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Iron amendments minimize the first-flush release of pathogens from stormwater biofilters☆

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ABSTRACT

First flush or the first pore volume of effluent eluted from biofilters at the start of rainfall contributes to most pollution downstream because it typically contains a high concentration of bacterial pathogens. Thus, it is critical to evaluate designs that could minimize the release of bacteria during a period of high risk. In this study, we test the hypothesis of whether an addition of iron-based media to biofilter could limit the leaching of *Escherichia coli* (*E. coli*), a pathogen indicator, during the first flush. We applied *E. coli*-contaminated stormwater intermittently in columns packed with a mixture of sand and compost (70:30 by volume, respectively) and iron filings at three concentrations: 0% (control), 3%, and 10% by weight. Columns packed with a mixture of sand and iron (3% or 10%) without compost were used to examine the maximum capacity of iron to remove *E. coli*. In columns with iron, particularly 10% by weight, the leaching of *E. coli* during the first flush was 32% lower than the leaching from compost columns, indicating that the addition of iron amendments could decrease first-flush leaching of *E. coli*. We attribute this result to the ability of iron to increase adsorption and decrease growth during antecedent drying periods. Although the addition of iron filings increased *E. coli* removal, the presence of compost decreased the adsorption capacity: exposure of 1 g of iron filings to 1 mg of DOC reduces *E. coli* removal by 8%. The result was attributed to the alteration of the surface charge of iron and blocking of adsorption sites shared by *E. coli* and DOC. Collectively, these results indicate that the addition of sufficient amounts of iron media could decrease pathogen leaching in the first flush effluent and increase the overall biofilter performance and protect downstream water quality.

1. Introduction

Pathogens and their indicator bacteria in stormwater, particularly in urban areas, are one of the leading causes of surface water impairments (EPA 2017). Urban runoff often contains a wide range of bacterial pathogens (*Salmonella*, *Campylobacter*, and *Escherichia coli*), viruses (norovirus, adenovirus, and rotavirus), and protozoa (*Cryptosporidium* and *Giardia*), which can pose a health risk (McBride et al., 2013; Sauer et al., 2011; Sidhu et al., 2012). To indicate pathogen presence in stormwater, however, fecal indicator bacteria such as *Escherichia coli* (*E. coli*), total coliform, fecal coliform, and enterococci are typically measured (Galfi et al., 2016; Hathaway and Hunt 2011; Surbeck et al., 2006). To manage nonpoint source pollution from bacterial pollutants, stormwater treatment systems such as biofilters have been implemented (Tirpak et al., 2021). Biofilters are designed by replacing a section of soil with conventional biofilter media—a mixture of sand and organic amendments—that increases infiltration of water and removal of contaminants (Zhang et al., 2021). However, these systems are often ineffective in removing bacterial pollutants to the permittable limit. Sometimes, the release of bacterial pollutants from biofilters during intermittent infiltration of stormwater could make these biofilters act as a source of bacterial pollutants (Mohanty et al., 2013). The concentration of pollutants in stormwater is typically high during the first flush, which is the first pore volume of water exiting the biofilters at the start of a rainfall event (Ekanayake et al., 2019). The first-flush effluent typically contains a high concentration of bacterial pollutants, potentially due to the

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growth of sequestered bacteria (Mohanty et al., 2014). Thus, it is critical to improving the design of biofilters to limit pollution originated from the first flush.

One way to improve the design of biofilters is to add amendments that could either decrease the mobilization of pathogens during the first flush or limit the growth of pathogens during antecedent drying periods (Mohanty et al., 2014). The conventional filter media such as a mixture of sand and compost or other organic amendment has limited adsorption capacity for some contaminants including nutrients and pathogens (Kranner et al., 2019; Ulrich et al., 2017a). Several studies show that conventional filter media may serve as a net source of contaminants under certain conditions including extreme rainfall events (Stagge et al., 2012), variations in redox condition and pH (Kranner et al., 2019), and the presence of de-icing salt in stormwater (Huber et al., 2016). Furthermore, the presence of dissolved organic carbon (DOC) and other nutrients in stormwater could help previously sequestered pathogens grow during the drying period between rainfall events (Mohanty et al., 2014). Thus, amendments should be added to biofilters that could limit the growth or leaching of bacterial pollutants during the first flush.

To improve the adsorption capacity of the traditional filter media, amendments containing zero-valent iron or iron oxides have been added to filter media (Erickson et al., 2012; Rangsivek and Jekel 2005; Reddy et al., 2014; Tian et al., 2019; Trenouth and Gharabaghi 2015; Weiss et al., 2016). Iron amendments are typically derived from waste materials such as iron filings and water treatment residues, and thus their use in biofilters could decrease the overall design cost. The iron amendments could remove pathogens by adsorption, coagulation, and inactivation (Tirpak et al., 2021). Because most iron oxides exhibit a net positive surface charge above pH 6, they can adsorb bacteria, which have a net negative surface charge (Ingram et al., 2012; Kim et al., 2020; Xu et al., 2019). Also, oxyhydroxide iron flocs (FeOOH) produced after dissolution irons can increase the removal of bacteria by flocculation (Appenzeller et al., 2005; Sun et al., 2019; Zhu et al., 2005). Furthermore, zero-valent iron (ZVI) can produce reactive oxygen species such as hydroxyl radical, which can inactive pathogen via intracellular damage (Sun et al., 2019). On the other hand, iron is a micronutrient, and the addition of iron can also promote bacterial growth in certain conditions. In particular, bacteria can couple organic matter oxidation with iron reduction for their growth (Appenzeller et al., 2005). Although many studies have examined *E. coli* removal by scrap irons or iron-coated sand based on breakthrough curve (George and Mansoor Ahammed 2019; Ingram et al., 2012; Kim et al., 2020; Xu et al., 2019), no study to date has examined the fate of *E. coli* removed by iron media after the rainfall. In particular, it is not clear if the previously sequestered *E. coli* grow or die off in biofilters amended with iron.

Most biofilter contains organic amendments such as mulch and compost to support plants atop (Widyastuti et al., 2020). These amendments can leach a high concentration of DOC, which can compete with bacteria for attachment sites and exhaust removal capacity of iron amendments (Abudalo et al., 2010; Foppen et al., 2008; Mohanty et al., 2013). The presence of compost in iron-amended biofilters could help sequestered *E. coli* grow and leach in the subsequent rainfall event. DOC can alter the surface charge of iron oxides to a net negative surface, and limit the binding of cations (Vindedahl et al., 2016). However, previous studies have rarely examined the removal capacity of iron media in the presence of compost.

The objectives of this study are to quantify the removal capacity of iron amendments in stormwater biofilters in the presence of compost and evaluate the potential of iron amendments on minimizing first-flush leaching of bacterial pollutants. We hypothesize that the addition of iron filings could increase the removal capacity of biofilters and decrease first-flush leaching by limiting the growth of sequestered bacteria or pathogens during the drying period between rainfall events, but the effectiveness of iron would depend on the quantity of iron filings used in biofilter and the presence of compost. To test our hypotheses, we exposed stormwater contaminated with *E. coli* — a pathogen indicator — to iron filings in batch reactors and packed columns with and without compost and measured the fate of *E. coli* during and after the rainfall events.

2. Materials and methods

2.1. Stormwater collection and preparation

Stormwater was collected once a week in a 20-L HDPE container from the Ballona Creek (34° 0′ 4.9″ N 118° 24′ 27.1″ W) located in Los Angeles. The Ballona Creek watershed receives dry-weather and wet-weather runoff from a highly urbanized area spanning over 130 square miles. Stormwater particulates were separated by gravity settling and stored at 4 °C. The collected stormwater was characterized by pH (8.32 ± 0.82), electrical conductivity (1335.43 ± 87.07 μS cm^{-1}), and dissolved organic carbon (3.7 ± 0.3 mg L^{-1}). Previous studies have observed consistent concentrations of iron (524.7 μg L^{-1}), zinc (83.2 μg L^{-1}), copper (19.8 μg L^{-1}), and *E. coli* (207–1782 CFU mL^{-1}) in the Ballona Creek (LASanitation 2018; Stein and Tiefenthaler 2005). Before the experiment, the stormwater temperature was equilibrated to room temperature (22 ± 2 °C), and the pH was adjusted to 7.0 ± 0.2 using 1 M HCl.

Gram-negative bacterium *Escherichia coli* (ATCC 10798) with resistance to kanamycin (*E. coli* K-12) was used as a pathogen indicator to distinguish the applied *E. coli* from naturally occurring *E. coli* in stormwater (Mohanty et al., 2013). The *E. coli* was grown to a stationary phase concentration in Luria-Bertani broth media (Fisher Scientific). The *E. coli* was separated from the growing medium by washing with phosphate-buffered saline (PBS) solution and concentrated *E. coli* suspension was spiked into stormwater to achieve a concentration of $1.0 \pm 0.3 \times 10^4$ CFU mL^{-1}. The concentration of *E. coli* in stormwater can vary between 1-10^4 CFU mL^{-1} based on land use and source of *E. coli*. We used a high concentration of *E. coli* to ensure that potentially high *E. coli* removal by iron columns could be estimated using the agar-plate method.

2.2. Amendments

Organic compost was purchased (Whittier Fertilizer, CA), which was produced from the decomposition of plants and contained nearly 57% of organic carbon. Iron filings (Connelly-GPM Inc., Illinois, USA) were used as amendments to the quartz sand (ASTM C 778, the size range of 600–850 μm, Glison Company Inc.) to create the biofilter media. Zero-valent iron particles became corroded during storage. We have not measured the iron mineral types or valence of iron present in the corroded coating or iron oxides. However, it is expected that ferrihydrite or other iron (III) oxides to form in the oxic condition in stormwater (Furukawa et al., 2002). The iron filings have been used to remove phosphate from stormwater (Erickson et al., 2012), whereas compost or other organic amendments have been used to support vegetation and enhance biodegradation (Sigmund et al., 2018; Ulrich et al., 2017b). Compost and iron filings were sieved to eliminate large particles (>2 mm). The sand was homogenously mixed with compost or iron filings to form three mixtures: (a) sand and compost (25% v/v), referred to as compost biofilters, (b) sand and iron (3% or 10% by weight), referred to as iron biofilters, (c) a sand and compost mixture with 3% or 10%

iron filings by weight, which is referred to as iron-compost biofilters. A previous study tested the effect of 0.3, 2%, and 5% of the same iron filings in lab columns and 10% iron in a field study to examine the phosphate removal and found that at least 2% or more iron filings are needed to remove 79% of influent phosphate (Erickson et al., 2012). They did not observe clogging when 10% iron filings were used. Therefore, we tested 3% and 10% iron filings as the lower and upper limit for iron amounts to examine whether both amounts would be sufficient to minimize the first flush leaching of bacterial pollutants. Iron filings with and without exposure to DOC compost were characterized for the surface charge using ZetaPALS (ZetaPALS, Brookhaven Instruments), and Fourier Transform Infrared Spectrometer (FTIR) (Supplementary Information).

2.3. Biofilter design

Sixteen transparent polyvinyl chloride (PVC) columns (2.54 cm internal diameter, 30.48 cm length) were packed: 3 columns each for iron-compost mixture or iron-only, both with the iron content of 3% and 10% and 4 columns for control (compost only). To create a drainage layer, glass wool was placed in the bottom cap followed by a 3–4 cm layer of pea gravel. The specific media mixture was added incrementally at 2–3 cm height and compacted consistently using a plastic rod until the total depth of filter media became 15.2 cm. Another gravel layer (2–3 cm height) was added on top of the filter layer to prevent floating or erosion of the compost particles during the stormwater injection. The stormwater was delivered at the top of the filter media layer using a peristaltic pump with a multichannel pump head. Total pore volume (PV) was estimated based on the weight difference between dry columns and saturated columns. To estimate residual pore volume after gravity drainage, the columns were weighed after draining them under gravity for 2–3 h. The residual pore volume indicates the maximum amount of water available during the drying periods between successive stormwater applications. The effective pore volume during stormwater application—an indication of the fraction of filter media exposed to stormwater during injection—was estimated based on the weight difference between the columns during stormwater injection and dry columns.

2.4. E. coli removal capacity of iron filings with and without compost

To establish stable flow in columns and leach any particulates from pore water, synthetic stormwater (10 mM NaCl solution) was used, so that it has the same electrical conductivity as the natural stormwater used in the study. Ionic strength can affect the adsorption and release of particles or bacteria. The synthetic stormwater was applied at 2.5 mL min^{-1} on the top of packed filter media for 1.5 h, followed by 22.5 h of drying cycles when all columns were drained by gravity. The process was repeated three times. To estimate bacterial removal capacity, stormwater containing *E. coli* ($1 \pm 0.3 \times 10^4$ CFU mL^{-1}) was applied for 1.5 h followed by a drying period of 1, 2, or 4 days. The drying period is simulated by allowing columns to be drained by gravity and remained at room temperature (22 °C). During each injection, two effluent sample fractions were collected: the first 20–35 mL (the first flush) and the last 200–250 mL. The first flush sample represents the residual pore water from the previous injection. The last fraction (200–250 mL) represents the sample after the injection of 8–10 PV of contaminated stormwater, which is sufficient to achieve a breakthrough or maximum bacterial concentration in the effluent during an injection. Therefore, the last sample reflects the overall removal capacity of the biofilter during the injection. *E. coli* concentration in the column effluent samples was measured using the spread plate technique as described elsewhere (Mohanty et al., 2013). The removal capacity was estimated by comparing the effluent concentration (C) with the influent concentration (C_0): log removal = — log (C/C_0).

2.5. Quantifying the role of iron filings on first-flush leaching

During the drying period, *E. coli* trapped in biofilters could grow or die. For instance, compost can provide nutrients, help retain moisture during drying periods, and boost *E. coli* growth (Pietronave et al., 2004), whereas iron filings could limit the growth of *E. coli* (Zhang et al., 2010). It is not clear if the presence of iron media would help or inhibit *E. coli* growth with or without compost or organic amendments. To examine the effect of the drying period on growth or die-off of *E. coli* sequestered in biofilters, we compared the change in concentration of *E. coli* during the drying period by calculating the Growth-Die off Index (GDI) as presented elsewhere (Valenca et al., 2021). The GDI equation is as follows:

$$GDI = \frac{C_1^i / C_o^i}{C_2^{i-1} / C_o^{i-1}}$$

where C_0, C_1, and C_2 represents *E. coli* concentration in the influent, first-flush sample, and second or last sample in a rainfall event, respectively, and *i, and i-1* represent current and the previous rainfall event. Thus, GDI compares the relative concentration of bacteria before and after the drying duration to evaluate whether bacteria trapped in biofilters grow and die during the drying period. GDI >1 indicates *E. coli* grow during the drying period and GDI <1 indicates *E. coli* are removed or die off during the drying period.

2.6. E. coli adsorption capacity of iron filings in the presence of DOC

To quantify the effect of DOC from compost on the *E. coli* removal capacity of iron filings, DOC was isolated from compost by mixing 50 g of compost in 1 L of deionized water for 24 h. The solution was centrifuged (5000×*g* for 20 min) and the supernatant was measured for DOC using a Total Organic Carbon Analyzer (TOC-L series, Shimadzu). The concentrated DOC solution was diluted to prepare solutions at DOC concentrations between 3 and 293 mg L^{-1} Approximately 0.5 g of sieved iron filings (<2 mm) were mixed with 20 mL of DOC solution, which is equivalent to an exposure of 0.2–12 mg DOC per 1 g of iron filings. Briefly, iron filings and 20 mL of DOC solution containing *E. coli* (~10^6 CFU mL^{-1}) were mixed at 416 rpm for 3.5 h at 22 °C using a wrist action shaker (Burrell Scientific, USA), and the remaining suspended *E. coli* concentration was estimated by settling iron filings by gravity in 30 min.

3. Results

3.1. Addition of iron improved E. coli removal during stormwater infiltration

Comparing the removal in the first injection, we found that the clean-bed removal capacity—breakthrough concentration in a pristine column without prior exposure to polluted stormwater—of biofilters with iron was an order of magnitude higher than biofilters with compost. The removal capacity of biofilters decreased following the order: iron > iron and compost > compost (Fig. 1). However, the initial high removal observed in 3% iron columns compared to iron-compost biofilters with the same amount of iron or compost biofilters was quickly diminished after five injections. In contrast, 10% iron columns removed on average 61% more *E. coli* than the columns with iron and compost columns in all injection

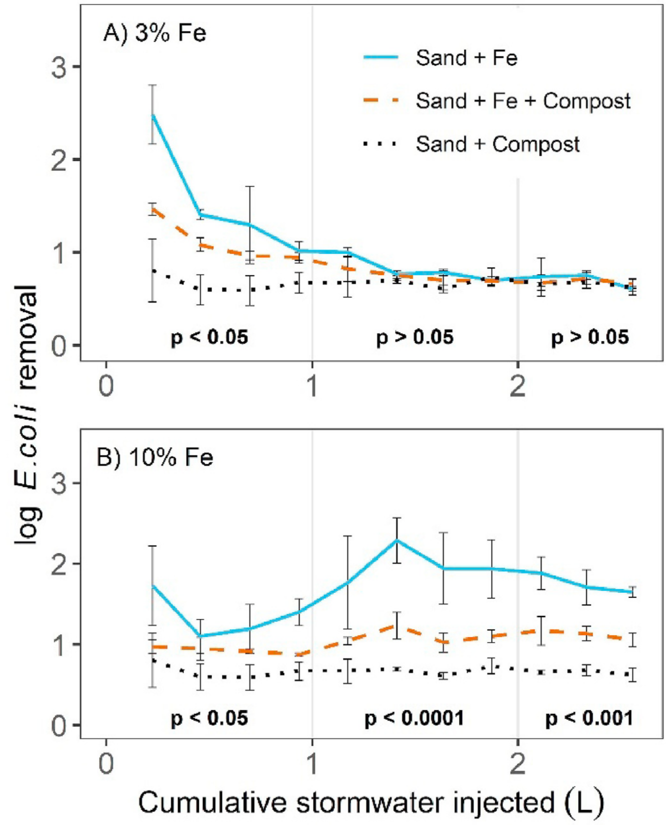

Fig. 1. Removal of *E. coli* in biofilters with (A) 3% iron and (B) 10% iron. Average log removal based on triplicate columns was calculated as $-\log(C/C_0)$, where C and C_0 represent the *E. coli* concentration in the effluent and influent water, respectively. p-values represent the comparison between the three biofilter types in each section (<1 L, 1–2 L, and >2 L).

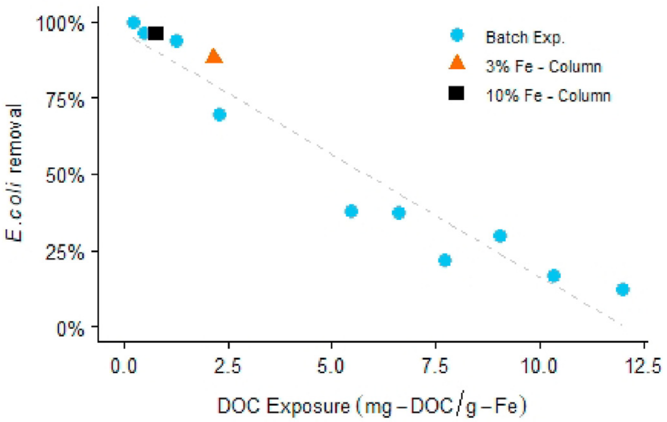

Fig. 2. Changes in *E. coli* removal with increases in DOC exposure. Duplicated batch experiments were conducted for each data point and the average removal is reported. The prediction of *E. coli* removal by biofilters amended with iron filings is indicated by the triangle (3% Fe) and square (10% Fe).

events. It should be noted that the log removal capacity of compost columns without iron (0.67 ± 0.02) was on average 35% lower than columns with compost and iron, indicating that the addition of iron is beneficial for bacterial contaminant removal. The increased log *E. coli* removal due to the addition of iron to compost is significant ($p < 0.05$) when iron content was 10% (Fig. 1-B).

3.2. DOC leached from compost diminish iron filings capacity to remove E. coli

An increase in DOC exposure decreased *E. coli* removal in batch experiments (Fig. 2). Based on linear regression, the removal of *E. coli* decreased by 8% with every 1 mg DOC exposure per gram of iron filings. The linear regression from batch studies slightly overpredicts the exhaustion of iron capacity in biofilters with 3% and 10% iron columns. We characterized the changes in surface properties of iron filings after exposure to DOC leached from compost and confirmed that surface charge of iron filings — measured as zeta potential — became more negative after the adsorption of DOC extracted from compost (Figure S1). Adsorption of DOC is further confirmed by a change in Fe–O peaks (Figure S2). Fe–O typically contributes to positive surface charge sites which increases the adsorption of bacteria and DOC (Gu et al., 1994).

3.3. Iron filing limits leaching E. coli during the first flush

The log removal of *E. coli* varies between the first-flush and last sample during the same rainfall event (Fig. 3). In biofilters with compost (Fig. 3-C) or 3% iron with compost (Fig. 3-D), *E. coli* concentration in the first-flush sample was similar or higher than the last sample. In contrast, *E. coli* concentration in the first-flush sample was consistently lower than the last sample in biofilters with 3% iron without compost (Fig. 3-A) and biofilters with 10% iron irrespective of compost presence (Fig. 3-B and 3-E).

The ability of iron to decrease the first flush release of *E. coli* changed with an increase in the injection sequences depending on the amounts of iron present or whether the compost was mixed with iron in the biofilters. While removal during the first flush decreased with an increase in injection in the 3% iron columns, it remained consistently high in the 10% iron columns. However, the addition of compost diminished *E. coli* removal during the first flush in columns with 10% iron, although the first-flush leaching from 10% iron columns with compost was still less than the first-flush leaching in compost only columns.

3.4. Iron filings affect the growth and die-off of sequestered E. coli during the antecedent drying period

Comparing the log removal during the first flush with the log removal in the previous injection, we estimated the Growth-Die off Index (GDI), which is an indicator of whether bacteria removed from infiltrating stormwater grow or die off during the drying period after the rainfall. The results show that GDI values are below 1 for all biofilters with iron, except for the biofilters containing a mixture of 3% iron with compost (Fig. 4). In contrast, GDI values of compost biofilters with or without 3% iron were near 1. GDI index in all columns did not significantly vary with the extent of the drying duration (Figure S3).

4. Discussion

4.1. Iron filings increase biofilter capacity to remove E. coli

Our results show that biofilters with 10% iron consistently removed more *E. coli* than conventional biofilters with compost. Although the presence of compost decreased the removal capacity of biofilters with iron filings, the removal remained similar to the capacity of compost columns. The long-term *E. coli* removal of 3% iron columns and compost columns are similar. The results indicate that the addition of sufficient iron can improve the bacterial removal capacity of biofilters, even in the presence of compost or

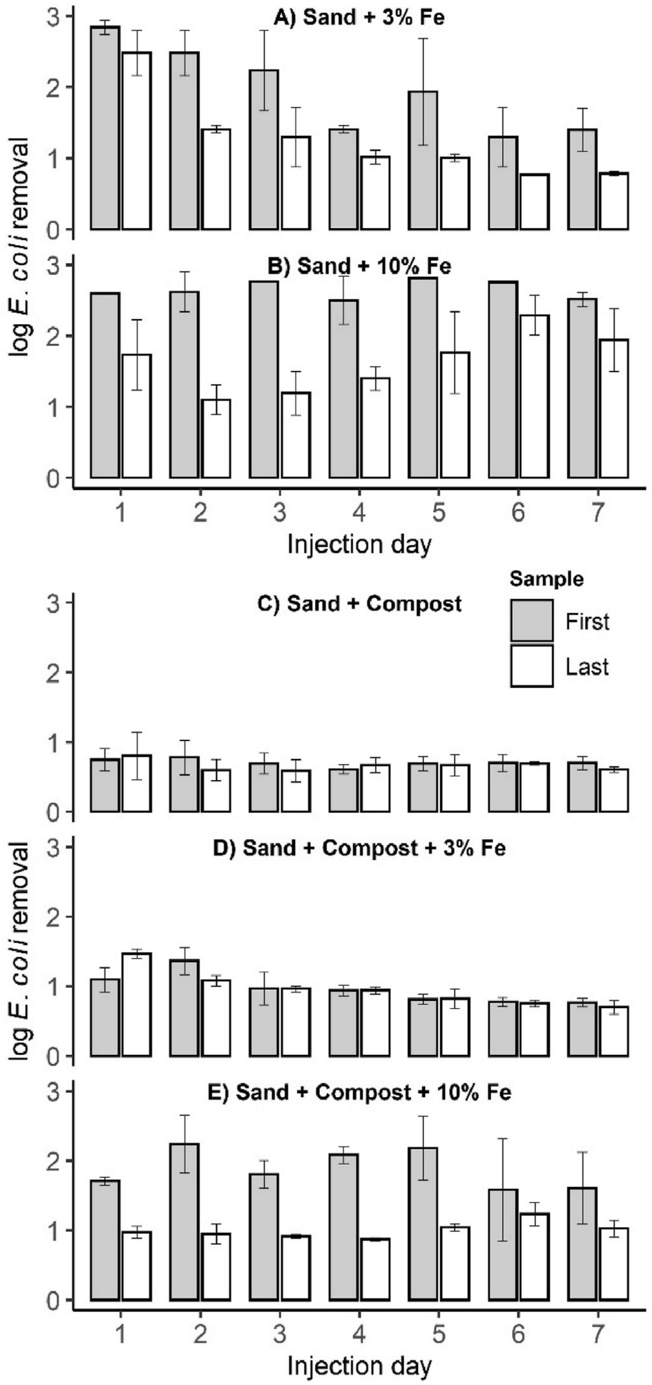

Fig. 3. Removal of *E. coli* in first-flush and last sample during 7 infiltration events in sand biofilters containing (A) 3% iron, (B) 10% iron, (C) compost without iron, (d) compost with 3% iron, and (e) compost with 10% iron. Error bars represent one standard deviation over mean removal based on at least triplicate columns.

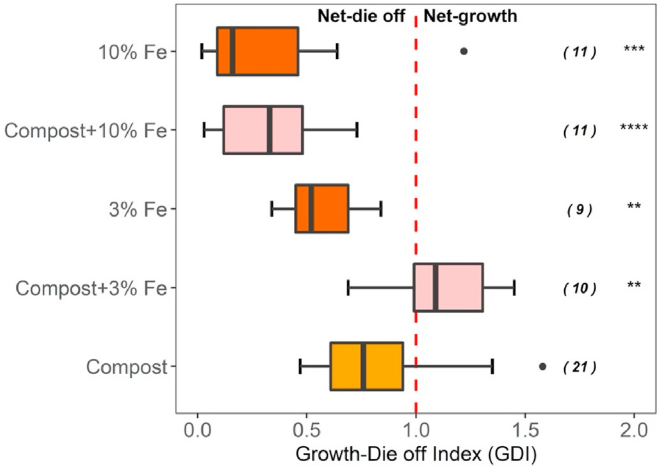

Fig. 4. GDI of biofilters containing different filter media. The vertical dashed line presents GDI = 1. GDI >1 presents the conditions when *E. coli* concentration increases in pore water potentially due to growth, whereas GDI <1 presents conditions when *E. coli* concentration decreases in pore water potentially due to removal by die off or adsorption. Numbers between parentheses represent the n-values used to construct each boxplot. Statistical difference was calculated using the Wilcoxon rank-sum test, and it compared the iron-containing biofilters with the compost columns. * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001, and **** p-value < 0.0001.

organic amendments. The result is attributed to the higher adsorption affinity of iron oxides for *E. coli* compared with conventional filter media such as sand or compost (Ingram et al., 2012). Other removal processes such as coagulation facilitated by iron precipitates and inactivation could have also helped increase removal (Sun et al., 2019). 3% iron columns have a lower capacity than 10% iron columns, indicating the increasing amount of iron can be beneficial for pathogen removal. The results are similar to previous studies where high bacterial removal was observed in iron-coated sand (Mohanty et al., 2013) and iron (oxyhydr)oxides mineral (You et al., 2005). However, an excessively high amount is not recommended, because of potential iron toxicity to plants (Zahra et al., 2021). Excess iron has been shown to increase clogging in permeable reactive barrier due to corrosion of zero-valent iron to iron oxides, which increase the volume of iron filings (Li et al., 2005). However, we did not observe clogging in columns within the duration of the experiment, potentially because iron was mixed with a sufficiently high amount of sand (Bilardi et al., 2015).

4.2. *Compost decreases adsorption capacity of iron filings*

The removal capacity of columns with iron filings decreased in the presence of compost, indicating iron filings lose their capacity to remove *E. coli* in the presence of compost. We attributed the results to adsorption of DOC leached from compost on sites that are otherwise used for adsorption of *E. coli*. Batch studies also confirmed that *E. coli* removal by iron filings decreased with increases in DOC exposure. Other studies also previously reported that the presence of organic carbon decreased the removal of pathogens in iron oxide-coated sand (Abudalo et al., 2010; Foppen et al., 2008; Mohanty et al., 2013). A decrease in removal capacity of iron particles in the presence of DOC can be attributed to several mechanisms: modifying surface charge of iron oxide from positive to negative (Abudalo et al., 2010; Foppen et al., 2008), competition for adsorption site by DOC (Li et al., 2010; Tratnyek et al., 2001; Wang et al., 2020; Yang et al., 2012; Yin et al., 2012), and increase in electrostatic hindrance by adsorbed DOC (Chen et al., 2011; Tanneru and Chellam 2012). Measurement of zeta potential confirmed that an increase in DOC exposure had altered the surface charge or made the iron oxides more negative, thereby lowering adsorption efficacy by electrostatic attraction. The result is similar to other studies that showed that adsorbed DOC could decrease the bacterial removal capacity of the iron media (Foppen et al., 2008). FTIR spectra of iron filings with and without exposure to DOC confirm organic carbon coating can occupy attachment sites, particularly Fe—O bond stretching. Natural organic matter especially higher molecular weight occupies or blocks adsorption sites of inorganic adsorbents such as sand (Yang et al., 2012) and metal oxide (Gu et al., 1994) and limits the adhesion of bacteria to the adsorption sites. Therefore, if the removal of bacterial pollutants is the primary goal of an iron-amended biofilter, it is not recommended to mix compost or other organic amendments with the iron amendment.

Compost decomposes and leaches a high concentration of DOC during the first flush, which can complex dissolved metals (Chahal et al., 2016). Thus, an increase in DOC concentration could increase the dissolution of iron from iron filings (Miller et al., 1986; Mladenov et al., 2010). On the other hand, the presence of iron could increase the microbial stability of organic carbon and prevent microbial decomposition of compost (Lalonde et al., 2012; Patzner et al., 2020; Riedel et al., 2013), although dissimilatory iron reduction could increase the dissolution of both iron (II) and DOC. Thus, future studies should examine the change in oxidation state and organic-Fe complex in biofilter amended with iron filings and organic amendment.

We showed that biofilters with 10% iron can consistently remove *E. coli*, despite the presence of compost, whereas the addition of 3% iron did not show any benefits in long term. The results indicate that amount of iron present in biofilter is critical to lower the detrimental effect of DOC. We estimated that exposure of 1 g of iron filings to 1 mg of DOC can reduce its bacterial removal capacity by 8%. This can help predict the reduction in removal capacity of iron-amended biofilters based on the typical loading of DOC in stormwater. As DOC is ubiquitous in stormwater, the capacity of iron-amended biofilters would decrease with time. Thus, a sufficiently high amount of iron should be added to overcome the negative impact of compost in biofilters.

4.3. Iron filings decrease the leaching of E. coli during the first flush

Our results show that bacterial concentration in the first flush from iron-amended biofilters was much lower than the sample taken later during the rainfall. Thus, the log removal estimated based on the first-flush sample was significantly higher than the last sample during the same rainfall. This result is opposite to what is expected for conventional biofilters, where the advancement of wetting front or disruption of air-water interfaces during the start of infiltration events typically mobilizes sequestered pathogens and increases their concentration in the effluent (Mohanty et al., 2013). The effect of the first flush is even more pronounced in the presence of DOC, potentially due to the growth of *E. coli* during the drying period (Mohanty et al., 2014). In this study, however, the presence of compost did not increase *E. coli* concentration during the first flush. In most samples, the concentration during the first flush was lower than the sample collected later during the same rainfall event. Bacterial removal in biofilters can vary by order of magnitude, and the cause of variability has been attributed to many factors including antecedent weather conditions, compaction of biofilter media (Ghavanloughajar et al., 2020; Le et al., 2020), media amendment type (Mohanty and Boehm 2015), and stormwater quality and dry duration (Nabiul Afrooz and Boehm 2017). It appears that the addition of iron could decrease the occurrence of highly polluted samples.

A decrease in the concentration of *E. coli* during the first flush in iron-amended biofilters was attributed to a lack of growth or die-off of *E. coli* trapped in the biofilter. The first flush effluent contains a larger fraction of pore water that was trapped from the previous rainfall, which therefore experiences longer residence time. An increase in residence time could increase adsorption (Mohanty and Boehm 2014) and increase the likelihood of predation or die-off of bacteria during antecedent drying (Zhang et al., 2010). When contaminated stormwater was injected, the pore water containing a low amount of *E. coli* was mixed with infiltrating stormwater with a high concentration of *E. coli*, thereby decreasing their concentration in the effluent.

4.4. Iron filings can affect the growth or persistent of E. coli sequestered in biofilters

Unlike chemical contaminants, biological pollutants can increase in biofilters due to their growth utilizing nutrients available in stormwater or biofilters media such as compost (Mohanty et al., 2014). Thus, it is important to use amendments that limit the growth of pathogens in biofilters during periods between rainfall events. We used the growth-die off index or GDI to evaluate the potential of compost and iron filings in supporting or inhibiting the growth of *E. coli* trapped in biofilters. We hypothesized that nutrients leached from the compost will provide a favorable condition for *E. coli* growth, whereas iron may limit growth by adsorption or inactivation. *E. coli* in compost columns showed a GDI close to 1, indicating no net growth or die-off in the columns. This is in contrast with our hypothesis that compost would increase the bacterial growth by providing a carbon source or nutrient for metabolic activities. A lack of net growth in the presence of compost can be attributed to predation or competition from the native bacterial community for nutrients. A previous study showed that conventional biofilter media consisting of sand, sandy loam soil, and mulch removed 99.98% *E. coli* by promoting the growth of heterotrophic bacteria and protozoa (Zhang et al., 2010). Thus, added *E. coli* in our study may not be able to compete for nutrients with the natural microorganisms present in compost or stormwater. Surprisingly, the median GDI values of biofilters with compost and 3% iron were greater than 1, indicating some of the sequestered *E. coli* reproduced or grew during the drying period between rainfalls. In 3% iron columns without compost, however, GDI values were consistently lower than 1, indicating that iron alone was not responsible for the growth of *E. coli* in the columns. A mixture of iron and compost could provide nutrients needed to increase the growth of the bacteria (Grandjean et al., 2006; Xiao et al., 2016). A previous study shows that bacteria can couple organic matter oxidation with iron reduction for their growth (Appenzeller et al., 2005). Iron filings can play a dual role: while solid iron can adsorb *E. coli* and remove them from pore water, dissolved iron leached from iron filings could provide micronutrients for microbial growth or cause toxicity. For instance, excess dissolved iron can be toxic to *E. coli* (Hantke 1997), whereas dissolved iron at low concentration (micronutrient) can support *E. coli* growth (Storz et al., 1990).

Our results show that the GDI of biofilters containing 10% iron was consistently below 1 irrespective of the presence of compost or antecedent drying duration. The result suggests that the presence of sufficiently high amounts of iron filings decreased the growth potential of *E. coli* trapped in biofilters. A possible reason could be the dual nature of iron and its oxides in the solution. Both zero-valent iron and iron oxides exhibit bactericidal properties, particularly if their size is in nanoscale (Lee et al., 2008; Schwegmann et al., 2010), which could prohibit *E. coli* growth in the drying periods. In our study, however, the size of iron filings is more than 2 μm and less than 2 mm. Thus, the bactericidal effect of these large iron particles is less likely. In this case, *E. coli* may adsorb on iron filings, where they may not grow due to lack of available nutrients. Suspended *E. coli* can also be removed by coagulation facilitated by oxyhydroxide iron flocs (FeOOH) produced from dissolved iron (Appenzeller et al., 2002; Sun et al., 2019; Zhu et al., 2005). Furthermore, some *E. coli* may be inactivated by hydroxyl radicals produced from zero-valent iron (Sun et al., 2019). However, we could not verify the extent to which each of these mechanisms could have contributed to the net decrease in *E. coli* concentration in biofilters with 10% iron filings.

5. Conclusions

Our results show that the addition of 10% iron filings by weight could not only increase *E. coli* removal capacity of stormwater biofilters but also significantly decrease the first-flush release of *E. coli* from biofilters, even in the presence of compost. The addition of 3% iron (by weight) was beneficial but mixing with compost completely diminished its capacity. A decrease in the *E. coli* removal capacity of iron filings by compost is attributed to adsorption of DOC leached from compost, alteration of surface charge on iron filings, and blocking of sites on iron filings by DOC. *E. coli* removed in biofilters can typically grow using nutrients in compost, but the addition of 10% iron diminished the growth or increase the removal, thereby lowering the concentration of *E. coli* in the first-flush effluent. Overall, the results indicate that the addition of iron amendments, preferably by 10% weight, can improve biofilter capacity to remove pathogen and limit net export of pathogens during the first flush by lowering the growth or increasing the die-off rate of the trapped pathogen in biofilters. Thus, biofilters should be amended with sufficient (~10% by weight) iron media to meet the total maximum daily load limit for the surface water bodies into which the effluent is discharged.

Credit author statement

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.116989.

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