Statistical Evaluation of the Factors causing Microbial Growth in Point-of-use Filters

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ABSTRACT

Due to the lead spike and its related health concern in the DC area, Point-of-Use (POU) filters were installed at public schools to reduce lead concentrations in water. However, the installation of POU filter could possibly lead to the growth of bacteria inside the filters, which could lead to health concerns. Therefore, the potential effects of POU filters on microbial growth was investigated. To explore the cause of filter effects on microbial growth, a sampling campaign was carried out between July and December 2017 from 25 outlets within 5 elementary schools in the DC area. The applicability of flow cytometry results as a quantification method was validated and then used to quantify the biological growth. Our results revealed that the installation of POU filters may lead to nitrification and an increase in microbial growth. Along with the increase in microbial growth, the microorganism community "fingerprints" based on flow cytometry data showed that the installation of filter could also shift the community distribution of bacteria based on their morphology. This study serves as a preliminary study to investigate the mechanics of microbial colonization on POU filters.

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GENERAL AUDIENCE ABSTRACT

Due to the lead spike and its related health concern in the DC area, Point-of-Use (POU) filters were installed at public schools to reduce lead concentrations in water. POU filters are usually installed at sinks and fountains and the Water Quality Association reported that 41% of American homes used POU filters by 2000. However, the installation of POU filter could possibly lead to the growth of bacteria inside the filters, which could lead to health concerns. Therefore, the potential effects of POU filters on microbial growth was investigated. To explore the cause of filter effects on microbial growth, a sampling campaign was carried out between July and December 2017 from 25 outlets within 5 elementary schools in the DC area. Flow cytometry is an optical technology that can measure and then analyze multiple physical characteristics of a single particle as it flows in a fluid stream. The applicability of flow cytometry results as a quantification method was validated and then used to quantify the biological growth. Our results revealed that the installation of POU filters may lead to nitrification and an increase in microbial growth. Along with the increase in microbial growth, the microorganism community “fingerprints” based on flow cytometry data showed that the installation of filter could also shift the community distribution of bacteria based on their morphology. This study serves as a preliminary study to investigate the mechanics of microbial colonization on POU filters.

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Chapter 1. Introduction

1.1 Water Quality Issues in Water Distribution Systems

Drinking water sources may contain a variety of contaminants that can impact human health. Sources can include microorganisms as well as inorganic and organic chemicals. There are over 80 contaminants regulated by the USEPA's National Primary Drinking Water Regulations, which cannot exceed concentration thresholds referred to as Maximum Contaminant Levels (MCLs) or should be treated through Treatment Techniques (TTs) (USEPA 2009, 2015a). Those contaminants could possibly be harmful to the human body, potentially resulting in learning disorders, endocrine disruption, and cancer (USEPA 2008).

Within those contaminants regulated by the USEPA, microbial water quality is addressed by TTs (USEPA 2009). Different bacteria or pathogens can survive in the drinking water distribution system and be harmful to health (USEPA 2015a). One method to control the microbial quality of water within distribution systems is by adding secondary disinfection to comply with the regulated TTs for mitigating the microbial growth in water systems. However, adding chlorine as a secondary disinfectant could possibly lead to different problems, such as the formation of disinfection byproducts (DBPs) (Tibbetts 2007).

To reduce the potential health hazards of DBPs produced by free chlorine, treatment plants (including the Washington Aqueduct Laboratory who is the supplier of drinking water to DC) are switching to chloramine as their secondary disinfectant due to its lower DBP formation potential. However, chloramine may be less effective as a disinfectant compared to free chlorine (Norton and LeChevallier 1997, Zhang et al. 2009). Thus, to ensure the same level of disinfection efficacy, higher doses of chloramine (compared to chlorine) are typically added to the water. During the decay of chloramine, ammonia is released (Eq. 1.1), which can trigger nitrification.

$$3NH_2Cl \rightarrow N_2 + NH_3 + 3Cl^- + 3H^+ \quad (1.1)$$

The process of nitrification reduces the pH and produces nitrite (Regan et al. 2003). This nitrification process causes water quality deterioration (Zhang et al. 2009), which could lead to various health concerns (USEPA 2015b). Hence, nitrification is an increasing concern for chloraminated systems within the U.S. and requires more attention.

1.2 Lead in the Washington DC (DC) area

In the United States, the development of drinking water distribution system started before the 1900s (AWWA 2017). There are several different materials used in the distribution system. For example, steel, iron, cement, concrete, and polyvinyl chloride have been used as the main materials for pipelines. However, increasing problems are associated with aging pipes to include water main breaks and other infrastructure failures. The replacement of those pipes is a top priority in the continual improvement and development of water distribution systems; however, due to the location of the pipe lines, cost of replacement, as well as other considerations, the replacement of aging pipes is a long-term economic challenge.

The history of using lead pipes in water distribution systems dates back to the late 1800s when the installation of lead pipes was popular in large cities with population larger than 30,000. Later, by the 1900s, at least 70% of those cities used lead water lines (Rabin 2008). Service lines were made of lead until the 1940s and, therefore, are still prevalent in some older houses in DC. As the health concern of lead was discovered later, many lead pipe lines have been replaced during renovation to copper or pipelines of other materials over recent years (Edwards et al. 2009)

Between 2001 and 2004, DC experienced alarmingly high levels of lead when the Washington Aqueduct switched from chlorine disinfection to chloramination in 2000 to address DBP formation and coliform issues within the system (Cohn 2004, Shaver and Hedgpeth 2016). The switch from chlorine to chloramine triggered the release of lead from the pipe walls (Dudi 2004, Vasquez et al. 2006) due to a change in the oxidation-reduction potential (ORP) in the disinfectant. Chloramine reduced the ORP of the water traversing the distribution system changing the redox chemistry. As shown in Figure 1-1, the ORP for chloramines is much lower compared to free chlorine at the same temperature in finished water.

The release of lead is a fundamental oxidation-reduction reaction where lead is oxidized to hydrocerussite or lead dioxide forming a passive layer of lead oxides lining the walls of the lead pipes. After the switch, the decrease in ORP changed the dominant oxidized species from hydrocerussite to lead dioxide, which is more soluble and resulted in higher lead releases into the drinking water (Renner 2004, Vasquez et al. 2006).

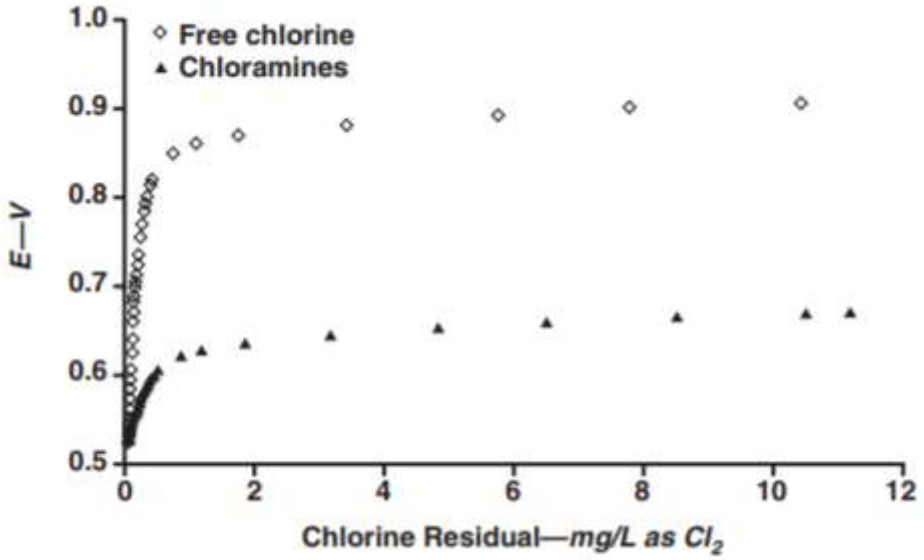


Figure 1-1 ORPs v.s. free chlorine and chloramine for finished RO at 25 ºC (Adapted from the work by Vasquez et al. 2006)

In early 2004, lead concentrations increased throughout the city such that 68% of addresses sampled had a lead concentration greater than the USEPA's lead action level (LAL) of 15 µg/L (Edwards et al. 2009) with some samples collected during this time period exceeding 300 µg/L (Edwards et al. 2009, Guidotti et al. 2007, Switzer et al. 2006).

Since 2003, DC Water has worked and continues to work with customers to replace existing lead service lines. DC Water also worked with the Washington Aqueduct to include the addition of orthophosphate to aide in corrosion control starting in 2004; however, lead remains a focus of concern for many DC-area residents.

1.3 Lead Health Effect and Regulated Limits

Lead is toxic if ingested. Several studies have shown that lead in drinking water could result in an increase in blood lead levels in young children and infants (Edwards et al. 2009, Guidotti et al. 2007, Watt et al. 2000). Elevated blood lead levels can damage nerves, hematopoietic stem cells, and renal systems especially for young children. Extremely high blood lead concentrations can cause neurologic problems (e.g., seizure, coma, and death) (Meyer et al. 2003). Lead is also harmful to adults and could result in reproductive problems, decreased kidney function, and cardiovascular effects.

The USEPA suggests that, since lead is a toxic metal harmful to human health, the maximum contaminant level goal for lead be set to zero while the MCL is controlled by a TT through corrosion control. By controlling the corrosivity of water, water systems are required to keep the lead level in water delivered to the tap under 0.015 mg/L. The USEPA also estimates that drinking water can make up at least 20% of one's total exposure of lead, which can bioaccumulate in one's body over time (USEPA 2018).

The lingering risk of lead exposure in a large distribution system like DC Water's has resulted in the installation of point-of-use (POU) filters in fountains and taps in public schools to further remove any lead in the water (DCPS 2017).

1.4 Point-of-Use (POU) Filters

Point-of-use (POU) filters are often installed at sinks and fountains for a variety of reasons including heavy metal and organic compounds removal as well as pathogen elimination (Sheffer et al. 2005, Sobsey et al. 2008). The Water Quality Association reported that 41% of American homes used POU filters by 2000 (Sheffer et al. 2005). Typically, POU filters designed for lead removal are made of granular activated carbon, powdered carbon block, or a mix of other agents capable of filtering particulates and adsorbing dissolved lead (Silverstein 2006, Su et al. 2009). When the surface of the filter is saturated with adsorbed pollutants, there will be no further purification effect of the filter, which could result in possibly higher containment concentration in the filtered water compared to unfiltered concentrations (Silverstein 2006).

Figure 1-2 The illustration of a carbon filter on the left taking in effluent from a sediment prefilter on the right

Figure 1-2 is an example of a POU filter included in this research. This type of filter consists a carbon-block filter to remove lead and a sediment filter to prevent possible clogging of the carbon-block filter.

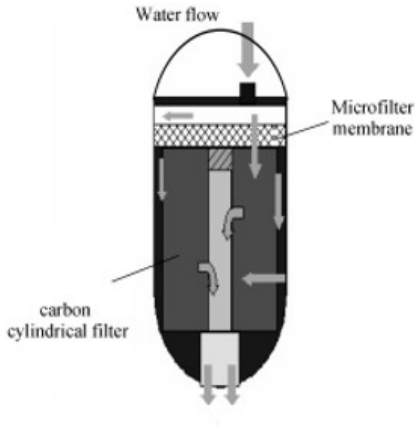


Figure 1-3 The illustration of the internal design of a carbon filter (adapted from the work by Su et al. 2009)

Figure 1-3 is an example of the flow of water through a typical carbon-block filter. The water comes from the top of filter, and flows from the outside of the block filter, through a microfiber membrane either located at the top of the filter or commonly wrapped around the filter block towards the inside of the filter unit. As the water passes through the carbon material, contaminants can be adsorbed into the carbon media. The filtrate then passes through the center of the filter before exiting at outlet. The efficiency of carbon filters also depends on the influent water quality, flow rate, and contact time (Knezev 2015).

Figure 1-4 shows the mechanism of the sediment prefilter. Like the carbon filter, water flows from the outside towards the inner core of the filter. For the sediment prefilter included in this study, the minimum pore size was 1 μm, which could intercept microorganisms before they enter to the carbon filter (AWS 2018).

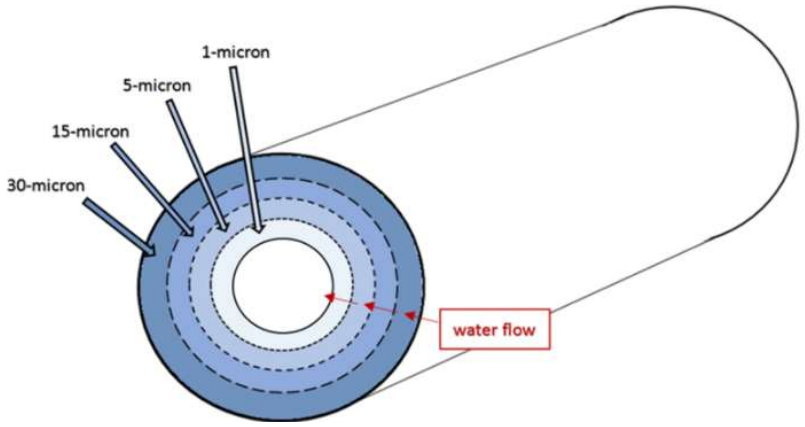


Figure 1-4 Illustration of internal design of a sediment prefilter (Aquaspace, APR 4-N-1, MD, USA, adapted from the work by AWS 2018)

1.5 Biological Growth within POU Filters

Some bacteria in the drinking water system could pose health risks. Installed POU filters may harbor bacteria and pathogens leading to increased growth and exposure (Chaidez and Gerba 2004, Wingender and Flemming 2011). It is possible that more biofilm forms inside the POU filters, which consequently could increase the bacteria concentration in the effluent water. For both granular activated carbon and carbon block filters, the attachment of bacteria to carbon granules occurs due to the large surface area, the hydrophobic nature of, and the presence of functional groups (Knezev 2015). Increased colony counts were observed using carbon filters in treatment plants (Schwartz et al. 1998, Stewart et al. 1990, Velten et al. 2007), which indicates this kind of material could increase microbial populations in POU filters as well. Bacteria can form biofilms on solid surfaces, which may cause further hygienic problems (Shen et al. 2016, Su et al. 2009).

Pathogens in the drinking water distribution systems have the potential to be captured by biofilms in POU filters and become embedded into to the extracellular polymeric matrix. Consequently, pathogens take advantage of biofilms as a protector from environmental stress (e.g., disinfectants, hydrodynamics, and low-nutrients). Therefore, newly grown bacteria could migrate from the filter (cells incorporated into biofilms) to the bulk water (existing as planktonic cells), which may lead to health concerns. Past studies have shown a variation in the bacteria removal capability of drinking water POU filters (Sheffer et al. 2005, Silverstein 2006, Sobsey et

al. 2008, Wilcox et al. 1983), which provide motivation to confirm the bacteria removal capability of POU filters.

1.6 Research Objectives

The objectives of this study are to:

1) Investigate the validity of new bacteria quantification methods such as flow cytometry and ATP analyses in replacing heterotrophic plate count (HPC) analyses in low chlorine systems.

   Hypothesis: Flow cytometer measurements will have strong correlations with both HPC and ATP, signifying its potential use in the field.
2) Identify process factors responsible for increased bacterial concentrations in filtered water.

   Hypothesis: The filter's ability to reduce total chlorine concentrations through catalytic reduction reaction will have the most significant impact on microbial concentrations.
3) Determine the impacts of installed POU filters on the microbial characteristics of the filtered water.

   Hypothesis: POU filters will foster biofilm growth and, thus, increase planktonic microbial concentrations in the filtered water as compared to the unfiltered water.

Chapter 2. Methods

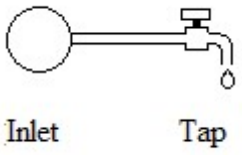
2.1 Sampling Methods and Sites

Drinking water samples were taken from public schools in Washington DC. A total number of five schools located in different areas of DC were included in the study. The schools were selected to capture water quality differences inherent in the distribution system such as varying disinfectant residual concentrations, approximate water age delivered to a location, and iron concentrations. Within each school, five outlets were selected to include two unfiltered (control) sources and three sources with installed carbon block filters rated to reduce lead levels to below 1 µg/L. here are three sampling scenarios for the sampling categories, diagrammed in Figure 2-1 a-c. These scenarios include:

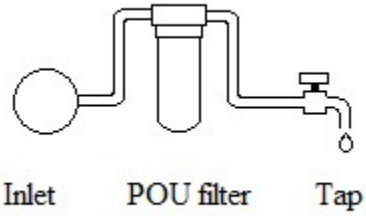
- Unfiltered water samples: the source water coming from the drinking water distribution system passes through the premise plumbing then directly to the tap (Figure 2-1a).

- Filtered water samples without a sediment prefilter: water from the distribution system travels through the premise plumbing and then to the lead-removal filters before it is sampled through the tap (Figure 2-1b).
- Prefiltered + filtered water samples: similar to the filter set up except that water travels through a sediment prefilter prior to the carbon block filter and then exits the tap (Figure 2-1c). The sediment prefilter reports to remove particles of 1 µm or greater. This sample is referred to simply as the "prefiltered" sample in the remainder of the text.

a) Scenario 1: Unfiltered Water Samples



b) Scenario 2: Filtered Water Samples (without prefilter)



c) Scenario 3: Sediment prefilter + filter water samples

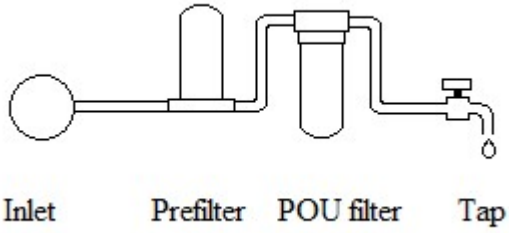


Figure 2-1 Illustration of three types of samples taken from this study: a) Unfiltered water samples, b) Filtered water samples without a prefilter, c) Prefiltered water samples

Table 2-1 Sampling locations

School	Filter Status	Date installed	Date	Sediment prefilter
School No.1	Filter	11/30/2016	10/11/2017	Prefilter
	Filter	11/30/2016	10/11/2017	Prefilter
	Filter	5/23/2017		Prefilter
	Unfiltered	---		---
	Unfiltered	---		---
School No.2	Filter	5/26/2017		No prefilter
	Filter	5/26/2017		No prefilter
	Filter	11/18/2016	10/12/2017	No prefilter
	Unfiltered	---		---
	Unfiltered	---		---
School No.3	Filter	6/14/2017		No prefilter
	Filter	6/14/2017		No prefilter
	Filter	6/14/2017		No prefilter
	Unfiltered	---		---
	Unfiltered	---		---
School No.4	Filter	6/19/2017		No prefilter
	Filter	11/21/2016	11/6/2017	Prefilter
	Filter	11/21/2016	11/6/2017	Prefilter
	Unfiltered	---		---
	Unfiltered	---		---
School No.5	Filter	12/9/2016	11/15/2017	Prefilter
	Filter	12/9/2016	11/15/2017	Prefilter
	Filter	6/28/2017		Prefilter
	Unfiltered	---		---
	Unfiltered	---		---

Within each school, the locations of each tap were dispersed within the building in order to capture water quality variation within the same school.

As shown in Table 2-1, schools No.1 and No.5 used prefilters while schools No.2 and No.3 were without prefilters. School No.4 contained both taps with and without prefilters. There were three models of filters included in this study. For scenario 2 in Figure 2-1, two models of filters were encountered: 1) Aquaspace AWR-2414 (Aquaspace Inc, Forestville, USA) for all the taps in this scenario except the one in School No.4, 2) and Everpure EF1500 (Pentair, Inc, Minneapolis, USA) which has similar design as the AWR2412, for one location in School No.4. For scenario 3 in Figure 2-1, the model included was Aquaspace AWR-4814 (Aquaspace Inc, Forestville, USA). This model is a twin unit with a sediment prefilter installed prior to a carbon block filter. All three models remove lead from the influent water and require replacement every 12 months.

Sources with prefilters were included to investigate the role these sediment prefilters play in biological growth on the downstream carbon-block filters. To capture the effects of aging filters and the installation of new filters, schools No.2 and No.4 were sampled until the end of December as they had filters replaced during October and November while the rest of the schools were sampled until the end of November.

Starting from July 2017, samples were taken each week from each outlet using the following sampling procedures:

1) Remove the aerator from the outlet (if one is present).
2) Run the water at maximum speed for 5 minutes.
3) Clean the inside of the outlets with Sani Cloth disinfection wipes.
4) Collect two microbial samples using sanitized 120 mL bottles containing sodium thiosulfate to quench residual chlorine. Collect an additional 500 mL of sample for bulk water quality in a clean, non-sterilized bottle as well as a sample in a 40 mL amber-glass vial for total organic carbon (TOC) analysis.
5) Analyze all water quality parameters (except TOC) within 8 hours of collection and process TOC samples within a week of collection.

2.2 Sampling Parameters

2.2.1 Measured Parameters

For biological growth parameters, HPC, adenosine triphosphate (ATP), as well as total and live cell counts were measured. Heterotrophic bacteria use organic nutrients for growth and are present in all types of water, soil, and air. HPC quantify heterotrophic bacteria and are broadly used to assess microbial growth on materials in drinking water (Allen et al. 2004, Lechevallier et al. 1991). ATP is the energy currency for all living cells and is a measurement for all active cells in a sample. It has been used as a microbiological parameter in drinking water (Bushon et al. 2009, van der Wielen and van der Kooij 2010, Vang et al. 2014) and is often suggested as a potential replacement for HPC measurements in the future. Total and live cells were measured using flow cytometry, which will be further discussed in the flow cytometry section (Section 3.1.2).

For the water chemistry parameters, monochloramine residual concentrations (as mg/L total Cl_2), NO_2^- (mg/L-N), pH, temperature (^oC), and TOC (mg/L-C) were measured using the methods tabulated in Table 2-2.

Table 2-2 Methods for the parameters measured in this study

Parameter	Method
Monochloramine (measured as Total Cl_2)	USEPA DPD Method: Method 10250 (equivalent to SM 4500-Cl G) (APHA 2012)
NO_2^-	USEPA Diazotization Method: Method 8507 (USEPA 1984)
pH	USEPA Electrode Method: Method 8156 (based on SM 4500-H+B) (APHA 2012)
Temperature	Measured by HQ40D Portable Multi Meter (Hach, Loveland, CO)
TOC	SM 5310 C (APHA 2012)
HPC	SM 9215 B (APHA 2012)

While temperature was measured, many of the water sources were water fountains that included cooling systems and, therefore, this parameter was primarily used to track the consistency of each source's temperature each week.

2.2.2 ATP

ATP is a measure of the viable biomass in the sample. Compared to HPC, it could give a better estimate of active biomass in a drinking water sample. Also, unlike HPC which requires seven days of incubation, the result of an ATP measurement is obtained within minutes. ATP measurements have been used in various aquatic environments to determine microbial activity and biomass concentration. ATP values were measured using a LuminUltra® Quench-Gone™ Aqueous Test Kit (LuminUltra, Fredericton, Canada) with a PhotonMaster™ Luminometer (LuminUltra, Fredericton, Canada). It serves as one of the quantification methods in our study to determine the usefulness of using such a technique to track biological growth on a short-term basis (over several weeks). Aliquots of 50mL were taken from the sterile, chlorine-quenched samples and filtered through a Quench-Gone filter. Samples were then processed according to the LuminUltra analytical procedures (LuminUltra 2017).

2.2.3 Flow Cytometry

Flow cytometry is a technology that can measure and then analyze multiple physical characteristics of a single particle as it flows in a fluid stream. Data are collected for every single particle that the flow cytometer captures using light scattering and fluorescent properties by the interaction between the laser beam and particles (BD 2000). The combination of flow cytometry with fluorescent stains has been used to quantify the bacterial regrowth in aquatic samples in Europe (Berney et al. 2008, De Roy et al. 2012, Liu et al. 2013, Nescerecka et al. 2014, Siebel et al. 2008). The model of flow cytometer used in our study is BD Accuri™ C6 (Becton Dickinson, Franklin Lakes, USA)

By using different dyes, this method can show specific bacterial communities in the water sample. The dyes used in this study included SYBR (r) Green to capture all of the cells (total cells) and also a combination of SYBR Green and Propidium Iodide to measure only the live cells. The total cell and live cell counts are calculated based on the raw output of the flow cytometry while the dead cell counts for samples are calculated by the subtraction of live cell

counts from the total cell counts. Flow cytometry is used both for the analysis of total and live cell counts as well as identifying phenotypic fingerprints associated with the microbial community of the sample based on the cell morphology.

Figure 2-2 is an example of a phenotypic microbial community output depicting the community augmentation after contamination, disinfection, and then later flushing with tap water, where we can clearly see the composite community change even though the fingerprint uses cell morphology instead of genetic differences. Figure 2-2 can be seen as a diversity analysis where principal coordinate analysis is applied and will be further explained in section 2.2.4.4.

Compared to HPC, this method is 1) rapid: the results can be delivered within minutes, 2) easy to use: the training of this method can be done in days, and 3) cultivation independent: there is no need to culture the biomass in the water.

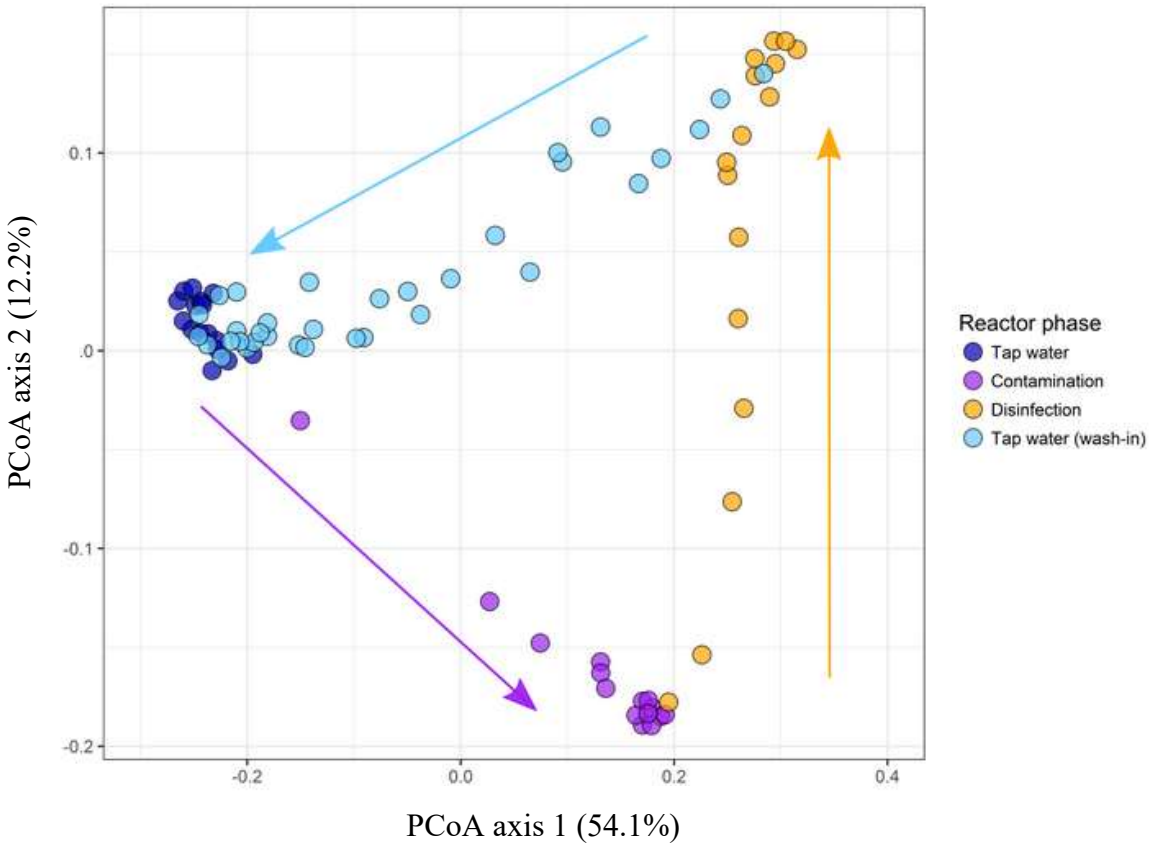


Figure 2-2 An example of flow cytometry fingerprint showing community morphology shift (Adapted from the study by Besmer et al. 2017)

2.2.4 Statistical Analysis

All the statistics calculation/analysis were conducted using RStudio version1.1.419 (RStudio Inc, Boston, USA).

2.2.4.1 Filter Impact

The filter impact on the water quality was defined in Eq. 3.1 by calculating the concentration difference between the filtered and unfiltered water within the school on a particular day.

$$f_{t,i} = F_{t,i_j} - \overline{C_{t,J}} \tag{2.1}$$

$f_{t,i}$: Filter impact (difference of concentration) at time t, for outlet i

F_{t,i_j}: Concentration in filtered water concentration measured at time t from POU setup illustrated in Figure 2-1b and c for outlet i located in school j

$\overline{C_{t,j}}$: Mean of concentration for unfiltered water sample (Figure 2-1a) in school j at time t

The filter impact was calculated for each parameter to quantify the contamination impacts attributed to the filter and to account for variations in bulk water quality received by each school on any given day. Positive filter impact values signify increases in concentrations due to the filter (influent concentrations are less than effluent concentrations) while negative values indicate removal by the filter (influent values are greater than effluent concentrations). Since each school had varying influent concentrations, the use of the filter impact calculation helped to normalize the data and better quantify the concentration fluctuations in the effluent that were attributable to the filter units.

2.2.4.2 Spearman's Correlation

Spearman's correlations were performed for each parameter. Statistically, Spearman's rank correlation coefficient is a rank-related non-parametric measure. It evaluates the relationship between two variables which can be described by a monotonic function (Lehman 2005). As it is a non-parametric measure, it removes the effects of the outliers and does not require the sample to have a normal distribution (Hauke and Kossowski 2011, Zar 1972).

2.2.4.3 Mann–Whitney–Wilcoxon (MWW) Test

Statistically, the Mann-Whitney-Wilcoxon (MWW) test is also a non-parametric test of null hypotheses (De Winter and Dodou 2010, Mann and Whitney 1947, Wilcoxon 1945). Unlike a t-test, the MWW test does not assume a normal distribution, but has the same effective result as the t-test with normal distributions.

The MWW test has multiple applications. For example, it can determine whether two independently selected samples from a population have the same distribution (Fay and Proschan 2010). In our study, $\alpha=0.05$ is chosen to determine if the result was significant.

2.2.4.4 Phenotypic Community Analysis

Community analysis computations based on flow cytometry data were performed. The difference and similarity related to each day of sampling were calculated by Principal Coordinates Analysis and then clustered using the k-means clustering method. A threshold concentration of 500 events (10 counts/μL) was used for the phenotypic community analyses to reduce uncertainty in the results.

Principal Coordinates Analysis

Principal coordinate analysis, usually called PCoA and known as classical scaling, is a projection based metric multi-dimensional scaling method that uses spectral decomposition to estimate the distance or dissimilarity matrices by the distance between a set of points in a multidimensional space. These points can be used for picturing sample distribution (Cox and Cox 2000, Gower 2005). By converting multi-dimensional dataset into a lower-dimensional dataset, the distance between the two data points could be used as a similarity measure. In other words, the closer two points are on the PCoA graph, the more similar they are. Also, the higher sum of variance on two axes indicates the results are more significant, i.e., the larger the sum of two axes percentage is, the better the variance of samples is represented.

K-Means Clustering

K-means clustering is a vector quantization method originally derived from signal processing and commonly used in data mining analyses. K-means clustering is designed to divide the

number of n observations into k clusters, where each observation belongs to a cluster with the nearest mean and is used as a prototype for clustering (MacQueen 1967).

As a simple unsupervised machine-learning algorithm, k-means groups the data into k clusters, although k is not the true number of clusters to classify. Therefore, it is important to determine the right number of clusters to group. In other words, before clustering, we need to identify the k value to get meaningful results.

Elbow Method

The elbow method is a method to validate the number of clusters. The basic idea of the elbow method is to test k-means clustering with data for a range of values of k (for instance, from 1 to 10) and calculate the sum of squared errors (SSE) for each value of k. The number of k clusters is chosen when the change of SSE reaches the largest value. In other words, k is the point where it has the steepest slope, and the point is seen as the elbow point. After identifying the elbow point, select a k which has smallest SSE in the range k (Goutte et al. 1999, Ketchen Jr and Shook 1996).

Silhouette Method

Another measure to select an appropriate k for a k-means analysis is based on the silhouette value which is determined by how similar a dataset is to its own cluster (cohesion) compared to other clusters (separation). The range of the silhouette value is from −1 to +1, where +1 means that the data is well fitted to its own cluster and poorly included to other neighboring clusters. So, it is optimal if the data has a value at or close to +1 (Rousseeuw 1987).

Gap Statistic Method

Gap statistic is a new method which was developed in 2001. The main idea is to find a way to normalize the comparison with the data that has a null reference distribution, especially when a distribution has no obvious clustering (Tibshirani et al. 2001). It imports a logistic function to calculate the cluster distance, and makes the difference between estimation value and sample expectation as large as possible, which can obtain better results for grouping. The number of k clusters is chosen when the increase of gap statistic reaches the largest value.

Chapter 3. Results and Discussion

For the objectives that are included in this research, the results are addressed below. The applicability of flow cytometry in our case is validated in Section 3.1 and then used to quantify biological growth in subsequent sections. Analysis of filter effects on nitrification and biological growth are discussed in Section 3.2. Finally, analysis of the microbial communities within each type of filter is presented in Section 3.3, bringing together many of the conclusions and findings from the entire research effort.

3.1 Quantification Methods and Biological Growth on Filter

Table 3-1 The ranges of microbial water quality parameters measured in this study

	HPC (CFU/ml)	ATP (pg/mL)	Total cell (counts/mL)
Max	27000	210	1700
Min	15	0.41	2.1
Mean	1500	6.3	130
Median	690	1.9	53
Standard deviation	3200	16	205
Coefficient of variation	208%	260%	152%

Table 3-1 shows the range of microbial water quality parameters in our study. The coefficient of variation (CV), which a measurement of variability, is the ratio of standard deviation and mean. The CV for HPC and ATP (208% and 260%) is higher than the CV for total cell concentrations measured by flow cytometry (152%). The high variance in HPC along with the fact that the HPC method is labor intensive has impelled us to use the Spearman correlation to determine if there is another method that could replace HPC as a microbial quantification method in this study.

3.1.1 Spearman Correlation

As discussed in section 2.2.4.2, a Spearman correlation could be used for non-parametric analysis, which does not require normal distribution. In environmental analysis, the relationship between two parameters is often nonlinear. Thus, this method could be more suitable for finding the correlations between two parameters. For this reason, Spearman correlations were calculated

in this study to identify the correlations between HPC, ATP as well as the total and live cell counts from the flow cytometry with regard to corresponding water quality parameters.

Spearman correlation coefficients are shown in Table 3-2. As we can see from Table 3-2, HPC, ATP, and total cells are well correlated with correlation coefficients larger than 0.5 which further supports the applicability of ATP and the flow cytometry method used in this study.

Table 3-2 Spearman correlation coefficients (r_s)

Parameters	HPC	ATP	total cell	live cell
HPC	1.00	0.51*	0.55*	0.58*
ATP		1.00	0.77*	0.88*
total cell			1.00	0.94*

Notes: * indicates the significance at $\alpha = 0.05$

ATP is also closely correlated with live cell counts ($r_s = 0.88$) from flow cytometry compared to ATP to total cell counts and HPC ($r_s = 0.51$, $r_s = 0.55$), which is expected as ATP is energy currency and thus only quantifies the live cells.

Total cell counts outputted by flow cytometry seem to have the largest correlation coefficients with ATP and HPC ($r_s = 0.55$, $r_s = 0.77$) as compared to the correlation coefficient between ATP and HPC ($r_s = 0.51$). It also has very high correlation coefficients with live cell counts, indicating that most of the cells captured in the sampling procedure maintained a similar percentage of live cells.

Spearman's correlations show that flow cytometry is promising as the main biological growth indicator in this research even though this method is rarely applied in the United States (De Roy et al. 2012, Gillespie et al. 2014, Liu et al. 2013, Props et al. 2016).

3.1.2 Filter Impact on Biological Growth

Due to the reduction of chlorine by the carbon-block filters (which will be further discussed in section 3.2.1), the installation of filters could have a potential impact on cell growth within the

filters and planktonic cell concentrations in the effluent. As discussed in section 3.1.1, flow cytometry could be used as the main biological growth indicator in our research; therefore, for the remainder of the analyses, the impact of filters on biological growth will be discussed mainly based on the output of flow cytometry. A plot of total cells measured in the unfiltered samples and in the filtered samples is shown in Figure 4-1 and shows higher cell counts (in terms of median = 71 counts/µL) in the filtered water.

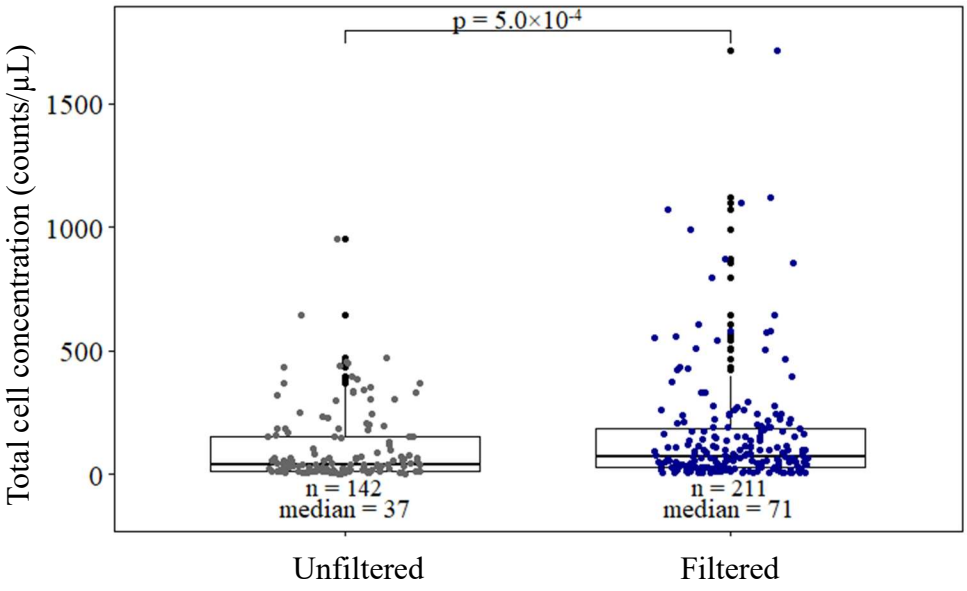


Figure 3-1 Comparison of total cells detected by flow cytometry in filtered and unfiltered water

From Figure 3-1, we can observe that total cell concentration is higher in filtered water sample (median = 71 counts/µL) than in unfiltered water samples (median = 37 counts/µL), indicating that the filter can promote biological growth. MWW tests indicate these differences are statistically significant ($p = 5\times10^{-4}$, n = 353).

Comparing the flow cytometry live cells counts in Figure 3-2, we can see that there are much higher live cell concentrations in the bulk filtered water than in the unfiltered water (median = 52 counts/µL and 6.1 counts/µL, $p = 3.5\times10^{-11}$, n = 177). This further strengthens the argument that these carbon filters are capable of harboring cell communities; however, there is more to consider like the installation of the sediment prefilters in the treatment scheme and the varying influent water quality. The following sections will explore these considerations in greater detail.

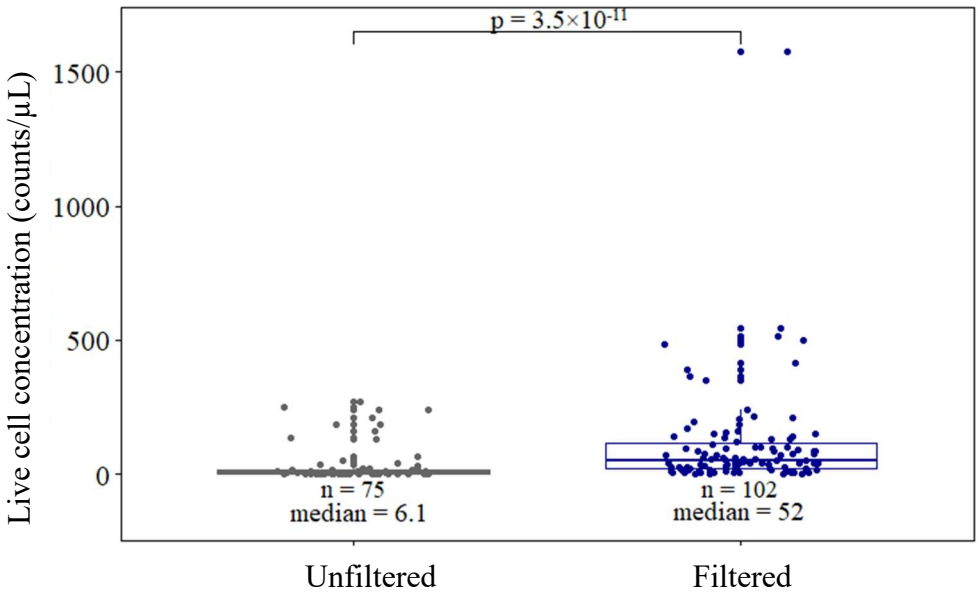


Figure 3-2 Comparison of the concentrations of live cells in filtered and unfiltered water samples

3.1.3 Sediment Prefilter Effects on Biological Growth

One filter feature that has the potential to impact the biological growth on these carbon filters is the presence of a sediment filter prior to the carbon-block unit. This prefilter, as described earlier (Figure 2-1c), has a pore size of approximately 1 µm.

Again, looking at the impacts of a prefilter on the total population of samples, box plots for all the filtered data with and without a prefilter are shown in Figure 3-3 a and b (total and live cell counts respectively). If the filter impact from prefiltered samples is a negative value, it means that the filters remove microorganisms. If the filter impact is positive, it is contributing planktonic cells to the effluent. Immediately apparent in these two plots is that there is a difference in filtrate concentrations related to biological activity as measured by total cell counts and live cell counts (MWW tests $p = 0.00025$ and 0.0040, and $n = 202$ and 102, respectively). The negative filter impact on total cell concentrations indicates that the prefilters reduced the total cell concentration from the effluent while the positive filter impact on live cell concentration indicates that prefilters are contributing to the live cell concentration in the effluent. Those results indicate that the installation of a carbon-block filter could increase both the total and live cell concentrations in the filtrate. Meanwhile, the prefilter could possibly remove certain

bacteria from the influent drinking water, resulting in the lower filtrate concentrations than in the to carbon-block filter only scenarios (Scenario 2 in Figure 2-1).

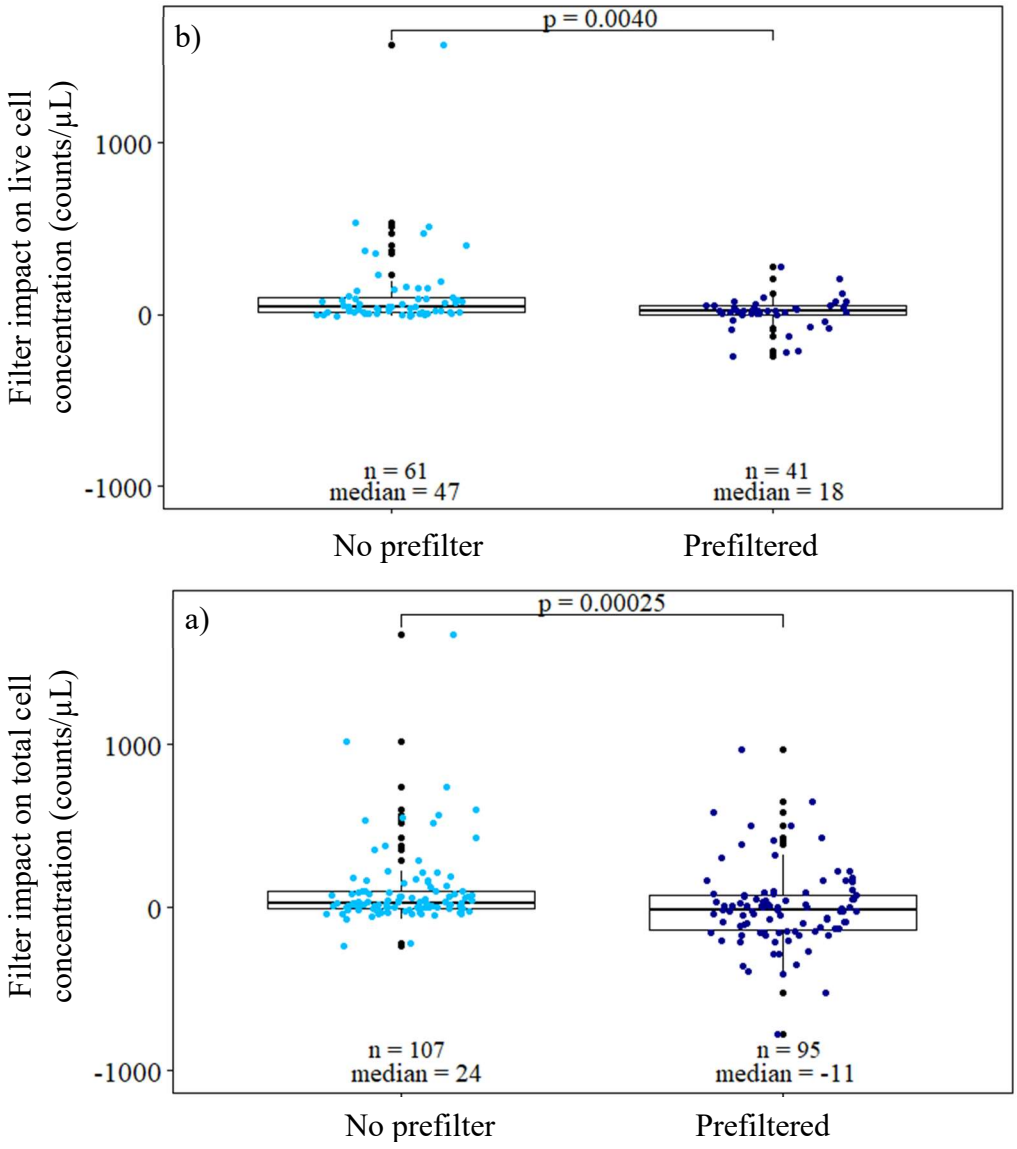


Figure 3-3 Filter impact as defined in Eq. 3.1 on a) total and b) live cells concentration with and without the installation of sediment prefilter

3.1.4 Filter Age Impact on Microbial Growth

Filter age could also possibly influence microbial growth on the filter units. As filter age increases, it is possible that there will be more microbial growth inside the filter. Hence, the

effect based on filter age was examined and is shown in Figure 3-4.

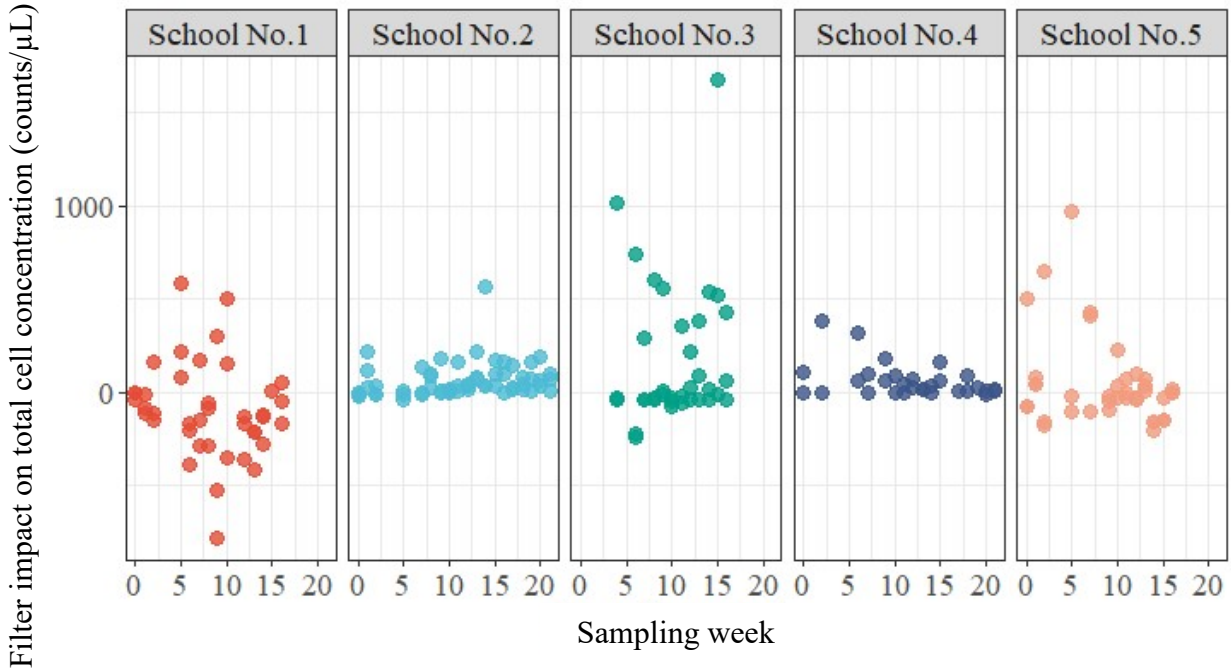


Figure 3-4 Filter impact as defined in Eq. 3.1 on total cells concentration by school over the 22 sampling weeks

As shown in Figure 3-4, within each school, the filter impacts (Eq. 3.1) for microbial growth are quite different. For schools No.1 (prefilter), No. 3 (no prefilter), and No. 5 (prefilter), we can see some variation of microbial growth during the middle of the study, (sampling weeks 5 to 10) whereas schools No. 2 (no prefilters) and No. 4 (mixed prefilter and no prefilter) seem relatively consistent in their cell numbers. It is not quite clear if the carbon-block filters alone or those paired with a sediment prefilter consistently increase or decrease biological activity of the filtrate. The variations inherent in the data presented in Figure 3-4 are more closely related to the influent water chemistry and this is discussed further in section 3.2.1.

Visually looking at filter impact by filter age, as plotted in Figure 3-4, it is hard to tell the difference. To better understand the overall impact of filter age on microbial growth and effluent cell concentrations, a boxplot of filter impact by filter age is presented in Figure 3-5.

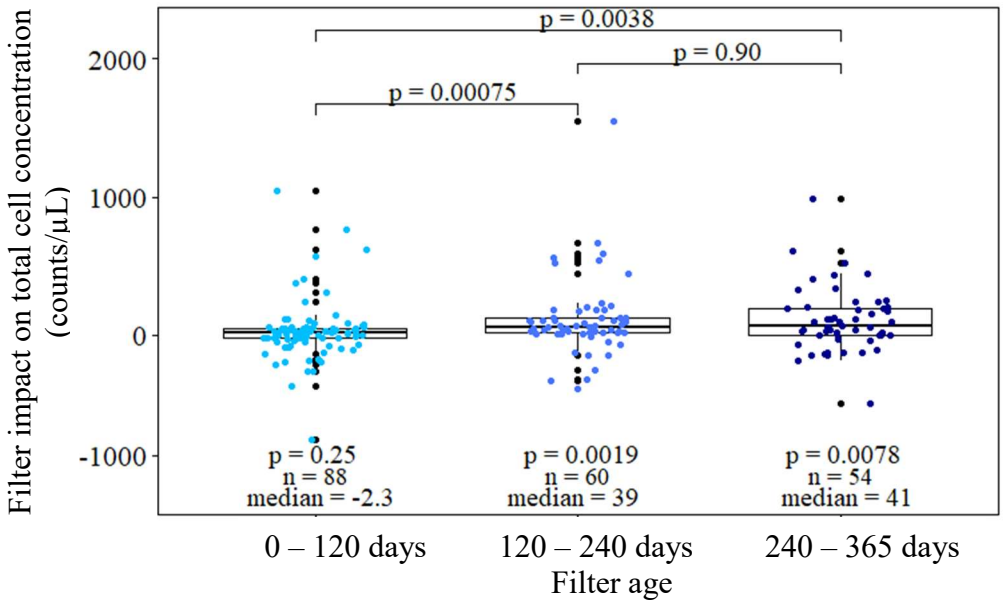


Figure 3-5 Filter impact as defined in Eq. 3.1 on total cell concentration over different age groups

Figure 3-5 shows that increases in filter age could lead to higher concentrations of total cells within the filters (median = -2.3 counts/µL, 39 counts/µL, and 41 counts/µL, respectively) since positive increases in filter impact signify concentrations higher in the effluent than the influent. MWW tests show that the difference between younger filter (filter age less than 120 days) and mid-aged and older filters are significant (p = 0.00075 and 0.0038 respectively). For young filters, MWW tests show that the filter impact on total cell concentration is not significantly different from zero (p = 0.25), indicating that there is no increase or decrease in total cell concentrations. This is also true for the difference between the mid-age and older filters where no significant difference (p = 0.90) is again determined. It is possible that at the early age of the filter, there was no bacteria accumulation and then, as the filter age increased, bacteria accumulated in the filter leading to higher effluent concentrations (Figure 3-5).

Section 3.1 supported the use of flow cytometry for measuring the biomass in our study. The output from flow cytometry shows that carbon-block filters, irrespective of the inclusion of a prefilter, indeed play a significant role in increasing effluent cell concentrations (Figure 3-1 and Figure 3-2).

3.2 Nitrification

Nitrification parameters including monochloramine (measured as total Cl_2), nitrite, and pH are discussed in this section. Those parameters are described in Section 2.2.

3.2.1 Filter Impacts on Nitrification Parameters

As discussed in section 3.1, the installation of filters could lead to biological growth inside of the filters. The possible reason for the increase in effluent cell concentrations could be the reduction of the total chlorine (primarily as monochloramine) by the filters.

Figure 3-6 a shows the monochloramine concentrations within each school over time for unfiltered and filtered water samples. From Figure 3-6 a, the monochloramine concentrations within each school are observed to have higher concentrations in the unfiltered taps than filtered taps. The decrease in monochloramine residual concentrations are possibly due to the decomposition of chloramine accelerated by the carbon-block filter, or the utilization of ammonia by nitrifiers growing in the POU filter. From Figure 3-6 b, the filter effects on the monochloramine residual concentrations indicate that the filters were capable of eliminating nearly all the influent chlorine, even at levels greater than 3 mg/L Cl_2. All the schools showed decreases from the influent concentrations in the filtrate (represented by filter impacts with negative values in Figure 3-6 b). For school No.1, a smaller impact (close to zero) was observed, which is due to the lower influent monochloramine concentrations as shown in Figure 3-6 a. Overall, the effluent monochloramine concentrations are relatively low for all filters.

Carbon-block filters accelerate the decomposition process of chloramine (Eq 1.1) by acting as a catalyst (WQA 2014).

First, carbon (C*) breaks monochloramine down into ammonia and chloride ions while forming a carbon intermediate (CO*).

$$NH_2Cl + H_2O + C^* = NH_3 + Cl^- + H^+ + CO^* \quad (3.1)$$

In the second step, the carbon intermediate breaks more monochloramine down into nitrogen gas and chloride.

$$2NH_2Cl + CO^* = N_2 + 2Cl^- + 2H^+ + H_2O + C^* \quad (3.2)$$

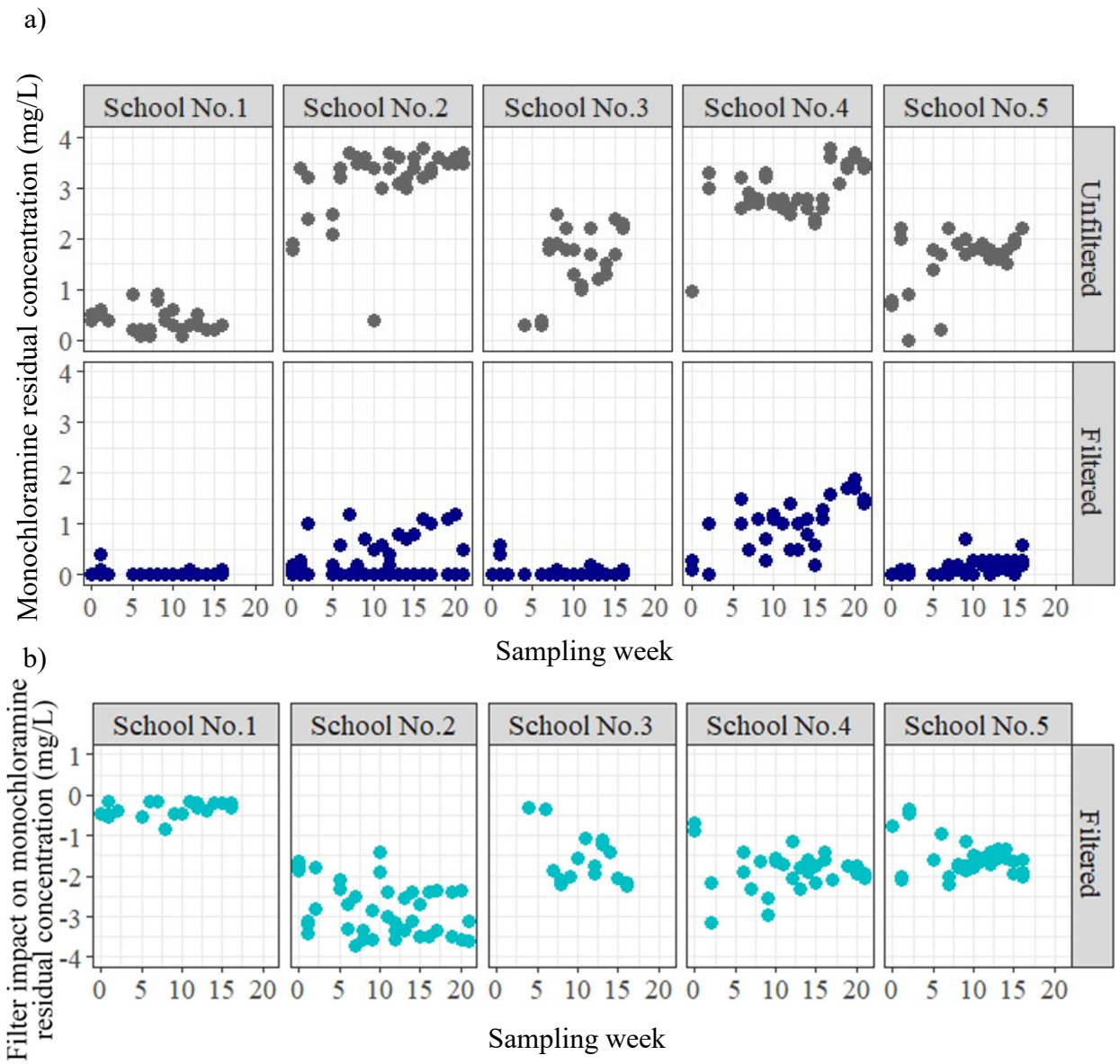


Figure 3-6 a) Monochloramine residual concentration (as mg/L total Cl_2) in schools for filtered and unfiltered taps; b) Filter impact as defined in Eq. 3.1 on monochloramine residual concentration by school over sampling weeks

The delivery of chloramine to the carbon-block filters and the subsequent removal indicates decomposition of monochloramine on the filter or potential consumption by microbial growth since significant doses of ammonia were delivered to the filters in the form of chloramine. Unfortunately, residual free ammonia was not measured as part of this study, however, we can approximate the amount of ammonia delivered to the filters in bound monochloramine form. To

avoid breakpoint chlorination, monochloramine is typically delivered in a Cl_2:NH_3 ratio in the range of 4.5:1 to 5:1 (NH_3 as mg/L N) or 2.5:1 to 4.1:1(NH_3 as mg/L NH_3) (Schippert 2014). The range of NH_3 is summarized in Table 3-3. The delivery of ammonia in form of chloramines as shown in Table 3-3 indicates that there is sufficient level of nitrification potential.

Table 3-3 NH_3 concentration

	Cl_2 (mg/L)	NH_3 (mg/L N)	(mg/L NH_3)
Max	3.8	0.64 - 0.84	0.93 - 1.52
Min	Below 0.05	Below 0.01	Below 0.02
Mean	1.0	0.20 - 0.22	0.24 - 0.40
Median	0.3	0.06 - 0.07	0.07 - 0.12

Chlorine consumption by the filter (through decomposition or microbiological consumption) is one water quality parameter that can serve as an indicator of nitrification.

Nitrification is a two-step process. In the first step, NH_3 is oxidized to NO_2^- by ammonia-oxidizing bacteria (AOBs).

$$NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^- \quad (3.3)$$

In the second step, NO_2^- is oxidized to NO_3^- by nitrite oxidizing bacteria (NOBs).

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^- \quad (3.4)$$

During nitrification, there is a reduction in pH (as signified by the H^+ production in the two-step nitrification process) (Eqs. 3.3 and 3.4). To track overall nitrification effects imparted by the filters, box plots of the total dataset were analyzed (Figure 3-7).

As can be seen from Figure 3-7a, the filter impact on the effluent NO_2^- is slightly higher than zero. By MWW test, the sample median (0.0055 mg/L N) is significantly higher than zero ($p = 3.0\times10^{-6,}$). From Figure 3-7b, the filter impact on monochloramine concentrations (as mg/L total chlorine) was observed to be lower than zero. A total number of 212 data points were plotted with no outliers. A MWW test was performed, and the sample median (-1.7 mg/L Cl_2) is significantly lower than zero ($p = 5.0\times10^{-16}$). In Figure 3-7c, the filter impact on H^+ shows a

slight increase (median = 5.8×10^{-9} mol/L [H^+], MWW test p = 2.2×10^{-6}, n = 216), indicating a slight decrease in pH associated with the filtrate.

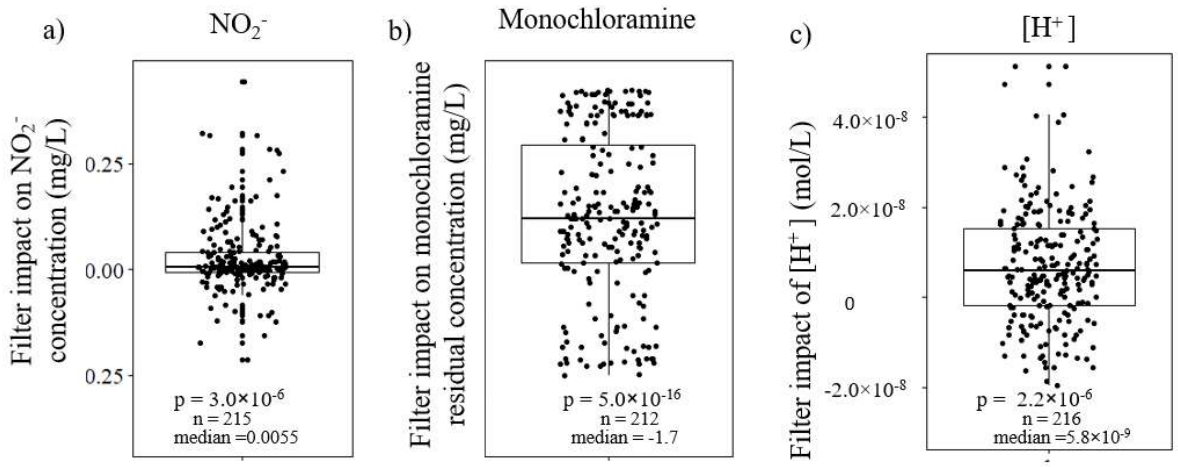


Figure 3-7 Filter impact as defined in Eq. 3.1 on the concentration of a) NO^{2-}, b) monochloramine and c) H^+

As shown in Figure 3-7, the installation of the filter leads to overall increases of NO_2^-, decreases of monochloramine, and decreases in pH. It is possible that there might be nitrifiers growing in the filter, leading to the production of NO_2^-, which further leads to the decrease of pH. The reason for the decrease of monochloramine, however, may be the consumption of the ammonia by the nitrifiers, decomposition of chloramine accelerated by the POU filters, or a combination of both mechanisms.

Investigating the impacts of the POU filters by a total sample composite, as analyzed above, provides only a limited understanding of the nitrification potential. To effectively evaluate the situation, a location-based approach is necessary since the influent water chemistry varied between schools. Figure 3-8 shows a plot of the filter impact on nitrite production by school.

A positive filter impact indicates NO_2^- concentration increases after influent water passed through the filter while a negative NO_2^- concentration value indicates the decrease after influent water passed through the filter. Figure 3-8 showed NO_2^- increased in School No.2 (median filter impact= 0.019 mg/L N), School No.4 (median filter impact= 0.0045 mg/L) and School No.5

(median filter impact= 0.025 mg/L) and NO_2^- decreases in School No.1 (median filter impact= -0.009 mg/L N) and School No.3 (median filter impact= - 0.00063 mg/L N). MWW tests examining the statistical difference of the median value from zero for each location results in only the median value for school No.3 not being statistically different than zero (p = 0.98). For the rest of the schools, there are indeed statistically different from zero (No. 1: p= 0.0024; No. 2: $p = 2.0\times10^{-9}$; No. 4p = 0.0035; No. 5: p = 0.0042).

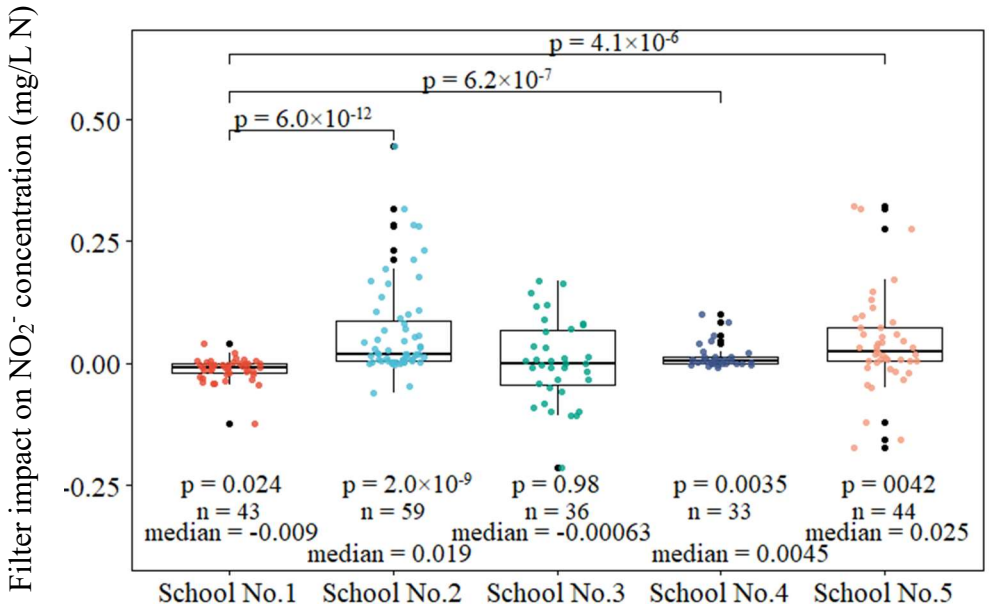


Figure 3-8 Filter impact as defined in Eq. 3.1 on NO_2^- by school

The increase of NO_2^- in school No.2, No.4 and No.5 also indicates there could be possible nitrification. The positive filter impact, or the increases in nitrite concentrations imparted by biological activity within the filters, might be small in some cases (i.e., School No. 4); however, the filter impact is significant and indicates that nitrification is occurring, even if it is small.

Comparing school No.1 to school No.2, school No.4, and school No.5, we observed that the difference of the filter impact on NO_2^- is also significant by the MWW test ($p = 6.0\times10^{-12}$, 2.0×10^{-9}, 4.1×10^{-6} respectively), which means School No.1 might have a different nitrification profile. As shown in Figure 3-9, the NO_2^- concentration varied greatly by school and by sampling week. Overall, for schools No.2, No. 3, and No.5, there are some peaks at sampling weeks 5-10. It is possible that there was a community shift of bacteria along with the NO_2^-

increase. Meanwhile for schools No.1 and No.4, the NO_2^- concentration did not have a lot variance.

For school No.1, a decrease of NO_2^- due to the installation of filter was observed and will be further discussed.

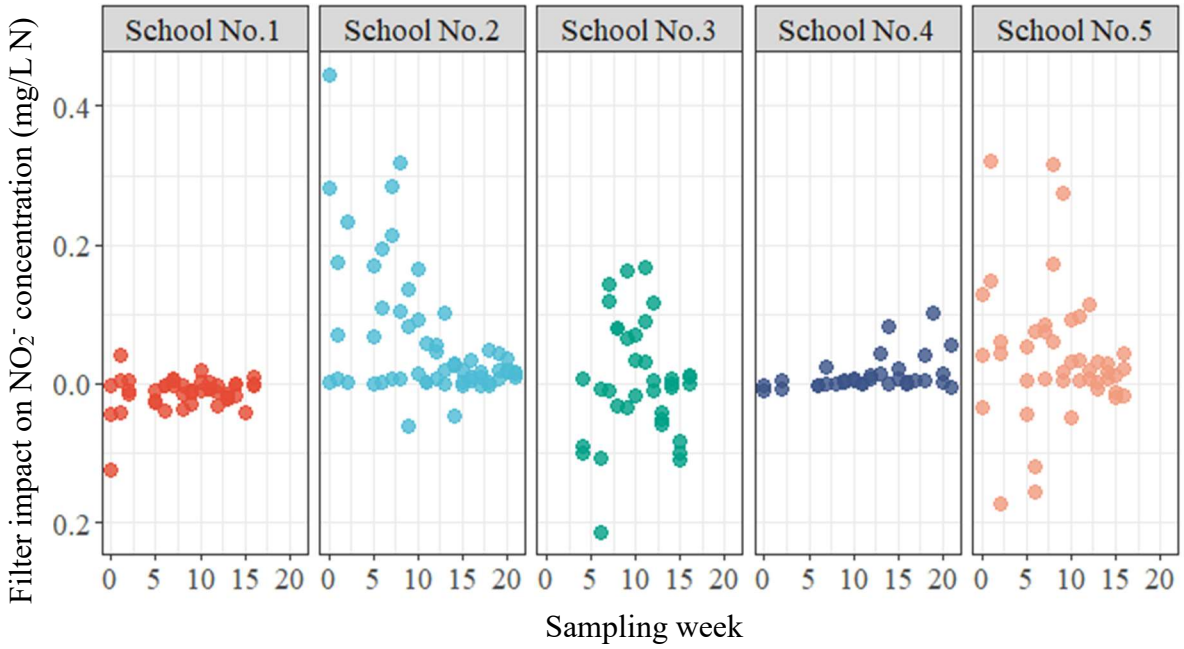


Figure 3-9 Filter impact on NO_2^- by school over 22 weeks of sampling duration

As shown in Figure 3-10, the filter impact on influent chlorine within each school is quite different as expected since influent monochloramine concentrations varied by school location (Figure 3-6). Comparing the filter impact on NO_2^- and influent monochloramine within School No.1 and School No.2, it is clear that School No. 2 had higher influent monochloramine (3.4 mg/L Cl_2) as shown in Table 3-3 and resulted in increases in NO_2^- in the effluent (Figure 3-11).

Intuitively, one would assume higher influent chlorine concentrations would lead to lower biological growth as more disinfectant would create a more difficult environment for biological growth; however, in this case, higher influent total Cl_2 concentrations correlated with the higher production of NO_2^- . Therefore, the higher disinfectant doses are supplying ammonia. It is proposed that as the carbon-block filter accelerated monochloramine decomposition, the ammonia becomes available for consumption by nitrifiers growing within the filter structure (Eq. 1.1). Because monochloramine concentration was lower in school No.1 (0.4 mg/L Cl_2), the

overall growth of nitrifiers would be much slower. Also, previous research has shown that carbon could possibly adsorb nitrite (Gierak and Łazarska 2017, Hanafi and Azeema 2016). For these two reasons, low nitrite production within the filters due to lower influent monochloramine concentration as well as well potential adsorption of the any produced influent nitrite, could possibly lead to the decrease of nitrite in school No.1's filter effluent. The increase in nitrite at the other schools is due to the nitrite production exceeding the adsorption capabilities of the carbon filter.

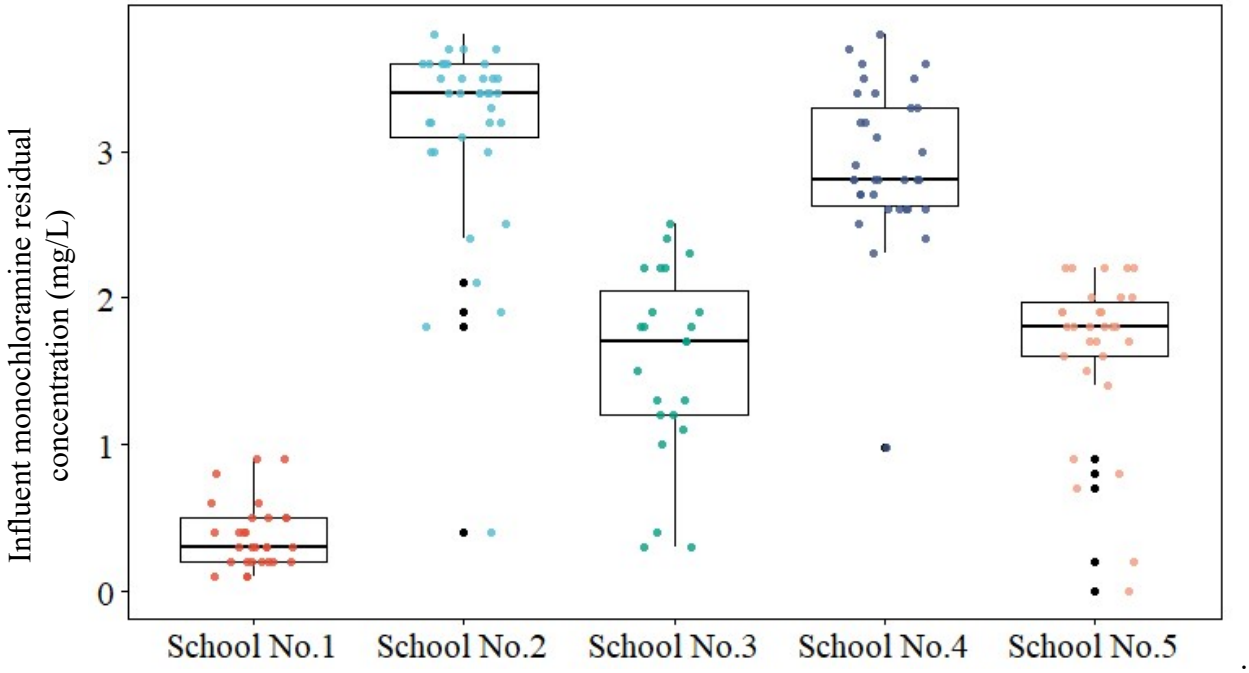


Figure 3-10 Total influent monochloramine (as in Total Cl_2) by school

Table 3-4 Average influent monochloramine by school

School	Average influent monochloramine
School No.1	0.4 mg/L
School No.2	3.4 mg/L
School No.3	1.7 mg/L
School No.4	2.8 mg/L
School No.5	1.8 mg/L

3.2.2 Prefilter Impact on Nitrification

As noted section 2.1 and Table 2-1, approximately half of the filtered sources within this study contained a sediment prefilter preceding the carbon-block filter. The sediment prefilter is reported by the manufacturer to contain a pore size of 1 µm, thereby trapping particles and microorganisms of approximately 1 µm or greater. To understand the impacts of the sediment prefilter on nitrification, total population filter impacts on nitrite are plotted in Figure 3-11.

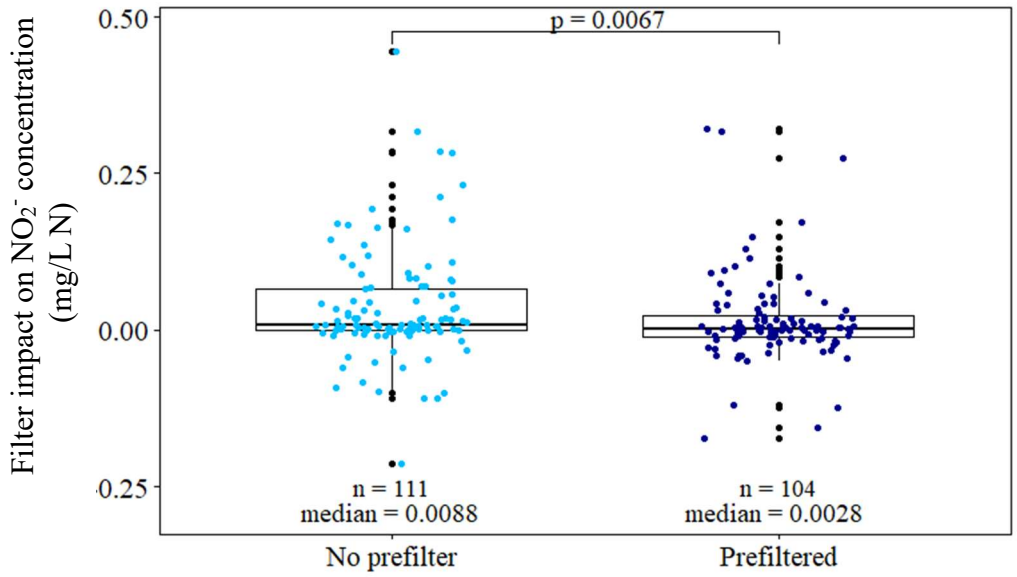


Figure 3-11 Filter impact on NO_2^- with and without using sediment prefilters

As shown in Figure 3-11, the inclusion of a prefilter could potentially decrease NO_2^- production (MWW test, p = 0.0067) indicating that the prefilter might remove some nitrifiers. The reduction of the nitrifying communities further could reduce the filter impact on NO_2^-. In other words, upon the installation of prefilters and the carbon-block filters, NO_2^- does not increase as much as where only carbon-block filters were installed. Examining the time series nitrite filter impact data (Figure 3-9) for two schools with similar average influent monochloramine concentrations, School No.2 (3.4 mg/L Cl_2) only contains taps without prefilters (Scenario 2 in Figure 2-1) and School No.5 (1.8 mg/L Cl_2) only has taps with prefilters (Scenario 3 in Figure 2-1). From these two schools, we can see the installation of the prefilter does not change the peak of NO_2^- produced during the sampling period. It is possible that with sufficient ammonia supplied, the installation of prefilter could have a limited effect on the nitrite production.

3.2.3 Filter Age Impact on Nitrification

Another potential variable influencing water quality is the relative age of the filter units. Filters are currently exchanged every year with some replaced early on an as-needed basis. It was hypothesized that as the filter age increased, the removal capability of filters would decrease, leading to deteriorated water quality as filters became incapable of removing containments in the water. This decrease in water quality includes the impact of nitrification on the effluent bulk water properties.

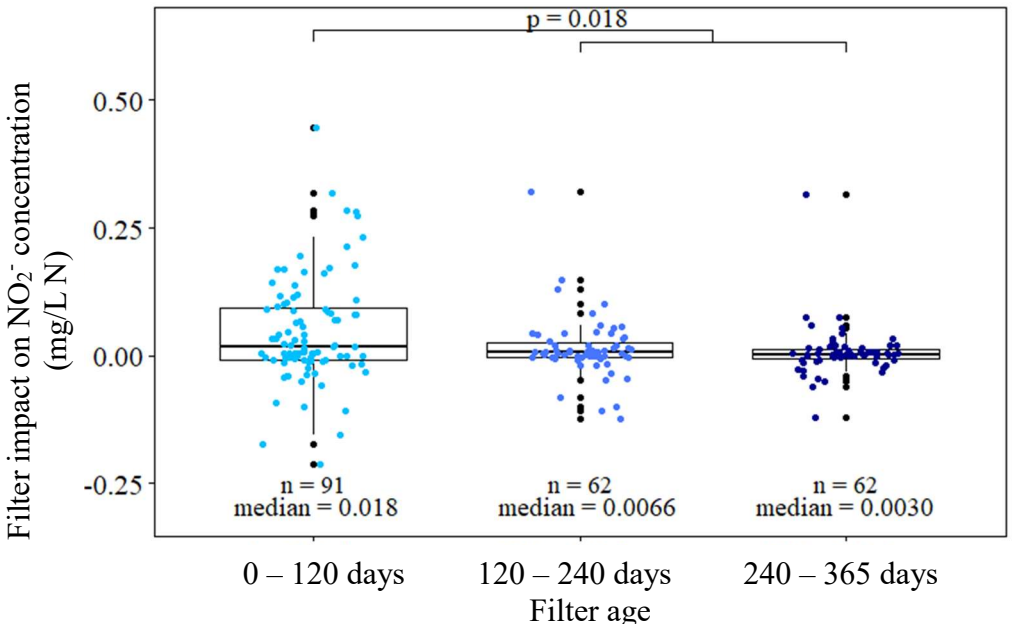


Figure 3-12 Filter impact on NO_2^- concentrations by filter age

Plotting nitrite filter impacts grouped by filter age in Figure 3-12 reveals that filter age has an impact on effluent NO_2^- concentrations contrary to what was hypothesized. MWW test confirms that there are indeed significant differences in nitrite production within the filter columns as the filter ages from a younger filter (where filter age is less than 120 days) and more mature filter ($p = 0.018$), however, this difference is the inverse of what was hypothesized. Younger filters, not older ones, have higher NO_2^- effects on effluent water quality. This indicates that nitrite increases resulting from the filters is not steady throughout a filter's life and, perhaps, decreases as varied, more stable biofilm organisms colonize the filter. To examine this further, filter impacts related

to nitrite formation and the associated week-by-week filter age of the study filters are plotted in Figure 3-13.

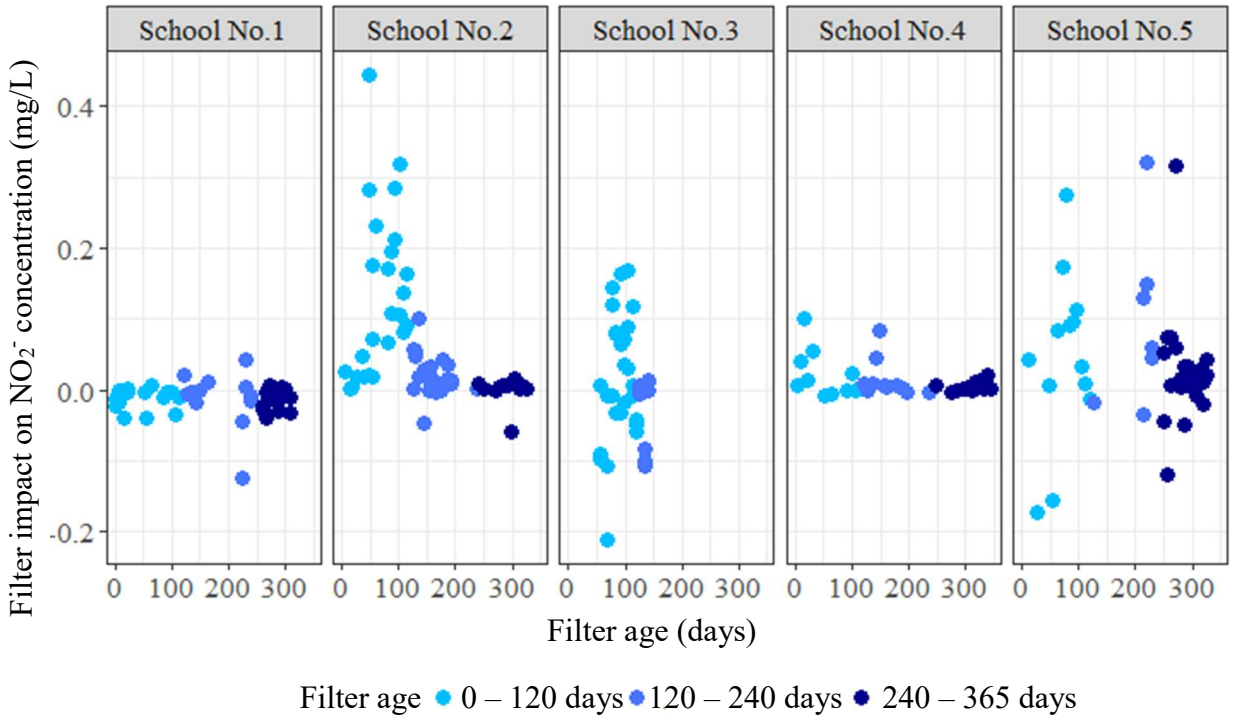


Figure 3-13 Filter impact on NO_2^- by filter age over time

From Figure 3-13 we can see that the filter impact on NO_2^- for each school does have a different profile. For school No.2 especially, there is a clear peak of NO_2^- around day 50 to day 100, which indicates filters with filter age below 120 days might have more influence on the increase of NO_2^-. For School No.3 and No.5, the peak also occurs around day 100. The difference is that for No.5, there is another peak or two, or possibly outliers, around day 240, which is different from our data for the other schools. Figure 3-14 shows the influent total Cl_2 associated with filter age. There is also a peak of Cl_2 around day 100 and day 240 for school No.5, which corresponds to the increase of NO_2^-, indicating that the increase in monochloramine was providing more nutrients.

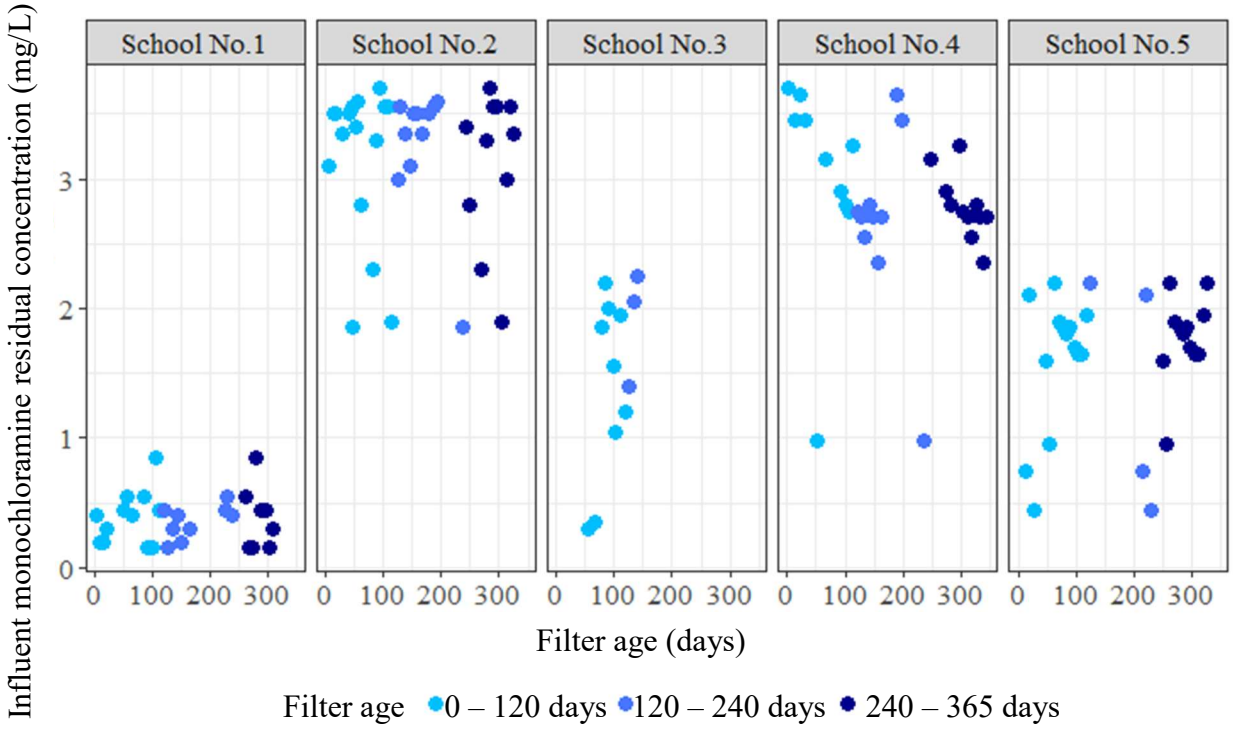


Figure 3-14 Influent total monochloramine concentration (as total Cl_2) by school

For school No.1 and No.4, there are no apparent trends related to the increase of NO_2^- as we discussed in the analysis associated with Figure 3-8. Overall, for schools which do show clear increases of NO_2^-, they all have peaks during the first third of the filter's life. One hypothesis regarding this nitrite increase at the early age of the filter is that, upon installation, filters are rapidly colonized by nitrifiers and later experience a change in or diversification of microbial communities. To explore this concept of changing microbial community populations, fingerprint analyses were conducted using the morphology data collected by the flow cytometer.

3.3 Fingerprint Analysis

As mentioned in section 2.2.4.4, fingerprint analysis was done using PCoA analysis and then data were grouped into different clusters. Only the live cell count data points were used in this analysis for sampling events that were greater than or equal to 10 cells/µL (approximately 500 counts on the flow cytometer) to increase the statistical robustness of the data (n=111).

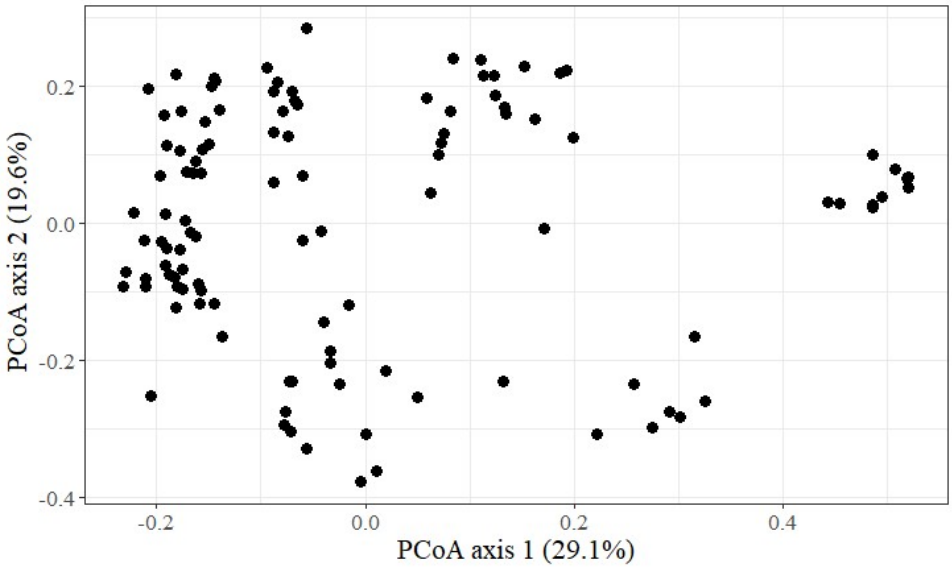


Figure 3-15 Live cell morphology fingerprints distribution in PCoA analysis

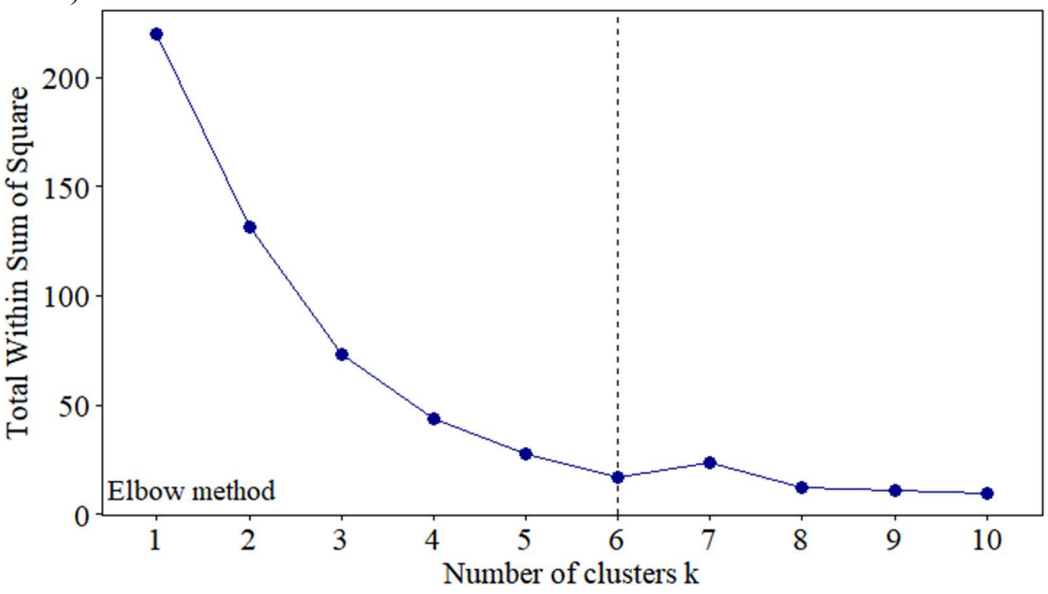
Figure 3-15 is an example of phenotypic fingerprints. The two axes represent the two main components that best explain the variance. As shown in Figure 3-15, the two components explain about 48.7% of the variance (sum of variance explained by two axes). Each data point on the graph represents a dataset for the flow cytometry based on its fingerprint. If two data points are close to each other, this indicates that those two phenotypic profiles have similar biomass based on its morphology and are, therefore, considered to have similar community signatures or "fingerprints". These similar fingerprints are considered to be similar microbiological communities.

3.3.1 Clustering Analysis of Entire Sample

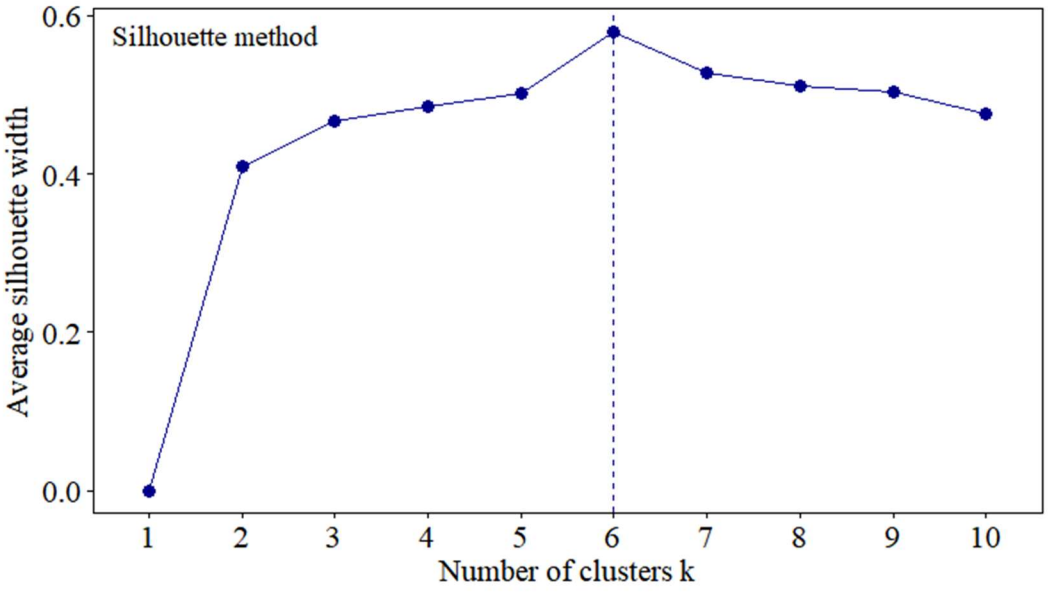
Before the clustering analysis, the first step is to define how many clusters should be used. The Elbow Method assists in defining the point when the increase of cluster numbers no longer creates large decreases in the sum of squared errors. For this set of data, this point is when the number of clusters, k, is equal to six, as shown in Figure 3-16 a. Another method is the Silhouette method where the optimal number of clusters is defined by the point at which the average Silhouette width is at its maximum. For this set of data, this point is also k = 6 as shown in Figure 3-16 b. A final method used for defining the number of clusters is the gap statistic method which also resulted in the best cluster number at k = 6 as increase of gap statistics starts

to slow down (Figure 3-16 c). Therefore, a total number of six clusters was chosen. The clustering analysis was applied to further fingerprinting graphs.

a)



b)



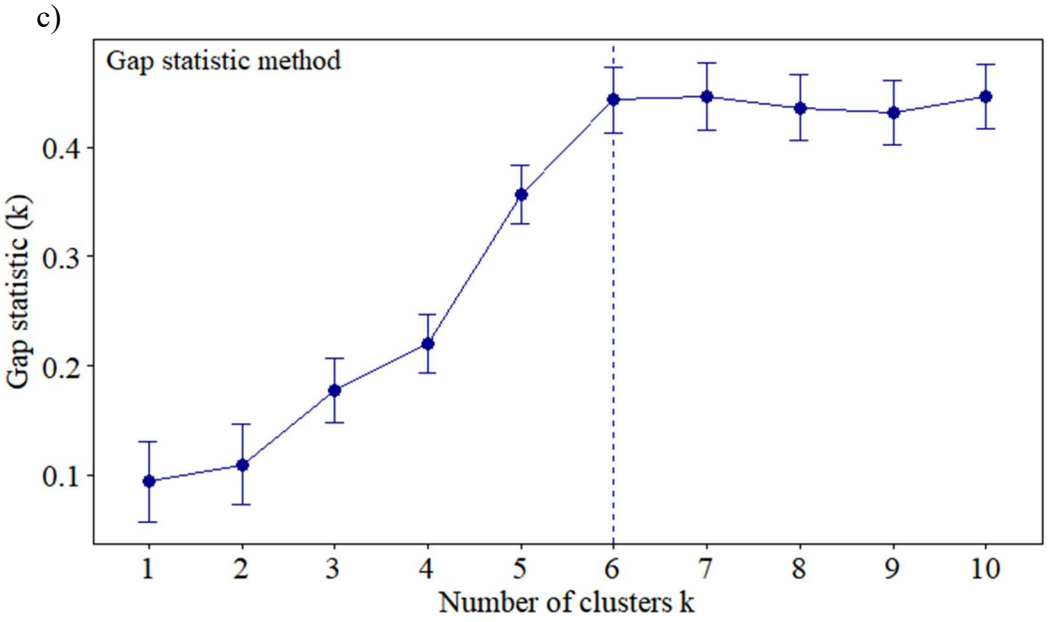


Figure 3-16 Optimal number of clusters determined by the a) Elbow method, b) Silhouette method, and c) Gap statistic method for entire sample

3.3.2 Filter Impact on the Ordination of Phenotypic Fingerprints

To further investigate the impact of carbon block filters on the microbial community, the phenotypic fingerprint of the data was categorized by those taps that contain a carbon-block filter (filtered) and those that were the unfiltered taps within the study (unfiltered). In Figure 3-17, delineations between filtered and unfiltered samples are present. To further explore these groups, k-means clustering was applied to the data using k=6 as determined from the tests in Section 2.2.4.4.

As shown in Figure 3-17, a total number of 6 clusters were identified. For filtered and unfiltered water samples, there is a clear distinction. Two out of six clusters are fully comprised of filtered data while the remaining four are a mix of both. The three clusters on the left top are comprised of a smaller amount of unfiltered data (2 to 4 data points) while the one on the bottom of the figure is mainly unfiltered data points.

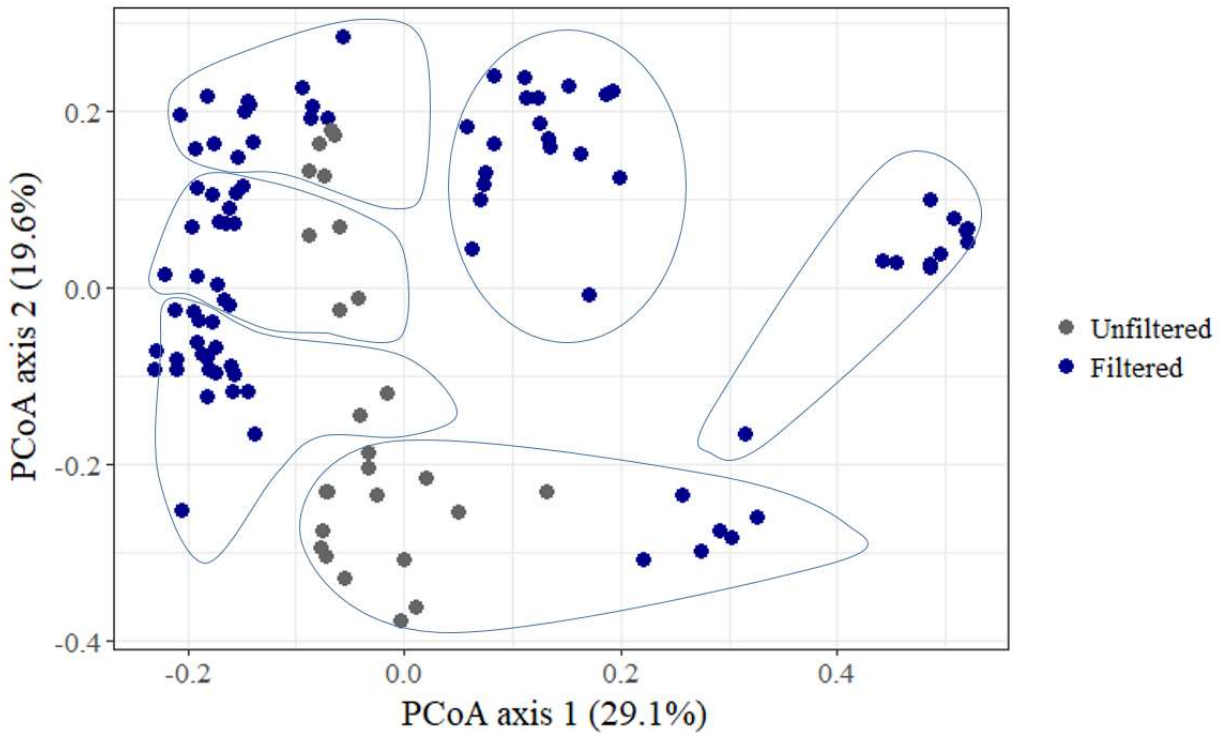


Figure 3-17 Effect of carbon block filter installation on the morphology fingerprints of effluent live cells as determined by PCoA analysis

These data show a distinction between the filtered and unfiltered water sample fingerprints, indicating that the installation of the filter changed the community component of the bacteria. Many of the clusters contain both filtered and unfiltered communities, yet the data points still have distinct gaps between them. It is highly probable that the filtered sources contain many of the same microbial community members as present in the unfiltered sources along with increased concentrations of certain microbes that are growing on the filters. This might not necessarily indicate different microbes being present, but a different ratio. The groups that are completely comprised of the filtered sources indicate different microbes being present as the communities are more distinct and clustered separately.

3.3.3 Fingerprint and Clustering Analysis of Filtered Samples

To better understand the prefilter impact and filter age impact, fingerprint analysis and clustering analysis is conducted again with filtered water samples only.

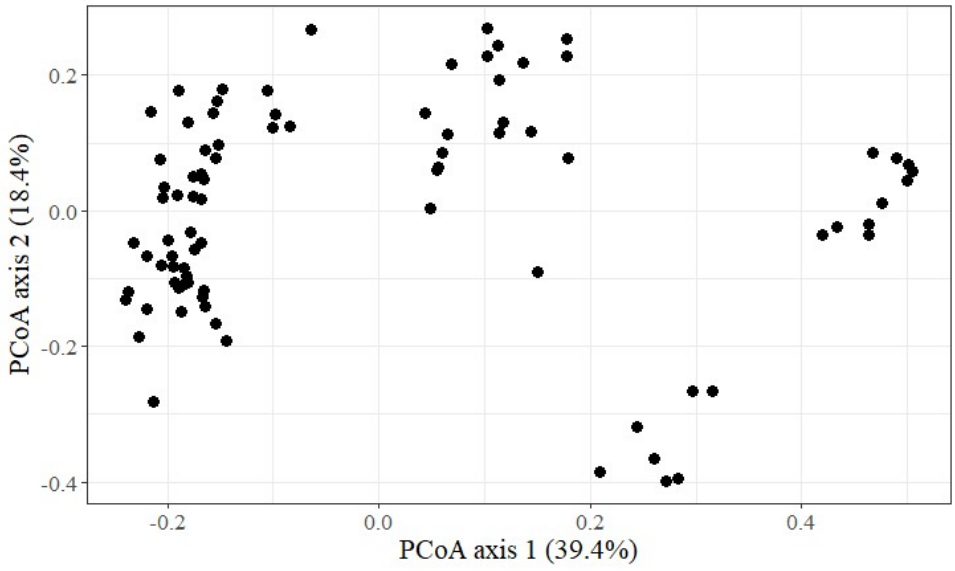
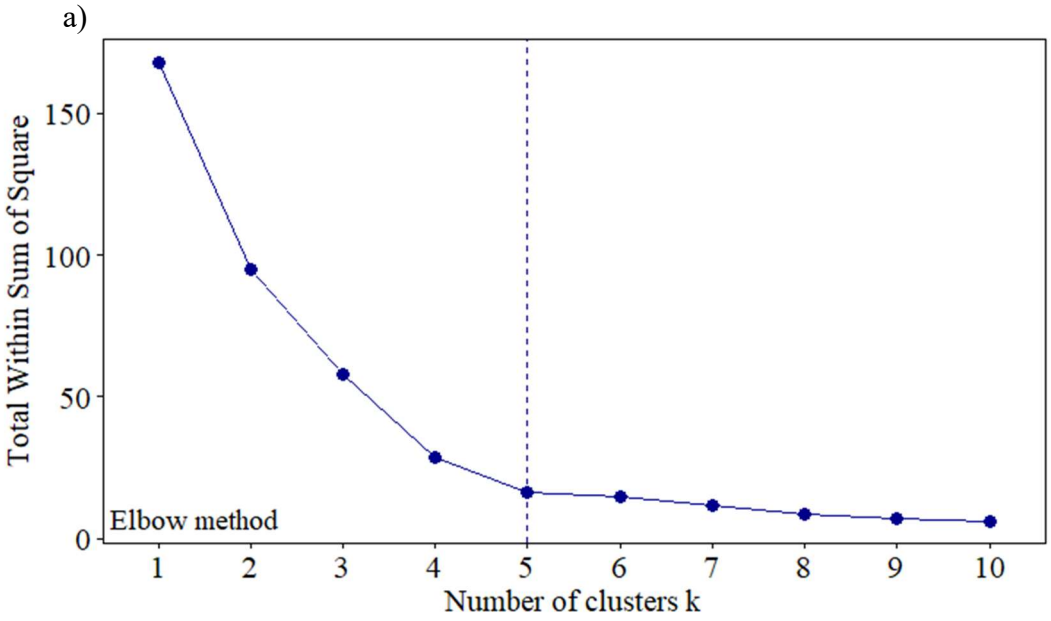


Figure 3-18 Live cell morphology fingerprints distribution for filtered water samples in PCoA analysis

Figure 3-18 is the phenotypic fingerprint for filtered water samples only and, in this plot, the two components explain 57.8% of the variance which is higher compared to the variance explained by two axes in Figure 3-15. This higher variance explanation indicates that the removal of the unfiltered data from the whole dataset could help us better understand the fingerprints for filtered water samples.



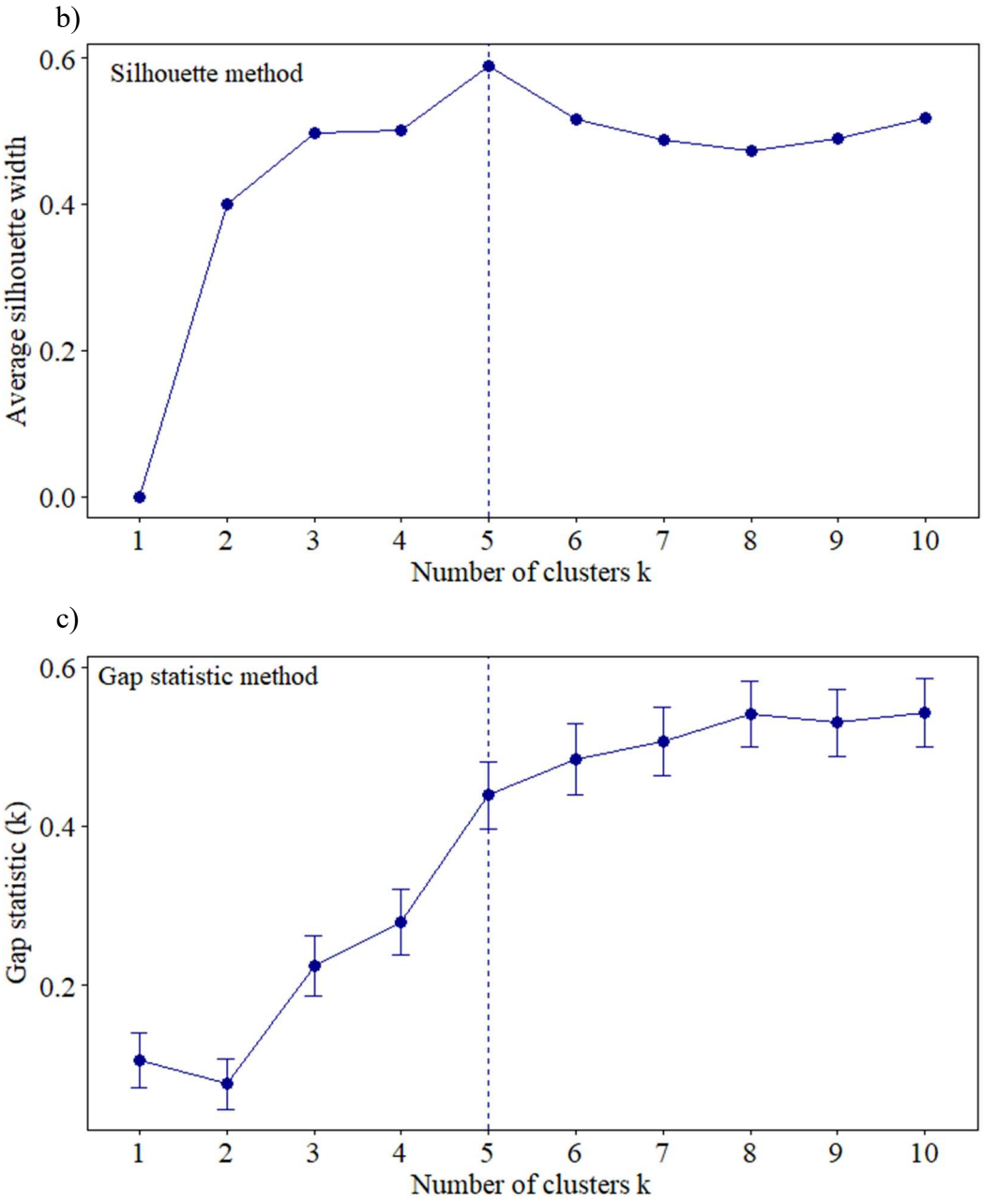


Figure 3-19 Optimal number of clusters determined by the a) Elbow method, b) Silhouette method, and c) Gap statistic method for filtered water samples

To identify the best number of clustering when analyzing only the filtered water samples, elbow method, Silhouette method, and gap statistic method were again used. For this set of data, all three statistical methods resulted in five clusters as the optimal number (Figure 3-19 a-c); thus, k-means clustering was performed on this data using k=5.

3.3.4 Prefilter Impact on the Ordination of Phenotypic Fingerprints

As discussed in sections 3.1.3 and 3.2.2, the sediment prefilter could possibly remove some communities from the influent to the carbon-block filter. A closer look at prefilter effects on bacteria fingerprints was examined using data in Figure 3-20.

As shown in Figure 3-20, there are differences between prefiltered and non-prefiltered water samples. It is important to note that those communities in the two clusters on the top right of Figure 3-20 are solely from the carbon block filters without a prefilter, indicating that distinct communities are present within the carbon-block filters. This also indicates that the prefilter may eliminate some bacteria from the influent (also supported by the lower bacteria counts in prefiltered samples, as shown in

Figure 3-3), reducing the ability of these distinct communities to form.

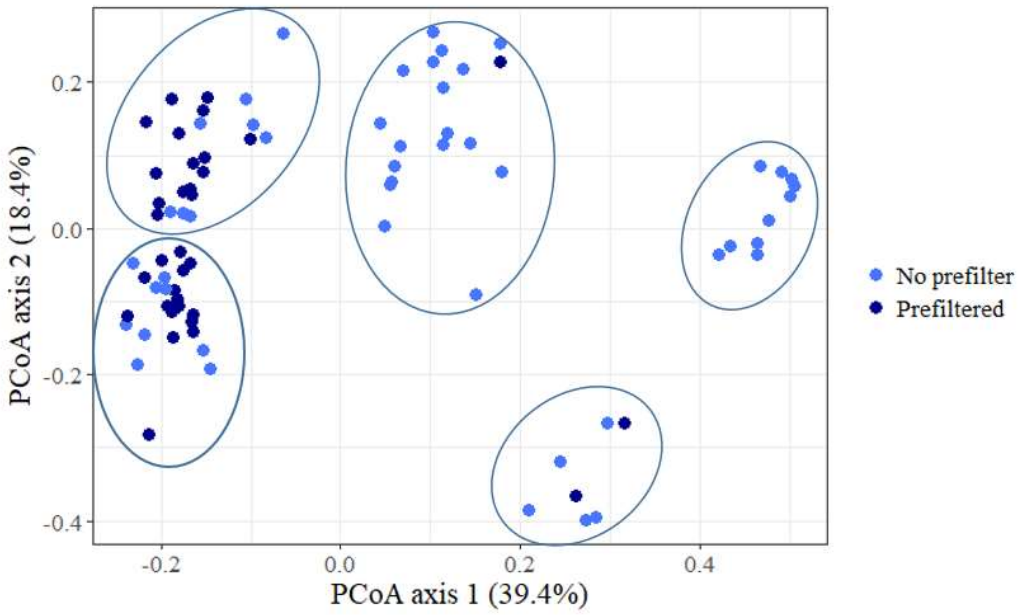


Figure 3-20 Effect of sediment prefilter installation on morphology fingerprints of effluent live cells as determined by PCoA analysis

From the two clusters on the left in Figure 3-20, we also see some communities from the carbon-block filters both with and without prefilters clustering together. It is possible that due to the

location difference and other considerations, there are no communities larger than the sediment prefilters' pore size to be removed or the prefilter became exhausted.

3.3.5 Ordination of Phenotypic Fingerprints by School

As observed in Figure 3-6, the influent water quality within each school can vary significantly, especially with regard to chlorine concentrations. Within each school, the age of the carbon block filters can also vary.

According to Table 2-1, both schools No.1 and No. 5 contain the sediment prefilters while schools No. 2 and No. 3 do not use these prefilters. School No. 4 contains a mixture of sources with and without this extra prefilter. Comparing School No.1 and No.5 to school No.2 and No.3 in Figure 3-21, it is quite clear that fingerprints for school No.1 and No.5 (prefilters installed) are more clustered while the school No.2 and No.3 (no prefilters) are much more scattered. In the absence of a prefilter, the microbial communities become more diverse, suggesting that some microorganisms are removed or reduced by the prefilters.

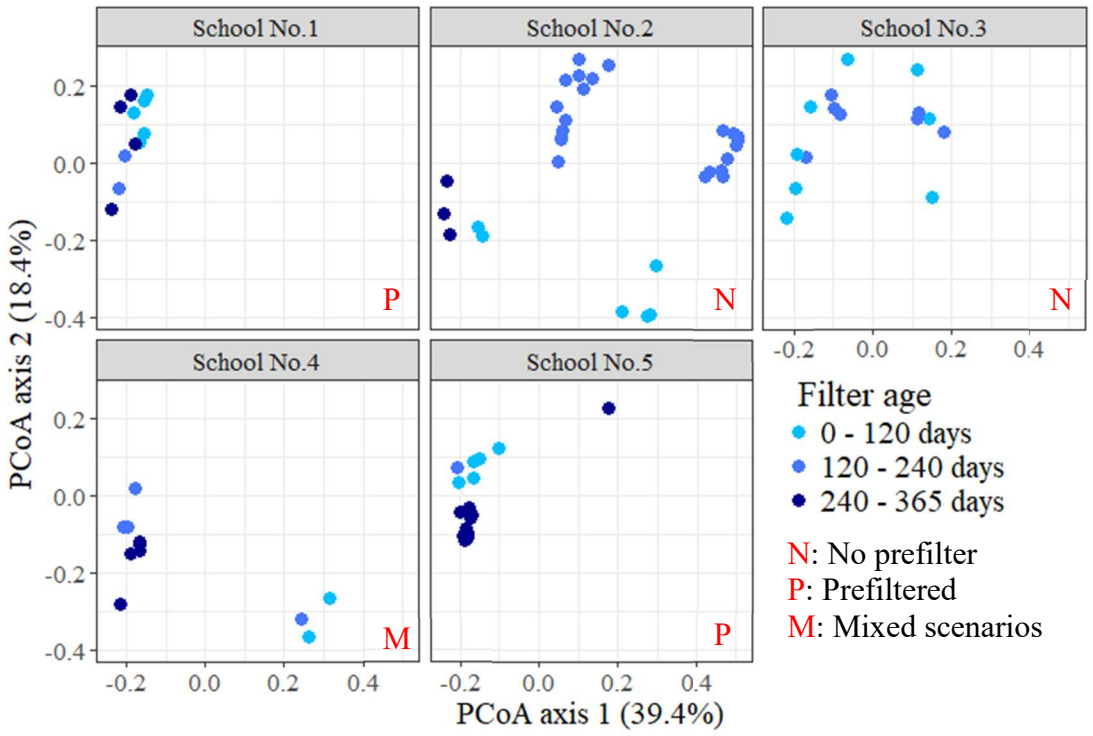


Figure 3-21 Effect of filter age on the morphology fingerprints of effluent live cells in each school as determined by PCoA analysis

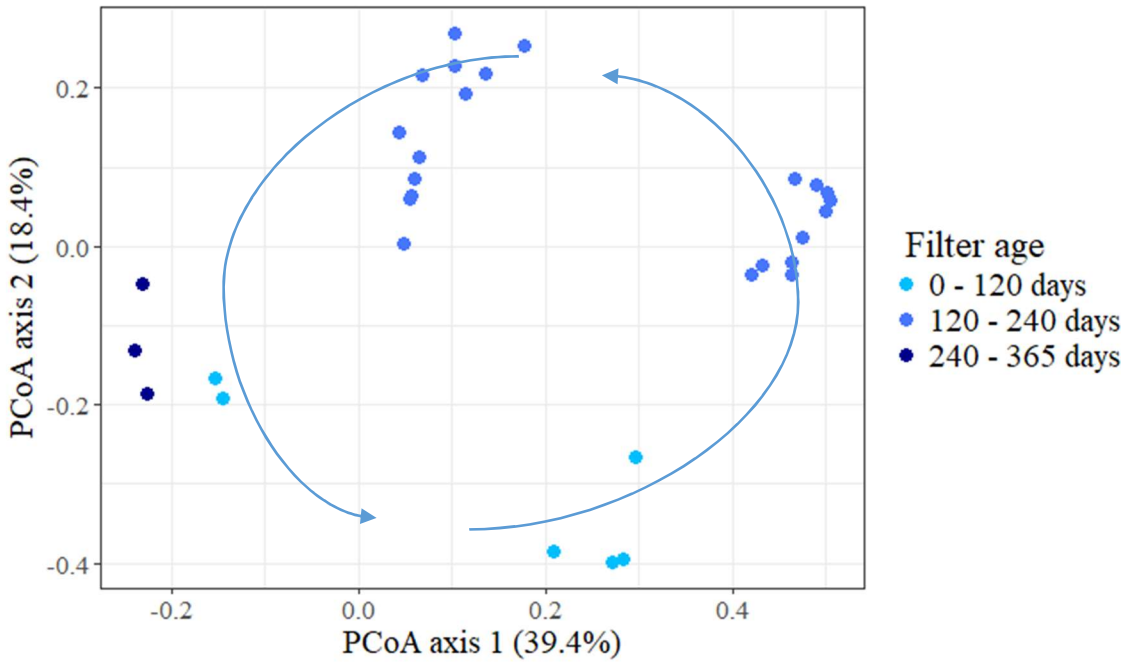


Figure 3-22 Effect of filter age on the morphology fingerprints of effluent live cells in school No. 2 as determined by PCoA analysis

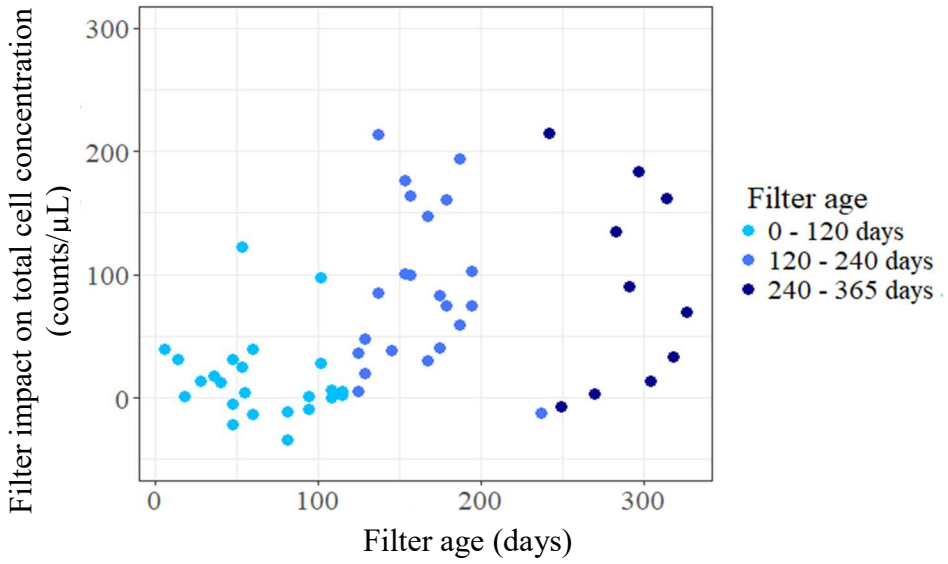


Figure 3-23 Filter impact on effluent total cells concentration in school No.2 within various filter age ranges

For school No.2, it is quite clear that there is a trend of movement related to the filter age (Figure 3-21), which could be related to the maturation of the filters. This also corresponds to the increase of the total cells counts in school No.2 by filter age (Figure 3-22).

Table 3-5 Monochloramine and filter impact on NO_2^- concentration in school No.2

School No.2	
Average influent monochloramine	3.40 mg/L
Filter impact on NO_2^-	
0-120 days	0.11 mg/L
120-240 days	0.014 mg/L
240-365 days	0.0030 mg/L

As the nitrifiers are then replaced by other communities, this shift is captured by the shift in School No.2 (Figure 3-21).Since nitrifiers are being replaced by other communities, the total amount of the bacteria concentration increases. The decrease in filter impact on NO_2^- as shown in Table 3-5 also confirms that there could be a decrease or replacement of nitrifying bacteria. Thus, filter age could possibly influence the fingerprints of the bacteria in the case without prefilter.

Chapter 4. Conclusions

The following are the conclusions from this study:

1. Due to its ease of use, accuracy, cost effectiveness, and labor efficiency, along with high correlations with ATP and HPC, flow cytometry appears to be the most appropriate biological growth indicator for this study. The applicability of flow cytometry in systems using chloramine as a secondary disinfectant was verified and deserves further consideration within utilities and with regulators for microbial compliance.

2. The POU filters fostered the biofilm growth as shown by increases in all the biological growth parameters in the filtrate from carbon block filters. The installation of a prefilter and the maturation of the filter age also influenced the planktonic concentrations in the filtrate.

3. The installation of the carbon-block filters led to nitrification in a chloraminated system where chloramine supplied the ammonia. The increase in nitrite for School No.2, which contained a high total influent chloramine concentration (measured as mg/L total chlorine), supports this conclusion. This school also saw increased concentrations of total cells. It is likely that these nitrifiers were not only supplied with the food source (ammonia) but were provided with an environment free of chlorine given that the carbon-block filters removed the influent chlorine to near 0 mg/L. The increase in effluent nitrite concentration from the installed filters confirmed the growth of nitrifying bacteria in the POU filters.

4. Fingerprint analysis shows there is a clear trend regarding the shift of bacteria morphology, indicating that nitrifiers were replaced with other slow-growing communities in the system. The decline in filter impact on NO_2^- also confirmed the shift of microbial communities.

5. While removing lead from the water, the installation of POU filters is indeed fostering microbial growth and nitrification. Increased nitrite associated with nitrification occurring on the carbon-block filters may lead to water quality deterioration.

Chapter 5. Future Work

This study should provide the basis for future investigations into the mechanics of microbial colonization within carbon-based POU filters. Future work should measure the nitrate and ammonia concentrations along with nitrite to better characterized the observed nitrification processes. Subsequent studies are also suggested to place an emphasis on controlled, laboratory investigations of the filters to reduce the variabilities inherent in the current investigation. Future research should also expand the utility of the flow cytometry fingerprint analysis by including more dyes to target specific groups of bacteria to better understand the movement of fingerprints as well as couple these measurements with specific DNA sequencing analyses.

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