4} m/s in the biofilm tube sections) and also facilitated easy sampling of biofilm by snipping segments as desired during operation without disrupting biofilm in the larger pipe segments. The outer surface of the tubes was thoroughly disinfected with 70% (v/v) ethanol and DNA Away (MBP, Inc., San Diego, CA) to avoid DNA contamination from outside the tube. Four sample ports were located on each pipe corresponding to calculated hydraulic residence time of 0-d (P0), 1-d (P1), 2.5-d (P2) and 5-d (P5) (Figure 1a). All six RWDSs were initially acclimated with unfiltered source water without disinfectants for six months at room temperature (25°C) to allow the establishment of a consistent and mature biofilm, as previously suggested by Zhou et al.[33] and Fish et al.[34] Afterwards, each RWDS was fed water from the designated reservoir and operated for 4-5 months at each target temperature to acclimate the RWDSs prior to sampling. A temperature cycle (14°C→22°C→30°C→22°C→14°C) was externally imposed in a constant temperature room to simulate a range of relevant seasonal temperatures (Climate-data.org). During the second 22°C phase, the first segment of the unfiltered chloramine RWDS had to be replaced due to leaking and was operated for 2 months before sampling in Nov 2016 to allow acclimation (Figure 1b).

2.2 Water chemistry and microbial sampling and analysis

At the end of each temperature phase, paired comprehensive water chemistry and biological sampling were conducted, with three independent water chemistry and biological sampling events at the end of the 30°C phase to assess reproducibility of the measurements. (Figure 1b).

Monitored water chemistry parameters included free chlorine and total chlorine residuals (HACH DR 2700 Spectrophotometer Method 8021), dissolved oxygen (DO) (Orion Star A 326 DO meter), pH (Oakton pH 110), ammonia (Hach Method 8155), nitrite and nitrate (Dionex® DX-120 Ion Chromatograph), and total organic carbon (TOC) (Sievers 5310C Laboratory TOC Analyzer). Total cell counts (TCC) were measured with a BD Acccuri C6® flow cytometer [34] starting with the first 22°C phase. Five hundred microliter of sample was collected in 2-ml Eppendorf tube and stained with 5 µl of SYBR Green I (100x diluted from stock in DMSO) by gentle vortexing and incubating in a water batch at 37°C for 10 mins. The fast flow rate setting on the BD Accuri C6 flow cytometer at 66 µl/min was selected to run samples. Fluorescence from the stained samples were collected at the green fluorescence at 520 ± 10 nm (FL1 channel) and the red fluorescence above 800 nm (FL3 channel). A fixed gating, established by trial runs on positive control samples, was used across all the TCC samples to ensure consistence and comparability in the measurements across different runs. A negative control sample using deionized water produced from Nanopure water purification system (Thermo Scientific, IL, USA) was included in each run to determine the detection limit.

For microbial analysis, bulk water was collected from each sample port and 3-cm length of the 3/8-in. diameter PVC connecting tubing (external surfaces sterilized with ethanol) were clipped in duplicate for biofilm analysis. One half liter of bulk water was collected in sterile bottles from each sample port and disinfectant residual was immediately quenched with sodium thiosulfate prior to splitting into duplicate 250 mL samples and filtering through sterile 0.22-µm mixed-cellulose ester filter (Millipore, Billerica, MA). Microbial DNA was extracted using the FastDNA® SPIN Kit (MP Biomedical, Inc., Solon, OH) and FastPrep®

198 Instrument (MP Biomedical, Inc., Solon, OH) and stored at -80°C. A total of 84 DNA
199 extracts were selected for shotgun whole-genome metagenomic sequencing, in three separate
200 sequencing runs carried out by the Biocomplexity Institute of Virginia Tech (Blacksburg,
201 VA). The first two runs were sequenced with the Illumina HiSeq 2500 rapid run mode with
202 2×100 bp pair-ended reads. The last run was sequenced with the Illumina NextSeq with 100
203 bp pair-ended reads. The change in sequencing platform was unavoidable due to evolving
204 next-generation DNA sequencing technology and the data were normalized to account for
205 potential differences. Sequencing libraries for all samples were prepared using the Illumina
206 Nextera XT DNA library Prep Kit (San Diego, CA). The sample matrix of the selected
207 samples for sequencing (detailed in SI Table 1) included two levels of water age – P1 or P2
208 samples for low water age and P5 for high water age and three temperature events - the first
209 14°C and 22°C unfiltered at all three disinfectant conditions and three independent sampling
210 events at 30°C at all three disinfectant conditions, both unfiltered and filtered.

211 2.3 Data analysis and statistical tools

212 Raw metagenomic forward and reverse reads were merged with FLASH.[35] Merged reads
213 were trimmed and filtered by TRIMMOMATIC to remove low quality reads according to
214 default parameters.[36] Taxonomy annotation of the merged reads were performed via the
215 MetaStorm platform[37] using the MetaPhlAn2 database with best-hit-alignment and identity >
216 90% for taxonomic mapping.[38] Taxonomic annotations were performed at the genus-level
217 and shown as percent relative abundances. Functional profiling of the trimmed metagenomic
218 reads was performed using the HUMAnN2 pipeline[39], which mapped reads to a universal
219 protein reference database, UniRef50,[40] at >50% amino acid sequence identity over 90% of
220 the translated query sequence and 50% of the translated subject sequence. Functional gene

abundances were expressed in copies per million and included an additional step to remove low abundance functional gene families to account for differences in sequencing depth between HiSeq and NextSeq sequencing platforms (SI Figure 3).

Water chemistry profiles were plotted in JMP Pro 14.0 and an effect test in standard least squares method was applied to the water chemistry matrix to identify significance of tested parameters. Correlation matrices analysis was performed in R (Version 3.4.1) with the "Corrr" and "PerformanceAnalytics" packages to assess correlations between water chemistry parameters, total cell counts and microbial diversity indices,[41,42] A first-order decay model was used to fit data and interpolate/extrapolate water ages at which targeted threshold levels of disinfectant residuals would be achieved. Kruskal-Wallis test was used to determine differences in relative abundances of nitrifier genus across water chemistry conditions. One-way and crossed two-way analysis of similarities (ANOSIM) from Bray-Curtis resemblance matrix was conducted in PRIMER_E (Version 6.1.13) to determine dissimilarity in microbial communities across experimental conditions. Non-metric multidimensional scaling (NMDS) plots of metagenomic taxonomy annotations were produced in R (Version 3.4.1) using the Vegan package.[43] Differences in metagenomic functional gene family composition were analyzed by performing an unsupervised transformation of the functional gene dataset to a high-dimensional sparse representation using Random Trees Embedding (number of trees = 500, maximum depth of each tree = 5, minimum number of samples required to split an internal node = 5, minimum number of samples required to be at a leaf node = 5) followed by Truncated SVD[44,45] using scikit-learn v0.21.3 in Python v3.7.3.[46]

3. Results

3.1 Study Overview: Unique Insights Gained from Simulated RWDSs

This study employed a unique simulated RWDS design that enabled sampling over a range of relevant water ages (0-d, 1-d, 2.5-d and 5-d) and sacrificial sampling of biofilms formed under higher flow velocity conditions in connecting tubes during five temperature phases (14°C – 22°C -30°C – 22°C - 14°C). This made it possible to compare effects of feed water treatment (without and with BAC-filtration) and residual disinfectant (chlorine, chloramines, or no residual) in parallel (i.e., six RWDSs). Testing two of the temperatures (14°C and 22°C) at the beginning and near the end of the experiment, allowed comparison of ambient temperature effects versus aging of the biofilm and pipe network. Comprehensive physicochemical analysis was performed at all time points, along with metagenomic analysis over a cross-section of key conditions (SI Table 1), providing insights into the interplay of water chemistry and microbial activity in determining the quality of water delivered by RWDSs.

3.2 Disinfectant decay in RWDSs

Using the standard least square test to identify dominant experimental factors that impacted the disinfectant decay trends, we found that chlorine profiles were significantly affected by temperature ($p<0.0001$), water age ($p<0.0001$), and BAC-filtration ($p=0.0086$), while temperature ($p<0.0001$) and water age ($p<0.0001$) significantly affected chloramine profiles. Disinfectant decay kinetics were characterized by estimating the water age at which a threshold of 0.5 mg/L disinfectant residual would be achieved ($T_{0.5}$) (Figure 2). This was done assuming a first order decay model and interpolating/extrapolating from disinfectant residuals measured at two consecutive sampling locations. In the following subsections, we first focus on the three RWDSs receiving unfiltered water, and thereafter describe the effects of BAC-filtration, focusing on distinguishing effects.3.2.1 RWDSs receiving unfiltered water

30°C – 30°C was by far the worst case scenario among the three temperature conditions in terms of disinfectant loss rates as revealed by triplicate sampling events (Figure 3). Chloramine levels dropped to 0.05 ± 0.03 mg/L versus 0.35 ± 0.49 mg/L for chlorine at the

end of the first pipe segment (P1), and chloramine levels averaged 0.05 ± 0.02 mg/L while corresponding chlorine levels were still slightly higher at 0.17 ± 0.16 mg/L at the end of the second pipe segment. Both disinfectants were nondetectable (< 0.02 mg/L) at the end of the third pipe segment (P5).

14°C – Much better persistence of both chlorine and chloramine residuals was observed at 14^0C, the lowest temperature tested. The $T_{0.5}$ for chloramine was > 100× that measured at 30°C (Figure 2). During the first 14°C phase, higher levels of chloramine than chlorine were observed at both P2 and P5. There was 1.5 mg/L of chloramine versus 0.14 mg/L of chlorine remaining at P2, whereas at P5, chloramine dropped to 0.11 mg/L and chlorine to 0.06 mg/L (Figure 3). During the second 14°C phase, while chloramine levels were still higher than corresponding chlorine levels, differences were less striking, with 1.16 mg/L of chloramine and 0.72 mg/L of chlorine remaining at P2 and 0.47 and 0.41 mg/L of chloramine and chlorine remaining at P5. The noticeably faster time to $T_{0.5}$ was observed in the second 14°C phase relative to the first phase, especially in the chloramine condition (Figure 2, 1st phase $T_{0.5}$ = 107 hr versus 2nd phase $T_{0.5}$ = 47 hr), presumably due to accelerated nitrification from aging of the biofilm, which has been reported in literature.[47,48]

22°C - Chloramine was also more persistent than chlorine during the first 22°C phase than the second 22°C phase (chloramine 1st phase $T_{0.5}$ = 221 hr versus 2nd phase $T_{0.5}$ <1 hr), whereas the rates of chlorine decay were more comparable between the second and the first phases (chlorine 1st phase $T_{0.5}$ = 45 hr versus 2nd phase $T_{0.5}$ = 103 hr). The net result was that the relative persistence of chlorine and chloramine were reversed in the second phase, with chloramine having 5× higher $T_{0.5}$ than chlorine during the first 22°C phase, but 2 orders of

magnitude lower $T_{0.5}$ during the second phase (Figure 2). Thus, the relative stability of each disinfectant was dependent on temperature and age of the distribution system.

3.2.2 RWDSs receiving BAC-filtered water

In potable water treatment, BAC-filtration can remove nutrients and improve biological stability,[49] potentially increasing the persistence of disinfectant. The benefits of BAC-filtration were the most pronounced during the first 14°C phase, where the $T_{0.5}$ was estimated at 3,720 hr in the filtered chorine condition and 2,190 hr in the filtered chloramine condition (Figure 2), both of which are more than an order of magnitude higher than the corresponding unfiltered conditions. This effect of filtration in enhancing disinfectant stability was observed for all conditions at 14 and 22°C, with the exception of the first 22°C phase for which the chloramine $T_{0.5}$ was about 4× higher for unfiltered than filtered condition. Comparing the decay rate between chlorine and chloramine, chlorine was always more persistent than chloramine, with the exception of the second 14°C for the BAC-filtered conditions, which had nutrient levels more similar to those common for drinking water.[9]

3.3 Biological Stability in the RWDSs

Four key indicators of biological stability were quantified: (a) TOC - a measure of organic carbon used by heterotrophic microbes; (b) DO loss - a measure of aerobic microbial respiration (here "DO loss" is defined as difference from the Reservoir to P5); (c) TCC - a direct measure of microbial biomass in bulk water; and (d) shifts in ammonia, nitrite, and nitrate driven by nitrifiers and/or denitrifiers. Comparison of the chlorine and chloramine conditions to the no residual conditions provided a means to distinguish relative effects of disinfectants in suppressing microbial activity.

318 **(a) TOC:** Source water TOC levels of 6.2 ± 0.80 mg/L were comparable to an average of 7.0
319 mg/L reported in a prior field study of full-scale RWDSs.[9] TOC levels in all three simulated
320 RWDSs receiving unfiltered water remained relatively uniform across water age and
321 temperature, with a mean of 5.6 ± 1.0 mg/L (SI Figure 1). BAC-filtration was effective at
322 reducing influent TOC to 1.3 ± 0.76 mg/L in the reservoir. In the three RWDSs receiving
323 BAC-filtered water, TOC was stable across all water ages along the systems, but the influent
324 level did vary throughout the study, as expected due to seasonality[50-52] and slight changes in
325 performance of BAC-filtration in removal of organic matter (SI Figure 1).[49]
326 **(b) DO loss:** DO profiles in the simulated RWDSs exhibited distinct trends across the six
327 RWDSs (Figure 3). Overall, chlorinating the feed source and BAC-filtration reduced DO
328 loss. DO losses were greatest at 30°C, with more loss in RWDSs receiving unfiltered versus
329 BAC-filtered water. In terms of effects of disinfectant, at 30°C DO loss was greatest in
330 RWDSs with chloraminated feed, followed by no residual and chlorinated systems. DO loss
331 in unfiltered and filtered chlorinated conditions averaged 3.82 ± 1.30 mg/L and 1.56 ± 1.62
332 mg/L, respectively (SI Table 2). Corresponding losses were 6.43 ± 0.81mg/L and 4.16 ± 2.54
333 mg/L in the chloraminated RWDSs receiving unfiltered and filtered water and 5.26 ± 1.71
334 mg/L and 3.19 ± 1.22 mg/L in the no residual RWDSs receiving unfiltered and filtered water.
335 DO losses were lowest at 14°C in all six RWDSs, with a maximum loss of 2.54 mg/L. At
336 22°C, DO losses were still low in RWDSs fed chlorinated (unfiltered and filtered) water with
337 0.085 - 3.13 mg/L loss. In contrast, both chloraminated and no residual systems experienced
338 greater overall losses of 4.24 – 6.3 mg/L in unfiltered and 2.42 - 4.87 mg/L in BAC-filtered
339 systems.

(c) TCC: Chlorine was effective in maintaining low/undetectable TCC at 14°C and 22°C, but at 30°C there was a consistent trend of increasing TCC from the reservoir to P5 in both filtered and unfiltered chlorinated systems, resulting in TCC levels higher than those measured in the chloramine and no residual RWDSs at P5 ($p=0.0425$) (Figure 3). In contrast, RWDSs receiving feed that was chloraminated or that contained no residual consistently yielded detectable levels of TCC at all temperatures. Aggregating measurements across temperature conditions, TCC in the chlorinated RWDSs were found to be significantly lower than in the chloraminated (Kruskal Wallis test, $p=0.00011$) and no residual (Kruskal Wallis test, $p=1.1\times10^{5}$) RWDSs, while chloramine and no residual TCC were comparable (Kruskal Wallis test, $p=0.438$). BAC-filtration only appeared to be effective at keeping TCC levels low in chlorinated systems at 14°C and 22°C, with no detectable TCC in the filtered condition.

(d) Ammonia, nitrite, and nitrate levels: Nitrification is a well-documented problem in DWDSs and is typically assessed by monitoring increase in nitrite level and accelerated loss of chloramine residuals,[53] especially in the warmer summer months. In the RWDSs, ammonia loss was found to be consistent with the loss of chloramine, with a 4:1 chloramine to ammonia mass ratio as N at each sample port (Figure 4). Ammonia loss increased with increasing temperature and was most pronounced at 30°C. Nitrite, which reacts directly with any chloramine that is present, only accumulated between the reservoir and P5 during the first 22°C phase in both BAC-filtered and unfiltered conditions (Kruskal Wallis $p = 0.00213$, compared to all other temperature phases). For all other temperature phases, nitrite was low/non-detectable and did not vary along the length of the distribution systems. Final nitrate

362 levels, measured in all six RWDSs, averaged 9.17 mg/L ± 2.51 mg/L across all RWDSs and
363 was not strongly affected by experimental conditions (SI Figure 1).

364 3.4 Taxonomic and functional gene-based indicators of nitrification and denitrification
365 Shotgun metagenomic sequencing served to comprehensively profile taxonomic and
366 functional gene indicators of nitrifiers and complementary measurements of ammonia, nitrite
367 and disinfectant decay. At the taxonomic level, five known nitrifier genera curated in the
368 MetaPhlAn2 database were screened (Figure 5). Concomitant with the nitrification trends
369 observed based on increase in nitrite and decrease in ammonia during the first 22°C phase,
370 total nitrifier genera percent abundance (relative to all other genera annotated) also spiked
371 during the first 22°C phase. An increase in nitrifier abundance was particularly notable in P5
372 biofilm in the unfiltered condition (>80% of the total annotated genera) (Figure 5). At 30°C,
373 which is in the middle of the optimum nitrifier growth temperature range of 25 – 35°C,[54]
374 relative abundances of nitrifiers were lower in the unfiltered condition. This is consistent
375 with the unfiltered conditions exhibiting a faster drop in ammonia and nitrite levels along the
376 RWDS, reaching the detection limit at P1, P2 and P5 (Figure 4) and creating a less conducive
377 environment for the growth of nitrifiers.

378 The genera of nitrifiers detected also varied between water and biofilm and across
379 disinfection treatment (Kruskal-Wallis, $p_{biofilm} = 0.01176$ and $p_{disinfection} = 4.77\times10^{-14}$). At the
380 same location, biofilm samples always had higher relative abundance of nitrifiers compared to
381 corresponding water samples (with the exception of the filtered no residual P5 location)
382 (Figure 5). Nitrifiers were rarely detected in chlorinated RWDSs. In contrast, *Nitrospira* and
383 *Nitrosomonas*, were frequently detected in chloraminated RWDSs. The no residual conditions
384 were found to harbor more diverse nitrifier genera, including *Nitrosopira* and

385 *Nitrosoarchaeum* (an Archaeae). Distinct nitrifier communities were detected in unfiltered
386 versus filtered conditions at 30°C, where *Nitrosoarchaeum* was found to be dominant in the
387 filtered no residual RWDSs, indicating that disinfection and filtration incurred a selection
388 pressure on the types of nitrifiers encountered.

389 Functional gene profiling was used to describe the metabolic potential of RWDS bacterial
390 communities. Here we examined two genes involved in nitrification: ammonia
391 monooxygenase (*amoA*) and nitrite oxidoreductase (*nxr*) and two genes involved in
392 denitrification (which could potentially be occurring as nitrate builds up as a by-product of
393 nitrification): the copper-containing nitrite reductase (*nirK*) and Cd1-type nitrite reductase
394 (*nirS*). Based on UniRef50 gene functional family annotation of all metagenomic samples, six
395 different *amoA*, three different *nirK* and one each of *nxr* and *nirS* were detected (Figure 6).
396 Temperature affected the relative abundances of *amoA*, *nxr* and *nirK*, with an increase in
397 abundance with increasing temperature (Figure 6). Notably, chlorination suppressed levels of
398 these three genes at all three temperatures, but chloramination did not. In fact, consistent with
399 the increase in *Nitrospira* in the chloraminated RWDS biofilm at 22°C, a pronounced spike in
400 *amoA* and *nirK* were noted in biofilm samples in this system (Figure 6). Also remarkable were
401 the increased levels of *amoA* and *nirK* in chloramine and, particularly, in no residual BAC-
402 filtered RWDSs at 30°C, compared to unfiltered RWDSs, which coincided with high relative
403 abundances of *Nitrosoarchaeum* in filtered RWDSs.

404 3.5 Reflection of RWDS Experimental Conditions in Microbial Community Profiles

405 The broader microbial community composition, beyond nitrifiers, was profiled via
406 metagenomic sequencing to gain deeper insight into the effects of the operational conditions
407 on RWDS water quality. Diversity was significantly higher in biofilm than in bulk water

408 based on Shannon and Simpson (Kruskal-Wallis p= 0.05×10^{-4} and p=1.216×10^{-5}
409 respectively) indices. Shannon and Simpson diversity indices were also significantly (p=
410 0.004461) higher in water samples from the chlorine and chloramine compared to the no
411 residual conditions, while diversity in biofilm was comparable across the three disinfection
412 conditions (SI Figure 3). No significant differences were observed based on water age,
413 filtration or temperature.

414 Consistent with observations that temperature was a significant operational parameter in
415 defining disinfectant decay and biological stability, NMDS analysis revealed that the
416 microbial community taxonomic composition diverged as the experiments progressed from
417 14°C→22°C→30°C (Figure 7). At 14°C and 22°C, no strong selective effects of disinfectants
418 were observed. As temperature increased to 30°C, the microbial community composition of
419 the chlorine condition become more distinct from that of chloramine (ANOSIM R= 0.326, p=
420 0.001) and no residual (ANOSIM R= 0.326, p= 0.0001) conditions. Filtration (ANOSIM R=
421 0.133, p= 0.007) and water age (ANOSIM R= -0.043, p= 0.914) of P1/P2 versus P5 had no
422 distinguishable impacts on overall microbial community taxonomic composition. Distinct
423 composition of water versus biofilm was noted at 14°C (ANOSIM R= 0.381, p= 0.006) and
424 30°C (ANOSIM R= 0.321, p= 0.0271), while generally the selective effect of disinfectant
425 conditions was more apparent in the bulk water than in the biofilm according to a two-way
426 crossed ANOSIM of disinfectants (ANOSIM R= 0.419, p= 0.21). ANOSIM results for all
427 tested experimental factors are included in SI Table 3 a-c.

428 Shifts in microbial community functional gene composition paralleled shifts in taxonomy
429 (Figure 8). The effect of disinfection became more pronounced as temperature increased.
430 Truncated SVD of Random Trees Embedding of functional gene relative abundances shows

that during the first 14°C there was no clear separation based on disinfection; however, at 22°C all the bulk water samples separated based on disinfection condition (22°C ANOSIM R = 0.428, p = 0.011). At 30°C, the combined effect of filtration and disinfection treatment resulted in distinct functional gene composition of microbial communities (ANOSIM R = 0.311, p = 0.001). Bulk water and biofilm samples from the unfiltered chloramine and no residual RWDSs clustered together, while the unfiltered chlorinated condition RWDS samples formed a separate cluster (Figure 8 and SI Figure 5). Filtration increased separation between the no residual and chloramine water samples and shifted the filtered chloraminated water samples closer to the filtered chlorinated water samples. Overall, the combined effect of filtration and disinfection treatment resulted in distinct functional gene composition of microbial communities at 30°C (ANOSIM R = 0.311, p = 0.001).

3.6 Discussion

3.6.1 Management Implications for RWDSs

In contrast to prior field surveys, this study provided controlled, direct comparison of multiple factors impacting RWDSs over an extended operational period to help inform improved management strategies.[9] Here we observed that RWDSs are particularly vulnerable to disinfectant depletion compared to DWDSs, where detectable levels of disinfectant residuals are required, especially at the higher temperatures that are common in water-stressed areas. Chloramine decayed rapidly, likely due to nitrification, and chlorine performed better as a disinfectant. Interestingly, the chloramine condition was often indistinguishable from that of the no disinfectant residual control condition in terms of DO loss, TCC, and microbial community composition. Based on a parallel study, after decommissioning and analysis of these pipe rigs, extensive amounts of sediment and biofilm accumulated in this system with time, especially in the chloramine and no residual

conditions.[30] Jar tests revealed that the biofilm and sediment exerted high disinfectant demand, which helps explain why the ability to maintain residual worsened with time in the current study. Sediment and biofilm are also known to be problematic in DWDSs, and this and the parallel study both indicate that they are likely to cause even greater water quality deterioration concerns in RWDSs.[30]

3.6.2 Choice of disinfectant for RWDSs: chlorine vs. chloramine

A critical decision for RWDS operators is choice of disinfectant.[55,56] This study emphasizes how ambient temperatures may ultimately affect this choice. Although higher temperature generally increases microbial and chemical reaction rates affecting disinfectant decay, herein, chloramine was more strongly affected by temperature than chlorine. Specifically, chloramine was more persistent than chlorine during the initial low temperature phase, but the trend was reversed as temperatures increased (Figure 2 and 3). Chloramine decay remained high even as temperatures decreased, suggesting that, once nitrifiers were established in a system, nitrification can still persist and cause chloramine loss at lower temperatures. On the other hand, chlorine was found to be a superior disinfectant in these simulated RWDSs and was able to better maintain consistent residual levels over a range of conditions, while also maintaining high DO levels and reducing biological activity. Nonetheless, at 30°C, chlorine also decayed extensively and was frequently below 0.2 mg/L at higher water ages. Thus, at very warm temperatures, such as 30°C, it will be very difficult to manage water quality of RWDSs in terms of disinfectant residuals. The reduced relative persistence of chloramine versus chlorine in RWDS is a key example of how experiences with some RWDSs will likely diverge from the experiences in DWDSs, where chloramine is often selected for better residual stability.[2,9]

3.6.3 Influence of BAC-filtration on feed water quality

Use of BAC-filtration markedly reduced TOC levels and DO losses in the RWDSs when compared to unfiltered water, but it did not consistently enhance disinfectant stability. BAC filtration did lower disinfectant losses during the initial low temperature phase as was generally expected,[7,8] but benefits of BAC-filtration were less apparent as temperature rose and RWDS operational conditions changed, including buildup of biofilm and sediment.[30] In particular, BAC-filtration was not protective against nitrification as seen by the drop in ammonia levels at 22°C and 30°C. The BAC-filter itself might have supported the growth and release of nitrifying bacteria, with RWDS in filtered condition carrying a different variety (such as *Candidatus_Nitrosoarchaeum*) than those found in the RWDSs receiving unfiltered water. Higher levels of nitrification functional genes, such as *amoA* and *nirK*, were also measured in chloramine and no residual RWDSs fed BAC-filtered water compared to the unfiltered conditions. Similar observations of the high ammonia-oxidizing archaea abundances associated with biofilters and bioreactors have been documented.[57-59] Existing research has considered various traits that make ammonia-oxidizing archaea more well-adapted to the filter media environment, including tolerance to lower pH,[60-61] lower DO[62], or even anoxic condition[19], and energy-efficient autotrophic lifestyle[63]. And it is important to evaluate the underlying ecology and chemistry in each case to understand the unique factors that selects for the ammonia-oxidizing archaea. Furthermore, the performance of BAC-filters has been shown to change as the microbial communities on the filters mature, resulting in less capacity to absorb and degrade organic compounds, increased bacterial counts in the filtered effluent due to biofilm sloughing and general release of bacteria,[6,48] and altered disinfectant demand in BAC-filtered source water.

3.6.4 Nitrification potential in RWDSs

At higher temperatures, nitrifiers were abundant in both chloramine and no residual RWDSs, with and without BAC-filtration, while chlorine suppressed nitrifiers. The dominant nitrifier group in the unfiltered condition was the *Nitrospira* genus. *Nitrospira* spp. harbor comammox members capable of converting ammonia to nitrite and nitrite to nitrate,[58,64] which have now been observed in a variety of environmental systems.[64-67] Detection of *Nitrospira*, which indicates potential presence of comammox, in the simulated RWDSs as the most abundant nitrifier genus may explain why nitrite, as it is commonly applied as an indicator for nitrification in DWDSs,[29,32] was not a particularly accurate indicator of nitrification in this study. This could be attributed to high abundance of *Nitrospira* spp. and other genera capable of denitrification, such as *Sphingopyxis* and *Afipia*. Also interesting were the detection of ammonia-oxidizing archaea and high disinfectant losses in BAC-filtered systems.

3.6.5 Managing Biofilm in RWDSs

The relative impact of biofilm on water quality is likely to be exacerbated by operational features inherent to many RWDSs.[2,67-69] Biofilm accumulation with time was likely a major driver for the increase in overall disinfectant decay rates, resulting in greater disinfectant loss and poorer water quality during the second 14°C and 22°C phases compared to the first 14°C and 22°C phases. Biofilm formed a more microbially-diverse environment than bulk water and served as a niche for nitrifiers, as evidenced by the spike in nitrifier taxonomic and functional genes in biofilm samples at 22°C. Biofilm continued to be the preferred environment of nitrifiers at 30°C (Figure 4 and Figure 5). Since disinfectants are known to be limited in their capability to penetrate biofilms,[70-72] once nitrifiers are established in the biofilm, RWDSs could become permanently susceptible to nitrification.

4. Conclusions

This multiyear study, with parallel analysis of water chemistry and metagenomic profiles, revealed distinct operational challenges for RWDSs:

- Maintenance of disinfectant residuals, including both chlorine and chloramine, was challenging at higher temperatures and as the distribution systems aged.
- Chlorine was a more persistent disinfectant relative to chloramine under most conditions tested and resulted in better water quality with higher DO levels and lower microbial regrowth, while biological stability indicators were comparable between the chloramine and no disinfectant residual conditions.
- Chloraminated provided a favorable environment in the RWDSs for nitrifier growth, making them susceptible to nitrification, which in turn drives rapid chloramine depletion and biological instability.
- BAC-filtration did not prevent chloramine loss over the course of the long-term operation.

Based on this study, distinct management guidelines are needed for RWDSs versus DWDSs, particularly in terms of water treatments and disinfectant conditions. Insights gained from operating the RWDSs through a cycle of temperature phases simulating seasonal effects highlighted the overarching role of temperature in disrupting biological stability of RWDSs. Considering that water reclamation facilities tend to be located in regions with warmer climates, active and comprehensive monitoring of RWDSs, especially during vulnerable seasons, can serve to inform and tailor appropriate treatment strategies to the local conditions.

548

549 Declaration of interests

550 The authors declare that they have no known competing financial interests or personal
551 relationships that could have appeared to influence the work reported in this paper.

552

553 Acknowledgements

554 This work is supported by the National Science Foundation (NSF) Collaborative Research grant
555 (CBET 1438328), and Partnership in International Research and Education (OISE 1545756), The
556 Alfred P. Sloan Foundation Microbiology of the Built Environment program, the Water
557 Environment & Research Foundation Paul L. Busch award, Virginia Tech NanoEarth (NSF
558 NNCI Award 1542100) the Advanced Computing Center at Virginia Tech, and the Virginia Tech
559 Institute for Critical Technology and Applied Science Center. Any opinions, findings, and
560 conclusions or recommendations expressed in this material are those of the author(s) and do not
561 necessarily reflect the views of the National Science Foundation. The authors thank Robert
562 Bielitz, Kandace Donaldson, Kris Mapili and Haniyyah Majeed for assistance in maintaining the
563 simulated RWDSs. Figure 1(a) was modified from a prior publication in Environ. Sci. Water
564 Res. Technol (doi:10.1039/C9EW00978G) and included with permission.

References

1. Y.Wada, M.F.P. Bierkens, Sustainability of global water use: past reconstruction and future projections. Environ. Res. Lett. 2014, 9, 104003. https://doi.org/10.1088/1748-9326/9/10/104003
2. E. Garner, N. Zhu, L. Strom, M. Edwards, A. Pruden, A human exposome framework for guiding risk management and holistic assessment of recycled water quality. Environ. Sci. Water Res. Technol. 2016, 2. https://doi.org/10.1039/C6EW00031B
3. P.K. Jjemba, L.A. Weinrich, W. Cheng, E. Girald, M.W. Lechevallier, Regrowth of potential opportunistic pathogens and algae in reclaimed-water distribution systems. Appl Env. Microbiol, 2010, 76, 4169–4178. https://doi.org/10.1128/AEM.03147-09
4. J. Bryk, R. Prasad, T. Lindley, S. Davis, G. Carpenter. National Database of Water Reuse Facilities Summary Report. Alexandria, VA. 2011.
5. California State Water Resources Control Board. Title 22 Code of Regulations. 2018.
6. L. Sbardella, J. Coma, A. Fenu, I. Rodriguez-Roda, M, Weemaes M, Advanced biological activated carbon filter for removing pharmaceutically active compounds from treated wastewater. Sci. Total Environ. 2018, 636, 519–529. https://doi.org/10.1016/J.SCITOTENV.2018.04.214
7. P. Jin, X. Jin, X.Wang , Y.C. Feng, Biological Activated Carbon Treatment Process for Advanced Water and Wastewater Treatment, in: Biomass Now - Cultivation and Utilization. InTech. 2013. https://doi.org/10.5772/52021
8. J. Reungoat, B.I. Escher, M. Macova, F.X. Argaud, W. Gernjak, J. Keller, Ozonation and biological activated carbon filtration of wastewater treatment plant effluents. Water Res. 2012, 46, 863–872. https://doi.org/10.1016/j.watres.2011.11.064
9. E. Garner, J. McLain, J. Bowers, D.M. Engelthaler, M.A. Edwards, A. Pruden, Microbial Ecology and Water Chemistry Impact Regrowth of Opportunistic Pathogens in Full-Scale Reclaimed Water Distribution Systems. 2018. https://doi.org/10.1021/acs.est.8b02818
10. S. Potgieter, A. Pinto, M. Sigudu, H. du Preez, E. Ncube, S. Venter, Long-term spatial and temporal microbial community dynamics in a large-scale drinking water distribution system with multiple disinfectant regimes. Water Res. 2018,139, 406–419. https://doi.org/10.1016/J.WATRES.2018.03.077
11. E.I. Prest, F. Hammes, M.C.M. van Loosdrecht, J.S. Vrouwenvelder, Biological Stability of Drinking Water: Controlling Factors, Methods, and Challenges. Front. Microbiol. 2016, 7, 45. https://doi.org/10.3389/fmicb.2016.00045
12. J.M. Regan, G.W. Harrington, D.R. Noguera, Ammonia- and nitrite-oxidizing bacterial communities in a pilot-scale chloraminated drinking water distribution system. Appl. Environ. Microbiol. 2002, 68, 73–81. https://doi.org/10.1128/aem.68.1.73-81.2002
13. J. Schullehner, L. Stayner, B. Hansen, Nitrate, Nitrite, and Ammonium Variability in Drinking Water Distribution Systems. Int. J. Environ. Res. Public Health, 2017, 14, 276. https://doi.org/10.3390/ijerph14030276
14. M.T. Lipponen, M.H. Suutari, P.J. Martikainen, Occurrence of nitrifying bacteria and nitrification in Finnish drinking water distribution systems. Water Res., 2002, 36, 4319–4329. https://doi.org/10.1016/S0043-1354(02)00169-0
15. A. Nescerecka, T. Juhna, F. Hammes, Identifying the underlying causes of biological instability in a full-scale drinking water supply system. Water Res. 2018, 135, 11–21. https://doi.org/10.1016/J.WATRES.2018.02.006

16. C.R.Proctor, F. Hammes, Drinking water microbiology—from measurement to management. Curr. Opin. Biotechnol. 2015,33, 87–94. https://doi.org/10.1016/j.copbio.2014.12.014
17. A.J. Pinto, C. Xi, L. Raskin, Bacterial Community Structure in the Drinking Water Microbiome Is Governed by Filtration Processes. Environ. Sci. Technol. 2012, 46, 8851–8859. https://doi.org/10.1021/es302042t
18. C.D. Norton, M.W. LeChevallier, Chloramination: its effect on distribution system water quality. J. Am. Water Works Assoc. 1997, 89, 66–77. https://doi.org/10.1002/j.1551-8833.1997.tb08260.x
19. Y. Zhang, M. Edwards, Accelerated chloramine decay and microbial growth by nitrification in premise plumbing. J. Am. Water Works Assoc. 2009, 101, 51–62. https://doi.org/10.1002/j.1551-8833.2009.tb09990.x
20. M. Deborde, U. von Gunten, Reactions of chlorine with inorganic and organic compounds during water treatment—Kinetics and mechanisms: A critical review. Water Res. 2008, 42, 13–51. https://doi.org/10.1016/J.WATRES.2007.07.025
21. I, Fisher, G. Kastl, A. Sathasivan, New model of chlorine-wall reaction for simulating chlorine concentration in drinking water distribution systems. Water Res. 2017, 125, 427–437. https://doi.org/10.1016/j.watres.2017.08.066.
22. M.J. Plewa, E.D. Wagner, S.D. Richardson, TIC-Tox: A preliminary discussion on identifying the forcing agents of DBP-mediated toxicity of disinfected water. J. Environ. Sci. 2017, 58, 208–216. https://doi.org/10.1016/J.JES.2017.04.014
23. R.A. Li, J.A. McDonald, A. Sathasivan A, Khan SJ. Disinfectant Residual Stability Leading to Disinfectant Decay and By-product Formation in Drinking Water Distribution Systems: A Systematic Review. Water Res.2019. https://doi.org/10.1016/J.WATRES.2019.01.020
24. N.I. Lieu, R.L. Wolfe, Means EGI. Optimizing Chloramine Disinfection for the Control of Nitrification. J. Am. Water Works Assoc. 1993, 85, 84–90. https://doi.org/10.1002/j.1551-8833.1993.tb05942.x
25. A. Sathasivan, J. Chiang, P. Nolan, Temperature dependence of chemical and microbiological chloramine decay in bulk waters of distribution system. Water Sci. Technol. Water Supply, 2009, 9, 493–499. https://doi.org/10.2166/ws.2009.387
26. P, Thayanukul, F. Kurisu, I. Kasuga, H. Furumai, Evaluation of microbial regrowth potential by assimilable organic carbon in various reclaimed water and distribution systems. Water Res. 2013, 47, 225–232. https://doi.org/http://dx.doi.org/10.1016/j.watres.2012.09.051
27. W.A.M. Hijnen, E.F. Beerendonk, G.J. Medema, Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. Water Res. 2006, 40, 3–22. https://doi.org/10.1016/j.watres.2005.10.030
28. G. Liu, F.Q. Ling, E.J. van der Mark, X.D. Zhang, A. Knezev, J.Q.J.C.Verberk, W.G.J. van der Meer, G.J. Medema, W.T. Liu, J.C.van Dijk, Comparison of Particle-Associated Bacteria from a Drinking Water Treatment Plant and Distribution Reservoirs with Different Water Sources. Sci. Rep. 2016, 6, 20367. https://doi.org/10.1038/srep20367
29. P.L.Rantanen, M.M. Keinänen-Toivola, M. Ahonen, I. Mellin, D. Zhang, T. Laakso, Vahala, R., 2017. The Spatial Distribution of Nitrite Concentrations in a Large Drinking Water Distribution System in Finland. J. Water Resour. Prot. 09, 1026–1042. https://doi.org/10.4236/jwarp.2017.98068

30. N. Zhu, K. Mapili, H. Majeed, A. Pruden, M.A. Edwards, Sediment and biofilm affect disinfectant decay rates during long-term operation of simulated reclaimed water distribution systems. Environ. Sci. Water Res. Technol., 2020, 6,6, 1615-1626, https://doi.org/10.1039/C9EW00978G
31. K.F.Makris,J. Langeveld, F.H.L.R.Clemens, A review on the durability of PVC sewer pipes: research vs. practice. Struct. Infrastruct. Eng. 2019. https://doi.org/10.1080/15732479.2019.1673442
32. S. Masters, H. Wang, A. Pruden, M.A. Edwards, Redox gradients in distribution systems influence water quality, corrosion, and microbial ecology. Water Res. 2015, 68, 140–9. https://doi.org/10.1016/j.watres.2014.09.048
33. B. Zhou, Y. Li, P. Song. Z. Xu, A kinetic model for biofilm growth inside non-PC emitters under reclaimed water drip irrigation. Agric. Water Manag. 2016, 168, 23–34. https://doi.org/10.1016/J.AGWAT.2016.01.007
34. M. Vital, M. Dignum, A. Magic-Knezev, P. Ross, L. Rietveld, F. Hammes. Flow cytometry and adenosine tri-phosphate analysis: alternative possibilities to evaluate major bacteriological changes in drinking water treatment and distribution systems. Water Res. 2012. 46, 4665–76. https://doi.org/10.1016/j.watres.2012.06.010
35. T. Magoc, S.L. Salzberg. Next-generation genome annotation: we still struggle to get it right. Bioinformatics. 2011. https://doi.org/10.1186/s13059-019-1715-2
36. A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014, 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170
37. G. Arango-Argoty, G. Singh, L.S. Heath, A. Pruden, W. Xiao, L. Zhang. MetaStorm: A Public Resource for Customizable Metagenomics Annotation. PLoS One. 2016. https://doi.org/10.1371/journal.pone.0162442
38. D.T.Truong, E.A.Franzosa, T.L.Tickle, M. Scholz, G.Weingart, E. Pasolli, A. Tett, C. Huttenhower, N. Segata, MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat. Methods 2015, 12, 902–903. https://doi.org/10.1038/nmeth.3589
39. E.A.Franzosa, L.J. McIver, G. Rahnavard, L.R. Thompson, M. Schirmer, G. Weingart, K.S. Lipson, R. Knight, J.G. Caporaso, N. Segata, C. Huttenhower, Species-level functional profiling of metagenomes and metatranscriptomes. Nat. Methods 2018 15, 962–968. https://doi.org/10.1038/s41592-018-0176-y
40. B.E. Suzek. H. Huang, P. McGarvey, R. Mazumder, C.H. Wu, UniRef: Comprehensive and non-redundant UniProt reference clusters. Bioinformatics 2007, 23, 1282–1288. https://doi.org/10.1093/bioinformatics/btm098
41. B.G. Peterson, P. Carl, K. Boudt, R. Bennett, J. Ulrich, E. Zivot, D. COrnilly, E. Hung, M. Lestel, K. Balkissoon, D. Wuertz. Econometric Tools for Performance and Risk Analysis [R package PerformanceAnalytics version 1.5.3]. 2019.
42. E. Ruiz, S. Jackson, J. Cimentada, Correlations in R [R package corrr version 0.4.0].2019.
43. J. Oksanen, F.G Blanchet, M. Friendly, R. Kindt, P. Legendre, D. Mcglinn, P.R. Minchin , R.B. O'hara, G.L. Simpson, P. Solymos, M. Henry, H. Stevens, E. Szoecs, H.W. Maintainer, Package "vegan" Title Community Ecology Package Version 2.5-6. 2019.
44. N. Halko, P.G. Martinsson, J.A. Tropp, Finding structure with randomness: Probabilistic algorithms for constructing approximate matrix decompositions. SIAM Rev. 2011, 53, 217–288. https://doi.org/10.1137/090771806

45. P. Geurts, D. Ernst, L. Wehenkel, Extremely randomized trees. Mach. Learn. 2006, 63, 3–42. https://doi.org/10.1007/s10994-006-6226-1
46. F. Pedregosa, G.Varoquaux, A. Gramfort, V. Michel, B. Thirion, O. Grisel, M. Blondel, A. Müller, J. Nothman, G. Louppe, P. Prettenhofer, R. Weiss, V. Dubourg, J. Vanderplas , A. Passos, D. Cournapeau, M. Brucher, M. Perrot, E. Duchesnay, Scikit-learn: Machine Learning in Python. 2012.
47. L.Lee, S. Ong, W. Ng, Biofilm morphology and nitrification activities: recovery of nitrifying biofilm particles covered with heterotrophic outgrowth. Bioresour. Technol. 2004, 95, 209–214. https://doi.org/10.1016/J.BIORTECH.2003.05.004
48. J.P. Bassin, R. Kleerebezem, A.S. Rosado, M.C.M. van Loosdrecht, M. Dezotti, Effect of Different Operational Conditions on Biofilm Development, Nitrification, and Nitrifying Microbial Population in Moving-Bed Biofilm Reactors. Environ. Sci. Technol. 2012, 46, 1546–1555. https://doi.org/10.1021/es203356z
49. L.G.Terry, R.S. Summers, Biodegradable organic matter and rapid-rate biofilter performance: A review. Water Res.2018, 128, 234–245. https://doi.org/10.1016/J.WATRES.2017.09.048
50. W. Liu, X. Xu, N.M. McGoff, J.M. Eaton, P. Leahy, N. Foley, G. Kiely, Spatial and Seasonal Variation of Dissolved Organic Carbon (DOC) Concentrations in Irish Streams: Importance of Soil and Topography Characteristics. Environ. Manage. 2014, 53, 959–967. https://doi.org/10.1007/s00267-014-0259-1
51. M.R.Teixeira, L.M. Nunes, The impact of natural organic matter seasonal variations in drinking water quality. Desalin. Water Treat. 2011, 36, 344–353. https://doi.org/10.5004/dwt.2011.2524
52. S.E. Baer, T.L. Connelly, R.E. Sipler, P.L. Yager, D.A. Bronk, Effect of temperature on rates of ammonium uptake and nitrification in the western coastal Arctic during winter, spring, and summer. Glob. Biogeochem Cycles 2013, 28. https://doi.org/10.1002/2013GB004765
53. K.K.Fleming, G.W. Harrington, D.R. Noguera, Nitrification potential curves: a new strategy for nitrification prevention. J. Am. Water Works Assoc.2005, 97, 90–99. https://doi.org/10.1002/j.1551-8833.2005.tb07453.x
54. S. Zhu, S. Chen. The Impact of Temperature on Nitrification Rate in Fixed Film Biofilters. Aquac. Eng.2002, 26
55. H. Sanawar, Y. Xiong, A. Alam, J.P. Croué, P.Y. Hong, Chlorination or monochloramination: Balancing the regulated trihalomethane formation and microbial inactivation in marine aquaculture waters. Aquaculture, 2017, 480, 94–102. https://doi.org/10.1016/J.AQUACULTURE.2017.08.014
56. S.M.Acharya. F. Kurisu, I. Kasuga, H. Furumai, Chlorine Dose Determines Bacterial Community Structure of Subsequent Regrowth in Reclaimed Water. J. Water Environ. Technol. 2016, 14, 15–24. https://doi.org/10.2965/jwet.15-063
57. S. Bagchi, S.E. Vlaeminck, L.A. Sauder, M. Mosquera, J.D. Neufeld, N. Boon, Temporal and Spatial Stability of Ammonia-Oxidizing Archaea and Bacteria in Aquarium Biofilters. PLoS One 2014, 9, e113515. https://doi.org/10.1371/journal.pone.0113515
58. R.P. Bartelme, S.L. McLellan, R.J. Newton, Freshwater Recirculating Aquaculture System Operations Drive Biofilter Bacterial Community Shifts around a Stable Nitrifying Consortium of Ammonia-Oxidizing Archaea and Comammox Nitrospira. Front. Microbiol. 2017, 8, 101. https://doi.org/10.3389/fmicb.2017.00101

59. H.D. Park, G.F. Wells, H. Bae, C.S. Criddle, C.A. Francis. Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors. Appl. Environ. Microbiol. 2006, 72, 5643–7. https://doi.org/10.1128/AEM.00402-06
60. R. Hatzenpichler, Diversity, physiology, and niche differentiation of ammonia-oxidizing Archaea. Appl. Env. Microbiol. 2012, 78 (21), 7501–7510.
61. K. Isobe, K. Koba,Y. Suwa, J. Ikutani, Y. Fang, M. Yoh, J. Mo, S. Otsuka, K. Senoo, High abundance of ammonia-oxidizing archaea in acidified subtropical forest soils in southern China after long-term N deposition. FEMS Microbiol. Ecol., 2012, 80, 190-193
62. T.J. Erguder, N. Boon, L. Wittebolle, M. Marzorati, W.Verstraete, Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. FEMS Microbiol. Rev. 2009, 33 (5), 855–869.
63. M. Könneke, D.M.Schubert, P.C. Brown, M. Hügler, S. Standfest, T. Schwander, L. Schada von Borzyskowski, T.J. Erb, D.A. Stahl, I.A. Berg, Ammonia-oxidizing archaea use the most energy-efficient aerobic pathway for CO2 fixation. Proc. Natl. Acad. Sci. U. S. A. 2014, 111, 8239–44. https://doi.org/10.1073/pnas.1402028111
64. H. Daims, S. Lücker, M. Wagner, A New Perspective on Microbes Formerly Known as Nitrite-Oxidizing Bacteria. Trends Microbiol. 2016, 24, 699–712. https://doi.org/10.1016/j.tim.2016.05.004
65. M.A.H.J. van Kessel, D.R. Speth, M. Albertsen, P.H.Nielsen, H.J.M. Op den Camp, B. Kartal, M.S.M. Jetten, S. Lücker, Complete nitrification by a single microorganism. Nature 2015, 528, 555–9. https://doi.org/10.1038/nature16459
66. C. Gruber-Dorninger, M. Pester, K. Kitzinger, D.F. Savio, A. Loy, R. Rattei, M.Wagner , H. Daims, Functionally relevant diversity of closely related Nitrospira in activated sludge. ISME J. 2015, 9, 643–655. https://doi.org/10.1038/ismej.2014.156
67. Y. Chao, Y. Mao, K. Yu, T. Zhang, Novel nitrifiers and comammox in a full-scale hybrid biofilm and activated sludge reactor revealed by metagenomic approach. Appl. Microbiol. Biotechnol. 2016, 100, 8225–8237. https://doi.org/10.1007/s00253-016-7655-9
68. T. Wang, Y. Li, T. Xu, N. Wu, M. Liang, P. Hynds, Biofilm microbial community structure in an urban lake utilizing reclaimed water. Environ. Earth Sci. 2016, 75, 314. https://doi.org/10.1007/s12665-015-5197-6
69. H. Wang, C. Hu, X. Hu, M. Yang, J.Qu, Effects of disinfectant and biofilm on the corrosion of cast iron pipes in a reclaimed water distribution system. Water Res. 46, 1070–8. https://doi.org/10.1016/j.watres.2011.12.001
70. H. Wang, X. Hu, C. Hu, Effects of chlorine and pipe material on biofilm development and structure in a reclaimed water distribution system. Water Sci. Technol. Water Supply, 2012, 12, 362. https://doi.org/10.2166/ws.2012.005
71. J. Liu, H. Ren, X. Ye, W. Wang, Y. Liu, L. Lou, D. Cheng, X. He, X. Zhou, S. Qiu, L. Fu , B. Hu, Bacterial community radial-spatial distribution in biofilms along pipe wall. Appl MIcrobiol Biotechnol 2017, 101, 747–759. https://doi.org/10.1007/s00253-0160-7887-8
72. Chen X, Stewart PS. Chlorine penetration into artificial biofilm is limited by a reaction-diffusion interaction. Environ. Sci. Technol. 1996, 30, 2078-2083. https://doi.org/10.1021/es9509184

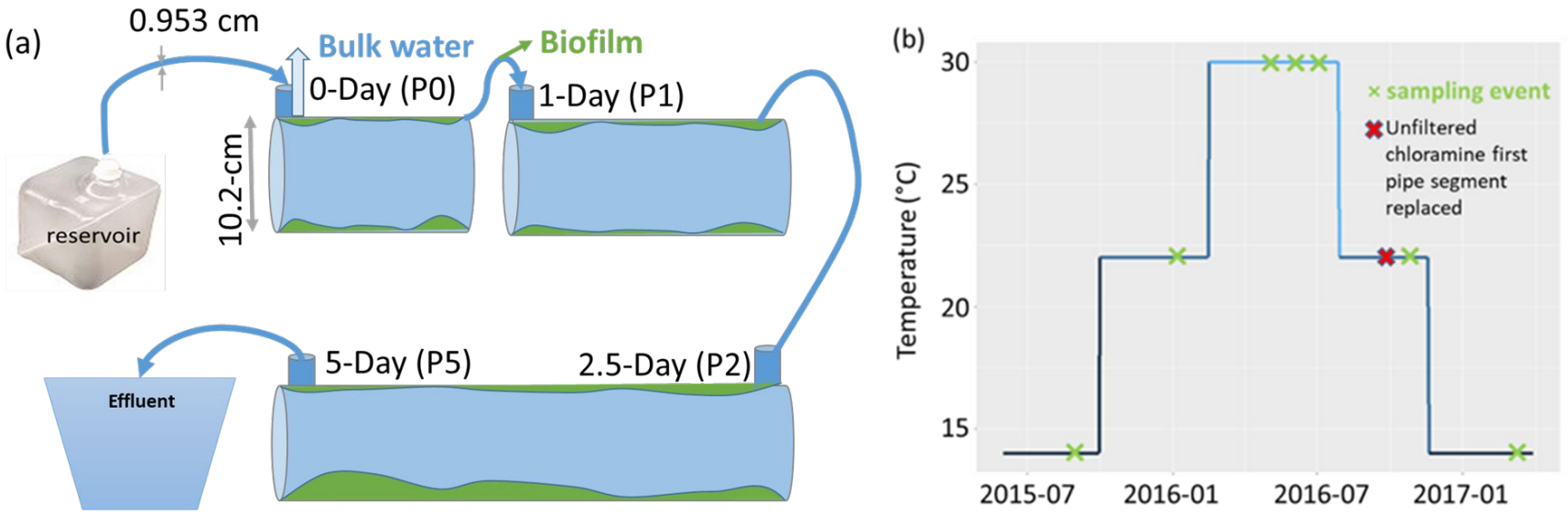

Figure 1 Simulated RWDS rig design, temperature phases, and operational timeline. (a) Each rig consisted of three 10.2 cm (4 in.) diameter pipe segments connected with 0.953 cm (3/8 in.) in diameter PVC tubes and was fed influent from a reservoir with a designated pretreated feed. Four sample ports, P0, P1, P2 and P5, were located along the length of the rigs. (b) The rigs underwent five temperature phases 14°C→22°C→30°C→22°C→14°C by adjusting ambient temperature. Prior to operating the rigs at the designated conditions, all rigs were acclimated at 25 °C for six months by running untreated wastewater effluent.

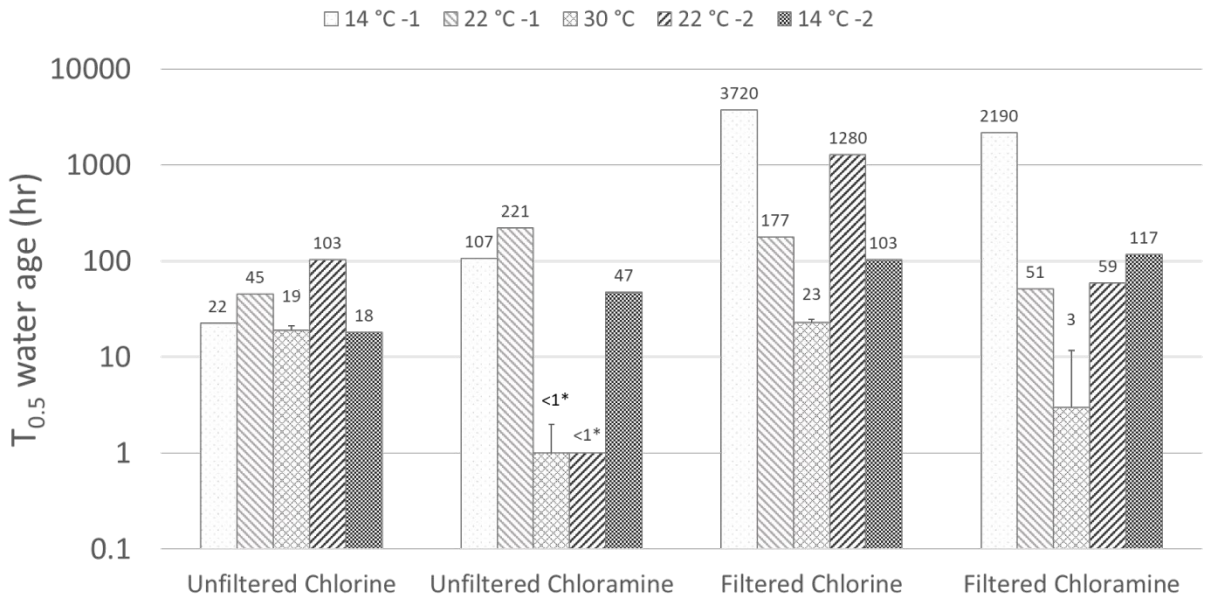

Figure 2 Estimated disinfectant decay rate ($T_{0.5,}$ defined as water age required for disinfectant residual along the pipe to drop to 0.5 mg/L) as a function of the water treatments (Unfiltered Chlorine, Unfiltered Chloramine, Filtered Chlorine, and Filtered Chloramine) and temperature phases (14°C-1, 22°C-2, 30°C, 22°C-2, 14°C-2). $T_{0.5}$ was estimated from the slope of disinfectant decay residuals measured between two consecutive sampling locations, expressed in terms of calculated hydraulic retention time (hr) in the pipes. When disinfectant residuals remained higher than 0.5 mg/L at the final sampling location (calculated water age = 120 hr), $T_{0.5}$, was interpolated using the decay gradient of the last pipe segment. Error bars at 30°C phase were standard deviation of triplicate biological samplings at that temperature. Labels on top of the bars indicate the actual calculated $T_{0.5}$. *Due to extremely rapid decay in the unfiltered chloramine pipe condition at 30°C and 22°C-2 temperature phase, an estimation of <1 hr $T_{0.5}$ value is indicated.

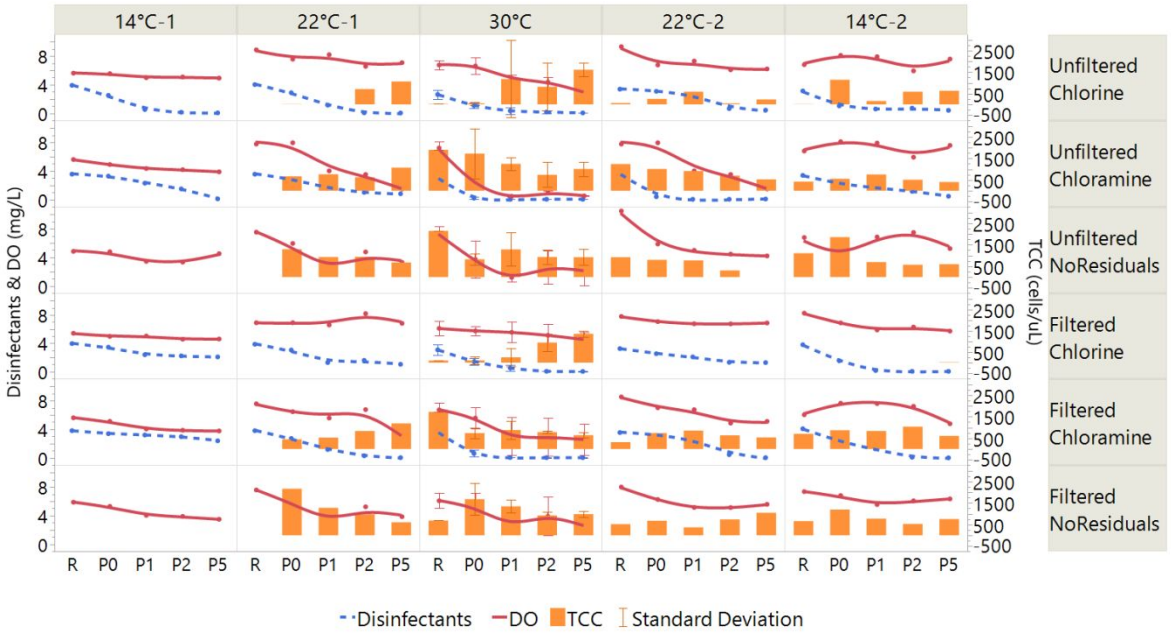

Figure 3 Disinfectant residuals, DO, and total cell counts (TCC) measured along the length of the six RWDSs at the end of each temperature phase. The x-axes represent the feed reservoir and the four sampling ports (Figure 1). 30°C data are mean and standard deviation of three sequential sampling events, other measurements are single measurements. Disinfectant residuals data represent free chlorine in the two chlorinated RWDSs and total chlorine in the two chloraminated RWDSs. TCC measurements were initiated at the first 22°C phase and therefore are not available for the first 14°C phase.

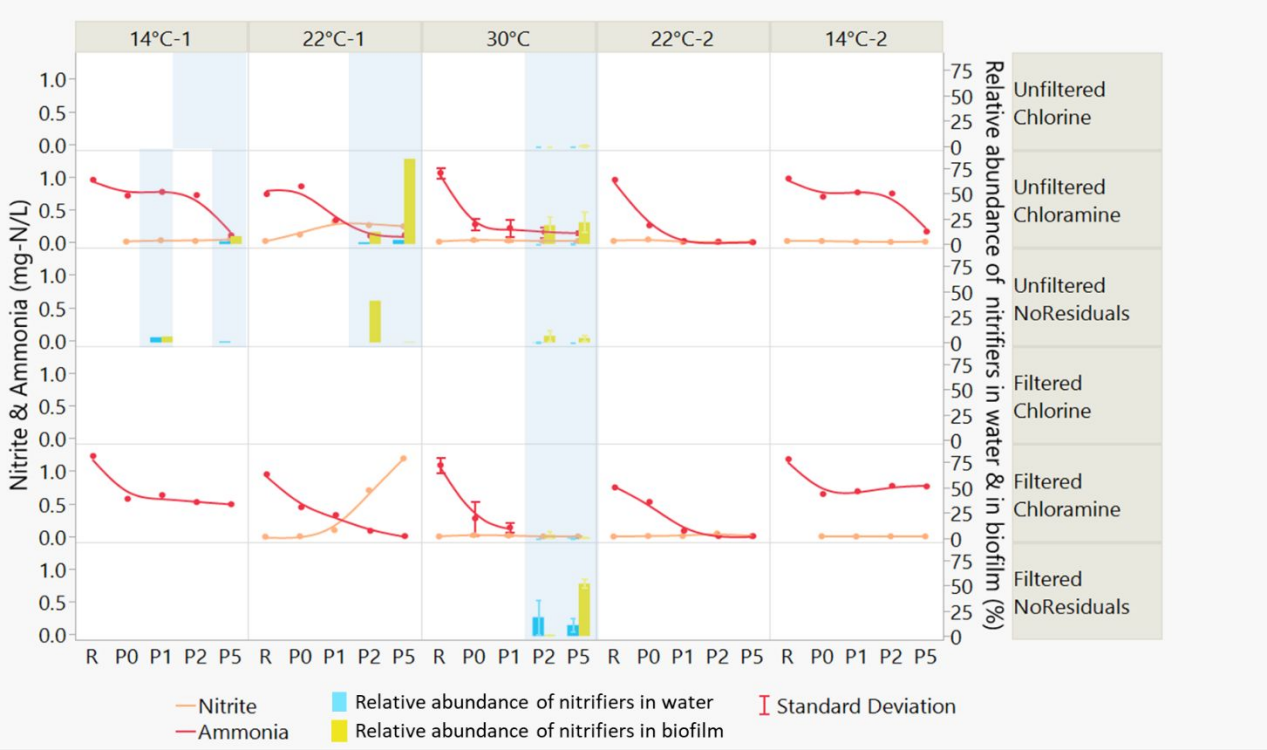

Figure 4 Ammonia and nitrite measurements in the two chloramine RWDSs for each temperature phase and relative abundance of nitrifiers in bulk water and biofilm in the six RWDSs. Sampling locations and time points where metagenomic data were available are shaded. The x-axes represent the reservoir (R) and the four sample ports (P0, P1, P2, P5) (Figure 1). Nitrite and ammonia data at 30°C are mean and standard deviation of three sequential sampling events, other measurements are single measurements. Nitrite level at all sample ports during the 14°C_1 phase was below detection. Colored bars indicate the compositional relative abundance (%) of taxonomically-annotated known nitrifier genera using the MetaPhlAn2 database (Truong et al. 2015). Nitrifier abundance data at 30 °C are mean and standard deviation of three independently sequenced samples. Details of samples included in each of the three sequencing runs is reported in SI Table 1.

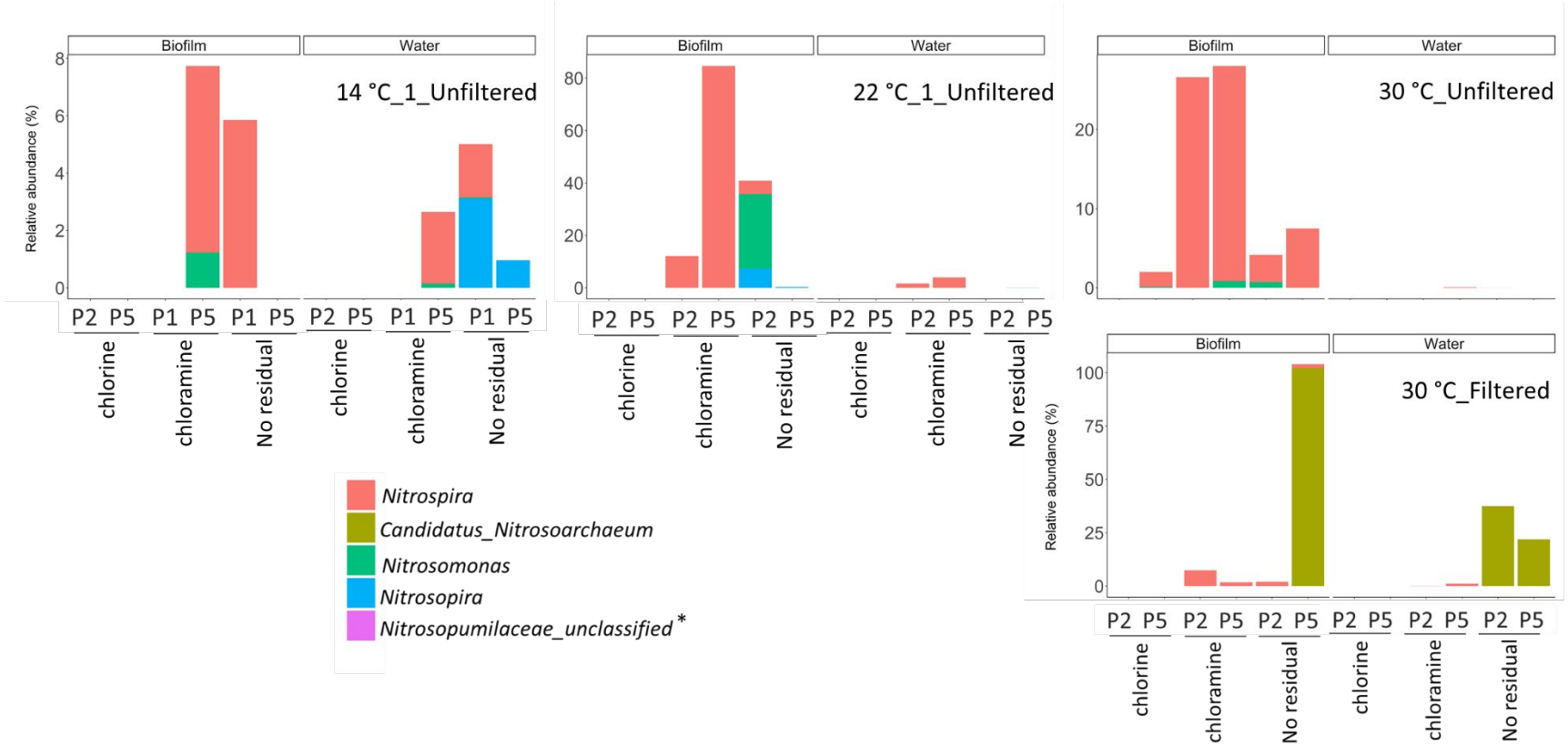

Figure 5 Relative abundance of taxonomically-annotated known nitrifier genera using the MetaPhlAn2 database (Truong et al. 2015) for the three disinfectant conditions at low (P1/P2) and high (P5) water ages at all three temperature phases. Chloramine and No Residual samples at 14 °C_1 were sampled at P1 instead of P2. Comparison of filtered versus unfiltered conditions is available for 30°C. Note the differences in y-axis ranges. * *Nitrosopumilaceae* was only detected at levels between 0.009% - 0.067%, hence not easily visible on this figure. The full dataset is attached in Supplementary Spreadsheet I.

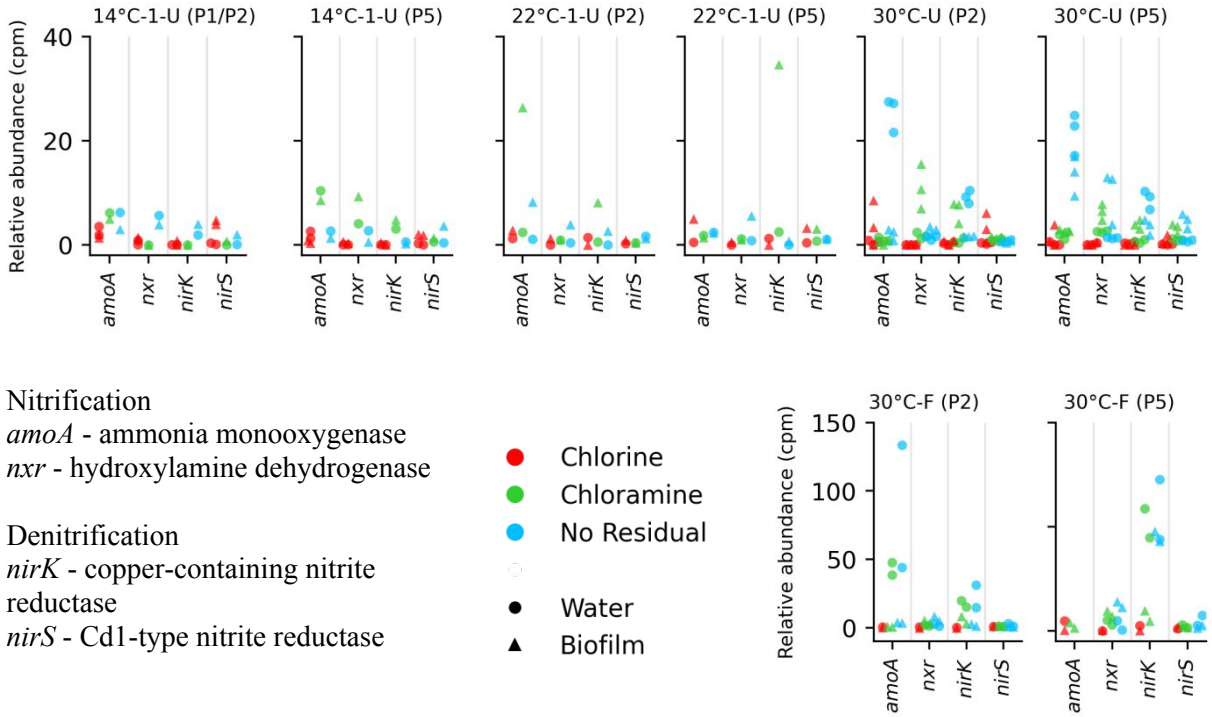

Figure 6. Relative abundances of genes in copies per million (cpm) involved in nitrification and denitrification detected through functional annotation of metagenomic data during the first 14°C and 22°C phases and 30°C at low (P1/P2) and high (P5) water ages. Chloramine and No Residual samples at 14 °C_1 were sampled at P1 instead of P2. Metagenomic reads were mapped to the UniRef50 database for functional gene family annotations using the HUMAnN2 pipeline (Franzosa *et al.* 2018). Note the spikes in *amoA* and *nirK* in chloramine biofilm at 22°C-1-U. y-axis scales are different for unfiltered (U) and BAC-filtered (F) conditions.

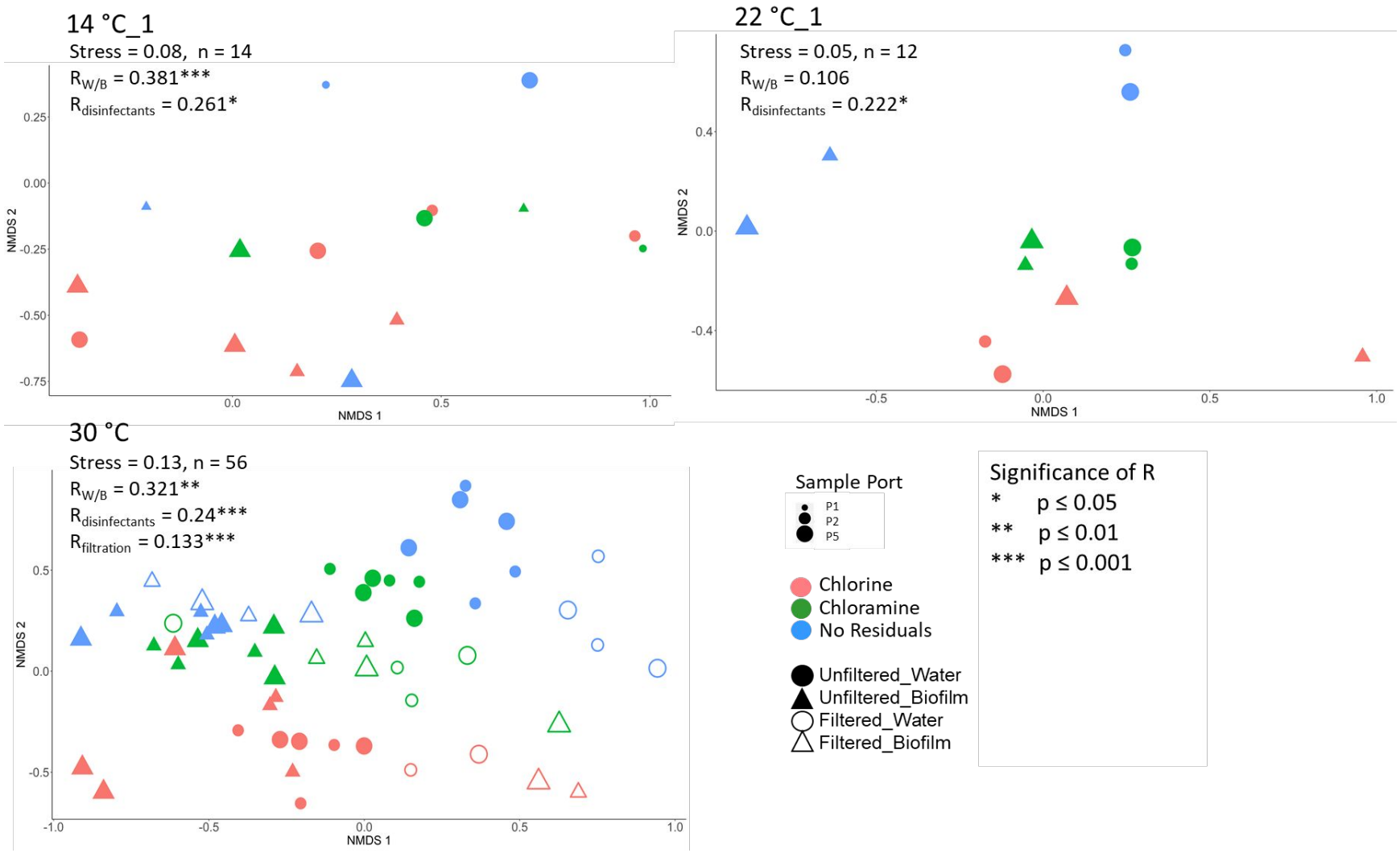

Figure 7. NMDS plots of microbial community genus-level taxonomic composition in the RWDSs. Metagenomic sequencing was performed for unfiltered RWDS samples (solid symbols) at 14°C-1 and 22°C-1 and for both filtered (solid symbols) and unfiltered (open symbols) conditions at 30°C. The number of samples subject to metagenomics sequencing (n) is indicated for each temperature

phase. ANOSIM was conducted for each experimental condition and significant R values are noted in each figure panel. Metagenomic reads were taxonomically-annotated using the MetaPhlAn2 database (Truong et al. 2015).

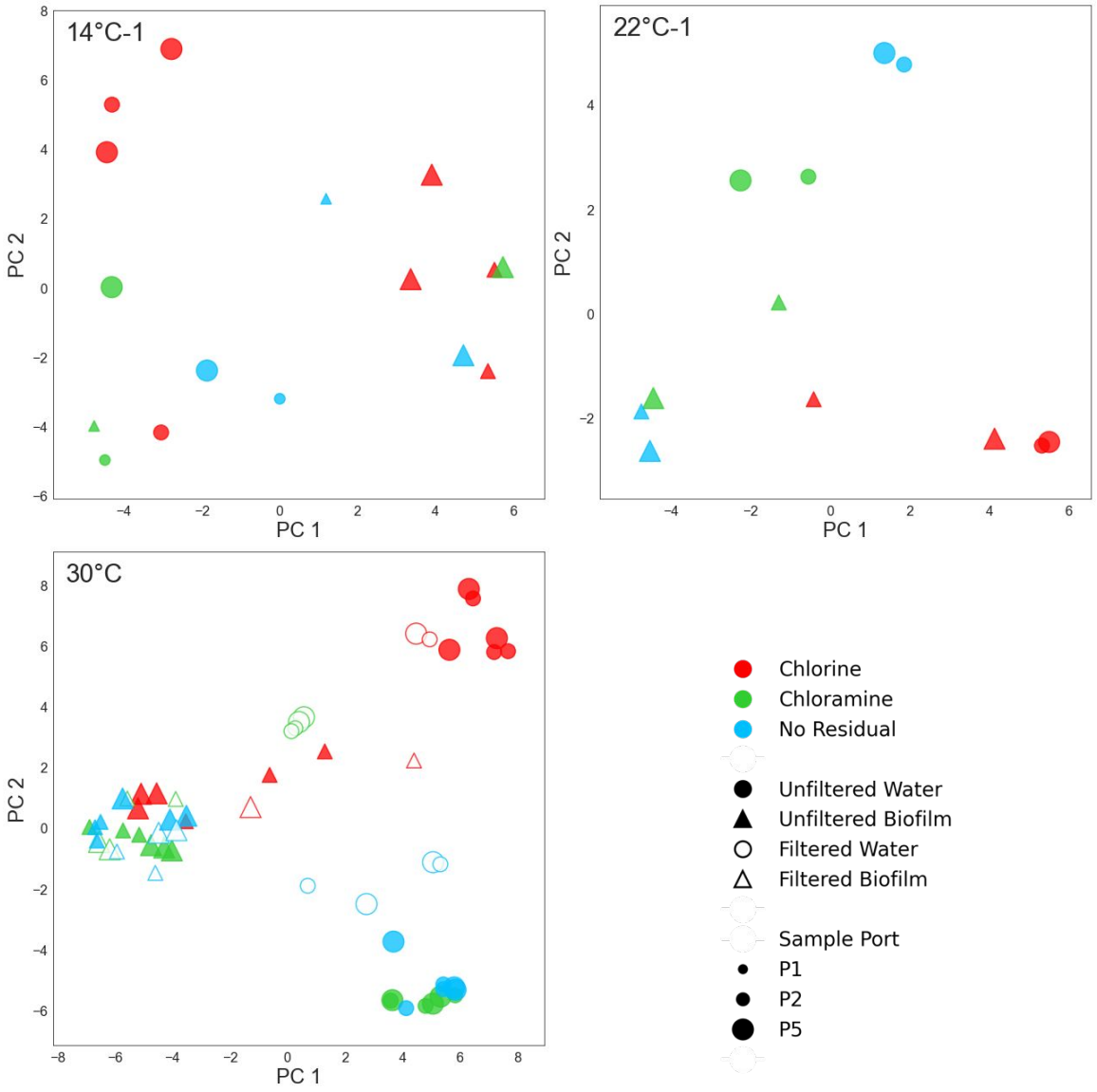

Figure 8. Comparison of RWDS water and biofilm microbiomes based on composition of functional gene family UniRef50 annotations. Metagenomic sequencing was performed for unfiltered RWDS microbiome samples (solid symbols) at 14°C-1 and 22°C-1 and for both filtered (solid symbols) and unfiltered (open symbols) conditions at 30°C. The plots indicate that the largest two components, PC1 and PC2, of truncated SVD on Random Trees Embedding of annotated functional gene relative abundances. 14°C-1: PC1 and PC2 explain 41% of variance among samples, 22°C-1: PC1 and PC2 explain 50% of variance among samples, and 30°C: PC1 and PC2 explain 30% of variance among samples. Metagenomic reads were mapped to the UniRef50 database for functional gene family annotations using the HUMAnN2 pipeline (Franzosa et al. 2018).

Effects of BAC-filtration, disinfection, and temperature on water quality in simulated reclaimed water distribution systems

Ni Zhu[1], Sudeshna Ghosh[1], Laurel Strom[2], Amy Pruden[1], Marc A. Edwards[1*]

[1] Via Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, VA 24061, USA

[2] CDM Smith, 14432 SE Eastgate Way, Suite 100, Bellevue, WA, 98007

* Corresponding author. Tel.: +1 540 231 7236. Email address: edwardsm@vt.edu (M.A. Edwards).

Graphical Abstract