Focus Article

Meeting the criteria: linking biofilter design to fecal indicator bacteria removal

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The capture, treatment, and reuse of storm-water runoff are win–win propositions that can lead to improvements in both human water security and ecosystem health. Although not all treatment technologies facilitate the capture, treatment, and reuse of water, biofilters do. Biofilters are engineered analogues of natural systems that use low energy, natural processes to treat stormwater. Biofilter design is closely linked to treatment efficiency. As such, specific design components, such as submerged zones (SZs: saturated, organic-rich layers near the base of biofilters), can significantly affect contaminant removal. Of particular interest, is the utility of SZ biofilter designs for removing indicators of pathogens, the so-called fecal indicator bacteria (FIB). FIB exist at high concentrations in stormwater, sometimes several orders of magnitude above recreational, nonpotable reuse, or drinking water standards, and have been identified as one of the primary barriers to stormwater reuse. A comparison of FIB removal values from literature indicates that SZ systems significantly enhance FIB removal (~10-fold) relative to other design configurations ($p < 0.05$). Processes that may contribute to this effect include physicochemical filtration, biofilm formation, and protistan grazing, amongst others. A high degree of synergy exists between processes, and many unknowns remain. Model frameworks developed for evaluation of similarly synergistic systems, including biofilter analogues like the vadose zone, may be useful for addressing these unknowns and informing future biofilter design.

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INTRODUCTION

Stormwater biofilters (also known as bioretention systems or rain gardens) are vegetated, vertical infiltration systems that decrease runoff volumes and contaminant loads from the urban environment.[1–4] These systems can be configured to treat, infiltrate, and reuse urban runoff, increasing water security and protecting downstream receiving waters.[5,6] Biofilters frequently contain: (1) a detention area (or ponded zone) that retains water prior to infiltration; (2) a biological component (including vegetation) with traits that facilitate pollutant uptake; (3) filter media (sand, sandy loam, or loamy sand) with high infiltration and pollutant removal capacity; (4) a coarse sand transition layer; and (5) a drainage layer, typically coarse sand or fine gravel[7,8] (Figure 1(a)). Drainage layers can be lined or unlined, and with or without collection pipes. Unlined, unpiped systems promote infiltration of water into soils while lined, piped systems allow for stormwater reuse.

In addition to the above-specified design criteria, biofilters can also be configured with a submerged zone (SZ, also called a saturated or internal water storage zone, Figure 1(b)). SZs are saturated layers near the base of biofilters that retain moisture between storms.[3,8,9] Configuring a biofilter with an SZ requires (1) reducing the filter media depth and raising the

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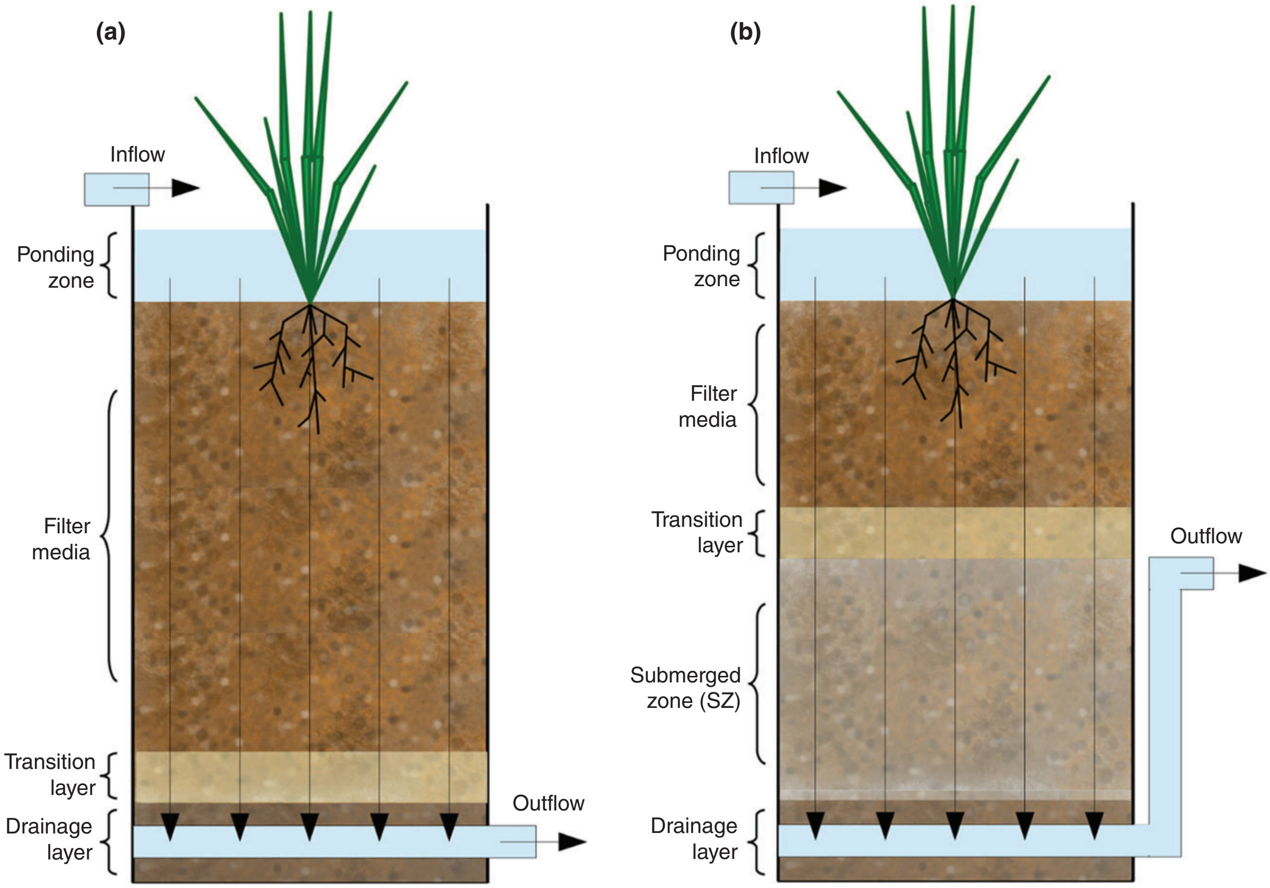

FIGURE 1 | Biofilter design schematics: (a) standard biofilter configuration without a submerged zone (SZ) and (b) advanced biofilter configuration with a SZ.

transition layer to make space for the zone, (2) piping the drainage layer, and (3) raising the outlet of the collection pipe to the level of the transition layer so that water only exits the pipe when the SZ is fully saturated.[3,4,8] The result is a biofilter with vertically variable flow and redox conditions (unsaturated and aerobic at the surface and saturated and anaerobic near the base).

SZs were initially proposed as a way to reduce nitrate leaching in biofilters. As such, they often contain organic carbon amendments that serve as electron donors for anaerobic metabolic processes such as denitrification.[4,10,11] SZs primarily affect nitrate by promoting microbially mediated denitrification or nitrate assimilation by biofilter vegetation.[9–12] Other contaminants of concern in stormwater, however, including bacterial pathogens and pathogen proxies (e.g., fecal indicator bacteria; FIB) are particle-associated or colloidal (1 nm to ~1 μm in size). For colloid and larger-sized particles, treatment and removal in biofilters may be affected more by physics and chemistry (e.g., particle–sediment grain interactions) than biological transformations. In short, FIB may be impacted by SZs differently than nitrate.

To better understand the potential risks and benefits of a SZs for treating stormwater runoff, this manuscript reviews available literature on FIB removal (fecal coliforms: FC, *Escherichia coli*: EC, and enterococci: ENT) in biofilters. The review contains four parts: (1) a description of FIB, their relevance for public health, and their abundance in stormwater runoff; (2) an overview of removal processes in porous media, focusing on those that are likely to affect biocolloids such as FIB; (3) a synthesis of available literature on FIB removal in biofilters with different design configurations; and (4) a discussion of key processes by which SZ may affect FIB capture or survival, informed by biofilter-specific studies, packed column experiments, and fieldwork from analogous natural systems (e.g., beach sands and the vadose zone). The review concludes with a recommendation regarding the suitability of SZ designs for FIB removal in biofilters, and a brief discussion of remaining unknowns in these systems, some of which may be best addressed using mathematical models informed by vadose zone literature.

THE FECAL INDICATOR BACTERIA

FIB are enteric bacteria that typically exhibit high concentrations in feces (human and animal). They are used, by proxy, to track human pathogens in aquatic systems, including stormwater. Proxies are required because pathogens can have high infectivity at low doses, as well as low abundance and high variability in stormwater, making it difficult to use them for stormwater quality monitoring purposes.[13] As such, stormwater monitoring tends to focus on three groups of FIB: FC (Gram-negative rod-shaped bacteria including *Klebsiella* and *Escherichia* genera);[14] EC (a subset of FC that contains nonpathogenic and pathogenic members);[15,16] and ENT (36 species of Gram-positive diplococcoid bacteria, a subset of which are human pathogens).[17]

FIB concentrations in stormwater are often high, sometimes several orders of magnitude above recreational, nonpotable reuse, or drinking water standards.[13,18,19] Although these high concentrations may reflect a combination of human (sewage) and animal or nonfecal sources (e.g., growth on stormwater infrastructure, soils, or vegetation),[17] co-occurrence of FIB, human pathogens, and chemical source tracking markers (e.g., caffeine) in urban stormwater, suggest that sewage contamination is a serious concern.[13,18,19] Furthermore, genetic markers for EC pathogenesis and shiga-like toxins (linked to enteropathogenic and enterohemorrhagic diarrheal disease) can be elevated in runoff-impacted urban streams.[16] This presents a challenge for stormwater treatment measures such as biofilters, which must be both effective (remove high concentrations of FIB/pathogens) and consistent, in order to address public health concerns. Indeed, meeting existing FIB criteria has been cited by some as the primary barrier to stormwater treatment and reuse.[13]

REMOVAL PROCESSES IN POROUS MEDIA

Removal of particulate contaminants in biofilters occurs by four main processes: mechanical filtration, straining, physicochemical filtration, and transformation (or growth/mortality in the case of FIB).[13,20] The specific processes affecting individual contaminants will depend in part on their initial state (particle-associated or in solution), as some mechanisms act on a limited range of particle sizes (e.g., mechanical filtration, straining, and physicochemical filtration).[20–22] Physical processes affecting capture and biological processes affecting either capture or survival are discussed separately in the following sections.

Physical Processes Affecting Capture

Mechanical Filtration

Mechanical filtration is important for large particles. Contaminants with diameters that exceed the maximum diameter of filter media pores cannot infiltrate the media and become trapped at the surface, forming a filter cake[20] (Figure 2(a)). For biofilter media with median grain diameters ranging from approximately 150 to 1000 μm, this corresponds to particles >75–500 μm in diameter. Heavy metals and particulate phosphorus are commonly removed by mechanical filtration, and can become highly concentrated in the filter cake.[2,23]

Straining

Straining traps particles too small to be removed by mechanical filtration at narrow pore throats (‘true straining’) or grain junctions (‘wedging’) within the filter media[22] (Figure 2(a)). True straining can affect particles with particle to median grain size ratios >0.18; for filter media grain sizes between 150 and 1000 μm, this corresponds to particles 27–180 μm in diameter.[24] Wedging has been implicated in the removal of smaller contaminant particles (particle to median grain size ratios > 0.005).[20,22] This corresponds to particles 0.75–5 μm in diameter, suggesting that wedging can affect biocolloids such as FIB.[20,21,25,26] The importance of straining for capturing submicron particles, however, is hotly debated, as straining may not be independent of physicochemical filtration for true colloids.[20–22,27,28]

Physicochemical Filtration: Classical

Physicochemical filtration acts on all size classes of particles, but is particularly important for colloids. Under classical filtration theory, physicochemical filtration involves two distinct steps: (1) the transport of particles to sediment grains (represented by a dimensionless transport rate) and (2) particle attachment to sediment grain surfaces (represented by a dimensionless attachment efficiency).[29] For particles <1 μm, the transport step occurs primarily by Brownian diffusion[30] (Figure 2(a)). For particles >1 μm, however, the transport step occurs primarily by interception (particles following a streamline impact a sediment grain) and gravitational sedimentation[29,30] (Figure 2(a)). Because FIB fall in the 1 μm size range, all three processes may affect the dimensionless transport rate.

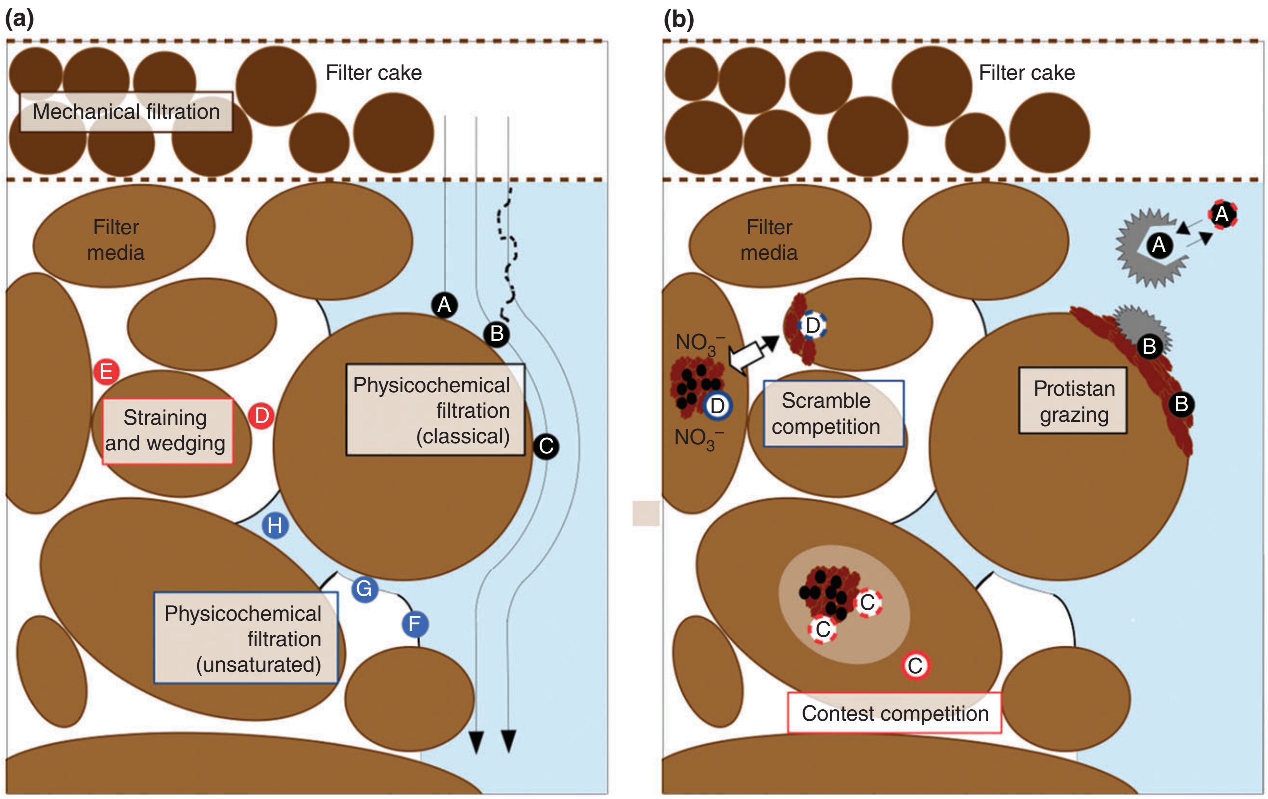

FIGURE 2 | A close-up of unsaturated sediments: filter cake layer (top) and filter media layer (bottom). (a) Size-dependent processes that contribute to particle capture. Large particles (dark brown) that cannot pass through filter media are captured in the filter cake by mechanical filtration. Small black particles illustrate classical modes of capture via physicochemical filtration. Particle (A) has been captured by sedimentation, particle (B) by diffusion, and particle (C) by interception. Physicochemical processes that are specific to unsaturated media are shown in blue. Particle (F) has been trapped at the air–water interface, particle (G) against a sediment grain by thin film straining, and particle (H) in a pendular ring of water between grains. Small red particles illustrate capture by straining in narrow pore throats (D) and wedging at grain junctions (E). (b) A subset of the biological processes affecting bacterial survival. Black particles represent bacteria grazed by protozoa (shown in grey). Particles (A) are engulfed by phagocytosis: (A-solid) is ingested, digested, and then excreted as waste (A-dashed). Particles (B) are grazed by a protozoan that specializes in biofilm bacteria. Intraspecific microbial competition is illustrated via particles (C) and (D). Particles (C) show contest competition, whereby a native microbial biofilm (brown and black plaque) excretes a substance that harms nearby competitors (C-dashed) but not distant cells (C-solid). Particles (D) show scramble competition, whereby native biofilm communities acquire nutrients (in this case nitrate) more efficiently than species D): this harms distant cells (D-dashed), but may aid nearby neighbors (D-solid).

Under 'favorable' attachment conditions, every time a particle collides with a sediment grain (referred to as a 'collector') it sticks, and therefore the rate of particle attachment is controlled by the dimensionless transport rate alone (i.e., the attachment efficiency is unity). Given that colloids, FIB, sands, and soil particles tend to be negatively charged under typical environmental pH,[13] not every particle collision is likely to stick. These so-called 'unfavorable' attachment conditions may be common in stormwater biofilters. Under unfavorable conditions particle removal is controlled by system chemistry (e.g., ionic strength, pH, and presence of dissolved or adsorbed organic molecules).[13,29,30] Particles may stick to collectors via weak, reversible interactions (secondary energy minimum) or, in some cases, strong, irreversible interactions (primary energy minimum).[31–33]

Whether or not a particle sticks depends on the forces it experiences as it approaches a collector. Derjaguin–Landau–Verwey–Overbeek (DLVO) theory assumes that interactions between particle and collector result from the additive effects of attractive Van Der Waals forces and attractive or repulsive electric double layer forces[27,29] Additional pertinent forces include: (1) Born repulsion: short range repulsion caused by interpenetration of electron shells, (2) hydration effects: repulsion due to the retention of water at colloid surfaces, (3) steric repulsion: weak repulsion caused by interpenetration of hydrophilic polymer chains on particle surfaces, (4) hydrophobic interactions: attraction between particles with hydrophobic surface groups, and (5) polymer bridging: long-range attractive forces between polymers on the surface of particles with low (or patchy) polymer concentrations.[27,31–33]

Physicochemical Filtration: Unsaturated Conditions and Flow Transients

In unsaturated sediment columns, additional processes contribute to attachment due to the presence of an air phase, which creates novel attachment surfaces (e.g., sediment–air and air–water).[31,32,34,35] The following attachment processes are unique to unsaturated media: (1) air–water interface attachment: binding occurs via strong capillary forces;[35] (2) thin film straining: colloids are pinned to sediment grains when water films become thinner than their diameter;[35] and (3) capture in pendular rings: colloids are trapped in stagnant water zones between grains, and are disconnected from bulk flow[35,36] (Figure 2(a)).

Under steady-flow conditions, unsaturated systems may exhibit higher colloid retention than saturated systems due to the combined effects of the above-mentioned processes.[34,35] The opposite may be true, however, when systems fill and drain (e.g., transient flow) because propagating wetting fronts mobilizes colloids trapped in pendular rings or attached at the air–water interface.[35] Wetting or drying fronts may also re-entrain colloids that are weakly attached in secondary energy minima.[32,34,35] Common mobilization processes include air–water interface scouring, thin film expansion, and reconnection of formerly stagnant water zones, amongst others.[32]

Biological Processes Affecting Capture or Survival

Micro- and Mesofaunal Grazers

Micro- and mesofaunal grazers, including protozoans and nematodes, exhibit high diversity in soils, and can exert significant control over microbial biomass, activity, and community structure.[37–39] However, because no studies to date have evaluated nematode activity in stormwater biofilters (let alone their effects on FIB capture/survival), the remainder of this section will focus on protozoans. Protozoan ciliates and flagellates typically ingest bacteria via phagocytosis. This process occurs in three steps: (1) encounter and cell recognition, (2) engulfment of bacterial prey, and (3) digestion and excretion from food vacuoles[39] (Figure 2(b)). Grazing rates in porous sediments range from 5 to 73 bacteria per protist per hour. Low grazing values come from experiments with free-living bacteria whereas larger values are reported for experiments with both particle-associated and free-living bacteria.[40] These results suggest that at least a subset of protozoans in soil are well adapted to grazing on attached microorganisms[36,40] (Figure 2(b)).

Protistan grazing has been observed to affect FIB in sediment column experiments and beach sands, although this pattern is not consistently detected.[41,42] Notably, grazing may impact survival of EC more than ENT, because ENT has a thick Gram-positive cell wall that can reduce or inhibit digestion by protists.[43] While grazing by heterotrophic nanoflagellates and ciliates have both been reported to decrease FIB abundance in aquatic systems,[44,45] heterotrophic nanoflagellates may exert more control in sediments, as standing stocks of ciliates in soils tend to be low.[39,40]

Invertebrate Macrofauna

Although soil invertebrate macrofauna are diverse, and perform a variety of important ecosystem functions (e.g., decomposition, litter transformation, and system 'engineering', including bioturbation),[37] little is known regarding their role in stormwater biofilters. This said, earthworms have been shown to increase microbial diversity/activity in sludge biofilters through selective digestion and transformation of large sludge particles into bioavailable forms.[46] Similarly, bioturbation (and soil oxygenation) by tubificid worms has been linked to increased aerobic microbial activity and dissolved organic carbon processing in soil columns amended with stormwater sediments.[47] These studies suggest that invertebrate macrofauna, such as worms, have the potential to impact microbial communities (including FIB) in biofilters. Further research evaluating the effects of specific macrofaunal groups in stormwater biofilters is clearly warranted, and is in fact under way as part of a large mesocosm study at Monash University, Australia, facilitated by the University of California Irvine Water PIRE program (a National Science Foundation funded Partnerships for International Research and Education project).

Vegetation

Vegetation–microbe interactions play an important role in the abundance, diversity, and activity of microorganisms in soil systems. Plant root characteristics can affect soil moisture content (a limiting factor for microbial activity in unsaturated soils),[36,48,49] and hydraulic residence time (which may impact capture/survival of particulate contaminants).[48–50] Roots also release oxygen, amino acids, and sugars that can stimulate aerobic metabolic processes in rhizosphere bacteria.[49] However, plants also compete directly with microorganisms for nutrients such as nitrate or ammonia. Indeed, plant community nitrogen preferences have been shown to alter rates of nitrification and denitrification by soil bacteria (and vice versa).[4,51]

FIB removal efficiency has been evaluated in biofilters containing a variety of plant morphotypes (grasses and sedges, climbing/scrambling dicots, and shrubs or trees).[12,49–51] While some studies report higher FIB removal in unplanted biofilters,[50] others suggest that certain plants increase removal efficiency.[49] For instance, the shrubs *Melaleuca incana* and *Leptospermum continentale* have been shown to facilitate higher FIB removal than the sedge *Carex apressa*, an industry standard in Australia due (primarily) to its efficient nutrient removal.[49] In some instances, high FIB removal has also been reported for biofilters planted with grasses, including *Stenataphrum secundatum*[49] and *Buchloe dactyloides*[12]. Notably, *S. secundatum* (and the above-mentioned shrubs), exhibit high nutrient removal in addition to FIB removal.[1,12,49] This suggests that these plants represent ideal candidates for future co-optomized biofilter designs that focus on simultaneous treatment of FIB and nutrients.

Microbial Competition

Microbial competition is frequently viewed through the resource ratio model of competition, whereby nutrient availability, demand, and relative consumption rates act in consort to determine the predominance of different microbial taxa. This framework, however, fails to take into account the ways in which bacteria 'manipulate the odds' (e.g., secretion of antibiotics, quorum sensing molecules, or quorum blockers), and shift competitive balance in their favor.[52] In this view, competition that involves direct, antagonistic interactions between individual bacterial cells or groups of cells is considered contest competition, whereas efficient uptake of limiting resources in the absence of cell to cell interactions is called scramble competition[52] (Figure 2(b)). Different microbial functional groups in soils (e.g., aerobic or anaerobic heterotrophs, nitrifiers, denitrifiers, sulfate reducers, and others) frequently compete via scramble competition. For instance, aerobic heterotrophs have been shown to outcompete nitrifiers in freshwater sediments with high C:N ratios, due to rapid assimilation of ammonium.[53] Less is known about contest competition between functional groups in soils, although some aerobic heterotrophs can produce quorum blocking substances that disrupt virulence expression or biofilm formation in other rhizosphere aerobes.[54,55]

Although competition amongst native microorganisms (and native microorganisms and FIB) has been observed in natural sediment systems, the nature and magnitude of these interactions vary, and the specific mechanisms involved remain an important topic of research.[36,42,56] It should be emphasized that, to date, assessment of FIB–microbe interactions in stormwater biofilters is rare,[57] and that the effects of microbe diversity in augmenting or ameliorating FIB–microbe competition is unknown. In fact, the biofilter microbiome itself remains (by and large) a black box. Even functional group activity (e.g., denitrification) is frequently inferred rather than quantified directly.[9,58] Microbiome formation and evolution in response to stormwater inputs is also understudied. However, there is some evidence that stormwater may increase bacterial abundance, biogeochemical activity (e.g., respiration, nitrification, and denitrification rates), metabolic diversity, and/or community structure,[59,60] all of which have the potential to impact FIB–microbe interactions.

Given how little is known about intramicrobial interactions in stormwater biofilters, all subsequent discussion of competition amongst biofilter microorganisms will be general, with functional groups or competition types (scramble vs contest) noted only if mentioned specifically in the literature.

Abiotic Factors

Although abiotic stressors in porous media impact all microorganisms to some degree, FIB are likely to be more sensitive than native soil flora because most strains lack adaptations for survival in sediments.[41,57] Indeed, the inability of some EC strains to down-regulate metabolic rates in response to low nutrient availability in soils, has been cited as a dominant factor contributing to mortality in sediments.[41] A variety of abiotic stressors may impact FIB survival in sediments, including pH, temperature, salinity, and moisture content.[56,57,61–63] Salinity and pH effects may be most notable for EC, as ENT are halotolerent and can survive at pH 4 to 10.[61,64] EC and ENT are both sensitive to soil moisture, although the magnitude and direction of response varies.[56,57,62] Temperature effects on FIB may also vary, with cold frequently reported to enhance survival.[56,57,65] Elevated FIB growth rates have been observed in warm, presterilized sediments, however, prompting speculation that the above-noted relationship between FIB and cold is driven by reductions in competitive or predatory microorganism abundance during cold weather, and not temperature itself.[57,65]

FIB are also sensitive to elevated concentrations of certain metals, which has prompted exploration of antimicrobial filter media containing copper for use in stormwater biofilters.[13,66] While these media show promise for FIB removal (median 3 $\log_{10}$ removal for EC), metal leaching can occur, and in some cases exceed recreational standards for marine and freshwater quality.[66] Because metal leaching appears to increase with salinity,[66] copper-based antimicrobial amendments may not be suitable for use in biofilters located in cold climates, where road salting elevates stormwater salinity (and is necessary for public protection).[60]

META-ANALYSIS OF FIB REMOVAL IN STORMWATER BIOFILTERS

A meta-analysis of FIB removal in stormwater biofilters was performed in order to evaluate: (1) overall variability in FIB removal performance and (2) differences in FIB removal amongst biofilters configured with or without SZs. The analysis was performed on February 2015 using the following search criteria in both Web of Science (all hits retained) and Google Scholar (top 50 hits retained): biofilter, biofiltration, bioinfiltration, bioretention, or rain garden, and stormwater or runoff, and bacteria, *Escherichia coli*, *Enterococcus*, total coliforms, or fecal coliforms.

This search procedure recovered 54 articles, 13 of which were retained for analysis. The remainder were excluded for one (or more) of the following reasons: (1) experiments were performed using chromatography columns, where column conditions (column length and cross-sectional area, influent ionic strength, degree of saturation, packing media, and/or vegetation type) were unlikely to be representative of biofilter conditions, (2) FIB removal data were co-reported in multiple articles (in these cases only one article was retained), (3) FIB removal was not evaluated under the unsaturated and transient flow conditions expected in practice, (4) multi-component treatment system performance was reported without separate consideration of individual components such as biofilters, or (5) FIB removal by biofilters was mentioned only anecdotally.

Of the 13 studies retained, 5 were classified as laboratory mesocosms (medium-sized biofilter experiments performed in a greenhouse or laboratory, total volume: 0.02–0.10 m^3) and 8 as field systems (permanent outdoor biofilters or large field-deployed mesocosms, total volume: 4–297 m^3). All laboratory mesocosm studies (except Ref 12) included three to five replicate biofilters per treatment. Field studies involved one to four biofilters, most of which were evaluated over more than seven storms (exceptions include Refs 50, 67, 68, which were evaluated over three or less storms). Data from all studies were screened and quality controlled as follows. First, unvegetated treatments were removed, as they are more representative of sand filtration systems than biofilters. Second, biofilter runs where outflow water was not detected (and thus FIB concentrations could not be quantified directly) were excluded from the analysis. Average $\log_{10}$ FIB removal was calculated for each study and reported in Table 1.

Average FIB removal efficiency varied across biofilter studies, with some reporting net leaching[51,65,70] and others >2 $\log_{10}$ FIB removal inlet to outlet[3,12,71] (Table 1). This variability may reflect differences in biofilter design as well as prevailing experimental or climactic differences. For instance, pollutant loading regimes varied both within and across studies, with reported antecedent dry periods (ADPs) ranging from <4 [3,49–51,65] to >30 days.[63,65,69] Biofilter age also varied (between <2 months[12,65,69] and >1 year),[3,51,65,67] as did filter media composition (silt and clay content ranged between 3[49–51,71] and 46%[65] across studies). All three factors have been reported to affect FIB removal, with higher removal often (although not always) associated with older biofilters,[72] short ADPs,[3,49] and high filter media silt and clay content, which enhances removal at the risk of system clogging.[13,67] Other factors that could have contributed to variable FIB removal performance across studies include inflow volume (especially variable in field systems), vegetation species/type (1–2 species in laboratory mesocosms vs 2–12 species in field systems), and drainage configuration (e.g., SZ presence/absence), discussed in detail later on.

Overall, average $\log_{10}$ removal of EC and FC was comparable across studies. It is unclear, however, if ENT removal was more or less efficient than other FIB, as cross-study averages imply less ENT were removed, and case-by-case comparisons reveal similar (or greater) removal efficiencies (Table 1).[67,70] Most studies focused on the removal of environmental FIB assemblages (e.g., FIB in natural stormwater, synthetic stormwater mixtures, or animal manure), and relied on culture-based techniques to quantify group-specific removal efficiencies (e.g., $\log_{10}$ removal of total FC, EC, or ENT) (Table 1). In a few cases, however (3 of 13 in Table 1), biofilter experiments were performed using pure FIB cultures (e.g., nonpathogenic EC O1:K1:H7, EC K12, or EC strain ATCC 13706) allowing strain-specific removal to be evaluated.[50,67,68] Additional strain-specific removal experiments have been performed, but only in unvegetated chromatography columns. The specific strains assessed include pathogenic EC (O157:H7 and O55:K59(B5):H-),[31,41] nonpathogenic EC (strain K12 and K12 mutants with differing lipopolysaccharide structures),[32,33] and *Enterococcus faecalis* (strains NCTC 6782 and V583, which is vancomycin resistant),[31,32] amongst others. Future studies would benefit from a more comprehensive evaluation of FIB removal that resolves species

TABLE 1 | Log_{10} FIB Removal in Biofilters With or Without Submerged Zones

		Enumeration	Bacterial	Log EC Removal		Log ENT Removal		Log FC Removal	
		Method	Source	NSZ	SZ	NSZ	SZ	NSZ	SZ
Laboratory mesocosm	Rusciano and Obropta[63]	MF	Horse manure	—	—	—	—	1.70 (0.19)[1]	—
	Chandrasena et al.[51]	DST	EC O1:K1:H7 (with/without stormwater sediments)	0.75 (0.16)[1]	—	—	—	—	—
	Li et al.[3]	DST	Natural stormwater + EC (unreported strain)	1.42 (0.12)[1]	2.63	—	—	—	—
	Barrett[12]	DST	Natural stormwater	1.78 (0.58)[1]	2.56 (0.43)[1]	—	—	1.07 (0.64)[1]	1.72 (0.36)[1]
	Chandrasena et al.[49]	DST	EC strain O1:K1:H7 and stormwater sediments	1.47 (0.07)	1.83 (0.08)	—	—	—	—
Field systems	Hunt et al.[69]	DST	Natural stormwater	0.54	—	—	—	0.51	—
	Davies et al.[68]	DST	EC ATCC 13706	1.63 (0.09)[1]	—	—	—	—	—
	Passeport et al.[58]	MF	Natural stormwater	—	—	—	—	—	1.06 (0.24)[1]
	Hathaway et al.[70]	DST	Natural stormwater	0.09 (0.49)[1]	—	0.43 (0.49)[1]	—	—	—
	Zinger et al.[71]	MF	Natural stormwater	—	3	—	—	—	2.5
	Chandrasena et al.[67]	MF	Raw sewage + synthetic stormwater	0.76	1.07	0.7	1.07	—	—
	Kim et al.[50]	qPCR	EC K-12	0.55 (0.14)[1]	—	—	—	—	—
	Zhang et al.[65]	MF	Natural stormwater	0.00 (0.24)[1]	—	—	—	0.11 (0.20)[1]	—
Overall average (SE)		—	—	0.86 (0.2)	2.22 (0.3)	0.57 (0.14)	1.07	0.85 (0.35)	1.76 (0.4)

NSZ, no submerged zone; SZ, submerged zone; MF, membrane filtration; DST, defined substrate technology.
The following studies are included in the analysis presented in Figure 3(b): Refs 3, 12, 49, 50, 63, 65, 67.
Numerical superscripts link specific studies to their full citation in the reference list.
[1] Average removal (standard error) from different system designs: e.g., column length, vegetation type, and media formulation.

and/or strain diversity (in addition to overall FC, EC, or ENT behavior) in complex, vegetated biofilters. FIB viability assessment may also be important, as EC and ENT can both enter a viable but nonculturable (VBNC) state, where they retain metabolic activity, antibiotic resistance, pathogenic traits (for pathogenic strains), and express/exchange genes, but are not detectable by culture-based methods.[73] Resuscitation of VBNC bacteria may pose problems from a regulatory perspective; e.g., inexplicable exceedances of water quality standards downstream of stormwater biofilters.

Despite the above-noted cross-study variability in FIB source, biofilter type (field vs lab), size, age, ADP, filter media composition, inflow volume, and vegetation, average log_{10} removal of FC, EC, and ENT was consistently larger in biofilters containing SZs (Table 1). This pattern was marked for EC, where removal was >20-fold higher on average in SZ configurations. To determine if the effects of SZ design on average FIB removal were significant, a nonparametric bootstrap analysis of pooled, study-averaged FC, EC, and ENT removal values from Table 1 (grouped by biofilter design; SZ vs no SZ) was performed. A significant effect of SZ design was detected (see nonoverlapping 95% confidence intervals in Figure 3(a)), with approximately 10-fold higher removal observed in SZ biofilters on average.

It is important to point out, however, that because this analysis was performed on study-averaged data, true variability in FIB removal performance could have been underestimated, making differences between biofilter design configurations appear overly distinct. This concern was addressed by repeating the nonparametric bootstrap analysis described above using only studies where the results of individual biofilter runs were presented (laboratory mesocosm studies 3, 12, 49, 63, and field systems 50, 65, 67, Table 1). In total, 358 biofilter runs were compiled, 89 from designs with SZs and 269 from designs without. The results of this analysis were consistent with those reported previously (i.e., FIB removal was significantly higher on average [~eightfold] in biofilters with SZs), supporting the importance of this design feature as a control on FIB removal performance (see Figure 3(b)).

In the following text, I address a subset of processes that may explain (at least in part) the improved FIB removal detected in biofilters configured with SZs (all processes are detailed in Figure 4).

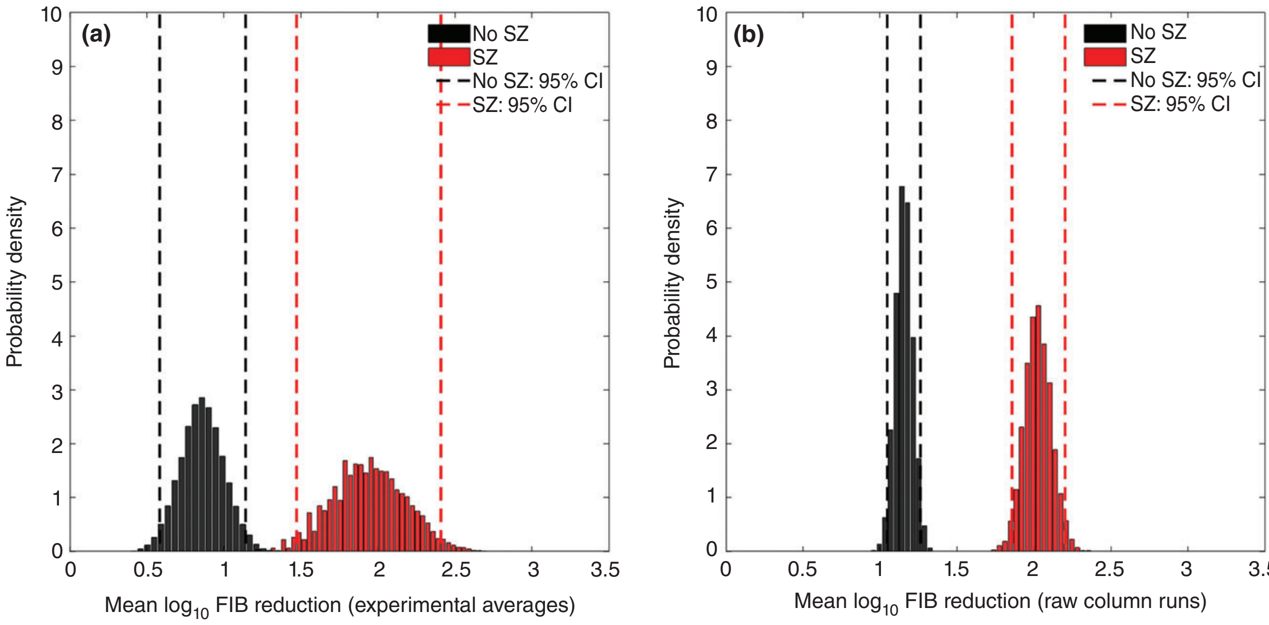

FIGURE 3 | Probability density functions of mean $\log_{10}$ fecal indicator bacteria (FIB) reduction (fecal coliforms, *Escherichia coli*, and enterococci combined) in systems with (red) or without (black) a submerged zone (SZ). 95% Confidence intervals for each distribution are shown using dashed lines of corresponding color. (a) Study-averaged FIB removal data (reported in Table 1) were used for this analysis. True average $\log_{10}$ FIB removal (the mean of the distribution of possible means) was ~1.9 for SZ designs and 0.9 across other designs. (b) FIB removal data from 358 individual biofilter runs were used for this analysis. These data were compiled from a subset of studies noted in Table 1. True average $\log_{10}$ FIB removal was ~2 for SZ designs and ~1.1 across other designs.

FIB REMOVAL IN BIOFILTERS WITH SUBMERGED ZONES

Flow Velocity (Capture)

In biofilters with SZs, flow velocity may decrease across the interface between filter media (unsaturated) and the SZ (fully saturated). This pattern has been observed in experiments simulating transport of low density contaminant plumes between unsaturated vadose zone sediments and underlying groundwater.[74] Because the total dimensionless transport rate of particles to grain collectors increases with decreasing flow velocity (classical filtration theory, Figure 5), the inclusion of SZs in biofilters may enhance FIB capture via physicochemical filtration.[20,29,30] This effect is expected to be more pronounced in filter media with larger sediment grains[30] (1000 vs 150 μm) (Figure 5). Note, however, that if grain sizes become too large, overall bacterial removal may decline due to reduced specific surface area of the media (an effect captured by overall particle mass balance, but not the dimensionless transport rate).

Particle deposition by straining is also predicted to increase with decreasing flow velocity.[20] At low-flow velocities, the volume of stagnant water zones between sediments increases, and the magnitude of hydrodynamic shear acting on particles near sediment–grain surfaces may decrease.[75] Both of these conditions are favorable for straining, which suggests that SZs have the potential to enhance FIB capture by this mechanism in addition to physicochemical filtration.

Saturation and Flow Transients (Capture)

Biofilters are transient flow systems that are wetted during storm events, and subsequently drain. Because saturated porous media tends to retain more colloids (including FIB) under transient flow conditions than unsaturated media,[34,35] biofilters with SZs (which increase overall system saturation[3]) may have greater FIB capture than those without. Mobilization of EC and ENT by propagating wetting and/or drying fronts has been observed in packed sediment columns.[32] Similarly, models simulating FIB detachment rate as a function of changing water content have been shown to accurately predict ENT mobilization in experiments mimicking transiently wetted beach sands.[76]

Transient flow conditions may impact removal of EC more than ENT because EC typically exhibit weaker physicochemical attachment than ENT (possibly due to attachment in a secondary vs primary energy minimum), which may make them more susceptible to scouring during intermittent flow. [31,32]

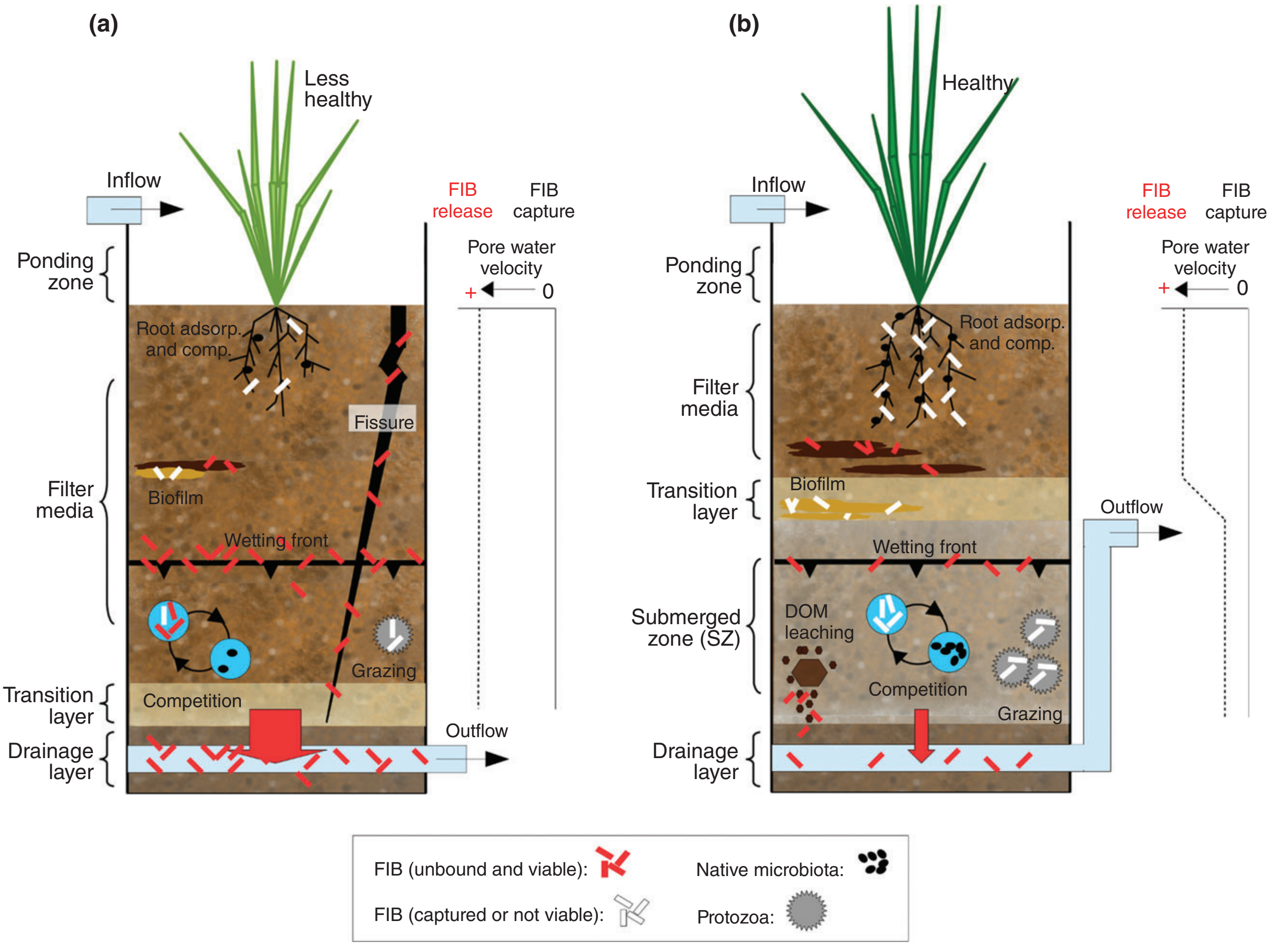

FIGURE 4 | Processes affecting fecal indicator bacteria (FIB) removal in (a) biofilters without submerged zone (SZ), and (b) biofilters with SZ. (1) *Pore Water Velocity*: Systems with or without SZ may have different vertical profiles of pore water velocity (shown schematically to the right of each filter column). Lower velocities exist in SZ designs and facilitate FIB capture. (2) *Saturation and Flow Transients*: Biofilters without SZ are unsaturated throughout, while systems with SZ are saturated near the base (see saturated SZ in panel (b)). Because they are less saturated, propagating wetting fronts may release more FIB in biofilters without SZ than those with SZ (compare unattached FIB [red rectangles] along the wetting front [black line with arrows] in biofilter (a) vs (b)). (3) *Fissure Formation*: Biofilters with SZ have a higher moisture content during dry periods between storms. This prevents media cracking and fissure formation, which can promote FIB leaching during subsequent rain events (see fissure [black gash] and FIB leaching [red rectangles] in schematic (a). (4) *Vegetation*: SZ biofilters promote plant health, including rapid growth rates and root development (see differences in plant color, size, and root length in biofilter (a) vs (b)). Well-developed vegetation can increase FIB removal via root capture and competition. Thus, more unviable FIB (white rectangles) attach to plant roots near native microbial competitors (black circles) in biofilter (a) vs (b)). (5) *Biofilm*: Moist SZ conditions may increase biofilm formation: higher biofilm abundance (brown and yellow streaks) is present in SZ biofilter (b). Biofilm may increase (yellow) or reduce (brown) FIB capture/mortality, depending on the species. (6) *DOM Release*: The carbon source in SZ biofilters can leach DOM, and reduce FIB attachment to sediment grains (see unattached FIB [red rectangles] surrounding the carbon source [brown polygon] in biofilter (b)). This problem is not expected in standard, unamended biofilter designs. (7) *Protozoan Grazing*: Moist conditions increase the survival and motility of protozoa, as shown via the larger number of protists (gray balls) in SZ biofilter (b). This may increase grazing pressure and FIB mortality (note the number of unviable FIB [white rectangles] contained within protozoa in (b) vs (a)). (8) *Microbial Competition*: Moist, carbon-rich SZ may increase the abundance and activity of native bacteria. This can enhance competition between natives and FIB, increasing FIB mortality (compare competition schematics between biofilter (a) and (b): note the higher abundance of microbial natives [black circles] and unviable FIB [white rectangles] in SZ biofilters (b)).

However, the strength of EC attachment in sediments can vary substantially due to differences in strain hydrophobicity,[31,41] electrokinetic properties,[31,33,41,77] and/or cell surface lipopolysaccharide (LPS) composition,[33] suggesting that EC may not always be more susceptible to scouring than ENT. In fact, average $\log_{10}$ removal values from Table 1, suggest that overall EC removal is higher than ENT across biofilter designs (note that very little data are available regarding ENT removal). Further evaluation of attachment

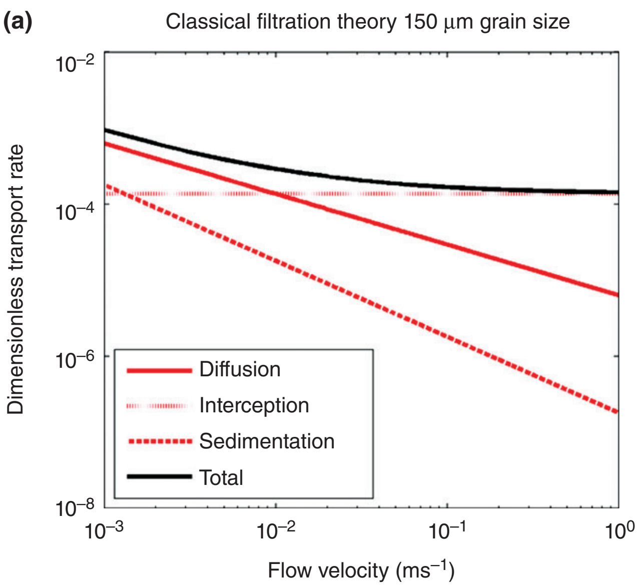

FIGURE 5 | Plots showing the change in dimensionless transport rate with pore water flow velocity (m s^{-1}) that is predicted for 1 μm colloids under classical filtration theory. Sediment grains are assumed to be (a) 150 or (b) 1000 μm, spanning the range of grain sizes in biofilter media. The diffusion (solid red), sedimentation (dashed red), and interception (fine dashed red) components of dimensionless transport are calculated as in Yao et al.[30] Their sum is the total dimensionless transport rate (shown in black). The total dimensionless transport rate is predicted to increase with decreasing velocity. This effect is more pronounced in systems with 1000 μm sediment grains than those with 150 μm sediment grains.

and mobilization mechanisms for different bacterial groups and/or strains is required to determine if manipulating system saturation via the presence or absence of SZ is a reliable means of controlling capture of FIB (or pathogens) in stormwater biofilters.

Fissures and Preferential Flow Paths (Capture)

An additional way in which SZs may enhance FIB capture is by maintaining moisture content between storms, preventing media desiccation, and the formation of preferential flow paths or fissures in filter media that facilitate leaching.[3,34] Desiccation may be especially problematic in short biofilter columns (filter media: 300 mm), as fissures can more readily traverse filter media.[3] Long vegetated columns (filter media: 700 mm), however, are also subject to desiccation, with declines in moisture content exceeding 50% for dry periods in excess of 2 weeks; note that moisture content was relatively constant in systems containing SZs.[3]

Vegetation Type (Capture/Survival)

Because SZs retain/store water, they may (1) provide a buffer for biofilter vegetation during dry periods and (2) increase plant growth rates or biomass, traits that are positively correlated with contaminant removal.[1,9,48] Although few studies have evaluated the effects of vegetation type on FIB removal in biofilters with or without a SZ, recent work by Chandrasena et al. (2014), suggests that SZs can significantly increase FIB removal performance across many vegetation types (sedges, grasses, and shrubs). This said, the degree to which SZs enhance FIB removal varies, and is reported to be higher (up to 10-fold) in treatments planted with *Melaluca incana*, *Leptospermum continentale*, *Dianella tasmanica*, *Stenataphrum secundatum*, and *Poa labillardieri* than those planted with *Carex appressa* or *Sporobolus virginicus*.[49] Similar (but not statistically significant) interaction effects between SZs and vegetation have been observed in other studies: for instance, Barrett et al.[12] observed elevated EC and FC retention in SZ biofilters planted with one species of native north American grass (*Muhlenbergia lindheimeri*), but not the other (*Buchloe dactyloides*).

The importance of SZ–vegetation interactions for FIB removal may be higher in regions that frequently experience dry periods of >2 weeks. While SZs may[3] or may not[49] prevent declines in biofilter performance during dry weather, they do appear to promote rapid (and more complete) recovery, especially in systems planted with shrubs.[49] It has been postulated that this effect is linked to reduced plant stress and root damage in SZ designs during dry periods, under the assumption that root damage impacts FIB survival (through perturbing microbial flora in the rhizosphere) or FIB capture (through decreased adsorption or formation of preferential flow paths along damaged root structures).[49] Further research is required to evaluate these hypotheses and identify specific mechanisms through which SZs and vegetation act to regulate FIB removal performance in biofilters.

Biofilm Formation (Capture/Survival)

Increases in moisture content and organic carbon can fuel biofilm development in sediments. To date, the species composition and organization of biofilm bacteria in stormwater biofilters are unknown. However, research on bioclogging suggests that extensive biofilms will preferentially develop in nutrient-rich sediment pore spaces or those with increased water flow, which favors nutrient resupply.[36] Discrete (single colony) biofilms are expected in narrow pore throats or at grain junctions.[36] Because SZs contain carbon amendments (and enhance moisture content, see above), biofilm formation may be greater in SZ biofilters. Biofilm growth in porous media may promote bacterial removal by straining[3,41,78] or physicochemical interactions (e.g., altering grain surface roughness, hydrophobicity, or electrostatic charge[53]). This suggests that SZs may promote FIB capture in biofilters. Notably, the effects of biofilm on FIB removal may depend on the microbes composing the biofilm and/or the FIB strain involved.[78,79] Careful evaluation of biofilms in biofilters with or without a SZ is warranted moving forward, as biofilms have the potential to impact FIB in contradictory ways: in some cases, incorporating FIB and increasing their survival,[79] and in other cases enhancing FIB removal via capture (addressed here) or mortality (e.g., microbial competition, discussed in a later section).

Dissolved Organic Matter 'DOM' (Capture)

It is important to point out that SZs could actually decrease FIB retention in biofilters if the organic carbon amendments they contain leach dissolved organic matter (DOM). DOM may reduce FIB attachment to sediment grains by creating an electrosteric repulsive force or by reducing or eliminating secondary or primary energy minima.[32,41,77,80] Carbon amendments such as newspaper, wood chips, and sulfur–limestone release low concentrations of total carbon ($<5\,mg\,L^{-1}$) to biofilter outflow water, and may be suitable for use in SZs.[10] Thus, careful carbon source selection, with an eye toward minimizing DOM leaching, may help mitigate negative effects of organic carbon amendments on FIB capture in biofilters.

Protozoan Grazing (Survival)

Although most of the mechanisms discussed thus far relate to FIB capture, SZs may also impact FIB survival. The stable, moist conditions promoted by SZs may increase abundance and mobility of protistan grazers.[39,41,72] Grazing has been shown to dominate FIB removal in packed column experiments; EC loss was observed in natural sediment (first-order decay rate of 0.9 day^{-1}), growth was observed in presterilized, irradiated sediments (first-order growth rate of 0.18 day^{-1}), and loss resumed when microbes (protozoa and bacteria) were reintroduced (first-order decay rate of 0.7 day^{-1}).[41] Notably, removal was lower in amended sediments when reintroduced microbial communities were protistan poor (first-order decay rate of 0.56 day^{-1}).[41] Elevated FIB removal has also been observed in mature (>1-year-old) filter columns, and has been attributed to elevated protist abundance (10-fold higher than in fresh media).[72] Importantly, while SZs may promote proliferation of native soil protozoa, the same is not expected for parasitic protozoan pathogens (e.g., *Giardia* and *Cryptosporidium*), which have complex life cycles and require a host to replicate.

Microbial Competition (Survival)

Natural sediment and rhizosphere microbial communities (so-called native communities) may impact FIB removal in biofilters via competition. Competition effects may be exacerbated in systems with SZs if increases in soil moisture, carbon content, or anaerobic conditions favor proliferation of native bacteria and/or different (more antagonistic) species compositions. Some soil carbon amendments can cause changes in microbial community composition and activity (both linked to increased intramicrobial competition). For instance, compost amendments are readily colonized by biocontrol bacteria (including *Bacillus sp*), known to antagonize pathogenic plant bacteria via secretion of quorum-blocking molecules.[54,55,81] This suggests that certain carbon amendments in biofilters with SZs could increase FIB mortality by augmenting competitive effects. However, elevated organic carbon could have the opposite effect, as dissolved organic carbon concentrations $>7\,mg\,L^{-1}$ are associated with regrowth of FIB in urban runoff.[82]

It has also been suggested that degree of saturation strongly regulates competition in sediments, with competition increasing alongside saturation.[36] This could be caused by proliferation of native microorganisms in moist conditions and/or higher aqueous connectivity, which enhances cell to cell contact.[36] Regardless, the above-noted pattern is consistent with increased competition in biofilters with SZs.

CONCLUSIONS AND FUTURE DIRECTIONS

Overall, it appears that biofilters with SZs are superior to other non-SZ designs for FIB removal (Table 1, Figure 3). This bodes well for our ability to maintain existing biofilter functionality (e.g., nitrate removal; also enhanced in SZ configurations) while maximizing FIB removal, which is requisite for the acceptance of biofilters as stormwater treatment technologies.[10] However, SZs may not always increase FIB removal, and trade-offs, whereby removal is enhanced via one process and decreased via another, are possible. An example of this is SZ carbon amendments, which have the potential to (1) reduce FIB capture by releasing DOM and decreasing physicochemical attachment to sediment grains,[32,41,77,80] (2) induce FIB growth,[82] or (3) enhance FIB mortality by increasing the activity/abundance of native soil bacteria or protozoa, promoting intraspecies competition and/or predation.[41,81] In addition to possible trade-offs, biofilter design is still peppered with unknowns. Limited information is available regarding the effects of biofilter physicochemistry, straining, and the 'bio' component of biofilters on FIB removal (e.g., vegetation type, bacterial diversity, protozoan grazing, bioturbation/grazing by invertebrate macrofauna, and intraspecific microbial competition). The black-box treatment of soil and rhizosphere bacteria in biofilters is particularly concerning, as microbiome formation and evolution in response to stormwater inputs sets the stage (so to speak) for subsequent FIB treatment. Addressing unknowns in biofilter systems is complicated by the fact that many processes are connected (e.g., DOM leaching can affect both FIB capture and survival). Questions regarding capture mechanisms like straining and physicochemical filtration cannot be viewed independently of system biology, as they may be impacted by biological processes such as biofilm formation and root growth/architecture.[3,41,78] In practice, this can make deconstructing biofilter function and evaluating the relative importance of mechanisms across design configurations, difficult.

Mathematical models can assist in this regard, especially those that resolve complex 3D structures such as roots. Most models currently used to evaluate vegetated biofilters model flow across three to four layers (ponding zone, unsaturated zone, saturated zone, and ±drainage layer).[82] The pollutant removal equations involved are contaminant specific, but frequently limited to filtration, adsorption, desorption and first order decay.[83,84] Fate and transport models in analogous systems such as the vadose zone, however, can be quite complex, and include a variety of colloid-related process (physicochemical filtration, straining, mechanical filtration, air–water interface scouring, and thin film straining and expansion[35]). As such, vadose zone models may provide a useful starting point for the development of more complex biofilter models that integrate hydraulics, colloidal, and biological processes. Models of this sort may prove useful for identifying dominant mechanisms controlling the removal of specific contaminant types (including FIB), thereby informing, and improving, future biofilter design.

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FURTHER READING

Davis AP, Hunt WF, Traver RG, Clar M. Bioretention technology: overview of current practices and future needs. *J Environ Eng* 2009, 135:109–117.

REFERENCES

1. Read J, Wevil T, Fletcher T, Deletic A. Variation among plant species in pollutant removal from stormwater in biofiltration systems. *Water Res* 2008, 42: 893–902.
2. Bratieres K, Fletcher TD, Deletic A, Zinger Y. Nutrient and sediment removal by stormwater biofilters: a large-scale design optimization study. *Water Res* 2008, 42:3930–3940.
3. Li YL, Deletic A, Alcazar L, Bratieres K, Fletcher TD, McCarthy DT. Removal of *Clostridium perfringens*, *Escherichia coli*, and F-RNA coliphages by stormwater biofilters. *Ecol Eng* 2012, 49:137–145.

4. Payne EGI, Fletcher TD, Cook PLM, Deletic A, Hatt BE. Processes and drivers of nitrogen removal in stormwater biofiltration. *Crit Rev Environ Sci Technol* 2014, 44: 796–846.
5. Walsh CJ, Fletcher TD, Ladson AR. Stream restoration in urban catchments through redesigning stormwater systems: looking to the catchment to save the stream. *J N Am Benthol Soc* 2005, 24:690–705.
6. Grant SB, Saphores J, Feldman DL, Hamilton AJ, Fletcher TD, Cook PLM, Stewardson M, Sanders BF, Levin LA, Ambrose RF, et al. Taking the "waste" out of "wastewater" for human water security and ecosystem health. *Science* 2012, 337:681–686.
7. U.S. Environmental Protection Agency (US EPA). Stormwater best management practice design guide volume 2 vegetative biofilters. EPA/600/R-04/121A. Washington, DC: US EPA; 2004, 194.
8. Facility for AdvancingWater Biofiltration (FAWB). *Adoption Guidelines for Stormwater Bioinfiltration Systems*. Melbourne: Monash University; 2009, 1–38.
9. Zhang Z, Rengel Z, Liaghati T, Antoniette T, Meney K. Influence of plant species and submerged zone with carbon addition on nutrient removal in stormwater biofilter. *Ecol Eng* 2011, 37:1833–1841.
10. Kim H, Seagren EA, Davis AP. Engineered bioretention for removal of nitrate from stormwater runoff. *Water Environ Res* 2003, 75:355–367.
11. Payne EGI, Fletcher TD, Russell DG, Grace MR, Cavagnaro TR, Evrard V, Deletic A, Hatt BE, Cook PLM. Temporary storage or permanent removal? The division of nitrogen between biotic assimilation and denitrification in stormwater biofilters. *PLoS ONE* 2014, 3:e90890.
12. Barrett ME, Limouzin M, Lawler DF. Effects of media and plant selection on biofiltration performance. *J Environ Eng* 2013, 139:462–470.
13. Grebel JE, Mohanty SK, Torkelson AA, Boehm AB, Higgins CP, Maxwell RM, Nelson KL, Sedlak DL. Engineered infiltration system for urban stormwater reclamation. *Environ Eng Sci* 2013, 30:437–454.
14. Edberg SC, Rice EW, Karlin RJ, Allen MJ. *Escherichia coli*: the best biological drinking water indicator for public health protection. *J Appl Microbiol* 2000, 88: 106S–116S.
15. McLellan SL. Genetic diversity of *Escherichia coli* isolated from urban rivers and beach water. *Appl Environ Microbiol* 2004, 70:4658–4665.
16. Higgins JA, Belt KT, Karns JS, Russell-Anelli J, Shelton DR. tir- and stx-positive *Escherichia coli* in stream waters in a metropolitan area. *Appl Environ Microbiol* 2005, 71:2511–2519.
17. Byappanahalli MN, Nevers MB, Korajkic A, Staley ZR, Harwood VJ. Enterococci in the environment. *Microbiol Mol Biol Rev* 2012, 76:685–706.
18. Sidhu JPS, Hodgers L, Ahmed W, Chong MN, Toze S. Prevalence of human pathogens and indicators in stormwater runoff in Brisbane, Australia. *Water Res* 2012, 46:6652–6660.
19. Sidhu JPS, Ahmed W, Gernjak W, Aryal R, McCarthy D, Palmer A, Kolotelo P, Toze S. Sewage pollution in urban stormwater runoff as evident from the widespread presence of multiple microbial and chemical source tracking markers. *Sci Total Environ* 2013, 463: 488–496.
20. Bradford SA, Simunek J, Bettahar M, van Glenuchten MT, Yates SR. Significance of straining in colloid deposition: evidence and implications. *Water Resour Res* 2006, 42:W12S15-1–W12S15-16.
21. Foppen JW, von Herwerden M, Schiven J. Measuring and modeling straining of *Escherichia coli* in saturated porous media. *J Contam Hydrol* 2007, 93:236–254.
22. Johnson WP, Pazmino E, Ma H. Direct observations of colloid retention in granular media in the presence of energy barriers, and implication for inferred mechanisms from indirect observations. *Water Res* 2010, 44:1158–1169.
23. Blecken G, Zinger Y, Deletic A. Impact of a submerged zone and a carbon source on heavy metal removal in stormwater biofilters. *Ecol Eng* 2009, 35:769–778.
24. Stevik TK, Aa K, Ausland G, Hanssen JF. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. *Water Res* 2004, 38:1355–1367.
25. Tufenkji N, Miller GF, Ryan JN, Harvey RW, Elimelech M. Transport of Cryptosporidium Oocysts in porous media: role of straining and physicochemical filtration. *Environ Sci Techol* 2004, 38:5932–5938.
26. Foppen JWA, Mporkoso A, Schijven JF. Determining straining of *Escherichia coli* from breakthrough curves. *J Contam Hydrol* 2005, 76:191–210.
27. Elimelech M, Gregory J, Jia X, Williams R. *Particle Deposition and Aggregation: Measurement, Modeling and Simulation*. Oxford: Butterworth-Heinemann Ltd; 1995, 33–63.
28. Johnson WP, Ma H, Pazmino E. Straining credibility: a general comment regarding common arguments used to infer straining as the mechanism of colloid retention in porous media. *Environ Sci Techol* 2011, 45:3831–3832.
29. Elimelech M, O'Melia CR. Kinetics of deposition of colloidal particles in porous media. *Environ Sci Techol* 1990, 24:1528–1536.
30. Yao K, Habibian MT, O'Melia CR. Water and waste water filtration: concepts and applications. *Environ Sci Techol* 1971, 5:1105–1112.
31. Chen G, Walker SL. Fecal indicator bacteria transport and deposition in saturated and unsaturated porous media. *Environ Sci Techol* 2012, 46:8782–8790.
32. Mohanty SK, Torkelson AA, Dodd H, Nelson KL, Boehm AB. Engineering solutions to improve the removal of fecal indicator bacteria by bioinfiltration systems during intermittent flow of stormwater. *Environ Sci Techol* 2013, 47:10791–10798.

33. Walker SL, Redman JA, Elimelech M. Role of cell surface lipopolysaccharides in *Escherichia coli* K12 adhesion and transport. *Langmuir* 2004, 20:7736–7746.
34. McCarthy JF, McKay LD. Colloid transport in the subsurface: past, present, and future challenges. *Vadose Zone J* 2004, 3:326–337.
35. Flury M, Qiu H. Modeling colloid-facilitated contaminant transport in the vadose zone. *Vadose Zone J* 2008, 7:682–697.
36. Or D, Smets BF, Wraith JM, Dechesne A, Friedman SP. Physical constraints affecting bacterial habitats and activity in unsaturated porous media—a review. *Adv Water Resour* 2007, 30:1505–1527.
37. Brussaard L. Soil fauna, guilds, functional groups and ecosystem processes. *Appl Soil Ecol* 1998, 9: 123–135.
38. Bardgett RD, Cook R, Yeates GW, Denton CS. The influence of nematodes on below-ground processes in grassland ecosystems. *Plant Soil* 1999, 212:23–33.
39. Kirchman DL. *Processes in Microbial Ecology*. Oxford: Oxford University Press; 2012, 123–128.
40. Starink M, Krylova IN, Bar-Gilissen M, Bak RPM, Cappenberg TE. Rates of benthic protozoan grazing on free and attached sediment bacteria measured with fluorescently stained sediment. *Appl Environ Microbiol* 1994, 60:2259–2264.
41. Zhang L, Seagren EA, Davis AP, Karns JS. The capture and destruction of *Escherichia coli* from simulated urban runoff using conventional bioretention media and iron oxide-coated sand. *Water Environ Res* 2010, 82: 701–714.
42. Feng F, Goto D, Yan T. Effects of autochthonous microbial community on the die-off of fecal indicators in tropical beach sand. *FEMS Microb Ecol* 2010, 74: 214–225.
43. Gonzalez JM, Iriberri J, Egea L, Barcina I. Differential rates of digestion of bacteria by freshwater and marine phagotrophic protozoa. *Appl Environ Microbiol* 1990, 56:1851–1857.
44. Hartke A, Lemarinier S, Pichereau V, Auffray Y. Survival of *Enterococcus faecalis* in seawater microcosms is limited in the presence of bacterivorous zooflagellates. *Curr Microbiol* 2002, 44:329–335.
45. Gonzalez JM, Iriberri J, Egea L, Barcina I. Characterization of culturability, protistan grazing and death of enteric bacteria in aquatic ecosystems. *Appl Environ Microbiol* 1992, 58:998–1004.
46. Zhao L, Wang Y, Yang J, Xing M, Li X, Yi D, Deng D. Earthworm-microorganism interactions: a strategy to stabilize domestic wastewater sludge. *Water Res* 2010, 44:2572–2582.
47. Nogaro G, Mermillod-Blondin F. Stormwater sediment and bioturbation influences on hydraulic functioning, biogeochemical processes, and pollutant dynamics in laboratory infiltration systems. *Environ Sci Techol* 2009, 43:3632–3638.
48. Read J, Fletcher TD, Wevill T, Deletic A. Plant traits that enhance pollutant removal from stormwater in biofiltration systems. *Int J Phytoremediation* 2009, 12: 34–53.
49. Chandrasena GI, Pham T, Payne EG, Deletic A, McCarthy DT. *E coli* removal in laboratory scale stormwater biofilters: influence of vegetation and submerged zone. *J Hydrol* 2014, 519:814–822.
50. Kim MH, Sung CY, Li MCK. Bioretention for stormwater quality improvement in Texas: removal effectiveness of *Escherichia coli*. *Sep Purif Technol* 2012, 84: 120–124.
51. Chandrasena GI, Deletic A, Ellerton J, McCarthy DT. Evaluating *Escherichia coli* removal performance in stormwater biofilters: a laboratory scale study. *Water Sci Technol* 2012, 66:1132–1138.
52. Hibbing ME, Fuqua C, Parsek MR, Peterson SB. Bacterial competition: surviving and thriving in the microbial jungle. *Nat Rev Microbiol* 2010, 8:15–25.
53. Strauss EA, Lamberti GA. Regulation of nitrification in aquatic sediments by organic carbon. *Limnol Oceanogr* 2000, 45:1854–1859.
54. Loh J, Pierson EA, Pierson LS, Stacey G, Chatterjee A. Quorum sensing in plant-associated bacteria. *Curr Opin Plant Biol* 2002, 5:285–290.
55. Molina L, Constantinescu F, Michel L, Reimmann C, Duffy B, Defago G. Degradation of pathogen quorum-sensing molecules by soil bacteria: a preventive and curative biological control mechanism. *FEMS Microbiol Ecol* 2003, 45:71–81.
56. Halliday E, Gast RJ. Bacteria in beach sands: an emerging challenge in protecting coastal water quality and bather health. *Environ Sci Techol* 2011, 45:370–379.
57. Chandrasena GI, Deletic A, McCarthy DT. Survival of *Escherichia coli* in stormwater biofilters. *Environ Sci Pollut Res* 2014, 21:5391–5401.
58. Passeport E, Hunt WF, Line DE, Smith RA, Brown RA. Field study of the ability of two grassed bioretention cells to reduce storm-water runoff pollution. *J Irrig Drain Eng* 2009, 135:505–510.
59. Nogaro G, Mermillod-Blondin F, Montuelle B, Boisson J, Bedell J, Ohannessian A, Volat B, Gibert J. Influence of a stormwater sediment deposit on microbial and biogeochemical processes in infiltration porous media. *Sci Total Environ* 2007, 377:334–348.
60. Endreny T, Burke DJ, Burchhardt KM, Fabian MW, Kretzer AM. Bioretention column study of bacteria community response to salt-enriched artificial stormwater. *J Environ Qual* 2001, 41:1951–1959.
61. Rozen Y, Belkin S. Survival of enteric bacteria in seawater. *FEMS Microbiol Rev* 2001, 25:513–529.
62. Oliver DM, Haygarth PM, Clegg CD, Heathwaite AL. Differential *E. coli* die-off patterns associated with agricultural matrices. *Environ Sci Techol* 2006, 40: 5710–5716.

63. Rusciano GM, Obropta CC. Bioretention column study: fecal coliform and total suspended solids reductions. *Trans Am Soc Agric Biol Eng* 2007, 50: 1261–1269.
64. Fisher K, Phillips C. The ecology, epidemiology and virulence of *Enterococcus*. *Microbiology* 2009, 155: 1749–1757.
65. Zhang L, Seagren EA, Davis AP, Karns JS. Effects of temperature on bacterial transport and destruction of bioretention media: field and laboratory evaluations. *Water Environ Res* 2012, 84:485–496.
66. Li YL, Deletic A, McCarthy DT. Removal of *E coli* from urban stormwater using antimicrobial-modified filter media. *J Hazard Mater* 2014, 271:73–81.
67. Chandrasena GI, Filip S, Zhang K, Osborne CA, Deletic A, McCarthy DT. Pathogen and indicator microorganism removal in field scale stormwater biofilters. In: *Proceedings of the 7th International WSUD Conference*, Melbourne, Australia, 2012, 9.
68. Davies CM, Mitchell VG, Petterson SM, Taylor GD, Lewis J, Kaucner C, Ashbolt NJ. *Water Sci Technol* 2008, 57:843–847.
69. Hunt WF, Smith JT, Jadlocki SJ, Hathaway JM, Eubanks PR. Pollutant removal and peak flow mitigation by a bioretention cell in urban Charlotte N.C. *J Environ Eng* 2008, 134:403–408.
70. Hathaway JM, Hunt WF, Graves AK, Wright DJ. Field evaluation of bioretention indicator bacteria sequestration in Wilmington, North Carolina. *J Environ Eng* 2011, 137:1103–1113.
71. Zinger Y, Deletic A, Fletcher TD, Breen P, Wong T. A dual-mode biofilter system: case study in Kfar Sava, Israel. In: *Proceedings of the 12th International Conference on Urban Drainage*, Porto Alegre, Brazil, 2011.
72. Zhang L, Seagren EA, Davis AP, Karns JS. Long-term sustainability of *Escherichia coli* removal in conventional bioretention media. *J Environ Eng* 2011, 137: 669–677.
73. Lleo MM, Bonato B, Tafi MC, Signoretto C, Pruzzo C, Canepari P. Molecular vs culture methods for the detection of bacterial faecal indicators in groundwater for human use. *Lett Appl Microbiol* 2005, 40:289–294.
74. Simmons CT, Pierini ML, Hutson JL. Laboratory investigation of variable-density flow and solute transport in unsaturated-saturated porous media. *Transp Porous Media* 2002, 47:215–244.
75. Bradford SA, Torkzaban S, Walker SL. Coupling of physical and chemical mechanisms of colloid straining in saturated porous media. *Water Res* 2007, 41: 3012–3024.
76. Russell TL, Yamahara KM, Boehm AB. Mobilization and transport of naturally occurring enterococci in beach sands subject to transient infiltration of seawater. *Environ Sci Techol* 2012, 46:5988–5996.
77. Foppen JW, Liem Y, Schijven J. Effect of humic acid on the attachment of *Escherichia coli* in columns of goethite-coated sand. *Water Res* 2008, 42:211–219.
78. Liu Y, Yang C, Li J. Adhesion and retention of a bacterial phytopathogen *Erwinia chrysanthemi* in biofilm-coated porous media. *Environ Sci Techol* 2008, 42:159–165.
79. Liu Y, Li J. Role of *Pseudomonas aeruginosa* biofilm in the initial adhesion, growth and detachment of *Escherichia coli* in porous media. *Environ Sci Techol* 2008, 42:443–449.
80. Torkelson AA, da Silva AK, Love DC, Kim JY, Alper JP, Coox B, Dahm J, Kozodoy P, Malboudian R, Nelson KL. Investigation of quaternary ammonium silane-coated sand filter for the removal of bacteria and viruses from drinking water. *J Appl Microbiol* 2012, 113:1196–1207.
81. Hoitink H, Boehm M. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. *Annu Rev Phytopathol* 1999, 37:427–446.
82. Surbeck CQ, Jiang SC, Grant SB. Ecological control of fecal indicator bacteria in an urban stream. *Environ Sci Techol* 2010, 44:631–637.
83. Roy-Poirier A, Champagne P, Filion Y. Review of biortention system research and design: past, present, and future. *J Environ Eng* 2010, 136:878–889.
84. Chandrasena GI, Deletic A, McCarthy DT. Evaluating *Escherichia coli* removal performance in stormwater biofilters: a preliminary modeling approach. *Water Sci Technol* 2013, 67:2467–2475.

