

Conventional and Oxidant-amended Biofiltration to Improve Treatment Resilience in Drinking Water Systems Vulnerable to Wildfire

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Climate-change-exacerbated landscape disturbances can create obstacles for the provision of safe drinking water. The threats posed by wildfires are of particular concern due not only to the potentially extreme nature of their effects, but also because millions of people worldwide depend on water sourced from forested watersheds. Wildfire may lead to major shifts in the concentration and character of dissolved organic carbon (DOC), which may impair drinking water treatment processes to the point of causing service disruptions or outages. Resource-limited small systems can be especially vulnerable to variable water quality, underscoring a need for wildfire-resilient treatment strategies that are cost-effective and less operationally demanding than conventional processes. Biofiltration technologies—especially slow sand filtration—have been proposed as sustainable, low-cost strategies to remove DOC; however, their effectiveness has not been thoroughly investigated during periods of extreme source water quality change such as the episodic deteriorations possible following severe wildfire. Despite the operational simplicity of biofiltration, it is often described as a “black box” process due to our limited knowledge of its biological treatment mechanisms. A better understanding of biofiltration response to wildfire-associated source water disturbances at both the treatment performance and microbial community levels may increase the adaptability of biofiltration processes to climate change effects. Furthermore, biofiltration enhancement is an emerging area of research focused on the customization of biofiltration processes through exerting greater control at the design, process, and influent stream levels. Potential benefits include increased biodegradation of organics and lower incidences of hydraulic challenges. For many of these strategies, there has been little investigation in slow sand filtration. This work aims to advance knowledge in these areas through the execution of two main objectives: 1) assess DOC removal capacity in conventional and peroxide-amended biological sand filters when treating wildfire-ash-impacted water at bench-scale, and 2) evaluate the impacts of wildfire-associated disturbances and peroxide exposure on biofilter bacterial communities.

Challenge testing with severely wildfire-ash-impacted water was conducted on biofilters operated in duplicate under conditions closely resembling slow sand filtration. Filters were subjected to two-, four-, and seven-day disturbance periods, each followed by a five-day return to “baseline” source water quality. One of these pairs, as well as another pair not undergoing ash challenge testing, received intermittent low-dose hydrogen peroxide amendment. Effluent DOC concentrations were elevated, and DOC removal declined during challenge periods; however, DOC characterization analysis showed this was likely the result of a higher proportion of slowly biodegradable humic and aromatic organic matter in the ash-impacted water. No significant evidence of impairment to biodegradation was observed. Biofilter performance was consistent within each disturbance period and recovered within hours of the return to baseline conditions. Over the 30-day experimental phase, the impacts of hydrogen peroxide amendment on organic matter accumulation and DOC removal were not significant to practice.

Amplicon sequencing was carried out on filter media samples collected throughout the experimental phase. Community composition and diversity were compared across experimental conditions and were assessed alongside biofilter performance to identify potential connections. DNA sequencing was also conducted on media samples collected from a similar set of biological sand filters in a previous ash challenge experiment, which used a distinctly different source water. The lack of compositional differences between microbial communities in filters under different experimental conditions supported the assertion that ash-impacted water and peroxide amendment did not severely disrupt the biological communities in the long-term. Comparison of the two filter sets, however,

demonstrated the significant impact of source water character on biological filter community characteristics and dynamics. Collectively, the two components of this work provided process insights into biofilter disturbance response and resilience from multiple perspectives.

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List of Abbreviations

µg	microgram
µm	micrometre
ANOVA	Analysis of variance
ASV	Amplicon sequence variant
ATP	Adenosine triphosphate
BAC	Biological activated carbon
cm	Centimetres
DADA2	Divisive Amplicon Denoising Algorithm 2
DBP	Disinfection byproduct
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DOC	Dissolved organic carbon
dPCR	Digital polymerase chain reaction
EBCT	Empty bed contact time
EPS	Extracellular polymeric substances
g	Grams
GAC	Granular activated carbon
h	Hours
HLR	Hydraulic loading rate
HMW	High molecular weight
ICV	Inter-column variability
L	Litres
LC-OCD	Liquid chromatography – organic carbon detection

LMW	Low molecular weight
m	Metres
mg	Milligrams
min	Minutes
mL	Millilitres
mm	Millimetres
MSA	Multiple sequence alignment
N	Normality
NGS	Next-generation sequencing
nm	Nanometres
NMDS	Non-metric multidimensional scaling
NOB	Nitrite-oxidizing bacteria
NPP	Normal probability plot
NTU	Nephelometric turbidity units
OTU	Operational taxonomic unit
PAH	Polycyclic aromatic hydrocarbon
PCA	Principal component analysis
PCR	Polymerase chain reaction
PERMANOVA	Permutational analysis of variance
PES	Polyethersulfone
QIIME 2	Quantitative Insights Into Microbial Ecology 2
qPCR	Quantitative (or “real-time”) polymerase chain reaction
rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid

SDWS	Small drinking water systems
SM	Standard method
spp.	Species
SSF	Slow sand filter
SUVA	Specific ultraviolet absorbance
TE-NBS	Techno-ecological nature-based solution
TOC	Total organic carbon
US EPA	United States Environmental Protection Agency
UVA ₂₅₄	Ultraviolet absorbance at a 254 nm wavelength
VS	Volatile solids
W	Watt
WAIW	Wildfire-ash-impacted water

Chapter 1

Introduction

1.1 Background

Climate change threatens the continued provision of safe drinking water by making it difficult to anticipate the extent of future changes in water quality. Climate-change-associated impacts to drinking water systems may include more abrupt and extreme shifts in source water characteristics (Rust et al., 2018). Notably, wildfire-associated source water quality disturbances can be among the most extreme of any climate-change-exacerbated landscape disturbance (Dahm et al., 2015). Millions of people worldwide depend on forested catchments as sources of high-quality drinking water. Forests cover 42% of the source watersheds supplying the drinking water for the 100 largest cities in the world (McDonald & Shemie, 2014). In the US and parts of Canada, where studies show that wildfires are increasing in frequency and size (Hanes et al., 2019; Robinne et al., 2016; Robinne et al., 2019; Westerling et al., 2016), forested watersheds provide drinking water for 2/3 or more of the population (National Research Council, 2008; Robinne et al., 2019). Thus, the possible impacts of wildfires on water quality and treatability are of significant concern in many parts of the world.

Wildfires can lead to increased erosion and greater runoff volumes by altering soil properties and reducing vegetation cover (Moody et al., 2009; Moody & Martin, 2001; Scott, 1997; Williams et al., 2019). Wildfires also generate ash, which is often described as the easily mobilized mixture of particulate material remaining on the landscape after the burning of wildland fuels (Bodí et al., 2014). Even at relatively small spatial scales, the composition of wildfire ash can vary considerably depending on factors such as fire severity, fuel type, and combustion completeness (Bodí et al., 2014; Goforth et al., 2005; Pereira et al., 2012). Ash is generally composed of several fractions, including organic and inorganic material as well as exogenous chemicals, in varying proportions (Bodí et al., 2014). Substantial volumes of ash may be exported to watercourses by runoff.

Post-fire runoff events can affect stream temperature (Wagner et al., 2014) and may lead to substantial shifts in several water quality parameters, including turbidity (Chen & Chang, 2023; Emelko et al., 2011; Silins et al., 2009), heavy metals (Abraham et al., 2017; Kelly et al., 2006), and nutrient concentrations (Emelko et al., 2011; Emelko et al., 2016; Oliver et al., 2012; Ranalli & Stevens, 2004). These effects have even been observed at large basin scales (Emmerton et al., 2020) and over longer periods. For example, longer-term releases of bioavailable phosphorus from sediments to the water

column—i.e., internal loading—have been observed and can lead to biostabilization and more variable water quality in impacted streams (Emelko et al., 2016; Stone et al., 2011). They promote primary productivity (Silins et al., 2014) and broader ecosystem responses (Martens et al., 2019). These effects can be generated from a relatively small fraction of the landscape (Stone et al., 2014) and are exacerbated when they converge with those from anthropogenic landscape disturbances (Watt et al., 2021).

In addition to affecting stream ecosystems (Martens et al., 2019; Silins et al., 2014; Spencer et al., 2003), post-fire water quality fluctuations may impact drinking water treatability. For example, several studies have demonstrated that periodic changes in both the concentration and character of dissolved organic carbon (DOC) may occur for several years following a wildfire (Emelko et al., 2011; Hohner et al., 2019; Rhoades et al., 2019). These changes can present ongoing operational challenges for drinking water utilities, including increased infrastructure and operating costs, service disruptions, or outages (Emelko et al., 2011; Price et al., 2017). Though not considered a direct risk to human health, excess DOC can reduce the efficiency of treatment processes (Health Canada, 2020). Natural organic matter can also react with chlorine to form disinfection byproducts (DBPs), several of which are suspected carcinogens (Li et al., 2022; Mitch et al., 2023; Richardson et al., 2007). DOC that reaches the distribution system promotes biofilm growth that may contribute to taste and odour problems or even shield pathogens from disinfectant residuals (Health Canada, 2020). For these reasons, it is important for treatment systems to be equipped to manage fluctuations in DOC.

Resilient treatment strategies and technologies can help increase utility preparedness; however, responding to post-fire spikes in DOC may require expensive adjustments like increased chemical doses, expansions to sludge management capacity, and even new or modified treatment infrastructure (Emelko et al., 2011); shouldering the associated costs could place a disproportionate burden on resource-limited small systems. Thus, DOC-associated post-fire treatment concerns emphasize the need for water supply and treatment resilience. The promise of techno-ecological nature-based solutions such as slow sand filtration and biofiltration have been identified to mitigate these threats at the source and/or in treatment plants (Blackburn et al., 2023; Blackburn et al., 2021; Emelko & Sham, 2014).

Small drinking water systems (SDWSs) in Canada face numerous financial, operational, and technical obstacles to sustainable operation (Maras et al., 2004) that may increase their vulnerability to failure events (Edwards et al., 2012). A small system may be designated as such based on the number

of connections, the population served, or the nature (i.e., public or private) of ownership and operation (Moffat & Struck, 2011). Health Canada (2021) generally defines small systems as those serving less than 5000 people. A study of residential SDWSs in British Columbia found that serviced population size, source water type, treatment processes, and governance structure were among the key determinants of drinking water advisory frequency and duration (Edwards et al., 2012). Additionally, multiple North American surveys have found that more than half of confirmed and suspected waterborne disease outbreaks in the late 20th and early 21st centuries occurred in systems defined by the respective authors as SDWSs (Pons et al., 2015; Schuster et al., 2005; Wilson et al., 2009). These findings highlight the need to remove or reduce the obstacles facing SDWSs in order to safeguard public health. Without economies of scale and access to large pools of trained operators, employing state-of-the-art processes used in larger treatment facilities may be financially and operationally infeasible for small systems (Boisvert & Schmit, 1997; Logsdon et al., 1990; Male et al., 1991). Furthermore, climate change is among several factors that are rapidly driving up the costs of drinking water treatment and distribution in North America, and a portion or all of this rise in costs is passed down to households (Baird, 2010; Federation of Canadian Municipalities and National Research Council, 2006). With many North Americans already facing water affordability concerns (around 10% of U.S. households currently spend at least 4.5% of their annual income on water and sewer services; Cardoso & Wichman, 2023), small systems have a critical need for effective treatment approaches that are low-cost, low-maintenance, and operationally simple. However, technologies that meet these criteria can lack the optimal treatment efficiency and high operational control that more sophisticated treatment processes offer; this trade-off may compromise the ability of SDWSs to manage deteriorated or variable water quality associated with wildfire and other climate-change-exacerbated landscape disturbances (Blackburn et al., 2021; Emelko et al., 2011).

Biofiltration has been proposed as a cost-effective and relatively “green” strategy to remove DOC from drinking water (Blackburn et al., 2021, Brown et al., 2020; Lauderdale et al., 2011). Slow sand filtration, a type of biofiltration and one of the earliest drinking water treatment technologies, has received renewed interest for use in small systems (Agrawal et al., 2021; Graham & Collins, 2014; Maiyo et al., 2023). Slow sand filters (SSFs) are relatively straightforward to operate; they are often gravity-driven, require little to no energy or chemical inputs, and no pretreatment is needed if the source water meets certain quality criteria (Huisman & Wood, 1974; Logsdon et al., 2002). As with other biofiltration processes, the ability of SSFs to achieve partial (but significant) degradation of low

molecular weight (LMW) DOC has been well documented for a variety of source waters (Collins et al., 1992; Demir et al., 2018; Grace et al., 2016). These consistent findings indicate that SSFs may offer some treatment resilience for small systems affected by wildfire. However, wildfires may alter the character of DOC on the landscape (Knicker et al., 2005), potentially increasing the export of slowly biodegradable compounds to watercourses (Hohner et al., 2019; Peticrew et al., 2006). These compounds present a greater challenge for biological treatment (Collins et al., 1992). Thus, adequate DOC removal after wildfire cannot be assumed based on biofilter performance at baseline source water conditions. Assessing the utility of biofiltration technologies for increased drinking water treatment resilience will require evaluations of their performance during wildfire-associated source water disturbances. To date, investigations of biofiltration performance in treating wildfire ash-impacted water are scant (Blackburn et al., 2023).

Operational control has been highlighted as an important consideration for climate change adaptation strategies; treatment processes designed to provide higher levels of control (e.g., mechanical controls like flow rate adjustment) may allow better responses to variable water quality (Blackburn et al., 2021). Traditionally, biofilters have been operated in a passive manner; process design parameters are limited, and the removal of dissolved compounds relies predominantly on naturally occurring, biologically mediated processes (Blackburn et al., 2021; Brown et al., 2015). While this approach reduces operational and energy demands, it provides few to no opportunities for operator intervention. An emerging area of research seeks to actively “engineer” aspects of the biofiltration process; proposed strategies include oxidant amendment to enhance hydraulic performance and extend filter run time. Such techniques present opportunities not only to improve performance and operator control in biofiltration, but also to reduce treatment costs. For SSFs, filter “cleaning” has been identified as the most costly and labour-intensive phase of typical filter operations in most cases (Letterman & Cullen, 1985). Filter cleaning typically involves taking filters out of service for at least 24 hours to manually remove the accumulated layer of deposited matter and biomass at the bed surface (called the *schmutzdecke*) and allow for re-acclimation (Visscher et al., 1990). Strategies to reduce the frequency and duration of cleaning periods may help to reduce operational costs (Graham & Collins, 2014). Low-dose hydrogen peroxide amendment may offer an environmentally friendly method (Pesqueira et al., 2022) to reduce organic matter accumulation in-situ (Christensen et al., 1990). To date, studies on peroxide amendment have reported variable results on its ability to improve hydraulic performance (Friedman et al., 2020; Lauderdale et al., 2012; Lei et al., 2014; McKie et al., 2015; Noh et al., 2020;

Nyffenegger et al., 2013) and research has yet to be extended from rapid biofilters to slow sand filters. Furthermore, as maintaining filter function to achieve treatment goals is of the utmost importance to safeguarding public health, further investigation is needed to identify any performance-compromising effects associated with peroxide amendment. Several studies have monitored DOC removal performance in comparison to non-peroxide-amended biofilters, but few have examined changes at the microbial community level in detail.

Although the DOC removal capabilities of biofiltration have been validated in research and in practice (Collins et al., 1992; Emelko et al., 2006; Hozalski et al., 1995; Moona et al., 2021), the biological mechanisms responsible for DOC degradation have not been well described. Biodegradation is known to be imperative in the removal of dissolved organic compounds, but the connections between microbial community characteristics and DOC removal performance are not fully understood; this knowledge gap limits the potential for process control, adaptability, and enhancement (Haig et al., 2015). Fortunately, the rapidly falling cost of Next-Generation Sequencing (NGS) technologies is increasing the accessibility of metabarcoding and metagenomic research (the study of organisms in non-cultured samples through the analysis of specific genes or the analysis of genomes, respectively) (Wetterstrand, 2021). Many recent biofiltration studies have employed 16S rRNA gene sequencing to provide snapshots of bacterial community compositions in source water, filter beds, and filtrate (Chen et al., 2021; Haig et al., 2015; Pinto et al., 2012; Zhang et al., 2018). Areas of opportunity for further application of molecular biology tools include the evaluation of biofilter responses to source water disturbances and chemical enhancement strategies at the microbial level.

Providing safe drinking water in a changing climate will necessitate that drinking water systems be resilient to climate-change-exacerbated landscape disturbances like wildfire. SDWSs face additional financial and technical barriers that make them especially vulnerable to the effects of climate change. Slow sand filtration processes are well-established technologies that can augment treatment plant DOC removal capacity without exceeding the resources of smaller systems. However, previous studies have not specifically focused on the aspects of slow sand filtration and other biofiltration processes that may determine their effectiveness in post-fire conditions, including performance when challenged with fluctuations in DOC concentration and character, and microbial-level responses to wildfire-ash-impacted water. Such investigations are crucial to evaluating the potential for biofiltration processes to provide sustainable treatment strategies in a changing climate.

1.2 Research Objectives

The overall goal of this study was to provide scientific insights on the potential for conventional and oxidant-amended biofiltration processes to increase the resilience of drinking water treatment systems in wildfire-prone regions. This assessment involved examining biofilter microbial community composition and DOC removal performance when treating wildfire-ash-impacted source water. This work did not aim to evaluate biofilter performance from the perspective of meeting local regulatory requirements for treated drinking water, nor did it attempt to closely emulate operational aspects of full-scale biofiltration processes. The use of certain operating conditions that are typical of slow sand filtration make the findings especially relevant to SDWSs; however, as a proof-of-concept study, this work provides process insights that apply to biofiltration processes in general, and thus will have value for utilities of all sizes.

Specific objectives that supported the overall research goal are listed below:

- 1) Evaluate the capacity of conventional and oxidant-amended biofilters to maintain, or recover to, pre-disturbance levels of DOC removal when challenged with severely wildfire-ash-impacted water (WAIW),
- 2) Assess the viability of intermittent hydrogen peroxide amendment as a strategy to control organic matter accumulation at the surface of drinking water biofilters without compromising treatment performance,
- 3) Characterize microbial community composition in drinking water biofilters through the use of amplicon sequencing and investigate potential connections to DOC removal performance, and
- 4) Explore the opportunities, limitations, and challenges associated with applying Next-Generation Sequencing (NGS) techniques to advance our understanding of biofiltration processes.

1.3 Research Approach

A six-month comparative study of eight bench-scale biofilters (operated in duplicate pairs under conditions closely resembling slow sand filtration) was conducted to accomplish the research objectives above. The purpose of the experiment was to evaluate the performance effects of a source water challenge condition and an enhancement strategy of interest relative to control conditions. Filter replication was incorporated to demonstrate the degree of natural performance variability possible between biofilters operated under the same conditions. Few biofiltration studies have compared replicate filters, but this practice was found to be valuable in distinguishing meaningful performance trends from naturally occurring variability.

To understand the impacts of wildfire challenge conditions, two biofilter pairs were subjected to variable-length periods during which they received severely ash-impacted source water. Challenge periods were separated by recovery periods at baseline conditions. This approach simulated the extreme fluctuations in source water character that may occur following a high-intensity wildfire. To investigate hydrogen peroxide as an enhancement strategy, four filters received intermittent hydrogen peroxide amendment concurrently with ash challenge tests. Key indicators of biofilter treatment performance (DOC removal and effluent turbidity) were monitored regularly and compared to control conditions. Filters were cleaned prior to the start of each challenge period to assess filter response and recovery at peak vulnerability.

Amplicon sequencing was used to characterize bacterial communities present at the surface of biofilter beds. Samples were collected from the biofilters used in the experiment described herein, as well as a set of biofilters used in a similar experiment. Taxonomic data were examined to identify community composition differences possibly driven by experimental conditions or source water characteristics.

1.4 Thesis Organization

This thesis comprises four chapters. Chapters 2 and 3 are written as separate (currently unpublished) manuscripts for submission to refereed journals. Chapter 2 provides a concise literature review focused on the impacts of wildfire on source water quality and treatability, challenges facing small drinking water systems, and areas of opportunity in biofiltration research. This chapter also discusses the design,

methods, and results of a bench-scale biofiltration study. Chapter 3 discusses the motivations for the application of metabarcoding analysis and metagenomics in biofiltration research and the opportunities it presents for improving climate change adaptability and process enhancement. Insights gained from bacterial community analysis conducted on two sets of biofilters from similar experiments are also presented in this chapter. Potential connections between microbial community characteristics and biofilter performance are investigated. Chapter 4 concludes with recommendations for further biofiltration research, as well as for utilities employing biofiltration in practice. Considerations when applying NGS methods in biofiltration research and interpreting the associated gene sequence data are also highlighted.

Chapter 2

Conventional and Oxidant-Amended Biofiltration as Strategies for Improved Wildfire-Resilience in Drinking Water Treatment

2.1 Summary

Wildfires can lead to degraded or more variable water quality. Fluctuations in the concentration and character of dissolved organic carbon in drinking water sources after wildfire can present challenges for drinking water treatment. Resource-limited drinking water systems, such as those serving rural and remote areas, may face the greatest obstacles in adapting to the effects of climate change. Conventional and enhanced biological filtration processes may offer strategies to increase organic carbon removal capacity while also being accessible to small systems; however, biofilter performance testing during wildfire-associated source water disturbances has been limited. Here, the response of conventional and peroxide-amended biological sand filters was assessed in duplicate throughout a series of challenge tests, during which the filters received wildfire-ash-impacted water for periods varying duration. Elevated effluent dissolved organic carbon levels during challenge tests were attributed to the higher proportions of slowly biodegradable organic compounds present in ash-impacted water and did not necessarily indicate impairment of biological treatment mechanisms. This finding underscores the value of carbon characterization in the performance assessment of biological treatment processes. Filters recovered to baseline performance levels by the first sampling event post-disturbance, suggesting biofiltration may offer treatment resilience in the form of autonomous recovery. Column replication demonstrated the extent of natural performance variation possible between filters under identical conditions, validating the significance of observed performance changes in response to challenge conditions. Under the conditions tested, the impacts of intermittent peroxide amendment on biomass accumulation in the filter beds were found to be insignificant to practice. While this finding suggests that the selected amendment approach would not have offered the anticipated hydraulic benefits and extended run times, no evidence of impairment to organic carbon biodegradation was observed.

2.2 Introduction

Numerous studies have found that wildfires are increasing in frequency and size in North America (Hanes et al., 2019; Robinne et al., 2016; Robinne et al., 2019; Westerling et al., 2016). These extreme events present a growing challenge for sustainable drinking water provision. The impacts that wildfires can have on hydrological processes and water quality are among the most severe of any climate-change-exacerbated landscape disturbance. Vegetation losses may reduce evaporation (Scott, 1997) and allow more precipitation to reach the landscape (Williams et al., 2019), resulting in higher runoff volumes. Wildfire may leave a hydrophobic layer near the soil surface or expose soil that is naturally more water-repellent, reducing infiltration and increasing overland flow (DeBano & Krammes, 1966; Doerr et al., 2009). These changes can result in higher overall stream flows and peak flows (Scott, 1997). Reduced vegetation and thermal alteration make material on the landscape, including ash and soil, more susceptible to erosion (Moody & Martin, 2001; Silins et al., 2009). Ash is the mixture of particulate pyrogenic material remaining on the landscape following a wildfire (Bodí et al., 2014). Though the composition of wildfire ash depends on several factors (e.g., fire severity, fuel type, and combustion completeness) and can vary temporally and spatially on the landscape (Bodí et al., 2014; Goforth et al., 2005; Pereira et al., 2012), some studies have aimed to provide a general description of ash properties. Wildfire ash is typically composed of several fractions, including organic matter (in varying states of combustion), inorganic material, material originating from soil, and exogenous chemicals (e.g., mercury) (Bodí et al., 2014). The quantity of organic matter in ash has been found to correlate to combustion completeness; material that has been combusted more completely will generally form ash that has less organic material. Ash colour can be an indicator of combustion completeness, with darker ash often containing incompletely combusted organics and lighter-coloured ash consisting primarily of inorganic material (Goforth et al., 2005; White et al., 1973).

Post-fire exports of ash, soil, and other material from the landscape to watercourses can lead to major shifts in water quality. Substantial increases in turbidity, heavy metals, nutrients, and dissolved organic carbon (DOC) have been observed in streams following wildfire (Emelko et al., 2011; Kelly et al., 2006; Oliver et al., 2012; Ranalli & Stevens, 2004). The character of aquatic DOC may also be altered (Emmert et al., 2020; Hohner et al., 2019). The magnitude of these water quality changes can be highly variable, even in similar catchments; some studies have detected little to no change in key water quality parameters post-fire, while others have reported that turbidity, suspended solids, and nutrient concentrations increased by several times, or even by orders of magnitude, during and

(sometimes) after major runoff events (Dahm et al., 2015; Lane et al., 2008; Silins et al., 2014). These effects are magnified when they converge with those from anthropogenic landscape disturbances (Watt et al., 2021). Altered water quality can lead to changes in stream ecosystems such as algal proliferation or altered food webs (Silins et al., 2014; Spencer et al., 2003). Shifts in water quality may continue for several years post-fire (Emelko et al., 2016), and it has been shown that these effects can propagate downstream and consequently pose a threat to drinking water treatment plants (Dahm et al., 2015; Stone et al., 2011). In particular, fluctuations in DOC concentration and character can present challenges for utilities. While not a direct threat to human health, source water DOC can heavily influence unit process design and operation because its removal is critical for effective downstream treatment (Crittenden et al., 2012). Residual DOC exerts a demand for coagulants and oxidants, meaning elevated DOC levels may necessitate the use of higher chemical doses to achieve adequate removal of solids and contaminants (Crittenden et al., 2012). Furthermore, chlorine (a common drinking water disinfectant) reacts with DOC to form various disinfection byproducts (DBPs) several of which are potential carcinogens (Li et al., 2022; Mitch et al., 2023; Richardson et al., 2007). Residual DOC also increases the potential for microbiological regrowth in the distribution system (Rittman et al., 1989). With the risk of wildfire-associated source water disturbances increasing, utilities in fire-prone regions should consider strategies to ensure adequate DOC removal capacity as part of their climate change adaptation plans.

Adjusting treatment processes to manage abrupt or extreme shifts in DOC can be both complex and expensive (Emelko et al., 2011); the need for rapid responses in such situations is especially problematic for small drinking water systems (SDWSs) because they are often constrained by tight budgets and a limited pool of trained operators (Edwards et al., 2012; Logsdon et al., 1990; Scheili et al., 2016). Thus, there is a critical need to identify operationally simple and low-cost treatment technologies that are resilient to post-fire episodes of deteriorated source water quality. Biologically active filtration, or “biofiltration”, may offer a strategy to augment treatment facilities’ DOC removal capacity (Blackburn et al., 2023). Biofilters are cost-effective and energy-efficient treatment technologies that have been shown to achieve significant removals of turbidity and organic carbon without chemical inputs (LeChevallier et al., 1992; Persson et al., 2006); thus, they are often considered a techno-ecological nature-based solution (TE-NBS; Blackburn et al., 2021). Recently, there has been renewed interest in slow sand filtration as a reliable biofiltration strategy that is accessible to small and

decentralized drinking water systems (Agrawal et al., 2021; Graham & Collins, 2014; Maiyo et al., 2023).

Despite a long history of their use, evaluations of slow sand filter (SSF) performance under extreme conditions such as the DOC fluctuations that can occur post-wildfire have been limited. Challenge testing that simulates these situations is crucial to anticipate filter behaviour, as wildfire can lead to shifts not only in the concentration, but also the character, of source water DOC. Not all organic compounds are readily biodegradable within typical biofilter retention times, which range from less than 10 minutes to more than 10 hours (Huck et al., 2000; Brown et al., 2020; Servais et al., 1987; Visscher et al., 1990; Zheng et al., 2010). Several studies have found that humic substances are relatively poorly removed by biofiltration compared to other DOC fractions (Chen et al., 2016; Naidu et al., 2013; Simon et al., 2013). These compounds and other less bioavailable organics are often described as “slowly biodegradable”. Reduced vegetation and increased erosion on burned landscapes can lead to greater exports of humic material to waterways (Moody & Martin, 2001). It is anticipated that biofilters with the longest contact times will generally achieve the greatest removal of slowly biodegradable compounds (Huisman & Wood, 1974). Slow sand filtration is thus a prime candidate for evaluation as a strategy to improve post-fire treatment resilience in small systems.

Biofiltration enhancement research is an emerging field that has the potential to further improve treatment performance by allowing process customization for targeted outcomes (Kirisits et al., 2019). One drawback of conventional SSFs is the need for periodic cleaning, which is resource-intensive and disruptive to operations (Letterman & Cullen, 1985). During filter operation, a mat of biomass and deposited material forms at the bed surface (Weber-Shirk & Dick, 1997). This carbon-rich layer (i.e., the *schmutzdecke*) supports microbial communities that are vital to contaminant removal, but it must be scraped off periodically to maintain hydraulic performance (Huisman & Wood, 1974). Filters must then re-acclimate for 1–2 days before being brought back online. Treatment facilities often increase hydraulic loading to other filters during cleaning and re-acclimation (Visscher, 1990), which may impair DOC biodegradation and solids removal by reducing contact times and introducing shear forces that disturb the filter bed (Moona et al., 2021). Strategies to reduce SSF cleaning frequency and duration could lower associated costs (Graham & Collins, 2014) and potentially avoid additional pressure on other treatment infrastructure.

Peroxide amendment has been proposed as a method of reducing the rate of biofilter clogging. Low doses of hydrogen peroxide, which is regarded as a relatively eco-friendly and (when dilute) non-harmful oxidant (Gatemala et al., 2017; Zhao et al., 2021; Zou et al., 2021), may aid in maintaining hydraulic performance by reducing the quantity of organic material that accumulates in filter beds (Lauderdale et al., 2012; Mauclaire et al., 2004). Hydrogen peroxide has cytotoxic effects above a certain concentration threshold and contact time (Bayliss & Waites, 1980), but lower doses have been shown to degrade biopolymers and other organics without damaging cells (Christensen et al., 1990). Peroxide amendment may also boost biological activity by providing an additional source of dissolved oxygen (DO) for biological filter communities (Lauderdale et al., 2012). Previous studies on the hydraulic effects of peroxide amendment have focused primarily on rapid biofilters. Reported results have been variable, and the impacts on filter biodegradation are still unclear; further testing is therefore needed. Testing should also be extended to SSFs, in which biomass and biological activity can be more concentrated at the bed surface than in rapid biofilters (Chen et al., 2021; Demir et al., 2018; Urfer & Huck, 2001; Velten et al., 2011). Furthermore, most experiments have investigated the effects of continuous peroxide dosing. The findings of Yang et al. (2019) and Urfer (1998) suggest that intermittent oxidant dosing could have some effect on organic matter accumulation. An intermittent dosing approach could lower chemical costs and may reduce selective pressure for peroxide-resistant bacteria.

To better understand the potential of conventional and enhanced slow sand filtration processes to improve drinking water treatment resilience to wildfire-associated source water disturbances, this study 1) evaluated the capacity of biofilters operated under conditions resembling slow sand filtration to attenuate the effects of elevated and altered DOC when challenged with wildfire ash-impacted water (WAIW), 2) assessed the ability of these biofilters to return to pre-disturbance levels of DOC removal performance within a timeframe that is reasonable for drinking water treatment operations, and 3) investigated intermittent hydrogen peroxide amendment as a potential strategy to control the accumulation of organic matter in filter beds without compromising DOC removal performance.

2.3 Methodology and Methods

2.3.1 Overview of Methodology

This study was a proof-of-concept evaluation of conventional and peroxide-amended biological filter performance when treating WAIW. Four pairs of bench-scale duplicate biofilters were operated under conditions closely resembling slow sand filtration. The biofilters received a baseline synthetic water matrix intended to exhibit key characteristics (i.e., low turbidity, low DOC concentrations) of a high-quality source water from a forested watershed. Two filter pairs periodically received severely ash-impacted water to simulate the extreme degradation in water quality that can occur post-fire when ash and other materials from the burned landscape are exported to watercourses. Biofilters were exposed to WAIW to challenge them with abrupt increases in turbidity and DOC concentration, as well as shifts in DOC character. One ash-challenged filter pair and one filter pair treating baseline source water only were amended with low doses of hydrogen peroxide on an intermittent basis. The experiment was designed to replicate elements of a previous biofiltration project, which used a natural water matrix of deteriorated quality (Blackburn et al., 2023).

2.3.2 Preparation of Baseline Source Water

The synthetic water matrix used as baseline influent was prepared in batches. A synthetic source water was used to increase the degree of control over influent quality. Tap water was passed through a pre-filter containing Filtrasorb® 300 granular activated carbon (GAC) to remove any disinfectant residual (Calgon Carbon Corporation, Pittsburgh, Pennsylvania). The pre-filtered water was allowed to equilibrate to room temperature and was stored for no more than 4 days before use. Final preparation involved ultraviolet (UV) irradiation of each influent batch for 4 h using a 5W submersible UV lamp (Blue Ocean Aqua, Aluthgama, Sri Lanka) to suppress biological activity in the influent reservoir. Immediately before use, the dechlorinated water was supplemented with nutrients to promote and sustain biological growth in the filter beds. The background concentrations of dissolved organic carbon (DOC), ammonia-nitrogen, and orthophosphate in the GAC-filtered water were measured at the project outset. Nutrient amendment was performed to encourage biological growth in the filter beds, as the water was depleted of organic carbon and other nutrients after GAC filtration. As this strategy was not part of the experimental design, measurement of background nutrient concentrations was completed only once. Ammonium-nitrogen and orthophosphate were measured by a commercial laboratory using a modified version of a procedure from Watson et al. (2005) and Standard Method (SM) 4500-P

(American Public Health Association [APHA], American Water Works Association [AWWA], & Water Environment Federation [WEF], 2023), respectively. These analytes were selected as they are bioavailable forms of each nutrient (or have bioavailable components) and are often present in natural waters (Huck et al., 1989; Reynolds & Davies, 2000; Watson et al., 2005). Carbon and phosphorus levels were supplemented by direct addition of sodium acetate (12.6 mg/L) and potassium phosphate dibasic (0.25 mg/L) to batches of influent with manual mixing. These compounds have been used for nutrient amendment in several other biofiltration studies using high quality tap water or groundwater sources (Anderson et al., 2008; Xin et al., 2008); though they are not necessarily abundant in natural source waters, they provide readily bioavailable nutrients essential for microorganisms. A C:N:P ratio of 100:10:1 is often cited as being optimal for microbial growth (LeChevallier et al., 1992; Nyfennegger et al., 2013; Redfield, 1934); however, a C:N:P molar ratio of 10:1:1 was targeted to ensure phosphorus did not limit microbial activity (Noh et al., 2020). Phosphorus-limiting conditions can trigger increased microbial production of extracellular polymeric substances (EPS), which can accelerate filter clogging (Mauclaire et al., 2004).

2.3.2.1 Pre-filter Design

Sterilized GAC was loosely packed into two columns with an inner diameter of 5 cm and length of 30 cm. The pre-filters were operated intermittently targeting an empty bed contact time (EBCT) of about 7.5 min to remove any chlorine residual that may have been present in the tap water. Though shorter than the EBCT typically recommended for dechlorination when using non-catalytic GAC (Fairey et al., 2007), SM 4500-Cl D (APHA, AWWA, & WEF, 2023) was used to measure free and combined chlorine using an amperometric titrator (Wallace & Tiernan Products, Vineland, New Jersey) to confirm the absence of a residual throughout the experiment.

2.3.3 Biofilter Design and Acclimation Phase

Eight bench-scale biofilters were constructed as described in Blackburn et al. (2023) and operated under conditions closely resembling slow sand filtration. Silica sand, chosen for its non-adsorptive properties, was washed with tap water and dried at 105°C for 40 h. The sand was autoclaved at 121°C for 45 min to eliminate any surviving or heat-activated microorganisms. Details on column set-up are provided in Appendix A.

Buckets containing 18 L of prepared source water were connected directly to the biofilter system to act as influent reservoirs. Buckets were swapped out daily. Source water was pumped into the tops of the filter columns using an Ismatec™ IPC Digital Peristaltic Pump (Cole-Parmer, Vernon Hills, Illinois). Flow rate was regulated by adjusting the head difference between the filter “inlets” and “outlets”; this was accomplished by shifting the position of the effluent tubing connected to each column. A volumetric flow rate of 1 mL/min was targeted, which corresponds to an EBCT of about 6.2 h and a hydraulic loading rate of 0.11 m/h. Both parameters were within typical ranges for slow sand filtration (Crittenden et al., 2012; Maiyo et al., 2023). Biofilter columns were wrapped in aluminum foil to block light and prevent algal growth.

The filters were operated continuously for 135 days prior to the start of experimental testing. Effluent turbidity and DOC removal were tracked to monitor the development of biofilter treatment performance during this time; samples were collected and analysed three times per week as described in section 2.3.7. Due to the very low DOC concentrations in the synthetic source water, relatively small variations in influent DOC had a major impact on DOC removal when expressed as a percentage. Thus, DOC removal efficiencies were not consistent by the end of this period; however, effluent DOC was highly consistent in all filters. Linear regression was also used to confirm that no upward or downward temporal trend was present in influent DOC concentrations. Thus, the filters were considered to be fully acclimated by this point. Water quality data collected during a three-week period leading up to the experimental phase was used to establish a performance baseline for the biofilters.

2.3.4 Preparation of Wildfire-ash-impacted Water

The wildfire ash used in this experiment was collected in 19 L plastic carboys using soft-bristled brooms and snow shovels (Skwaruk, 2020). Collection took place on September 22, 2020 at a site in the Rocky Mountains in the southern region of British Columbia, Canada (50°05'00.2"N 116°03'52.6"W). This area, which is dominated by coniferous forest, was impacted by the 2020 Doctor Creek Wildfire (N21257, high burn severity) (Government of British Columbia, 2023). The collected material was sifted to remove debris. In this work, “wildfire ash” is considered to be the mixture of particulate matter that remains or is deposited on the landscape after the burning of wildland fuels (Bodí et al., 2014) which can be easily mobilized in major runoff events. This mixture may include pyrogenic material formed by the combustion of vegetation and forest floor material, as well as inorganic matter originating from soil. This mixture is representative of the material that is exported to watercourses by post-fire

runoff events and may subsequently impact downstream drinking water treatment (Skwaruk et al., 2020). The ash used in this experiment was dark grey in colour, suggesting a high likelihood that it contained organic material. The same ash was also used by Blackburn et al. (2023), which allows for a close comparison of the impacts of WAIW on biofilter performance that were observed in both studies.

WAIW was prepared daily during challenge tests using baseline synthetic source water prior to nutrient supplementation. UV-treated, dechlorinated tap water was mixed with wildfire ash at a concentration of 1 g/L, which corresponded to the high ash disturbance severity level evaluated by Blackburn et al. (2023). The water and ash were mixed in a PB-900 Series Programmable 6-Paddle Jar Tester (Phipps & Bird, Richmond, Virginia) for 2 h at 200 rpm, then for 16 h at 180 rpm to facilitate efficient leaching of water-extractable organic matter. The water was allowed to settle for 3 h to remove large solids that may cause biofilter clogging (as would be achieved by upstream treatment processes in many drinking water treatment facilities). Average turbidity after settling ranged from 35.4 NTU to 48.4 NTU, much higher than the average baseline source water turbidity of 0.66 NTU. Post-fire turbidity can vary widely (with spikes above 1000 NTU being reported in several studies), but the turbidity data were within a reasonable range for a high-quality source water following a moderate- to high-severity wildfire (Emelko et al., 2011; Rhoades et al., 2019).

To prevent clogging, the WAIW was pretreated with an intermittently operated gravel roughing filter (media sizes ranging from 1 to 5 mm, filter inner diameter = 15.2 cm, bed depth ~ 22 cm). The target roughing filter effluent turbidity before being fed to the biofilters was ≤ 8 NTU (Barrett et al., 1991). A rate of 55–65 mL/min (0.19–0.22 m/h) was generally sufficient, but flow rates as low as 35 mL/min (0.12 m/h) were sometimes required to stay below the 8 NTU threshold. The media were rinsed with de-ionized water between each use, then sterilized by autoclaving between challenge tests.

2.3.5 Ash Challenge Tests and Peroxide Amendment

The biofilters were considered to be acclimated once effluent DOC was stable, and no temporal trend was present in influent DOC concentration. After three weeks of near-steady-state operations (referred to herein as the baseline operation phase), the experimental phase of the project was started. To simulate periodic source water disturbances that may be associated with post-wildfire runoff events, two filter pairs were challenged by continuously receiving WAIW for two-, four-, then seven-day periods (Figure 2.1). These challenge tests were separated by five-day recovery periods at baseline source water conditions. A similar challenge test procedure was used by Blackburn et al. (2023); however, the nine-

to ten-day recovery periods used in that experiment were found to be longer than necessary for DOC removal performance to recover. Filter cleaning was carried out 27–30 h before the start of each challenge period. This ensured that all challenge tests started at the same point in the filter run and performance assessments were done at near-peak filter vulnerability. Concurrent with ash challenge testing, two filter pairs received intermittent hydrogen peroxide amendment every 3–4 days. Peroxide solutions were prepared by amending 175 mL of the appropriate influent with 0.7 mg/L of hydrogen peroxide. Influent reservoirs were temporarily disconnected from the biofilter system, and the peroxide solutions were pumped into the columns for a period of 1 h. A dose of 0.7 mg/L falls below the concentration threshold at which hydrogen peroxide has cytotoxic or inhibitory effects on microorganisms (Dahiya et al., 1968; Price & Lee, 1970).

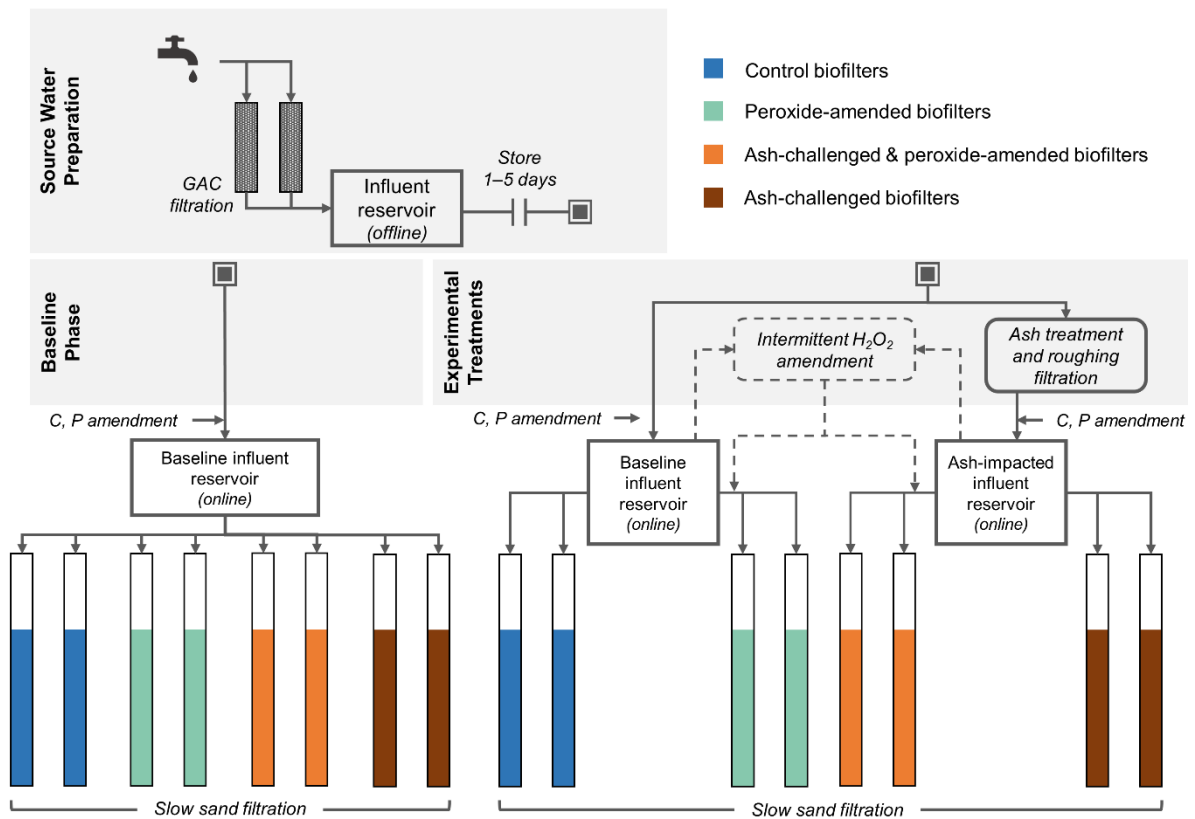


Figure 2.1 Graphical representation of the experimental approach. The top panel shows the technique used to prepare batches of synthetic baseline source water throughout the entire project. The bottom left panel indicates direct addition of nutrient-amended synthetic source water to all biofilter columns during the baseline phase (i.e., end of acclimation). The bottom right panel shows the process of preparing WAIW during challenge tests and applying intermittent hydrogen peroxide amendment. Note that the approach used during recovery periods is not specifically shown but would be the same as the baseline phase with the addition of intermittent peroxide amendment where applicable.

2.3.6 Organic Matter Characterization

To investigate possible effects of peroxide amendment on the accumulation of organic matter in the biofilter beds, samples of filter media (including attached and deposited material forming the schmutzdecke layer) were collected from the tops of the filter beds and analysed. Organic matter in the samples was quantified and characterized using Total Organic Carbon (TOC), Liquid Chromatography paired with Organic Carbon Detection (LC-OCD), and Volatile Solids (VS) analyses. Organic material

was separated from media by sonicating samples for 10 min, and samples of the slurry were characterized with LC-OCD. Combined alkaline and sonication treatment was applied to additional biomass slurry samples before TOC analysis to improve the recovery of particulate organics (Lee et al., 2020). VS retained on the media were measured as the mass lost on ignition when samples were heated at 550°C for 30 min. A detailed procedure is provided in Appendix B.

2.3.7 Water Sampling and Analysis Methods

Influent and effluent samples were analysed for some analytes on a daily basis during the experimental phase (DOC, turbidity, UV_{254}), while others were analysed 1–2 times during each challenge test or recovery period (pH, alkalinity, TOC, and LC-OCD analysis). Collection of composite effluent samples took 3–5 hours due to the very low filtration rates. Sample bottles were carefully fastened to the filter columns at the position of the effluent tubing and removed when sufficient volume was collected. Due to the instability of influent DOC concentrations, influent grab samples were collected and analysed at both the beginning and end of the effluent sample collection period. The data collected from these samples were treated as separate replicate measurements for statistical analyses, but plots presented in section 2.4 and Appendix C show the average of the two influent samples.

Standard Methods for the Examination of Water and Wastewater (APHA, AWWA, & WEF, 2023) were used for routine measurements of key water quality parameters. For DOC analysis, samples were vacuum filtered using 0.45 μm pre-rinsed polyether sulfone (PES) filter membranes (Millipore, Burlington, Massachusetts). Samples for DOC and TOC analysis were acidified to $\text{pH} < 2$ using 12 N hydrochloric acid (HCl) and processed according to SM 5310 B with a TOC-L total organic carbon analyser (Shimadzu, Kyoto, Japan). Turbidity was measured according to SM 2130 B using a 2100Q Portable Turbidimeter (Hach, Loveland, Colorado). SM 4500-H+ B was used to measure sample pH using an Accumet AB250 pH/mV/Ion meter (Thermo Fisher Scientific, Waltham, Massachusetts). Alkalinity was measured as per SM 2320 B by titrating with 0.1 N HCl to an endpoint of 4.5. A Cary 100 UV-Visible spectrophotometer (Agilent Technologies, Santa Clara, California) was used to measure UV absorbance at a wavelength of 254 nm (UV_{254}) in samples filtered with pre-rinsed 0.45 μm PES syringe filters (Agilent Technologies, Santa Clara, California). Specific UV absorbance (SUVA) was calculated as the ratio of UV_{254} to DOC and used as an indicator of the aromaticity of sample organic matter (Edzwald, 1993; Westerhoff et al., 1999). Hydrophilic DOC in vacuum-filtered samples was further characterized using a Liquid Chromatography – Organic Carbon Detection – Organic

Nitrogen Detection (LC-OCD-OND) system (DOC Labor GmbH, Karlsruhe, Germany). Chromatograms produced through LC-OCD analysis were integrated using ChromCALC software (DOC-Labor, Labor GmbH, Karlsruhe, Germany) following the manufacturer’s guidelines. Table 2.1 shows the detection and reporting limits applied to experimental data.

Table 2.1 Quantitation and detection limits used for quality assurance of experimental data

Instrument	Parameter	Method Detection Level (MDL)	Minimum Quantitation Level (MQL)
Accumet AB250 pH/mV/Ion meter (Thermo Fisher Scientific)	pH	-	-
TOC-L total organic carbon analyser (Shimadzu)	DOC/TOC	0.05 mg/L	0.1 mg/L
2100Q Portable Turbidimeter (Hach)	Turbidity	-	0.05 NTU
LC-OCD-OND (DOC-Labor GmbH)	Biopolymers	9 µg/L	27 µg/L
	Humic substances	9 µg/L	27 µg/L
	Building blocks	26 µg/L	78 µg/L
	Low molecular weight (LMW) acids	44 µg/L	132 µg/L
	LMW neutrals	7 µg/L	21 µg/L

2.3.8 Statistical Analysis

Data normality was determined by conducting Shapiro-Wilk tests (Appendix D) and examining normal probability plots (NPP) of the data. Influent samples collected on the same day were treated as separate replicates for statistical testing. Unpaired, one-sided t-tests were thus used to compare influent and effluent data. Paired, one-sided t-tests were used to compare data across different filter pairs. A significance level of 10% ($\alpha = 0.1$) was used for t-tests following the example of Emelko et al. (2016); a lower threshold for establishing significance was considered acceptable in this case because of the dynamic nature of biological systems, and the resultant natural performance variability that is characteristic of passive biological treatment processes. Data from each filter pair were lumped over relevant periods and treated as replicate measurements. Performance parameters were averaged over relevant periods of interest (e.g., individual ash challenge tests or recovery periods) and are expressed

as “mean \pm standard deviation”. Linear regression was used to evaluate performance stability during periods of interest.

Biofilters were assumed to be operating at steady state throughout this experiment, allowing water quality data to be handled as independent replicate measurements instead of as a time series. This is common practice in biofiltration studies. However, the assumption of steady state would presumably not be valid immediately following abrupt shifts in source water quality like those applied in this experiment. Further investigation may be needed to determine how quickly biofilter performance stabilizes following such shifts and thus inform optimal data analysis approaches. Further details on statistical analyses are provided in Appendix D.

2.4 Results and Discussion

2.4.1 Biofilter Performance During Baseline Phase

Measured water quality parameters were relatively consistent across all biofilters over the three-week baseline phase prior to challenge testing; all filters received non-deteriorated (i.e., baseline) source water during this period. Effluent turbidity in 97.5% of baseline phase samples was ≤ 0.3 NTU. UV absorbance at 254 nm was stable in all columns ($p > 0.35$) and, in general, no significant differences between baseline influent and effluent UV_{254} were observed ($p > 0.33$). Effluent SUVA exceeded influent SUVA on all sampling occasions (Appendix C), suggesting the ratio of aromatic DOC to total DOC was higher in filter effluent. Preferential removal of non-aromatic DOC was anticipated, as this has been observed in several previous biofiltration studies (Blackburn et al., 2023; Fu et al., 2017; Noh et al., 2020).

It should be noted that DOC removal efficiencies reported in biofiltration studies are highly variable, ranging from 0–25% for various types of biofiltration (Blackburn et al., 2023; Dhawan et al., 2017; McKie et al., 2015; Moona et al., 2021; Noh et al., 2020). Removals may be higher in “classical” biofiltration (Kirisits et al., 2019) when oxidative pretreatments like ozonation are used, as they have been shown to improve the biodegradability of DOC (Carlson & Amy, 1997; Emelko et al., 2006; Goel et al., 1995). Other factors affecting DOC removal variation between studies include filter media, EBCT, and overall source water DOC characteristics (Collins et al., 1992). Rapid biofiltration using adsorptive media like GAC has been shown to achieve higher DOC removal than sand biofilters;

however, pre-treatments and use of adsorptive GAC increase operational and maintenance demands. In the current study, effluent DOC concentrations were steady over time and were generally similar for all biofilters (0.5 ± 0.1 mg/L); however, DOC removal expressed as a percentage of influent DOC varied considerably, ranging from less than 15% to more than 80%. This result was largely due to the high degree of variability in influent DOC. The sodium acetate added to the synthetic water as an additional source of readily available carbon was not stable in the influent reservoir.

2.4.1.1 DOC Characterization

DOC characterization using LC-OCD provided insight into DOC removal trends observed in the biofilters. The baseline source water had very low concentrations (< 0.3 mg/L) of all DOC fractions except for low molecular weight (LMW) acids and, less frequently, LMW neutrals. Elevated LMW acid concentrations were the result of carbon supplementation with sodium acetate. The hydrolysis of sodium acetate produces an acetate ion, which is the conjugate base of acetic acid that exists near a neutral pH. Acetate does not require transformation for cell uptake (Jiang et al., 2022), making it a readily biodegradable compound; however, cells may release intermediate or end products formed during acetate metabolism (Barker & Stuckey, 1999). Thus, the biodegradation of acetate and other readily biodegradable organics such as biopolymers may have produced the elevated LMW neutrals concentrations.

The LC-OCD results obtained during the three-week baseline operation phase (Figure 2.2) showed that the biofilters were able to achieve statistically significant reductions of biopolymers ($p \leq 2.1 \times 10^{-3}$) and, in some cases, LMW neutrals ($p \leq 0.011$). Excellent removal of LMW acids (sometimes exceeding 90%) was observed when influent concentrations were substantial. The majority of the DOC in this category was acetate, so near-complete removal is not surprising. Urfer & Huck (2000), for example, consistently observed $>95\%$ removal of acetate in rapid biofilters with dual media beds of anthracite over sand. The readily biodegradable nature of biopolymers has been demonstrated in previous biofiltration studies using several water matrices (Blackburn et al., 2023; Chen et al., 2016; Zheng et al., 2010). In several sampling events, effluent LMW neutrals concentrations from individual columns exceeded influent concentrations, suggesting that short-term releases of DOC were occurring periodically. No major reductions of humic substances or building blocks (compounds of a humic nature formed by the breakdown of humic substances; Huber et al., 2011) were measured during baseline operation; these observations are generally consistent with several other biofiltration studies

(Chen et al., 2016; Noh et al., 2020). The data regarding the removal efficiency of LMW acids and neutrals by biofiltration are more variable, with some studies achieving significant reductions (Blackburn et al., 2023; Simon et al.; 2013) and others reporting very minor or insignificant reductions in one or both fractions (Chen et al., 2016; Simon et al., 2013). The DOC signatures of natural surface waters in forested and wildland landscapes are often dominated by humic compounds (Baghoth et al., 2011; Gray, 2023). As mentioned above, much of the DOC present in the synthetic source water used in this experiment was highly biodegradable acetate. As a result, even average bulk DOC removals from the baseline source water exceeded the range of values typically reported in biofiltration studies. These findings demonstrate that DOC fractionation can provide valuable insights into biofilter DOC removal efficiencies that would be missed if only bulk concentrations were measured.

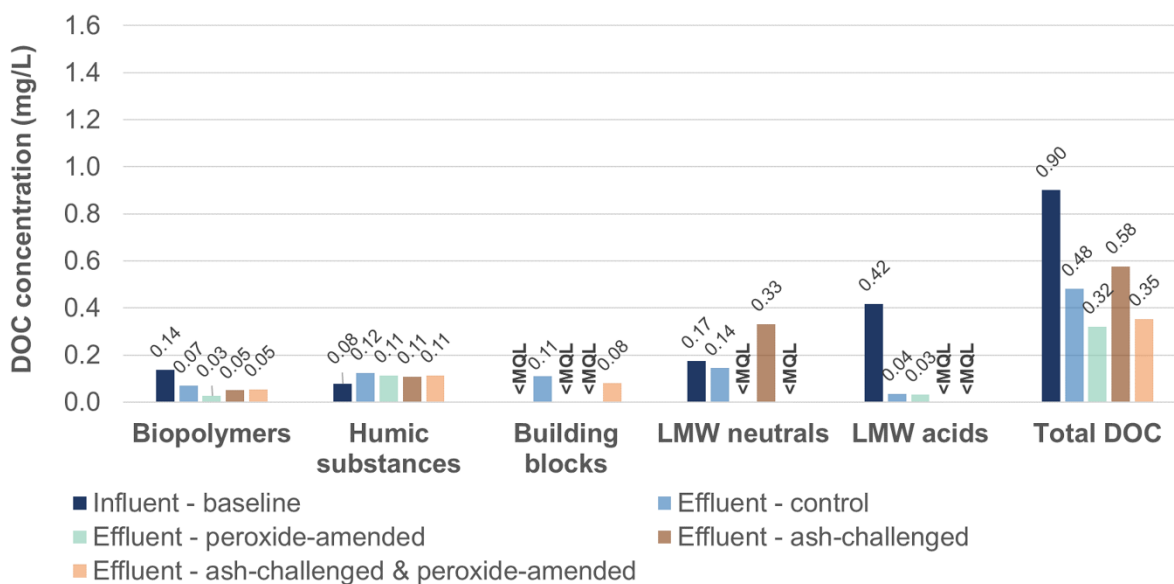


Figure 2.2 Hydrophilic DOC characterization in baseline phase samples using LC-OCD analysis. The average concentrations of samples from replicate columns (n=2) are shown by each bar. If the averaged concentrations fell below minimum quantitation levels, “<MQL” is shown in place of a bar. Error bars are not displayed, as the information they can provide for pairs of samples that are not exact replicates is limited.

2.4.2 Performance During Tubing Change

During the first ash challenge test and recovery period, elevated DOC was observed in the effluent from all columns (Figure 2.3). DOC concentrations in filter effluents exceeded that of the influent, indicating that DOC was being released from the biofilters. The greatest DOC release occurred in the ash-challenged filters, with effluent concentrations reaching as high as 16.4 mg/L compared to WAIW DOC concentrations of around 2.8 mg/L. In the subsequent recovery period, during which all filters received baseline source water from a common reservoir, effluent DOC concentrations dropped but remained elevated (6.0–7.5 mg/L) relative to baseline influent concentrations (~1.0 mg/L). In biofilters receiving only baseline source water, effluent DOC unexpectedly increased steadily throughout the duration of both the challenge test and recovery period, reaching between 1.8 and 4.9 mg/L. Troubleshooting led to the conclusion that the replacement of aged influent transfer tubing shortly before the first challenge test was linked to the disturbance. The tubing change was completed as a proactive step to reduce the risk of operational complications during challenge testing. It is hypothesized that the new tubing leached plasticizers or other chemicals that disrupted the biological communities in the filter beds. Effluent DOC returned to baseline levels within 24 h of the tubing change being reversed on day six of the experimental phase. LC-OCD data showed that the effluent DOC during this phase consisted predominantly of LMW neutrals (Appendix C). These organic compounds may have been products formed from the metabolism of acetate that accumulated in the filter beds. Acetate storage in sand filter beds was previously observed by Urfer & Huck (1997). DOC release following changes in experimental conditions have been observed in other studies. Moona et al. (2019) observed sudden increases in desorption of DOC from GAC media when under negative carbon gradients (such as following a decrease in source water DOC or backwashing with clean water). Moona et al. (2021) observed temporary releases of DOC from filters with non-adsorptive expanded-clay media following increases in hydraulic loading rates, which the authors hypothesized was the result of disturbances to the filter biomass; DOC removal was reported to have recovered within three days of the loading rate change. Thus, DOC release may be a symptom of biofilter disturbance responses.

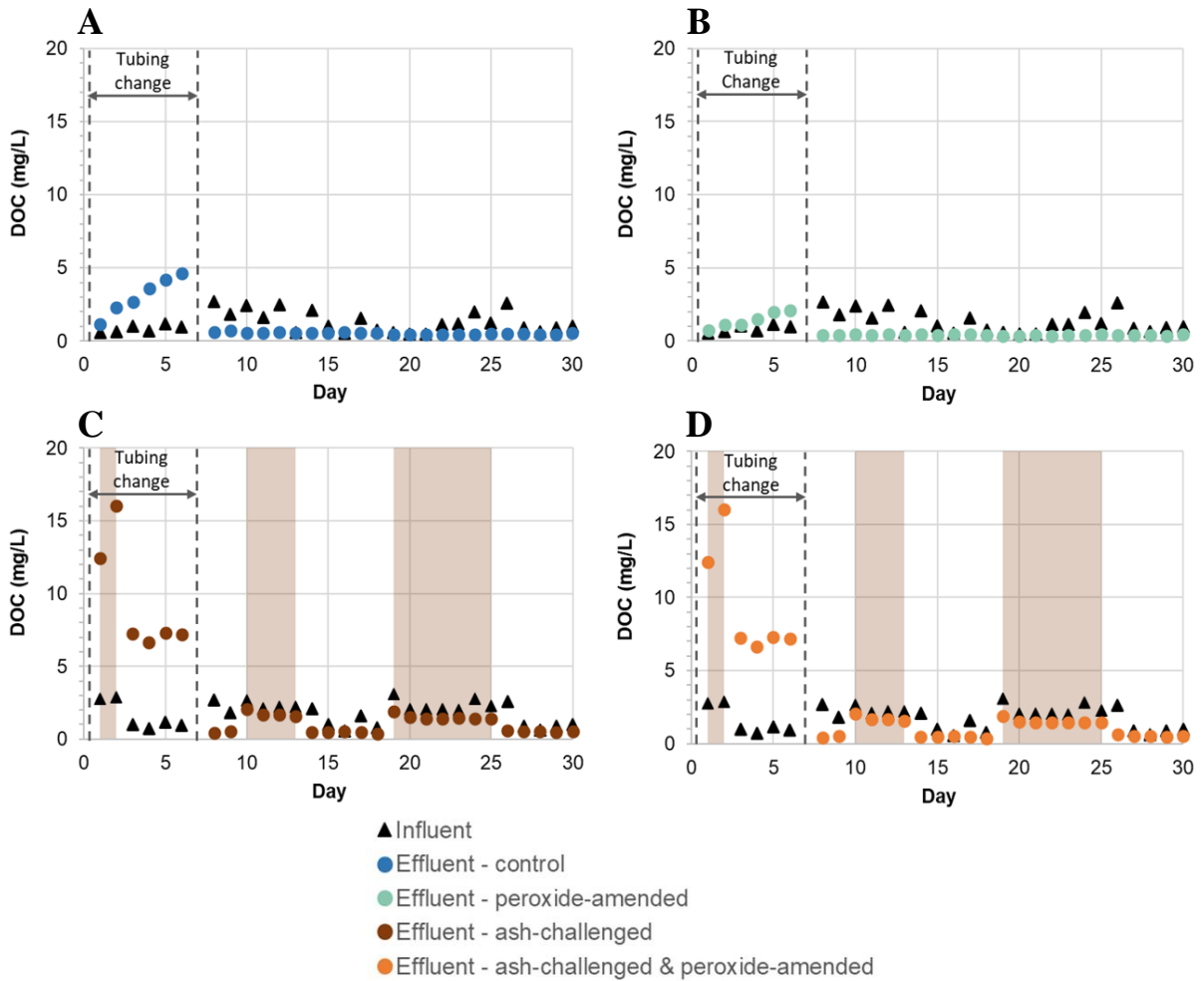


Figure 2.3 Influent and average effluent DOC concentrations for each of the four replicate filter pairs (A-D) over the 30-day experimental phase, highlighting the magnitude of the performance disruption observed from day 1–6. This disruption coincided with a change in influent tubing. Acclimation and baseline phases are not shown. Ash challenge periods are shown by the vertical brown shaded regions.

This disruption to filter performance provided valuable insight on the sensitivity of bench-scale biofilters and the compounding effects of source water disturbances. Changes in materials can lead to a severe decline in bench-scale biofilter function and the disruption may even exceed the filters' capacity to recover. The exacerbation of these effects at small scales underscores the potential for significant performance differences between bench-scale and full-scale biofilter systems. Furthermore,

the more severe nature of the performance disruption in ash-challenged biofilters compared to biofilters treating baseline source water indicated that the presence of ash may have exacerbated the disturbance.

2.4.3 Filter Performance During Ash Challenge Testing Phase

Filter performance was within the range of reasonable expectations (based on previous studies) during the remaining two sets of challenge tests and recovery periods. Effluent alkalinity was relatively stable and consistent across all biofilters throughout the entire 30-day experimental phase, including ash challenge tests (241 ± 2 mg/L as CaCO_3). This was also true for effluent pH (8.2 ± 0.1). Ash amendment did not cause a significant change in influent alkalinity ($p = 0.43$) but did cause a slight increase in average influent pH from approximately 8.3 to 8.6 ($p = 2.4 \times 10^{-4}$; Table 2.2). An increase in stream pH has been documented in several studies of wildfire-affected watersheds (Earl & Blinn, 2003; Oliver et al., 2012). Hamidi et al. (2020) found that TOC removal in biological activated carbon (BAC) filters declined above a pH of 7.5. Other studies have found that pH is not a significant factor in NOM removal (Granger et al., 2014).

Table 2.2 Comparison of baseline and ash-impacted source water properties over experimental phase

Parameter	Baseline source water	Wildfire-ash-impacted water
pH	8.3	8.6
Alkalinity	243 mg/L as CaCO_3	244 mg/L as CaCO_3
Turbidity	0.66 NTU	3.73 NTU
UV ₂₅₄	0.0105 cm^{-1}	0.0936 cm^{-1}
DOC	1.3 mg/L	2.4 mg/L
SUVA	1.2 L/(mg·m)	4.2 L/(mg·m)

Effluent UV₂₅₄ and SUVA were significantly elevated ($p < 2.2 \times 10^{-16}$ and $p \leq 2.5 \times 10^{-8}$, respectively) in the effluent of the biofilters receiving WAIW relative to controls, indicating greater aromaticity of DOC in the ash-challenged filter effluent. This result was not surprising, as wildfire has been shown to increase the concentration of aromatic DOC in soil and runoff from burned landscapes (Hickenbottom et al., 2023; Knicker et al., 2005). Effluent SUVA exceeded influent SUVA for most sample pairings, indicating that the DOC removed by the biofilters was disproportionately composed of non-aromatic compounds. This result was anticipated based on the lower observed bioavailability of aromatic and humic DOC (Chen et al., 2016; Naidu et al., 2013; Simon et al., 2013); an increase in SUVA from biofilter influent to effluent has been reported previously (Noh et al., 2020).

In biofilters treating WAIW, statistically significant increases in effluent turbidity were observed during challenge periods relative to control biofilters ($p \leq 1.0 \times 10^{-8}$). Average effluent turbidity reached 1.09 ± 0.18 NTU and 1.33 ± 0.17 NTU for conventional and peroxide-amended biofilters receiving WAIW, respectively (Figure 2.4); however, the average magnitude of the step increase diminished in the final challenge period (0.65 ± 0.23 NTU and 0.76 ± 0.22 NTU), a possible indication that the biofilters were gradually acclimating to the WAIW with repeated exposures. If true, this observation could challenge the assumption of steady state as noted in section 2.3.8. Spikes in effluent turbidity were detected in several filters (increases of 0.18–3.46 NTU) on the day following filter cleaning events. These observations reinforce the importance of allowing no less than one day of re-acclimation before putting filters back online for drinking water treatment.

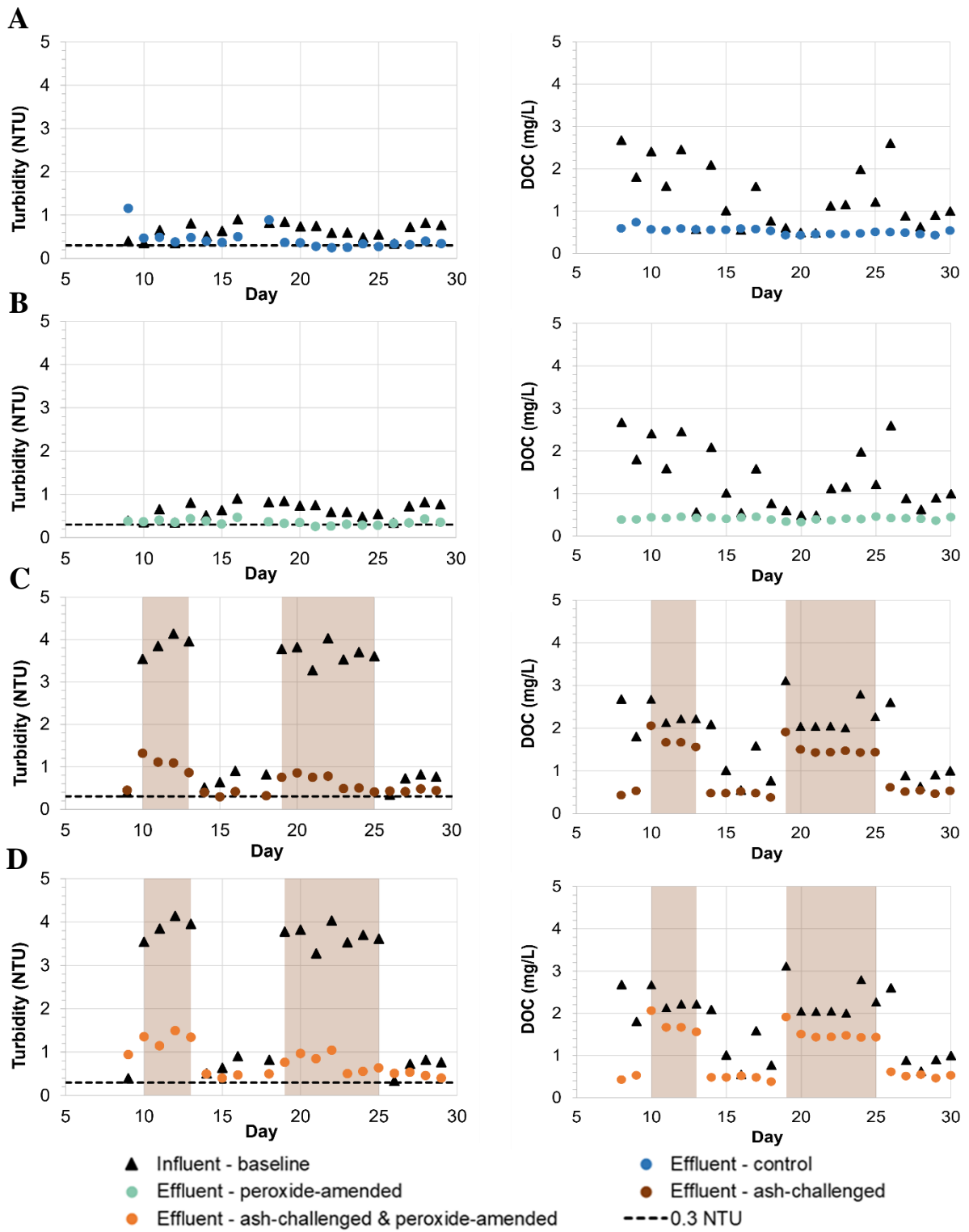


Figure 2.4 Influent and average effluent DOC concentrations and turbidity for each of the four replicate biofilter pairs (A-D) over the experimental phase. Acclimation and baseline phases are not shown. Plots exclude data collected during the first ash challenge test and recovery period, which coincided with a tubing change. Dashed lines indicate the 0.3 NTU threshold at or below which 95% of effluent turbidity measurements must fall each month for conventional filtration processes to meet protozoan cyst and virus removal requirements in Ontario, Canada (MECP, 2021). Ash challenge periods are shown by the vertical brown shaded regions.

In biofilters treating WAIW, effluent DOC was elevated significantly during challenge periods relative to control biofilters (1.7 ± 0.2 mg/L; $p < 2.2 \times 10^{-16}$) (Figure 2.4). Effluent DOC was typically highest on the first day of the ash challenge tests, then dropped slightly on the following day and appeared to stabilize. In filters receiving only baseline source water, effluent DOC remained stable throughout the second and third challenge tests and recovery periods (0.5 ± 0.1 mg/L and 0.4 ± 0.04 mg/L). No spikes in effluent DOC were observed following cleaning. Thus, any impact of schmutzdecke removal on DOC biodegradation capacity did not appear to persist beyond 20 h (the approximate time of the next sample collection).

In biofilters receiving WAIW, a significant reduction in bulk DOC removal was observed relative to control biofilters during all challenge tests ($p < 0.078$) (Figure 2.5). In addition to the use of paired t-tests, the significance of these performance disparities was assessed using the concept of inter-column variability (ICV). Biofilters with an identical design and operated under the same conditions can exhibit performance differences due to factors like differences in bed conditions (e.g., short-circuiting or clogging; Maurya et al., 2020). Comparing performance differences against ICV can help to determine whether they are the result of experimental conditions or natural variability. This practice is similar to the use of control charts to assess drinking water quality. The ICV expected to naturally occur between identical biofilters was calculated as plus or minus three times the standard deviation of the differences in DOC percent removal between the two control biofilters, following the example of Blackburn et al. (2023). Data points falling outside this region indicate variability in the performance of non-control biofilters that cannot be explained by natural ICV, and so are indicative of a significant impact from experimental conditions. Data collected during the second ash challenge test clearly indicate a difference in performance between the ash-challenged filters and controls (Figure 2.5);

however, data points for the third ash challenge test fall much closer to, or even within, the ICV region, suggesting the filters may have begun to biologically acclimate to the altered source water quality.

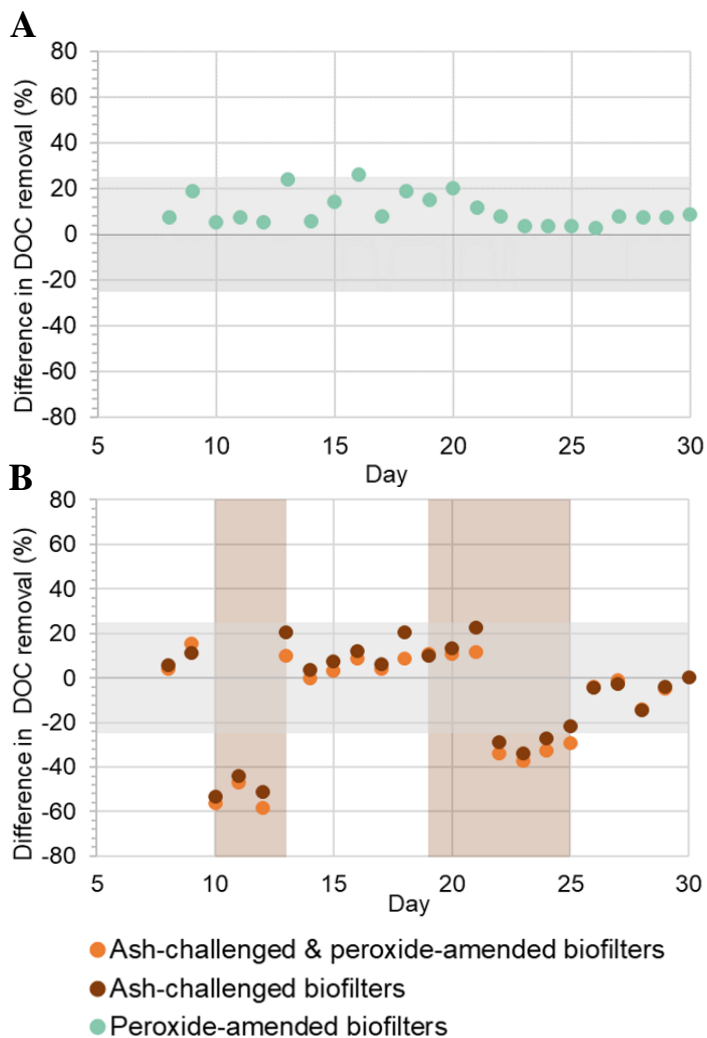
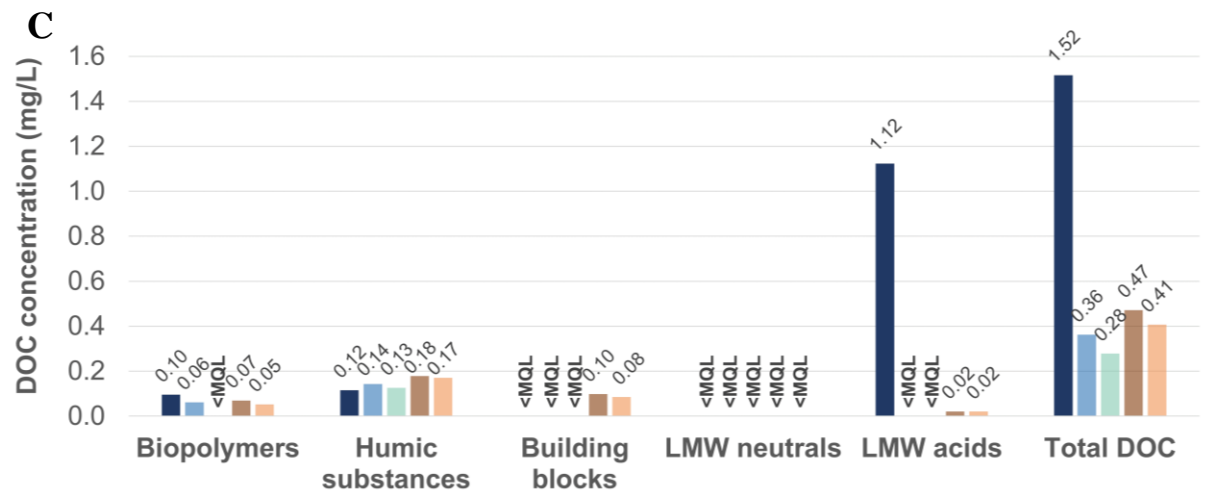
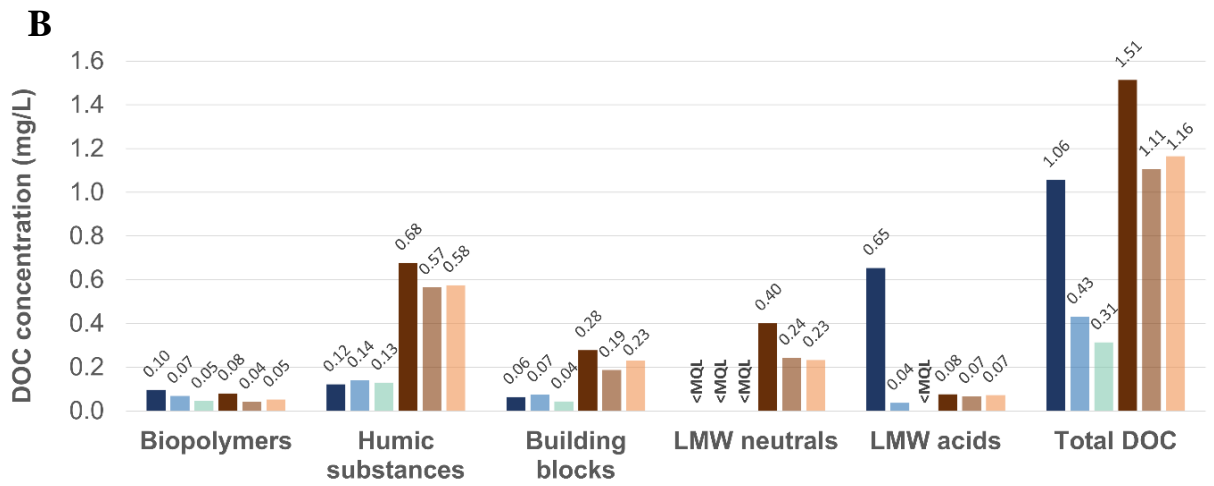
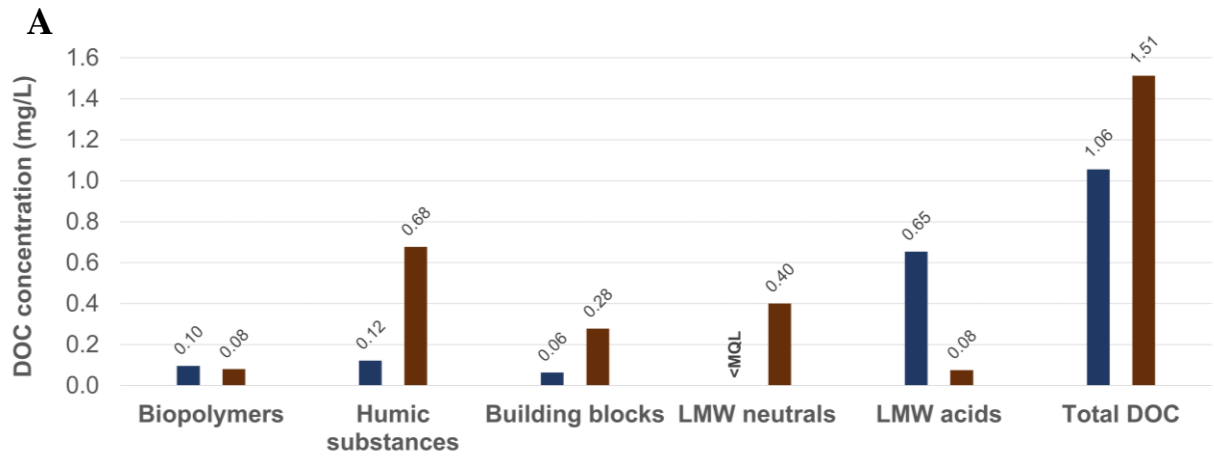


Figure 2.5 Differences between average percent DOC removals achieved by control biofilters and removals achieved by A) peroxide-amended biofilters and B) ash-challenged biofilter pairs over the experimental phase. Data points falling above the 0% line indicate higher DOC percent removal in the experimental biofilter pair relative to controls, and points below indicate lower removal relative to controls. Acclimation and baseline phases are not shown. Plots exclude data collected during the first ash challenge test and recovery period, which coincided with a tubing change. Regions of natural inter-column variability (ICV) are indicated by the horizontal grey shaded regions. Ash challenge periods are shown by the vertical brown shaded regions. The data demonstrate that ash-challenged biofilters removed a significantly smaller fraction of total DOC than control biofilters during challenge tests.

LC-OCD data collected during the third ash challenge period may offer explanations for some of the observed biofilter behaviour, including the lower percent DOC removal in filters receiving WAIW relative to controls. A visual comparison of the DOC signature in baseline source water and WAIW samples showed that ash leaching had a considerable impact on the overall “DOC signature” of the synthetic water matrix; concentrations of humic substances, building blocks, and LWM neutrals are significantly higher in the WAIW (Figure 2.6A). These changes were largely anticipated, as increases in humic material and aromatic compounds have been consistently observed in studies assessing the effects of wildfire ash leaching on aquatic DOC (Blackburn et al., 2023; Hohner et al., 2016; Skwaruk, 2020). Overall, slowly biodegradable humic material made up a greater fraction of total DOC in the WAIW, leading to lower DOC removal efficiencies in ash-challenged filters compared to controls. These data agreed with SUVA trends, which suggested that the WAIW had higher concentrations of humic and aromatic DOC. Furthermore, LC-OCD analysis showed that the baseline influent often had much higher concentrations of LMW acids than the WAIW, despite each influent batch being amended with the same concentration of sodium acetate (Figure 2.6B). The absence of this readily biodegradable carbon source in the WAIW samples may have caused an underestimation of the DOC removal in the ash-challenged filters. Additional sampling was completed to investigate acetate degradation after nutrient amendment; rapid DOC consumption began several hours after amendment, indicating that biological activity (and not instantaneous sorption to ash) was likely responsible.



■ Influent - baseline
 ■ Effluent - control
 ■ Effluent - peroxide-amended
 ■ Influent - ash-impacted
 ■ Effluent - ash-challenged
 ■ Effluent - ash-challenged & peroxide-amended

Figure 2.6 Hydrophilic DOC characterization using LC-OCD analysis in A) baseline influent and ash-impacted influent samples; B) samples collected during the seven-day ash challenge test; and C) samples collected during the third recovery period following the seven-day ash challenge test. The average concentrations of samples from replicate columns (n=2) are shown by each bar. If the averaged concentration fell below minimum quantitation levels, “<MQL” is shown in place of a bar. Error bars are not displayed, as the information they can provide for pairs of samples that are not exact replicates is limited.

Significant removals of humic substances and, in some cases, building blocks occurred in the biofilters treating WAIW ($p \leq 0.087$); in the third ash challenge period, ash-challenged filters achieved average reductions of 18% and 25%, respectively (Figure 2.6B). These removal efficiencies are higher than those seen for humic DOC fractions in several other biofiltration studies (McKie et al., 2015; Simon et al., 2013). Blackburn et al. (2023) frequently observed that biological sand filters reduced SUVA values when treating WAIW, despite humic fractions not being removed as efficiently as in the current study. A reduction in SUVA was thus anticipated here; however, as mentioned previously, effluent SUVA generally exceeded influent SUVA during challenge periods, suggesting that the biofilters may have preferentially removed the less aromatic fractions of humic material. Westerhoff et al. (1999) observed a correlation between SUVA and molecular weight of organic compounds; an increase in SUVA here may reflect the greater persistence of high molecular weight (HMW) compounds compared to LMW compounds, which are generally more bioavailable. Persistence of these DOC fractions despite the long contact times provided by slow sand filtration suggests that conventional biofiltration technologies may not be ideal primary treatment strategies for source waters with predominantly humic, aromatic, or HMW DOC fractions. Aromatic and HMW DOC is typically well-removed in coagulation processes, while LMW, non-aromatic DOC is harder to coagulate (Edzwald & Van Benschoten, 1990). Thus, while not a “silver-bullet” solution, biofiltration may still play a valuable role in systems treating such source waters especially if upstream coagulation is present.

Filters treating WAIW demonstrated rapid returns to pre-disturbance performance levels during recovery periods. Effluent turbidity and DOC concentrations were generally consistent with control filter concentrations by the first sampling event. Effluent turbidity in the ash-challenged filters (both conventional and peroxide-amended) was elevated relative to control filters in the final recovery period only ($p < 4.3 \times 10^{-3}$). DOC removals and effluent DOC signatures were generally consistent across all biofilters during recovery periods, indicating no lasting impairment to biodegradation mechanisms after the return to baseline conditions (Figure 2.6C).

2.4.4 Effect of Hydrogen Peroxide Amendment on Filter Performance

Organic matter in filter media samples collected at the end of the experimental phase was characterized and quantified to look for possible differences in the rate of schmutzdecke formation. The quantities of organic carbon extracted from each media sample by sonication were estimated from the TOC concentrations of the diluted biomass slurry. VS measurements quantified the amount of organic matter retained on the media. Both quantities were normalized by sample mass and compared across filter pairs. Samples from peroxide-amended biofilters did not have consistently lower quantities of organic matter than samples from conventional filters. Differences in organic matter quantities between replicate filter samples were often greater than the differences between samples from different filter pairs (Table 2.3). LC-OCD chromatograms obtained for the biomass slurries were similar across all biofilters. Thus, there was no evidence that peroxide amendment had a significant impact on the accumulation of organic matter in the filter beds. However, several factors could have obscured evidence of its oxidative effects. Excess phosphorus amendment was applied to avoid accelerated EPS production in the filter beds, thereby reducing the risk of clogging (Mauclaire et al., 2004). However, faster EPS production may have made the effects of peroxide amendment more apparent. The short intervals between cleaning, which were selected to replicate the approach of Blackburn et al. (2023), may have limited the potential to observe differences in organic matter accumulation. The small sample volumes also prevented measurement replication. Finally, error may have been introduced as a result of the difficulty of collecting fully representative media samples from the columns, as well as differential losses during sampling handling and analysis methods.

Table 2.3 Quantitative analysis of organic matter on biofilter media samples

	Non-ash-challenged filters				Ash-challenged filters			
	Control		<i>H₂O₂</i> amendment		Ash		<i>Ash + H₂O₂</i> amendment	
Column number	1	2	7	8	3	4	5	6
Total Organic Carbon								
TOC measurement of diluted biomass slurry (<i>mg-C/L</i>)	10.6	13.7	3.9	10.6	4.9	6.8	3.4	2.7
Estimated total TOC in full volume of concentrated biomass slurry (<i>mg-C</i>)	2.6	3.4	1.0	2.7	1.2	1.7	0.9	0.7
Estimated total TOC per gram of media (<i>mg-C/g-media</i>)	3.0	4.0	1.1	2.9	1.4	1.9	1.0	0.8
Replicate average of total TOC (<i>mg-C/g-media</i>)	3.5		2.0		1.7		0.9	
Volatile Solids								
Residual VS on media sample (<i>mg-organics</i>)	1.6	2.0	3.8	3.3	2.4	7.5	5.0	3.0
VS per gram of media (<i>mg-organics /g-media</i>)	1.8	2.3	4.1	3.6	2.8	8.4	5.7	3.4
Replicate average of VS (<i>mg-organics /g-media</i>)	2.1		3.9		5.6		4.5	

A statistically significant difference was detected between the effluent DOC levels in the control and non-ash-challenged peroxide-amended biofilters, with the peroxide-amended filters having slightly lower concentrations ($n = 42$, $p = 1.6 \times 10^{-14}$). However, the differences in mean effluent DOC concentrations during the baseline and experimental phases were of a similar magnitude (~ 0.1 mg/L). Furthermore, differences in DOC removal efficiency were within the range of ICV (Figure 2.5). Thus, the difference in DOC removal performance was not considered significant to practice. Investigations into the impact of peroxide amendment on DOC removal have reported variable results; some studies have found DOC removal increased by up to 50% (Lauderdale et al., 2012), while many others have detected no significant effect (McKie et al., 2015; Noh et al., 2020; Nyfennegger et al., 2013). This finding underscores the value of column replication in biofiltration studies. Meaningful interpretation of performance differences requires an understanding of the natural performance variability that can be expected between biofilters operated under the same conditions.

Though no clear enhancement was observed, there was also no evidence that hydrogen peroxide amendment had any negative effects on filter biodegradative capacity. Azzeh et al. (2015) observed a slight decrease in removal of biopolymers and DBP precursors in biofilters continuously

amended with ~1.0 mg/L of hydrogen peroxide, but most studies that have reported impairment of DOC removal used higher doses (> 2 mg/L). These results are compelling evidence that intermittent low doses of hydrogen peroxide do not 1) impair biological filter activity or 2) alter the biodegradability of aquatic DOC in ways that substantially affect its fate during biofiltration.

2.5 Conclusions

1. Elevated DOC may be observed in biofilter effluent following wildfire-associated shifts in source water quality. As humic and aromatic fractions of DOC are more likely to persist through the biofiltration process, the severity of the decline in biofilter effluent quality post-fire may be directly connected to changes in source water DOC character. However, if degraded water quality persists for several days or more, biofilters may begin to acclimate to the deteriorated conditions. Further research would be needed to determine whether biofilters may reach a new performance equilibrium in such situations and assess the degree of DOC removal impairment at the new equilibrium relative to pre-disturbance function.
2. Biological filters demonstrated a capacity to rapidly recover to pre-disturbance levels of DOC removal after source water quality returned to baseline conditions. No maintenance or remedial action was required to restore filter performance following the disturbance. Unit processes that can demonstrate this resilient behaviour may be highly beneficial to small drinking water utilities where operator availability is limited. In this way, biofilters may offer drinking water utilities some degree of resilience to wildfire-associated organic carbon fluctuations. Further challenge testing should be conducted to better monitor filter recovery behaviour. Capturing filter recovery would require a high-frequency sampling schedule following the return to baseline conditions. Daily sampling, which is frequently employed in biofiltration studies, would not be sufficient in this case.
3. Intermittent dosing of hydrogen peroxide amendment at ~0.7 mg/L did not result in measurable reductions in the accumulation of organic matter in biofilter beds under slow-sand-filtration-like conditions. No enhancement nor impairment of DOC biodegradation capacity that would be significant to practice was observed. Thus, there was no evidence of microbial community

inhibition or bactericidal effects from peroxide exposure. However, as statistically significant differences in effluent DOC and DOC removal did exist between peroxide-amended biofilters and control biofilters (with the former showing better performance), peroxide amendment merits further investigation in slow sand filters at higher doses, with greater frequency, and for a longer experimental phase. Continuous dosing of peroxide has been correlated with improved hydraulic performance in some previous biofiltration studies, and intermittent dosing may offer further cost reductions if similar results can be achieved.

4. A proper assessment of biofilter function and system-specific suitability requires characterization of source water DOC. Biofiltration may not be an appropriate treatment strategy for source waters with high concentrations of aromatic, humic, and high molecular weight carbon, especially if coagulation is not applied upstream of filtration. Bulk organic carbon measurements like DOC/TOC do not provide insights into DOC characteristics that impact treatability. LC-OCD, UV_{254} , and SUVA may be valuable tools for both researchers and utilities to understand DOC treatability. It is important to note, however, that the applicability of treatment performance indicators like SUVA and DOC percent removal may be less meaningful in highly controlled experiments using synthetic source waters. Bench-scale tests such as those described herein may provide a proof-of-concept but will not necessarily be comparable to other studies.

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Chapter 3

Investigating Biofilter Microbial Community Response to Wildfire-Ash-Impacted Water and Peroxide Amendment

3.1 Summary

Biological filters may offer a strategy to increase the resilience of drinking water systems to climate-change-exacerbated landscape disturbances such as wildfire; however, the black-box nature of biofiltration processes and the passive manner in which they are typically operated limit opportunities for process control, adaptability, and enhancement. Strategies such as peroxide amendment have been proposed to enhance various aspects of biofilter function, allowing for greater active operator control over the process. Higher levels of operational control may allow for better process responsiveness during periods of deteriorated water quality such as the dissolved organic carbon (DOC) fluctuations that can occur after severe wildfire. However, the impacts of peroxide enhancement on biofilter microbial communities have not been evaluated systematically. Here, amplicon sequencing was used to characterize filter bed bacterial communities in duplicate pairs of conventional and peroxide-amended biofilters challenged with wildfire-ash-impacted water. Samples from two sets of biofilters treating distinctly different baseline source waters were analysed. No significant differences in community composition were detected between samples from control, peroxide-amended, and ash-challenged biofilters at the sequencing depths used for this work. In some cases, significant differences in biofilter community composition were detected between sampling events and within filter pairs, underscoring the importance of filter replication in distinguishing between significant impacts of independent variables and natural community variability. In some cases, changes in biofilter DOC removal performance did not coincide with observed changes in the relative abundance of major taxa. Source water quality was found to be a significant factor in biofilter bacterial community composition and diversity, and it was noted that the filters with consistently lower species diversity also exhibited a greater change in performance when challenged with wildfire-ash-impacted water. This work provides insights on the sensitivity and resilience of biofilter bacterial communities to 1) episodic exposure to deteriorated source water quality resulting from wildfire ash and 2) intermittent peroxide amendment. Characterization of microbial-level responses to source water disturbance has not been widely studied and may help to inform biofiltration performance enhancement and optimization strategies. The inherent limitations of amplicon sequencing reduced the conclusiveness of observed correlations

between microbial characteristics, dynamics, and performance. These factors are explored to inform the application of metagenomics in future biofiltration studies.

3.2 Introduction

Climate change poses a range of threats to drinking water security. Climate-change-exacerbated landscape disturbances may cause abrupt changes in source water character within and downstream of affected areas. The impacts of severe wildfires can be especially extreme; periodic shifts in water quality may continue for several years post-fire, meaning drinking water treatment systems drawing water from burned catchments must be prepared to manage these fluctuations (Emelko et al., 2011; Emelko et al., 2016; Rhoades et al., 2019). Effects observed post-fire include changes in the concentration and character of dissolved organic carbon (DOC), a key design parameter for several drinking water treatment processes (Crittenden et al., 2012). Elevated or altered DOC can interfere with the removal of other target contaminants by exerting a demand for chemical coagulants and oxidants, sometimes prompting the use of increased chemical doses (Health Canada, 2020). Efficient removal of DOC itself is also of importance because it is a precursor to potentially harmful disinfection byproducts (DBPs) and can impair the biological stability of finished water (Prest et al., 2016; Stevens et al., 1990). Ensuring sufficient DOC removal capacity during extreme source water disturbances should therefore be a consideration in the planning of climate-resilient treatment systems.

Small, rural, and remote communities are likely to be disproportionately challenged in adapting drinking water treatment systems to the effects of wildfire and other climate-change-exacerbated events. These systems generally have tighter budgets and smaller pools of trained personnel (Edwards et al., 2012; Logsdon et al., 1990; Scheili et al., 2016). Biological filtration technologies may provide a sustainable and operationally simple treatment approach that does not exceed the financial and human resources available to small systems. Biofilters can be operated without chemicals or major energy inputs, reducing both their operational demands and environmental impact (Huisman & Wood, 1974; Logsdon et al., 2002). The simple design and passive operation of slow sand filters make this technology especially well-suited to small drinking water systems. However, because passive treatment strategies like slow sand filtration rely heavily on biologically mediated “black box” processes, they offer fewer opportunities for operator control than conventional treatment technologies (Blackburn et al., 2021). Thus, passive strategies may be less adaptable to source water disturbances associated with

climate-change-exacerbated events. A relatively new body of biofiltration research examines opportunities for active operator intervention that do not greatly increase operational demands. Proposed techniques include physical modifications as well as the use of chemical amendments to enhance aspects of biofilter function. Improving hydraulic performance and promoting biological activity are often among the objectives of these studies. For example, oxidant amendment has been proposed as a strategy to reduce the accumulation of organic matter in biofilter beds, thereby increasing filter run times and reducing costs associated with filter cleaning (Graham & Collins, 2014). However, the potential impacts of oxidant amendment on biofilter microbial communities and, by extension, biofilter performance have not been examined comprehensively. Such investigations are needed, as even short-term exposure to moderate doses of oxidants (e.g., chloramine) have been shown to alter biological communities in drinking water biofilters (Ma et al., 2020).

Although the use of biofiltration is well-established in parts of the world, the connections between microbial community characteristics, environmental conditions, and biofilter performance are not well described in the literature. This fundamental knowledge gap further constrains process optimization and adaptability (Haig et al., 2015). Previous studies have identified a need for further investigation into the impacts of water quality and other environmental factors on biological filter communities (Q. Li et al., 2017; Ma et al., 2020). The use of next-generation sequencing (NGS) to characterize filter communities is a nascent but rapidly evolving area of biofiltration research that may help to illuminate the biological mechanisms responsible for water treatment. As the cost of NGS technologies has fallen considerably in recent years, revolutionary tools like amplicon sequencing (targeted sequencing of a specific region of DNA) are now more practical for application in exploratory research (Satam et al., 2023). Sequencing the 16S rRNA gene can provide information about sampled communities, including the bacterial and archaeal taxa that are present and their relative abundance; previous studies have suggested that the number of taxa and evenness of their distribution may be connected to biofilter performance and resilience (Haig et al., 2015). Thus, 16S rRNA gene sequencing can be a valuable tool to identify high-level connections between overall bacterial community composition, environmental conditions, and biofilter performance (Kirisits et al., 2019).

Due to the multitude of factors that can potentially affect biofilter microbial communities, performance assessments across a wide range of experimental conditions are needed to understand their role in biofilter performance. Challenge testing to assess the response of biological filter communities to extreme water quality changes has been limited. These investigations may help to anticipate DOC

removal capacity and other aspects of biofilter performance during source water disturbances associated with wildfire.

This study 1) provided microbial-community-level insights on biofilter response and resilience to wildfire-associated source water disturbances by assessing changes in the composition of bacterial communities exposed to wildfire-ash-impacted-water (WAIW), 2) assessed potential impacts of intermittent hydrogen peroxide amendment on the composition of biofilter bacterial communities, 3) investigated connections between bacterial community diversity, dynamics, and changes in DOC removal performance, and 4) compared bacterial community composition and microbial-level responses to WAIW across filter sets treating distinctly different source water types.

3.3 Materials and Methods

3.3.1 Research Overview

Here, microbial communities in the filter beds of two sets of bench-scale drinking water biological sand filters were characterized using amplicon sequencing. All biofilters (16 in total) were operated in duplicate pairs under conditions closely resembling slow sand filtration (Figure 3.1). Non-adsorptive fine silica sand with an effective size of 0.2 mm was used as filter media. The filter columns were wrapped in foil to prevent growth of algae and the tops of the columns were loosely covered. The two filter sets treated source waters of distinctly different character at room temperature (18–22°C). Filter set A treated surface water collected from the municipally and agriculturally impacted Grand River in Waterloo, Ontario. Once acclimated, the filters were periodically challenged with source water impacted by low, medium, and high concentrations of wildfire ash to simulate post-fire water quality fluctuations (Table 3.1). Blackburn et al. (2023) designed the ash challenge test experiment and provided a performance assessment for filter set A. In that study, bench-scale biofilters were found to effectively buffer wildfire-associated fluctuations in low molecular weight (LMW) DOC; overall filter performance was generally consistent across control filters and filters challenged with WAIW. Filter set B (studied in the experiment described in Chapter 2) had the same physical design as set A (Table 3.2) but included both conventional and peroxide-amended biofilters. These filters treated a synthetic water matrix low in organics and other nutrients, which represented water sourced from a forested watershed. Two filter pairs were challenged with source water impacted by the high concentration of

wildfire ash used by Blackburn et al. (2023). The wildfire ash used in both experiments was collected on September 22, 2020 from a site in British Columbia, Canada (50°05'00.2"N 116°03'52.6"W) that was impacted by the 2020 Doctor Creek Wildfire (Government of British Columbia, 2023). Challenge tests lasted two, four, and seven days in both projects. Tests were separated by recovery periods at baseline source water conditions lasting nine to ten days for filter set A, and five days for set B. A performance assessment for filter set B was provided in Chapter 2. Peroxide-amended biofilters received source water with 0.7 mg/L of hydrogen peroxide for one hour, two times per week.

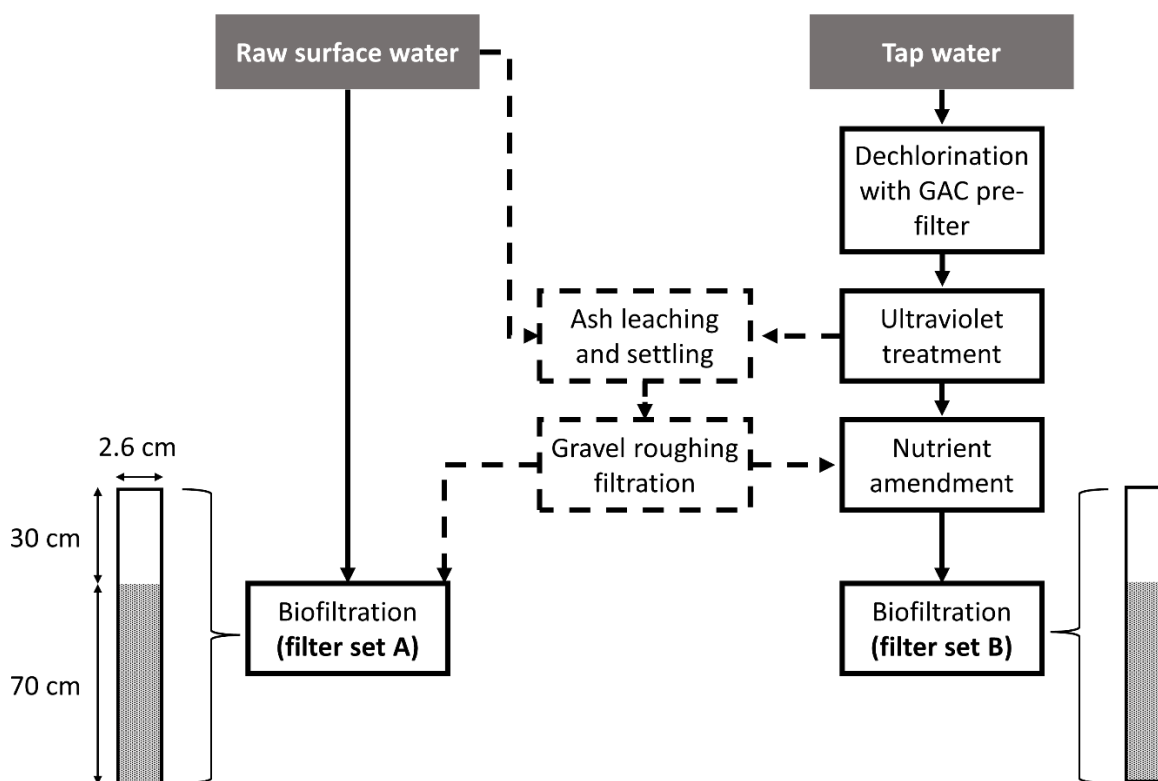


Figure 3.1 Source water preparation procedure for filter set A (left), as described by Blackburn et al. (2023), and filter set B (right). Steps used to prepare WAIW are shown in the middle of the diagram in dotted outlines. The same physical biofilter design was used in both experiments.

Table 3.1 Experimental conditions applied to duplicate biofilter pairs in each project

Filter no.	Experimental conditions		Notes
	Filter set A	Filter set B	
1, 2	Control (baseline source water – natural matrix)	Control (baseline source water – synthetic matrix)	
3,4	Challenge tests with source water impacted with low ash content	Challenge tests with source water impacted with high ash content	Filter set A pair not sampled for gene sequencing
5,6	Challenge tests with source water impacted with medium ash content	Challenge tests with source water impacted with high ash content, intermittent peroxide amendment	
7,8	Challenge tests with source water impacted with high ash content	Intermittent peroxide amendment	

Table 3.2 Filter design parameters and source water characteristics before and after ash addition

Design parameter	Filter set A			Filter set B	
Bed depth	0.7 m			0.7 m	
Empty Bed Contact Time (EBCT)	10 h			6.2 h	
Hydraulic loading rate (HLR)	0.07 m/h			0.11 m/h	
Filter media	Silica sand			Silica sand	
Filter media effective size	0.2 mm			0.2 mm	
Water quality parameter	Natural water matrix			Synthetic water matrix	
	Before ash addition	Medium ash content	High ash content	Before ash addition	After ash addition
Turbidity (NTU)	2.9–5.0	4.8–6.9	4.3–8.9	0.3–0.9	3.3–4.1
SUVA (L/(mg·m))	1.5–2.8	1.9–2.7	1.5–3.0	0.3–2.2 ¹	3.2–4.8
DOC (mg/L)	6.7–12.5	7.9–10.7	8.8–15.0	0.5–2.6	2.0–2.4
pH	8.2–8.5	8.6–8.7	8.6–8.7	8.3–8.4	8.5–8.6

¹Single outlier removed from range

3.3.2 Sample Collection

Mixed samples of filter media and biomass for microbial community analysis were collected during filter cleaning, which was carried out the day before each ash challenge period began. A final set of samples was collected at the end of the last recovery period (Table 3.3). The top ~0.5 cm of material in the filter beds (including the layer of deposited matter and biomass often referred to as the

schmutzdecke—Figure 3.2) were removed by manual scraping with metal scoops. For filter set A, cleaning was performed without draining the columns. For set B, water levels were dropped just below the bed surface (< 2 cm) to increase the ease of sample collection and reduce water content in the collected samples. Lower sample water contents avoided the need for additional centrifugation steps before DNA extraction. Samples were stored at -80°C until processing. After cleaning, columns were refilled with upward flow (without fluidizing filter media) to prevent the formation of air pockets or voids in the filter beds. Voids can create preferential flow pathways, which may reduce DOC biodegradation (Maurya et al., 2020) and thus introduce variability between biofilter columns.

Table 3.3 Timing of collection events for samples submitted for amplicon sequencing analysis

Filter set	Collection point in project sequence	Sample collection date (YYYY-MM-DD)
A	Before challenge tests (i.e., baseline samples)	2021-10-18
	End of first recovery period	2021-10-23
	End of third (final) recovery period	2021-11-25
B	Before challenge tests (i.e., baseline samples)	2022-11-20
	End of first recovery period	2022-11-29
	End of second recovery period	2022-12-08
	End of third (final) recovery period	2022-12-21

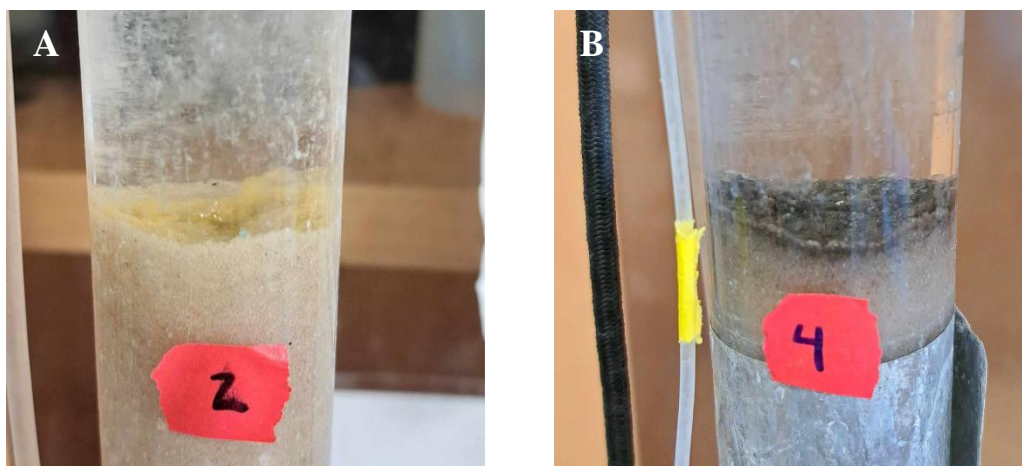


Figure 3.2 Filter bed surface in A) a control biofilter with yellow-pigmented, microbiologically active schmutzdecke that developed after approximately 9 days of operation and B) an ash-challenged biofilter showing clear discoloration from wildfire-ash-impacted water. Both filters were part of set B. The photographs were taken before the final sampling event at the end of the third and final recovery period. Aluminum foil used on all filters to block light is visible in photo B.

3.3.3 Methodology for Bacterial Community Analysis

The microbial community analysis conducted in this study aimed to provide a high-level characterization of biological filter communities based on relative taxonomic abundance data. A key focus was identifying any shifts in community composition that correlated with experimental treatments, source water disturbances, or changes in biofilter performance. The analysis focuses on predominant taxonomic groups. Phylum- and class-level data are presented to be consistent with and comparable to other biofiltration studies that provide metabarcoding analyses. Lower-level taxonomic groups are highlighted when exceptionally predominant, or in reference to environmental conditions or notable community shifts; however, it is important to note that there is evidence that non-abundant taxa can play functionally important roles in the biological treatment of water, such as contributing to the biotransformation of pharmaceuticals and trace organics (Cao et al., 2022; Falås et al., 2018). The analysis of rare taxa thus merits further investigation in future studies of biofilter DOC removal performance but was outside the scope of this work.

Multiple sequence alignments (MSA) and phylogenetic analyses were not carried out because of the broad and exploratory nature of this work; no specific taxon (but rather the entire bacterial community) was of interest at the experiment outset, making these analysis steps impractical. MSAs also serve the function of confirming the accuracy of taxonomic assignments made using the selected gene sequence database. The omission of this quality control step was considered in the interpretation of results.

3.3.4 DNA Isolation and 16S rRNA Gene Sequencing

DNA was extracted from samples collected from both filter sets using a DNeasy PowerSoil Pro Kit (QIAGEN N.V., Venlo, The Netherlands) and following the kit supplier instructions. Centrifugation steps were completed using a Sigma 1-14 microcentrifuge (Sigma Laboratory Centrifuges GmbH, Osterode am Harz, Germany). A Bead Ruptor Elite bead mill homogenizer (OMNI International Inc., Kennesaw, Georgia) was used to homogenize samples. The quantity and purity of extracted DNA were assessed using a NanoDrop 2000 spectrophotometer (ThermoFisher Scientific Inc., Waltham Massachusetts). DNA extracts were stored at -20 °C, then 20 µL volumes were submitted to a commercial laboratory (Metagenom Bio Inc., Waterloo, Ontario) for 16S rRNA gene sequencing. All DNA samples from the same biofilter set were sequenced in a single run. Universal polymerase chain reaction (PCR) primers targeting the V4 region of the 16S rRNA gene were used (515FB: 5'-

GTGYCAGCMGCCGCGGTAA, 806RB: 5'- GGACTACNVGGGTWTCTAAT) (Walters et al., 2015). DNA was sequenced with an Illumina MiSeq and a MiSeq Reagent Kit v2 (Illumina Inc., San Diego, California).

3.3.5 Sequence Data Processing

Demultiplexed paired-end reads generated by sequencing were processed using version 2022.8 of the bioinformatics pipeline, Quantitative Insights Into Microbial Ecology version 2 (QIIME 2; Bolyen et al., 2019). Quality control of the data involved dereplicating and denoising to remove sequencing errors such as chimeras, which are formed by the incorrect joining of DNA from separate organisms (Edgar et al., 2011). These steps were completed using the Divisive Amplicon Denoising Algorithm version 2 (DADA2) plugin in QIIME 2 (Callahan et al., 2016). PCR primers were trimmed from the start of each sequence read, then low-quality reads were filtered out by truncating at the ends. For data from filter set A, forward and reverse reads were truncated at 250 and 243 base pairs, respectively. Reads in both directions were truncated at 250 base pairs for data from filter set B. Paired end reads were then merged to produce a feature table containing Amplicon Sequence Variants (ASVs). Unlike operational taxonomic units (OTUs), which group sequences based on a selected similarity threshold (often 97% similarity), ASVs may be distinguished from one another by as little as a single nucleotide (Callahan et al., 2017). The use of OTUs has been commonplace in metabarcoding studies, but some researchers have shifted toward the use of ASVs due to their improved resolution, comparability across studies, and other benefits (Callahan et al., 2017). Taxonomic assignment of all ASVs was completed using a Bayes classifier pre-trained on the SILVA138 database (v. 132) (Quast et al., 2013).

3.3.6 Community Analysis

Data processed in QIIME 2 were imported into R for community-level characterization. A phyloseq object was created using the package, *qiime2R* (v. 0.99.6) (Bisanz et al., 2021). The package *phyloseq* (v. 1.22.0) (McMurdie & Holmes, 2013) was then used to remove unclassified ASVs, eukaryotic ASVs, and ASVs identified as mitochondrial or chloroplast DNA. Bar charts showing the relative abundance of taxonomic groups in each sample were created using the package *mirlyn* (Multiple Iterations of Rarefaction for Library Normalization; v 1.4.0) (Cameron & Tremblay, 2020). The filtered phyloseq object was then used to generate data frames with updated ASV counts. Library sizes ranged from 16 968–50 297 reads for filter set A and 26 514–90 221 reads for filter set B (Appendix E).

Larger library sizes do not necessarily indicate that a greater number of microorganisms or a greater number of species are present in a sample; thus, to accurately assess and compare sample diversity, differences in library sizes must be controlled. Rarefying data involves subsampling sequence reads such that all sample libraries are equal in size; rarefying repeatedly can help to reduce statistical bias that may be introduced as a result of omitting data (Cameron et al., 2021). The *mirlyn* package was used to generate rarefaction curves, which showed the number of unique ASVs observed in each sample as a function of library size (Figure 3.3). Visual inspection confirmed that values close to the smallest sample library sizes (16 968 reads for sample A-O18-1 in filter set A, 26 514 reads for sample B-N29-5 in filter set B) could be selected as normalized library sizes without omitting a large fraction of the ASVs obtained. Data in the phyloseq object were rarefied to this minimum sample depth 100 times.

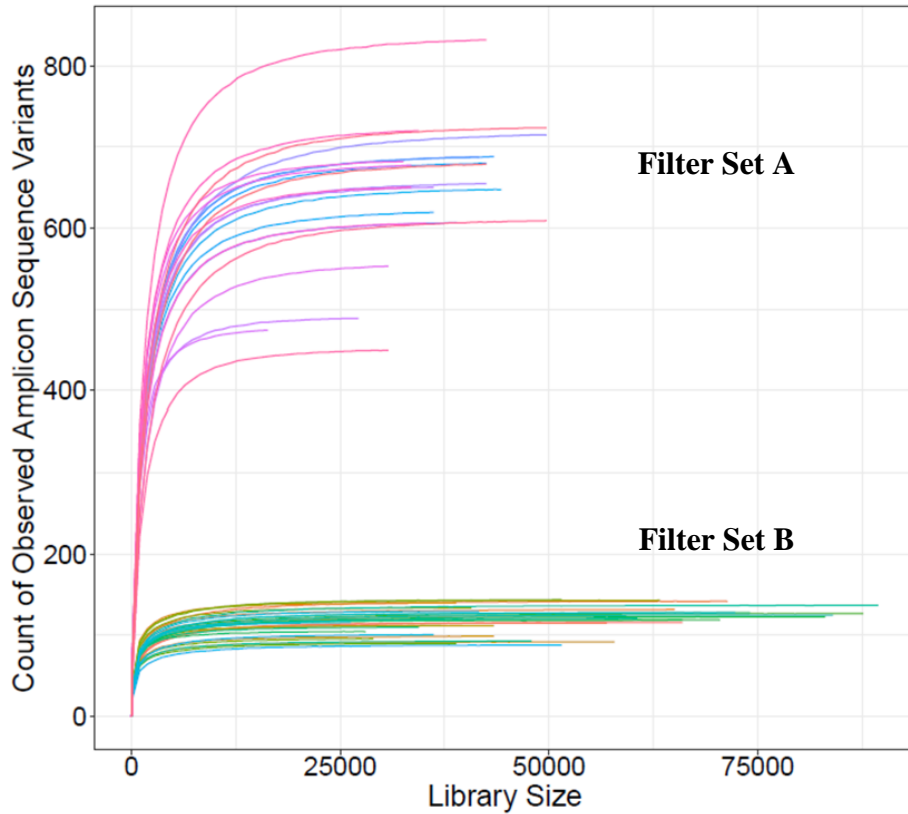


Figure 3.3 Rarefaction curves showing the number of unique amplicon sequence variants (ASVs) observed as a function of library size (i.e., number of reads) generated for merged sample sets from filter sets A and B.

Rarefied libraries were used for diversity analyses. Alpha diversity is a measure of species diversity in a single sample, which is a function of both the number of unique species present (richness) and the evenness of their relative abundances. The Shannon Index (Shannon & Weaver, 1949), a common alpha diversity metric, was determined for all samples using version 1.22.0 of the package *microbiome* (Appendix F; Lahti et al., 2017) and plotted with the *mirlyn* package. Calculation of the Shannon Index assumes that the studied population is large, that sampling is random, and all taxa are present in the sample (Shannon & Weaver, 1949). Pielou's evenness (Pielou, 1966) was assessed using the *microbiome* package as well. Because rarefying repeatedly did not have a significant impact on Shannon Index values (see section 3.4.2), the tabulated Shannon Index and Pielou's evenness values provided in Appendix E were calculated based on sample data sets that were rarefied to their respective minimum sample depths (16 968 and 26 514) only once without replacement. This step was completed using the *phyloseq* package. To verify that this approach was valid, Shannon Index values obtained after rarefying once were compared to several index values for the same samples obtained by averaging the output from all 100 rarefying iterations; the compared index values were found to be within ± 0.01 of each other, which was considered acceptable for the purposes of this work.

Beta diversity analyses provide measures of community similarity across multiple samples; principal component analysis (PCA) was conducted on Bray-Curtis dissimilarity matrices and used to create PCA ordinations with the package *mirlyn* (Appendix G). Bray-Curtis dissimilarities are often selected for the analysis of microbial community abundance data (Kers & Saccenti, 2022). Non-metric multidimensional scaling (NMDS) ordination, another method for beta diversity analysis, was performed with Bray-Curtis dissimilarities in two dimensions to compare samples based on all differentiating factors. Unlike PCA ordination, which was conducted on data that were repeatedly rarefied, original datasets were rarefied only once before NMDS ordination. This step was accomplished using the *phyloseq* package. Sample datasets were also merged to create an overall NMDS plot; in this case, data were rarefied to a sample depth of 16 968 after merging.

To avoid the overinterpretation of diversity data, it is important to understand the limitations of metabarcoding analysis and the potential for unavoidable error in the methods used to obtain and process amplicon sequence data. First, sequencing captures only a fraction of the bacteria present in the sampled environment at a single point in time. There is no guarantee that samples are fully representative of the communities from which they were collected. For example, some taxa will almost certainly go undetected either because they were not captured by random sampling or because of error

introduced by factors like differential losses and recovery during sample handling or differential amplification efficiency during PCR (Pinto & Raskin, 2012; Zhou et al., 2011). Therefore, the assumptions of the Shannon Index may not be fully valid in many situations. The interpretation of non-detects is not always given thorough consideration in microbiological studies, but it has been argued that mishandling of non-detects can lead to inaccurate representations of community characteristics, especially in enumerations studies (Chik et al., 2018). Furthermore, error and bias could be introduced in quality assurance steps applied by bioinformatics pipelines. For example, pipelines differ in the treatment of singletons (sequences with an abundance of 1). Singletons are often removed from sequence data under the presumption of high likelihood that they are the result of sequencing error; however, like the mishandling of non-detects, it has been argued that this practice is potentially problematic as it may discard legitimate data (Schmidt et al., 2022). The DADA2 plugin in QIIME 2 does not infer sequences of single reads, thus singleton omission may have impacted the results of diversity analysis in this study. In summary, statistical analysis of microbial community data will always have some degree of error that cannot be known with certainty, and thus diversity analyses can provide only an approximation of true community diversity.

3.3.7 Statistical Tests

PERMANOVA (permutational multivariate analysis of variance) tests using Bray-Curtis dissimilarities were conducted on non-rarefied microbial community data using the *vegan* package in R (v. 2.6-4) (Oksanen et al., 2022). This test is frequently used to detect significant differences between microbial communities in different sample groups (Anderson & Walsh, 2013). Application of the PERMANOVA test assumes exchangeability of samples. To confirm whether significant findings from PERMANOVA tests were the result of differences in community composition or were simply due to differences in sample group dispersion, an ANOVA test was performed on the Bray-Curtis dissimilarities from each sample point to the sample group centroid. These steps were also completed using the *vegan* package in R.

3.3.8 Water Quality Measurements

Water quality data for filter set B was used to examine biofilter performance alongside microbial community profiles. Effluent dissolved oxygen (DO) in flow through cells constructed at filter outlets was measured weekly using a SympHony DO meter and polarographic electrode (VWR International

LLC, Radnor, Pennsylvania). Details of the methods used to measure other analytes are provided in section 2.3.7.

3.4 Results and Discussion

Biofilter community composition was evaluated by analysing 16S rRNA gene sequence data with a focus on predominant phyla. Findings were compared across filter sets to assess potential effects of source water character on individual taxa. Community dynamics were examined through comparison across sampling events. Diversity analyses were used to identify impacts of experimental conditions and water quality on overall filter community composition, as well as to make potential connections to filter performance results obtained for filter set B.

3.4.1 Microbial Community Composition

Predominant phyla in the filter biomass for both filter sets showed consistent overlap with the findings of previous microbial community analysis studies (Lautenschlager et al., 2014; Liu et al., 2023; Pinto et al., 2012). Many of the detected phyla are frequently found in freshwater environments and can be well suited to oligotrophic conditions (Table 3.4; Humbert et al., 2009). A total of 14 183 unique ASVs were observed in samples from filter set A, which treated a natural surface water matrix. A total of 2667 unique ASVs were observed in the samples from filter set B, which treated a synthetic water matrix with low levels of nutrients. Only 209 ASVs were shared between filter sets. Figure 3.4A and Figure 3.4B show the taxonomic composition of samples by phyla. The plots show that many of the predominant phyla were common to both filter sets, but their relative abundances varied. Community composition was also found to diverge at lower taxonomic levels.

Table 3.4 Notable characteristics of major phyla and classes of interest detected in biofilter beds

PHYLUM Class (if applicable)	Relative abundance		Characteristics	References
	Filter set A	Filter set B		
PROTEOBACTERIA Alphaproteobacteria Gammaproteobacteria	31–79%	61–86%	<ul style="list-style-type: none"> • Well-suited to oligotrophic environments • Can break down complex organic compounds • Competitive in highly nutrient-deficient environments 	Eiler et al., 2003; Hutalle-Schmelzer et al., 2010 Q. Li et al., 2017
ACTINOBACTERIA	2–12%	2–15%	<ul style="list-style-type: none"> • Prevalent in fresh-water environments 	Glöckner et al., 2000; Newton et al., 2011
ACIDOBACTERIA	2–18%	<0.2%	<ul style="list-style-type: none"> • Often found in drinking water biofilms 	Martiny et al., 2005; Van Assche et al., 2019
BACTEROIDOTA	3–20%	5–20%	<ul style="list-style-type: none"> • Often found in drinking water biofilms 	Van Assche et al., 2019
CHLOROFLEXI	1–6%	<0.5%	<ul style="list-style-type: none"> • Often found in drinking water biofilms 	Chao et al., 2015
DEINOCOCCOTA	<0.03%	0.2–10%	<ul style="list-style-type: none"> • Genus <i>Meiothermus</i> can be resistant to environmental stressors like alkaline pH, high temperature, peroxide exposure 	Nobre et al., 1996
PLANCTOMYCETOTA	5–21%	1–9%	<ul style="list-style-type: none"> • Often found in drinking water biofilms • May be competitive in high-turbidity water • Often associated with macroalgal biofilms 	Martiny et al., 2005; Q. Li et al., 2017; Lage & Badoso, 2011
NITROSPIROTA	1–3%	1–6%	<ul style="list-style-type: none"> • Genus <i>Nitrospira</i> are nitrite-oxidizing bacteria 	Martiny et al., 2005
VERRUCOMICROBIOTA	1–8%	1–6%	<ul style="list-style-type: none"> • May be competitive in turbid and high-TOC waters 	Martiny et al., 2005; Q. Li et al., 2017; Van Assche et al., 2019

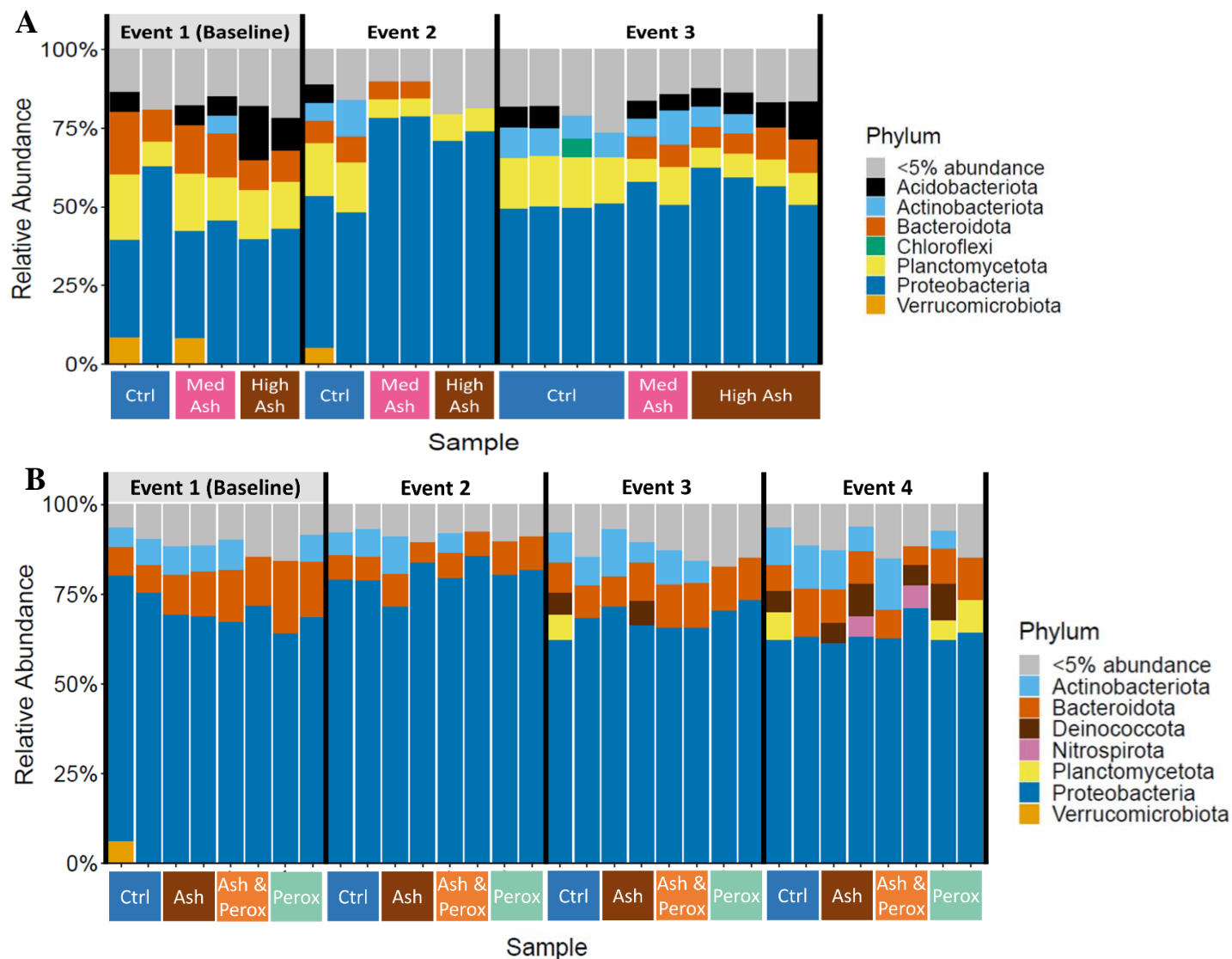


Figure 3.4 Taxonomic bar plots showing relative abundances of major phyla detected in A) filter set A, which treated a natural water matrix, and B) filter set B, which treated a synthetic water matrix. Samples are grouped by experimental condition and sampling event. Phyla accounting for less than 5% of the total sequenced community in each sample are lumped together for better visualization. One sample was collected and sequenced from each filter per sampling event, with the exception of the filters treating low ash content water in filter set A, which were not sequenced. Technical replicates (i.e., two volumes of DNA extracted from the same sample) were submitted for each control biofilter and each biofilter treating high ash content water in the final sampling event for filter set A.

Microorganisms in the phylum Proteobacteria were present in the highest abundance in both sample sets, ranging from 31%–79% of the total sequenced community in filter set A, and 61%–86% of the community in filter set B. Many previous studies of biological filter communities have also found that Proteobacteria are predominant. Moreover, they are frequently found in both water and biofilm samples collected at various other points throughout drinking water treatment systems (C. Li et al., 2017; Wakelin et al., 2011). Proteobacteria are known to thrive in low-nutrient conditions, making them especially competitive in engineered drinking water treatment environments, especially after well-operated coagulation (Eiler et al., 2003). Bacteria in the class Alphaproteobacteria were generally most predominant in filter set A samples. Some Alphaproteobacteria can break down complex organic compounds, which may give them an advantage over other taxa in waters such as the municipally and agriculturally impacted surface water treated by filter set A (Hutalle-Schmelzer et al., 2010). The class Gammaproteobacteria was also present in substantial abundance in biofilter set A samples. Gammaproteobacteria can proliferate in treated water after chlorine disinfection, showing that they are able to compete in nutrient-deficient environments (Q. Li et al., 2017).

In filter set B, Proteobacteria accounted for an even larger fraction of the overall bacterial community than in filter set A. Gammaproteobacteria had a higher relative abundance than Alphaproteobacteria in almost all filter B samples, as might be anticipated in water with low DOC where conditions may select for bacteria well suited to nutrient-poor environments. The synthetic water matrix feeding filter set B had DOC concentrations ranging from 0.5–2.5 mg/L, while the DOC concentrations of the natural water matrix treated by filter set A ranged from 6.7–12.5 mg/L (Blackburn et al., 2023). When analysing the microbial communities of slow sand filters treating a range of surface water types, Bai et al. (2023) also observed a greater abundance of taxa known to be well suited to low-nutrient environments in filters treating water with lower DOC. Liao et al. (2013) found Proteobacteria to be the most abundant phylum in three GAC filter beds, and the relative proportions of predominant classes—Alpha-, Beta-, and Gammaproteobacteria—varied based on the source water.

Members of the phyla Acidobacteriota, Bacteroidota, Chloroflexi, and Planctomycetota have previously been detected in drinking water biofilms (Allgaier & Gossart, 2006; Chao et al., 2015; Martiny et al., 2005; Van Assche et al., 2019). All four phyla were major taxa in filter set A, often accounting for close to or more than 5% of the total community. In filter set B, however, Planctomycetota were less predominant and Acidobacteriota and Chloroflexi were only present in extremely low relative abundance. The presence of algal material in the raw natural water matrix treated

by filter A may have encouraged the growth of Planctomycetota, as these bacteria are often found in the biofilm communities of macroalgae (Lage & Bandoso, 2011; Newton et al., 2011). Actinobacteria were relatively abundant in samples from both filter sets. Planctomycetota and Verrucomicrobiota were found to be more abundant in filter set A than filter set B. Q. Li et al. (2017) observed that bacteria in both phyla can be highly competitive in turbid waters, and that Verrucomicrobiota may be more abundant in higher-TOC environments. A greater relative abundance of these bacteria in biofilters treating higher-turbidity, higher-TOC matrices may have therefore been anticipated. Li et al. also observed higher relative abundance of Actinobacteria with higher turbidity and TOC; however, Actinobacteria had a slightly higher average relative abundance in filter set B, despite the substantially higher DOC/TOC and turbidity in the natural water matrix treated by filter set A.

In filter set B, bacteria belonging to the genus *Acinetobacter* (class Gammaproteobacteria) were often highly predominant, reaching a relative abundance of up to 38% of all bacteria in some samples. Relative abundance did not appear to be correlated with any experimental variables, though it was generally higher in earlier sampling events. Bacteria in the genus *Caulobacter* (class Alphaproteobacteria) were prevalent in several samples from set B filters that were not challenged by wildfire ash, and this genus appeared to have an inverse relationship with *Acinetobacter*. *Caulobacter* reached a relative abundance of more than 25% of total bacteria in some samples, but never accounted for more than 2.1% of bacteria in ash-challenged filters. Inverse relationships in relative abundance could be evidence of direct competition for resources between these two genera. Haig et al. (2015) found that a higher relative abundance of *Acinetobacter* was correlated with better treatment performance (i.e., higher effluent quality) in full-scale slow sand filters; this performance assessment was based on the number of drinking water quality targets (as defined by the European Union) that were achieved across a set of ten different parameters, including DOC, ammonia, nitrate, coliforms, and turbidity. It was speculated that improved performance in biofilters with more diverse communities may be the result of functional redundancy (Haig et al., 2015). This trend was not observed in filter set B; DOC and turbidity removal performance did not decline in later sampling events despite the lower relative abundance of *Acinetobacter*.

The only genus of the phylum Nitrospirota present in either filter set was *Nitrospira*; bacteria in this genus are often the predominant type of nitrite-oxidizing bacteria (NOB) present in natural aquatic environments as well as engineered water systems (Fujitani et al., 2020; Liu et al., 2020; Palomo et al., 2016; Yao & Peng, 2017). They have previously been detected in drinking water treatment plants

and distribution systems where nitrification consumes monochloramines (used for the maintenance of a disinfectant residual) and produces nitrate as an end-product (Martiny et al., 2005; Wang et al., 2014). *Nitrospira* have been found to account for up to 50% of the entire bacterial community in some drinking water biofiltration studies (Gulay et al., 2016), but here they accounted for no more than 5% in any sample. This suggests nitrification was not occurring to a great extent in these biofilters. Although source water microbiomes are often distinct from the microbiomes of the receiving biofilters (Pinto et al., 2012), the notable abundance of this genus in filter set B could be related to the use of monochloramines in municipal tap water (Scott et al., 2015). Low concentrations of organic carbon in the synthetic source water may have also selected for bacteria capable of utilizing non-carbon-containing compounds as electron donors. In addition to the many *Nitrospira* spp. that are NOB, others can use ammonia as an electron donor (Fujitani et al., 2020). Species in the genus *Hydrogenophaga* (phylum Proteobacteria) can oxidize hydrogen (Willems et al., 1989) and were also found in high abundance in filter set B. These bacteria have been detected in biofilters treating groundwater (Hu et al., 2020), and so could be expected to be competitive in other biofilters where oligotrophic conditions are present.

Finally, the phylum Deinococcota was present in extremely low abundance in filter set A but was a major taxon in many filter set B samples. Only one genus, *Meiothermus*, was present in notable relative abundance. *Meiothermus* can be resistant to environmental stressors, including hydrogen peroxide, high temperatures, and alkaline pH (Nobre et al., 1996). As they typically grow in environments with temperatures exceeding 50°C, *Meiothermus* have rarely (if ever) been reported as a predominant taxon in drinking water biofiltration studies. These bacteria may have been introduced to the filters from an external source where favourable growth conditions existed, such as the building hot water system. The ability of *Meiothermus* spp. to compete in low-nutrient, slightly alkaline conditions may have allowed them to temporarily survive in the biofilters.

In summary, the community compositions of samples from both filter sets were generally consistent with the findings of previous studies. Differences in source water quality are hypothesized to be a primary cause of the differences between sample sets. Although pronounced shifts in specific taxa were found to occur within a single filter over time (thus highlighting the potential for considerable temporal variability in biological communities as discussed below), clear differences between the two filter sets existed. This was largely evident when examining the number of taxa and the evenness of

their relative abundance; this concept is discussed further in section 3.4.2. Conditions in filter set B appeared to select for taxa competitive in very low-nutrient environments.

3.4.1.1 Notable Changes in Community Composition Over Time

Temporal changes in specific taxa were identified by visual analysis of taxonomic bar plots organized by sample collection date (Figure 3.4). Statistical significance of temporal changes at the overall community level are discussed in section 3.4.3. Although the experimental phase was relatively short (30 days), previous studies have shown that biological filter communities may shift in composition or exhibit other responses within days, or even hours, of environmental changes. Anderson et al., (2008) saw nearly instantaneous releases of endotoxins from biological communities in rapid biofilters after shutdown and subsequent restart. Zhao et al. (2021) observed significant changes in sand biofilter communities within 10 days of changing hydraulic loading rates and increasing source water concentrations of manganese (an essential mineral for many microorganisms). Using real-time PCR (also called qPCR), Li et al. (2010) detected changes in specific taxa within two days of starting phosphorus addition in biological activated carbon filters. Elevated levels of bioavailable phosphorus have been detected in studies of wildfire-affected streams (Emelko et al., 2016; Watt et al., 2021), and this phenomenon has been linked to increased biofilm growth on stream sediments after one week of exposure (Stone et al., 2011). Based on these findings, rapid change in biofilter communities exposed to phosphorus-rich ash could be possible. Furthermore, the schmutzdecke layer was removed from all filters the day before exposure to WAIW in this study. Community changes driven by the shift in source water quality could be expected to be more pronounced as a result. Haig et al. (2014) observed significant change in the composition of slow sand filter microbial communities in the first three weeks following cleaning and noted that filters operated under similar conditions were most likely to exhibit community composition differences in the early stages of the filter run. Thus, significant differences in the composition of the biofilter communities studied herein could have been possible within the 30-day experimental period.

In filter set A, communities in the control biofilters appeared to exhibit greater stability in phylum-level composition over time compared to ash-challenged filters. This result suggested the variability could be correlated with exposure to shifting source water quality (including fluctuations in readily biodegradable carbon and turbidity; Blackburn et al., 2023) during ash challenge testing. Conversely, in filter set B, control and experimental biofilters appeared to exhibit a similar degree of

variability in community composition over time, and were generally more variable than filter set A. This observation suggests that microbial communities in filter set B may have been less stable in general. Several biofiltration studies have found that biofilter community composition can be relatively steady over time once fully acclimated (LaPara et al., 2015; Morales-Medina et al., 2023). Others have detected significant temporal change in community characteristics (Haig et al., 2015; Ma et al., 2020) and speculated that seasonal changes in water quality parameters like DOC, ammonia, and temperature could be partly responsible (Ma et al., 2020). Even after reaching relatively steady-state performance, slow sand filters may show slight improvements with maturity (Huisman and Wood, 1974); temporal changes in microbial community composition may be the result of continuing gradual acclimation in some cases.

Abrupt increases in the relative abundance of Proteobacteria were observed in both filter sets in the second collection event. These changes may point to a disturbance in the filter communities. In filter set A, the considerable relative increase was restricted to ash-challenged filters and was largely attributed to bacteria from the genus *Acinetobacter* (class Gammaproteobacteria). *Acinetobacter* can survive in highly hostile environments as a result of their high biofilm formation ability (Vidal et al., 1996). While the cause cannot be identified with certainty, the occurrence of the community shift after the first ash challenge test and recovery period suggests that initial ash exposure may have been a contributing factor. As mentioned above, previous laboratory experiments have observed greater rates of stabilizing biofilm growth on wildfire-affected streambed sediments compared to undisturbed sediments (Stone et al., 2011); this phenomenon was linked to releases of bioavailable phosphorus post-fire (Emelko et al., 2016; Watt et al., 2021). Increased availability of phosphorus as a result of ash leaching may have led to the temporary shift in taxonomic composition observed in filters treating WAIW.

The slightly increased relative abundance of Proteobacteria in all set B filters also coincided with a major release of DOC from all biofilters following a change in influent tubing material (see section 2.4.2). Ash-challenged filters released more DOC during both the challenge test and subsequent recovery period, again providing evidence that the introduction of ash may have disrupted biofilter communities. Unlike filter set A, the change in Proteobacteria predominance was not due to the proliferation of a single taxon. In the ash-challenged filters, various less-abundant genera of Proteobacteria appeared to become more predominant. In non-ash-challenged filters, increases in the relative abundance of *Caulobacter* and *Hydrogenophaga* were primarily responsible.

The phylum Deinococcota (and more specifically, the genus, *Meiothermus*) appeared to increase in relative abundance over time in filter set B. Certain *Meiothermus* spp. have manganese catalases (Raposo et al., 2019). Catalases are enzymes that catalyse the reaction of hydrogen peroxide. Mn-catalases may provide an advantage over typical catalases and catalase-peroxidases in conditions of low peroxide stress (Whittaker et al., 2012). Some species in the genus *Meiothermus*, which accounted for most of the bacteria in this phylum, can also use nitrate as an electron acceptor (Nobre et al., 1996). The relative abundance of *Nitrospira* (which are capable of using ammonia and nitrite as electron acceptors) were also seen to increase over time in filter set B samples. Hydrogen peroxide amendment and slight declines in DO concentrations over the experimental phase may have caused selective pressure for these characteristics and allowed the survival of some *Meiothermus* despite unfavourable growth conditions. However, as mentioned previously, these bacteria may have been introduced via the filter influent. Thus, the higher relative abundance may have been the result of more *Meiothermus* being introduced by the source water.

3.4.2 Alpha Diversity

Shannon Index values for filter set A (treating the natural water matrix) ranged from 4.2–5.8 (Figure 3.5A). Shannon Index values for filter set B were substantially lower, ranging from 2.5–3.8 (Figure 3.5B), despite rarefying to larger library sizes that would retain greater diversity. While the procedure of rarefying repeatedly was applied in this work, it was noted that multiple iterations did not appear to have a major impact on the final values of the Shannon Index. The very large library sizes associated with all samples may have made repeated rarefying of data unnecessary in this case. As discussed in section 3.3.6, estimates of diversity can be biased by factors such as sample collection, sample handling procedures, and bioinformatics pipelines. Thus, comparisons of the absolute value of Shannon Index values across different studies may not provide meaningful insights. Values obtained in the current study were therefore not compared in detail to those found in other biofiltration studies, though it was noted that they were within the range of what has been previously reported for biological sand filters (Hunter et al., 2013; Q. Li et al., 2017). The utility of diversity indices for engineered systems such as biofilters has not been examined extensively; their application remains largely exploratory. If connections between biological diversity and the mechanisms affecting biofilter performance can be confirmed, however, diversity indices may play a role in informing biofiltration enhancement techniques (Haig et al., 2015).

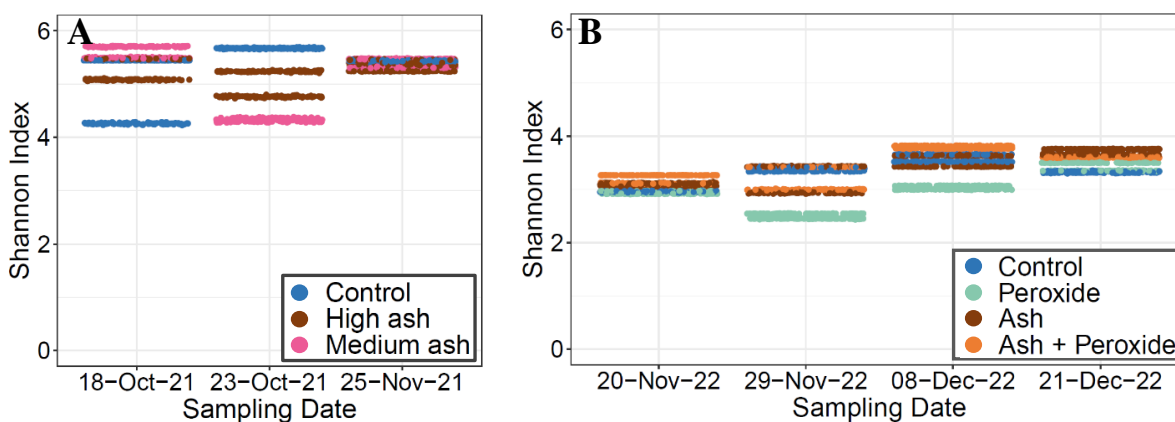


Figure 3.5 Shannon index values for samples collected from A) filter set A, which treated a natural water matrix with higher organic content, and B) filter set B, which treated a synthetic water matrix with low organic content. Samples are organized by sample collection date and colour coded by biofilter type (i.e., experimental condition). The bands corresponding to each sample are created by rarefying the data 100 times.

The disparity in Shannon Index values reported here is largely due to differences in species richness. Though bacteria are not usually classified to the species level by amplicon sequencing analysis, confidence in the finding of lower diversity in filter set B is high, as substantially fewer genera were detected; a total of 527 bacterial genera and 1 archaeal genus were detected in filter set A, compared to 130 bacterial and 0 archaeal genera in filter set B. Pielou's evenness values ranged from 0.68–0.88 for filter set A and 0.55–0.78 for filter set B (Appendix E), indicating the relative abundances of species were slightly more even in filter set A communities on average.

It is hypothesized that the difference in species richness between filter set A and B was related to source water characteristics (described in Table 3.2). The synthetic water matrix treated by filter set B had very low concentrations of organic compounds following GAC pre-filtration. Most of the available DOC was introduced through sodium acetate addition, which presumably would have favoured acetate-utilizing bacteria. In contrast, the natural water matrix contained a more heterogenous mixture of organic carbon in greater concentrations (Blackburn et al., 2023). This matrix was likely able to support a much wider variety of bacterial populations. Furthermore, DO levels in set B filters were low during the entire experimental phase. Huisman and Wood (1974) recommended keeping DO

concentrations at the surface of slow sand filter beds above 3 mg/L to avoid the development of anaerobic conditions. Though DO could not be measured at the bed surface, concentrations in the biofilter effluents were frequently well below the 3 mg/L threshold (falling as low as 1.8 mg/L) during the experimental phase. Thus, conditions at the filter bed surface may have been oxygen-limiting. If so, suppression of bacterial growth by oxygen deficits may have been a contributing factor to the reduced diversity in filter set B, though this theory is speculative as DO was not measured in filter set A.

3.4.3 Beta Diversity

NMDS ordination was used to visualize community relatedness. Figure 3.6 shows NMDS ordinations for the final two sampling events when the effects of the experimental conditions (WAIW and peroxide) would presumably be most evident. The sample sets were plotted individually (Figure 3.6A–B), then merged and plotted together (Figure 3.6C). No clustering of samples was observed based on sampling date or biofilter type (i.e., experimental conditions). Biofilters treating separate source waters were anticipated to have different microbial community compositions due to the introduction of different microorganisms and varying substrate diversity and availability. Multiple biofiltration studies have found source water type to be a significant determinant of microbial community characteristics (Hu et al., 2020; Liu et al., 2023; Pinto et al., 2012). It has also been suggested that filtration rate and EBCT may shape biomass quantities and biological activity due to the direct connection with DOC removal (Bai et al., 2022; Liu et al., 2023). While samples did not form clear clusters based on filter set, which would have indicated a clear effect of source water character on community composition, PERMANOVA tests indicated that bacterial communities in filter set A differed significantly from those in filter set B ($p = 0.041$). In Figure 3.6C, it appears that the two sample sets overlap almost entirely, but there is more dispersion visible in the filter set A samples. PERMANOVA can detect differences in both the location of sample cluster “centroids” as well as sample variance (Anderson & Walsh, 2013); however, an ANOVA test conducted on the two sample sets indicated that there was no significant difference in dispersion between the two groups ($p = 0.28$; Appendix H). Thus, the result of the PERMANOVA test points to differences in community composition between the filter sets; however, the extremely low stress values obtained for all NMDS ordinations must also be noted. Stress is a measure of how well the obtained ordination fits the data (Kruskal, 1964); values close to zero indicate that sample sets may have been too small or too similar to obtain a unique solution. Other NMDS ordinations that are equally correct could therefore exist. Ordinations were repeated several times to verify that no substantially different plots were obtained.

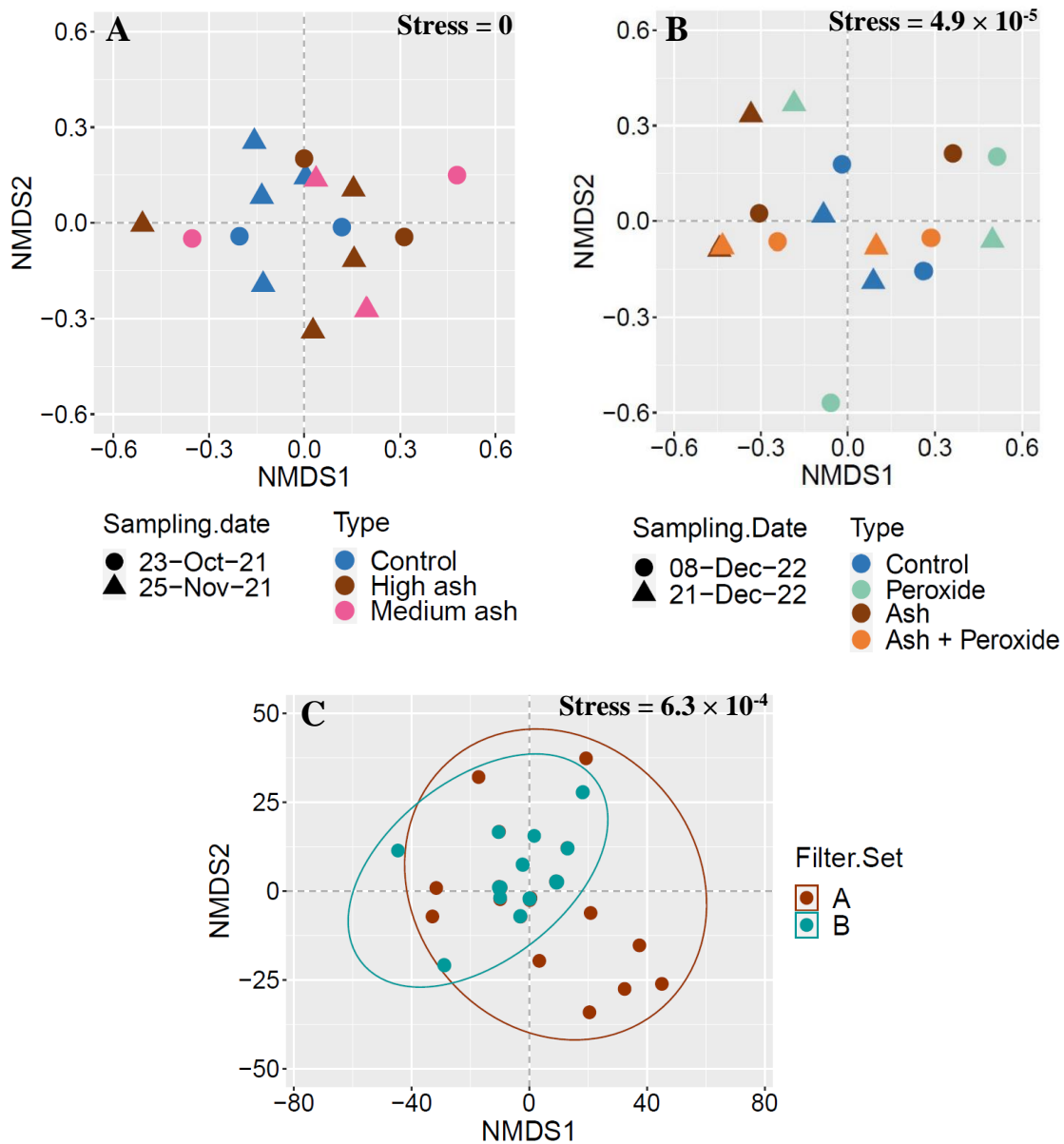


Figure 3.6 NDMS ordination plots comparing bacterial communities in mixed samples of filter media and biomass collected from A) filter set A, which treated a natural water matrix, and B) filter set B, which treated a synthetic water matrix. Only samples collected in the final two sample collection events are shown. Samples are organized by date (marker shape) and biofilter type (i.e., experimental condition; marker colour). Full sample sets were merged in a single NMDS plot (C), with samples organized by filter set. Datasets were rarefied to minimum library sizes only once before NMDS ordination, with the rarefying step being completed after dataset merging in the case of plot C).

In filter set A, no significant differences were detected between or within any biofilter pairs ($p = 1$ for all comparisons; Table 3.5). Community differences between samples when grouped by collection date were not significant ($p > 0.42$). These results agree with NMDS plots, which showed no clustering of samples based on date or experimental condition. In filter set B, significant differences were detected between the bacterial communities in the control biofilters, as well as the conventional ash-challenged biofilters ($p = 0.036$ and $p = 0.041$, respectively). Significant differences were also detected between samples when grouped by collection date ($p = 0.001$). Thus, there was more variation in bacterial communities over time and within biofilter pairs than there was across experimental conditions. Although source water DOC has been found to affect microbial community composition in biofilters and other freshwater environments (Allgaier & Grossart, 2006; Bai et al., 2023), the addition of organic compounds by ash leaching did not have a significant effect on biological filter communities in either filter set over the 30-day experimental phase. Ash leaching added a relatively small amount of DOC to both source water matrices (Table 3.2). The effect on the overall DOC signature was greater in the synthetic water matrix treated by filter set B; however, much of the leached carbon was humic material (see section 2.4.3). The relatively low bioavailability of the leached carbon may partly explain the negligible shift in bacterial community composition.

Table 3.5 Results of PERMANOVA tests comparing bacterial communities in biofilter beds

Data set	Data subset	Grouping (independent variable)	PERMANOVA <i>p</i> -value
Merged filter sets	All data	Filter set	0.041
Set A filters (natural water matrix)	All data	Type (Control, Medium ash, High ash)	1
		Sampling date	0.406
		Biofilter	1
	Final sampling event only	Type (Control, Medium ash, High ash)	1
		Biofilter	1
	Control filter samples only	Sampling date	1
		Biofilter	1
	Ash-impacted filter samples	Type (Control, Medium ash, High ash)	1
		Sampling date	1
		Biofilter	1
	High ash content filter samples only	Sampling date	1
		Biofilter	1
Set B filters (synthetic water matrix)	All data	Type (Control, Ash, Peroxide + Ash, Peroxide)	0.945
		Sampling date	0.001
		Biofilter	0.686
		Ash vs. non-ash	0.778
		Peroxide vs. non-peroxide	0.999
	Final two sampling events	Ash vs. non-ash	0.635
		Sampling date	0.008
		Type (Control, Ash, Peroxide + Ash, Peroxide)	0.734
		Peroxide vs. non-peroxide	0.981
	Final sampling event only	Biofilter	0.332
		Ash vs. non-ash	0.547
		Type (Control, Ash, Peroxide + Ash, Peroxide)	1
	Control biofilters only	Peroxide vs. non-peroxide	1
		Biofilter	N/A (too few degrees of freedom)
	Peroxide biofilters only	Sampling date	1
		Biofilter	0.036
	Ash & peroxide biofilters	Sampling date	1
		Biofilter	1
	Ash biofilters	Sampling date	1
		Biofilter	0.041

Bolded entries indicate a significant difference between sample groups was detected ($p < 0.05$)

In summary, ash challenge tests and peroxide amendment were not found to have a significant impact on biological community composition in either filter set. Some filter pairs showed significant changes over time, as well as between duplicate filters. This finding emphasizes the value of repeated sampling and filter replication in biofiltration studies. As discussed in Chapter 2 with the concept of inter-column variability, biofilters with the same design and operated under the same conditions can exhibit significant differences in average treatment performance, hydraulic performance, and bacterial community characteristics. Thus, experimental data from a single column (even if sampled repeatedly) is not sufficient to evaluate the extent of variability possible between replicate filters. Conclusions regarding the impacts of studied variables on community composition can be based on incomplete information without an assessment of natural filter variability. Filter replication is therefore critical to distinguish natural variability from performance differences that are the result of significant impacts from experimental conditions; unfortunately, the associated increase in work-intensity can make replication difficult in biofiltration research studies. Furthermore, despite the use of filter duplication in the current study, sample sizes were still relatively small for the purpose of statistical analysis when data were grouped by certain factors; PERMANOVA tests may not have been sufficiently powerful to detect minor differences in community composition when small sample groups were compared. Considering the evidence that rare taxa can play functionally important roles in biological communities, minor changes in taxonomic composition may impact filter performance even if statistically insignificant at a community level. Deeper sequencing could help to detect changes in minor taxa as well as differentiate between microbial communities in highly similar environments (Caporaso et al., 2012). Furthermore, factors such as differential losses during sample handling, and differential PCR replication efficiency may have increased the variance in community composition (Zhou et al., 2015). Amplified within-group variance could have obscured significant differences between groups.

3.4.4 Possible Connections Between Community Composition and DOC Removal Performance

The major DOC release event observed in filter set B provided an opportunity to investigate connections between DOC removal performance and changes in biofilter communities. The DOC release may have been due to a (presumably minor) shift in filter influent quality resulting from a change in influent tubing material. Tubing replacement was carried out as a proactive step to avoid clogging and other operational challenges during the experimental phase. When this change was reversed, effluent DOC returned to baseline levels within one day. All samples collected the day after

the return to pre-disturbance performance (sampling event 2) showed increased relative abundance of Proteobacteria. Increased predominance of Alpha- and Gammaproteobacteria, two classes well-suited to very low-nutrient conditions, largely accounted for the change. This shift may have been the result of the die-off of other taxa (and therefore a potential contributor to the DOC release) or it may have been a response to an abrupt decrease in available carbon substrate in the filter bed (and thus an effect of the DOC release). It is interesting to note that the shift in overall community composition was not statistically significant despite the extreme decline in effluent quality. This observation suggests that certain disturbance events may be able to substantially affect biofilter performance without major impacts on bacterial community composition. It may also provide some information regarding the nature of the mechanism(s) responsible for the DOC release. For example, if non-abundant bacterial species were responsible for the DOC release (though the magnitude of the release makes this seem unlikely), die-off of those taxa may not cause a statistically significant change in overall community composition. Furthermore, the DOC release may not have been the result of bacterial die-off at all, but instead a symptom of a bacterial stress response or other changes in biological processes. However, it must also be noted that DNA from inactive and dead biomass retained in the filter schmutzdecke could have been captured by amplicon sequencing (Wang et al., 2023). Thus, even if a substantial die-off event did occur during this period, it may not be evident from the analysis of amplicon sequence data. After the tubing change was reversed, effluent DOC quickly returned to pre-disruption concentrations and the relative abundance of Proteobacteria returned to baseline levels in subsequent sampling events. These observations suggest that rapid recovery of biological filter communities is possible, even following severe disturbances. Disturbance response characterization at the microbial level could be improved by using quantitative abundance methods like real-time and/or digital PCR (qPCR and dPCR). Metatranscriptomic techniques, which allow researchers to analyse gene expression, could also be used to provide a functional profile of microbial communities if samples can be collected during the disturbance response period.

It has been hypothesized that higher species richness and evenness may be correlated with improved biofilter performance and resilience (Haig et al., 2015). Greater community diversity can provide functional redundancy, making it less likely that disturbances affecting certain microbial taxa will impact overall performance. Blackburn et al. (2023) reported that DOC percent removal in ash-challenged filters was generally consistent with controls during challenge testing in filter set A. In filter set B, DOC removal (both percent and absolute) declined in ash-challenged filters relative to controls

(Figure 3.7). In section 3.4.2, it was also noted that filter set B communities had substantially lower species richness and a slightly lower range of evenness values than filter set A. The disparity in community diversity may be connected to the apparent differences in filter sensitivity to ash-associated disturbances. These results are aligned with observations made by Haig et al., (2015), supporting the hypothesis that higher community evenness may contribute to improved performance and response to source water disturbances.

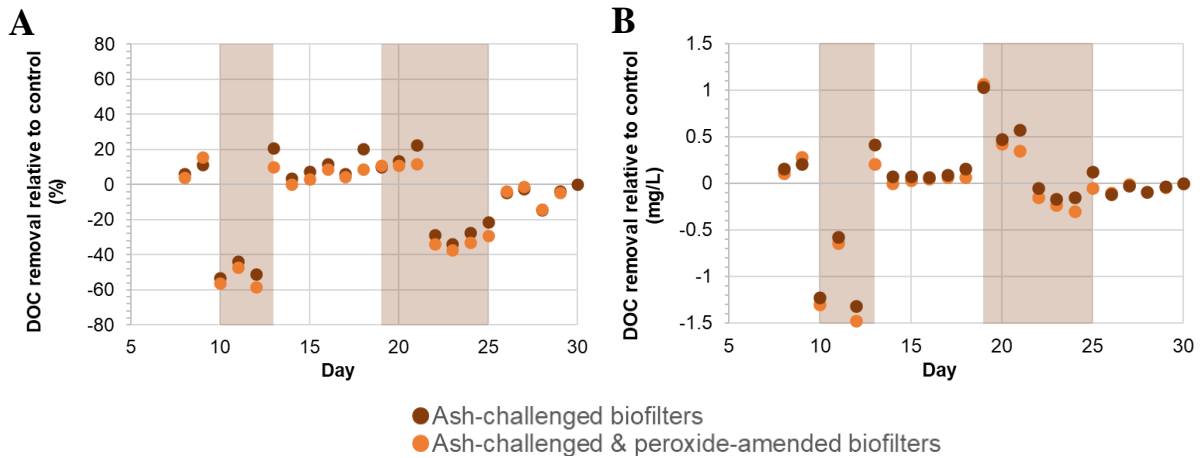


Figure 3.7 Average A) percent and B) absolute DOC removal in duplicate ash-challenged biofilters relative to control biofilters in filter set B during the experimental phase. Ash challenge periods are shown by the vertical brown shaded regions. Plots exclude data collected during the first ash challenge test and recovery period, which coincided with a tubing change.

Finally, two genera often highly abundant in set B ash-challenged filters were *Comamonas* and *Pseudomonas* (phylum Proteobacteria). Members of both genera have been found to degrade a wide range of aromatic organic compounds including polycyclic aromatic hydrocarbons (PAHs) (Goyal & Zylstra., 1996; Khlebnikov & Peringer, 1996). Several studies have found that incomplete combustion of organic material during wildfires can increase the presence of PAHs on the landscape; these compounds may then leach into water when material from the landscape is exported to watercourses (Olivella et al., 2006; Schäfer et al., 2010). The ability to utilize PAHs as substrates could give *Comamonas* and *Pseudomonas* a competitive advantage in WAIW, which was found to have substantially higher concentrations of aromatic organic material than the baseline synthetic matrix (section 2.4.3). It was noted that bacteria from both genera were also isolated from GAC biofilters in

another study that utilized the same tap water source as a base for synthetic biofilter influent (Anderson, 2004). The consistency of these findings increased confidence that detection of these bacteria was legitimate.

3.5 Conclusions, Implications & Recommendations

Bacterial communities in conventional and oxidant-amended biological sand filters were assessed over time as filters were challenged with WAIW. One set of filters treated a natural water matrix with high organic content, while the other treated a synthetic water matrix with low organic content. Insights into the relationships between biological filter community characteristics, source water quality, and biofilter performance were obtained. The results of community analysis highlighted limitations associated with the use of amplicon sequencing to characterize biofilter disturbance response at the microbial level; meaningful interpretation of microbial community data must take these limitations into consideration.

1. Significant differences in the compositions of bacterial communities in biofilters operated under different experimental conditions (severity of wildfire ash impact, peroxide amendment) were not detected; greater variation in community composition existed between replicate filters and sampling events. Thus, at the sequencing depths used in this work, there was no evidence of major or lasting changes to filter bacterial communities due to i) exposure to wildfire-ash-impacted water or ii) intermittent exposure to hydrogen peroxide at a dose of 0.7 mg/L. Comparing filter sets from two different projects indicated that source water character can have a significant impact on bacterial community composition.

Recommendation

Further studies should be conducted at higher peroxide doses and with different amendment schedules to evaluate possible microbial effects of this biofiltration enhancement strategy.

2. This study highlights the necessity of filter "replication" when applying metabarcoding analysis to biofiltration studies. Biofilters operating under identical conditions can have statistically significant differences in bacterial community characteristics. In this case, variation between

the composition of communities in replicate filters exceeded that observed between different filter pairs on several occasions.

Recommendation

Incorporating filter replication helps to validate that differences between communities can be meaningfully attributed to different experimental conditions and are not simply the result of natural variability.

3. Relative taxonomic abundance data may not be sufficient to identify the occurrence of disturbances in biological filter communities, even if severe. On some occasions, minor changes in bacterial community composition were observed to coincide with changes in experimental conditions or biofilter performance disruptions. A major release of DOC from set B filters suggested that a disturbance in the filter beds (possibly at the microbial level) occurred between the first and second sampling events. Unfortunately, the potential biological mechanisms involved could not be identified through the analysis of community composition and diversity alone.

Recommendations

Future challenge test studies should utilize methods like whole-genome sequencing, metatranscriptomics, ATP analysis, and qPCR/dPCR to understand how changes in taxon abundance, gene expression, metabolism, and other microbial functions may be linked to biofilter disturbance response. These tools may also help to identify changes in minor or rare taxa that are not statistically significant to overall community composition but have the potential to alter filter function. A better understanding of the mechanisms behind biofilter disturbance response may help drinking water utilities be better prepared for wildfire-associated source water disturbances.

4. Observations made in the current study support the hypothesis that connections exist between biofilter performance and community diversity. Bacterial communities in filter set A were found to have a considerably higher species diversity than communities from filter set B.

Blackburn et al. (2023) reported generally consistent DOC removal across all set A filters during ash challenge tests, while a decline in DOC removal performance relative to control biofilters was observed for set B filters treating ash-impacted water.

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Chapter 4

Conclusions and Implications

4.1 Overview

The primary goal of this research was to evaluate conventional and oxidant-amended biofiltration as strategies to increase drinking water treatment resilience to wildfire. The core of this work focused on advancing the understanding of biofilter responses to source water disturbance at both the treatment performance and microbial community level. The use of filter designs and hydraulic conditions that simulated slow sand filtration processes made the findings of this study especially applicable to small drinking water systems, which may be disproportionately challenged in adapting to the impacts of climate change. The main conclusions of this research are presented in this chapter. Additionally, limitations of the work and key insights gained from challenges encountered in its execution are presented and used as a basis for recommendations for future research studies. Possible implications of these key insights for drinking water utilities are also outlined.

4.2 Conclusions

1. Declines in biological filter effluent quality may occur during wildfire-associated source water disturbances; however, biofilters may still offer some resilience to drinking water utilities by exhibiting consistent responses and recovering rapidly post-disturbance without the need for operator adjustment. These traits may be desirable for small drinking water systems where operators may not always be available to respond to source water disturbances.
 - The DOC removal performance of biological sand filters was assessed throughout a series of challenge periods during which they received wildfire-ash-impacted water (WAIW). Effluent DOC was elevated relative to control biofilters and baseline performance when treating WAIW; however, effluent DOC concentrations were generally stable within each challenge test and recovery period.
 - While ash-challenged filters achieved lower bulk DOC removal relative to control biofilters during challenge periods, DOC characterization indicated that biodegradation was not necessarily impaired by exposure to WAIW. Results of

LC-OCD analysis showed that disparities in DOC removal performance could be explained by 1) higher concentrations of readily biodegradable acetate persisting in the baseline source water at the time of influent sampling and 2) the higher concentration of slowly biodegradable DOC in the WAIW.

- Amplicon sequencing was used to create taxonomic profiles for two different sets of biofilters subjected to ash challenge testing. Analysis of sequencing data did not indicate significant differences in bacterial community composition between ash-challenged biofilters and control biofilters. In one instance, a notable increase in the relative abundance of a predominant taxon (Proteobacteria) was observed in all ash-impacted filters following the introduction of WAIW, but not in subsequent samples. This observation suggests that biofilter bacterial communities may experience shifts in composition in response to source water disturbances but can subsequently return to pre-disturbance states.
 - Biofilters demonstrated an ability to consistently recover to pre-disturbance levels of effluent turbidity and DOC removal within 20 hours of returning to baseline source water conditions (i.e., by the end of the next effluent sample collection). In future challenge studies, more frequent sampling should be conducted post-disturbance to capture biofilter recovery rate and behaviour.
2. Intermittent hydrogen peroxide amendment using the selected methodology (one-hour treatments twice per week at doses of about 0.7 mg/L) is not likely to be an effective strategy to extend biofilter run time. There was no evidence that peroxide amendment affected the accumulation of organic matter in the filter schmutzdecke over the 30-day experimental phase. While peroxide did not have the anticipated benefits, it is also notable that no evidence of impaired DOC biodegradation was observed in peroxide-amended biofilters. Furthermore, peroxide-amendment did not lead to significant changes in bacterial community composition by the end of the 30-day experimental period. However, one notable observation was that both peroxide-amended biofilters treating baseline source water released substantially less DOC than all other biofilters (including controls) following a change in influent tubing material.

Further analysis to determine the significance of that observation was beyond the scope of this work.

3. Changes in biofilter performance, including those associated with short-term source water disturbances, may not be accompanied by significant shifts in bacterial community composition. Significant changes in community composition were not detected in filters challenged with WAIW for two, four, and seven days, despite substantial shifts in the DOC character, DOC concentration, and turbidity of the source water being treated.
4. A connection between bacterial community diversity and biofilter performance may exist. The filter set with less diverse bacterial communities (filter set B) showed a higher sensitivity to source water disturbances; effluent DOC was elevated in set B filters treating WAIW, while Blackburn et al. (2023) reported that performance was generally consistent across all set A filters during challenge tests. Differences in the quality of baseline source water are likely to have played a role in these performance disparities, however. Filter set B treated a water matrix of higher quality than filter set A. It is widely understood that several operational and technical factors put drinking water systems treating high quality source waters at greater risk of performance decline or failure during periods of deteriorated water quality (Smith et al., 2011; White et al., 2006).
5. A substantial degree of variability in both treatment performance and bacterial community composition can exist between biofilters with the same design and operating conditions. The variability in treatment performance between pairs of biofilters subjected to different experimental conditions was often similar to the variability between duplicate filters. Similar observations were made at the bacterial community level in filter set B, where significant differences in community composition were more often found between replicate filters than between filter pairs. While this variability may be less significant at larger scales, it is nevertheless an important consideration for utilities conducting pilot studies to determine whether biofilters meet treatment objectives for their systems, especially if those objectives are

stringent. Furthermore, understanding the degree of homogeneity possible within and between filter communities will be important if biofiltration enhancement strategies are to one day be “customized” based on microbial community characteristics. Finally, this finding also highlights the importance of implementing filter replication in biofiltration research.

6. Biofilters treating different source waters can have microbial communities with significantly different compositions. Previous biofiltration studies incorporating microbial community analysis have reported similar results.
7. 16S rRNA gene sequencing provided a high-level snapshot of biofilter community composition, diversity, and dynamics; however, the taxonomic data provided by amplicon sequencing techniques are not sufficient for applications such as characterizing biofilter responses to source water disturbances. Quantitative community analysis and functional profiling may be needed to understand the relationships between biofilter treatment performance and microbial-level responses to such events.

4.3 Challenges Encountered

Challenges encountered in this work demonstrated several pitfalls and possible limitations of bench-scale biological filtration research. If not controlled or accounted for in data analysis, these limitations have the potential to reduce the reproducibility of results, obscure significant findings, and lead to incorrect conclusions. Laboratory research must balance control of environmental conditions with practical relevance in a way that is aligned with the research objectives. In this proof-of-concept work, researcher control was prioritized over the simulation of real-world conditions. Discussed below are some of the problems experienced in the execution of this experiment. Potential steps to eliminate or mitigate these problems through strategic experimental design or modified laboratory methods are highlighted.

4.3.1 Influent Instability

Unstable source water DOC concentrations in the influent reservoir were a persistent problem throughout this experiment. Influent samples were collected from this reservoir at the start and end of effluent sample collection. A synthetic source water was used with the goal of having greater control over influent quality than if a natural water matrix had been used. However, the DOC concentration in the influent batches often decreased substantially from the time of nutrient amendment (immediately before connection to the biofilters) to the time of sample collection and analysis, making it difficult to confirm that the biodegradative capacities of the biofilters were being accurately measured. While acetate has been used as an influent carbon source in other biofiltration studies (Anderson et al., 2008; Xin et al., 2008), filters were typically operated at higher hydraulic loading rates with inline injection of sterile acetate solutions in these instances. The longer influent reservoir retention times in this experiment (which were needed due to the very low hydraulic loading rates) allowed for greater consumption of readily biodegradable acetate (Jiang et al., 2022). Sterilizing influent batches with UV light before connection to the biofilters and thorough cleaning of influent buckets between batches did not fully control this problem. Thus, it is hypothesized that biofilms on the transfer tubing placed in the influent buckets were the primary source of biological activity. While slower DOC consumption would be expected in natural water matrices with less biodegradable organic compounds, this observation nevertheless shows that biological activity outside of biofilter columns may have significant effects on influent quality. At larger scales and higher flow rates, these effects are anticipated to be much less pronounced and potentially more reflective of actual operations. For bench-scale research, however, smaller conduits and reservoirs lead to greater contact between water and biofilm-coated surfaces. With the extended contact times of slow sand filtration processes, the potential for influent DOC biodegradation increases even further. As a result, paired influent and effluent DOC measurements may not reflect the extent of DOC removal occurring in the filter bed itself.

4.3.2 Biofilter System Size

The small size of the bench-scale biofiltration system reduced operational demands of the project, but also complicated some experimental and analytical procedures.

4.3.2.1 Low Flow Rates

Achieving the high EBCTs characteristic of slow sand filtration processes required a low filtration rate. As a result, effluent sample collection took 3–5 h. Avoiding long sample collection periods is desirable

for the purpose of sample preservation. Identifying the optimal point at which to collect representative influent samples was also difficult due to the long EBCT and changing influent quality. Furthermore, the use of a low filtration rate precluded in-line chemical amendment. For this reason, nutrients were added manually to batches of influent, and peroxide amendment was performed by diverting influent lines to a separate reservoir and sending a pulse of pre-amended water into the top of the filter columns. Despite laminar conditions in the water column, longitudinal mixing would have decreased the concentration of hydrogen peroxide reaching the filter bed. Finally, low flow rates and a small water-air interface may have led to low influent dissolved oxygen levels, increasing the risk of anaerobic conditions developing at the filter bed surfaces (Huisman & Wood, 1974).

4.3.2.2 System Sensitivity

The small scale of the experimental setup also presented concerns about increased sensitivity of the filter system. For example, replacement of the influent transfer tubing was followed by a major release of DOC from all filter beds. It is hypothesized that the new tubing leached substances that were in some way harmful or inhibitory to microorganisms in the filters. The small tubing diameter would have allowed a high amount of contact with the influent water, possibly increasing the concentration of any leached substances beyond what would occur with larger tubing. Furthermore, smaller columns may be more likely to be disturbed by slight forces applied during maintenance or sampling, especially if the filter height-to-diameter ratio is very large. Resultant settling of the bed or dislodging of biofilms may impact biofilter performance.

4.3.2.3 Media Sampling

Small bed sizes also constrained options for media sampling. Media samples for amplicon sequencing could not be collected between filter cleaning events, as this would have disrupted the schmutzdecke layer and affected filter performance (Weber-Shirk & Dick, 1997). Even when the entire schmutzdecke layer was removed, collected volumes of mixed media and biomass were less than 1.5 cm³. Thus, the number of analyses that could be conducted were limited and replication was often infeasible. Furthermore, a column diameter that was many times smaller than the water column height made filter cleaning difficult to perform in a consistent manner. Media sampling ports at bed surface may have reduced this challenge, but schmutzdecke removal caused slight changes to the bed height and may have negated the convenience of ports.

4.4 Lessons Learned

Several steps can be taken to prevent similar challenges in future biofiltration studies. Use of acetate and other readily biodegradable carbon compounds as primary influent carbon sources should be carefully considered, especially when working with low flow rates. To isolate and more accurately quantify the removal of dissolved constituents by biofiltration, influent samples should ideally be collected from the water column relatively close to the filter bed surface. Removal of particulate matter, however, may be more accurately quantified by collecting a sample from the influent reservoir after mixing or at the filter inlet due to the potential for sedimentation in the water column. Additional planning may be required to design an optimal sampling schedule. Key considerations include but are not limited to 1) the volume of effluent sample that can be collected at one time while ensuring sufficient preservation and 2) selecting the relative timing of influent and effluent sample collection (e.g., simultaneous or staggered) to ensure they are as close as possible to being paired.

Filter sizing should balance operational demands and compatibility with experimental procedures. As outlined in previous subsections, small column diameters and low flow rates can complicate influent quality control, source water amendment, and sample collection. Conversely, the use of higher flow rates can make biofiltration projects more challenging, particularly when collection and transportation of large quantities of source water are required. The work-intensive nature of biofilter experiments may be a factor in the lack of column replication in many bench- and pilot-scale studies; operating additional columns increases the time needed for source water collection and preparation, sample analysis, and routine maintenance. However, as demonstrated by the results of this work, there can be a considerable degree of variability between columns operated under near-identical conditions. Column replication is therefore critical for meaningful comparisons of biofilter performance and should be a priority in future biofiltration experiments.

4.5 Other Recommendations for Bench-Scale Biofiltration Experiments

Daily influent and effluent sampling are typical in long-term biofiltration studies. This work demonstrated that when conducting challenge testing, higher-frequency sampling regimens may be required to properly capture biofilter disturbance response and recovery behaviour. Here, effluent

turbidity and DOC concentrations showed that the biofilters had largely recovered to pre-disturbance performance levels by the first effluent sample collection (about 20 hours, or 3.2 EBCTs) following the return to baseline source water conditions. When targeting long filter EBCTs, fully capturing these phases may require sampling at inconvenient hours and for extensive periods.

Comparison of the current study with work done by Blackburn et al. (2023) shows that biofilters treating different source waters may exhibit different disturbance responses at both the treatment performance and microbial community levels. The disparities in experimental observations underscore the need for further biofilter challenge testing using 1) baseline source waters with a diverse range of characteristics and 2) a range of disturbance types and severities.

4.6 Implications for Metagenomics in Biofiltration Research

Amplicon sequencing was selected for this project due to the broad focus and exploratory nature of the work; though this method provides less detailed information than other sequencing techniques, it is less costly and thus allowed for a greater number of samples to be sequenced. Amplicon sequencing involves sequencing a targeted region of the genome that can provide sufficient information to classify taxonomic groups (Zhou et al., 2011). For bacteria and archaea, the 16S rRNA gene is typically targeted for this technique; this gene has both conserved and variable regions that allow the identification of different microorganisms when sequences are compared to a reference database (Greay et al., 2019). In the last decade, a surge of experiments applying NGS technologies to engineered drinking water environments have provided valuable insights into treatment technologies and the drinking water microbiome. While the heightened interest in using molecular biology tools to study biofilter function is therefore well-founded, it is important that the inherent limitations of these methods are not neglected in the interpretation of gene sequence data and the assessment of their value. Many biofiltration studies that have used NGS techniques have not discussed the scope, technical biases, and overall confidence in the data. As they are often published in journals that are not focused on biology, it can be assumed that a substantial proportion of readers would not have detailed prior knowledge of molecular biology tools. A brief discussion about the meaningful interpretation of amplicon sequencing data is provided here to give context to the findings of this work and support recommendations for future metabarcoding and metagenomic studies of biofilters.

Zhou et al. (2011) demonstrated that amplicon sequencing results are not highly reproducible. Even with thorough sample homogenization and careful sample handling procedures that avoid differential losses, several factors are known to introduce error to amplicon sequencing results, including inadequate random sampling methodologies, differential amplification efficiencies during PCR, and sequencing errors (Schmidt et al., 2022). Genetic sequencing provides a snapshot of a small portion of a microbial community at a single point in time. Even in bench-scale biofiltration research where conditions may be relatively controlled and the sampled community may be contained in a small area, there is no guarantee that randomly collected samples are fully representative of entire communities. DNA is extracted from small quantities of material and only a small fraction of this extract is sequenced. The PCR process is not guaranteed to amplify all sampled DNA evenly and can result in highly biased read counts (Pinto & Raskin, 2012). Thus, it is important to understand that not all members of biological communities are captured in sequencing processes.

For these reasons, amplicon sequencing data are not reliable enough to provide quantitative estimates of organism abundance; only the approximate relative abundance of various taxa can be assessed (Zhou et al., 2011). Furthermore, data processing techniques can affect final taxonomic profiles. The accuracy of taxonomic assignment depends on the quality of data in the 16S rRNA database used, however, errors and disagreements between databases are known to exist (Edgar, 2018). As sequencing of a single amplicon in the bacterial genome is often insufficient to provide species-level identification (Greay et al., 2019), researchers perform a final sequence grouping step. Many metabarcoding studies group sequences into operational taxonomic units (OTUs) based on similarity to a selected reference sequence and use these units as surrogates for unique species. In this work, unique amplicon sequence variants (ASVs) were quantified; ASVs, by contrast, can differ by as little as one nucleotide. Callahan et al., (2017) provides a detailed argument for the benefits of replacing OTUs with ASVs; one of the major advantages is increased comparability across studies, as ASVs are not associated with a particular selected reference sequence present in the studied dataset.

The limitations of amplicon sequence data were considered when drawing conclusions in the current study and discussing levels of confidence. At the sequencing depths used, no significant differences in community composition were detected between filter pairs that treated the same baseline source water but were operated under different experimental conditions. Sequence depth (also referred to as read depth or library size) refers to the number of times that a given nucleotide in a genome is read during an experiment (Sims et al., 2014). Results of a study by Caporaso et al. (2012) indicated

that sequencing to relatively shallow depths is often sufficient to detect community differences that would be meaningful in most applications; the additional information provided by deeper sequencing may not justify the higher costs in many studies. However, Caporaso et al. noted that detecting changes in rare taxa or differences between communities in highly similar environments may require greater read depths. Higher sequencing resolution may therefore be desirable in some applications, especially given the increasing recognition of the functional importance of rare taxa.

As mentioned in Chapter 3, the use of techniques that allow quantitative analysis of taxa abundance (e.g., qPCR, dPCR) and functional profiling (metatranscriptomics) could play a critical role in illuminating the connections between microbial communities and filter performance. Meaningful disturbance response characterization was found to be infeasible using only amplicon sequence data obtained in the current study. Nevertheless, amplicon sequencing can still provide valuable information in biofiltration research. For example, conducting high-level surveys of a large number of samples may help to narrow down locations, points in time, or even particular taxa on which to focus higher-resolution techniques. If particular taxa of interest are identified, sequence alignments can be performed to validate the accuracy of taxonomic assignments; applying these techniques to full community datasets is impractical due to the required computational time and resources (Bazinet et al., 2018).

Sample replication is not always incorporated into microbial community studies, presumably due to the considerable costs of sequencing. While the non-quantitative nature and inherent bias of amplicon sequence data may limit the power of statistical comparison tests regardless of replication, Prosser (2010) argues the practice is still critical and highlights the need for both technical replicates and biological replicates. Technical replication involves the sequencing of multiple aliquots of the same DNA extract to assess the variability introduced by sequencing methods. Biological replication would involve sequencing multiple samples from the same location and collection event to assess community variation between theoretically identical samples. Replication is particularly important when assessing differences between sample groups (i.e., beta diversity analysis). The results of the current study demonstrated that bacterial communities in replicate biofilters can have statistically significant differences in composition at moderate sequencing depths.

4.7 Implications for the Drinking Water Industry

While pilot- and full-scale trials using the source water treated by a specific utility are needed to properly anticipate biofilter performance in that treatment system, some process and analysis insights from this study are generally applicable to full-scale unit processes. Therefore, they may aid utilities with climate change strategic planning.

- Though the exploitation of natural biologically mediated processes allows biofilters to remove DOC from drinking water with relatively low environmental impact and cost (Brown et al., 2015), this study demonstrated the potential challenges associated with biological treatment technologies with low operational control. Anticipating and troubleshooting performance declines proved to be especially complicated due to the “black box” nature of biofiltration processes. As wildfire and other climate-change-exacerbated landscape disturbances may result in water quality changes that are unprecedented for treatment facilities, the need to safeguard public health may require increased monitoring of biological treatment processes when there is a risk of deteriorated water quality. In the current study, biofilters demonstrated an ability to achieve consistent removals of the LMW fraction of DOC in WAIW, indicating that biological function was maintained during exposure to ash. However, given the variable nature of ash on burned landscapes (Goforth et al., 2005) and the potential for toxic agents (e.g., heavy metals) to be mobilized post-fire (Harper et al., 2019; Kelly et al., 2006), biofilter responses to water impacted by different types of wildfire ash may vary.
- The examination of DOC removal during ash-challenge testing highlighted the importance of characterizing source water DOC. LC-OCD analysis showed that the increased effluent DOC concentrations during ash challenge periods were not evidence of impaired biodegradation but were largely the result of preferential removal of non-humic DOC fractions. However, the specialized instrumentation and time required for LC-OCD analysis make this technique impractical for real-time monitoring and potentially cost-prohibitive for small utilities. Monitoring source water UV_{254} may be a more cost-effective alternative for small utilities to assess the impacts of wildfire on source water quality. Though less specific and detailed than LC-OCD data, UV_{254} can provide information about both concentration and aromaticity of

DOC. Influent and effluent UV_{254} showed a clear response to the presence of WAIW in the current study (Appendix C).

- Molecular biology tools may provide value to drinking water utilities as investigative or diagnostic tools for biofiltration and various other components of the drinking water system. For example, analysing taxonomic and functional profiles of biofilter communities may help to better understand shifts and variability in biofilter performance, as well as detect evidence of undesirable conditions like oxygen depletion or the occurrence of nitrification. If financial resources available for microbial community analysis are limited, amplicon sequencing may help utilities to identify high-priority locations and times for more thorough metagenomic study. Short-term monitoring of biological filter communities may be desirable during particular events like the introduction or modification of upstream treatment processes or a change in the source water supply; however, at present, molecular biology tools are likely unsuitable for routine monitoring and other long-term applications. High costs and the need for specialized expertise make the frequent use of these technologies impractical for many utilities (Hooper et al., 2019). Furthermore, technical limitations reduce their value for certain monitoring objectives. For example, the inability of amplicon sequencing to achieve species-level identification makes this technique unsuitable for pathogen detection (Greay et al., 2019). Additionally, sequencing technologies are useful only for retroactive analysis; processing may take weeks to months from the time of sample submission to the return of sequence results. Microbial communities in biological filters can change substantially over such timescales, as demonstrated in the current study as well as studies by Haig et al. (2015) and Ma et al. (2020). In addition to temporal variability, the spatial heterogeneity of microbial communities in biological filters means that multiple samples may be needed from each biofilter unit for sufficient characterization.
- Though still in the research phases, biofiltration engineering and enhancement strategies may be valuable tools for climate change adaptation in drinking water treatment. By advancing knowledge of the biological functions that are integral to biofiltration, metagenomics may help to guide the development of these strategies and, in the future, allow customization of biofilter function for utility-specific conditions and objectives (Kirisits et al., 2019).

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Appendix A

Column Set-up Procedure

The following procedure was used to assemble biofilter columns. Steps 2 and onward were developed by Emma Blackburn with input from Dr. Monica Emelko and Dr. Sarah Dickson-Anderson.

1. Silica sand, chosen for its non-adsorptive properties, was washed with tap water and dried at 105°C for 40 h. The sand was autoclaved at 121°C for 45 min to eliminate any surviving or heat-activated microorganisms. Glass liquid chromatography columns (Cytiva Life Sciences, Marlborough, Massachusetts) were autoclaved at 121°C for 15 min. Sand was packed into the columns to a depth of 70 cm while agitating the columns continuously using a handheld massage tool. Agitation eliminated larger voids in the sand bed to minimize differential settling and help prevent formation of preferential flow pathways.
2. Sand columns were purged with up-flow of carbon dioxide at a maximum flow rate of 100 mL/min to expel other gases present in the filter bed. Six bed volumes of carbon dioxide passing through the beds was assumed to be sufficient to fully purge the beds. Because carbon dioxide is highly soluble in water, entrapment of gas bubbles would be unlikely during column filling.
3. Dechlorinated (i.e., GAC-filtered) tap water was sparged with helium for approximately 20 min to eliminate any dissolved gases. The sand columns were subsequently filled with the degassed water through up-flow at a flow rate of 1 mL/min.

Appendix B

Organic Matter and Biomass Analysis Procedure

Samples of filter media collected after the final recovery period were analysed to quantify and characterize organic matter present at the filter bed surface. In many biofiltration studies, extracellular polymeric substances (EPS) are measured as a surrogate for biofilm quantity. Here, an alternative method using TOC analysis and volatile solids (VS) measurements was developed to quantify biomass due to the selectivity of EPS quantification methods, as well as the anticipated difficulty of preventing cell lysis during extraction.

Samples consisting of biomass and sand were mixed thoroughly mixed. One gram (1 g) of sample as measured and combined with 10 mL of ultrapure water, Biomass was separated from filter media by immersing the mixture in an ultrasonic bath for 10 min, vortexing for 5 s, and decanting the slurry (Lauderdale et al., 2011; Lee et al., 2020). Compared to other physical techniques, sonication is more effective at extracting both soluble (or “free”) and bound EPS; however, the high likelihood of cell lysis if sonication is applied for any substantial period of time reduces its utility for targeted EPS quantification (D’Abzac et al., 2009; Pellicier-Nàcher et al., 2013). Here, cell lysis was desirable as breakdown of microbial biomass would improve particulate organic carbon (POC) recovery in TOC measurements. Media were rinsed, then dried and retained for solids analysis. A 1 mL volume of the biomass slurry was diluted with ultrapure water, filtered with a 0.45 µm syringe filter (Agilent Technologies, Santa Clara, California) and characterized with LC-OCD analysis (Elhadidy et al., 2017). A 2 mL volume of the decanted slurry was combined with 5 mL of 0.01 M sodium hydroxide and diluted to 50 mL with ultrapure water. Combined use of sonication and alkaline chemical treatment has been shown to significantly improve the recovery of POC in TOC analysis by reducing particle size and increasing solubility (Lee et al., 2020). The mixture was sonicated for a further 10 min before DOC and TOC analysis.

Dried filter media samples were weighed, then heated at 550°C for 30 min in a muffle furnace. The volatile solids masses lost on ignition were taken as the masses of organic matter remaining on the media. The total masses of organic matter per gram of filter media in the original mixed samples were estimated from the TOC concentrations of diluted slurry, combined with the masses of volatile solids on the filter media.

Appendix C

Supplementary Plots – Water Quality Data

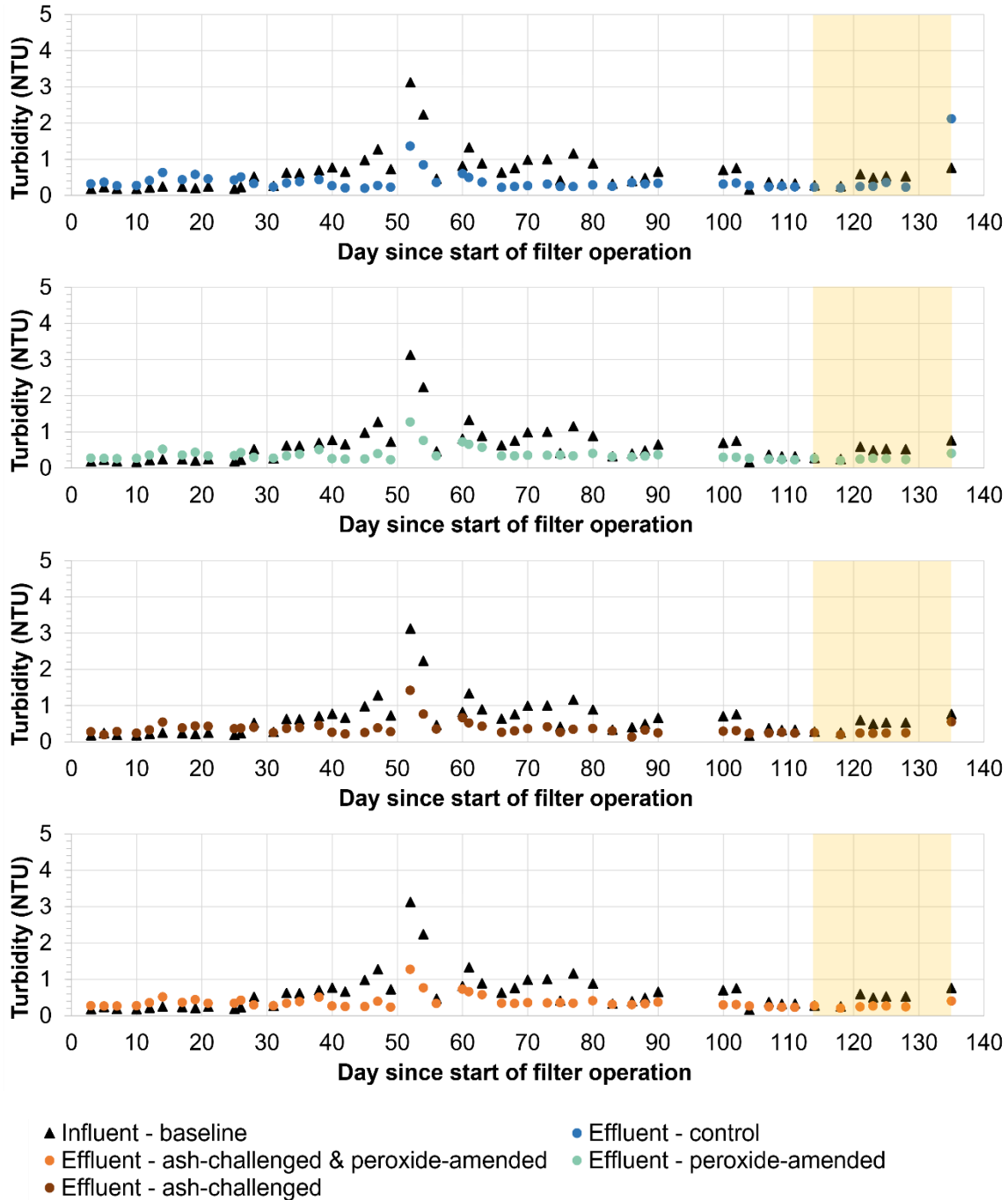


Figure C.1 Influent and effluent turbidity over the column acclimation period and baseline period. Vertical yellow shaded regions show the baseline period.

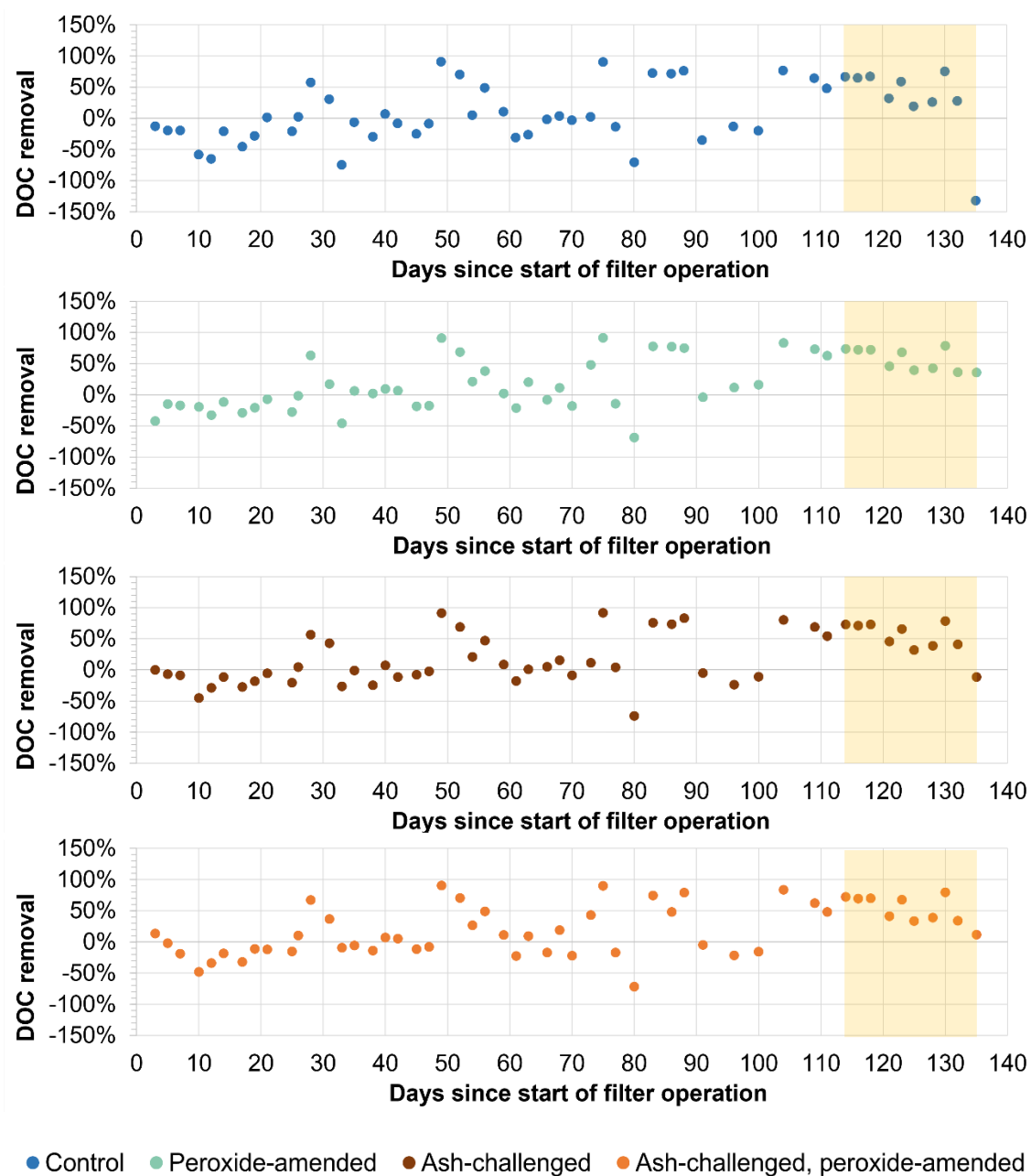
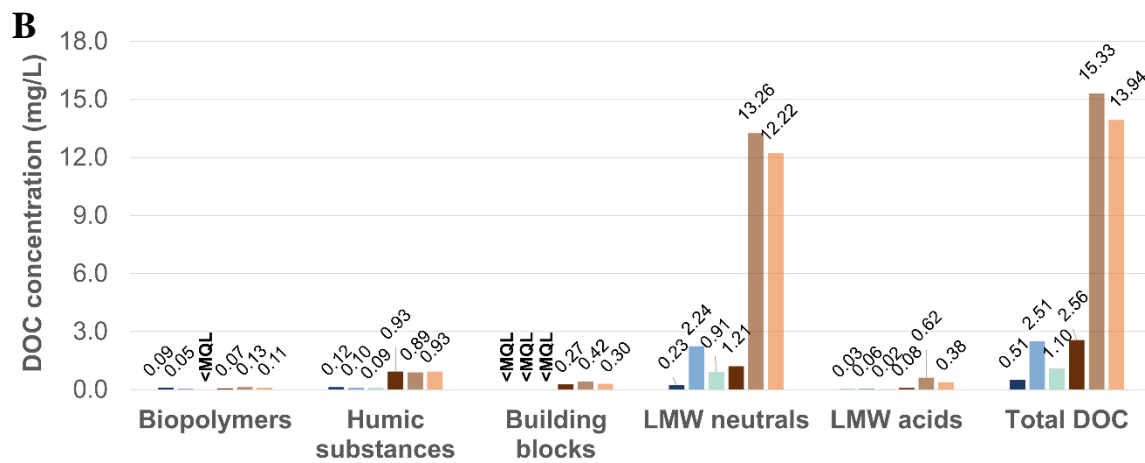
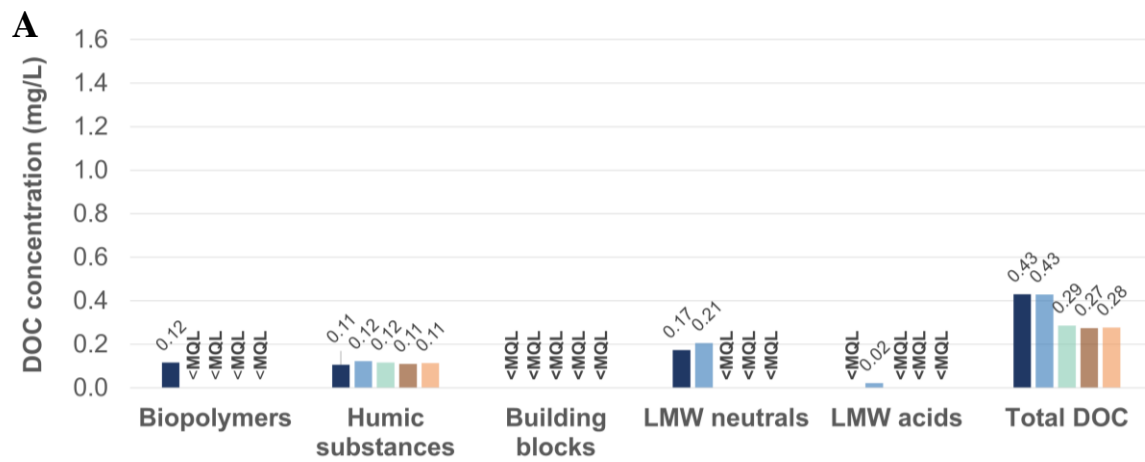
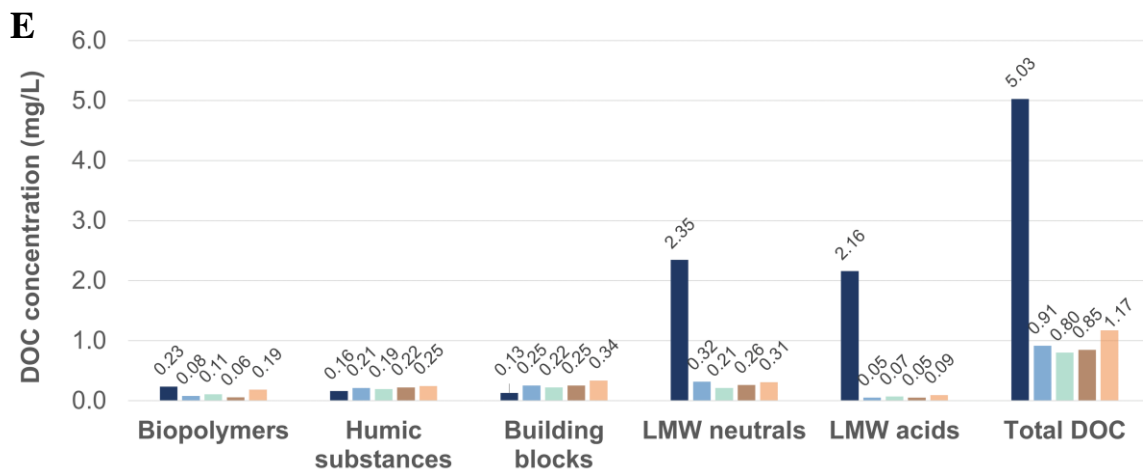
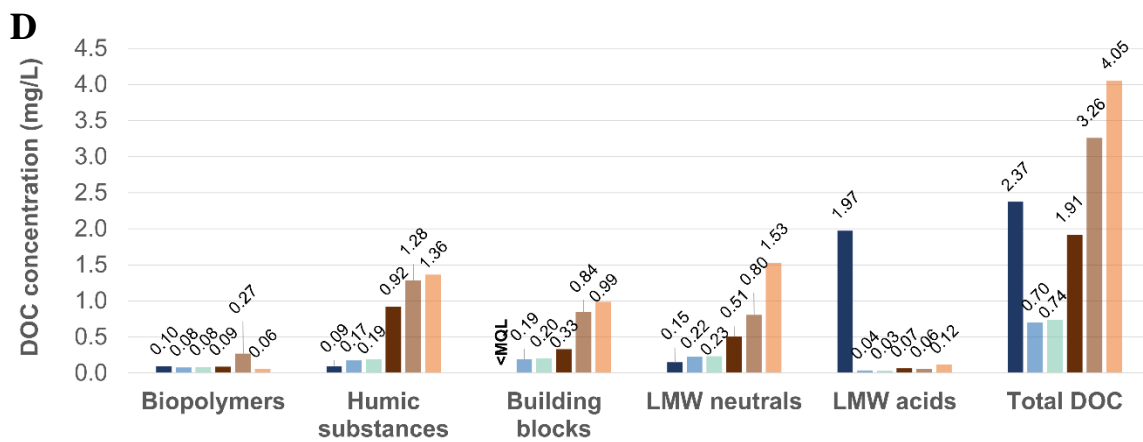
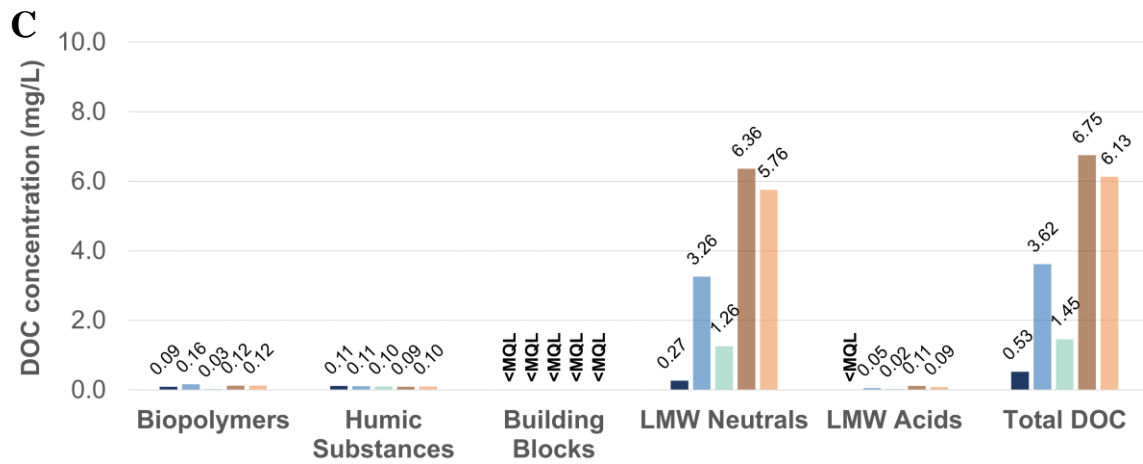


Figure C.2 DOC removal (%) over the column acclimation period and baseline period. Vertical yellow shaded regions show the baseline period.





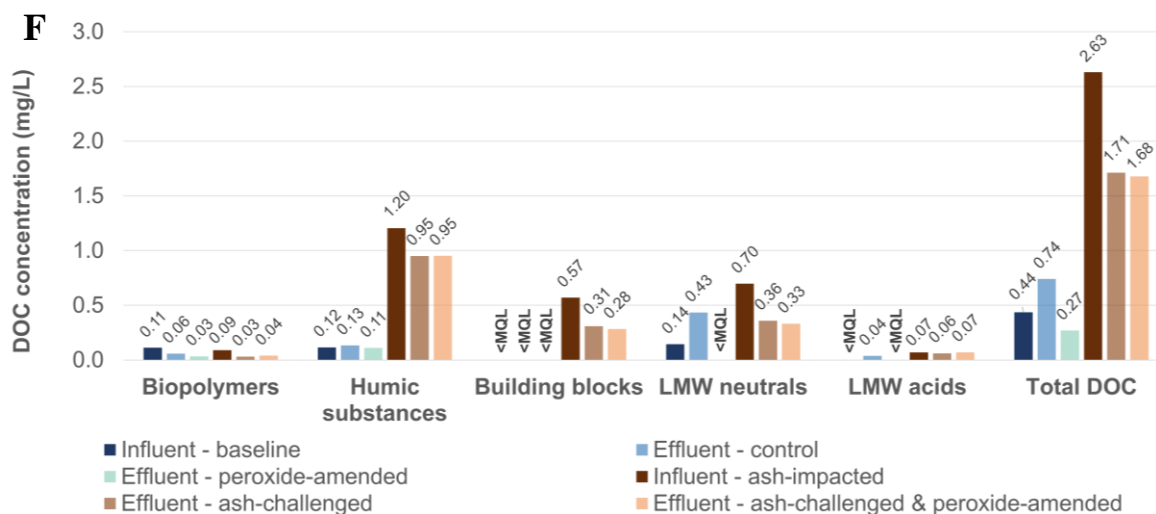


Figure C.3 Hydrophilic DOC fractionation of additional samples collected during the A) baseline period, B) first ash challenge test, C) first recovery period, D) second ash challenge test, E) second recovery period, and F) third ash challenge test. The average concentrations of samples from replicate columns (n=2) are shown by each bar. If the averaged concentrations fell below minimum quantitation levels, “<MQL” is shown in place of a bar. Error bars are not displayed, as the information they can provide for pairs of samples that are not exact replicates is limited. Note that the sampling events conducted during the first ash challenge test and recovery period occurred during a performance disruption that coincided with a change in influent tubing. Evidence of possible contamination is visible in effluent samples from the ash-challenged filters in the second ash challenge test (D), as the elevated effluent DOC is not consistent with bulk DOC measurements conducted on the same samples.

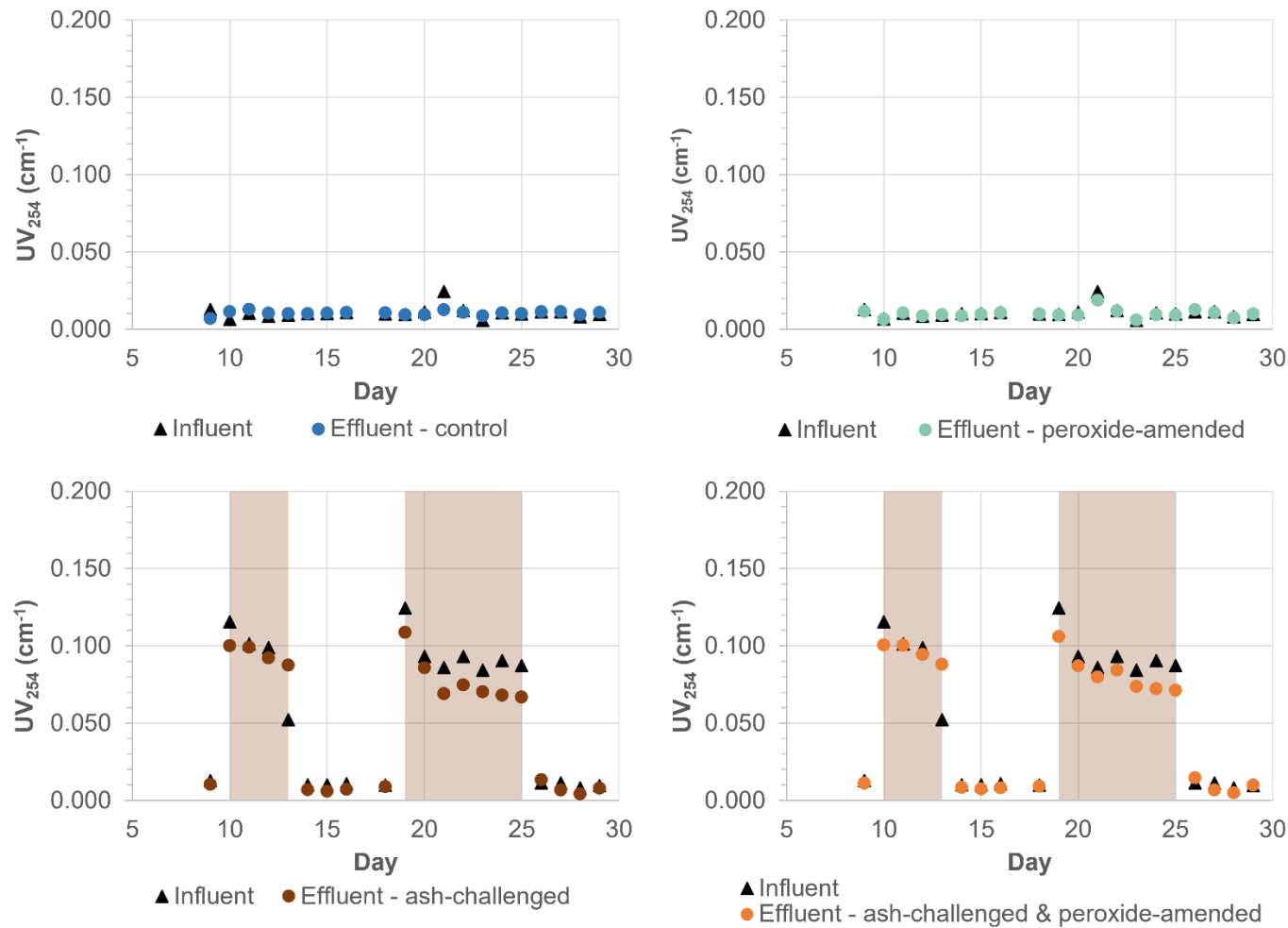


Figure C.4 Influent and average effluent UV₂₅₄ for each replicate biofilter pair over the 30-day experimental phase (acclimation and baseline phases not shown). Plots exclude data collected during the first ash challenge test and recovery period, which coincided with a performance disruption possibly associated with a change in influent tubing. Ash challenge periods are shown by the vertical brown shaded regions.

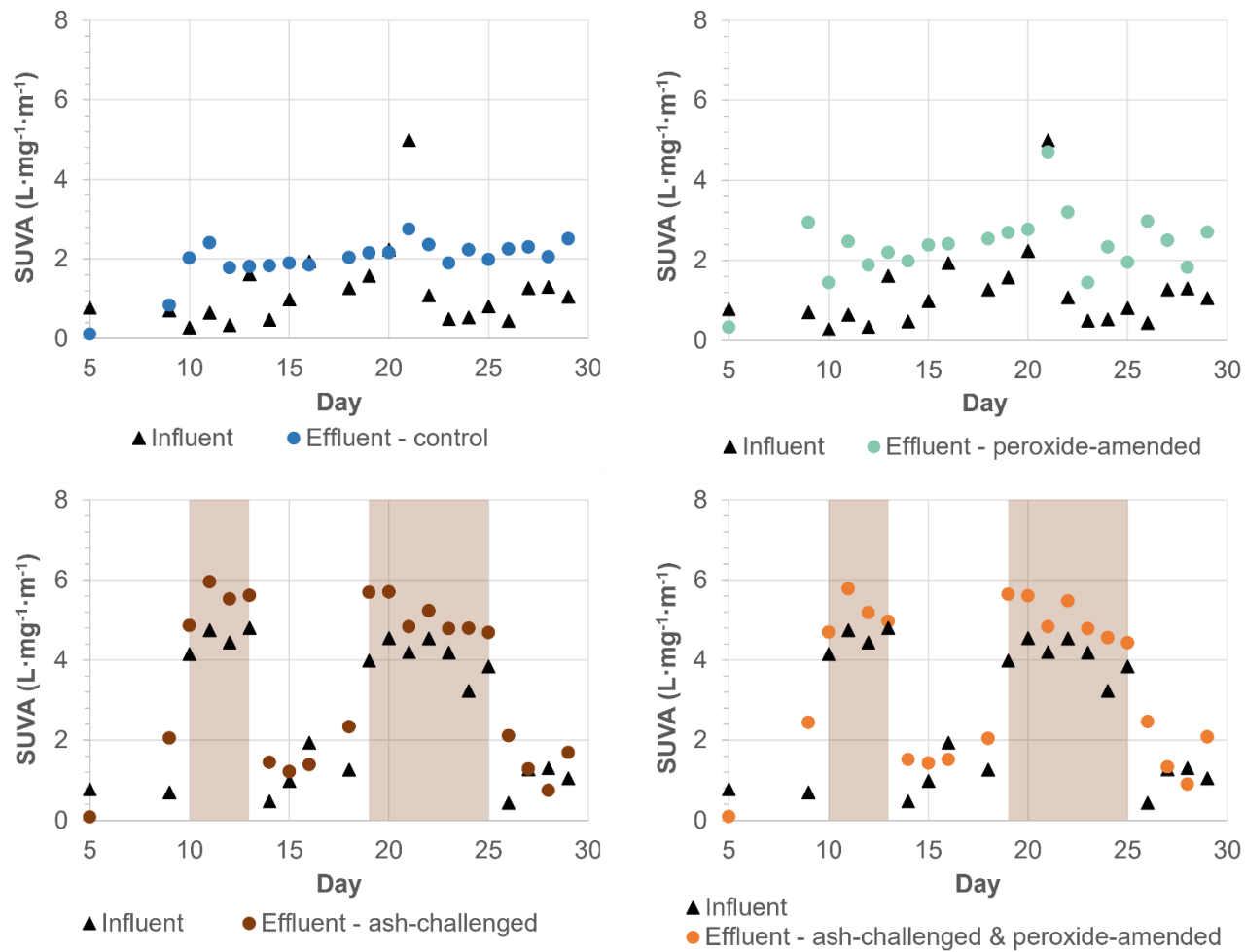


Figure C.5 Influent and average effluent SUVA for each replicate biofilter pair over the 30-day experimental phase (acclimation and baseline phases not shown). Plots exclude data collected during the first ash challenge test and recovery period, which coincided with a performance disruption possibly associated with a change in influent tubing. Ash challenge periods are shown by the vertical brown shaded regions.

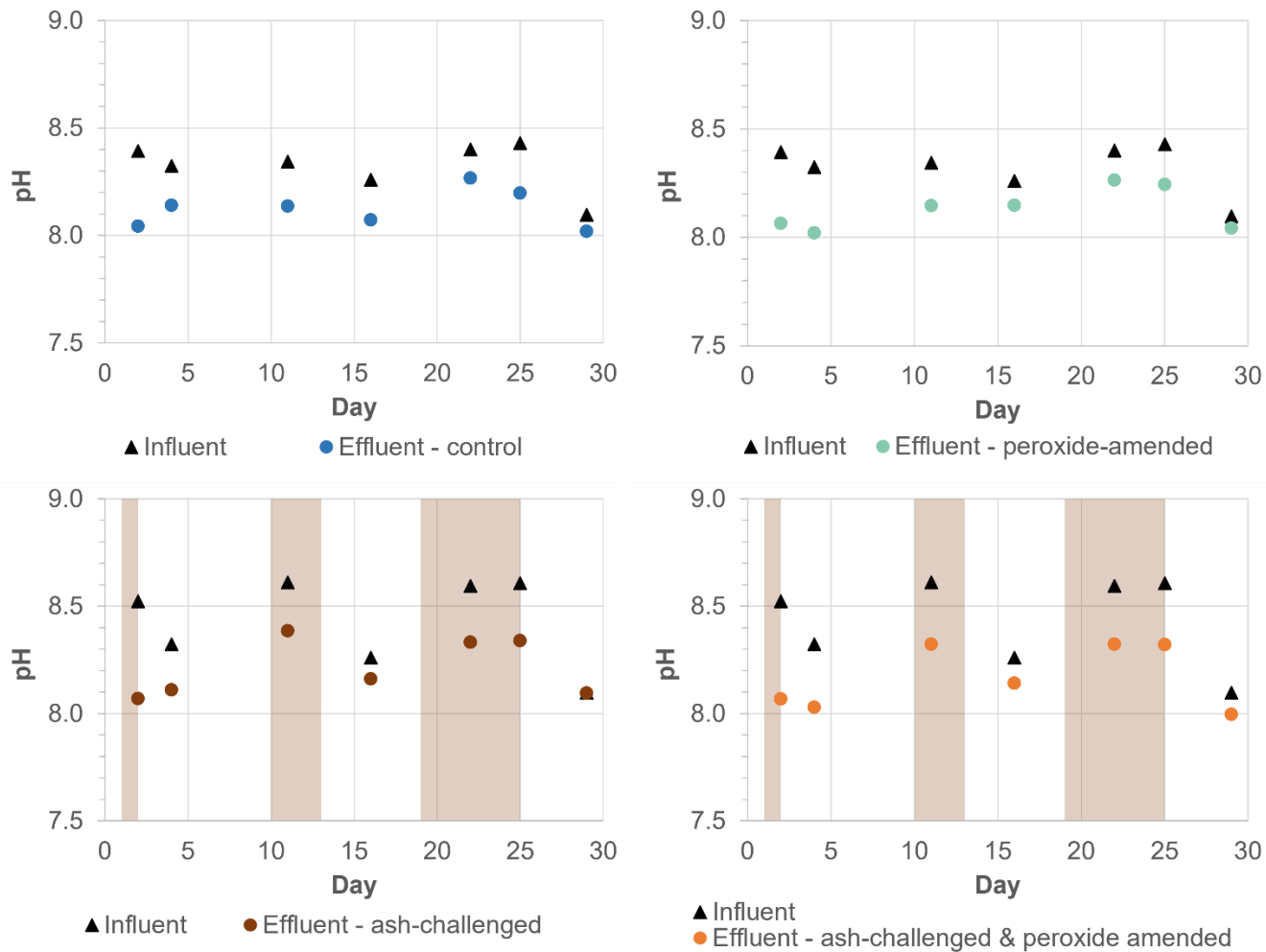


Figure C.6 Influent and average effluent pH for each replicate biofilter pair over the 30-day experimental phase (acclimation and baseline phases not shown). Ash challenge periods are shown by the vertical brown shaded regions.

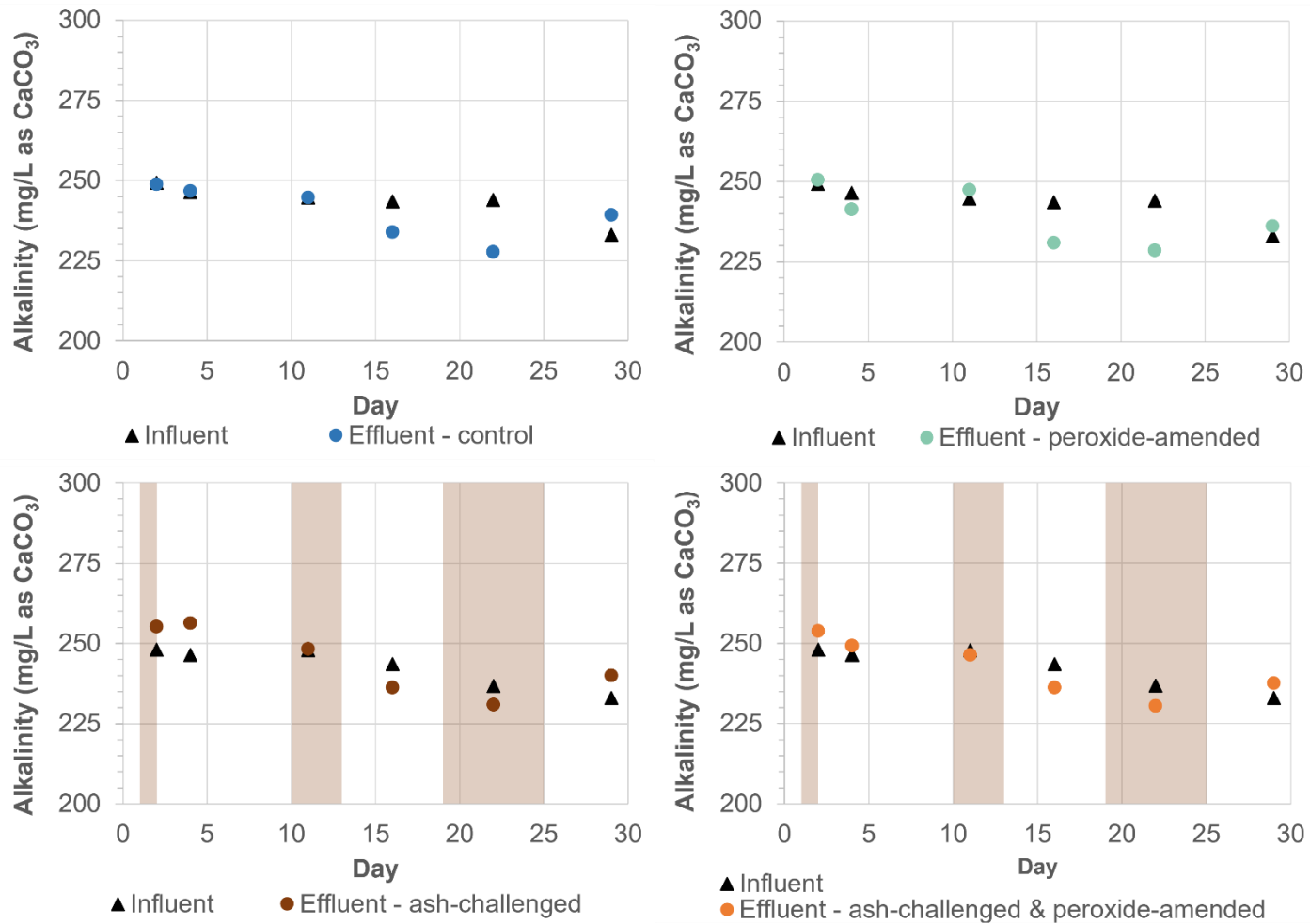


Figure C.7 Influent and average effluent alkalinity for each replicate biofilter pair over the 30-day experimental phase (acclimation and baseline phases not shown). Ash challenge periods are shown by the vertical brown shaded regions.

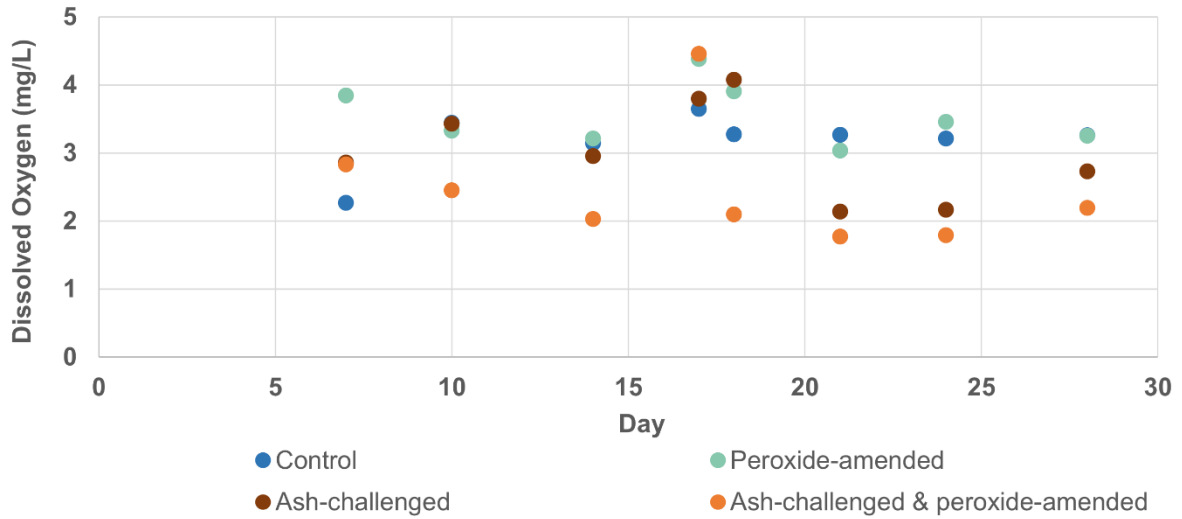


Figure C.8 Effluent dissolved oxygen concentrations throughout the experimental phase.

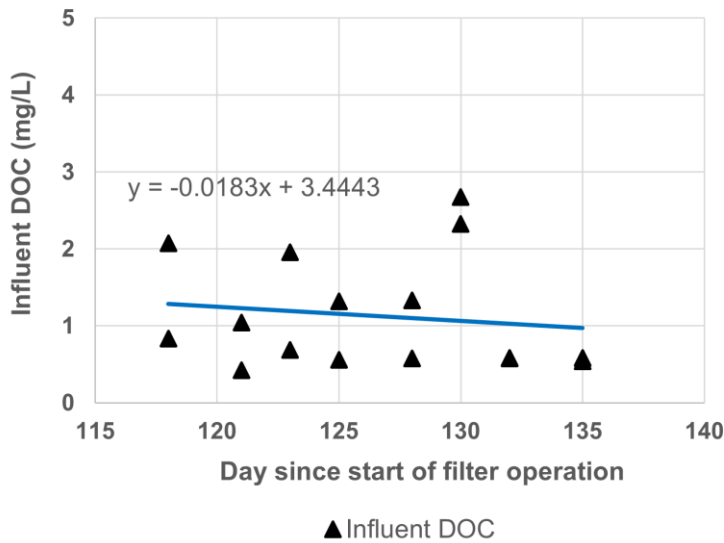


Figure C.9 Influent dissolved organic carbon concentrations during the three-week baseline period leading up to ash challenge testing. Samples collected at the start and end of the effluent collection period are plotted separately. Linear regression was conducted to confirm that no upward or downward temporal trend was present in the influent DOC ($p = 0.61$).

Appendix D

Statistical Test Results – Water Quality Data

Paired t-tests with a significance level of about 10% ($\alpha = 0.1$) were used to compare biofilter effluent quality data across filter pairs, as measurements were taken from each filter on the same schedule (i.e., the data were paired temporally). A lower threshold for establishing significance was considered acceptable in this case because of the degree of natural performance variability possible in passive biological treatment processes, as well as the proof-of-concept nature of the work (Emelko et al., 2016). Data from both biofilters in each pair were aggregated and treated as replicate measurements for the same experimental condition, or “treatment group”. Combining data from replicate columns ensured that the “within-group” variability assessed in statistical tests incorporated not just performance variability of a single filter column, but also inter-column variability. Variances were assumed to be unequal.

Unpaired t-tests with a significance level of 0.1 were used to compare influent and effluent quality data. Two influent samples were collected daily due to the variability in influent DOC over the effluent collection periods; one sample was collected and analysed at the start of the collection period, the other was collected and analysed at the end. Statistical tests were conducted on the raw data, meaning influent and effluent data could not be considered as pairs. Variances were assumed to be unequal.

Assumptions of the t-test include that data are approximately normally distributed or (for the paired test) that the differences between data pairs are approximately normally distributed. Normality was assessed with normal probability plots (NPP) in combination with the Shapiro-Wilk test using a significance level of 0.01. The null hypothesis is that data are normally distributed; a *p*-value below the significance level would result in rejection of the null hypothesis and conclusion that data are likely not normally distributed. A lower significance level was used in this case because a degree of normality can be tolerated in t-tests without issue. Data were grouped 1) period-by-period, 2) by lumping periods (e.g., all recovery period data were aggregated), or 3) across the entire experimental phase excluding the first ash test and recovery period when a performance disruption occurred. The final grouping was only tested for filters that did not undergo ash challenge testing. Shapiro-Wilk test results are shown below for primary performance parameters (effluent DOC, DOC removal, and turbidity). Certain data groupings showed evidence of non-normality. A bootstrapping technique could be used in such cases to assess the significance of differences between non-normal datasets, but this analysis was beyond the scope of this work. Here, to avoid the use of multiple test types, t-tests were applied to all data.

A second assumption of t-tests is data independence. The independence of measurements collected from the same biofilter at different times is debatable, however, data independence is frequently assumed in biofiltration studies that use a daily sampling regimen so that comparative tests can be applied. The same approach was therefore taken in this study.

Table D.1 Determination of DOC data normality using Shapiro-Wilk test

Period	Sample type	Shapiro-Wilk test <i>p</i> -value	Normal (Y/N)
Experimental phase (excludes first ash test and recovery period)	Influent – Baseline	4.6E-05	N
	Effluent – Control	3.3E-04	N
	Effluent – Peroxide	0.10	Y
PERIODS LUMPED			
Baseline (n=20)	Influent – Baseline	0.016	Y
	Effluent – Control	5.5E-07	N
	Effluent – Ash-challenged	0.067	Y
	Effluent – Ash-challenged, peroxide-amended	0.11	Y
	Effluent – Peroxide	0.037	Y
Ash tests (2 and 3) (n=22)	Influent – Baseline	6.7E-04	N
	Influent – Ash (i.e., WAIW)	1.6E-03	N
	Effluent – Control	1.8E-03	N
	Effluent – Ash-challenged	0.10	Y
	Effluent – Ash-challenged, peroxide-amended	0.016	Y
	Effluent – Peroxide	0.26	Y
Recovery periods (2 and 3) (n=20)	Influent – Baseline		Y
	Effluent – Control	0.086	Y
	Effluent – Ash-challenged	0.77	Y
	Effluent – Ash-challenged, peroxide-amended	0.45	Y
	Effluent – Peroxide	0.13	Y
PERIOD BY PERIOD			
Ash test 2 (n=8)	Effluent – Control	0.12	Y
	Effluent – Ash-challenged	0.045	Y
	Effluent – Ash-challenged, peroxide-amended	0.070	Y
	Effluent – Peroxide	0.79	Y
Recovery period 2 (n=10)	Effluent – Control	0.074	Y
	Effluent – Ash-challenged	0.76	Y
	Effluent – Ash-challenged, peroxide-amended	0.019	Y
	Effluent – Peroxide	0.19	Y

Ash test 3 (n=14)	Effluent – Control	0.65	Y
	Effluent – Ash-challenged	0.043	Y
	Effluent – Ash-challenged, peroxide-amended	8.3E-03	N
	Effluent – Peroxide	0.56	Y
Recovery period 3 (n=10)	Effluent – Control	0.68	Y
	Effluent – Ash-challenged	0.30	Y
	Effluent – Ash-challenged, peroxide-amended	0.99	Y
	Effluent – Peroxide	0.37	Y

Table D.2 Unpaired t-tests comparing influent and effluent DOC

	<i>p</i> -value in comparison test to influent (regular or ash)				Test notes
	Control	Ash-challenged	Ash-challenged, peroxide-amended	Peroxide-amended	
Experimental phase (excludes first ash test and recovery period) (n=42)	4.5E-07	N/A	N/A	3.9E-08	Unpaired t-test, one-sided
PERIODS LUMPED					
Baseline (n=20)	4.1E-03	3.3E-04	3.0E-04	1.9E-04	Unpaired t-test, one-sided
Ash tests (n=22)	1.2E-04	1.4E-08	3.0E-07	3.4E-05	Unpaired t-test, one-sided
Recovery periods (n=20)	8.2E-04	6.6E-04	8.4E-04	2.6E-04	Unpaired t-test, one-sided

1. Significant test results ($p < 0.1$) indicate removal of DOC occurs (effluent DOC < influent DOC)

Table D.3 Paired t-tests comparing DOC of control biofilters to experimental biofilters

	<i>p</i> -value in comparison test to control			Test notes
	Ash-challenged	Ash-challenged, peroxide-amended	Peroxide-amended	
Experimental phase (excludes first ash test and recovery period) (n=42)	N/A	N/A	1.6E-14 [†]	Paired t-test, one-sided
PERIODS LUMPED				
Baseline (n=20)	0.042 [†]	0.054 [†]	0.027 [†]	Paired t-test, one-sided
Ash tests (n=22)	<2.2E-16*	<2.2E-16*	1.8E-08 [†]	Paired t-test, one-sided
Recovery periods (n=20)	0.24	0.45	1.3E-07 [†]	Paired t-test, one-sided
PERIOD BY PERIOD				
Ash test 2 (n=8)	9.0E-07*	2.0E-07*	2.4E-04 [†]	Paired t-test, one-sided
Recovery period 2 (n=10)	0.024 [†]	0.053 [†]	5.9e-05 [†]	Paired t-test, one-sided
Ash test 3 (n=14)	4.8E-11*	4.1E-14*	7.7E-07 [†]	Paired t-test, one-sided
Recovery period 3 (n=10)	0.026*	0.020*	4.0E-06 [†]	Paired t-test, one-sided

[†]Effluent DOC was higher in control biofilters

*Effluent DOC was lower in control biofilters

Table D.4 Determination of DOC removal (%) data normality using Shapiro-Wilk test

Period	Sample type	Shapiro-Wilk test <i>p</i>-value	Normal (Y/N)
Experimental phase (n=42)	Control	0.014	Y
	Peroxide	8.8E-03	N
PERIODS LUMPED			
Baseline (n=20)	Control	1.7E-07	N
	Ash-challenged	9.0E-03	N
	Ash-challenged, peroxide-amended	0.066	Y
	Peroxide	7.8E-03	N
Ash tests (2 and 3) (n=22)	Control	5.2E-03	N
	Ash-challenged	0.039	Y
	Ash-challenged, peroxide-amended	4.9E-03	N
	Peroxide	9.7E-03	N
Recovery periods (2 and 3) (n=20)	Control	0.34	Y
	Ash-challenged	0.17	Y
	Ash-challenged, peroxide-amended	0.087	Y
	Peroxide	0.37	Y
PERIOD BY PERIOD			
Ash test 2 (n=8)	Control	4.9E-03	N
	Ash-challenged	0.57	Y
	Ash-challenged, peroxide-amended	0.89	Y
	Peroxide	1.7E-03	N
Recovery period 2 (n=10)	Control	0.47	Y
	Ash-challenged	0.098	Y
	Ash-challenged, peroxide-amended	0.10	Y
	Peroxide	0.22	Y

Ash test 3 (n=14)	Control	0.033	Y
	Ash-challenged	0.82	Y
	Ash-challenged, peroxide-amended	0.12	Y
	Peroxide	0.21	Y
Final four days of ash test 3 (n=8)	Control	0.026	Y
	Ash-challenged	0.93	Y
	Ash-challenged, peroxide-amended	0.16	Y
	Peroxide	0.16	Y
Recovery period 3 (n=10)	Control	0.12	Y
	Ash-challenged	0.64	Y
	Ash-challenged, peroxide-amended	0.57	Y
	Peroxide	0.16	Y

Table D.5 Paired t-tests comparing DOC removal of control biofilters to experimental biofilters

	<i>p</i> -value from comparison test to control			Test notes
	Ash-challenged	Ash-challenged, peroxide-amended	Peroxide-amended	
Experimental phase minus A1, R1 (n=42)	N/A	N/A	2.3E-13	Paired t-test, one-sided
PERIODS LUMPED				
Baseline period (n=20)	0.098 [†]	0.11	0.071 [†]	Paired t-test, one-sided
Ash periods (n=22)	8.1E-03*	1.0E-03*	4.7E-06 [†]	Paired t-test, one-sided
Recovery periods (n=20)	0.24	0.47	2.3E-05 [†]	Paired t-test, one-sided
PERIOD BY PERIOD				
Ash test 2 (n=8)	0.030*	0.013*	0.011 [†]	Paired t-test, one-sided
Recovery period 2 (n=10)	0.052 [†]	0.050 [†]	1.6E-03 [†]	Paired t-test, one-sided
Ash test 3 (n=14)	0.078*	0.019*	8.3E-05 [†]	Paired t-test, one-sided
Final four days of ash test 3 (n=8)	0.016*	2.1E-03*	N/A	Paired t-test, one-sided
Recovery period 3 (n=10)	0.030*	0.045*	2.6E-05*	Paired t-test, one-sided

[†]DOC removal was lower in control biofilters

*DOC removal was higher in control biofilters

Table D.6 Determination of turbidity data normality using Shapiro-Wilk test

Period	Sample type	Shapiro-Wilk test <i>p</i> -value	Normal (Y/N)
PERIODS LUMPED			
Baseline (n=14)	Influent – Baseline	0.63	Y
	Effluent – Control	6.4E-07	N
	Effluent – Ash-challenged	4.4E-03	N
	Effluent – Ash-challenged, peroxide-amended	4.8E-05	N
	Effluent – Peroxide	6.1E-03	N
Ash tests (2 and 3) (n=22)	Influent – Baseline	0.049	Y
	Influent – Ash (i.e., WAIW)	0.85	Y
	Effluent – Control	0.33	Y
	Effluent – Ash-challenged	0.54	Y
	Effluent – Ash-challenged, peroxide-amended	0.84	Y
	Effluent – Peroxide	0.40	Y
Recovery periods (2 and 3) (n=16)	Influent – Baseline	0.097	Y
	Effluent – Control	7.5E-04	N
	Effluent – Ash-challenged	0.33	Y
	Effluent – Ash-challenged, peroxide-amended	0.53	Y
	Effluent – Peroxide	0.14	Y
PERIOD BY PERIOD			
Ash test 2 (n=8)	Influent – Baseline	0.030	Y
	Influent – Ash (i.e., WAIW)	0.72	Y
	Effluent – Control	0.34	Y
	Effluent – Ash-challenged	0.64	Y
	Effluent – Ash-challenged, peroxide-amended	0.63	Y
	Effluent – Peroxide	0.65	Y
Recovery period 2 (n=8)	Influent – Baseline	0.87	Y

	Effluent – Control	0.010	Y
	Effluent – Ash-challenged	0.60	Y
	Effluent – Ash-challenged, peroxide-amended	0.73	Y
	Effluent – Peroxide	0.50	Y
Ash test 3 (n=14)	Influent – Baseline	0.065	Y
	Influent – Ash (i.e., WAIW)	0.31	Y
	Effluent – Control	0.27	Y
	Effluent – Ash-challenged	0.63	Y
	Effluent – Ash-challenged, peroxide-amended	0.28	Y
	Effluent – Peroxide	0.52	Y
Recovery period 3 (n=8)	Influent – Baseline	0.027	Y
	Effluent – Control	0.39	Y
	Effluent – Ash-challenged	0.26	Y
	Effluent – Ash-challenged, peroxide-amended	0.41	Y
	Effluent – Peroxide	0.09	Y

Table D.7 Unpaired t-tests comparing influent and effluent turbidity

	<i>p</i> -value from comparison test to influent (regular or ash)				Test notes
	Control	Ash-challenged	Ash-challenged, peroxide-amended	Peroxide-amended	
PERIODS LUMPED					
Baseline (n=14)	0.48 <i>1.6e-04</i> ⁽²⁾	7.5E-05	2.3E-04	1.4E-04	Unpaired t-test, one-sided
Ash tests (n=22)	2.0E-06	< 2.2E-16	< 2.2E-16	3.5E-07	Unpaired t-test, one-sided
Recovery periods (n=16)	1.6E-03	9.5E-05	1.4E-03	4.6E-05	Unpaired t-test, one-sided

1. Significant test results ($p < 0.1$) indicate removal of turbidity occurs (effluent turbidity < influent turbidity)
2. Significant p-value resulted after removing one outlier point corresponding to a post-cleaning turbidity spike

Table D.8 Paired t-tests comparing effluent turbidity of control biofilters to experimental biofilters

	<i>p</i> -value from comparison test to control			Test notes
	Ash-challenged	Ash-challenged, peroxide-amended	Peroxide-amended	
Experimental phase minus A1, R1 (n=42)	N/A	N/A	-	Paired t-test, one-sided
PERIODS LUMPED				
Baseline (n=20)	0.14	0.14	0.16	Paired t-test, one-sided
Ash tests (n=22)	1.0E-08*	2.3E-09*	0.032 [†]	Paired t-test, one-sided
Recovery periods (n=20)	0.22	0.27	0.085 [†]	Paired t-test, one-sided
PERIOD BY PERIOD				
Ash test 2 (n=8)	1.3E-05*	4.3E-06 *	8.2E-03 [†]	Paired t-test, one-sided
Ash test 3 (n=14)	1.6E-05*	2.4E-06*	0.42	Paired t-test, one-sided
Recovery period 2 (n=8)	0.035 [†]	0.20	0.053 [†]	Paired t-test, one-sided
Recovery period 3 (n=8)	2.5E-04*	4.3E-03*	0.22	Paired t-test, one-sided

[†]Effluent turbidity was higher in control biofilters

*Effluent turbidity lower in control biofilters

Table D.9 Paired t-tests comparing effluent dissolved oxygen of control biofilters to experimental biofilters

	<i>p</i> -value from comparison test to control			Test notes
	Ash-challenged	Ash-challenged, peroxide-amended	Peroxide-amended	
Full experimental phase (n=14)	0.25	6.4E-03 [†]	0.032*	Paired t-test, one-sided

[†]Dissolved oxygen was higher in control biofilters

* Dissolved oxygen was lower in control biofilters

Table D.10 Unpaired t-tests comparing pH and alkalinity in baseline source water and ash-impacted source water (WAIW)

Parameter	<i>p</i> -value from comparison test between influent types	Test notes
pH (n=14)	2.4E-04	Unpaired t-test, one-sided
Alkalinity (n=12)	0.43	Unpaired t-test, one-sided

Table D.11 Unpaired t-tests comparing influent and effluent UV₂₅₄

	<i>p</i> -value in comparison test to influent (regular or ash)				Test notes
	Control	Ash-challenged	Ash-challenged, peroxide-amended	Peroxide-amended	
Experimental phase minus A1, R1 (n=38)	0.461	N/A	N/A	0.2072	Unpaired t-test, one-sided
LUMPED PERIODS					
Baseline (n=10)	0.33	0.69	0.65	0.78	Paired t-test, one-sided
Ash tests (n=22)	0.45	1.3E-03*	5.7E-03*	0.25	Paired t-test, one-sided
Recovery periods (n=16)	0.29	5.7E-05*	3.0E-05*	0.50	Paired t-test, one-sided

1. Significant test results ($p < 0.1$) indicate reduction of UV₂₅₄ occurs (effluent turbidity < influent turbidity)

Table D.12 Paired t-tests comparing effluent UV₂₅₄ of control biofilters to experimental biofilters

	<i>p</i> -value in comparison test to control			Test notes
	Ash-challenged	Ash-challenged, peroxide-amended	Peroxide-amended	
Experimental phase minus A1, R1 (n=38)	N/A	N/A	0.033	Paired t-test, one-sided
PERIODS LUMPED				
Baseline (n=10)	0.051*	0.093*	0.014*	Paired t-test, one-sided
Ash tests (n=22)	< 2.2e-16*	< 2.2e-16*	0.12	Paired t-test, one-sided
Recovery periods (n=16)	6.7E-05*	3.5E-05*	0.30	Paired t-test, one-sided
PERIOD BY PERIOD				
Ash test 2 (n=8)	1.7e-10*	2.2E-10*	2.9E-03 [†]	Paired t-test, one-sided
Ash test 3 (n=14)	1.9e-10*	6.4e-12*	0.36	Paired t-test, one-sided
Recovery period 2 (n=8)	1.6E-04 [†]	7.1E-04 [†]	0.013 [†]	Paired t-test, one-sided
Recovery period 3 (n=8)	0.039 [†]	0.16	0.15	Paired t-test, one-sided

[†]Effluent UV₂₅₄ was higher in control biofilters

*Effluent UV₂₅₄ was lower in control biofilters

Table D.13 Paired t-tests comparing effluent SUVA of control biofilters to experimental biofilters

	<i>p</i> -value in comparison test to control			Test notes
	Ash-challenged	Ash-challenged, peroxide-amended	Peroxide-amended	
Experimental phase minus A1, R1 (n=38)	N/A	N/A	7.7E-04*	Paired t-test, one-sided
PERIODS LUMPED				
Baseline (n=10)	1.9E-03*	0.051*	2.5E-03*	Paired t-test, one-sided
Ash tests (n=22)	2.5E-08*	1.6E-08*	2.4E-03*	Paired t-test, one-sided
Recovery periods (n=16)	2.1E-03*	9.4E-04*	3.4E-04*	Paired t-test, one-sided

*Effluent SUVA was lower in control biofilters

Table D.14 Unpaired t-tests comparing DOC fractionation of influent and effluent

	<i>p</i> -value in comparison test to influent (regular or ash)				Test notes
	Control	Ash-challenged	Ash-challenged, peroxide-amended	Peroxide-amended	
LMW Acids					
Baseline and experimental phase minus A1, R1 (n=14)	3.7E-03	-	-	-	Unpaired t-test, one-sided
PERIODS LUMPED					
Biopolymers					
Baseline (n=4)	2.1E-03	1.4E-03	3.5E-04	6.5E-04	Unpaired t-test, one-sided
Humic Substances					
Ash test 2 (n=2)	-	5.3E-03	0.013	-	Unpaired t-test, one-sided
Ash test 3 (n=2)	-	0.087	0.080	-	Unpaired t-test, one-sided
Building Blocks					
Ash test 2 (n=2)	-	0.054	0.056	-	Unpaired t-test, one-sided
Ash test 2 (n=2)	-	0.10	0.16	-	Unpaired t-test, one-sided
LMW Neutrals					
Baseline (n=4)	0.51	0.60	0.011	0.011	Unpaired t-test, one-sided

1. Significant test results ($p < 0.1$) indicate reduction of the respective DOC fraction occurs (effluent < influent)

Appendix E

Additional Information on Bacterial Communities in Biofilter Media Samples

Table E.1 Metadata and alpha diversity indices for sequenced bacterial communities in biofilter media samples

Sample ID	Biofilter # (filter set)	Collection date	Experimental condition type	Ash quantity added (g/L)	Total ASV abundance (library size)	Species richness	Shannon Index	Pielou's evenness index
A-O18-1	1 (A)	Oct 18, 2021	Control	0	16968	477	5.44	0.882
A-O18-2	2 (A)	Oct 18, 2021	Control	0	31004	548	4.25	0.674
A-O18-5	5 (A)	Oct 18, 2021	Medium ash content	0.5	34071	669	5.70	0.876
A-O18-6	6 (A)	Oct 18, 2021	Medium ash content	0.5	33390	670	5.49	0.844
A-O18-7	7 (A)	Oct 18, 2021	High ash content	1.0	36085	599	5.09	0.795
A-O18-8	8 (A)	Oct 18, 2021	High ash content	1.0	33546	649	5.47	0.845
A-O23-1	1 (A)	Oct 23, 2021	Control	0	34685	712	5.67	0.863
A-O23-2	2 (A)	Oct 23, 2021	Control	0	42973	815	5.67	0.846
A-O23-5	5 (A)	Oct 23, 2021	Medium ash content	0.5	30777	445	4.31	0.706
A-O23-6	6 (A)	Oct 23, 2021	Medium ash content	0.5	50297	588	4.33	0.680
A-O23-7	7 (A)	Oct 23, 2021	High ash content	1.0	50148	699	5.23	0.799
A-O23-8	8 (A)	Oct 23, 2021	High ash content	1.0	43007	654	4.75	0.732
A-N25-1	1 (A)	Nov 25, 2021	Control	0	44403	640	5.42	0.839
A-N25-1*	1 (A)	Nov 25, 2021	Control	0	37058	607	5.44	0.848
A-N25-2	2 (A)	Nov 25, 2021	Control	0	43105	662	5.43	0.836
A-N25-2*	2 (A)	Nov 25, 2021	Control	0	43494	670	5.45	0.837
A-N25-5	5 (A)	Nov 25, 2021	Medium ash content	0.5	41806	677	5.46	0.838
A-N25-6	6 (A)	Nov 25, 2021	Medium ash content	0.5	39573	590	5.30	0.831
A-N25-7	7 (A)	Nov 25, 2021	High ash content	1.0	49759	684	5.37	0.823
A-N25-7*	7 (A)	Nov 25, 2021	High ash content	1.0	43225	648	5.41	0.836
A-N25-8	8 (A)	Nov 25, 2021	High ash content	1.0	36200	644	5.47	0.845
A-N25-8*	8 (A)	Nov 25, 2021	High ash content	1.0	27174	490	5.24	0.847
B-N20-1	1 (B)	Nov 20, 2022	Control	0	87880	124	2.94	0.609
B-N20-2	2 (B)	Nov 20, 2022	Control	0	66388	119	2.96	0.620

B-N20-3	3 (B)	Nov 20, 2022	Ash-challenged	1.0	83856	121	3.11	0.649
B-N20-4	4 (B)	Nov 20, 2022	Ash-challenged	1.0	70721	119	3.05	0.639
B-N20-5	5 (B)	Nov 20, 2022	Ash-challenged, peroxide-amended	1.0	28241	105	3.27	0.702
B-N20-6	6 (B)	Nov 20, 2022	Ash-challenged, peroxide-amended	1.0	74220	127	3.12	0.645
B-N20-7	7 (B)	Nov 20, 2022	Peroxide-amended	0	35268	110	2.97	0.632
B-N20-8	8 (B)	Nov 20, 2022	Peroxide-amended	0	48673	93	2.93	0.645
B-N29-1	1 (B)	Nov 29, 2022	Control	0	90221	134	3.41	0.695
B-N29-2	2 (B)	Nov 29, 2022	Control	0	60972	121	3.35	0.699
B-N29-3	3 (B)	Nov 29, 2022	Ash-challenged	1.0	84617	124	3.42	0.710
B-N29-4	4 (B)	Nov 29, 2022	Ash-challenged	1.0	73096	126	2.94	0.609
B-N29-5	5 (B)	Nov 29, 2022	Ash-challenged, peroxide-amended	1.0	26514	116	3.42	0.719
B-N29-6	6 (B)	Nov 29, 2022	Ash-challenged, peroxide-amended	1.0	41643	130	3.00	0.617
B-N29-7	7 (B)	Nov 29, 2022	Peroxide-amended	0	36301	101	2.54	0.551
B-N29-8	8 (B)	Nov 29, 2022	Peroxide-amended	0	51887	87	2.45	0.548
B-D8-1	1 (B)	Dec 8, 2022	Control	0	43421	112	3.65	0.774
B-D8-2	2 (B)	Dec 8, 2022	Control	0	55262	124	3.51	0.729
B-D8-3	3 (B)	Dec 8, 2022	Ash-challenged	1.0	37176	112	3.43	0.727
B-D8-4	4 (B)	Dec 8, 2022	Ash-challenged	1.0	63269	141	3.63	0.733
B-D8-5	5 (B)	Dec 8, 2022	Ash-challenged, peroxide-amended	1.0	41483	134	3.77	0.770
B-D8-6	6 (B)	Dec 8, 2022	Ash-challenged, peroxide-amended	1.0	52308	144	3.82	0.769
B-D8-7	7 (B)	Dec 8, 2022	Peroxide-amended	0	29456	96	3.07	0.673
B-D8-8	8 (B)	Dec 8, 2022	Peroxide-amended	0	39425	89	3.00	0.668
B-D21-1	1 (B)	Dec 21, 2022	Control	0	66182	114	3.32	0.701
B-D21-2	2 (B)	Dec 21, 2022	Control	0	57689	114	3.34	0.706
B-D21-3	3 (B)	Dec 21, 2022	Ash-challenged	1.0	71490	141	3.74	0.756
B-D21-4	4 (B)	Dec 21, 2022	Ash-challenged	1.0	65526	131	3.65	0.748
B-D21-5	5 (B)	Dec 21, 2022	Ash-challenged, peroxide-amended	1.0	35760	120	3.61	0.755
B-D21-6	6 (B)	Dec 21, 2022	Ash-challenged, peroxide-amended	1.0	39148	140	3.60	0.729
B-D21-7	7 (B)	Dec 21, 2022	Peroxide-amended	0	43597	99	3.34	0.727
B-D21-8	8 (B)	Dec 21, 2022	Peroxide-amended	0	58442	91	3.50	0.775

*Denotes technical replicates (two volumes of the same DNA extract submitted for sequencing)

Table E.2 Taxonomic composition of sequenced bacterial communities in biofilter set A media samples by phyla present in $\geq 1\%$ relative abundance. Set A biofilters treated a natural water matrix of deteriorated quality.

Sample ID	Relative abundance of phyla (%)														
	Acidobacteriota	Actinobacteriota	Armatimonadota	Bacteroidota	Chloroflexi	Cyanobacteria	Dependentiae	Firmicutes	Gemmatimonadota	Latescibacterota	Myxococcota	Nitrospirota	Planctomycetota	Proteobacteria	Verrucomicrobiota
A-O18-1	6.36	3.30	0.18	19.91	1.10	1.10	0.25	0.24	0.22	0.08	2.05	1.96	20.61	31.31	8.43
A-O18-2	3.04	4.55	0.24	10.52	0.73	0.99	0.17	0.21	0.40	0.20	3.20	0.80	7.59	62.90	3.05
A-O18-5	6.55	4.11	0.33	15.19	3.35	1.20	0.48	0.41	1.06	0.50	1.04	3.21	18.39	34.17	8.17
A-O18-6	6.56	5.35	0.86	14.07	2.62	1.39	0.71	0.70	0.35	0.16	0.97	1.19	13.51	45.79	4.28
A-O18-7	17.55	2.62	0.76	9.23	2.47	1.68	0.58	0.50	0.17	0.05	2.06	1.45	15.49	39.91	4.01
A-O18-8	10.55	3.24	0.98	9.76	4.05	1.39	0.92	1.09	0.22	0.06	2.62	1.72	14.93	43.14	3.18
A-O23-1	6.18	5.36	0.83	7.06	2.58	0.77	0.56	0.59	0.34	0.11	1.37	2.69	16.72	48.50	5.17
A-O23-2	3.16	11.83	0.70	7.91	2.60	1.21	0.37	0.99	0.37	0.41	1.63	0.76	15.79	48.42	2.51
A-O23-5	1.71	2.72	0.14	5.69	1.72	0.35	0.23	0.25	0.45	0.22	0.26	0.51	5.81	78.38	1.06
A-O23-6	2.24	2.51	0.23	5.74	1.07	0.36	0.13	0.24	0.12	0.08	0.53	0.52	5.45	78.84	1.44
A-O23-7	2.65	2.74	0.32	4.74	1.65	1.24	0.37	0.37	0.08	0.03	1.96	1.22	8.60	70.98	1.69
A-O23-8	2.90	1.96	0.29	4.16	2.17	0.62	0.31	0.48	0.14	0.04	1.94	0.98	7.20	74.19	1.14
A-N25-1	6.65	9.85	0.05	3.07	4.53	0.64	0.97	1.45	0.34	0.52	0.23	2.62	15.99	49.50	2.52
A-N25-1*	6.94	8.75	0.09	3.12	4.60	0.58	1.12	1.58	0.32	0.53	0.29	2.26	16.08	50.25	2.41
A-N25-2	4.75	7.61	0.15	4.12	5.59	1.16	0.66	1.49	0.32	2.03	1.06	0.87	16.10	49.85	2.43
A-N25-2*	4.47	7.76	0.07	4.53	4.85	1.31	0.77	1.94	0.30	1.85	1.06	0.85	14.67	51.14	2.53
A-N25-5	5.65	5.76	0.32	7.40	4.32	0.79	0.36	1.19	0.69	1.14	1.18	1.35	7.00	58.06	2.54
A-N25-6	5.31	10.96	0.19	6.87	3.00	0.35	0.67	1.01	0.14	0.28	0.28	2.15	12.05	50.71	4.57
A-N25-7	5.73	6.78	0.74	6.24	2.81	0.83	0.48	1.06	0.12	0.20	0.55	2.73	6.34	62.61	1.52
A-N25-7*	6.99	5.98	1.22	6.38	3.02	1.11	0.47	0.96	0.07	0.20	0.37	2.88	7.48	59.50	2.13
A-N25-8	8.04	4.04	0.32	10.40	3.25	0.66	0.56	0.64	0.44	0.14	1.06	1.49	8.20	56.66	1.89
A-N25-8*	12.35	3.44	0.69	10.68	2.75	1.01	0.53	0.60	0.53	0.24	0.97	1.33	9.93	50.67	2.27

Table E.3 Taxonomic composition of sequenced bacterial communities in biofilter set B media samples by phyla or groups present in $\geq 1\%$ relative abundance. Set B biofilters treated a synthetic water matrix with low organics.

Sample ID	Relative abundance of phyla (%)								
	Acidobacteriota	Actinobacteriota	Bacteroidota	Deinococcota	Nitrospirota	Planctomycetota	Proteobacteria	Verrucomicrobiota	Unclassified bacteria
B-N20-1	0.23	5.56	7.96	0.97	0.51	2.55	74.24	5.95	0.95
B-N20-2	0.07	7.68	7.47	1.24	1.32	1.84	75.42	2.40	1.50
B-N20-3	0.06	8.00	10.95	1.14	1.25	2.62	69.44	3.56	1.47
B-N20-4	0.07	7.29	12.50	2.90	0.88	2.20	68.99	2.97	1.37
B-N20-5	0.16	8.46	14.51	1.15	1.21	2.96	67.36	1.75	1.44
B-N20-6	0.08	3.24	13.74	1.03	1.39	1.94	71.81	2.64	3.08
B-N20-7	0.06	4.93	20.33	1.49	1.65	2.39	64.13	2.14	1.76
B-N20-8	0.15	7.44	15.42	0.18	0.94	2.50	68.72	2.64	0.87
B-N29-1	0.13	6.43	6.82	1.32	0.66	1.46	79.07	1.96	1.41
B-N29-2	0.10	7.86	6.30	1.57	1.33	0.84	78.97	0.83	1.32
B-N29-3	0.05	10.77	8.84	1.67	1.71	2.24	71.61	1.56	0.82
B-N29-4	0.05	4.34	5.60	1.59	0.55	0.71	83.91	1.11	1.77
B-N29-5	0.07	5.49	7.12	1.92	1.20	1.35	79.55	0.53	2.10
B-N29-6	0.07	2.34	6.75	0.91	0.87	1.22	85.78	0.86	0.77
B-N29-7	0.04	4.12	9.41	1.37	0.72	0.92	80.48	0.65	1.03
B-N29-8	0.09	2.73	9.17	0.24	0.50	2.05	81.96	0.69	0.34
B-D8-1	0.14	8.77	8.27	5.95	2.56	7.12	62.28	2.43	1.57
B-D8-2	0.09	7.94	9.24	4.59	3.50	1.27	68.37	1.59	1.82
B-D8-3	0.02	13.44	7.99	1.18	1.31	0.79	71.73	1.27	1.11

B-D8-4	0.06	5.59	11.00	6.52	3.25	1.12	66.43	2.70	1.72
B-D8-5	0.08	9.46	12.10	3.38	2.97	1.38	65.75	1.99	1.61
B-D8-6	0.14	6.11	12.62	3.27	4.43	2.07	65.68	1.64	1.87
B-D8-7	0.06	4.20	12.22	4.19	1.94	2.27	70.61	1.27	2.14
B-D8-8	0.07	4.25	11.71	0.85	2.48	2.74	73.57	1.23	1.59
B-D21-1	0.12	10.62	7.29	5.81	2.89	7.56	62.39	2.43	0.58
B-D21-2	0.07	12.11	13.45	3.24	2.95	0.97	63.19	1.79	0.99
B-D21-3	0.07	11.16	9.38	5.42	4.56	3.65	61.39	1.59	1.75
B-D21-4	0.02	6.84	9.08	9.34	5.33	2.25	63.28	2.23	0.91
B-D21-5	0.15	14.53	7.66	4.45	4.05	3.00	62.90	1.62	1.04
B-D21-6	0.08	4.88	5.21	6.01	5.95	2.89	71.29	1.56	1.29
B-D21-7	0.08	5.03	9.88	10.37	3.83	5.26	62.28	1.28	1.05
B-D21-8	0.07	4.25	11.91	2.69	4.50	8.82	64.48	1.48	0.98

Note that eukaryotic phyla and sequences not assigned to a kingdom are not shown

Appendix F

Diversity Index Calculations

Shannon-Wiener index of species diversity (H)

$$H = - \sum_{i=1}^s p_i \cdot \ln p_i$$

Pielou's evenness index (J)

$$J = H / \ln s$$

Where

s = number of species in community (i.e., sample species richness)

p_i = proportion of total community abundance made up of i^{th} species

Appendix G

Supplementary Plots – Bacterial Community Analysis

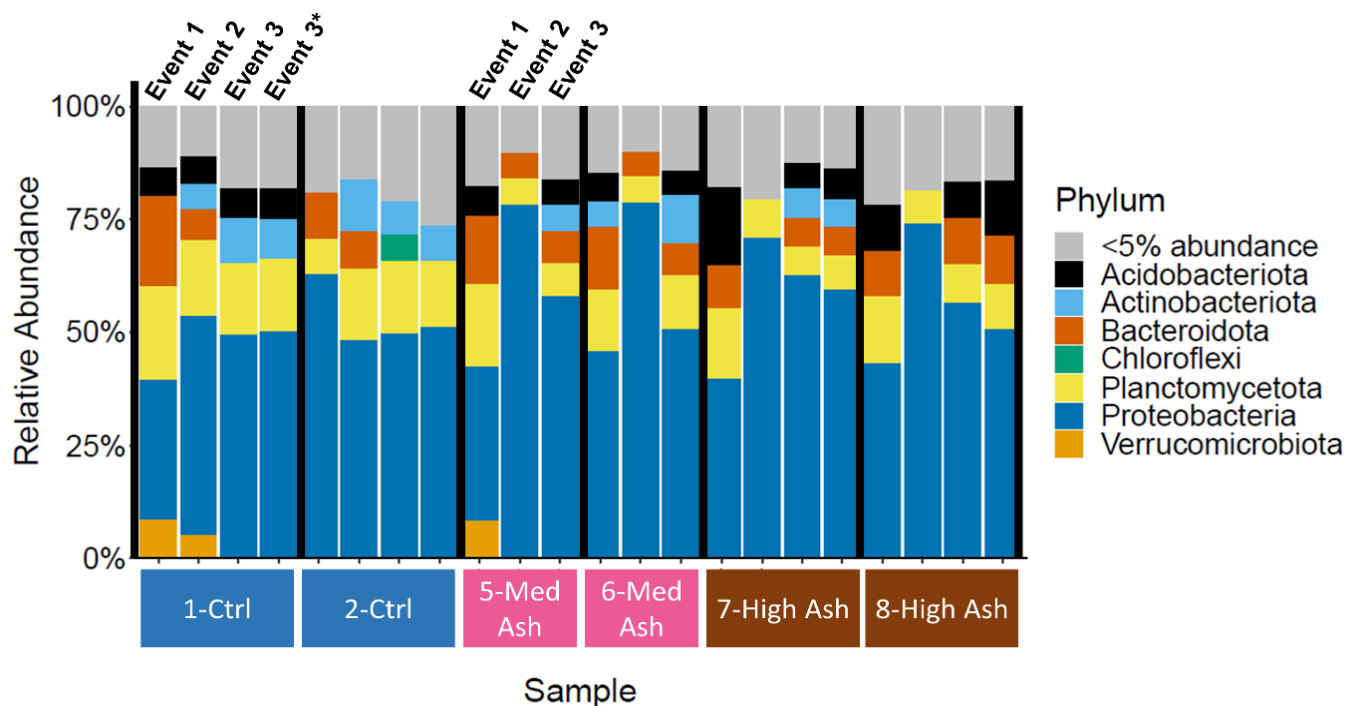


Figure G.1 Taxonomic bar plot showing relative abundances of major phyla detected in filter set A, which treated a natural water matrix. Samples are grouped by experimental condition and ordered by sampling event. Phyla accounting for less than 5% of the total sequenced community in each sample are lumped together for better visualization. One sample was collected and sequenced from each filter per sampling event, with the exception of samples from filters treating low ash content water in filter set A, which were not sequenced. Technical replicates (i.e., two volumes of DNA extracted from the same sample) were submitted for each control biofilter and each biofilter treating high ash content water in the final sampling event for filter set A (denoted as Event 3*).

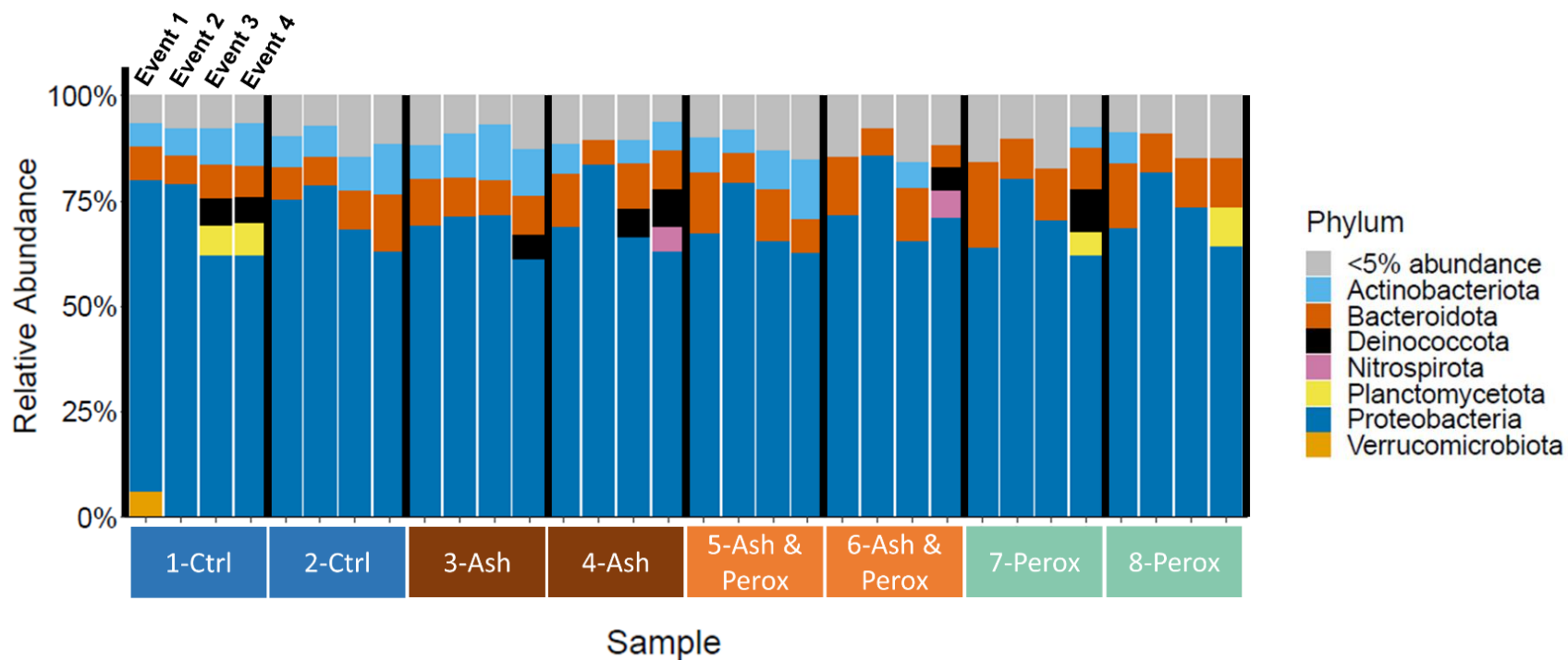


Figure G.2 Taxonomic bar plot showing relative abundances of major phyla detected in filter set B, which treated a synthetic water matrix. Samples are grouped by experimental condition and ordered by sampling event. Phyla accounting for less than 5% of the total sequenced community in each sample are lumped together for better visualization. One sample was collected and sequenced from each filter per sampling event.

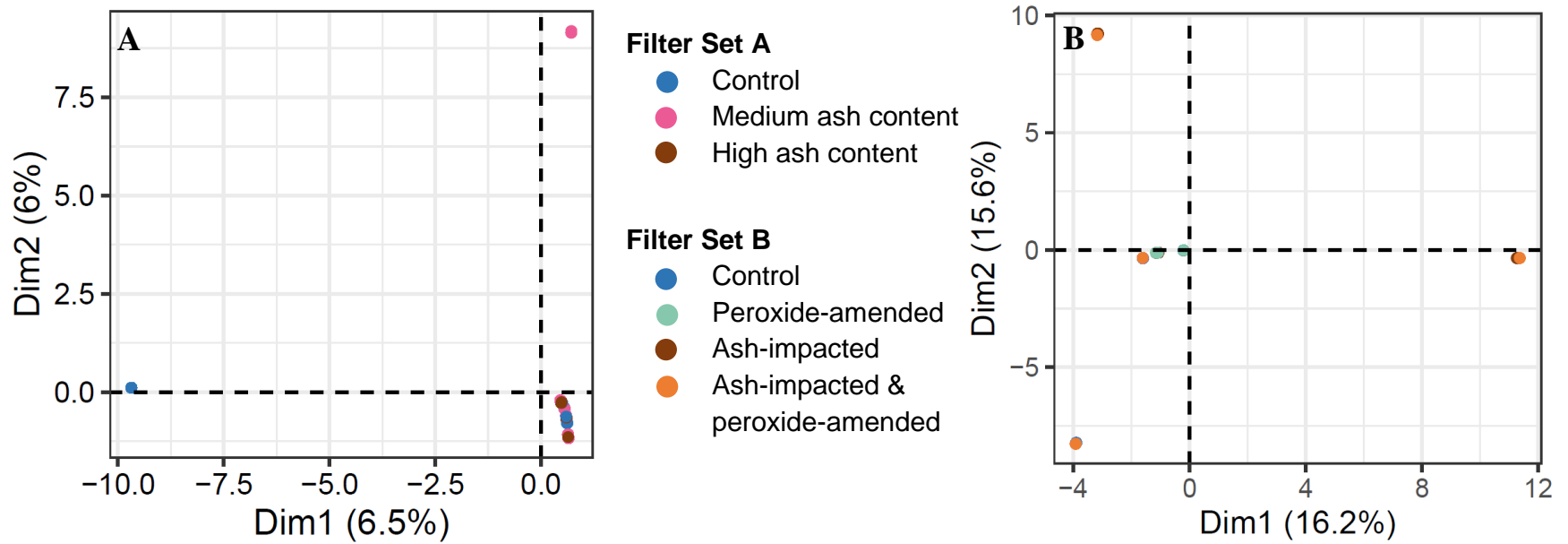


Figure G.3 Principal Component Analysis (PCA) plots comparing bacterial communities in samples collected in the final two sampling events from A) filter set A, which treated a natural water matrix, and B) filter set B, which treated a synthetic water matrix. Technical replicates are removed from the data set for filter set A. Data were rarefied 100 times before ordination using Bray-Curtis dissimilarities.

Appendix H

Statistical Test Results – Bacterial Communities

Table H.1 Results of ANOVA tests comparing dispersion of sequenced sample groups

Data set	Data subset	Grouping (independent variable)	ANOVA <i>p</i> -value (testing data dispersion)
Merged filter sets	All data	Filter set	0.28
Set B filters	All data	Sampling date	0.99
	Final two sampling events	Sampling date	0.94
	Control biofilters only	Biofilter	0.46
	Ash biofilters	Biofilter	3.1×10^{-4}

Sample groups above were found to be significantly different in PERMANOVA tests

Bolded entries indicate that a significant difference in sample group dispersion was detected ($p < 0.05$)