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Biochar increases nitrate removal capacity of woodchip biofilters during high-intensity rainfall

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ABSTRACT

Stormwater biofilters have been increasingly used to mitigate the impact of climate change on the export of contaminants including nitrate to water bodies. Yet, their performance is rarely tested under high-intensity rainfall events, which are predicted to occur more frequently under climate change scenarios. We examined the potential of biochar to improve the resilience of woodchip biofilters under simulated high-intensity rainfall events and linked denitrification to biochar-mediated changes in hydrological (physical), chemical, and biological properties of woodchip biofilters. Results showed that nitrate removal capacity of woodchip biofilters decreased with increases in rainfall intensity or duration and decreases in antecedent drying time. However, adding biochar to woodchips significantly decreased the exhaustion rate of woodchips, only when the hydraulic residence time (HRT) was less than 5 h. At longer HRT (>5 h), the benefits of biochar became less apparent. We attributed the improved denitrification during high nitrate loading to biochar's ability to decrease dissolved oxygen in pore water and increase water holding capacity and retention of dissolved organic carbon and nitrate—all of which could increase nitrate utilization. Biochar increased the net microbial biomass but did not affect the relative abundance of denitrifying genes, which indicates that a shift in microbial biomass could not fully explain the observed increase in nitrate removal in biochar-augmented woodchip biofilters. Overall, the results showed that biochar could increase the resiliency of woodchip biofilters for denitrification in high-intensity rainfall events, a worst-case scenario, thereby mitigating the water quality degradation during climate change.

1. Introduction

Eutrophication or algal blooms are expected to intensify during climate change, partly due to an increasing frequency of high-intensity rainfall. These climate-driven changes increase the conveyance of nutrients to water bodies and warm surface waters, which accelerates algal growth (Whitehead et al., 2009). In particular, rainfall intensity can be a critical indicator of nutrient pollution because runoff generated during high-intensity rainfall disproportionally exports more nutrient per runoff volume to surface waters than runoff generated during low-intensity rainfall (Michalak et al., 2013; Jenny et al., 2016). To treat runoff, low-impact development infrastructure such as biofilters have been increasingly incorporated into urban and agricultural landscapes. Yet, the performance of these natural treatment systems is rarely tested under high-intensity rainfall events (Norton et al., 2017). The resilience of a treatment system can be indicated by the number of events in which the treatment system fails to remove specific pollutants to below a desired standard or by the duration it takes to restore the removal rate after a failure has occurred (Juan-García et al., 2017). Therefore, it is critical to evaluate the impact of high-intensity rainfall on nitrate removal potential of conventional biofilters and improve their design to increase their resilience.

Passive treatment systems such as stormwater biofilters offer cost-effective solutions to treat nitrate in runoff originated from nonpoint sources. Biofilters are typically designed by replacing a portion of surface soil with soil amendments that serve multiple functions: to increase stormwater infiltration, to remove pollutants,

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and to support plants growth. For nitrate removal, carbon amendments such as woodchips, sawdust, and crop residues are typically used (Shrestha et al., 2018; Jia et al., 2019). These amendments release dissolved organic carbon (DOC), which the denitrifying microbial communities use as an electron donor to reduce nitrate to nitrogen gas via a series of intermediate steps (Ambus and Zechmeister-Boltenstern, 2007). Among these amendments, woodchips are popular (Bruun et al., 2016; Hoover et al., 2016) because they typically last longer and consistently provide DOC (Lopez-Ponnada et al., 2017). For efficient denitrification, four conditions should be met: (1) dissolved oxygen (DO) concentration should be low (preferably below 3 mg L^{-1}) as high DO can deactivate the enzymes involved in denitrification (Gómez et al., 2002); (2) a sufficient amount of DOC must be present (Newcomer et al., 2012); (3) the conditions in biofilters should favor growth of denitrifying microorganisms (Falkentoft et al., 2000); (4) the hydraulic retention time (HRT) should be low enough for maximum utilization of nitrate by microorganisms (Damaraju et al., 2015; Hoover et al., 2016; Halaburka et al., 2017). Thus, the nitrate removal capacity of woodchip biofilters is sensitive to rainfall characteristics and antecedent weather conditions (Lynn et al., 2015), which may affect all four of the conditions for denitrification.

Rainfall characteristics are expected to change with climate change. In particular, high-intensity rainfall and prolonged dry periods are projected to occur more frequently (IPCC, 2007; Prein et al., 2017), which may decrease the denitrification potential of woodchip-based biofilters. Biofilters typically bypass water when the runoff loading rate exceeds the maximum infiltration capacity of the biofilter, but woodchip-biofilters have high hydraulic conductivity due to large pore sizes. Thus, HRT of the biofilter under high-intensity rainfall event could decrease rapidly. However, most studies with woodchip biofilters (Damaraju et al., 2015; Hoover et al., 2016; Halaburka et al., 2017) examined their performance at long HRT (>48 h) and under steady-state condition, thereby overestimating denitrifying capacities of woodchip biofilters. Most stormwater biofilters are passive and subjected to an unsteady condition, when nitrate concentration in pore water is expected to change because of either high loading during high-intensity rainfall or no loading during antecedent dry conditions. While high loading can decrease nitrate removal, antecedent drying could increase nitrate removal (Lynn et al., 2015; Wang et al., 2018). Thus, the overall performance of woodchip biofilters under unsteady conditions is unclear.

To increase the resiliency of woodchip biofilters under heavy rainfall conditions, biochar—a porous black carbon produced by pyrolysis of organic materials — can be added to biofilters. The effect of biochar on nitrate removal capacity of stormwater biofilters has been reported to vary widely (See review by Mohanty et al., 2018), but the cause of the wide variation is unclear. Biochar can alter physical, chemical, and biological processes in the soil, but how these changes can affect denitrification is not well understood (Bock et al., 2016, 2018). While some studies have demonstrated that biochar addition can improve nitrate removal by increasing storage volume and residence time (Bock et al., 2015) or by shrinking of the denitrifying microbial community (Chen et al., 2015), others have shown contrasting results including the release of nitrate into infiltrating water (Sarkhot et al., 2013).

The objective of this study is to link the nitrate removal capacity of biochar-augmented woodchip biofilters under unsteady flow conditions to biochar-mediated alteration in physical, chemical, and biological properties of the biofilter. We compared the nitrate removal capacity of woodchip biofilters with and without biochar amendments under simulated rainfall events with increasing intensities and antecedent drying durations.

2. Materials and methods

2.1. Stormwater

Stormwater was collected twice a week in 20-L HPDE 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 km^2 urban area with 82% developed and 61% impervious surface (Gold et al., 2015). Stormwater was characterized for pH, DO, and turbidity within 1 h of the sample collection. Large particulates were removed from stormwater by gravitational settling for 1 h, and the supernatant was stored up to 1–2 days in a refrigerator at 4 °C to minimize bacterial growth. Before injection, stormwater was warmed to room temperature (~23 °C), and concentrated nitrate stock solution was spiked to 18 L of stormwater to achieve a targeted initial nitrate concentration of 20 ± 2 mg L^{-1}.

2.2. Biofilter media

Pine woodchips without chemical treatment (Whittier Fertilizer Company, CA) were sieved (sieve # 20) to remove woodchips larger than 1.27 cm. We used commercially available biochar (Biochar Supreme, Everson, WA), which has been demonstrated to remove heavy metals and organic contaminants from stormwater (Miles et al., 2016). The biochar, produced by high-temperature (900–1000 °C) gasification of a softwood, has following characteristics: high BET surface area (690–720 m^2/g) (based on nitrogen adsorption), low ash content (4%), and low moisture content (10%). Both woodchips and biochar were dried in an oven at 80 °C until the dry weight of the media remained constant. Oven drying was necessary to kill most of the bacteria from woodchips and biochar. This permits us to compare the microbial community developed in columns with and without biochar. Biochar was mixed with woodchips to prepare woodchip-biochar mixtures: 5, 10, and 20% biochar by volume. Woodchips without biochar were used as a control media.

2.3. Biofilter media characterization

The particle size distribution of the biochar was determined using a laser diffraction particle size analyzer (model LS 13 320, Beckman Coulter, Brea, CA, USA). For woodchips, the size distribution was determined using an optoelectronic particle size analyzer (model CAMSIZER, Retsch Technology Gmbh, Germany). D_{10}, D_{50}, and D_{90} of woodchips were 3.7, 8.4, and 13.3 mm, respectively; D_{10}, D_{50}, and D_{90} of biochars were 0.02, 0.7, and 1.7 mm, respectively (Fig. S1). After experiments, saturated hydraulic conductivity was measured in all columns using the falling head method.

The water retention characteristics of the woodchips and woodchips-biochar mixtures were determined using a benchtop instrument, UMS HYPROP (METER Group Inc., Pullman, WA). The HYPROP measures the unsaturated hydraulic conductivity based on the evaporation method by simultaneously recording the sample weight and soil matric potential during the sample drying cycle from saturation to around pF 2.5 (*pF* is the $\log_{10}$ h, where h is the suction in cm), which is below wilting point. We utilized the bimodal soil water retention model (Durner, 1994), which is ideal to describe changes in physical properties of a medium having a heterogeneous pore structure. In this case, the water retention curve is expressed as a linear superposition of sub-curves of a homogeneous pore structure using a unimodal model (van Genuchten, 1980), and effective saturation was estimated using the equation below.

$$S_e = w_1\left[\frac{1}{1+(\propto_1 h)^{n_1}}\right]^{m_1} + (1-w_1)\left[\frac{1}{1+(\propto_2 h)^{n_2}}\right]^{m_2}$$

where S_e is effective saturation; w_1 is the weighing factor for the first subcurve subject to $0 < w_i < 1$; $\propto$ is a scaling factor that determines the position of the pore size maximum; h is the suction (cm); n and m empirical shape parameters and $m = \left(1 - \frac{1}{n}\right)$. S_e is related to volumetric water content as $\theta = \theta_r + (\theta_s - \theta_r)S_e$, where θ is the volumetric water content, θ_s is saturated, and θ_r is the residual water content (cm^3 cm^{-3}). The model was implemented using a nonlinear fitting program (Seki, 2007) and the modeled water retention curve was fitted with experimental data using the derived parameters. The details of the method were described in a previous study (Trifunovic et al., 2018). It should be noted that drying of biochar in the oven may have removed moisture from micropores or nanopores, which likely reduced the amount of intraparticle pore space that could be wet. Nevertheless, multiple rewetting cycles prior to the experiment is expected to refill some of the pores.

2.4. Biofilter design

To examine the effect of biochar concentration on denitrification potential of woodchip biofilters, woodchips without (control) and with biochar at 5%, 10%, or 20% by volume were packed in plastic columns (5 cm inner diameter × 61 cm length) and fittings made up off polyvinyl chloride (PVC). Triplicate columns were used for each mixture (Fig. S2). To create a drainage layer, gravel was first filled in bottom 5-cm layer. A nylon screen (100 μm pore size) was placed on the top of the gravel to prevent washout of biochar particulates. About 100 g of woodchips or woodchip-biochar mixture was added and compacted at 5 cm incremental heights until the total filter media depth was 45.7 cm. Gravel was added on the top of the filter layer to prevent suspension of biochar particles. A submerged layer was created by raising the outlet 30.5 cm above the top surface of the bottom gravel layer. All columns were wrapped with aluminum foil to prevent algal growth. To displace air from pores in the submerged layer, deionized (DI) was injected from the bottom (up-flow) until the filter media layer was completely submerged Once the submerged layer was established, stormwater was applied on the top of the filter media using a peristaltic pump, and the effluent was collected at regular intervals through the raised outlet using 300 mL amber bottles. The effective pore volume of each column was estimated based on the volume of water required to achieve 50% of the concentration of influent bromide, a conservative tracer (Method details in Supplementary Material). Total pore volume measured by the bromide breakthrough curve does not account for the intraparticle pore volume, where bromide transport is diffusion-limited. Thus, estimated pore volume only represents the pores or flow paths through which stormwater infiltrated during the experiment.

2.5. Nitrate removal experiments

Because both woodchips and biochar were oven-dried before the experiment, they were expected to have a limited microbial activity. To grow biofilms or denitrifying communities, the columns were first conditioned by applying stormwater on the top of columns periodically (0–4 d) for three months. Following conditioning of the columns, stormwater spiked with nitrate was applied on the top of the columns at a specific rate to simulate different rainfall intensities. Effluents were collected at regular intervals (~0.5 pore volume fraction) in 300-mL amber bottles and analyzed for nitrate, DO, pH, and UV_{254} absorbance—an indicator of DOC concentration in pore water.

To determine the effect of rainfall duration on nitrate removal, stormwater with 24.7 mg L^{-1} nitrate was injected at 18.5 cm h^{-1} for 12 h following 4 days of antecedent drying. Effluents were collected at 1 h intervals, and selected samples (1, 2, 4, 6, 8, 10, and 12 h) were analyzed for nitrate and other water quality parameters. To examine the effect of rainfall intensity and antecedent drying duration on nitrate removal in biochar-augmented woodchip biofilters, contaminated stormwater was applied at a specific rate ranging between 19 and 296 mm h^{-1}. To test the changes in nitrate removal during rapid infiltration of stormwater, 1.2 L of stormwater containing nitrate was injected through each column at 5 rainfall intensities varying from 19 mm h^{-1} to 296 mm h^{-1}, which correspond to HRT between 16.6 ± 0.5 to 1.04 ± 0.03 h. HRT was calculated by dividing the pore volume of each column type—estimated from bromide breakthrough data (Fig. S3)—by infiltration rate through pores. Infiltration rate was assumed to be the discharge rate at the bottom, which was observed to be same as rainfall application rate. To examine the effect of antecedent drying duration on denitrification, stormwater with nitrate was applied at 148 mm h^{-1} for 4 h on biofilters subjected to first increasing and then decreasing antecedent drying duration in the following order: 1, 2, 4, 8, 30, 8, 4, 2, and 1 day.

2.6. Water sample characterization

Effluents and influents were analyzed immediately after the experiment for the following water quality parameters: pH (Fisher Scientific #9107BN), DO (Fisher Scientific 087010MD), and UV_{254} absorbance (PerkinElmer Lambda 365 UV–Visible Spectrophotometer). UV absorbance was used as a surrogate measurement for dissolved organic carbon, assuming specific UV absorbance of DOC leached from woodchips remained constant during the experimental period (Abusallout and Hua, 2017). UV absorbance and nitrate concentrations were measured in samples centrifuged at 5000×g for 10 min to remove particulates or bacteria. Following centrifugation, the supernatant was acidified (pH ~3) and stored at 4 °C, to minimize any microbial activity. The samples were analyzed for nitrate concentration using ion chromatography (Dionex Integrion HPIC, ThermoScientific) within 1–2 days of sample storage.

2.7. Modeling exhaustion rate of woodchip biofilters

During intermittent rainfall events with high-intensity rainfall, nitrate loading rate can exceed the nitrate removal rate of woodchip biofilters. Thus, effluent nitrate concentration is expected to rise rapidly due to exhaustion of the nitrate removal capacity of woodchip biofilter. To estimate the rate at which the nitrate removal capacity of woodchip biofilters was exhausted with an increase in nitrate loading (contaminated water volume) during high-intensity rainfall, we used the following model to describe the concentration of nitrate in pore water:

$$dC / dV = k(C_0 - C)$$

Where, C and C_0 are effluent and influent nitrate concentrations, respectively; k is exhaustion rate; V is the volume of stormwater passed through the columns. The model assumes that change in concentration in woodchip biofilters is a function of the difference in nitrate concentrations between pore water and influent water. Solving the first-order linear differential equation (Supporting Material), the effluent concentration can be expressed as:

$$C = C_0\left(1 - e^{-kV}\right)$$

Nitrate removal ($1 - C/C_0$) was calculated for each column type at all intervals. The exhaustion rate was calculated based on the exponential fit of the data showing a decrease in removal with increases in nitrate loading (pore volume). To compare the total removal of nitrate in a rainfall event as a function of antecedent drying duration, we calculated the ratio of the total mass of nitrate removed $\left(\sum_i C_i V_i\right)$ and total mass of nitrate injected $\left(C_0 \sum_i V_i\right)$ during a rainfall event, where *i* is sample number. All samples were collected and analyzed for nitrate concentration to complete the mass balance.

2.8. Denitrifying functional gene analysis

After the experiment, columns were dismantled to remove media mixture from the center of the column. The total nucleic acids were extracted from the 10 g media mixture using DNeasy PowerMax Soil Kit (Qiagen), and the concentration of extracts was measured on NanoDrop® ND-2000 (NanoDrop Technologies, Willmington, DE, USA). Target genes encoding nitrate reductases (*napA* and *narG*) and nitrite reductases (*nirK* and *nirS*) were amplified using real-time quantitative polymerase chain reactions (qPCR) conducted on a StepOnePlus thermocycler (Life Technologies, Carlsbad, CA). The primer sets for each denitrifying functional genes and details methodology were described in a previous study (Miao et al., 2018). The qPCR reaction system was 20 μL, which included 2 × Luminaris Color HiGreen-HiROX qPCR Master Mix (10 μL, Thermo Scientific, Waltham, MA), 0.25 μM forward and reverse primers (0.5 μL with 10 μM), 2 μL of DNA (5–20 ng μL^{-1}) templates, and 7 μL double-distilled water. The cycling program for each target gene started with 95 °C for 5 min, ran for 40 cycles with respective annealing temperatures (Table S1). All reactions had the amplifying efficiency from 85 to 97%, accompanied by a melt-curve analysis to confirm the specificity of qPCR products. The gene copies numbers in each sample were normalized to the total weight of biochar-woodchip samples (10 g), with units of "copies g^{-1}".

2.9. Statistical analysis

Statistical analysis of data (ANOVA and Tukey HSD) was performed using R v3.4. Pearson correlation was used to evaluate if an increase in the addition of biochar to woodchip biofilters resulted in changes in nitrate removal capacity, exhaustion rate, 16S rRNA, and functional gene abundances. Differences were considered significant at a p-value less than 0.05.

3. Results

3.1. Effect of biochar amendment on hydraulic properties of woodchip biofilters

Based on bromide tracer study, the pore volume of columns with 0%, 5%, 10% and 20% biochar (by volume) were estimated to be 649.1 ± 64.8, 623.4 ± 10.8, 616.0 ± 34.0, and 607.8 ± 23.2 mL, respectively (Fig. S3). Thus, the addition of biochar did not significantly ($p > 0.05$) alter the pore volume of woodchip biofilters. Saturated hydraulic conductivity of woodchip columns decreased with increases in biochar fraction. Saturated hydraulic conductivities of woodchip columns with 0, 5, 10, and 20% biochar (by volume) were 6048 ± 1080, 5364 ± 936, 2304 ± 864 and 1476 ± 72 mm h^{-1}, respectively (Fig. S4). Although the addition of biochar (20%) decreased hydraulic conductivity of woodchip columns by 75%, the hydraulic conductivity was still more than five times the observed infiltration rates. The infiltration rates were measured based on the discharge rate: volume of stormwater collected in each sample divided by sampling time. Difference between discharge rates from columns with and without biochar was insignificant ($p > 0.5$).

Soil water retention curves of woodchips with and without biochar showed that the addition of biochar increased the water holding capacity of woodchips (Fig. 1). Addition of biochar to woodchips increased water retention in macropores and decreased water retention in micropores.

3.2. Effect of biochar addition on pore water chemistry in woodchip biofilters

Biochar addition affected effluent water chemistry including pH, DO, and UV_{254} absorbance (Fig. S5). Effluent pH from all columns remained consistent at 7.5 ± 0.1, irrespective of biochar fractions and stormwater application rates or hydraulic residence time (HRT). In all columns, effluent DO decreased from 9.2 ± 0.4 mg L^{-1} (influent) to 3.3–4.9 mg L^{-1} as more stormwater passed through the columns. However, the lowest effluent DO value depended on HRT. The effluent DO decreased with increases in HRT up to 18 h; any further increase in HRT did not decrease DO any further. Addition of biochar appears to decrease DO in pore water. The UV absorbance of effluents, an indicator of DOC leaching from the system, in columns typically decreased with increases in biochar fraction and decreases in HRT.

Drying duration changed the pore water chemistry (Fig. S6). Comparing changes in porewater chemistry of the effluent before and after drying, we showed that an increase in drying duration decreased DO of pore water. Addition of biochar resulted in greater DO removal for 1, 2, and 4 d drying, but not for 8 d. Increasing drying duration increased UV_{254} absorbance of effluents across all columns indicating an increase in DOC concentration in the pore water during drying. In general, woodchip columns without biochar had pore water with the highest UV_{254} absorbance or DOC concentration.

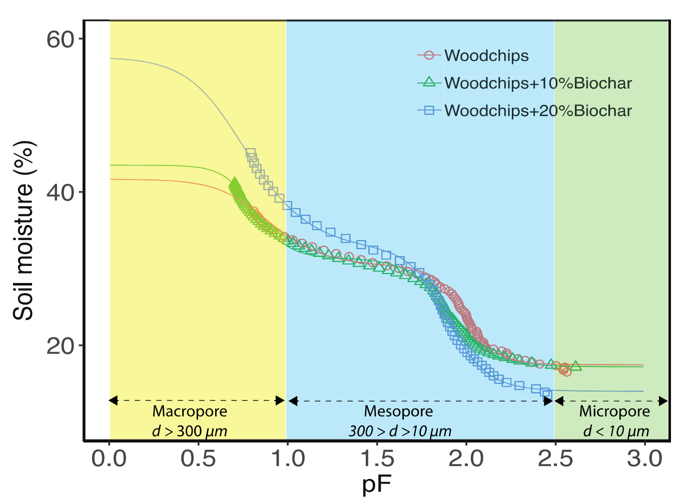

Fig. 1. The soil water retention curve for the woodchips and woodchips - biochar mixtures. The solid lines indicate the Durner (1994) bimodal model fit for the experimental data. pF is suction pressure ($\log_{10}$ suction in cm).

3.3. *Effect of biochar fraction and stormwater loading on nitrate removal*

Nitrate removal decreased with increases in the applied volume of stormwater or nitrate loading (Fig. 2). The model fits the decrease in removal capacity of biofilters. Comparing the nitrate removal capacity (worst case scenario) of all columns at the end of the experiment, we estimated that biochar-amended columns can treat at least 25% more stormwater than biochar-free woodchip biofilters before being exhausted to the same level of effluent nitrate concentration.

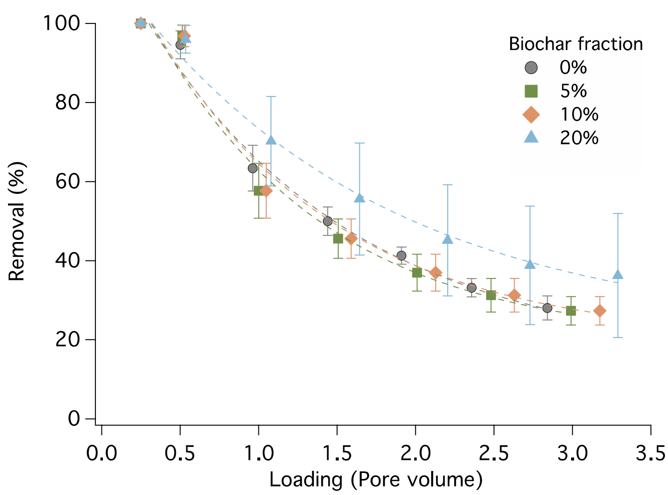

Fig. 2. Effect of stormwater loading (pore volume) on nitrate removal. Removal percentages were averaged across same column types: 0%, 5%, 10%, and 20% biochar. Error bars represent one standard deviation over mean in triplicate columns of each type. The dashed lines represent best fit to estimate exhaustion rate constant (PV^{-1}).

3.4. *Effect of infiltration velocity on nitrate removal*

Nitrate removal decreased with increasing infiltration velocity, which increases with an increase in rainfall intensity (Fig. 3). Nitrate was completely removed (100%) in all columns at low infiltration rates (19 and 37 mm h^{-1}). With increases in infiltration velocity, nitrate removal declined. A decrease in nitrate removal with an increase in contaminated stormwater exposure is defined as the rate of exhaustion (k) of the nitrate removal capacity of biofilters. The exhaustion rate decreased with increases in biochar fraction (Fig. 4a), although the positive impact of biochar was significant ($p < 0.05$) when the infiltration rate was 146 mm h^{-1} or higher. At low infiltration rate, nitrate was completely removed (or no exhaustion) in all columns irrespective of biochar fractions.

3.5. *Effect of drying duration on nitrate removal*

Increase in antecedent drying duration before stormwater application decreased ($p < 0.05$) the rate at which nitrate removal capacity of columns was exhausted during stormwater application (Fig. 4). Increase in biochar fraction decreased the exhaustion in nitrate removal capacity. Increases in antecedent drying duration increased nitrate removal for all columns irrespective of biochar content (Fig. 5).

3.6. *Effect of biochar percentage on bacterial functional gene abundance*

An increase in biochar fraction in the woodchip column was associated with increased total 16S RNA copy numbers (coefficient = 0.989, *p*-value<0.05, Table S2), but did not have any significant ($p > 0.05$) effect on the relative abundance of denitrifying genes including *napA* and *narG* (Fig. 6). During incubation, the total bacterial population had grown, which was originally at 10^7 and 10^4 copies g^{-1} on woodchips and biochar, respectively. Among the functional genes, *napA*, *narG,* and *nirK* showed significantly positive

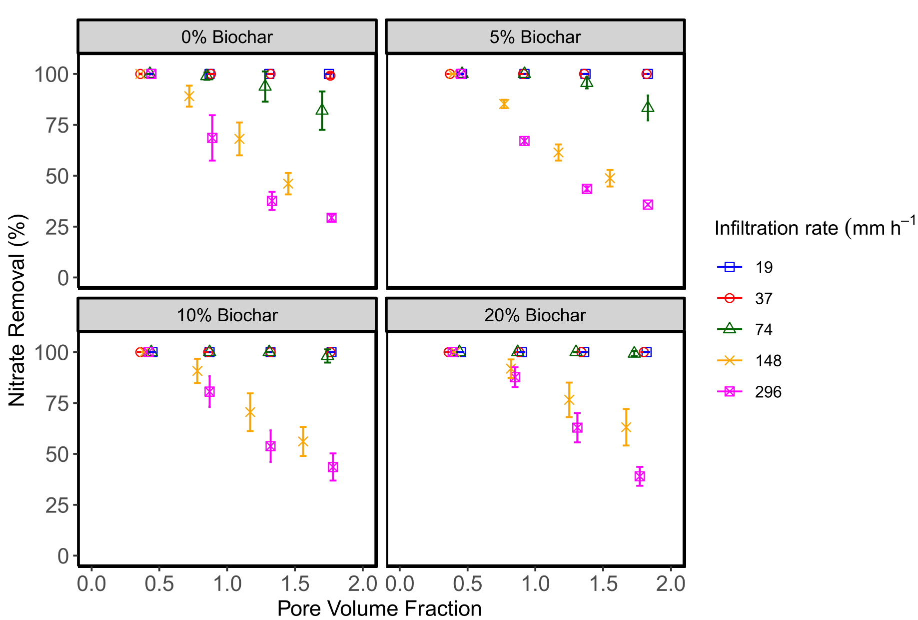

Fig. 3. Effect of stormwater application rates on mean nitrate removal in woodchip biofilter with different fractions of biochar: 0%, 5%, 10%, and 20% biochar by volume. Nitrate removal, $\left(1-\frac{C}{C_0}\right)\times 100$, after passage of different pore volumes of water during a rainfall event was compared between rainfall events with infiltration rates (loading rate). Error bars represent one standard deviation over mean in triplicate columns.

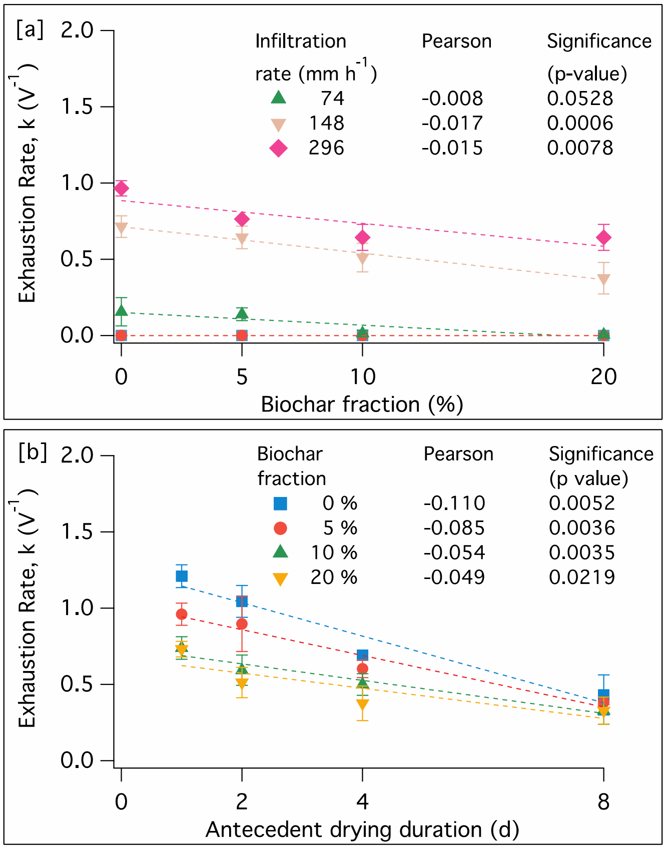

Fig. 4. (a) Increase in biochar fraction significantly decreased the exhaustion rate of woodchips biofilters at high infiltration rates (>140 mm h^{-1}). (b) Increase in antecedent drying duration significantly decreased exhaustion rate of nitrate removal capacity of woodchips biofilters.

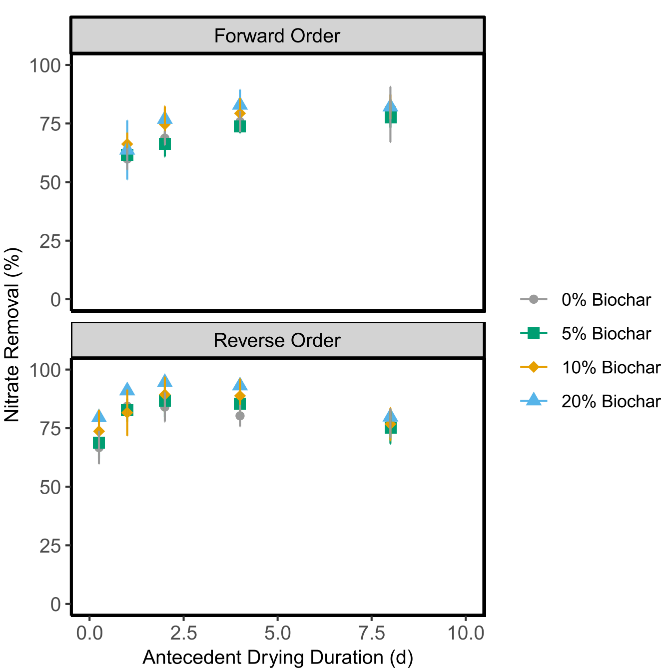

Fig. 5. Effect of increases (A) and then decreases (B) in antecedent drying durations on nitrate removal in woodchip biofilters with different fraction of biochar: 0%, 5%, 10%, and 20% biochar by volume. Error bars represent one standard deviation over mean removal in triplicate columns.

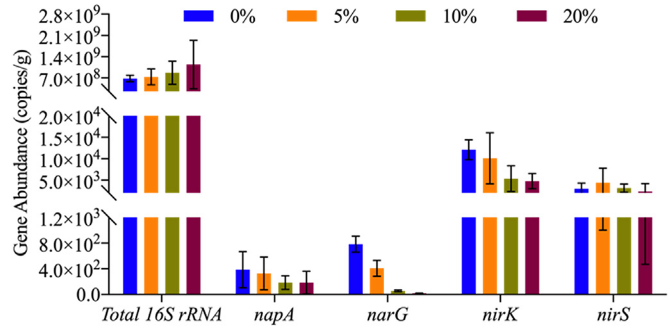

Fig. 6. Abundances of 16S rRNA, two nitrate reductases genes (napA and narG), and two nitrite reductases genes (nirK and nirS) in columns with different biochar fractions. The absolute abundance was determined using quantitative real-time PCR by normalizing the copies of the genes to the total weight of biochar-woodchip samples. Note two breaks in the y-axis to show the gene abundances in linear scale for comparison.

correlations with each other (Table S2), demonstrating their potential critical functions in the columns.

4. Discussion

4.1. Effect of rainfall characteristics and antecedent drying on nitrate removal in biofilters

In nature, heavy rainfall events deliver more nitrogen than smaller events (Higashino and Stefan, 2014; Jones et al., 2018). Intense rainfall events increase not only the runoff volume that create ponding but also the infiltration rate by increasing the moisture content of the filter layer. We found that both factors—longer duration of an infiltration event with infiltration above a threshold value and high infiltration rate—decreased the nitrate removal capacity of woodchips biofilters. The removal was high initially (<2 PV injection) due to the mixing of contaminated stormwater with less contaminated pore water remained in the submerged zone during antecedent dry periods (Wang et al., 2018). This result indicates that the performance of biofilter where HRT of stormwater is low (<5 h) can be highly variable depending on the volume of stormwater passing through the biofilter during a rainfall. Volume of stormwater runoff depends on rainfall intensity and characteristics of the catchment area that contributes to the runoff volume. Thus, biofilter size and thickness of the filter layer should be determined during design based on the catchment characteristics and historic and projected precipitation pattern.

Comparing average nitrate removal with increases in HRT, we found that nitrate removal decreased with decreases in HRT up to a certain critical value (Fig. S8). However, when HRT was longer than 18 h, nitrate removal did not change because the removal was at its maximum capacity (100%). The lowest removal was observed in woodchip columns at HRT 1.13 h. At lower HRT (<5 h), which applies to most passive stormwater biofilter, nitrate removal was higher in biochar-augmented woodchip biofilters than woodchips only biofilters. We estimated that HRT was a function of infiltration rate, not biochar fraction. Addition of biochar did not change HRT significantly ($p > 0.05$). Thus, a difference in nitrate removal between columns with and without biochar at high infiltration rate excluded HRT as a potential cause of increased nitrate removal in biochar columns. This result indicates that a change in HRT by addition of biochar could not explain observed improvements in nitrate removal.

4.2. Effect of antecedent drying on nitrate removal in biofilters

With climate change, the frequency of persistent dry condition is expected to increase. Our results showed that increases in drying duration could be beneficial for nitrate removal in biofilters with a submerged layer as observed in other studies (Lynn et al., 2015; Wang et al., 2018). We attributed the positive impact of drying duration to changes in favorable pore water chemistry during drying periods. DO concentration decreased and $UV_{254\ nm}$ absorbance of pore water increased during drying. Increase in UV absorbance of pore water after drying suggests that an increase in drying duration caused more DOC to leach into pore water. However, it should be noted that drying did not affect the moisture content in the submerged layer—the layer that potentially removes most nitrate. Thus, DOC increased during drying duration because of accumulation of leached DOC in pore water over a long period. Addition of biochar retained DOC leached from woodchips during drying conditions and helped remove nitrogen trapped inside the pores.

4.3. Effect of biochar fraction on nitrate removal

Addition of biochar to woodchip biofilters improved their nitrate removal capacity, and the effect was more apparent when the fraction of biochar added was 10% by volume or greater. Overall biochar-amended woodchip biofilters treated more stormwater than woodchip-only biofilters until the effluent concentration reached a similar value. Because mean pore volumes of biochar-amended columns were similar ($p > 0.05$) to pore volume of woodchip biofilters, pore volume did not explain an increase in nitrate removal in biochar-amended columns. Biochar could occupy the void spaces between woodchips and can decrease the porosity, similar to how it was observed in a recent study that used a mixture of coarse sand and biochar (Trifunovic et al., 2018). The positive effect of biochar on denitrification was more apparent at lower HRT (<5 h). This result confirmed that biochar addition could improve nitrate removal in one of the worst-case conditions—high loading, during high-intensity rainfall events—thereby contributing to an increase in resilience of woodchip biofilters.

4.4. Cause of increased resilience of woodchip biofilter following biochar addition

Our results showed that denitrification capacity of woodchip biofilters decreased under high-intensity rainfall events and that the addition of biochar could mitigate this concern. An increase in the denitrification capacity of woodchip biofilters by biochar can be attributed to three factors: (1) changes in hydraulic properties of a filter layer that increase the contact time of contaminated water, (2) changes in pore water chemistry that favor denitrification, and (3) increase in microbial biomass that enhances denitrification.

Addition of biochar increased net biomass but did not significantly affect denitrifying genes. A lack of correlation between biochar fraction and denitrifying genes suggests that denitrifying genes concentration did not increase despite an increase in the concentration of biomass after biochar addition. Thus, biomass increase should not be used as an indicator of the denitrifying potential of woodchips biofilters.

Biochar affected the hydraulic and pore water chemical properties of woodchip biofilters and consequently affected contaminated water availability and efficiency of the microbial communities to carry out denitrification processes. The mean pore volume, estimated by bromide tracer, did not significantly change due to biochar addition. However, bromide concentration in the effluent reflected bulk pore water concentration, which typically is affected by advection (Reedy et al., 1996). It does not account for the water within the diffusion-dominated intraparticle pore space, where a temporal change in concentration of bromide or any dissolved solute could be slow due to slow transport of solute by diffusion. Water-retention curves of woodchips with and without biochar showed that the addition of biochar increased water retention in macropore and mesopore regions. We attribute this result to the formation of intra-particle macro- or mesoporosity (Sun and Lu, 2014). Thus, the volume of immobile water could increase due to the addition of biochar, which could trap DOC leached from woodchips. Because bacteria concentration is expected to be higher near the surface than the bulk liquid due to biofilm formation, increase in the concentration of DOC or nitrate in diffusion-dominated intraparticle pore space increases utilization of DOC and consequently the removal of nitrate. Biochar also shifted the dominant pore sizes for water retention from micropores to macropores. Microbial communities typically utilize nutrients trapped in macropores or pores larger than their size. Thus, water retention in larger pores could have contributed to increasing microbial utilization of nitrate in columns with biochar.

Biochar altered the chemical properties of pore water: increased retention of DOC and decreased DO. A decrease in DO, an inhibitor for denitrification, can help denitrification. Similarly, retention of DOC in biofilters or decreasing the loss of DOC into infiltration stormwater could increase their utilization for denitrification. A high concentration of DOC near the surface of biochar or woodchips could increase the kinetics of denitrification that highly depends on the concentration of dissolved organic carbon (Hartz et al., 2017). Biochar could trap DOC in pore water or in the thin film near its surface, which can be utilized by the microbial community (Ulrich et al., 2017). Thus, we conclude that biochar-mediated alteration of the physical and chemical properties of biofilters creates more beneficial conditions for the denitrifying activity, although the microbial community remained unchanged.

5. Conclusion

Our results showed that increases in rainfall intensity and duration decrease nitrate removal in the woodchip biofilter, but the addition of biochar could improve its nitrate removal capacity by altering water holding capacity of the biofilter and chemical properties of pore water. Specific conclusions of this study are:

- Increases in storm duration or stormwater loading decreased nitrate removal in the woodchip biofilters due to exhaustion of their removal capacity. Increases in rainfall intensity decreased hydraulic residence time and decreased in nitrate removal. At high-intensity rainfall, the decrease in nitrate removal is attributed to decreases in the contact time of nitrate with biofilms, increases in DO, and decreases in DOC of pore water.
- Addition of biochar improved nitrate removal capacity of woodchip biofilters, but the improvement was sensitive to rainfall intensity or hydraulic residence time. If the hydraulic retention time was less than 5 h, then the addition of biochar helped decrease the exhaustion rate of the biofilter's capacity. But at HRT above 5 h, biochar did not provide any additional benefit on nitrate removal because woodchips alone were sufficient to remove all nitrate.
- Increase in nitrate removal by biochar addition is attributed to change in the water-holding capacity of biofilters and pore water chemistry—decrease in DO and increase in DOC retention. Biochar addition increased microbial biomass in woodchip biofilters, but it did not fully explain the increased nitrate removal as the abundance of denitrifying genes was not affected by biochar.

- Increase in antecedent drying duration increased nitrate removal in biofilters irrespective of the amount of biochar fraction. Longer drying duration helped deplete DO of pore water and increase the concentration of DOC in pore water; both conditions favor nitrate removal.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2019.115008.

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