Effects of submerged zone, media aging, and antecedent dry period on the performance of biochar-amended biofilters in removing fecal indicators and nutrients from natural stormwater

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27   **1. Introduction**

28   Biofilters are common green stormwater infrastructure that can be used to reduce, capture,
29   and treat urban stormwater runoff. Currently, a majority of these infiltration systems uses a
30   conventional soil mix as the biofilter soil media (BSM), consisting of either loamy sand,
31   sand/compost or sand/native soil (Sage et al. 2015, Hsieh and Davis 2005a). Yet, the
32   performance of conventional BSM is inconsistent and inadequate for removing contaminants
33   from urban stormwater runoff that pass through the biofilters. For example, in multiple studies,
34   conventional stormwater biofilters have been reported to leach both microbial and chemical
35   contaminants into the stormwater as it exits the biofilters (García-Albacete et al. 2014,
36   Hathaway et al. 2009, Chandrasena et al. 2012, Zhang et al. 2012). Such findings motivate
37   ongoing work (Bratieres et al. 2008, Li et al. 2012, Hsieh and Davis 2005b, Li et al. 2014,
38   Mohanty et al. 2013, Mohanty and Boehm 2014, Mohanty et al. 2014, Li et al. 2016, Poresky et
39   al. 2016) to identify effective BSM for removing stormwater contaminants, including pathogens,
40   pathogen indicator bacteria, and nutrients.

41   One of the major stormwater biofilter design goals is to maximize the removal efficiency of
42   microbial contaminants and nutrients, primarily due to the associated public and ecological
43   health implications, respectively. Runoff-associated fecal indicator bacteria (FIB) contamination
44   is one of the major sources of surface water impairment in the U.S (Cahoon et al. 2016). On the
45   other hand, capturing nitrogen and phosphorus from stormwater is crucial for preventing algal
46   blooms in receiving waters, as well for boosting soil fertility to support the vegetation in

stormwater best management practices (BMPs) (Mallin et al. 2009). Additionally, engineered stormwater biofilters with enhanced FIB and nutrient removal-capacity may help cities in the US comply with total maximum daily load (TMDL) and municipal separate storm sewer system (MS4) regulatory requirements. Stormwater biofilters remove contaminants primarily via BSM where the targeted contaminants undergo a combined process of physical, chemical, and biological removal (Subramaniam et al. 2015).

In addition to effectively removing contaminants, an ideal BSM needs to be cost-effective, easily sourced, and sustainable. Using a bulk aggregate (i.e., sand), modified with either organic or mineral additives, can be effective for removing contaminants, maintaining hydraulic characteristic, and relatively inexpensive (Subramaniam et al. 2015). Sourcing the organic and mineral additives from recycled materials could lead to a sustainable stormwater management system (Hengen et al. 2016). Other efforts have been made to source BSM from recycled rubber (e.g., tire crumb), scrap iron (e.g., zero-valent iron, iron-oxides), scrap wood (e.g., wood chips), industrial by-products (e.g., coconut coir, high-carbon wood ash, biochar) and waste biomass (e.g., biochar) (Mohanty et al. 2013, Seelsaen et al. 2006, Joyner and Waldrop 2008, Lim et al. 2015, Chang et al. 2012). Note that pyrogenic carbonaceous materials, such as biochar, are good candidates for BSM not only because of their low material cost but also due to their porous structures, high specific surface areas, functional groups, and hydrophobicity (Inyang and Dickenson 2015).

Biochar—a pyrolyzed biomass produced from waste biomass—shows particular promise as a low-cost, sustainable BSM. The production of biochar from the anoxic pyrolysis of discarded biomass makes it a carbon negative material due to low greenhouse gas (GHG)

emissions and potential for long-term carbon sequestration (Lehmann and Joseph 2015). Biochar can also be produced as a by-product of biodiesel, and used for amending agricultural soil to enhance crop yields (Inyang and Dickenson 2015, Chan et al. 2008). The addition of biochar to sand for use as BSM in biofilters has reportedly improved both FIB and nutrient removal capacity of the BSM. FIB removal has been reported to increase by more than 3-log depending on the particle size, feed stock, and pyrolysis temperature of the biochar as well as type of the FIB (Mohanty and Boehm 2014). While complete mechanistic understanding of enhanced microbial removal in biochar-amended media is yet to be achieved, increased removal has been attributed to higher surface area, lower surface charge, and stronger hydrophobicity of biochar compared to sand (Mohanty et al. 2014, Abit et al. 2012). Additionally, research has also been conducted on biochar derived from various feedstock and pyrolyzed at different temperature to evaluate its nutrient recovery capacity from aqueous solution (Mohan et al. 2014). Biochar addition also has demonstrated potential to increase nutrient removal from stormwater in at least two studies. One study—performed using gasified biochar derived from wood waste pallet—reports up to 47 and 85% of phosphorus and nitrogen from stormwater, respectively (Reddy et al. 2014). Another study used two types of biochar (i.e., derived from wood and poultry litter) to investigate ammonium-nitrogen ($\text{NH}_4^+\text{-N}$) absorption in biochar particles: 74 and 37% $\text{NH}_4^+\text{-N}$ removal was observed by wood and poultry litter biochar, respectively (Tian et al. 2014). These results underscore the potential of biochar as a promising amendment for BSM in removing FIB and nutrients from stormwater.

However, utilizing biochar in a field-scale biofilter first requires more field representative performance evaluations to gauge its efficacy under complex environmental

conditions. There are no published studies that investigate the performance of the biochar-augmented BSM in removing contaminants from natural stormwater for an extended period of time. The durations used in the previous studies range from 3-70 h (Abit et al. 2012, Reddy et al. 2014, Mohanty and Boehm 2015). However, stormwater biofilters in the field will be exposed to natural stormwater for extended periods of time: reported operational life of decades and beyond (Flynn and Traver 2013, Payne et al. 2014a). The physical, chemical, and biological properties of a BSM are, thereby, likely to change over time, which, in turn, could potentially influence capture and removal of FIB and nutrient in biochar-modified BSMs (Hunt et al. 2008, Zhang et al. 2011, Chandrasena et al. 2016, Passeport et al. 2009). Therefore, it is necessary to evaluate the effects of field conditions (e.g., biological aging and antecedent dry days) on biochar-modified sand biofilter performance. Moreover, the literature has yet to discuss the effects of biofilter design parameters—for example, a saturation zone or vegetated cover—on the performance of biochar-augmented stormwater biofilters. While providing a saturation zone has been reported to increase FIB and nutrient removal efficiency of sand/compost biofilters (Li et al. 2012, Barrett et al. 2012), it remains unknown if the presence of a saturation zone would have a similar effect on the performance of biochar-modified sand filters.

In this study, we evaluated the long-term (i.e., simulated one water-year of hydraulic loading considering average annual rainfall in San Francisco, USA) performance of biochar-augmented stormwater biofilters in removing FIB and nutrients from natural stormwater runoff. While field-scale experiments where test-biofilters are installed in situ would be useful, there are legal barriers to doing so due to the potential for negative impacts of experimental

designs on flooding and effluent water quality that precluded us from carrying out such studies. We used laboratory scale biofilters that were exposed to natural stormwater flushes--once - or twice-weekly --to investigate the effects of antecedent dry days (ADD) on performance. In addition, the performance of biofilters with a saturation zone (SZ) was compared with biofilters with no SZ. Moreover, we quantified the amount of active biomass formed on the BSM, caused by natural stormwater exposure, and its effects on the biofilter FIB and nutrient removal performance. Our results address the gap in the data concerning the performance of biochar-augmented biofilters under representative field conditions. This research informs the design of full-scale bioinfiltration systems with biochar-augmented sand used as the BSM.

2. Materials and Methods

2.1 Porous media

Ottawa sand (0.6-0.8 mm Fisher Scientific, IL, USA) was mixed with biochar (Swallow Valley Farm, Valley Ford, CA) at a 7:3 proportion (by volume) to prepare the BSM for the laboratory scale biofilters. This volumetric ratio was selected based on the current recommended proportion (20-40%) for organic matter in biofilter soil media (California State Waterboard 2011). The sand was washed, dried, autoclaved (121°C, 100 KPa, 15 min), and stored in a sterile container. The details of the preparation procedure are presented in the supplementary material (SM) section: M1.

The biochar particles were crushed, sieved (to obtain sizes smaller than the 0.6 mm), and autoclaved (121°C, 100 KPa, 15 min) for sterilization. The biochar feedstock consisted of a mix of 60% Monterey Pine, 20% Eucalyptus, 10% Bay Laurel, 10% mixed hardwood and softwood.

The pyrolysis was performed over a 6 h period at temperatures ranging from 180-395°C. Based on the feedstock and the pyrolysis temperature, this biochar represents a wood-derived low-temperature biochar.

2.2 Model biofilters

Polyvinyl chloride (PVC) pipes with a 2.5 cm internal diameter and a 15 cm length were glued to end fittings to build model biofilters. Nylon wool was inserted at both ends of a biofilter to prevent the porous media from washing out. The biofilters were dry-packed layer-by-layer, where 2-cm layers were poured in and compacted with a wooden rod. The biofilters were tapped by hand after each layer was poured to ensure there was no stratification (or short-circuiting) in the packed media. After packing, 30 pore volume (PV) of DI water was fed into the biofilters (arranged in a down-flow configuration) using a peristaltic pump (Masterflex, L/S, Cole-Parmer, IL). The PV of each biofilter was measured gravimetrically by weighing the biofilter to determine the difference between a dry and a saturated biofilter.

2.3 Influent stormwater

Natural stormwater was collected from Sierra Creek (37°25'40.5"N 122°09'25.6"W, Stanford University, Stanford, CA). The creek receives runoff from the surrounding areas which include parking lots, roadway, and residences. The stormwater was collected twice every week between 28 January 2016 and 16 June 2016 —directly prior to conducting the column experiments (as described in section 2.4)—for 20 weeks. Samples collected after May 16, 2016 represent dry weather flow (defined as no rain for the previous 11 days). These samples represent 25% of the total samples collected from the creek during the abovementioned

experimental period. pH, total non-purgeable organic carbon (NPOC), total nitrogen (TN), enterococci, and *Escherichia coli* were quantified in the stormwater each time it was collected. pH and FIB concentrations were measured within 4 hours of stormwater collection. At the same time, an aliquot of stormwater was filter-sterilized by filtering through 0.22 µm pore size PVDF syringe filters (Fisher Scientific, IL, USA), stored at -15°C for 2 weeks, and then TN and NPOC were measured. Detailed description of the stormwater characterization procedures (which are standard methods) are provided in the SM (M2). After each collection event, a portion of the collected stormwater was autoclaved (and allowed to cool to room temperature) to flush through the control columns (described in section 2.4)

2.4 Column experiments

Porous media column experiments were conducted using the model biofilters configured in a down-flow arrangement. While a down-flow configuration has a possibility of causing preferential flow in the biofilters, this arrangement was chosen to represent direction of stormwater flow and gravitational draining in the biofilters under field conditions. The biofilters were packed carefully to minimize the possibility of preferential flow paths. Details of the biofilters with relevant operating conditions are presented in Table 1. The SZ in a biofilter was achieved by raising the outlet of the corresponding biofilter by 10 cm forming a U-tube (Chandrasena et al. 2014). Biofilters are named by indicating if they had any SZ (U if operated without a SZ; S if operated with a SZ) followed by the ADD (7 for 7 days ADD; 3 for 3 days ADD). For example, U7 represents the group of biofilters that were operated without a SZ with 7 days ADD. We added the suffix “C” for the control-biofilters that received autoclaved stormwater. For example, U7C biofilters were operated under the same condition as U7; however, unlike U7

biofilters, U7C biofilters received a flush of autoclaved stormwater every 7 days. Schematics are presented in the SM to further illustrate the timeline of the experiments (Figure S1) and weekly stormwater flushing procedure (Figure S2).

Biofilter performance was evaluated in three experimental phases (Figure S1): 1) baseline removal: investigation of biofilter FIB removal performance in week 1 – experiment conducted the day after packing the biofilters; 2) FIB removal during the conditioning of the biofilters: effluents from the biofilters sampled during weeks 2, 4, 7, 8, 9, 10, 11, 13, 14, 16, and 18; 3) FIB and nutrient removal during challenge experiment: challenge tests performed in week 20 on the test-biofilters that had been aged by the natural stormwater flushed through the columns once- or twice- weekly for 20 weeks. In addition, the FIB and nutrient removal of 6 control-biofilters— exposed to the same stormwater but after autoclaving the stormwater— were tested in week 20.

Table 1: Descriptions of test- and control-biofilters used in the experiment.

Biofilter Type	Injected Stormwater Type	Biofilter ID	Saturation Zone (absent = U; present = S)	Antecedent Dry Period (days)	No. of biofilter replicates tested
Test-biofilters	Natural stormwater	U7	U	7	3
		U3	U	3	3
		S7	S	7	3
Control-biofilters	Autoclaved Natural stormwater	U7C	U	7	2
		U3C	U	3	2
		S7C	S	7	2

For all the experiments, a Masterflex L/S precision variable-speed peristaltic pump (Cole-Parmar, IL) was used to pump influent into the biofilters. The approach velocity of the water was maintained at 12.7 cm/h which represents the minimum hydraulic conductivity that has been suggested by California Water Board for stormwater biofilters (California State WaterBoard, 2011). The duration of natural stormwater injection was less than 3.5 h and the influent sample was collected at the beginning and the end of the experiment: to identify any change in FIB concentrations within this time period. For determining influent NPOC and TN concentrations, samples were collected at the end of the column experiments. Using this value as the influent nutrient concentrations assumes the change during the length of the experiment (3.5 hr) in the influent NPOC and TN concentration was negligible (Ryzhakov et al. 2010). All experiments were conducted at the room temperature (~20°C).

2.5 Baseline removal in week 1:

Enterococci and *E. coli* removal observed during week 1 column test are termed as baseline FIB removal in stormwater biofilters. Before the baseline removal performance tests, 4 PV of autoclaved stormwater was fed into the biofilters to condition the biofilters to the chemistry of the natural stormwater. Following the autoclaved stormwater injection, 6 PV (approximately 46.5 cm rainfall event) of natural stormwater was pumped into the biofilters and the effluent was collected in two fractions: 0-3 PV and 3-6 PV. The effluent was collected in two fractions to separate the rising limb of the breakthrough curve (i.e., 0-3 PV) from the breakthrough plateau (3-6 PV) (Mohanty et al. 2013, Chen and Walker 2012). In equation i, we used the FIB concentrations in the second portion of the effluent (3-6 PV) to avoid overestimating biofilters' FIB removal performance for a storm event bigger than 3 PV. While we acknowledge that 6 PV

(46.5 cm rainfall) is much larger than what we typically observe in a single storm event in San Francisco, this dosing volume was selected considering the following: a) it provides enough sample volume (>3 PV) to capture the plateau region of the breakthrough curve; and b) under the given flow velocity (12.7 cm/h), this allows for conducting the experiments within 6 h of natural stormwater collection with large enough effluent sample volume for FIB (i.e., *E. coli* and enterococci) and nutrient characterization. FIB log removal was determined using the following equation:

$$\text{Log removal} = -\log_{10} (C/C_0) \dots\dots\dots (i)$$

where, C= FIB concentration in 3-6 PV effluent (MPN/100 mL), and C₀= influent FIB concentration (MPN/100 mL)

The log removal values observed in replicate biofilters (3 under each category for test-biofilters) were used to calculate average log removal in the biofilters and reported as (arithmetic average ± standard deviation). Similar reporting procedures were followed for removal during conditioning and challenge tests.

Also note that 6 additional columns were also packed on the same day and saturated with DI water, and labeled as U7C, U3C, and S7C (2 columns under each category, Table 1). These columns served as the control-biofilters and received autoclaved stormwater in the first and the subsequent weeks during the conditioning period. We describe the details of the conditioning in the following section.

2.6 Removal from week 2 to week 18

Biofilters were aged for 20 weeks (Table 1 and Figure S1 and S2). A total of three types of biofilters were maintained during this period: U7, U3, and S7. The U7 and S7 biofilters were flushed with 6 PV of natural stormwater once weekly. However, the bottom 10 cm of the S7 biofilters were maintained as saturated at all times, while the U7 biofilters were allowed to drain in between weekly storm events. The U3 biofilters were allowed to drain after each flush and received 9 PV of stormwater weekly: 6 PV on the first day and another 3 PV on the 4th day of the week. Applying 6 PV weekly is equivalent to approximately 46.5 cm rainfall event. Over the length of the experiment, this equates to a total 930 cm of rain passed through the biofilters. This amount of stormwater equates to estimated total precipitation in San Francisco, California, USA in 18 years (i.e., assumed design life of the biofilters) (NOAA, 2016). Considering a 6% biofilter area (of a catchment), total volume of flushed stormwater represents total hydraulic loading for a full-scale stormwater biofiltration over a typical water-year in San Francisco (Ambrose and Winfrey 2015). The 3 PV flush received mid-week by U3 provides a 3 day ADD for comparison to 7 day ADD. These ADDs were selected considering the climate of northern California where majority of the precipitations occurs from December to April with frequent storm events (NOAA, 2016). In addition, as mentioned in section 2.5, 2 control-biofilters were set up for each of the three types of test-biofilters: U7C, U3C, and S7C. During the conditioning period, these control-biofilters received autoclaved stormwater at the same as the test-biofilters. This allowed the control-biofilters to be aged by the natural stormwater with similar chemistry without exposure to the stormwater microorganisms.

During the stormwater flush events, effluent from the biofilters was collected (composite samples combining 3-6 PV effluent) and the log removal for enterococci and *E. coli*

was computed using the equation i. Comparing the performance of the biofilters receiving stormwater once weekly (U7) *versus* to those receiving stormwater twice weekly (U3) provides insight on the effect of ADD on the performance of the biofilters. Finally, a comparison between U7 and S7 biofilters provides insight into the effects of SZ on biofilter performance.

2.7 Challenge test on the test- and control-biofilters in week 20

After 20 weeks of natural stormwater exposure, challenge tests were performed on the biofilters to compare their FIB removal performance with their baseline performance and that of control-biofilters. 6 PV of Sierra Creek natural stormwater was fed into all the test- and control- biofilters. Effluents were collected in 0-3 and 3-6 PV fractions, and the log removal of enterococci and *E. coli* was calculated using the equation i.

In addition to the FIB concentrations, influent and effluent from the biofilters were also characterized for their pH, non-purgeable organic carbon (NPOC), and nutrients (total nitrogen, total phosphorus, nitrate-nitrogen, and ammonium-nitrogen). The removal for NPOC and nutrients was calculated using the following equation:

$$\% \text{ Removal} = \left(\frac{C_0 - C}{C_0} \right) * 100 \dots\dots\dots (ii)$$

where, C= concentration of the target chemical species in the 3-6 PV effluent (mg/L), and C₀= influent concentration of the target species (mg/L).

The percent removal values observed in replicate biofilters were used to calculate average percent removal in the biofilters and reported as (arithmetic average ± standard deviation). Comparison of FIB log removal in control-biofilters (i.e., U7C, S7C, and U3C) and the

test-biofilters (i.e., U7, S7, and U3) provides insight into the role of biological aging (that is, the growth of biofilm on the filter media) on the performance of stormwater biofilters.

2.8 Falling head hydraulic conductivity test

Prior to the baseline tests and after the challenge tests, the hydraulic conductivity of the biofilters was determined using a falling head permeability test. The details of the test is presented in the SM (M3).

2.9 Quantifying the biomass in the BSM

At the end of the challenge experiment, the BSM in each biofilter was characterized using an ATP-based biomass quantification method described elsewhere (Velten et al. 2007) with some modification. The details of the method has been presented in the SM (M4).

2.10 Statistical analysis

Significant difference of microbial and nutrient removal between biofilters under different operating conditions was analyzed using two sample t-tests. We tested the hypothesis that the length of the ADD (3 days vs. 7 days), the presence of a SZ (presence vs. absence), aging (baseline log removal vs. challenge test log removal), and presence of biofilm (week 20 removal in test-biofilters vs. week 20 removal in control-biofilters) significantly affected log removal of FIB. Results were considered statistically significant if $p < 0.05$. All analyses were performed using SAS (SAS Institute Inc, Cary, NC). Spearman's rank coefficients were used (in MSExcel) to determine the strength of the correlation between biofilter age and FIB log removal.

3. Results

3.1 Baseline FIB removal in biochar-modified biofilters

FIB removal data, obtained from the column experiments performed in the first week, are termed as baseline capacity of the biofilters in removing *E. coli* and enterococci from natural stormwater. There is no significant differences ($p > 0.05$) among the biofilters with respect to baseline FIB removal capacity, however the removal of *E. coli* is significantly higher ($p < 0.05$ for S7, U7 and U3 biofilters) than enterococci (Figure 1a-1d). Average log removal across all the biofilters for *E. coli* and enterococci are 2.15 ± 0.20 and 1.74 ± 0.22 , respectively.

3.2 FIB removal during conditioning of the biofilters

The stormwater biofilters received once or twice weekly flushing of natural (test-biofilters) or autoclaved natural (control-biofilters) stormwater (Table 1). The chemical composition of the influent stormwater (NPOC, TN, and pH) are presented in the Figure S3b. Average and standard deviation NPOC, TN, and pH is 8.8 ± 3.8 mg/L, 2.4 ± 1.8 mg/L, and 7.4 ± 0.3 , respectively. Based on the 19 weeks of data, the geometric mean of influent FIB concentrations are 554 and 152 most probable number (MPN)/100 mL for *E. coli* and enterococci, respectively (Figure S3a).

On the other hand, the geometric mean across the conditioning phase of *E. coli* concentration in the biofilter effluent is 34, 35, and 52 MPN/100 mL for U3, U7, and S7 biofilters, respectively (Figure 1). The effluent concentrations for enterococci are 26, 22, 34 MPN/100 mL for U3, U7, and S7 biofilters, respectively. Over the course of the experiment, the log removal in U7 biofilters decreases from 2.08 ± 0.23 (week 1) to 0.99 ± 0.28 (week 18), and 1.63 ± 0.18 (week 1) to 0.60 ± 0.07 (week 18) for *E. coli* and enterococci, respectively (Figure 1a).

A similar reduction occurs in U3 biofilters where *E. coli* removal changes from 2.32 ± 0.12 (week 1) to 1.24 ± 0.08 (week 18) and enterococci removal changes from 1.76 ± 0.09 (week 1) to 0.67 ± 0.07 (week 18) (Figure 1b). In S7 biofilters, the observed log removal changes from 2.06 ± 0.08 (week 1) to 1.16 ± 0.01 (week 18) for *E. coli* and from 1.73 ± 0.17 (week 1) to 1.00 ± 0.14 (week 18) for enterococci over 18 weeks, respectively. Statistical analysis reveals little or no correlation (Spearman's $\rho < 0.2$) between influent FIB concentration and FIB log removal in the biofilters.

Figure 1: Change in FIB removal in biochar-augmented biofilters during prolonged exposure to natural stormwater: a-b) effect of antecedent dry days (ADD); b-d) effect of the presence of saturation zone (SZ). U3 and U7 indicate biofilters without a SZ that experience 3 days and 7 days ADD, respectively. S7 biofilters represent biofilters with a SZ and 7 days ADD. Biofilters were kept and tested for their FIB removal capacity at the room temperature. Error bars represent one standard deviation.

It appears from the Figure 1 that FIB log removal decreases with time with some exceptions. For example, FIB log removal in all the biofilters increases between weeks 13 and 14. Statistical analysis indicates a significant, negative correlation between the age of the biofilters and their FIB removal capacity ($p < 0.05$ for all): the values for Spearman's rho for *E. coli* removal and biofilter age are -0.4, -0.64 and -0.39 for U3, U7, and S7 biofilters respectively; the rho values for enterococci removal and biofilter age comparison are -0.62, -0.56, and -0.35 for U3, U7, and S7 biofilters, respectively. All of these correlations are statistically significant ($p < 0.05$) except for S7 biofilters (both *E. coli* and enterococci removal) and U3 biofilters (in case of *E. coli* removal).

3.3 FIB removal during challenge Test

During challenge tests, control-biofilters achieve significantly greater FIB removal compared to the corresponding test-biofilters conditioned at a similar ADD or saturation condition (Figure 2): average difference 0.27 log units *E. coli* and 0.22 log unit enterococci; $p < 0.05$ for both). Specifically, *E. coli* log removal in U7 biofilters (i.e., 1.14 ± 0.04) is significantly lower ($p < 0.05$) compared to 1.38 ± 0.02 log removal in U7C biofilters; *E. coli* log removal of 1.03 ± 0.16 in U3 biofilters is significantly lower ($p < 0.05$) than the 1.36 ± 0.07 log removal in U3C biofilters; and *E. coli* log removal in S7 biofilters (1.07 ± 0.09) is significantly smaller ($p < 0.05$) than that of S7C biofilter (1.30 ± 0.02). Further, the log removal observed for *E. coli* in the

control- and test-biofilters during the challenge test (week 20) are significantly lower (average difference 0.8 log for control- and 1.08 log for test-biofilters, $p < 0.05$) than their baseline removal observed in the week 1.

Figure 2: Removal of *E. coli* and enterococci in biochar-augmented biofilters after 20 weeks of stormwater exposure. U3 and U7 indicate test-biofilters without a saturation zone (SZ) that experienced 3 day and 7 day antecedent dry period (ADD), respectively. S7 represents test-biofilters with a SZ and 7 day ADD. U3C, U7C, and S7C are control-biofilters for U3, U7, and S7, respectively. Control- and test-biofilters were exposed to the stormwater with a similar chemistry: control- columns received autoclaved natural stormwater water while test-biofilters received natural stormwater without autoclaving. Biofilters were kept and tested for their FIB removal capacity at the room temperature. Error bars represent one standard deviation.

The biofilters have smaller enterococci log-removal ($p < 0.05$) compared to *E. coli* during the challenge test (Figure 2). Average *E. coli* removal in S7 biofilters (1.07 ± 0.09) is significantly 0.33 log unit higher than average enterococci removal in S7 biofilters (0.74 ± 0.07). The enterococci removal capacity is the same among the U7, U3, and S7 biofilters during the

challenge test ($p>0.05$). Similarly, there is no difference in enterococci removal among the control-biofilters (i.e., U7C, U3C, and S7C) ($p>0.05$). However, enterococci removal is significantly greater in the control biofilters ($p<0.05$) compared to the test-biofilters during the challenge test: the observed log removal values are 0.90 ± 0.01 in U7C vs. 0.64 ± 0.03 in U7; 0.92 ± 0.03 in U3C vs. 0.79 ± 0.16 in U3; 1.00 ± 0.05 in S7C vs. 0.74 ± 0.07 in S7.

3.4 Nutrient and NPOC removal during challenge test

The removal of nitrogen in biofilters varies depending on the type of nitrogen species and biofilter operating conditions (Figure 3). NO_3^- -N removal in control-biofilters ranges from net leaching in U7C biofilters (i.e., $-10.3\pm 17.3\%$) to $18.3\pm 5.2\%$ removal in S7C biofilters. The removal of NO_3^- -N in the test-biofilters varies from 36.1 ± 6.9 in U7 biofilters to $61.4\pm 3.4\%$ in S7 biofilters. The presence of a SZ significantly increases NO_3^- -N removal; percent removal of NO_3^- -N in S7 biofilters is significantly greater than that of U7 biofilters (average difference = 25%, $p<0.05$). However, there is no difference in NH_4^+ -N removal in biofilters with and without a SZ (U7 versus S7, $p > 0.05$) or with and without biofilm (U7 versus U7C; S7 versus S7C, $p>0.05$); all exhibited 50-58% removal efficiency of NH_4^+ -N. The nutrient removal data in U3C biofilters are not available due to a mishap during sample handling. The test-biofilters demonstrate significantly better TN removal performance compared to control-biofilters ($P < 0.05$): based on our comparison between U7 and U7C biofilters and S7 and S7C biofilters. While average TN removal in control-biofilters ranges from net leaching ($-6.8\pm 16.6\%$ in S7C) to very low removal ($7.7\pm 4.3\%$ in U7C), test-biofilters do not leach any TN and average removal observed ranges between $19.0\pm 8.5\%$ (in U3) to $26.8\pm 7.2\%$ (in S7).

Figure 3: Removal of nutrients in biochar-augmented biofilters with different operating conditions after 20 weeks of operation: a) removal of nitrogen; b) removal of total dissolved phosphorus. The legends U3 and U7 indicate test-biofilters without a saturation zone (SZ) that experienced 3 day and 7 day dry period (ADD) respectively. S7 represents test-biofilters with a SZ and 7 day ADD. U7C and S7C are control-biofilters for U7 and S7, respectively. Control- and test-biofilter were exposed to the stormwater with a similar chemistry: control- biofilters received autoclaved water while test-biofilters received natural stormwater without autoclaving. Biofilters were kept and tested for their nutrient removal capacity at the room temperature. Error bars represent one standard deviation.

There is no significant difference ($p < 0.05$) between U7 ($18.8 \pm 10.7\%$) and S7) or U7 and U3 biofilters with respect to their TP removal performance (Figure 3b, ortho-phosphate removal not measured): observed values are $18.8 \pm 10.7\%$ in U7, $19.7 \pm 6.9\%$ in S7, and $21.9 \pm 14.5\%$ in U3 biofilters. Similarly, no significant difference ($p < 0.05$) is observed neither between U7 and U7C biofilters nor between S7 and S7C biofilters in terms of their TP removal capacity: observed removal values are $13.3 \pm 10.9\%$ in S7C to $15.8 \pm 7.9\%$ in U7C biofilters. Biofilters demonstrate negligible NPOC removal during the challenge test regardless of their operating conditions (Figure S4). Note that the nutrient removal results presented in this study assumes that natural attenuation of the nutrients in the influent stormwater during the duration of the daily experiments are negligible (Section 2.4).

3.5 Growth of biofilm in the biofilters and changes in hydraulic conductivity

We report the presence of active biomass in the biofilters by quantifying the ATP density in the BSM: mass of ATP/mass of dry collectors (Figure 4). The exposure of test-biofilters to natural stormwater results in the presence of active biomass in the BSM and the density of accumulated biomass near the inlet is significantly higher ($p < 0.05$): inlet versus outlet ATP densities comparison within the same group of test-biofilters, e.g., U7, S7, and U3 (Figure 4). ATP densities (average $\pm$ standard deviation) at the inlet of the biofilters range from 37.2 ± 4.9 (U3 biofilters) to 56.7 ± 5.3 (S7 biofilters) ng/g-dry collectors compared to the outlet densities of 10.7 ± 3.9 to 19.9 ± 3.2 ng/g-dry collectors. The biofilters with a SZ (i.e., S7 biofilters) have a significantly higher ($P < 0.05$) amount of biomass compared to the biofilters without a SZ (U7). Note that active biomass within the control-biofilters is small relative to that observed in the test-biofilters, ranging from 1.1 ± 0.2 to 2.0 ± 0.5 ng/g-dry collector.

Figure 4: Changes in a) amount of active biomass within and b) hydraulic conductivity of the biofilters due to a prolonged exposure to stormwater. U3 and U7 indicate test-biofilters

without a saturation zone (SZ) that experienced 3 day and 7 day antecedent dry period (ADD), respectively. S7 represents test-biofilters with a SZ and 7 days ADD. Control- and test-biofilters were exposed to the stormwater with a similar chemistry: control- biofilters received autoclaved natural stormwater water while test-columns received natural stormwater without autoclaving. Hydraulic conductivity of the biofilters was measured using falling head permeability tests. Error bars represent one standard deviation.

The average hydraulic conductivity, K_{sat} ($\pm$ standard deviation) of the BSM in the control-biofilters is 18.7 ± 4.9 cm/h during week 1 that increases to 23.3 ± 3.3 cm/h over 20-weeks of exposure to autoclaved stormwater. However, this difference between the K_{sat} of the control-biofilters in week 20 and the K_{sat} of the control-biofilters in week 1 is not statistically significant ($p > 0.05$). Similarly, there is no significant difference ($p > 0.05$) between U7 and S7 or U7 and U3 biofilters with respect to their K_{sat} . The average K_{sat} in test-biofilters ranges from 16.6 ± 1.7 (S7 biofilters) to 20.6 ± 3.0 cm/h (U7 biofilters).

4. Discussion

4.1 *E. coli* removal in biofilters is higher than enterococci removal

Biochar-augmented biofilters more efficiently remove *E. coli* than enterococci, regardless of the biofilter-operating conditions, i.e., test- versus control-biofilters, 7-day versus 3-day ADD, or the presence versus the absence of an SZ. Our findings corroborate previous studies conducted in simplified systems that report that enterococci are retained less (15-34%) retention in porous media than *E. coli* (67-88% retention) (Mohanty et al. 2013, Chen and Walker 2012). The distinct mobility of *E. coli* and enterococci in saturated porous media can be attributed, in part, to differences in their physicochemical properties including cell membrane

composition, motility, shape, surface charge, hydrophobicity, and surface macromolecules length and composition (Abu-Lail and Camesano 2003a, Becker et al. 2004, Fontes et al. 1991, Silliman et al. 2001). These properties may control various physicochemical mechanisms of bacterial attachment to porous media (Tufenkji 2007) including hydrophobic attachment, electrostatic deposition, and steric interaction. Quantifying the relative contributions of these mechanisms to the observed removal is challenging due to the complexity of our experimental system. This complexity includes highly varied stormwater chemistry, biofilm-coated BSM, and the inherent complexity in characterizing the physicochemical characteristics of environmental strains of the target FIB.

Nonetheless, previous reports on the comparable hydrophobic characteristics of *E. coli* and *Enterococcus faecalis* and the strong hydrophobicity of biochar imply “hydrophobic attachment” as a likely mechanism for both *E. coli* and enterococci removal in biochar-amended biofilters (Mohanty et al. 2013, Abit et al. 2012). However, in the case of *E. coli*, additional mechanisms, such as electrostatic deposition onto the BSM surface sites with “charge heterogeneity”, are possible because of their relatively lower negative surface charge compared to enterococci (Chen and Walker 2012, Tufenkji and Elimelech 2005, Elimelech et al. 2000). Under low ionic strength conditions, Gram-negative bacteria like *E. coli* can also be removed via “steric interaction” or the “polymer bridging” between the cell surface macromolecules and the BSM surfaces (Chen and Walker 2012, Abu-Lail and Camesano 2003b, Katsikogianni and Missirlis 2004). Further studies may help rank the significance of the aforementioned mechanisms, and may also explore possible additional mechanisms (e.g.,

grazing or microbial-competition) to delineate the observed differences between the two FIB in regard to their mobility in biofilters.

4.2 Aging of the BSM reduces FIB removal in biofilters

The removal of *E. coli* and enterococci in the biochar-modified biofilters decreases with the repeated exposure of the biofilters to natural stormwater. While this is the first study investigating FIB removal in biochar-modified biofilters for an extended period of time, several studies have reported significant changes in the performance of both sand and sand-compost biofilters, stemming from prolonged exposure to natural stormwater (Chandrasena et al. 2012, Zhang et al. 2011). We must note that the evidence presented in the literature is conflicting regarding the exact nature of the influence of biofilm on microbial transport through porous media. Multiple studies have discussed an increase in the bacteria-removal capacity due to biofilm formation (Liu and Li 2007), but several others reported a decrease in FIB removal in biofilm-coated porous media (Liu et al. 2007). Altered bacterial attachment in biologically aged biofilters may be attributed to the changes in physicochemical properties of the BSM over time in several ways (Rittmann 1993, Sheng et al. 2010, Pintelon et al. 2012, Mitzel et al. 2016): a) altered surface charge, due to biofilm EPS; b) reduced porosity, due to biofilm formation onto the interstitial pore-spaces; c) increased macroscopic diffusion, resulted from higher retention time and tortuosity; d) altered surface hydrophobicity of the collectors, due to biofilm coating; and e) altered surface interaction, due to absorption of natural macromolecules.

We observed no change in the hydraulic conductivity of the biofilters over the course of the experiment indicating that the biological aging do not affect biofilters porosity or hydraulic

retention time. Thus, a reduction in the hydrophobicity and enhancement in surface charge of the biochar-surfaces due to the formation of biofilm may have contributed to reduced microbial removal. We are also aware that the accumulation of active biomass within the biofilters may introduce additional pathways of FIB reduction, i.e., protozoan grazing or straining (Zhang et al. 2010). In this study, however, the role of such mechanisms in FIB removal appears to be either insignificant or outplayed by other mechanisms resulting from weaker hydrophobic forces and less available surface sites, as log removal was always lower in biofilters with biofilms compared to those without.

4.3 Presence of saturation zone and length of antecedent dry period does not influence FIB removal

Physical weathering, e.g., wet and dry cycles, has been reported to influence the efficacy of stormwater biofilters (Li et al. 2012). Multiple studies have discussed the reduced performance of sand-compost biofilters after a dry period primarily stemming from fine fissures and macropore formation within the BSM (Chandrasena et al. 2012, Li et al. 2012, Rusciano and Obropta 2007). However, in our study, we observe no significant difference in FIB removal in biochar-augmented biofilters when the ADD changed from 3 to 7 days. We attribute this to the high water-holding capacity of the biochar-modified BSM (Liu et al. 2016) due to strong capillary forces within the residual nano-pores of the biochar particles (Gray et al. 2014). Note that significant effects of ADD on the performances of sandy loam biofilters was reported for after a dry period of 2 weeks: which probably was a long enough time for fissures or macropores to form within the sandy loam BSM. In contrast, we used shorter ADDs, which are

probably not long enough to induce any significant changes in the biochar-modified BSM with increased water-holding capacity.

Our results suggest that the presence of SZ in biochar-amended biofilters does not affect their FIB removal capacity. However, the presence of a SZ resulted in a slightly higher amount of active biomass when compared to biofilters without an SZ. Based on the discussion presented in section 4.2 on the effect of the presence of biofilm on FIB removal, one would expect that the FIB removal in biofilters with a SZ would be lower than biofilters without any SZ. We explain this anomaly by hypothesizing that the observed difference between biofilters with and without SZ in terms of biofilm (as inferred from ATP densities) are not pronounced enough to cause a difference in their FIB removal capacity. Alternatively, presence of a SZ in biofilters may affect the diversity of the microbial community within the biofilters. For example, the presence of SZ has been reported to support the growth of denitrifying bacteria in biofilters (Singleton et al. 2007). Such unique microbial composition in biofilters with a SZ may introduce unique effects of biofilm on the FIB removal in biofilters in the presence of a SZ. In addition, the presence of an SZ in biofilters may reduce the remobilization of bacteria (Chandrasena et al. 2014), thereby offsetting the minor reduction (if any, due to the presence of biomass) in microbial attachment. "Dilution effect" is another mechanism that has been reported in the literature to explain a decrease in FIB concentration in effluent of sand biofilters with a SZ (Chandrasena et al. 2014). In biofilters with a SZ, dilution of influent stormwater occurs by the water present in the SZ that contains little or no FIB. The result is an overall decrease in FIB concentration in the exiting effluent. Note that, our experimental design does not allow for

testing the effects of dilution on FIB removal in biochar-augmented biofilters with SZ: the sampled effluent for measuring FIB concentration is not likely to contain any SZ water.

4.4 Biological aging and the presence of a saturation zone enhance nitrate removal in biofilters

While nitrate removal in control- biofilters varied from net leaching to poor removal, we observe an average nitrate removal of nearly 60% in the test-biofilters. A very low nitrate removal in the control-biofilters indicates that the pristine biochar particles have a limited nitrate-absorption capacity; however, microbial activity in the test-biofilters is able to augment this capacity to a great extent. Such an increase in nitrate removal in the presence of biofilm can be attributed to either biological uptake or microbial conversion of nitrate (i.e., denitrification or anaerobic ammonium-oxidation) or both in the anoxic micro-pockets within the biofilters (Payne et al. 2014b, Payne et al. 2014c). This finding corroborates several earlier studies that were performed on conventional BSM (e.g., sand and sand-compost), and emphasized a need to provide a SZ to boost nitrate removal in biofilters (Payne et al. 2014d, Zinger et al. 2013). Ultimately, the results of our study suggest that biochar-modified biofilters with a SZ can be an effective tool to enhance nitrate removal from stormwater runoff.

4.5 Biochar-augmented sand is inefficient in removing NPOC, organic nitrogen, and dissolved phosphorus

Relatively lower removal of TN than inorganic nitrogen (i.e., NH_4^+ -N and NO_3^- -N) in biochar-amendment biofilters suggests biochar is an inefficient amendment for removing dissolved organic nitrogen (assuming, organic nitrogen equals TN minus inorganic nitrogen). Organic

nitrogen removal in biofilters is primarily associated with the processes of ammonification, hydrolysis, or amination (Saeed and Sun 2012). These processes are, however, often suppressed in stormwater biofilters when heterotrophic bacteria predominantly drives denitrification (followed by nitrification by nitrifying bacteria) processes over ammonification: as evidenced by repeated reports of low organic nitrogen removal in biofilters consisting of conventional BSM (Fletcher et al. 2007, Lloyd et al. 2001). In case of biochar-amended biofilters, biochar surfaces may act as “electron shuttles” and enhance the transfer of electrons to denitrifying microorganisms (Cayuela et al. 2013). However, further studies are needed to identify the specific factors dictating the organic nitrogen removal in stormwater biofilters and their role on the biofilter performance.

The removal of total phosphorus in biochar-augmented biofilters is relatively low compared to nitrogen removal: the highest phosphorus removal being around 30%. Dissolved phosphorus removal by biofilters has been shown to be high if BSM is augmented with metal oxides (Erickson et al. 2011, Erickson et al. 2007, Henderson et al. 2007). The primary mechanism involved in phosphorus removal from water by porous media filtration is complexation with alkaline metals (Ca^{2+} , Mg^{2+} , K^{+}) and precipitation (Genz et al. 2004). The dissolved phosphorus may also be removed by the process of anion exchange; however, the anion exchange capacity of the wood-derived low-temperature biochar—used in this study—is reportedly low (Fang et al. 2013). The formation of organo-phosphorus complexes and their depositions onto the collector surfaces can be an additional mechanism of phosphorus removal in biochar-augmented biofilters (Sudhakar and Dikshit 1999, Yao et al. 2013). Biochar, known exceptionally efficient in sorbing polar or non-polar organic molecules (Ahmad et al. 2014), may

sorb metal chelating organics and subsequently develop sites for soluble phosphorus removal via absorption (Yao et al. 2013). However, our results describing low phosphorus and organic carbon removal suggest that this mechanism is not dominant in wood-derived biochar made by slow pyrolysis. Therefore, if phosphorus is the primary contaminant of concern, BSM with higher phosphorus removal efficiency should be explored to complement the high inorganic nitrogen and FIB removal capacity of biochar.

The influent natural stormwater and effluent from the biofilters have similar concentrations of NPOC. The limited removal of dissolved organic carbon is one of the drawbacks of using biochar as an amendment for BSM. Insignificant removal of NPOC in the biochar-amended biofilters may have occurred due to the leaching of fine biochar particles (which subsequently contributed to the NPOC signal in the effluent), a competition between humic substances and other contaminants (i.e., microbes) for the available surface absorption sites, or breakdown and re-suspension of the absorbed carbonaceous particles (Liu et al. 2016).

5. Conclusion

Our results suggest that biochar-augmented biofilters are capable of removing *E. coli* and enterococci from natural stormwater over approximately 5 months. Effluent concentrations complied with water quality standard set by USEPA for recreational use. However, the microbial removal in biofilters decreases with a repeated exposure to natural stormwater possibly due to the accumulation of active biomass onto the BSM. The presence of active biomass negatively influences FIB removal by weakening hydrophobic interaction among biochar surfaces and bacterial cells. FIB removal can also decrease due to a potential reduction in the number of available surface sites for bacterial attachment. However, based on the log-removal calculated

using equation i, we observe no significant influence of antecedent dry days of 3 or 7 day length or the presence of a submerged zone on the FIB removal capacity of the biofilters.

Biochar-augmented biofilters are also able to capture a considerable amount of inorganic nitrogen from natural stormwater. While ammonium removal does not depend on the operating conditions (i.e., 3 days versus 7 days ADD, the presence of biofilm, and SZ), the presence of SZ and biofilm significantly increase nitrate removal in biofilters. However, the biochar-augmented biofilters are not very efficient in phosphorus removal irrespective of the operating conditions of the biofilters.

We would like to note here that, in utilizing our findings one has to keep it in mind that these results could be exclusive to wood-derived biochar produced via the stated pyrolysis procedure. In addition, the biofilters used in this study lacked indigenous soil microorganisms as the BSM was autoclaved prior to commencing the experiment. Soil microbes may influence the performance of biochar-augmented biofilters. Therefore, further studies involving a) biochar produced from a wider set of feedstock, pyrolysis temperature; b) additional microbial contaminants, including human pathogens; c) a longer aging period, in the order of years; and d) the effects of indigenous soil microbial communities and vegetation may provide additional complementary information.

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Note: The authors declare no competing financial interests.

Supplementary Material (SM)

Details of sand preparation (M1); Details of Water Quality Analyses (M2); Details of falling head hydraulic conductivity test (M3); Details of biomass characterization protocol (M4); Timeline of the column experiments (Figure S1); Overview of weekly flushing events (Figure S2); Chemical and microbiological quality of influent stormwater (Figure S3); Non-purgeable organic carbon removal in biofilters (Figure S4).

References

- Abit, S.M.; Bolster, C.H.; Cai, P.; Walker, S.L. Influence of feedstock and pyrolysis temperature of biochar amendments on transport of *Escherichia coli* in saturated and unsaturated soil. *Environmental science & technology*. 46:8097-8105; 2012
- Abu-Lail, N.I.; Camesano, T.A. Role of Ionic Strength on the Relationship of Biopolymer Conformation, DLVO Contributions, and Steric Interactions to Bioadhesion of *Pseudomonas putida* KT2442. *Biomacromolecules*. 4:1000-1012; 2003a
- Abu-Lail, N.I.; Camesano, T.A. Role of lipopolysaccharides in the adhesion, retention, and transport of *Escherichia coli* JM109. *Environmental science & technology*. 37:2173-2183; 2003b
- Ahmad, M.; Rajapaksha, A.U.; Lim, J.E.; Zhang, M.; Bolan, N.; Mohan, D.; Vithanage, M.; Lee, S.S.; Ok, Y.S. Biochar as a sorbent for contaminant management in soil and water: a review. *Chemosphere*. 99:19-33; 2014
- Ambrose, R.F.; Winfrey, B.K. Comparison of stormwater biofiltration systems in Southeast Australia and Southern California. *Wiley Interdisciplinary Reviews: Water*. 2:131-146; 2015
- Barrett, M.E.; Limouzin, M.; Lawler, D.F. Effects of media and plant selection on biofiltration performance. *Journal of Environmental Engineering*. 139:462-470; 2012
- Becker, M.W.; Collins, S.A.; Metge, D.W.; Harvey, R.W.; Shapiro, A.M. Effect of cell physicochemical characteristics and motility on bacterial transport in groundwater. *Journal of contaminant hydrology*. 69:195-213; 2004
- Bratieres, K.; Fletcher, T.; Deletic, A.; Alcazar, L.; Le Coustumer, S.; McCarthy, D. Removal of nutrients, heavy metals and pathogens by stormwater biofilters. 11th International Conference on Urban Drainage; 2008

646 Cahoon, L.B.; Hales, J.C.; Carey, E.S.; Loucaides, S.; Rowland, K.R.; Toothman, B.R. Multiple modes of
 647 water quality impairment by fecal contamination in a rapidly developing coastal area: southwest
 648 Brunswick County, North Carolina. *Environmental monitoring and assessment*. 188:1-13; 2016
 649 Cayuela, M.L.; Sánchez-Monedero, M.A.; Roig, A.; Hanley, K.; Enders, A.; Lehmann, J. Biochar and
 650 denitrification in soils: when, how much and why does biochar reduce N₂O emissions? *Scientific*
 651 *reports*. 3; 2013
 652 Chan, K.; Van Zwieten, L.; Meszaros, I.; Downie, A.; Joseph, S. Agronomic values of greenwaste biochar
 653 as a soil amendment. *Soil Research*. 45:629-634; 2008
 654 Chandrasena, G.; Deletic, A.; Ellerton, J.; McCarthy, D. Evaluating *Escherichia coli* removal performance
 655 in stormwater biofilters: a laboratory-scale study. *Water Science and Technology*. 66:1132-1138;
 656 2012
 657 Chandrasena, G.; Deletic, A.; McCarthy, D. Biofiltration for stormwater harvesting: Comparison of
 658 *Campylobacter* spp. and *Escherichia coli* removal under normal and challenging operational
 659 conditions. *Journal of Hydrology*. 537:248-259; 2016
 660 Chandrasena, G.; Pham, T.; Payne, E.; Deletic, A.; McCarthy, D. *E. coli* removal in laboratory scale
 661 stormwater biofilters: Influence of vegetation and submerged zone. *Journal of Hydrology*.
 662 519:814-822; 2014
 663 Chang, N.; Islam, M.; Wanielista, M. Floating wetland mesocosm assessment of nutrient removal to
 664 reduce ecotoxicity in stormwater ponds. *International Journal of Environmental Science and*
 665 *Technology*. 9:453-462; 2012
 666 Chen, G.; Walker, S.L. Fecal indicator bacteria transport and deposition in saturated and unsaturated
 667 porous media. *Environmental science & technology*. 46:8782-8790; 2012
 668 Elimelech, M.; Nagai, M.; Ko, C.-H.; Ryan, J.N. Relative insignificance of mineral grain zeta potential to
 669 colloid transport in geochemically heterogeneous porous media. *Environmental science &*
 670 *technology*. 34:2143-2148; 2000
 671 Erickson, A.; Gulliver, J.; Weiss, P. Removing dissolved phosphorus from stormwater. 12th Int Conf on
 672 Urban Drainage, International Water Association (IWA), London; 2011
 673 Erickson, A.J.; Gulliver, J.S.; Weiss, P.T. Enhanced sand filtration for storm water phosphorus removal.
 674 *Journal of Environmental Engineering*. 133:485-497; 2007
 675 Fang, Q.; Chen, B.; Lin, Y.; Guan, Y. Aromatic and hydrophobic surfaces of wood-derived biochar enhance
 676 perchlorate adsorption via hydrogen bonding to oxygen-containing organic groups.
 677 *Environmental science & technology*. 48:279-288; 2013
 678 Fletcher, T.; Zinger, Y.; Deletic, A.; Bratières, K. Treatment efficiency of biofilters; results of a large-scale
 679 column study. *Rainwater and Urban Design* 2007:266; 2007
 680 Flynn, K.M.; Traver, R.G. Green infrastructure life cycle assessment: A bio-infiltration case study.
 681 *Ecological engineering*. 55:9-22; 2013
 682 Fontes, D.; Mills, A.; Hornberger, G.; Herman, J. Physical and chemical factors influencing transport of
 683 microorganisms through porous media. *Applied and Environmental Microbiology*. 57:2473-
 684 2481; 1991
 685 García-Albacete, M.; Tarquis, A.M.; Cartagena, M.C. Risk of Leaching in Soils Amended by Compost and
 686 Digestate from Municipal Solid Waste. *The Scientific World Journal*. 2014:565174; 2014
 687 Genz, A.; Kornmüller, A.; Jekel, M. Advanced phosphorus removal from membrane filtrates by
 688 adsorption on activated aluminium oxide and granulated ferric hydroxide. *Water research*.
 689 38:3523-3530; 2004
 690 Gray, M.; Johnson, M.G.; Dragila, M.I.; Kleber, M. Water uptake in biochars: The roles of porosity and
 691 hydrophobicity. *Biomass and Bioenergy*. 61:196-205; 2014
 692 Hathaway, A.; Hunt, W.; Wright, J.; Jadlocki, S. Field evaluation of indicator bacteria removal by
 693 stormwater BMPs in North Carolina. *World Environmental and Water Resources Congress*; 2009

Henderson, C.; Greenway, M.; Phillips, I. Removal of dissolved nitrogen, phosphorus and carbon from stormwater by biofiltration mesocosms. *Water Science and Technology*. 55:183-191; 2007
 Hengen, T.J.; Sieverding, H.L.; Stone, J.J. Lifecycle Assessment Analysis of Engineered Stormwater Control Methods Common to Urban Watersheds. *Journal of Water Resources Planning and Management*:04016016; 2016
 Hsieh, C.-h.; Davis, A.P. Evaluation and optimization of bioretention media for treatment of urban storm water runoff. *Journal of Environmental Engineering*. 131:1521-1531; 2005a
 Hsieh, C.H.; Davis, A.P. Evaluation and optimization of bioretention media for treatment of urban storm water runoff. *Journal of Environmental Engineering-Asce*. 131:1521-1531; 2005b
 Hunt, W.; Smith, J.; Jadlocki, S.; Hathaway, J.; Eubanks, P. Pollutant removal and peak flow mitigation by a bioretention cell in urban Charlotte, NC. *Journal of Environmental Engineering*. 134:403-408; 2008
 Inyang, M.; Dickenson, E. The potential role of biochar in the removal of organic and microbial contaminants from potable and reuse water: A review. *Chemosphere*. 134:232-240; 2015
 Joyner, B.L.; Waldrop, R.J. Method of treating stormwater runoff and domestic waste with high carbon ash. Google Patents; 2008
 Katsikogianni, M.; Missirlis, Y. Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. *Eur Cell Mater*. 8:37-57; 2004
 Lehmann, J.; Joseph, S. Biochar for environmental management: science, technology and implementation: Routledge; 2015
 Li, Y.; McCarthy, D.T.; Deletic, A. Escherichia coli removal in copper-zeolite-integrated stormwater biofilters: Effect of vegetation, operational time, intermittent drying weather. *Ecological Engineering*. 90:234-243; 2016
 Li, Y.L.; Deletic, A.; Alcazar, L.; Bratieres, K.; Fletcher, T.D.; McCarthy, D.T. Removal of Clostridium perfringens, Escherichia coli and F-RNA coliphages by stormwater biofilters. *Ecological Engineering*. 49:137-145; 2012
 Li, Y.L.; Deletic, A.; McCarthy, D.T. Removal of E. coli from urban stormwater using antimicrobial-modified filter media. *Journal of hazardous materials*. 271:73-81; 2014
 Lim, H.; Lim, W.; Hu, J.; Ziegler, A.; Ong, S. Comparison of filter media materials for heavy metal removal from urban stormwater runoff using biofiltration systems. *Journal of environmental management*. 147:24-33; 2015
 Liu, C.; Wang, H.; Tang, X.; Guan, Z.; Reid, B.J.; Rajapaksha, A.U.; Ok, Y.S.; Sun, H. Biochar increased water holding capacity but accelerated organic carbon leaching from a sloping farmland soil in China. *Environmental Science and Pollution Research*. 23:995-1006; 2016
 Liu, Y.; Li, J. Role of Pseudomonas aeruginosa biofilm in the initial adhesion, growth and detachment of Escherichia coli in porous media. *Environmental science & technology*. 42:443-449; 2007
 Liu, Y.; Yang, C.-H.; Li, J. Adhesion and retention of a bacterial phytopathogen Erwinia chrysanthemi in biofilm-coated porous media. *Environmental science & technology*. 42:159-165; 2007
 Lloyd, S.; Fletcher, T.; Wong, T.; Wootton, R. Assessment of pollutant removal in a newly constructed bio-retention system. *Proceedings of the 2nd South Pacific stormwater conference*; 2001
 Mallin, M.A.; Johnson, V.L.; Ensign, S.H. Comparative impacts of stormwater runoff on water quality of an urban, a suburban, and a rural stream. *Environmental Monitoring and Assessment*. 159:475-491; 2009
 Mitzel, M.R.; Sand, S.; Whalen, J.K.; Tufenkji, N. Hydrophobicity of biofilm coatings influences the transport dynamics of polystyrene nanoparticles in biofilm-coated sand. *Water Research*. 92:113-120; 2016

740 Mohan, D.; Sarswat, A.; Ok, Y.S.; Pittman, C.U. Organic and inorganic contaminants removal from water
741 with biochar, a renewable, low cost and sustainable adsorbent—a critical review. *Bioresource*
742 *technology*. 160:191-202; 2014

743 Mohanty, S.K.; Boehm, A.B. Escherichia coli Removal in Biochar-Augmented Biofilter: Effect of
744 Infiltration Rate, Initial Bacterial Concentration, Biochar Particle Size, and Presence of Compost.
745 *Environmental science & technology*. 48:11535-11542; 2014

746 Mohanty, S.K.; Boehm, A.B. Effect of weathering on mobilization of biochar particles and bacterial
747 removal in a stormwater biofilter. *Water Research*. 85:208-215; 2015

748 Mohanty, S.K.; Cantrell, K.B.; Nelson, K.L.; Boehm, A.B. Efficacy of biochar to remove Escherichia coli
749 from stormwater under steady and intermittent flow. *Water research*. 61:288-296; 2014

750 Mohanty, S.K.; Torkelson, A.A.; Dodd, H.; Nelson, K.L.; Boehm, A.B. Engineering solutions to improve the
751 removal of fecal indicator bacteria by bioinfiltration systems during intermittent flow of
752 stormwater. *Environmental science & technology*. 47:10791-10798; 2013

753 NOAA. Annual Precipitation for selected locations. 2016

754 Passeport, E.; Hunt, W.F.; Line, D.E.; Smith, R.A.; Brown, R.A. Field Study of the Ability of Two Grassed
755 Bioretention Cells to Reduce Storm-Water Runoff Pollution. *Journal of Irrigation and Drainage*
756 *Engineering*. 135:505-510; 2009

757 Payne, E.G.; Fletcher, T.D.; Cook, P.L.; Deletic, A.; Hatt, B.E. Processes and drivers of nitrogen removal in
758 stormwater biofiltration. *Critical Reviews in Environmental Science and Technology*. 44:796-846;
759 2014a

760 Payne, E.G.; Fletcher, T.D.; Russell, D.G.; Grace, M.R.; Cavagnaro, T.R.; Evrard, V.; Deletic, A.; Hatt, B.E.;
761 Cook, P.L. Temporary storage or permanent removal? The division of nitrogen between biotic
762 assimilation and denitrification in stormwater biofiltration systems. *PloS one*. 9:e90890; 2014b

763 Payne, E.G.; Pham, T.; Cook, P.L.; Fletcher, T.D.; Hatt, B.E.; Deletic, A. Biofilter design for effective
764 nitrogen removal from stormwater—influence of plant species, inflow hydrology and use of a
765 saturated zone. *Water Science and Technology*. 69:1312-1319; 2014c

766 Payne, E.G.I.; Fletcher, T.D.; Russell, D.G.; Grace, M.R.; Cavagnaro, T.R.; Evrard, V.; Deletic, A.; Hatt, B.E.;
767 Cook, P.L.M. Temporary Storage or Permanent Removal? The Division of Nitrogen between
768 Biotic Assimilation and Denitrification in Stormwater Biofiltration Systems. *PLoS ONE*. 9:e90890;
769 2014d

770 Pintelon, T.R.; Picioreanu, C.; van Loosdrecht, M.; Johns, M.L. The effect of biofilm permeability on
771 bio-clogging of porous media. *Biotechnology and bioengineering*. 109:1031-1042; 2012

772 Poresky, A.L.; Allen II, V.P.; CPSWQ, L.-A.; Reynolds, S.K. Biofiltration Equivalency: Assessing the Relative
773 Performance of Innovative and Conventional Designs. *World Environmental and Water*
774 *Resources Congress* 2016; 2016

775 Reddy, K.R.; Xie, T.; Dastgheibi, S. Evaluation of biochar as a potential filter media for the removal of
776 mixed contaminants from urban storm water runoff. *Journal of Environmental Engineering*.
777 140:04014043; 2014

778 Rittmann, B.E. The significance of biofilms in porous media. *Water Resources Research*. 29:2195-2202;
779 1993

780 Rusciano, G.; Obropta, C. Bioretention column study: Fecal coliform and total suspended solids
781 reductions. *Transactions of the ASABE*. 50:1261-1269; 2007

782 Ryzhakov, A.; Kukkonen, N.; Lozovik, P. Determination of the rate of ammonification and nitrification in
783 natural water by kinetic method. *Water resources*. 37:70-74; 2010

784 Saeed, T.; Sun, G. A review on nitrogen and organics removal mechanisms in subsurface flow
785 constructed wetlands: Dependency on environmental parameters, operating conditions and
786 supporting media. *Journal of Environmental Management*. 112:429-448; 2012

Sage, J.; Berthier, E.; Gromaire, M.C. Stormwater Management Criteria for On-Site Pollution Control: A Comparative Assessment of International Practices. *Environmental Management*. 56:66-80; 2015

Seelsaen, N.; McLaughlan, R.; Moore, S.; Ball, J.; Stuetz, R. Pollutant removal efficiency of alternative filtration media in stormwater treatment. *Water science and technology*. 54:299-305; 2006

Sheng, G.-P.; Yu, H.-Q.; Li, X.-Y. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. *Biotechnology advances*. 28:882-894; 2010

Silliman, S.; Dunlap, R.; Fletcher, M.; Schneegurt, M. Bacterial transport in heterogeneous porous media: Observations from laboratory experiments. *Water Resources Research*. 37:2699-2707; 2001

Singleton, M.; Esser, B.; Moran, J.; Hudson, G.; McNab, W.; Harter, T. Saturated zone denitrification: potential for natural attenuation of nitrate contamination in shallow groundwater under dairy operations. *Environmental science & technology*. 41:759-765; 2007

Subramaniam, D.N.; Egodawatta, P.; Mather, P.; Rajapakse, J. Stabilization of stormwater biofilters: Impacts of wetting and drying phases and the addition of organic matter to filter media. *Environmental management*. 56:630-642; 2015

Sudhakar, Y.; Dikshit, A. Kinetics of endosulfan sorption on to wood charcoal. *Journal of Environmental Science & Health Part B*. 34:587-615; 1999

Tian, J.; Yi, S.; Imhoff, P.; Chiu, P.; Guo, M.; Maresca, J.; Beneski, V.; Cooksey, S. Biochar-amended media for enhanced nutrient removal in stormwater facilities. *World Environ Water Resour Congr*. 2014:197-208; 2014

Tufenkji, N. Modeling microbial transport in porous media: Traditional approaches and recent developments. *Advances in Water Resources*. 30:1455-1469; 2007

Tufenkji, N.; Elimelech, M. Breakdown of colloid filtration theory: Role of the secondary energy minimum and surface charge heterogeneities. *Langmuir*. 21:841-852; 2005

Velten, S.; Hammes, F.; Boller, M.; Egli, T. Rapid and direct estimation of active biomass on granular activated carbon through adenosine tri-phosphate (ATP) determination. *Water Research*. 41:1973-1983; 2007

Waterboard CA. Attachment H. Bioretention / biofiltration design criteria 2011

Yao, Y.; Gao, B.; Chen, J.; Yang, L. Engineered biochar reclaiming phosphate from aqueous solutions: mechanisms and potential application as a slow-release fertilizer. *Environmental science & technology*. 47:8700-8708; 2013

Zhang, L.; Seagren, E.A.; Davis, A.P.; Karns, J.S. The capture and destruction of *Escherichia coli* from simulated urban runoff using conventional bioretention media and iron oxide-coated sand. *Water Environment Research*. 82:701-714; 2010

Zhang, L.; Seagren, E.A.; Davis, A.P.; Karns, J.S. Long-term sustainability of *Escherichia coli* removal in conventional bioretention media. *Journal of Environmental Engineering*. 137:669-677; 2011

Zhang, L.; Seagren, E.A.; Davis, A.P.; Karns, J.S. Effects of temperature on bacterial transport and destruction in bioretention media: Field and laboratory evaluations. *Water Environment Research*. 84:485-496; 2012

Zinger, Y.; Blecken, G.-T.; Fletcher, T.D.; Viklander, M.; Deletić, A. Optimising nitrogen removal in existing stormwater biofilters: Benefits and tradeoffs of a retrofitted saturated zone. *Ecological Engineering*. 51:75-82; 2013

Stormwater runoff

- + Biofilm
- + Saturated zone
- + Dry period

FIB, Nutrient

FIB	Nutrient
↑	↓
↔	↓
↔	↔

No change: ↔
Increase: ↑
Decrease: ↓