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Performance and Longevity of Stormwater Biofilter Media Under Hydrologic and Physical Stressors

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Performance and Longevity of Stormwater Biofilter Media Under Hydrologic and Physical Stressors

A dissertation in partial satisfaction of the requirements for the degree Doctor of Philosophy in Civil and Environmental Engineering

by

Maryam Ghavanloughajar

2021

ABSTRACT OF THE DISSERTATION

Performance and Longevity of Stormwater Biofilter Media
Under Hydrologic and Physical Stressors

by

Maryam Ghavanloughajar

Doctor of Philosophy in Civil and Environmental Engineering
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Professor Sanjay K. Mohanty, Chair

Stormwater treatment systems such as biofilters have been increasingly incorporated in urban areas because they can alleviate water scarcity issues in urban areas, decrease pollutions from contaminated runoff, and provide an alternative source of water. Despite all benefits, large-scale application of biofilters is limited, partly due to limited knowledge of how the anthropogenic stressors (e.g., compaction) and filter media design could decrease their longevity. The overall objective of this dissertation is to investigate the processes that affect the longevity of stormwater biofilter media. Specific objectives include: (1) to examine the effect of one of the physical stressors—compaction—on the longevity of biofilter media and performance of biofilters; (2) to evaluate the effect of chemical stressors—stormwater constituents such as dissolved organic carbon—on longevity and performance of iron amendment; (3) to examine the persistence of antibiotic resistance genes, an emerging contaminant, in stormwater biofilters with different design configurations.

To examine the effect of compaction on the capacity of biochar-augmented roadside biofilters to infiltrate stormwater and remove pollutants, columns were packed with a mixture of sand and amendments (biochar or compost) using energy equivalent to the standard Proctor test. The effects of compaction on hydraulic conductivity and removal of *Escherichia coli (E. coli)* were evaluated. The results revealed that the negative impact of compaction on hydraulic conductivity can be alleviated by using moist biochar. The addition of moisture during compaction can increase contaminant removal, initial particle release, and infiltration capacity of biochar-augmented sand filters for road runoff treatment. To evaluate the designs that could increase the long-term removal of bacterial pathogen in biofilters and minimize their leaching in the first flush, conventional biofilter media, a mixture of compost and sand, was augmented with iron fillings. The results show that the addition of iron amendments could increase removal and decrease first-flush leaching of *E. coli.* However, the presence of compost decreased the adsorption capacity: exposure of 1 g of iron filings to 1 mg of dissolved organic carbon (DOC) from compost reduces *E. coli* removal by 8%, potentially due to the alteration of the surface charge of iron and blocking of adsorption sites shared by *E. coli* and DOC. To examine the effect of two design features—addition of iron amendment and submerged layer—on biofilter ability to mitigate the dissemination of antibiotic-resistant genes (ARGs), conventional biofilter media was augmented with iron filings with and without a submerged layer. Natural stormwater containing ARGs was applied intermittently, and the effluent was analyzed for ARGs. The results show that conventional biofilter design including organic compost, without any submerged layer, removed nearly all antibiotic resistance genes, whereas the presence of submerged layer in the same column significantly ($p<0.05$) reduced the removal or increased the relative gene abundance in the effluent. While the addition of iron improved compost's ability to remove ARGs in the partially submerged biofilter, it decreased removal in the unsubmerged biofilter. Collectively, all these results help engineers improve the design of stormwater biofilters by modifying filter media and increase their longevity.

The dissertation of Maryam Ghavanloughahar is approved.

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2021

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Mohanty, S.K.; **Ghavanloughajar, M.;** Stenstrom, M.K.waste-derived amendments in stormwater biofilters: do the benefits outweigh the risks? *ACS National Meeting.* August 25-29, 2019. San Diego, CA. (Oral presentation)

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1. Longevity of Stormwater Treatment Biofilters: Opportunities, Challenges, and Research Gaps

1.1. Water scarcity and opportunities for stormwater management in urban areas

Freshwater, which accounts for less than 3% of the total water on Earth's surface, is the most critical resource to support human civilization. However, an increase in water demand from the growing population has depleted the available water resources and increase water scarcity. In fact, water use has grown at more than twice the rate of population increases (Zhao et al. 2018) resulting in a net depletion of freshwater resources in water-stressed areas. By 2025, 1.8 billion people are projected to experience absolute water scarcity, and two-thirds of the world population will be living under water-stressed conditions(Connor et al. 2014).

By 2050, nearly 70% of the world's population will live in urban areas, where the water scarcity issue is becoming severe. Rapid urbanization has also adversely affected both water quality and quantity. For example, an increase in impervious surfaces from urban developments has increased stormwater runoff volumes and peak flows. The increase in the overland flow -runoff and stormwater- not only depletes the natural recharge of groundwater but also wash out pollutants to receiving water bodies(Barbosa et al. 2012), thereby making them less likely to be used without extensive treatments. While an increase in stormwater volume is inevitable due to ever-expanding urban areas, it is critical to limit the contamination of water bodies by stormwater.

Historically, stormwater management in urban areas has been used for flood control by lowering the peak flow when design to the removal of pollutants was not considered. However, treated stormwater can be a resource instead of a nuisance. Using proper treatment systems, stormwater can be captured and reused locally, thereby simultaneously minimizing contamination from runoff and increasing water availability (Grebel 2013). Many cities are increasingly integrating stormwater harvest technologies into their water management plan. For example, the Los Angeles Department of Water and Power is projected to capture 180,000 acre-feet of stormwater by 2035 using both centralized and distributed systems (LADWP, 2015). Low impact development (LID) can be integrated into existing infrastructure such as buildings, roadways, or parking lots (Raček et al. 2020). While harvesting stormwater via LID meets the dual objective of stormwater reduction and water quality improvement, over-harvesting which leaves downstream with low water levels especially in more arid areas must be avoided (Fletcher et al. 2007).

1.2. Stormwater treatment systems

Typically, decentralized low impact development (LID) systems are onsite design or catchment-wide approaches for stormwater capture without incorporation of the conveyance system(Fletcher et al. 2015). LID practices such as infiltration and bioretention systems not only mitigate flood risk but also provide a source of water for reuse(Walsh et al. 2012). Among different types of LID, biofilter systems are popular due to their versatile size, configuration, (Bratieres et al. 2008), and higher pollutant removal capacity. Biofilters are typically designed by replacing the soil with a mixture of soil, sand, or compost to increase the infiltration and removal of pollutants (**Figure 1-1**). Figure 1-1 depicts the main features of a typical biofilter. Stormwater and runoff from the catchment area enter the biofilter and treated products either flows beneath the filter to recharge the groundwater or exits the filter via outlet ports for reuse purposes. Main filter media such as sand, improves the hydraulic conductivity of biofilters. Traditional filter media such as mulch or compost primarily facilitate the biodegradation of pollutants by providing dissolved organic carbon as a substrate for the microbial community (Ulrich et al. 2017). Contaminants are removed by physical filtration, adsorption, and biotransformation (Davis et al. 2003). While filter media is the main contributor to biofilter performance, topsoil and drainage layer add to the biofilter functionality by protecting the media. Vegetation is planted at the top for aesthetic purposes, although plants also help in maintaining high infiltration capacity and facilitate pollutant removal in the root zone.

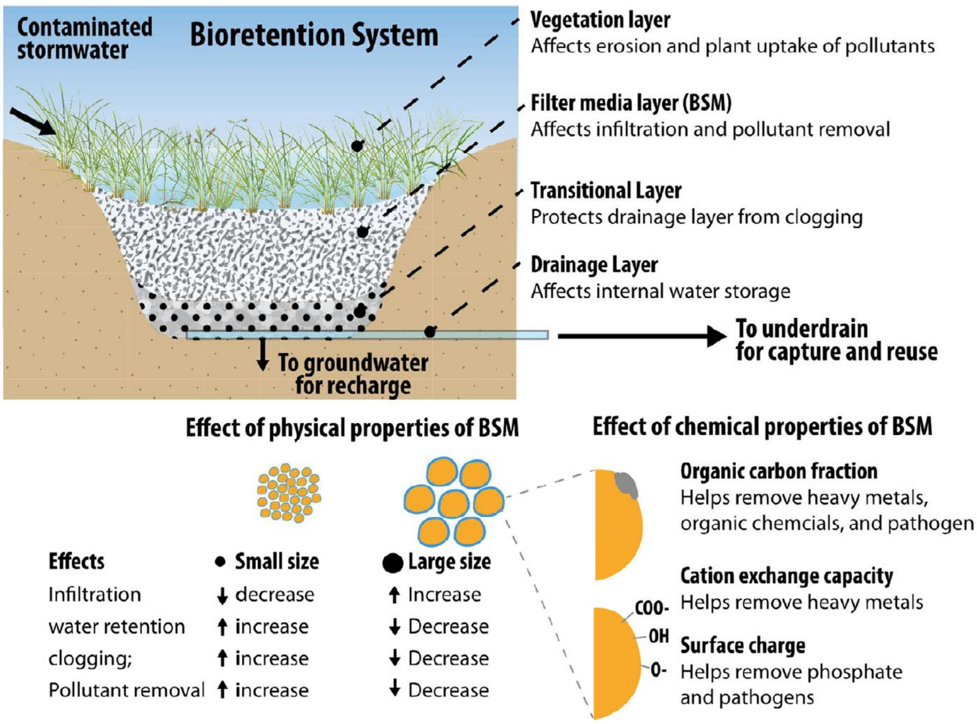

Figure 1-1. Stormwater biofilter without submerged zone schematics (Tirpak et al. 2021).

1.3. Longevity and performance of stormwater treatment biofilter: Challenges and literature review

Despite the multiple advantages of stormwater treatment systems, their adoption in urban areas has been slow, partially because of several challenges. First, successful implementation of the LID system must meet a large number of hydrological, environmental, and socioeconomic constraints(Zhang et al. 2018). Second, the long-term effectiveness of stormwater biofilters to consistently remove pollutants has not been evaluated. Biofilters are rarely monitored beyond the first 3 years of operation, even though they are expected to be operational for 20 years or longer. Third, stormwater biofilters are subjected to natural weather cycles, and some of the conditions can be harsh (e.g., long drought, flooding). These conditions could rapidly deteriorate the filter media and change their physical, chemical, and biological properties, which could lower the infiltration capacity of biofilters and decrease contaminant removal. For example, compaction of filter media over time or by surface activity could alter the hydraulic conductivity of filter media and increase the physical loss of filter media. Table 1-1 summarizes the physical, chemical, and biological processes that could affect the performance lifetime of biofilter media. Sections 1.4 to 1.6 discuss each of these processes in more detail. Finally, filter media can be exhausted with time due to an increase in exposure to contaminants and other stormwater constituents that compete with contaminants for attachment sites on filter media.

Table 1-1. Processes that could decrease the lifetime of the filter media in stormwater biofilters.

Processes		References
Physical	Erosion of filter media	(Han et al. 2019)
	Clogging of filter media by deposition of sediments	(Kandra et al. 2014, Seo et al. 2005)
	Compaction can both increase erosion and clogging of media	(Mohammadshirazi et al. 2016)
Chemical	Deterioration of filter media by chemical processes on the surface	(Lynn et al. 2015)
	Exhaustion of adsorption sites	(Hamisi et al. 2019), (Charbonnet et al. 2018, Kandra et al. 2014, Pratt et al. 2011, Klausen et al. 2003)
Biological	Deterioration of filter media by biological processes on the surface (e.g., wood rotting)	(Singh et al. 2018)

Biofilm formation that could block the adsorption sites	(Freidman et al. 2017, Le Bihan et al. 2000, Nabiul Afrooz et al. 2017)

1.4. Physical processes affecting the longevity of filter media

One of the primary barriers in the universal adoption of the stormwater biofilter is processes that induce biofilter blockage in which pore space between media grains decreases drastically (Le Coustumer et al. 2012). Reduction in pore space will be manifested in hydraulic conductivity reduction when biofilter loses its ability to infiltrate stormwater. This will result in the creation of a ponding zone (Le Bihan et al. 2000). If adequate provisions have not been made for the maintenance of the ponding zone or if hydraulic conductivity is so low that flow pass is negligible, then biofilter is deemed as failed. For instance. erosion of particles of the catchment area with subsequent increase in total suspended solid load to biofilters can drastically reduce the pore capacity (Liebens 2001).

1.4.1. Factors affecting erosion of filter media or mobilization of particles

Rainfall and subsequent runoff erode particles from the soil surface. The rate of erosion and the extent of soil disintegration and formation of macroaggregate sediments is a function of soil composition, catchment area gradient, type of vegetation, rainfall intensity, and kinetic energy of raindrops (El Kateb et al. 2013, Li et al. 2021). Besides rainfall, variability in stormwater frequency and extreme temperature changes such as dry-wet and freeze-thaw cycles can affect erosion in the catchment area or biofilter media. Changes in flow regime can induce detachment of constituents or media. Extended drying durion also results in a reduction of soil moisture content and negative capillary pressure in filter media, which induces collapse in porous media and consequently colloid generation (Mohanty et al. 2015). During antecedent drying duration, cracks develop and further facilitate the migration of fine particles from the top of the biofilter to the deeper level where their accumulation cause clogging (Zinger et al. 2021). Placement of stormwater control measures such as biofilter near areas that are susceptible to erosion not only can greatly decrease the net flux of eroded soil to the adjacent water bodies but also can promote more distributed and less clustered areas with a high risk of erosion(Yang et al. 2018).

Despite the beneficial impact of biofilter on erosion reduction, erosion can affect the longevity of biofilters in two ways. First, surface runoff can carry a high load of different constituents such as suspended solids or *E.coli* (Boithias et al. 2021) from the eroded catchment area to the biofilter and ultimately overwhelm the filter media. For instance, the concentration of heavy metals in eroded sediments is higher compared with the background soil and is highly correlated with clay, silt, and organic carbon fraction in eroded soil (Quinton et al. 2007). Typical biofilter media such as compost are vulnerable when exposed to consecutive loads of sediment and might lose more than half of their capacity after a few filtration events (Taleban et al. 2009). Second, if erosion happens at biofilter media topsoil, media loss leads to lower biofilter adsorption capacity. Incorporation of the saturation zone or geomedia that promotes stronger attachment can mitigate the adverse effect of intermittent flow. For instance, the saturation zone slows down the formation of preferential path flow, promotes the capillary moister rise (Zinger et al. 2021), and hinders air phase development(Mohanty et al. 2013).

1.4.2. Clogging of filter media

Physical clogging and ultimately loss of permeability of filter media is a direct consequence of particles deposition from stormwater on filter media grains often at the top layer of the biofilter. Filter media can be clogged before their adsorption capacity is exhausted (Pitt et al. 2018). In general, an increase in the residence time of stormwater enhances removal capacity. However, if the increase in residence time is a result of clogging and ponding zone, it will not be beneficial for contaminant removal (Hatt et al. 2006).

Suspended solid from road runoff is a major contributor to the degraded quality of filtrate stormwater compared with eroded particles from biofilter media (Flanagan et al. 2019). Depending on the size of filter media grains, stormwater carries sediments to different levels of biofilters. For instance, in the fine media, the cake layer of filtrate develops more rapidly at the top of the biofilter due to straining whereas, in coarse media, the higher sheer force of stormwater can carry particles to a deeper level (Kandra et al. 2014). Similarly, in only gravel media, clogging occurs further at the interface between media and surrounding soil (Siriwardene et al. 2007).

Hydraulic loading of stormwater affects clogging in different ways. Increased stormwater loading not only carries a considerable amount of sediment but also tends to compact the filter media (Le Coustumer et al. 2012). Also, stormwater flow in the porous biofilter media, can potentially detach fine particles from media grains and deposit them on pore throats which causes clogging. The detachment and redeposition of particles increase with the increase in flow velocity (Han et al. 2019).

Some modifications can ameliorate clogging and increase longevity. For instance, thick roots improve physical clogging by creating macropores and therefore preferential path for stormwater and then maintaining conductivity even with deposited sediments (Le Coustumer et al. 2012). Changes in geometry such as an increased proportion of lateral infiltration compared with vertical infiltration in deep biofilters maintain a higher infiltration rate and increase the life span (Conley et al. 2020). Another intervention such as pretreatment of stormwater in the sedimentation basin has been suggested to remove total suspended solids before entering biofilter (Kabenge et al. 2018).

1.4.3. Compaction of filter media

Common soil preparation practices such as compaction can adversely affect soil properties. Like erosion, compaction influences biofilter longevity in different ways. At the catchment area scale, compaction decreases infiltration and contributes to runoff increase(Alaoui et al. 2018). For instance, compaction has been shown to reduce the infiltration rate of natural and planted forest soil by 77-90% (Gregory et al. 2006). At the biofilter scale, compaction increases soil bulk density and mechanical penetration resistance (Keller et al. 2019) and decreases soil hydraulic conductivity (Mossadeghi-Bjorklund et al, 2016).

The severity of the compaction effect depends on soil structure, moisture, and methods of compaction (Jones et al. 2003). Compaction methods vary based on exerted mechanical force or initial soil content. Optimum initial soil moisture binds soil particles via a cohesive force, therefore, reduces the impact of compaction. However, at higher moisture beyond optimum level, water acts as a lubricant between particles and reduces friction (Canillas et al. 2001). Media modification such as the addition of biochar can improve soil bearing capacity. However, the extent to which biochar size distribution affects soil properties including water holding capacity and bulk density varies (Verheijen et al. 2019). Therefore, despite the beneficial use of biochar in stormwater biofilters, their performance under physical stressors such as compaction is not clear.

1.5. Chemical processes affecting the longevity of filter media

Chemical clogging follows physical and biological clogging. Similar to physical processes, attachment sites on biofilter media can be exhausted rapidly due to interaction with common stormwater constituents or alteration of surface properties by chemical processes. For instance, in the absence of total suspended solids, adsorption of phosphorous on dewatered aluminum-based amendment produces solid precipitation that contributes up to 6% to media clogging (Yang et al. 2021). Chemical clogging is not as troublesome as the other two types of clogging since biofilter media can be potentially recovered. For instance, hypochlorous acid completely regenerates manganese oxide-coated sand after its exhaustion by bisphenol A adsorption(Charbonnet et al. 2018). However, it must be noted that although regeneration of adsorbents is a common practice in water and wastewater treatment, it is not routinely practiced in stormwater management because it would kill the helpful bacteria responsible for the biodegradation of pollutants.

1.5.1. Alternation of surface properties

Multiple factors alter the surface properties of biofilter media that can adversely affect their performance. First, natural aging such as oxidation, dissolution, and precipitation can adversely affect biofilter capacity. Natural aging can cause the formation and subsequent leaching of organic acid from organic media such as wood chips over time (McLaughlan et al. 2009). This dissolved organic matter is well correlated with a decrease in pH, which can induce immobilization of previously retained heavy metals (Trenouth et al. 2015). Second, prolonged drying duration can cause an alternation in surface properties. For instance, the antecedent dry duration can alter the surface properties of media and reduces its affinity for different constituent adsorption (Li et al. 2012). The breakdown of organic matter media over-drying duration releases phosphate from compost media which then leaches out from the biofilter (Bratieres et al. 2008). Finally, stormwater characteristics can significantly affect biofilter media properties. Stormwater is subjected to temporal and seasonal variations therefore fluctuation in the concentration of its constituents is inevitable. For example in areas with a long dry season, the first flush of runoff contains a higher concentration of all pollutants including total organic carbon (Lee et al. 2007). The high concentration of organic matter can interfere with media capacity in degrading and removing pollutants. For instance, adsorption of natural organic matter (NOM) can alter the surface charge of goethite and reduce its capacity towards *E. coli* removal(Foppen et al. 2008).

It must be noted that chemical alteration of surface properties is very specific to media type. For instance, iron-containing amendments are prone to mineral precipitation on their surface (Lin et al. 2005). They can become passive due to the corrosion and dissolution of iron (Carniato et al. 2012). Another example would be biochar in which aging increases its O:C ratio either due to oxidation of biochar or attachment of oxygen from soil mineral and dissolved organic matter (Sorrenti et al. 2016). Oxidation of biochar not only increased the pore volume and surface area of biochar (Fan et al. 2018) but also promotes the development of C functional groups such as hydroxyl, carbonyl, and carboxyl on biochar surfaces that eventually increases the negative surface charges of biochar (Wang et al. 2020). Alteration of surface charge of biochar can affect its capacity to remove contaminants including pathogens (Suliman et al. 2017).

1.5.2. Exhaustion of adsorption sites

The longevity of biofilter decreases if media components lose their capacity because of changes in surface properties as discussed in 1.2.1. However, in some scenarios, loss of removal capacity of media is due to the exhaustion or coverage of adsorption sites by competing and larger pollutants or gas phase development. Stormwater contains a wide range of pollutants and other benign constituents such as dissolved organic carbon, and they all can compete for the same sites on filter media (Rangsivek et al. 2005). Gas-phase also can propagate within the media and create immobile water regions (Vikesland et al. 2003). Although the effect of these new regions on the reduction of porosity is limited compared with an accumulation of precipitation on the surface, they can contribute up to 14% to the decrease of porosity (Vikesland et al. 2003).

The presence of natural organic matter (NOM) in the influent to sand biofilter can compete with a bacteria for adsorption site or cover the media pores by an adsorbed blanket of NOM(Mohanty et al. 2013). NOM can form complexation with iron oxide in iron-coated sand media that increases the remobilization of retained bacteria upon its dissolution (Mohanty et al. 2013). In another column transport study with three types of bacteria including non-motile gram-positive and gram-negative and motile gram-negative on quartz sand at the presence of humic acid, it was shown that the presence of humic acid does not alter the surface properties of either sand or bacteria. However, the presence of humic acid as low as 1 mg L^{-1} in the packed porous decreased deposition of bacteria. This observation was attributed to competition between humic acid and bacteria for the adsorption site and repelling force of suspended humic acid (Yang et al. 2012).

1.6. Biological processes affecting the longevity of filter media

Biological processes are integral to biofilter functionality. Variation of indigenous microbial communities is present in soil or compost media that generally compromise a majority of filter media (Tirpak et al. 2021). Moreover, stormwater is one of the major contributors of microbial pollutants to water bodies. Biofilters utilize bacterial metabolism to remove pollutants from stormwater. However, bacteria simultaneously degrade or dissolve biofilter media including compost and iron filings, therefore, reduce the lifespan of filter media. Also, the synthesis of biofilm or extracellular polymer substance (Faucette et al.) is inevitable when the bacteria community is present.

1.6.1. Biological deterioration of filter material

Bacteria can enhance pollutant removal (especially organic contaminants) in redox reaction via direct or exogenous electron transfer, while their growth on media surface and subsequent leaching from biofilter can create challenges for the long-term application of biofilter (Park et al. 2009). Microorganisms can prevent the attachment of some pollutants on the media surface by blocking access or competition for nutrients. For instance, while biofilm can enhance nutrient removal, its formation reduces fecal indicator bacteria removal (Nabiul Afrooz et al. 2017). Dissolution and development of corrosion products on the iron surface are more prevalent in the presence of iron-reducing bacteria which can further increase nitrobenzene removal up to 50%(Yin et al. 2015). Incorporation of other macrofaunae such as earthworms or plants (Maurya et al. 2020) or the addition of amendments specific to bacteria retention such as iron filings (Park et al. 2009) or biochar (Mohanty et al. 2014) can further improve biofilter capacity for bacterial removal.

1.6.2. Biological Clogging

Similar to physical clogging, biological processes such as the production of biofilms can reduce hydraulic conductivity and initiate bioclogging (Zhou et al. 2018). Biofilm is formed as strained microorganisms accumulate on the surface of the particles. Biosynthesized Extracellular Polymer Substance (EPS) further enhances the integrity of biofilm in pores (Costa et al. 2018) and appears to be the best predictor of biofilm effectiveness(Zhang et al. 2021). EPS and biofilm formation linearly correlate with saturated hydraulic conductivity reduction (Xia et al. 2016). However, compared with the high loading of suspended solid, biomass growth is not a major cause of clogging (Langergraber et al. 2003).

Unlike other types of clogging, biofilm not only affects the permeability but also directly contributes to the removal capacity of biofilter based on the characteristic of pollutants. For instance, biofilms were shown to be more effective compared with sand to retain collide in porous media (Zhang et al. 2021). Both hydrophobic and hydrophilic biofilm coated on sand can significantly increase nanoparticle retention(Mitzel et al. 2016). In the column study with different types of media, the formation of biofilm enhanced retention of iron and manganese-oxidizing bacteria in the media which could further increase iron and manganese removal from groundwater up to 95% (Li et al. 2016).

The ability of biofilm and its associated EPS to interact with pollutants highly depends on the composition of the biofilm. For instance, two strains of *P. aeruginosa* have very different *E.coli* removal capabilities (Liu et al. 2008). Changes in flow regime such as dry duration when water head declines can recover media from biofilter. In this situation, biofilm and EPS are dewatered and shrunk and therefore become dispersive (Zhou et al. 2018).

Like physical clogging, biological clogging is dependent on media grain size. In uniform homogeneous fine media, biofilm is generated at the top layer and eventually, aged biofilm detaches from the surface and penetrates to the deeper level to create deep clogging. In the multilayer biofilters, the interface between media is a hotspot of microbial activity which promotes biofilm formation and maintenance and minimizes its propagation to a deeper level (Perujo et al. 2019).

1.7. Research Gap

As mentioned in section 1.3, there are few obstacles in the way of sustainable implementation of stormwater biofilters including lack of proper prediction for the long-term performance, adverse effect of operational conditions, and exhaustion of media. To resolve the long-term effectiveness challenge of the biofilter and increase its adaptability, the desirable filter media must effectively and consistently remove a variety of pollutants from stormwater. Although sand and organic substances such as compost compose most of the filter media, their inability to remove all ranges of pollutants necessitates supplementing filter media with organic and inorganic amendments. However, the choice of proper amendment with the appropriate amount, mechanistic understanding of interactions between amendments and other media components, and mitigating probable unfavorable consequence of amendments application such as inducing premature clogging is still a place for discussion.

For example, biochar is among the most favorable organic amendments that offer a high specific surface area and adsorption capacity for bacteria and organic contaminant removal (Mohanty et al. 2018). However, biochar breaks by disintegration under compaction – a common practice during the construction of roadside biofilters- and exponentially decreases the hydraulic conductivity (Le et al. 2020). Although compaction is practiced routinely for road construction, its effect on roadside biofilters is not thoroughly investigated. We have addressed the effect of compaction on different properties of biofilters in the second chapter.

Another example is Iron-based materials that are classified as an inorganic amendment and are a good candidate for removing bacteria with net negative charge due to their positive surface properties (Kim et al. 2020). However, their application is extremely limited by the presence of other constituents such a dissolved organic matter (Mohanty et al. 2013). Although iron amendments are promising for removing a variety of contaminants, their exhaustion capacity for fecal indicator bacteria is not fully explored. This longevity and bacterial removal capacity of biofilters amended with zero-valent iron filings when exposed to dissolved organic carbon is the core of the third chapter.

Biofilters are a complex and interrelated environment of air, water, and soil in which promotes the growths of plants and other microorganisms. Depending on the source origin, stormwater carries a complex mixture of organic and inorganic contaminants. These contaminants have different and sometimes competing fate pathway that influences their interaction with biofilter media. Therefore, it is critical to improving the understanding of how physical, chemical, and biological stressors can affect the longevity of stormwater treatment systems by inducing clog formation and develop appropriate design controls to extend the performance lifetime of stormwater biofilters.

1.8. Objectives

The overall aim of this dissertation to investigate the processes that affect the longevity of stormwater biofilter media so that their performance lifetime can be improved by engineering controls or design modifications. Specific objectives include:

(1) To examine the effect of compaction on particle release, hydraulic conductivity and *E. coli* removal of model biofilters augmented with biochar or compost. This section focuses on one of the physical stressors—compaction— on the longevity of biofilter media.

(2) To evaluate if iron amendments remain effective at removing bacteria in the presence of compost in a biofilter. This section focuses on one of the chemical stressors—exhaustion of attachment sites by stormwater constituents—on the longevity of a popular amendment: iron.

(3) To examine if the addition of iron filings or submerged layer can decrease the concentration of ARGs in biofilter effluent. This chapter examines whether or not specific design factors may make biofilters a source of ARGs.

All sections evaluate the effect of specific designs—media moisture content during construction, and amendments types and quantity—on the overall performance of stormwater biofilters in environmentally relevant conditions. The result of this work will advance the understanding of how physical, chemical, and biological processes in biofilters could deteriorate biofilter media over time. Furthermore, the potential of engineering controls to extend the performance lifetime will be tested.

1.9. Chapter organization

This dissertation consists of three research chapters as follows:

Chapter 2 quantifies the effect of compaction, one of the physical stressors for filter media deterioration, on the performance of biochar augmented sand biofilters. The immediate effect of compaction on biofilters includes leaching of a particle from the media, change in hydraulic conductivity, changes in flow paths, and consequently change in the filter media interaction with stormwater. This chapter will also evaluate potential engineering methods to minimize the detrimental effect of compaction on biofilters so that their performance lifetime is extended. Chapter 3 evaluates whether the longevity and performance of iron filings are affected by the presence of organic amendments such as compost. In particular, this chapter quantifies changes in bacterial removal capacity of iron-augmented biofilters in the presence of compost or dissolved organic carbon.

Chapter 4 investigates the extent that biofilter design factors such as the presence of a submerged layer or the addition of iron amendment contribute to the persistence or discontinuation of ARGs in the biofilter effluent.

Chapters 2 and 3 of this dissertation have been previously published in the journals, respectively: *Science of the Total Environment* and *Environmental pollution*. The third one is under preparation:

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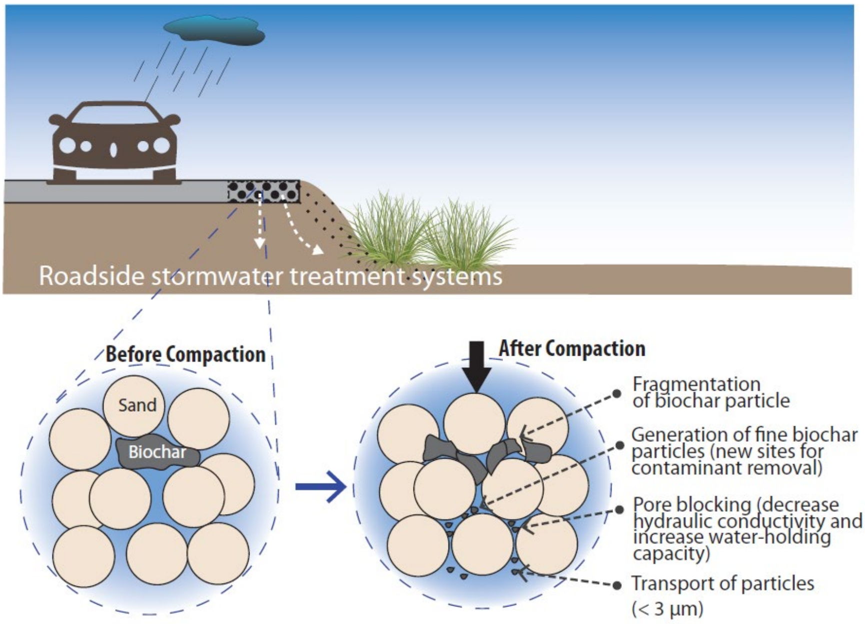
2. Compaction conditions affect the capacity of biochar-amended sand filters to treat road runoff

Graphical abstract:



Journal Article:

Ghavanloughajar, M., Valenca, R., Le, H., Rahman, M.D., Borthakur, A., Ravi, S., Stenstrom M.K., and Mohanty, S.K. Compaction conditions affect the capacity of biochar-amended sand filters to treat road runoff. *Science of the Total Environment.* **2020.** https://doi.org/10.1016/j.scitotenv.2020.139180

Abstract

Amending roadside soil with adsorbents such as biochar can help remove pollutants from road runoff. To maintain soil stability, the roadside soil requires compaction. However, it is unknown how compaction conditions affect the capacity of biochar-augmented roadside biofilters to infiltrate stormwater and remove pollutants. This work examines the effect of compaction conditions on the release of biochar particles disintegrated during compaction and the change in their capacity to infiltrate stormwater and remove *E. coli*. The net loss of biochar particles by mobilization with stormwater was insignificant compared to the biochar that remained in the filters. The initial release of biochar particles in wet-compacted biochar columns was greater than that in dry-compacted biochar. The results revealed that compaction can affect the release of biochar particles in a series of three-step processes: generation of particles by the disintegration of large biochar under compaction, diffusion of particles deposited near grain walls to bulk pore water, and transport and retention of particles in constricted pore paths based on pore water connectivity. Under similar conditions, compost columns released more particles than biochar columns, suggesting biochar is more stable than compost under compaction. *E. coli* removal in wet compacted columns was greater than removal in dry-compacted columns, owing to greater pore path connectivity in wet-compacted columns. These results indicate that the addition of moisture during compaction can increase contaminant removal, initial particle release, and infiltration capacity of biochar-augmented sand filters for road runoff treatment. The results would help develop design guidelines for roadside stormwater treatment systems that require compaction of filter media.

2.1. Introduction

Road infrastructures help economic growth, but road runoff is also one of the major sources of pollutants. Stormwater runoff washes off pollutants deposited on impervious surfaces on and around the road during dry weather and conveys them to waterbodies (Cambi et al., 2015). To minimize pollution from road runoff, roadside green infrastructures can be implemented where pollutants can be removed from road runoff as it infiltrates through roadside soil mixed with amendments. These roadside green infrastructures could also provide additional benefits including groundwater recharge, carbon sequestration, and ecological habitat restoration (Wong et al., 2000). To prevent landslides and increase soil stability, the roadside soils, however, are compacted—a practice not recommended for infiltration-based green infrastructure in order to maintain its infiltration capacity. Thus, it is critical to examine whether or how compaction may affect the performance of infiltration-based green infrastructure, so that a design guideline for roadside green infrastructure with compaction can be developed (Pereira et al., 2017; Sax et al., 2017).

Only a couple of studies have examined the effect of compaction on the performance of stormwater treatment systems (Pitt et al., 2008; Mossadeghi-Bjorklund et al., 2016), and these studies showed that compaction can reduce hydraulic conductivity of soil and lower stormwater infiltration. Amendments such as compost and biochar have been used to improve the contaminant removal capacity of stormwater biofilters (Boehm et al., 2020; Sun et al., 2020). Biochar, a carbonaceous porous medium produced by pyrolysis of wood or biomass, has been shown to exhibit higher removal capacity than any other conventional biofilter media for a wide range of pollutants, including heavy metals, organic contaminants, nutrients, and pathogens (Lau et al., 2017; Mohanty et al., 2018). High capacity of biochar has been attributed to high carbon content, porous structure with high surface area, high cation exchange capacity, and redox-active surfaces to aid transformation of attached pollutants.

Treatment capacity biochar subjected to compaction has not been evaluated, partly because compaction may not be recommended in most cases. In some cases, however, compaction of biochar-amended media may naturally occur or is required (e.g., road bank or permeable pavement). The extent to which compaction may affect their physical integrity of biochar and their ability to treat stormwater is unknown (Mohanty et al., 2018). Many studies have examined the effect of biochar addition on hydraulic and mechanical properties of soil after compaction (Ahmed et al., 2017; Liu et al., 2017; Ahmed and Raghavan, 2018; Menon et al., 2018), but these studies rarely examined how compact would affect biochar properties (e.g., size) or how compaction would affect biochar properties. For example, biochar size could change under compaction due to fragmentation of biochar particles; similarly, biochar size and quantity can influence the dissipation of energy from compaction.

The long-term performance of biochar in biofilters depends on the physiochemical integrity of biochar, which can be changed by compaction. As biochar particles have lower load-bearing capacity than other media such as woodchips (Reza et al., 2012), compaction could break biochar grains, release small suspended particles, and increase the loss of these biochar particles by erosion during stormwater infiltration. On the other hand, compaction could also constrict the flow paths and minimize the mobility of biochar particles. Thus, the net leaching potential of biochar particles during compaction could depend on two competing factors: the quantity of biochar particles created during compaction (source-limited factor) and the fraction of the particle pool mobilized during the infiltration of stormwater (transport-limited factor). Initial moisture content affects the degree of compaction of soil (Henderson et al., 2005). Increases in moisture content until a critical moisture value initially help compacting the soil because water helps slide soil particles under compaction stress; increases in moisture content beyond the critical moisture value prevent soil particles to come closer under compaction because of the presence of the excess water, an incompressible fluid, between soil particles. Thus, presence of moisture during compaction could be a critical factor in affecting the release of biochar particles after compaction. It is unclear how compaction affects the erosion potential of biochar and other types of particles such as compost in biofilters (Kumar et al., 2019). As biochar particles could contain a high concentration of adsorbed contaminants, the release of particles may lead to an increase or even exceedance of the concentration of contaminants in the effluent (Mohanty et al., 2013). Because compaction can reduce the net porosity of filter media, it may decrease the infiltration capacity of biofilter (Sileshi et al., 2016). As compaction can alter the way water moves through pore spaces, it could affect the removal of contaminants such as bacterial pathogens. To what extent compaction may affect infiltration and contaminant removal capacity of biochar-augmented biofilters is unknown.

This work aims to quantify the effect of compaction on the performance of biochar-augmented biofilters, which includes changes in hydraulic conductivity, flow-path heterogeneity, leaching of biochar particles, and removal of pathogen-indicator bacteria. We used a reductionist approach using a sand filter augmented with biochar or compost to isolate specific processes and examine the impact of compaction on specific factors. We hypothesized that the effect of compaction on the net loss of biochar particles is dependent on media type and moisture content during compaction. Furthermore, we evaluated the utility of engineering controls such as compost and initial moisture content to test their role in minimizing the detrimental effect of compaction on biochar-augmented biofilter.

2.2. Materials and Methods

2.2.1. Stormwater

To discern the effect of physical factors such as compaction on the release of biochar particles during infiltration events, the ionic strength and pH—the factors that affect particle release—were kept constant. Natural stormwater was collected twice a week in 20-L HDPE plastic carboys from Ballona Creek in Los Angeles, CA (34° 0'36" N, 118° 23'29" W), which receives dry-weather irrigation runoff from a 318 km² urban area with 82% developed and 61% impervious surface (Gold et al., 2015). Stormwater was characterized for pH (8.0±0.5) and conductivity (900±100 μS cm^{-1}) within 1 h of sample collection. Synthetic stormwater was prepared by adding 10 mM NaCl to DI water so that the electrical conductivity (900 ± 50 μS cm^{-1}), as an indicator of ionic strength or total dissolved solid, is similar to that of the natural stormwater from Los Angeles. pH was adjusted to 7.8±0.2 by adding a small volume of concentrated HCl or NaOH. pH and electrical conductivity are key water quality parameters that could affect release of particles from filter media. Unlike synthetic stormwater, the natural stormwater may contain other pollutants and constituents (McPherson et al., 2002; Brown et al., 2013).

A kanamycin-resistant *Escherichia coli* strain (NCM 4236) was used to distinguish the applied *E. coli* from other *E. coli* strains present naturally in stormwater. *E. coli* were suspended in stormwater following a method outlined elsewhere (Mohanty et al., 2014). Briefly, *E. coli* were cultured to a stationary phase, centrifuged and washed with phosphate buffer solution to remove the growth medium, and suspended in the collected stormwater to achieve an initial concentration of nearly 10^5 colony forming units (CFU) mL^{-1}.

2.2.2. Biofilter media

Commercially available biochar (Black Owl Biochar™, Biochar Supreme, WA), sand (20-30 Ottawa Sand, Certified Material Testing Products, FL), and garden compost (Whitter

Fertilizer, CA) were used in this study. The biochar, produced by gasification of a softwood at high temperature (900-1000 ^{0}C), exhibits high BET surface area (800 m^2 g^{-1}) and low ash content (5.8%) (**Table 2.1**). Typically pyrolysis of biomass can alter its physical properties and decreases its mechanical strength (Reza et al., 2012). Thus, it is expected biochar would be more susceptible to breakage under compaction than the biomass used for biochar production. Sand was used because it is commonly applied to increase stormwater infiltration, whereas compost was used to increase pollutant removal with added benefit of their ability to alleviate the effect of compaction (Sax et al. 2017). To remove silica colloids from sand, sand was washed in deionized (DI) water and dried in an oven. Biochar and compost were sieved to remove particles smaller than 833 μm—the mean size of sand particles— to ensure that any fine particles released in the effluent were generated during the packing of media under compaction in the columns. Particles larger than 2 mm were also removed to minimize preferential flow.

Table 2-1. Physical and chemical properties of the Black Owl Biochar™ provided by the company

Black Owl Biochar™		
Property	**Value**	**Unit**
Water Holding Capacity	874	g H_2O/100
Surface area	800	$m^2\ g^{-1}$
Organic Carbon	80.1	%
Inorganic Carbon	0.39	%
H-$C_{organic}$ (molar Ratio)	0.56	%
Hydrogen	3.7	%
Nitrogen	0.4	%
Oxygen	9.6	%
Ash	5.8	%
Calcium	1.28	%
Magnesium	0.1	%
Sulfur	0.01	%
Iron	0.05	%
Manganese	0.03	%
Ammonia NH_4-N Mineral	6.7	mg kg^{-1}
Nitrate NO_3-N	6.3	mg kg^{-1}
Phosphorous Total	0.086	mg kg^{-1}
Phosphorous Available	14	mg kg^{-1}
K-Potassium Total & Available	1.17	%
Bulk Density	4.94	lb ft^{-3}

The typical biofilter media may contain 10-30% amendment to increase pollutant removal. We mixed sand (85% by volume) with biochar or compost (15% by volume) in a sterile 4-L bucket, to compare the effect of compaction on biochar and compost—the widely used soil amendment to alleviate compaction effect. The media was mixed manually for 5 minutes to ensure a uniform mixture. We mixed same volume of compost and biochar (15%

of each) with sand (70 % by volume) to examine whether addition of biochar would affect the treatment capacity of compost-biofilter after compaction. We compacted biochar-sand mixture without and with water (15% by weight) to examine the presence of moisture during compaction affects the treatment capacity of compacted biochar. We chose 15% moisture content because it is the optimum moisture content for maximum dry density after compaction of poorly grade sand (Deb, K. 2010), although the critical moisture content for biochar-sand mixture could be different. The mixtures were immediately used to pack the columns after preparation. It should be noted that we did not include sand-only column because, unlike biochar or compost or soil, sand is not affected by compaction. Physical properties of compacted mixtures are described in **Table 2.2**. Additionally, the particle size distributions of the mixtures were determined using a laser diffraction particle size analyzer (model LS 13 320, Beckman Coulter, CA).

We used a reductionist approach to eliminate the effect of confounding factors and isolate the effect of specific variables on particle leaching after compaction or removal of *E. coli*. While the reductionist approach may not simulate all conditions in natural biofilters, it is helpful to improve the understanding of selected processes or mechanisms such as mobilization of particles and bacterial pollutants in stormwater biofilters (Mohanty et al., 2013).

Table 2-2. Hydraulic characteristics and design parameters of laboratorial biofilters. Each biofilter type was constructed in triplicated (n = 15). Mean and standard deviation over mean for each parameter was estimated from measurements in triplicate columns

Biofilter type	Sand (%)	Compost (%)	Biochar (%)	Moisture content (w/w) (%)	Compaction	Bulk density (g cm^{-3})	Pore volume of filter layer (mL)	Pore volume including drainage layer (mL)	Porosity (%)
Uncompacted biochar (dry)	85	0	15	0	No	1384.4 ± 39.8	205.23 ± 6.2	284.3 ± 1.5	33.2 ± 1.0
Compacted biochar (dry)	85	0	15	0	Yes	1579.2 ± 42.3	192.27 ± 2.2	253.5 ± 5.1	31.1 ± 0.4
Compacted biochar (wet)	85	0	15	15	Yes	1384.3 ± 72.5	232.07 ± 9.3	305.7 ± 10.9	37.6 ± 1.5
Compacted compost and biochar (dry)	70	15	15	0	Yes	1547.8 ± 79.9	187.70 ± 11.6	266.4 ± 5.0	30.4 ± 1.9
Compacted compost (dry)	85	15	0	0	Yes	1377.3 ± 50.1	204.75 ± 8.6	279.0 ± 9.3	33.2 ± 1.4

2.2.3. Packing of filter media in columns

To design a laboratory column setup, a transparent PVC tube (5.1 cm ID and 61.0 cm length) was glued with PVC fittings and connected to a control valve to regulate the effluent flow. A total of 15 columns were packed with the following media mixture with sand under different compaction conditions: uncompacted biochar, compacted biochar under dry condition, compacted biochar under wet condition, compacted compost, compacted biochar, and compost. Each mixture was packed in triplicate columns (**Figure 2.1**).

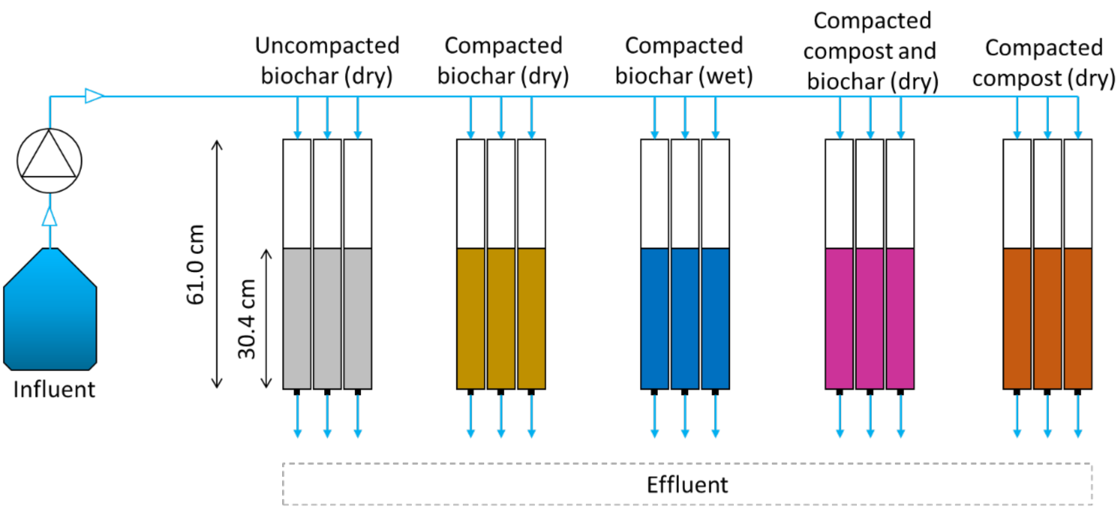


Figure 2-1.Schematic of 5 different types of biofilters.

Before packing the filter media in each column, a 6-cm drainage layer was created in the bottom of the column using nylon membranes (100 µm pores) and pea gravels. The media mixture was added incrementally and compacted to a height of a 3.8-cm layer using a standard Proctor hammer (2.5 kg). To ensure that comparable energy of a standard Proctor test was applied on the filter media, the hammer was dropped 7 times from 30.4 cm height per layer. The procedure was repeated for 8 layers so that the total height of the filter layer became 30.4 cm. The bulk density was estimated after compaction of each layer to ensure uniform distribution of filter media by depth (**Figure 2.2**). A 2.5-cm layer of pea gravels was added on the top of filter media to prevent biochar or compost particles to float in the event of ponding. The total pore volume (PV) of the filter layer and the porosity of each column are summarized in **Table 1**. More information on packing methodology and determination of pore volume and porosity can be found in Supplementary Material.

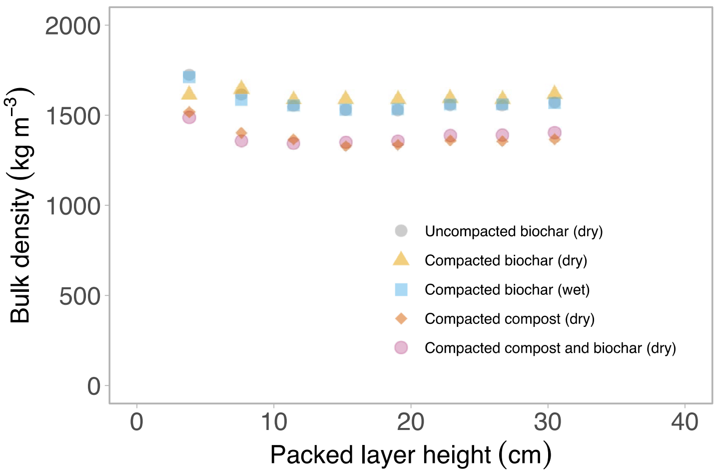


Figure 2-2. Bulk density of different layer of media during packing.

Experiments were conducted using the same columns in the following order to determine the effects of compaction on (1) initial release of particles, (2) hydraulic conductivity of packed media, (3) flow-path connectivity via tracer study, and (4) *E. coli* removal capacity of the columns. The particle release experiment was conducted at the beginning in order to examine the immediate effect of compaction on particle release. The next two experiments helped determine the change in hydraulic properties of filter media following compaction. The *E. coli* experiment was conducted at the end to correlate any change in removal capacity to changes in hydraulic properties of columns after compaction. Between each experiment, the columns were drained by gravity and kept it for 2-3 days.

2.2.4. Release of particles during the infiltration of stormwater

To examine the release of biochar particles during application of stormwater, synthetic stormwater (10 mM NaCl) was used so that the influent water would be particulate free. Synthetic stormwater was applied on the top of the filter media at a flow rate of 5 mL min^{-1} or flux of 14.8 cm h^{-1} for 2 pore volume, following which the filter media was drained by gravity. We expect that increases in flow rate can increase particle release (Shang et al., 2008) and decrease bacterial removal (Mohanty and Boehm, 2014b). We used a fixed velocity to rule out the effect of flow fluctuation on the results. Effluents were collected in 250 mL amber bottles at 0.5 PV fractions and analyzed for particle concentration and volume. The experiment was repeated for five consecutive days beyond which particle release did not change with successive infiltration events. Particle concentration in the effluent samples was estimated by measuring the absorbance at 890 nm—the wavelength used to determine turbidity—using a UV-Vis Spectrophotometer (Lambda 365, PerkinElmer) and correlating the absorbance with concentration using a calibration curve. The particle size distribution of the effluent was measured using a single-particle optical sensor instrument (AccuSizer 780, Entegris Company). More information on sample measurement can be found in Supplementary Material.

2.2.5. Measurement of hydraulic conductivity

Hydraulic conductivity (*K*) of each column was measured using a falling head method using the equation: $K = \frac{L}{t} ln\left(\frac{h_0}{h_t}\right)$, where *L* is the depth of filter media, *t* is the time to drain water from initial height h_0 to final height h_t after drainage of a specific volume of water. Briefly, 2 PV of synthetic stormwater (without particulates) was applied on the top of packed media to permit free drainage of stormwater at the bottom valve. The effective hydraulic conductivity of biofilters was estimated by recording the time required to drain a specific volume of stormwater. The procedure was repeated 6 times to estimate the average hydraulic conductivity of each column.

2.2.6. Measurement of effective pore volume and flow path heterogeneity

We measured effective pore volume, the fraction of total pore space filled with stormwater during an infiltration event, by estimating the volume of stormwater injected to achieve 50% of the concentration of bromide (a conservative tracer) in the feed solution (Ptak et al., 2004). Briefly, synthetic stormwater containing bromide (9 mM NaCl + 1 mM KBr) was applied on the top of each column at 5 mL min^{-1} for 2 h before applying 10 mM NaCl for additional 2 h to flush bromide from pore water. The flow was interrupted for 12 h, followed by injection of 10 mM NaCl for additional 0.7 h to compare the extent of diffusion-limited regions in different media mixture (Brusseau et al., 1997). We used the ratio of bromide concentration before and after flow interruption as an indicator of the presence of diffusion-limited zones in the biochar-augmented sand filters, as compaction is expected to create low-permeable zones or flow-stagnant zones, where diffusion would be significant for the transport of solute in between rainfall events.

2.2.7. *E. coli* removal

Stormwater contains a wide range of pollutants. However, we chose *E. coli* to test removal of particulate pollutants as the removal process based on physical filtration is most likely to be affected by change in pore structure under compaction. Furthermore, presence of pathogen or indicator bacteria is the lead cause of water quality violation in Los Angeles area. Pathogens can be removed in biofilters by a combination of processes including physico-chemical filtration, inactivation, gazing, and natural die-off via starvation or drying (Rippy, 2015). We hypothesize that compaction could increase bacterial removal via physico-chemical filtration by decreasing pore size. To investigate the effect of compaction on removal of *E. coli*, 5 PV of natural stormwater spiked with *E. coli* (concentration ~ 10^5 CFU mL^{-1}) was applied at 5 mL min^{-1} on the top of each column and samples were collected at the bottom. The concentration of *E. coli* was measured in the last 0.5 PV fraction of the samples, which serves as an indicator for the steady-state removal capacity of the filter. We did not measure *E. coli* concentration during first-flush because the previous study with biochar (Mohanty et al., 2014) showed that the concentration of *E. coli* in the first flush (old water in the column) were lower than that of pore water collected in the last part of infiltration events, due to inactivation or increased adsorption of *E. coli* trapped in pore water during period between rainfall events. *E. coli* concentration in stormwater was quantified by a spread plate technique and reported as colony-forming units (CFU) per mL of effluent. The experiment was repeated 3 times with 2 days interval to estimate a change in the bacterial removal capacity of biofilters with an increase in exposure to contaminated stormwater.

2.2.8. Data and statistical analysis

The cumulative amount of biochar particles released during a rainfall event from a column was estimated based on the following equation: $m_{BC} = \sum_i C_i V_i$, where C_i and V_i are the concentration of biochar particles and volume of a sample fraction and *i* is the total number of sample fractions collected during a rainfall event. The log-removal of *E. coli* from stormwater during the attachment phase was calculated using the equation: log removal or removal capacity = $-\log(C/C_0)$, where *C* is the steady-state effluent concentration and C_0 is the influent concentration.

Statistical tests (one-way analysis of variance, ANOVA) were conducted to identify the differences between parameters measured under different experimental conditions. The significance of differences between two specific means was assessed with Tukey HSD post-hoc comparison test. Differences were considered significant at *p*-value less than 0.05. All statistical analyses were performed using the computing environment R, version 3.6.1.

2.3. Results

2.3.1. Compaction conditions affected hydraulic conductivity

Compaction decreased the hydraulic conductivity of biofilter, but the extent to which the hydraulic conductivity decreased depended on media type and the presence of water (**Figure 2.3**). All compacted columns had significantly ($p < 0.005$) lower hydraulic conductivity than uncompacted biochar columns. Compaction of biochar under dry condition lowered the hydraulic conductivity by 44%. Compaction of biochar in wet condition (15% moisture) decreased the hydraulic conductivity by only 12% of the uncompacted biochar. Compaction decreased the hydraulic conductivity of biochar columns to a greater extent than compost columns. An addition of biochar to compost columns did not significantly ($p = 0.99$) changed their hydraulic conductivity.

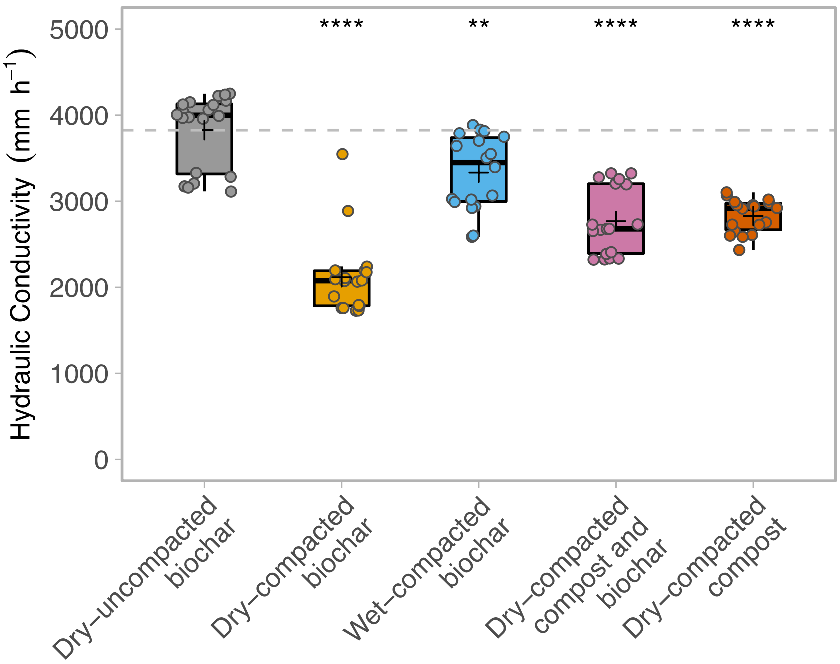


Figure 2-3. Effect of compaction on saturated hydraulic conductivity of biofilter packed with a mixture of sand and adsorbent (15% by volume) including biochar and compost. The vertical dashed line indicates the mean hydraulic conductivity of uncompacted biochar columns. Plus (+) signs indicate mean hydraulic conductivity of total 18 measurements between triplicate columns of each type. Hydraulic conductivities of compacted columns were significantly lower than that of the uncompacted columns. A p-value of 0.01 and 0 correspond to notation "**" and "****", respectively.

2.3.2. Compaction increased water-holding capacity and macropore-matrix interaction

Effective pore volume, estimated from bromide breakthrough curves in unsaturated biofilters, provides a quantitative estimate of the fraction of total biofilter media exposed to contaminated stormwater during infiltration events (**Figure 2.4-A**). The effective pore volume of uncompacted biochar and wet-compacted biochar were similar, which was significantly ($p < 0.05$) lower than that of dry-compacted biochar columns (**Figure 2.4-B**). It should be noted that the effective pore volume estimated from bromide breakthrough curves includes the volume of water in the drainage layer. Flow interruption during breakthrough tail increased the bromide concentration by a factor of 3 to 15 depending on the compaction conditions or media type (**Figure 2.4-B**). The increase in bromide concentration was more apparent in compacted columns than the uncompacted columns.

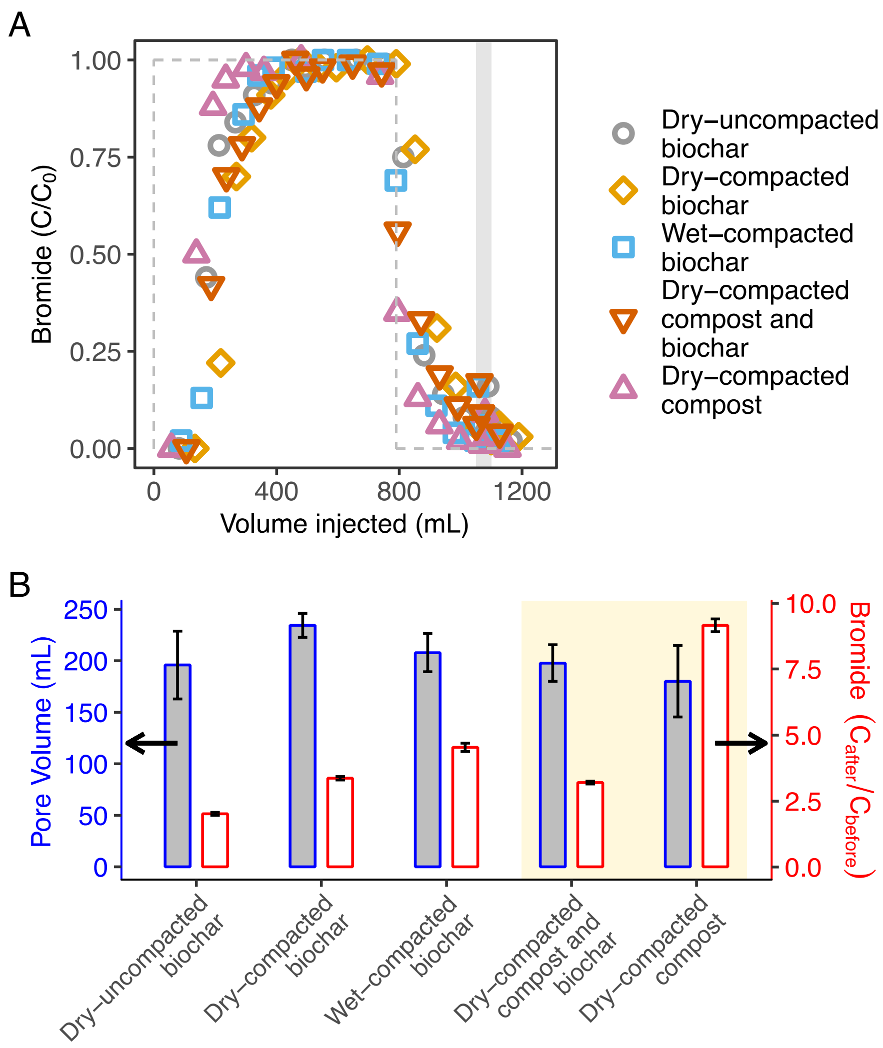


Figure 2-4. (A) Breakthrough curves of bromide through columns packed with uncompacted biochar, compacted biochar under dry condition, compacted biochar under wet condition, compacted compost, and compacted compost and biochar mixture. The arrow mark indicates flow interruption for 12 h. (B) Effective pore volume, which is estimated based on the volume of stormwater injected to achieve 50% of bromide breakthrough ($C/C_0 = 0.5$) and increase in bromide concentration as a result of flow interruption, an indicator of diffusion dominated region in all columns. Error bars indicate one standard deviation over mean between triplicate columns.

2.3.3. Compaction conditions affected the quantity of particles released

During the infiltration of stormwater after compaction, particles were released in the effluent, but the concentration and total quantity of released particles depended on the media type and compaction conditions (**Figure 2.5**). One of the concerns of compacting biochar is that biochar may break into smaller particles, which can be flushed out of the biofilter, thereby decreasing the concentration of biochar remained in the biofilter for intended application. However, we found that the total amount of particles released from each column were insignificant compared to the total amount of amendment (biochar or compost) added in the column. During each infiltration event, particle concentration peaked initially, but the particle concentration rapidly decreased with the passage of more stormwater. The peak in particle concentration decreased with successive rainfall events. The cumulative mass of particles released varied with column types. Among columns without compost, dry-compacted biochar columns released the least amount of biochar particles. The particle release potential in biochar columns increased with the following design conditions: dry compaction < no compaction < wet compaction. After dry compaction, compacted compost released more particles than compacted biochar.

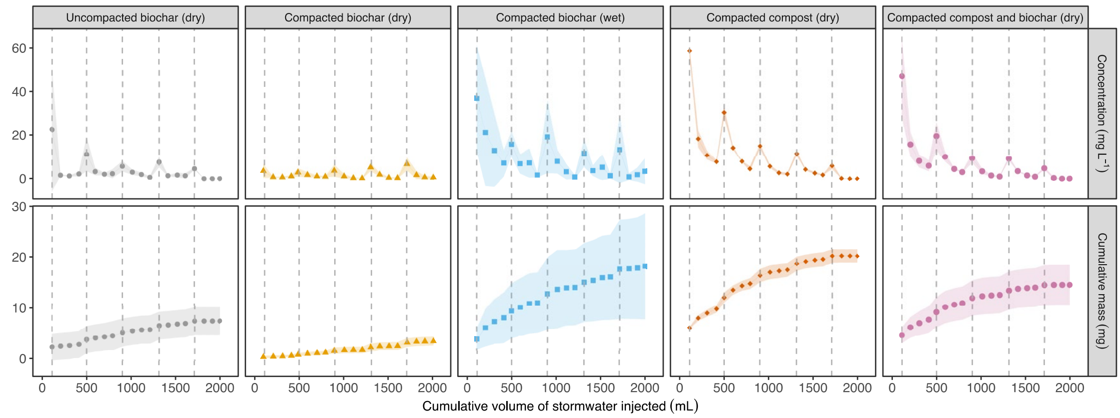


Figure 2-5. Concentration (top) and cumulative mass (bottom) of particles released from biofilters packed with biochar or compost under different compaction conditions. Dashed lines indicate the start of rainfall infiltration events. Shaded area indicates one standard deviation over mean value from triplicate columns.

Millions of particles (> 99% are smaller than 3 μm) were present in the effluent, and the number of particles was higher in the columns with compost than the columns with biochar (**Figure 2.6-A**). The size distribution of particles in the effluent was slightly different based on the compaction conditions and types of filter media used (**Figure 2.6-B**). The particle size corresponding to peak distribution was smaller for biochar columns than for compost columns. The compaction conditions did not significantly ($p = 0.13$) affect the concentration of biochar particles released, but it affected the particle size distribution: the wet compacted biochar columns released a greater number of large particles than dry compacted columns. Columns with biochar released significantly fewer particles than the column with compost, indicating biochar is less susceptible to physical loss than compost under compaction. An addition of 15 % (by volume) of biochar to compost columns significantly ($p < 0.05$) decreased the number of particles released and shifted the peak of the particle size distribution towards a smaller particle size, indicating that biochar can lower the loss of compost particles and block large particles. A decrease in particle released by replacing 15% of sand with biochar in compost-sand column is significant because sand has high load-bearing capacity than biochar and thus is expected to release less particles under compaction than biochar.

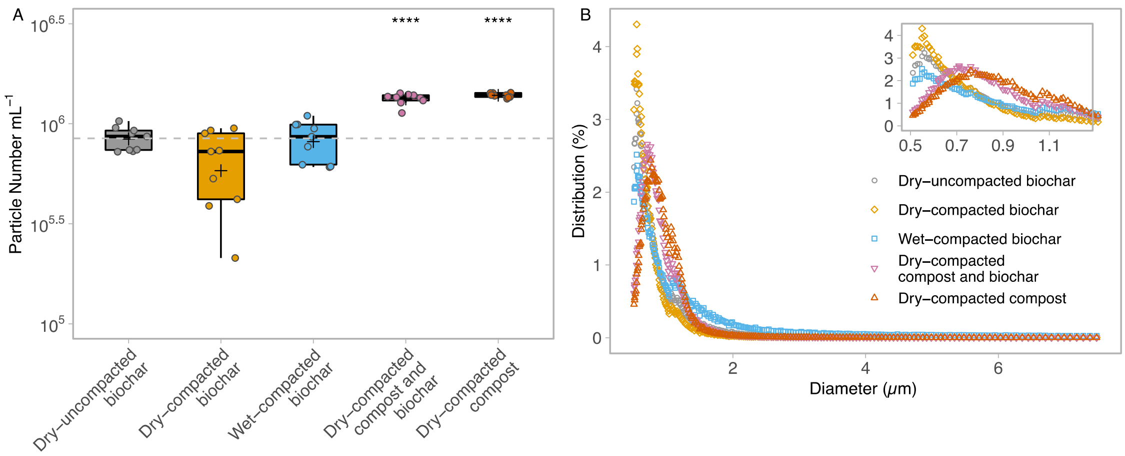


Figure 2-6. (A) Concentration of particles (number per mL) in the first sample during the infiltration event, and (B) the size distribution of the particles. The insert shows the peak in particle size distribution curves.

2.3.4. Compaction conditions affected *E. coli* removal

Compaction increased *E. coli* removal in biochar columns but the increase in removal depended on compaction conditions and the volume of contaminated stormwater injected (**Figure 2.7**). During the first infiltration event, compacted biochar columns removed more *E. coli* than uncompacted biochar columns, and wet-compacted biochar columns removed more *E. coli* than dry-compacted biochar columns. However, increases in the exposure of contaminated water in the successive rainfall events reduced the *E. coli* removal capacity of all columns. After the exposure to 20 pore volumes of contaminated stormwater, the *E. coli* removal capacity of wet-compacted biochar columns (95 ± 02 %) remained significantly ($p < 0.05$) greater than the removal capacity of dry-compacted (75 ± 06 %) or uncompacted biochar columns (70 ± 10 %), indicating wet compaction favors *E. coli* removal. The *E. coli* removal by dry-compacted compost columns was similar to the removal by dry-compacted biochar columns, and the addition of biochar to compost did not improve bacterial removal under the tested conditions. It should be noted that sand has significantly lower removal capacity than biochar (Mohanty and Boehm, 2014b; Mohanty et al., 2014); thus, amount of sand is not expected to influence the overall capacity of biochar-augmented columns.

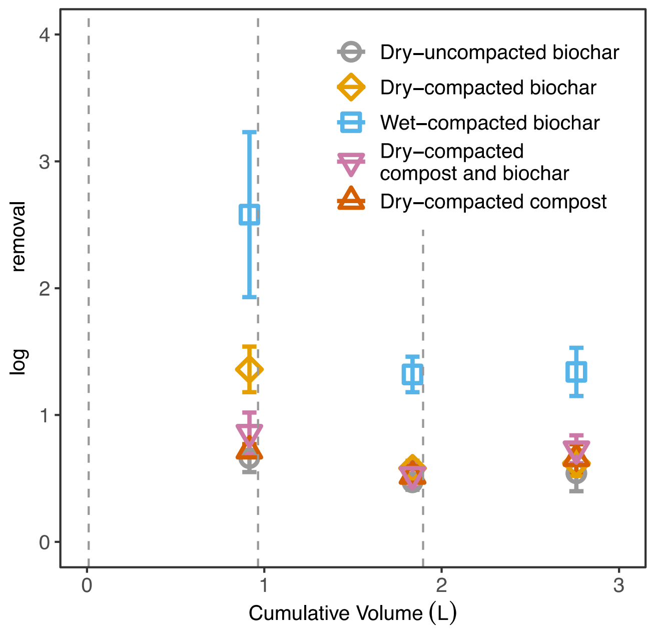


Figure 2-7. Average *E. coli* removal by columns packed with a mixture of biochar or compost under different compaction conditions. Dashed lines indicate the start of a rainfall event, where more than 5 pore volume of contaminated stormwater was injected through the columns. Error bars indicate one standard deviation over mean log *E. coli* removal by triplicate columns of each type.

2.4. Discussion

2.4.1. Cause of decrease in hydraulic conductivity of biochar after compaction

A decrease in saturated hydraulic conductivity of biofilters after compaction confirmed that compaction reduced the flow path permeability. The negative effect of compaction on hydraulic conductivity of biochar and sand mixture was more severe when the media was packed dry. This result confirmed that the presence of water in the filter media during compaction could reduce the negative impact of compaction on hydraulic conductivity. As water is an incompressible fluid, the presence of water in pores prevented compression of pore space. It should be noted that water content (15% by weight) used in this study can be nearly the field capacity of the mixture. At lower water contents, the presence of water could decrease friction between soil grains and increase compaction (Rollins et al., 1998). Thus, optimum moisture content for compaction could vary with filter media types and particle size distribution of filter media (Henderson et al., 2005). Future studies should determine the optimum water content for compaction of biochar-augmented soil or filter media. Compaction is expected to reduce the pore space between soil grains and decrease the flow path permeability (Gregory et al., 2006), which in turn could affect transport and removal of contaminants through biofilters (Hatt et al., 2008). Our results showed the extent to which flow paths could change after compaction of biofilter media.

Comparing the hydraulic conductivity of columns containing compacted biochar and compost, we showed that the hydraulic conductivity of biochar decreased to a greater extent than that of compost after compaction. We attribute this to low mechanical strength of biochar compared with compost. Biochar is produced by pyrolysis of biomass, which alters its physical properties and decreases its mechanical strength (Reza et al., 2012). Under stress, biochar could break and produce fine particles, which can further clog the constricted flow paths under compaction. Thus, biochar is less effective compared to compost in mitigating the impact of compaction on hydraulic conductivity. However, biochar offers a better contaminant removal capacity than compost. Thus, when high hydraulic conductivity of biofilter is desired, biochar should be mixed with compost to withstand the impact of compaction and to maintain both high infiltration and contaminant removal capacity.

2.4.2. Compaction increased stormwater interaction with filter media

Although biofilters are designed to quickly infiltrate stormwater, a high infiltration rate provides limited opportunity for contaminants to interact with the filter media, which consequently lowers the overall contaminant removal capacity of biofilters (Mohanty and Boehm, 2014a). Thus, conditions that could increase the interaction time without decreasing the infiltration capacity of biofilters could serve both purposes: reducing the quantity of runoff over land and contaminants in the effluent. Based on the bromide breakthrough time and exchange of bromide between macropores and micropores during flow interruption, we showed that compaction could increase the interaction of stormwater with filter media.

Effective pore volume, a measure of the fraction of filter media exposed to stormwater during an infiltration event, was slightly higher in compacted biochar columns than that of uncompacted biochar columns.

Based on bromide concentration change after flow interruption, we showed that compaction increased the solute interaction between diffusion-limited regions and the flow paths. Diffusion-limited regions are comprised of intraparticle pores, micropores, capillary gaps between grains, and water film on the surface of biochar particles (Kumari et al., 2014). These regions are critical in the removal of contaminants in stormwater biofilters (Ulrich et al., 2015). Our study showed that compaction can disintegrate biochar and produce small particles, which could occupy pores between sands. This process could increase diffusion-limited regions. A decrease in hydraulic conductivity after compaction further increased the water-holding capacity, similar to how it was observed in another study that used natural soil (Peake et al., 2014). Thus, compaction increased the retention time of water in biofilters, which could help improve contaminant removal from stormwater. Compaction of soils could increase infiltration through preferential flow paths (Etana et al., 2013). Sands were used in this study, which cannot be compacted to the extent that it would block water entirely. Because sand has higher hydraulic conductivity than the rainfall application rate, stormwater is expected to move through preferential flow paths in unsaturated columns. Adding biochar and compaction could minimize the preferential flow paths by blocking the large pores.

2.4.3. Mechanism of particle release after compaction

Filter media serves a critical role in removing the contaminants from stormwater. However, intermittent infiltration of stormwater can release particles from filter media (Mohanty and Boehm, 2015), which has consequences on the loss of filter media and particle-facilitated leaching of contaminants in biofilter (Mohanty et al., 2013). Our results revealed that compaction could affect the quantity of particles available for mobilization during infiltration of stormwater, although pools of particles available depletes with successive infiltration events due to exhaustion of particles generated during compaction. Fewer amount of biochar particles were released from dry-compacted columns than the wet-compacted columns. Based on the results, biochar release after compaction can be explained by a three-step process: (1) generation of particle by compaction, (2) diffusion of particles from grain wall to bulk liquid in flow paths, (3) transport in flow paths by advection and removal of large particles by filtration (**Figure 2.8**).

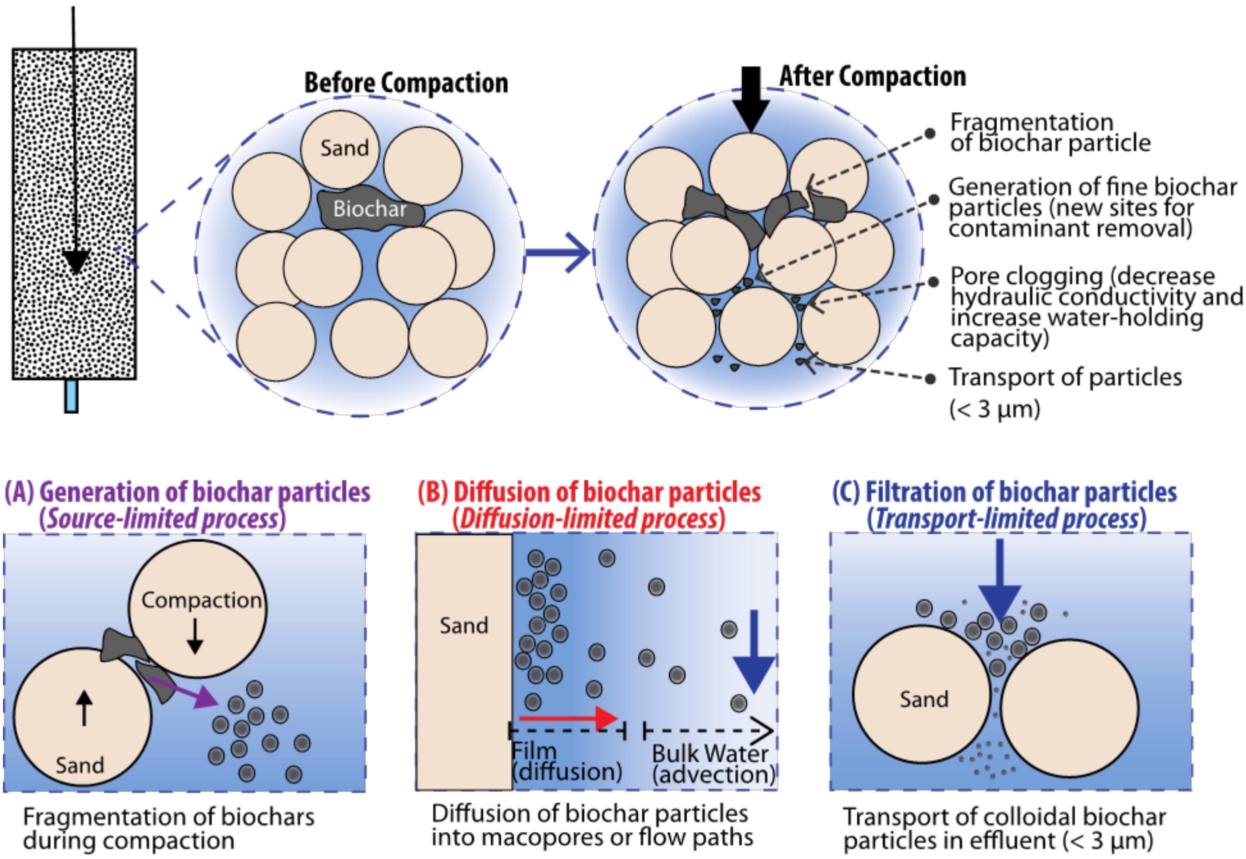


Figure 2-8. Suggested mechanisms of biochar particle release due to compaction. First, compaction causes disintegration of biochar particles, which generates fine biochar particles and decreases average biochar particle size in sand filter. Second, fine biochar particles deposited on grain surfaces diffuse from the film surrounding particles to bulk water in flow paths, which limits the amount of biochar particles available for transport during an infiltration event. Third, only a fraction of total available biochar particles based on size (< 3 μm) could transport as the pore path width, which may become constricted

during compaction, can limit the transport or leaching of biochar particles during infiltration events.

First, compaction can generate a high concentration of biochar particles due to the disintegration of biochar under pressure exerted during compaction. Increases in the percentage of particles smaller than 100 μm after compaction confirmed that compaction disintegrated biochar particles and produce smaller particles and increased the pool of particles in the biofilter (**Figure 2.9**). Thus, the source is not a limiting factor for the release of particles. Second, during infiltration events, the suspended particles in pore paths were quickly eluted, which contributes to the initial peak. A rapid decrease in particle concentration indicates that a limited supply of particles to pore paths during infiltration. A linear relationship between cumulative particle released during a rainfall event as a function of the square root of time (Jacobsen et al., 1997) suggested that diffusion is a limiting step in the mobilization of biochar particles from grain walls to bulk liquid in flow paths (**Figure 2.10**). A difference in leaching of particles in dry- and wet-compacted biochar columns indicates that the presence of water during compaction could help mobilization and transport of biochar particles. As the extent of diffusive transport would increase with the increase in water-filled pores, more biochar particles were mobilized in wet-compacted biochar, thereby increasing the availability of biochar particles for transport in flow paths. Third, the filtration of large biochar particles in narrow flow paths, which may be constricted during compaction, can limit the leaching of biochar particles.

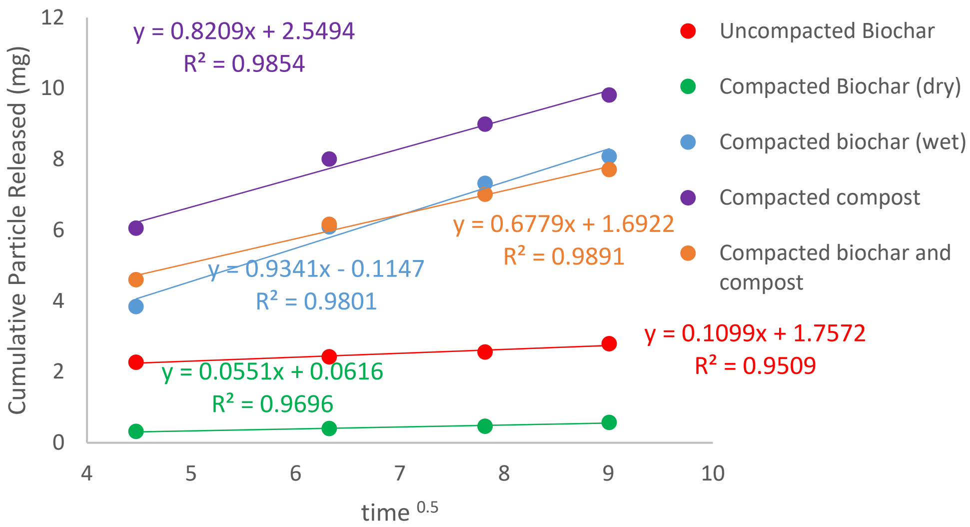


Figure 2-9. A decrease in the mean particle size of biochar in biofilter after compaction.

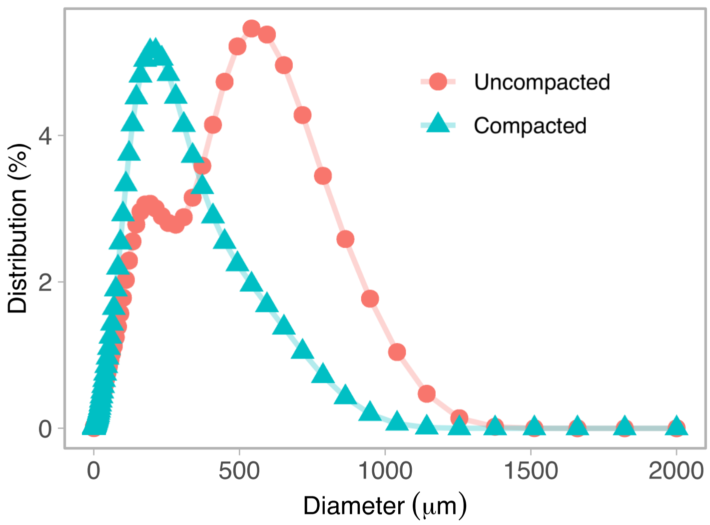


Figure 2-10. A linear relationship between cumulative particle released during a rainfall event as a function of square root of time suggested that diffusion is a limiting step in mobilization of particle.

We used hydraulic conductivity is a proxy measurement for flow path permeability. Higher hydraulic conductivity of wet-compacted biochar columns than that of dry-compacted biochar columns suggests that wet compacted biochar columns were more conducive to transport biochar particles. Consequently, a decrease in flow-path permeability in dry-compacted biochar columns resulted in a decrease in particle leaching. For the same reason, columns with compost leached more particles than biochar-only columns. These results confirmed that particle release processes after compaction is dominated by a transport-limited process, not a source-limited condition, and the transport of particles was dependent on the diffusion of particles from grain walls to bulk water in flow paths and physical filtration of particles when flow path width is smaller than particle size. As filter media containing wet-compacted biochar maintained a higher infiltration and *E. coli* removal capacity than the biofilter containing dry-compacted biochar, water should be added to biochar during compaction so that it maintains high hydraulic conductivity. The addition of water would also minimize the dust emission (Ravi et al., 2016) during installation. Biochar can also be mixed with compost to increase the hydraulic conductivity of the mixture.

2.4.4. Compaction conditions could affect *E. coli* removal

Our results showed that compaction of biochar improved *E. coli* removal during the first injection (clean bed), but repeated exposure of contaminated stormwater decreased removal of *E. coli*. We attributed the initial high removal in columns with compacted biochar compared to uncompacted biochar to fragmentation of biochar particles during compaction that expose additional surface areas for bacterial attachment and to increase in straining or physical filtration (Sasidharan et al., 2016) by pore clogged with fragmented biochar particles. Furthermore, compaction altered pore paths and decreased the pore space, which in turns increased the interaction *E. coli* in infiltrating stormwater with biochar, resulting increased removal. In our study, bacterial removal decreased with increases in the exposure of contaminated stormwater, which indicates that the attachment sites on particles generated after compaction were exhausted progressively. This is similar to observation in another field study with biochar, where the removal capacity decreased after initial high removal (Kranner et al., 2019).

Removal in wet-compacted biochar was more than that in dry-compacted biochar, which indicates that *E. coli* interaction with biochar was greater in the wet-compacted column than the dry-compacted columns. Based on bromide breakthrough data, pore-water connectivity was higher in wet-compacted biochar columns compared with dry-compacted biochar columns, indicating a greater fraction of wet-compacted biochar columns was in contact with *E. coli* in infiltrating stormwater. Overall, these results indicate that compacting biochar in the presence of water may increase *E. coli* removal in biofilters. It should be noted that road runoff typically contains high concentration of dissolved pollutants such as heavy metals and organic pollutants. Our result show that compaction can affect pore-water interaction with filter media and water retention in biofilter, which could change the geochemical and redox properties of pore water. As geochemical and redox properties are critical in removal of dissolved pollutants, future studies should examine the mechanistic link between compaction and removal of dissolved pollutant in roadside biofilters.

2.5. Conclusions

We examine the effect of compaction on physical processes of stormwater treatment including stormwater infiltration, particle release, and removal of particulate pollutants such as *E. coli*, and provide a mechanistic understanding of how compaction condition such as presence of moisture could affect the performance of roadside soil augmented with biochar. The results can inform the design guideline for the construction of roadside biofilters with biochar as an amendment. Specific conclusions are:

- Compaction decreased hydraulic conductivity of biochar-augmented biofilter, but the detrimental effect of compaction can be minimized by adding compost and water.
- Compaction disintegrated biochar particles, which can be released during intermittent infiltration of stormwater. Although compactions generated fine biochar particles, the net release of these particles was controlled by the flow-path permeability after compaction.
- Irrespective of the initial release of biochar particles after compaction, the total biochar amount released is insignificant compared to the total biochar remained in the system, and the extent of biochar release was less than that observed in compost columns.
- Compaction increased water holding capacity and interaction time of stormwater with filter media.
- Compaction increased *E. coli* removal in a short term but the benefit was short-lived in dry-compacted biofilters augmented with biochar. Wet-compacted biofilter consistently removes more *E. coli* than dry-compacted or uncompacted biofilter with biochar.

2.6. References

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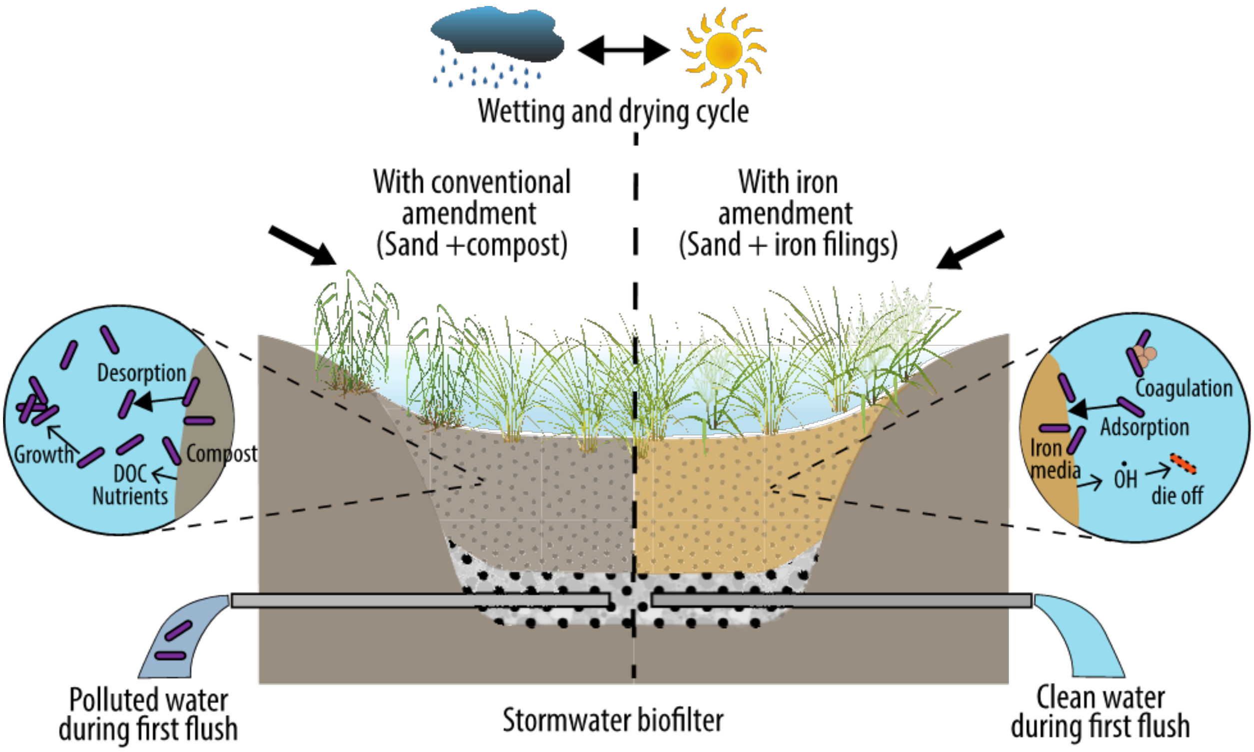
3. Iron amendments minimize the first-flush release of pathogens from stormwater biofilters

<u>Graphical abstract</u>



Journal Article:

Ghavanloughajar, M., Borthakur, A., Valenca, R., McAdam, M., Khor, C.M., Dittrich, T.M., Stenstrom M.K., and Mohanty, S.K. Iron amendments minimize the first-flush release of pathogens from stormwater biofilter. *Environmental Pollution.* 2021. https://doi.org/10.1016/j.envpol.2021.116989

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, 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 was tested. *E. coli*-contaminated stormwater was applied 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*. This result was attribute 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.

3.1. Introduction

Pathogens and their indicator bacteria in stormwater, particularly in urban areas, are one of the leading causes of surface water impairments (EPA 2017). To manage non-point 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 growth of sequestered bacteria (Mohanty et al. 2014b). 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. 2014b). 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 (Ulrich et al. 2017a, Kranner et al. 2019). 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 and other nutrients in stormwater could help previously sequestered pathogens grow during the drying period between rainfall events (Mohanty et al. 2014b). Thus, an amendment 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, iron-based amendments such as iron filings and water treatment residues have been tested in laboratory and field studies (Tian et al. 2019, Trenouth et al. 2015, Erickson et al. 2012, Weiss et al. 2016, Reddy et al. 2014, Rangsivek et al. 2005). Iron amendments are typically derived from waste materials, 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 (Xu et al. 2019, Ingram et al. 2012, Kim et al. 2020). 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 *Escherichia coli* (*E. coli*) removal by scrap irons or iron-coated sand based on breakthrough curve (Kim et al. 2020, Ingram et al. 2012, George et al. 2019, 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 dissolved organic carbon (Brisson et al.), 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. It was hypothesized 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 this hypotheses, exposed stormwater contaminated with *Escherichia coli (E. coli),* a pathogen indicator, was exposed to iron filings in batch reactors and packed columns with and without compost and the fate of *E. coli* during and after the rainfall events was measured.

3.2. Materials and Methods

3.2.1. 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±0.2 using 1 M HCl.

Gram-negative bacterium *Escherichia coli* (ATCC 10798) with resistance to antibiotic 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 potentially high *E. coli* removal by iron columns was detected using the agar-plate method.

3.2.2. 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 as iron biofilters, (c) a sand and compost mixture with 3% or 10% iron filings by weight, which is referred 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 amount 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).

Measurement of surface charge

To measure the change in surface charge of iron filings after the exposure to DOC from compost, the zeta potential of iron filings and E. coli with and without exposure to DOC was measured. Iron filings were sieved using a 45 μm sieve, and 2 g of sieved iron filings were mixed for 3 hours with 40 mL of 10 mM NaCl, with or without 250 mg/L of DOC leached from compost- The iron filings suspension were centrifuged (5000 rpm for 10 min), the pellets were resuspended in 50 mL 10 mM NaCl, and the pH was adjusted to 7 using HCl or NaOH. The iron suspension was dispersed using an ultrasonic probe and centrifuged at a speed based on Stoke's law to settle particles greater than 1 μm, and the supernatant was collected for zeta potential measurement.

The supernatant collected from the solution containing iron filings were divided into 4 centrifuge tubes. The pH of the centrifuge tubes were adjusted using HCl or NaOH to 4.7, 4.9, 6 and 6.6 (natural pH of solution). Similarly, the supernatant from the solution containing iron filings with 250 mg/L DOC were divided into 4 centrifuge tubes and the pH of the samples were adjusted to 4.1, 4.6, 5.8 and 7.1 (natural pH of solution).The zeta potential of each sample were analysed using a ZetaPALS instrument (Zetapals, Brookhaven Instruments, NY).

Fourier transform infrared spectroscopy measurment

To investigate the effect of DOC exposure on the surface properties of the iron filings, fourier transform infrared spectroscopy (FTIR) spectra of the iron filings (size < 75 μm) and iron filings exposed to dissolved organic carbon (DOC) were obtained using JASCO Model 420 FTIR instrument.

Compost was used to prepare the DOC solution to which iron filings were exposed. 10 g compost sieved through a 2 mm sieve was added to 100 mL DI water and organic carbon was allowed to leach from the compost for 6 hours. The mixture was then centrifuged at 5000 RPM for 15 minutes and the supernatant was transferred to a separate tube. The DOC content of the supernatant was measured to be 289.9 mg L^{-1} using UV-Vis spectrophotometry. 0.5 g iron filings passing through a 75 μm sieve were added to this DOC solution and the solution was shaken at 150 RPM for 18 hours. The solution was then centrifuged at 5000 RPM for 15 minutes and the supernatant was discarded. The iron filings were then dried at 110 ^{0}C for 1 hour.

Prior to analysis, the iron filings (Iron), Iron + DOC samples and potassium bromide (KBr) were dried in vacuum for 18 hours. The samples were first mixed with KBr and the mixtures were compressed into pellets at 8000-10000 psi using a hydraulic press. Each pellet was scanned atleast five times and best spectra with minimum noise was selected for the analysis. KBr pellets were scanned to determine the background intensity. From the analysis it was observed that infrared waves of wavenumber 570 cm^{-1}, corresponding to corresponds to the Fe-O bond in iron oxides (Ishii et al. 1972, Namduri and Nasrazadani 2008), were absorbed more in in Iron + DOC samples than in Iron samples.

3.2.3. 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 hours. 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.

3.2.4. *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 hours, 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_0/C).

3.2.5. 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, the changes in concentration of *E. coli* during the drying period based on the Growth-Die off Index (GDI) were compared, which is estimated using the equation:

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

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.

3.2.6. *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 to 293 mg L^{-1}. 0.5 gram 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 hours 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.

3.3. Results

3.3.1. Addition of iron improved *E. coli* removal during stormwater infiltration

Comparing the removal in the first injection, I 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 (Figure 3.1.A). 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 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% (Figure 3.1.B).

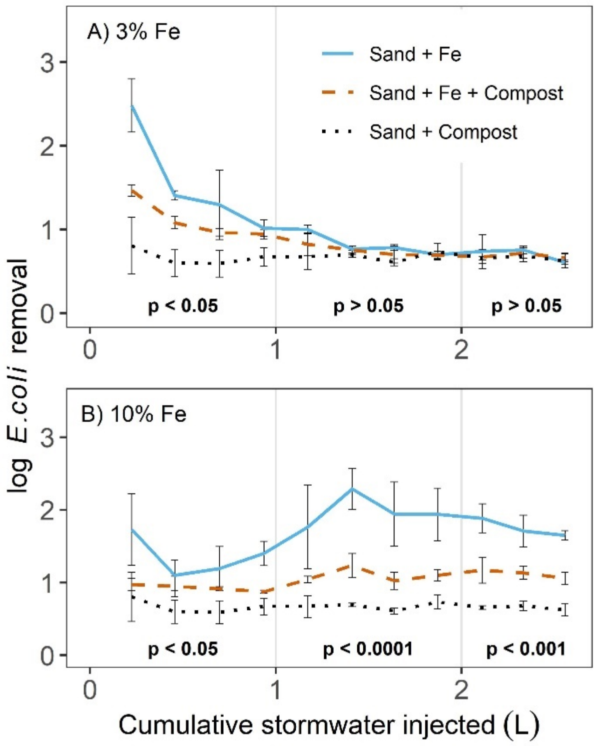


Figure 3-1. Removal of *E. coli* in biofilters with (A) 3% iron and (B) 10% iron. Average log removal based on triplicate columns was calculated as log (C_0/C), where C and C_0 represent the *E. coli* concentration in the effluent and influent water, respectively. p-values represent the comparison between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

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

An increase in DOC exposure decreased *E. coli* removal in batch experiments (Figure 3.2). 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. Changes in surface properties of iron filings were characterized after the exposure to DOC leached from compost and confirmed that surface charge, measured as zeta potential, of iron filings, became more negative after the adsorption of DOC extracted from compost (Figure 3.3). Adsorption of DOC is further confirmed by a change in Fe-O peaks (Figure 3.4). Fe-O typically contributes to positive surface charge sites which increases the adsorption of bacteria and DOC (Gu et al. 1994).

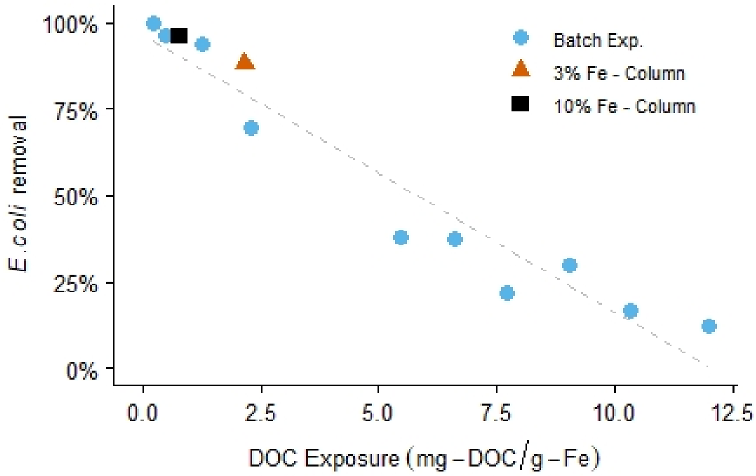


Figure 3-2. Changes in *E. coli* removal with increases in DOC exposure. Duplicated batch experiments were conducted for each data point and the average removal is reported. The prediction of *E. coli* removal by biofilters amended with iron filings is indicated by the orange triangle (3% Fe) and black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

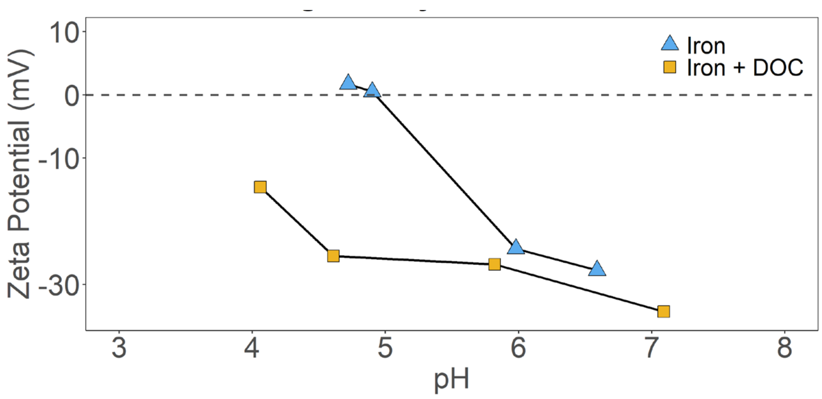


Figure 3-3. Change in Zeta potential of iron filings with and without DOC coating with pH.

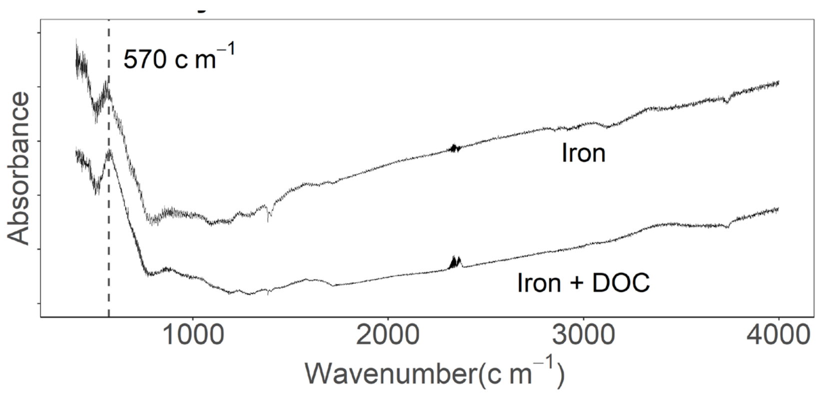


Figure 3-4. FTIR analysis of Iron and DOC coated Iron.

3.3.3. 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 (Figure 3.5). In biofilters with compost (Figure 3.5-C) or 3% iron with compost (Figure 3.5-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 (Figure 3.5-A) and biofilters with 10% iron irrespective of compost presence (Figure 3.5-B and 3.5-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.

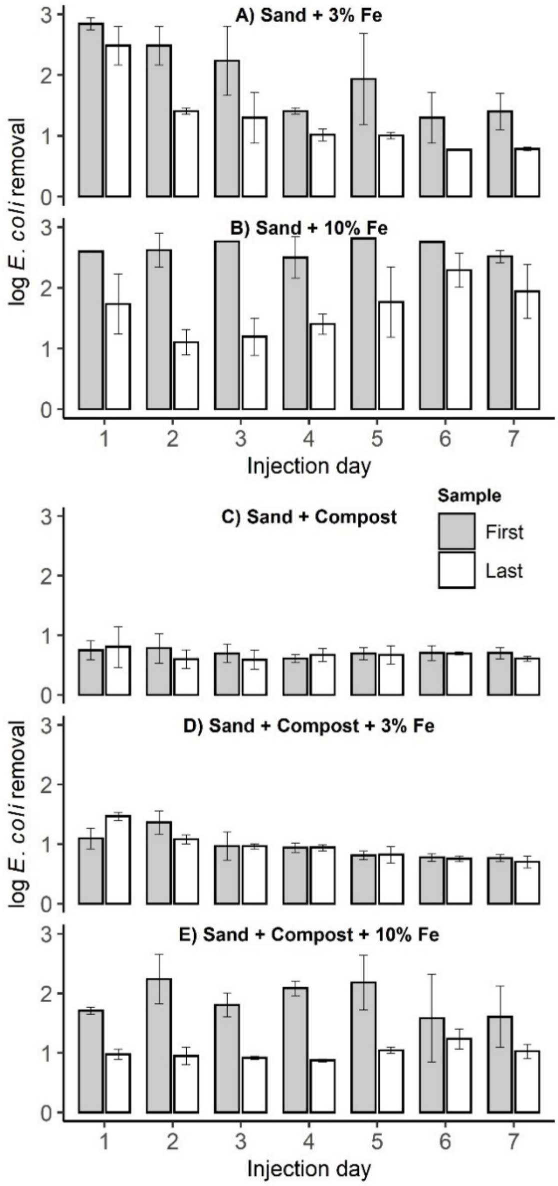


Figure 3-5. Removal of *E. coli* in first-flush and last sample during 7 infiltration events in sand biofilters containing (A) 3% iron, (B) 10% iron, (C) compost without iron, (d) compost with 3% iron, and (e) compost with 10% iron. Error bars represent one standard deviation over mean removal based on at least triplicate columns.

3.3.4. 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, Growth-Die off Index (GDI) was estimated the, 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 (Figure 3.6).

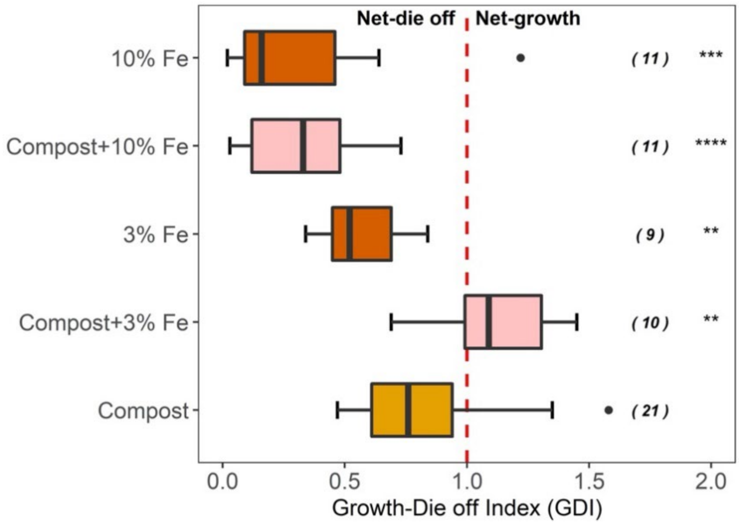


Figure 3-6. GDI of biofilters containing different filter media. The vertical dashed line presents GDI = 1. GDI > 1 presents a condition when *E. coli* concentration increases in pore water potentially due to growth, whereas GDI < 1 presents a condition when *E. coli* concentration decreases in pore water potentially due to 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.

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 3.7).

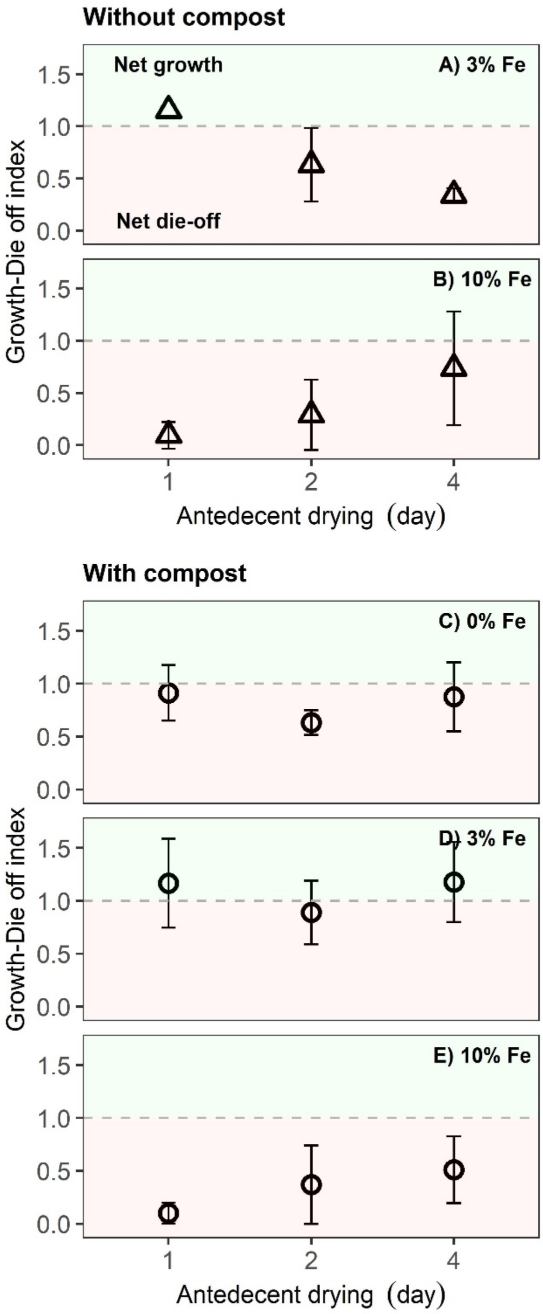


Figure 3-7. Effect of antecedent dry duration on the growth or die-off index for biofilters containing (a) 3% iron (by weight), (b) 10% iron, (c) compost, (d) compost with 3% iron, and (e) compost with 10% iron. The error bars indicate one standard deviation over average growth- die off index. Dashed line indicates where bacteria concentration does not change or growth is compensated by die off of bacteria in biofilters during drying period between rainfall events.

3.4. Discussion

3.4.1. 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 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, clogging was not observed in columns within the duration of the experiment, potentially because iron was mixed with a sufficiently high amount of sand (Bilardi et al. 2014).

3.5. Compost decreases adsorption capacity of iron filings

The removal capacity of columns with iron filings decreased in presence of compost, indicating iron filings lose their capacity to remove *E. coli* in the presence of compost. The results were attributed 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. 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.

The results 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. I 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.

3.6. Iron filings decrease 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.

3.7. 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. I 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. It was hypothesized that nutrients leached from the compost will provide a favorable condition for *E.* c*oli* 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.

3.8. 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.

3.9. References

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4. Stormwater biofilter designs affect the proliferation of antibiotic resistance genes

Abstract

Stormwater biofilters could limit or spread antibiotic resistant genes (ARGs) in urban environments. Stormwater biofilters are designed to remove both resistant and non-resistance bacteria, thereby creating an environment where they could become a source or sink based on the fate of ARGs in the biofilter. Yet, the design features that could either limit or increase the proliferation of ARGs in the environment are not clear. This study examines the effect of two design features—addition of iron amendment and submerged layer—on biofilter ability to mitigate ARGs dissemination. Columns were packed with sand and compost, with or without iron filings, and a submerged layer was created in replicate columns using raised outlets. Natural stormwater containing ARGs was applied intermittently, and the effluent was analyzed for ARGs. The results show that conventional biofilter design including organic compost, without any submerged layer, removed nearly all antibiotic resistance genes, whereas the presence of submerged layer in the same column significantly ($p<0.05$) reduced the removal or increased the relative gene abundance in the effluent. The addition of iron filings had a contrasting effect on ARGs abundance based on whether the submerged layer was present. While the addition of iron improved compost's ability to remove ARGs in the submerged biofilter, it decreased removal in the unsubmerged biofilter. We attributed the result to the potential increase in dissolved iron in a submerged layer that removes ARGs by coagulation and release of the iron-ARGs complex in the unsubmerged layer. The results will help design the stormwater biofilters to improve the removal of ARGs from urban stormwater.

Ghavanloughajar, M., Cira, M., Borthakur, A., Jay, J., and Mohanty, S.K (2020). Persistence of ARGs in stormwater biofilters: Effect of design controls. *Water Research*. (In preparation)

4.1. Introduction

Extensive use of antibiotics has transformed infectious disease treatment. However, overuse of antibiotics, especially in agriculture and livestock farming, promotes bacterial resistance towards common antibiotics (Piscitelli et al. 2018). After prolonged exposure to antibiotics, bacteria can evolve to survive in the presence of the same antibiotics and develop antibiotic-resistant traits(van Hoek et al. 2011). Bacteria with these resistance traits are referred to as antibiotic resistance bacteria (ARB). ARB harbor antibiotic resistance genes (ARGs) found on transposons, integrons, or plasmids (Allen et al. 2010). In the environment, other bacteria can acquire resistance via horizontal and vertical evolution (Tenover 2006). Thus, it is critical to design the natural system to minimize the proliferation or dissemination of ARGs in the environment.

ARGs and ARBs can disseminate throughout different environmental compartments via wastewater, stormwater, and air (Allen et al. 2010). Although numerous studies have examined the fate and transport of ARGs in recycled water (Luprano et al. 2016, Christou et al. 2017, Yuan et al. 2015, Jia et al. 2017, Yang et al. 2014, Zhang et al. 2015), far fewer studies have examined the fate and transport in stormwater (McLellan et al. 2007, Garner et al. 2017, Zhang et al. 2016, Ahmed et al. 2018). To treat stormwater from a variety of contaminants including heavy metals, antibiotics, and pathogens, stormwater biofilters are increasingly used (Askarizadeh et al. 2015). Biofilters incorporate natural design by replacing native soil with a mixture of soil, sand, or compost (Damodaram et al 2010). They can remove bacterial pollutants via straining and adsorption (Chandrasena et al. 2016, Hathaway et al. 2009). The same process can remove ARBs and other non-pathogenic bacteria (Kawecki et al. 2017), thereby increasing the chance of pathogenic or resistant bacteria to transmit the ARGs to non-pathogenic bacteria via horizontal gene transfer. Thus, it is critical to examine the conditions or biofilter design that may potentially proliferate ARGs in biofilters.

Studies examining the ARG removal capacity of biofilters are scarce. In particular, it is not clear how ARG removal is affected by biofilter designs such as the presence of amendments or submerged layers. Amendments such as have been shown to attenuate ARGs in soil biochar and iron media (Cui et al. 2016, Gao et al. 2017). However, they are rarely tested with a mixture of organic amendments present in biofilters. Traditional biofilter media consists of organic amendments such as mulch and compost, which facilitate the biodegradation of organic pollutants (Ulrich et al. 2017). The nutrient-rich compost can also contribute to ARGs' persistence in biofilter by providing a growth favorable environment (Jiao et al. 2017) or facilitating horizontal gene transfer (Hu et al. 2020). In a recent study (Ghavnloughajar et al. 2021), the addition of compost to iron decreased the removal of ARBs in a biofilter packed with iron filings, but the addition of a sufficient amount of iron still provided benefits of increased ARB removal. However, the study did not test the impact of the submerged layer, which is typically used to decrease the mobilization of bacteria during intermittent flow (Mohanty et al. 2013) and increased removal of nitrate (Wu et al. 2017). The presence of submerged zone in biofilters improves nutrient removal; however, it has shown inconsistent removal for indicator bacteria (Kranner et al. 2019). Furthermore, the submerged layer can dissolve iron and may affect its capacity to remove ARGs. Because biofilters are enriched with a high concentration of diverse bacterial communities, the accumulation of ARGs could increase the risk of proliferation via horizontal and vertical gene transfer. The submerged layer could also increase proliferation by providing a moist environment for bacteria to strive and exposed to ARGs in pore water. Thus, it is critical to determine the combined effect of the submerged layer on the ability of iron amendment to remove ARGs.

The current study aims to investigate the effect of iron amendment and submerged layer on ARG removal in stormwater biofilters. We hypothesized that compost in submerged conditions would increase the persistence of ARGs by providing a viable environment and substrate for bacterial growth, whereas iron filing amendment in the same condition would decrease the persistence of ARGs through adsorption and coagulation.

4.2. Materials and Methods

4.2.1. Stormwater collection

Stormwater could contain a significantly high concentration of ARGs in some regions and increase ARGs in receiving water bodies by 3 orders of magnitude (Garner et al. 2017, Jang et al. 2021). Unlike synthetic stormwater, natural stormwater contains a wide range of ARGs (Kawecki et al. 2017). To examine the removal and persistence of ARGs in biofilters with different types of amendments and design, natural stormwater was collected in 20 L containers from Ballona Creek in Los Angeles, which has been shown to contain ARGs (Kawecki et al. 2017). Stormwater was collected before sunrise on the day of the experiment to minimize UV light exposure to mobile genetic components and bacteria in stormwater. After collection, the pH of the stormwater was adjusted to 6.9±0.2 using 10 M NaOH and then used in the experiment the same day.

4.2.2. Biofilter Design

Eight laboratory-scale biofilters were designed using PVC tubes with 61 cm length and 5 cm internal diameter fitted with a cap at the bottom (Figure 4.1). First, gravel was filled up to 2 cm at the bottom to create a drainage layer. A plastic screen (pore size: 100 μm) was placed on top of the gravel layer to prevent the media mixture from top to fall into gravel. Three types of media mixture were packed in columns: a mixture of sand and compost (23% v/v) referred to as Compost (C) henceforth and a mixture of sand and compost with 5% iron filings by weight referred to as Compost-Iron (C+Fe). Well-mixed media were added incrementally at 5 cm height and compacted multiple times using a steel rod until the total filter media depth became 30 cm. Total 4 replicates were packed per media mixture type. To examine the effect of the submerged layer on ARG removal, two columns per media type were saturated up to the bottom 15 cm by raising the outlet tubes above 15 cm of the bottom media layer. Effluent for the other two columns was collected from the bottom outlet, thereby eliminating any submerged layer. Before the experiment, deionized (DI) water was injected from the bottom to flush out the air out of pore spaces and quantify the total pore volume. The water was drained by gravity, and DI water was injected several times to flush any suspended particles from pores.

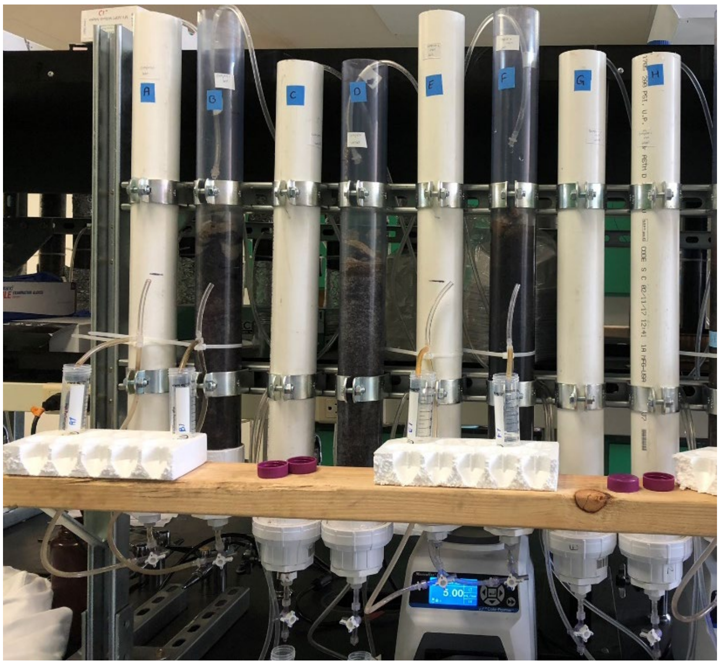

Figure 4-1. Biofilter setup featuring compost and compost + iron with and without a submerged layer.

4.2.3. Application of stormwater

Before the application of stormwater, all tubes connecting the pump to columns were flushed for 10 minutes with particulate-free stormwater to remove any possible residue from past experiments. Approximately 1200 mL of natural stormwater was injected into each column at 15.2 cm h^{-1} for 4 hours using a peristaltic pump connected with a multichannel head (Cole-Parmer, USA). The injected stormwater is equivalent to 3.6±0.1 and 6.8±0.4 pore volume of packed columns with and without submerged layer, respectively. To simulate intermittent flow in nature, the experiment was repeated 3 times with a 2-3 days drying period between injections when the biofilters were kept at room temperature (22 °C). The effluent was collected in 1000 mL pre-autoclaved HDPE bottles for analysis of ARGs and 16s rRNA following the method described in previous studies (Echeverria-Palencia et al. 2017).

4.2.4. DNA Extraction

The effluents were filtered by a vacuum manifold using a 47 mm diameter, 0.45 μm pore size mixed cellulose ester filter membrane (MilliporeSigma, Darmstadt, Germany) to retain microbial cell on the filter. Filter cups were flushed with phosphate-buffered saline (PBS) solution after effluent filtration for maximum recovery of DNA. Folded filters were placed in the preloaded 2mL screw cap tubes from the DNeasy, PowerWater kit, (Qiagen, Hilden, Germany). Tubes were either stored at -20 °C for the next day's DNA extraction or immediately prepared for DNA extraction following the manufacture's guidelines. The extracted DNA was aliquoted in a 1.7 ml low-binding plastic microcentrifuge tube for further gene analysis. The same filtration and DNA extraction procedure were carried out for the PBS solution, stormwater, and deionized water. In addition, one 2-ml bead tube with a sterile filter membrane was included in each series of extraction to be accounted for extraction blank. The purity and concentration of extracted nucleic acid were evaluated by ultraviolet absorbance spectrophotometry (Nanodrop 2000C Thermo Scientific, Waltham MA).

4.2.5. Quantitative Polymerase Chain Reaction (qPCR).

DNA samples were analyzed for 16S-rRNA gene, which presents a total bacteria surrogate measure, and abundance of ARGs via qPCR following the method outlined elsewhere (Gao et al. 2017). Relative gene abundance was reported as normalized to the 16S rRNA. Samples were initially diluted with different ratios for the determination of the minimum dilution required to minimize inhibition. All qPCR assays were tested in a 96-well reaction plate in Step One Plus (Applied Biosystems). Each assay includes a 7-point standard curve, molecular grade water for the negative control, diluted DNA extract and filter, and PBS blank. All of the assay components were performed in triplicate. The temperature cycle and proper primer for each ARG were chosen based on what has been validated in the previous peer-reviewed literature.

For all assays, the qPCR mixture consisted of 10 μL of PowerUp SYBR Green Master Mix (Thermo Fisher Scientific, Waltham, MA), 1.25 μL of each primer, 3 μL of template DNA, and 5 μL of RNase-free molecular biology grade water (Fisher Scientific, Pittsburgh, PA). Primers and primer concentrations were previously validated in the literature (Table 4.1).

Table 4.1. Overview of primer sequencing and concentration for antibiotic resistance gene and 16S rRNA

Target Gene	Primer	Concentration (nM)	Sequence (5'-3')	Amplicon Size (bp)	Reference
sul1[a]	*sul1*-F *sul1*-R	200	CGCACCGGAAACATCGCTGCAC TGAAGTTCCGCCGCAAGGCTCG	258	(Pei et al. 2006)
intI1[b]	*intI1*-F *intI1*-R	200	GGCTTCGTGATGCCTGCTT CATTCCTGGCCGTGGTTCT	424	(Luo et al. 2010)
16S rRNA[c]	*16S rRNA-F* *16S rRNA*-R	600	ATGGCTGTCGTCAGCT ACGGGCGGTGTGTAC	351	(Pan et al. 2018)

To determine the appropriate DNA concentration, serial dilution and well spike tests were performed for all samples. All assays were performed in 96-well reaction plates that contained triplicate: samples, applicable extraction blanks, an RNase-free molecular biology grade water negative control, and a 7-point standard curve positive control. The standard curve, covering 7 orders of magnitude, was constructed with 10-fold serial dilutions of known amounts of dsDNA (Integrated DNA Technologies, Coralville, IA). All assays were run in a StepOne Plus Real-Time PCR System (Applied Biosystems, Foster City, CA) with the reaction specifics in Table 4.2. The StepOne Plus Real-Time PCR System generated quantification thresholds and melt curves for each assay.

Quantification thresholds were converted into gene copies using the standard curve. Melt curves were used to further verify target gene amplification specificity. Standard curve efficiencies were at or above 90% and R^2 values were at or above 0.99 across all qPCR assays.

Table 4-1. Reaction temperatures and duration used to quantify the antibiotic resistant genes and 16SrDNA gene.

Target Gene	Holding		Denaturation		Annealing		Extension	
	Temp. (°C)	Time (min)	Temp. (°C)	Time (s)	Temp. (°C)	Time (s)	Temp. (°C)	Time (s)
*sul*1	95	10	95	15	65	30	72	30
*int*I1	95	10	95	15	55	30	72	30
16S rRNA	94	4	94	40	60	45	72	60

4.3. Result and discussion

4.3.1. Effect of the submerged zone

The results show that the presence of submerged layer increased the relative abundance of ARGs and mobile genetic element in the effluent (Figure 2), indicating submerged layer may increase ARGs in the biofilter effluent compared to unsaturated biofilters. This agrees with a previous study in which the ratio of retained to released bacteria in unsaturated soil was nine times greater than saturated soil (Balkhair 2017).

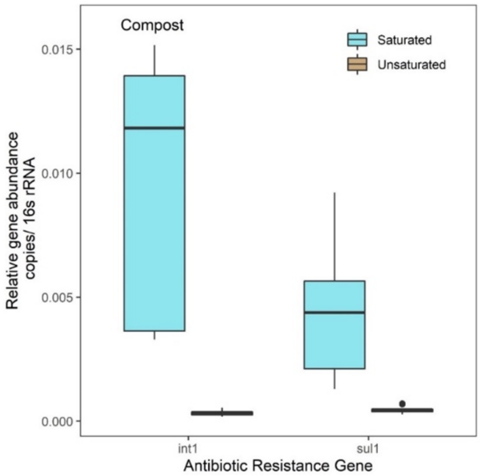

Figure 4-2. Relative gene abundance of *intI1* and *sul1* in the effluent from compost biofilters with and without a submerged layer.

We attributed our observation to multiple contributors that mainly cause a decrease in decay or increase in the persistence of ARGs when a submerged layer is present. First, the transport of genetic material can be facilitated in the biofilters' media with a saturation zone. The previous study (Poté et al. 2003) showed that biologically active DNA can be transported in water-saturated soil without significant degradation. Air-water interface contributes substantially to colloid transport in porous media (Balkhair 2017). In an unsaturated medium, a mobile air-water packet can remobilize hydrophilic and to a greater extent hydrophobic colloids from the solid surfaces (Sharma et al. 2008). However, the stagnant air-water pockets can immobilize bacteria and become the sites to retain ARGs (Jiang et al. 2006). Second, in addition to increased bacterial mobility, liberated substrate diffusion in saturated soil promotes bacterial community survival (Chowdhury et al. 2011).

Alternatively, degradation of genetic material can be enhanced in unsaturated soil and ultimately decrease their relative abundance in the effluent. Soil with less pore water (< 56%), could experience higher bacterial diversity(Carson et al. 2010). Therefore, it can be assumed that adsorbed ARGs to the compost in biofilters without submerged layer are more prone to antagonistic interaction with diverse indigenous bacteria that can ultimately inactivate bacteria. Another contributor to the significant decrease in relative gene abundance in unsaturated biofilter could be explained by the presence of small volume pore water in the columns. Small packets of pore water connect via extremely thin liquid film in unsaturated soil, promote the formation of extracellular polymeric substances (Faucette et al. 2009) that protect embedded bacteria via irreversible adsorption (Or et al. 2007) from flow fluctuation(Costa et al. 2018). Although biofilm or extracellular polymer was not measured in this study, their presence has been shown to extensively affect transport and deposition ARGs and ARBs (Guo et al. 2018). Lastly, exposure of bacteria in the soil to progressive desiccation in the unsaturated column can increase bacterial inactivate in unsaturated columns (Barrios et al. 2020). Relative rapid drying in unsaturated media increases salt concentration that could be another stressor for bacterial survival (Chowdhury et al. 2011). The submerged layer would limit particle leaching and thus would decrease the release of ARGs in association with particle or ARB (Liu et al. 2013). However, our results show that the addition of the submerged layer did not change the concentration of particles in the effluent. Thus, particle concentration is not a factor in explaining the observed increase in ARG abundance in the submerged layer as was assumed in previous studies.

4.3.2. Effect of iron amendment

Comparison between gene abundance in compost and mixture of compost and iron with and without submerged layer, we found that the addition of iron significantly decreased the relative abundance of both genes in effluent with submerged layer (Fig. 5.3-a), but the addition of iron had the opposite effect in columns without submerged layer (Fig. 5.3-b).

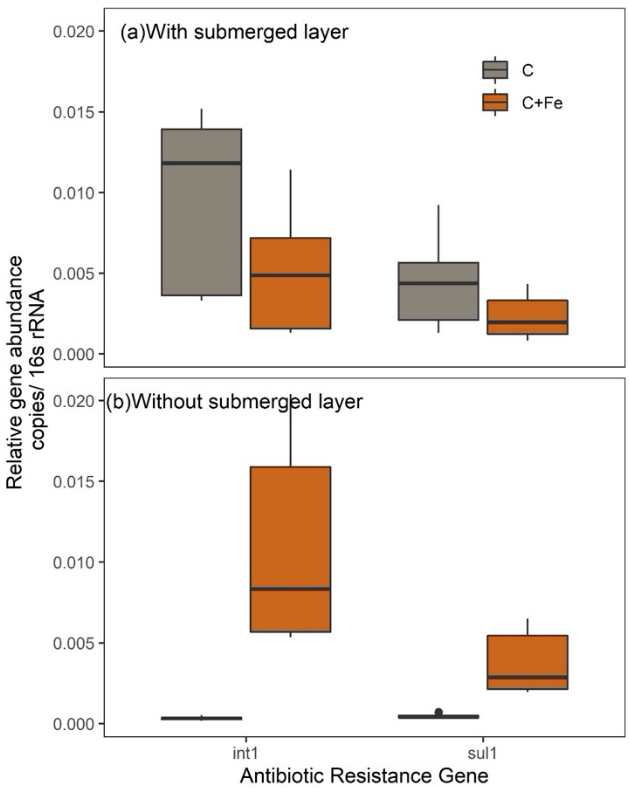

Figure 4-3. Relative gene abundance of *intl1* and *sul1* from compost(C) and compost and iron (C+Fe) (a) with and (b) without submerged layer.

Interestingly, biofilter removal efficiency in columns without submerged layer got worse when compost is mixed with iron. Without a submerged layer, the compost columns removed nearly 100% for both genes, but the addition of iron decreased the removal of ARGs, leading to increased ARG concentration in the effluent. The results were attributed to the effect of dissolved iron on the removal of ARGs by coagulation and deactivation. For instance, the backbone phosphate group in DNA can form complexation with iron. Iron dissolution could be higher in the submerged layer, and the dissolved iron can increase the removal of ARGs by coagulation (Li et al. 2017). Furthermore, an increase in dissolved iron could suppress the horizontal gene transfer and lower their proliferation (Xu et al. 2021). In the submerged layer, iron is more likely to dissolve by complexation and reduced by DOC in pore water. Without the submerged layer, the iron is exposed to oxygen and corrode, which can bind ARGs (Li et al. 2017). In unsaturated conditions, the aggregate of

Fe-ARG can be displaced upon injection of new stormwater, releasing the absorbed ARGs. This result indicates that the presence of iron can decrease ARGs released in columns with submerged layer but increase ARGs released in columns without submerged layer. Thus, if the biofilters do not have a submerged layer, then iron filings should not be used as compost is sufficient to remove most ARGs in the absence of a submerged layer.

4.4. Conclusion

Our results highlight the impact of the media type and amendment in addition to engineering modification such as submerged layer on the mobilization of *sul1* and *intl1* from stormwater using model biofilter. By examining relative gene abundance in the effluent of biofilters subjected to intermittent stormwater, we observed that the presence of the submerged layer with conventional compost media decreases the biofilter's ability to remove ARGs. If the presence of the submerged layer is inevitable for other purposes such as nutrient removal, then amending the compost media with zero-valent iron would improve the biofilter functionality. The addition of iron filings had a contrasting effect on ARGs abundance based on whether the submerged layer was present. While the addition of iron improved compost's ability to remove ARGs in the submerged biofilter, it decreased removal in the unsubmerged biofilter.

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5. Summary and conclusion

5.1. Summary

The dissertation aims to study the performance and longevity of stormwater biofilters that are integral in urban stormwater management practices. Biofilters not only reduce the peak volume of stormwater but also improve the water quality owning to their pollutant removal capacity. However, there are challenges in the large-scale application of stormwater biofilters mainly due to a lack of knowledge in the long-term performance of them. Stormwater biofilters are under constant physical and hydrological stressors from their environment and this work aims to explore the effect of some of these stressors on stormwater biofilters. This work recognizes some of the most important stressors that can jeopardize biofilter performance. It also discussed the engineering modifications that can potentially minimize those undesirable effects.

Chapter 2 examines the effect of a physical stressor, compaction on biofilter media performance. The results show that while compaction can decrease the infiltration capacity of biofilters, the addition of biochar in moist conditions could alleviate the negative effect of compaction. In Chapter 3, the intermittent nature of stormwater infiltration was recognized as a stressor on biofilter, which is known to release a high concentration of pollutants during the first flush, thereby compromising the biofilter function. The result shows that the addition of 10% iron filings to compost biofilter can reduce the high concentration of *E. coli* in the first flush of biofilters. In Chapter 4, biofilter longevity to remove ARGs from stormwater was tested. The results show that conventional biofilter media (sand and compost mixture) are capable to remove the two types of ARGs, namely *intI1* and *sul1* from the natural stormwater, but the removal depends on the presence of a submerged layer. The presence of submerged decreased the capacity of compost to remove

ARGs. In this case, adding iron to the submerged layer could increase ARG removal. Interestingly, the addition of iron without the submerged layer made the biofilter worse.

5.2. Recommendations for future studies

The current study shows that the application of wet biochar can alleviate the negative effect of compaction in the same way compost does with additional benefits of increased bacterial pollutant removal. It is not clear how the biological function of compacted biochar would change with time. The compacted biochar may change the microbiome of biofilter by altering the redox properties to more anoxic. Thus, future studies should examine the changes in redox properties, microbiome, and plant health in biochar-augmented biofilters under compaction.

In this study, the results proved that the addition of iron could decrease the negative effect of the first flush release of microbial pollutants. However, iron would continue to dissolve by complexing with DOC in the biofilter. The iron-DOC complex could affect the fate and transport of other organic pollutants. The study recommends the use of 10% iron (by weight). It is not clear if 10% iron would pose any toxicity to plants. In particular, it is not clear how the modification would change the root microbiome. Further study should examine the effect of iron addition on the biofilter microbiome and their functions.

Secondly, this study shows that conventional biofilter media may have enough capacity to remove some ARGs from stormwater, and the addition of a submerged layer may not be beneficial in this case. However, the addition of iron to submerged compost biofilters could increase their capacity to remove ARG. However, the mechanism by which iron may affect ARG removal or proliferation is not clear. Iron filings could also remove heavy metals, which can impose selective pressure on non-resistance bacteria. Future studies should examine the effect of accumulated pollutants on iron amendments on co-selective pressure in biofilters.

Finally, although this study includes promising results of laboratory work for enhanced removal capacity of biofilters, they did not examine the performance of these systems on the practical conditions relevant to fieldwork. Further fieldwork is essential to disclose interconnection among many components such as variation in rainfall intensity and dry duration as well as a wide range of pollutants in stormwater.