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Microbial quality of surface water and subsurface soil after wildfire

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ABSTRACT

Runoff from wildfire affected areas typically carries high concentrations of fine burned residues or eroded sediment and deposits them in surface water bodies or on subsurface soils. Although the role of wildfire residues in increasing the concentration of chemical contaminants in both environments is known, whether and to what degree wildfire residues may affect microbial contaminants is poorly understood. To examine the effect of wildfire residues on growth and die-off of *Escherichia coli* (*E. coli)* —a pathogen indicator, we mixed stormwater with *E. coli* and suspended particles from the pre- and post-wildfire area in batch reactors and monitored *E. coli* concentration. *E. coli* grew initially in the presence of all particles, but the relative *E. coli* concentration was 10 times lower in the presence of wildfire residues than in natural soil from unaffected areas. Wildfire residues also decreased the persistence of *E. coli* during a 15-day incubation period. These results indicate that the growth or persistence of *E. coli* in surface water in the presence of wildfire residues was less than that in the presence of unburned soil particles, potentially due to depletion of nutrient concentration and/or loss of viability of bacteria in the presence of wildfire residues. To examine the transport potential of wildfire residues and their ability to facilitate the transport of *E. coli* in the subsurface system, suspensions containing wildfire residues and/or *E. coli* were injected through unsaturated sand columns—a model subsurface system. Transport of wildfire residues in sand columns increased with decreases in the depth and increases in the concentration of particles, but increased transport of wildfire residues did not result in the increased transport of *E. coli*, suggesting wildfire residues do not facilitate the transport of *E. coli*. Overall, the results indicate that wildfire residues may not increase the risk of the microbial contamination of surface water or groundwater via subsurface infiltration.

1. Introduction

Wildfire frequency is likely to increase by more than 15% based on a 2000–2050 meteorology prediction (Huang et al., 2015). Wildfire removes vegetation, increases soil hydrophobicity, and reduces infiltration, thereby increasing the volume of stormwater runoff (Rodrigues et al., 2019). Furthermore, compared with pre-fire runoff, post-fire runoff could contain 1000 times more suspended particles and contaminants including traces of metals, nutrients, total suspended solids, and polycyclic aromatic hydrocarbons (Burke et al., 2013), and it could affect the water quality of receiving water bodies and affect aquatic species (Silva et al., 2016). Although numerous studies have examined the effect of wildfire on transport of chemical contaminants to surface waters (Burke et al., 2013; Earl and Blinn, 2003; Hernandez et al., 1997; Ilstedt et al., 2003; Stein et al., 2012; Tsai et al., 2019), no study to date has examined the effect of wildfire on microbial contamination of surface water and groundwater via subsurface infiltration of stormwater containing wildfire residues and pathogen.

Post wildfire runoff can carry wildfire residues and deposit them in surface waters or in the subsurface, from where they can infiltrate into groundwater (Fig. 1). Thus, wildfire residues could mix with pathogens present in surface waters or subsurface environment and affect their fate in these systems. The fate of pathogens in surface water depends on water chemistry (Wang et al., 2019), sunlight exposure (Nelson et al., 2018), and the presence of

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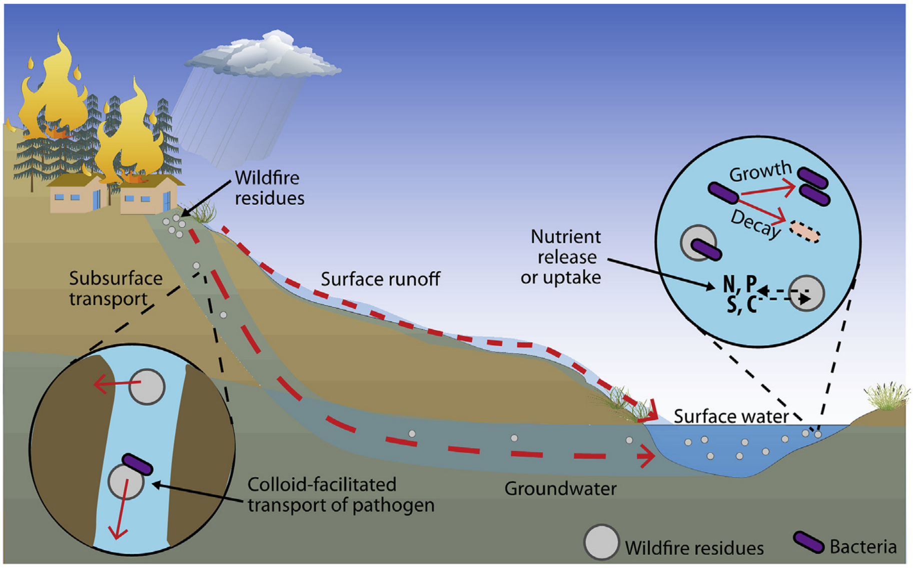

Fig. 1. Illustration of potential routes for the transport of wildfire residues in environments and their impact on microbial water quality.

particles, which may protect pathogens from inactivation by sunlight exposure (Bohrerova and Linden, 2006) or provide nutrients (Chua et al., 2009) for microbial growth. On the other hand, particles may release chemicals that are toxic to bacteria and kill them. Although wildfire events shift the microbial community in soil (Fultz et al., 2016), it is not clear whether wildfire residues could increase or decrease the survival of pathogens in surface waters. Wildfire residues primarily consist of burnt biomass such as ash, black carbon or charcoal, and soil minerals. All these particles have different chemical properties (Bodi et al., 2014; Preston et al., 2017), which can affect the growth or decay of pathogen in water. Bacteria can colonize on black carbon and form biofilm, which could protect them from disinfectants (Lechevallier et al., 1984). Small suspended solids (<20 μm) might induce the agglomeration of bacteria by acting as a condensation nucleus offering protection from antibacterial effects (Henao et al., 2018). Wildfire residues can also change the nutrient concentration in water. Thus, wildfire residues could either help increase or decrease the viability of pathogens in surface water based on the chemical composition of water containing wildfire residues.

Wildfire residues, like natural soil colloids, could be transported through the subsurface and facilitate the transport of pathogens to groundwater. Microbial contamination of groundwater has been associated with heavy rainfall events, particularly in the beginning of the wet season (Wu et al., 2016). Under this condition, the concentration of *E. coli* and coliform bacteria in groundwater could increase, partly due to particle-facilitated transport of bacteria (Zeleznik et al., 2011). The same process could be relevant after wildfire, where the concentration of particles in the runoff increases by orders of magnitude due to post-wildfire depletion of vegetation and intensification of erosion. For colloid-facilitated transport to be important in the subsurface environment, wildfire residues or colloids must be transported through the subsurface, and pathogens must remain attached and viable on wildfire residues. However, little is known about the effect of wildfire residues on growth or decay of pathogens in water.

Conditions that may affect the infiltration of pathogens through subsurface soils include soil chemical properties (Clark and Pitt, 2007), infiltration rate (Mohanty and Boehm, 2014), and pH or chemical composition of infiltrating water (Pitt et al., 1999). Suspended particle type and concentration are critical in determining the relevance of the facilitated transport of pathogens in subsurface soil (AbuSharar and Salameh, 1995). Post wildfire, the concentration of particles in runoff increases due to an increase in erosion (Lee et al., 2016). An increase in particle concentration could decrease pathogen removal in the subsurface (Muirhead et al., 2006), thereby facilitating infiltration through the subsurface (Fries et al., 2006; Jeng et al., 2005). Particles may also be filtered through subsurface soils. Thus, it is important to compare the effect of wildfire residues with that of natural soil particles to help determine the fate and transport of pathogens in surface water and subsurface environment, so that the effect of wildfire on the microbial quality of surface waters and groundwater can be assessed.

This study examines the effect of wildfire particles on bacterial viability and their transport through the subsurface. We hypothesized that the microbial transport through the subsurface would increase in the presence of particles, but the transport would depend on particle type due to the bacteria-particle association. Additionally, wildfire residues would decrease the viability of pathogens in surface waters due to the depletion of nutrients in wildfire-eroded soils. To test these hypotheses, bacteria-laden stormwater was injected at varying particle concentrations and subsurface depths, and the effluent was monitored for *E. coli* – a pathogen indicator – and total particle concentration. The viability of *E. coli* in stormwater was monitored over time in the presence of unburned soil, wildfire residues and biochar, a surrogate for black carbon generated during a wildfire.

2. Material and methods

2.1. Stormwater collection

Stormwater was collected using 20-L carboys from Ballona Creek located in Los Angeles, CA (34 0′36″ N 118 23′29″ W). The stormwater from urban areas is expected to have a very different composition than stormwater from forest watersheds or other type of catchments. Nevertheless, the Ballona Creek stormwater provides a natural water matrix for use in the study to examine the fate of *E. coli* when the stormwater is mixed with runoff from wildfire affected areas. The collected stormwater was left untouched for at least 24 h to settle large particles, and the supernatant was transferred into 1-L glass containers and autoclaved at 121 °C for 45 min.

The sterile stormwater was stored at 4 °C before being used in the experiment. We autoclaved the water to kill the native microorganisms to avoid competition with the added *E. coli* for the available nutrients. Although autoclaving water may change the nutrient composition of stormwater, the same water was used for all the experiments.

2.2. Post-wildfire residues collection

For the control study, natural unburned soil was collected from the Ballona Wetlands (33.9713, −118.4304), where there has been no fire occurrence in the last several decades. After the Woolsey Fire in November 2018, recently burned soil with wildfire residues were collected from Corral Canyon Park (34.0365, −118.7223) and from Malibu Lagoon (34.0341, −118.6812). The sampling locations were chosen based on the stormwater runoff route. The samples were collected from the top 10 cm of soil using a sterilized spatula and stored at 4 °C. Biochar particles (Biochar Supreme, Everson, WA) were used as a surrogate for black carbon without soil.

2.3. Characterization of wildfire residues

The postfire residues were characterized using Nuclear Magnetic Resonance (NMR) spectroscopy. Briefly, the ^{13}C Cross Polarization with Magic-Angle Spinning NMR spectrum was acquired on a Bruker AV-III HD 600 NMR spectrometer at a frequency of 150.9 MHz. The fine wildfire residue was mixed thoroughly, and a homogenous wildfire sample weighing 33.7 mg was packed in a 3.2 mm (outside diameter) zirconia rotor with a Vespel cap. A total of 59,657 scans were acquired with a sample spinning rate of 10 kHz using a variable amplitude cross-polarization sequence with a contact time of 2 ms and a recycle delay of 1 s. The data was processed with 50 Hz of line broadening. The analysis was repeated twice with another set of burnt residue from the homogenous mixture.

To analyze the nutrient concentration leached from soil samples, 4.0 g of different particle types were suspended into 40 mL of Milli-Q water using a 50 mL centrifuge tube, and the solution was shaken (Wrist Action Shaker, Burrel Scientific) for 24 h. The particles were removed from the supernatant by centrifugation (5000 g for 15 min), and the water chemistry of the supernatant was analyzed for nutrients (nitrate, nitrite, and phosphate), dissolved organic carbon, and total nitrogen. The results were reported in Supplementary Material.

2.4. Suspended particles solution preparation

To prepare suspended wildfire residues and unburned soil, samples were first sieved to remove particulates larger than 45 μm, and 2 g of the sieved sample was suspended in 1 L deionized water. The suspension was placed in an ice-bath and sonicated using a probe (Branson Digital Sonifier) to enhance the dispersion of particles for 15 min (on for 1.0 s, off for 3.0 s). The suspension was transferred into a 500-mL graduated cylinder, and particles with a size greater than 10 μm were settled based on Stokes Law (Details in the Supplementary Material). Particles with size lower than 10 μm were isolated for the transport study because larger particles have limited potential for subsurface transport due to filtration and gravitational settling and are expected to be deposited on the surface. 200 mL of the suspension was transferred into 50 mL centrifuge tubes, centrifuged at 4000 g for 15 min, and 45 mL of the supernatant was discarded, leaving behind 5 mL of concentrated particle suspension. The stock suspension was shaken by hand and then sonicated for 1 min prior to its use in the experiments. The particle size distribution of influent and effluent samples containing suspended solids was determined by analyzing 1.0 mL of the solution using a Particle Sizing Analyzer System (AccuSizer Model 770, Particle Sizing Systems), which determines the concentration of particles per mL and the diameter of each particle ranging from 0.55 μm to 500 μm.

2.5. E. coli K-12 suspension

Suspension of *E. coli* K-12 with resistance to kanamycin (CAS: 25389-94-0, Fisher BioReagents) was prepared following the method described in a previous study (Mohanty and Boehm, 2014). Although growth of bacteria could vary with strains (Foppen et al., 2010), and stormwater may contain a wide range of bacterial strain, we used this particular strain to eliminate growth of environmental *E. coli* or potential contamination from natural dust during the experiment. Briefly, a single colony of *E. coli* was grown in Luria-Bertani growth media (LB Broth, Miller, Fisher BioReagents), and the *E. coli* was separated from the media by centrifugation to remove the supernatant and washed with a phosphate-buffered saline (PBS) solution. The *E. coli* stock solution was added to stormwater containing particles to achieve the desired final concentration (10^3–10^5 CFU mL^{-1}). The range used in this study is within the expected concentration of *E. coli* in stormwater or surface waters (Grebel et al., 2013). For the column experiments, the suspension was mixed for 120 min using an automated shaker to ensure attachment of bacteria on particles (Vasiliadou and Chrysikopoulos, 2011).

2.6. Growth and decay of E. coli in the presence of post-wildfire residues

To examine if the presence of wildfire residues affects the growth and die-off of *E. coli* in the stormwater, 50 mL of autoclaved stormwater spiked with 10^3 CFU mL^{-1} of *E. coli* and 100 mg L^{-1} of suspended particles from different origins (a control soil, 3 soils with wildfire residues, or biochar) were mixed at 150 rpm in 100 mL glass flasks at 37 °C for 15 days. To identify the growth and die-off of *E. coli* in stormwater without particles, the experiment was repeated without particles. To monitor any change in concentration of *E. coli*, 500 μL samples were pipetted and analyzed for *E. coli* at the following time intervals: 0.3, 0.8, 1, 2, 3, 4, 7, 9, 11, and 15 days. Bacteria concentration was analyzed by inoculating 50 μL of the sample into LB agar plates with kanamycin, following spread plate and counting techniques (2 plates per sample). When the concentration was expected to be too high to count within range (>300 CFU), the PBS solution was used to dilute the sample to achieve bacteria counts between 30 and 300 CFU per plate. However, samples with low concentrations were not concentrated due to low sample volume, and the resulting low colony count below 30 was included to estimate the concentration.

2.7. Sand columns as a model for subsurface

Sand filters were used as a model to examine if the wildfire residues could migrate through the subsurface into groundwater. A coarse sand (20–30 Standard Sand, Certified MTP) with grain diameters between 0.6 and 0.85 mm was used in this study to examine the worst-case condition for subsurface infiltration. Sand was washed using deionized water for 10 min, soaked in 1M HCl solution for 6 h and then washed multiple times with Milli-Q water until the pH was near neutral. PVC pipes (2.0 cm diameter and 35 cm height) were used as columns. A screen (100 μm pore size) was placed at the bottom of the column before packing to prevent the sand particles from being washed away with the effluent. Columns were packed with sand in 15 g intervals to ensure they were packed uniformly. Deionized water was applied on the top of the sand surface at 9.0 mL min^{-1} for 4 h using a peristaltic pump (Masterflex L/S Digital Drive, Cole Parmer) in order to equilibrate the flow and wash out any small sand colloids generated during packing. Details about the pore volume (PV) estimation by the bromide tracer are provided in the Supplementary Material (Fig. S1 and Table S1, Supplementary Material).

2.8. Effect of sand filters depth and suspended particle concentration on particle removal

To examine the effect of subsurface depth on particle removal, ten sand columns with different heights (10, 15, 20, 25, and 30 cm) were assembled in duplicate and autoclaved stormwater was injected at 9.0 mL min^{-1}. To simulate pulse input, 1.0 mL of suspension containing control (unburned) soil (4.0 g L^{-1}) was injected on the top of the column using a pipette controller. 1.0 mL is sufficiently high to detect effluent concentration and low to prevent temporary ponding layer on filter layer, which could increase the flow rate and affect transport of particles or bacteria. Ten effluent samples were collected at the bottom of the column every 0.3 PV using 15-mL centrifuge tubes. The injection of suspended solids was repeated 5 times per column.

To investigate the change on particle removal due to particle type and concentration, duplicated sand columns with 20-cm depth were used, and suspensions of control soil and biochar particles were created at different concentrations: 0.01, 0.05, 1.0, 2.0, 3.0 and 4.0 g L^{-1}. The concentration range represents the concentration of particles measured in stormwater (Huey and Meyer, 2010). 1.0 mL of each particle concentration solution was injected per column, and samples were collected every 0.3 PV at the bottom of the column. Each injection was repeated 5 times per column. The volume and particle concentration of samples were measured in order to calculate the total mass of solids removed during the injection.

2.9. Transport of *E. coli* and suspended particles through sand columns

To examine the transport of bacteria with and without wildfire residues, 1 mL of suspension containing 10^5 CFU mL^{-1} of *E. coli* with 2.0 g L^{-1} of particles of either type was injected on top of the column using a pipette controller, while deionized water was continuously injected at 9.0 mL min^{-1} using a peristaltic pump. Effluent samples were collected at the bottom of the column. Influent and effluent samples were analyzed for volume and bacteria and particle concentration in order to calculate the mass balance for each contaminant during the infiltration process.

2.10. Water sample analysis

The pH of the solutions used for column and batch experiments was measured using an Ion-Selective Electrode (Fisher Scientific #9107BN), and the concentration of particles was measured using a spectrophotometer (PerkinElmer Lambda 365 UV–Visible Spectrophotometer) based on absorbance at 890 nm. The high wavelength is typically used for turbidity measurement (Mohanty et al., 2015) because at high wavelength the absorbance by color from dissolved organic carbon is negligible. Calibration curves were used for unburned and burned particles to accurately estimate the particle concentration based on the absorbance (Figs. S2 and S3, Supplementary Material). The concentration of nutrients (nitrate, nitrite, and phosphate) was analyzed using Ion Chromatography (Dionex™ Integrion™ HPIC™ System, ThermoFisher). The concentration of dissolved organic carbon, total nitrogen, and total organic carbon was analyzed using a Total Organic Carbon Analyzer (TOC-L, Shimadzu).

2.11. Data and statistical analysis

The bacteria concentration in samples was calculated by multiplying the average of colonies counted in two plates and presented as colony forming units (CFU) per mL. The relative concentration of bacteria during batch experiments was determined by calculating the ratio of the *E. coli* concentration (C) in the sample and the initial *E. coli* concentration (C_0). Total removal (R) of suspended solids through column experiments was calculated as $R = 1 - \frac{\sum C_e V_e}{C_i V_i}$ (%), where C = particle concentration (mg L^{-1}), V = volume (mL), i = influent and e = effluent. Statistical analysis was conducted using R (version 3.5.3).

3. Results

3.1. Growth and die-off of fecal bacteria is affected by particle types

Nutrient leaching results (Table S2, Supplementary Material) showed that more nutrients were leached from unburned soil than wildfire residues or fire depletes the nutrient availability in soil. NMR analysis of burned residues (Figs. S4 and S5, Supplementary Material) of wildfire residues confirmed these changes:presence of aliphatic (0–50 ppm), substituted aliphatic (50–110 ppm), aromatic/substituted aromatic (110–165 ppm), and carboxylic and carbonyl (165–215 ppm).

E. coli in stormwater grew in the presence of particles, but the extent of growth varied with particle origin (Fig. 2). Irrespective of particle types, *E. coli* concentration increased for 2–3 days (growth phase) and remained constant (stationary phase) for an additional 1–7 days based on particle types before a decrease in concentration indicating the die-off phase. The lag, growth and stationary phases (Table S3, Supplementary Material) of *E. coli* were determined following a method described elsewhere (Buchanan et al., 1997). The die-off phase was stipulated as the total concentration of bacteria started decreasing.

The extent to which the concentration increased initially or decreased after the stationary phase depended on the particle origin or type. In the absence of added particles in the stormwater, *E. coli* grew to 79 times its initial concentration by the end of 7 days; whereas in the presence of unburned soil particles, *E. coli* grew faster: the concentration increased by a factor of 250 by the end of 7 days. However, in the presence of wildfire residues or biochar particles, *E. coli* grew only by 20–30 times, which is nearly 10 times less than that observed in the presence of unburned soil particles. Additionally, the growth phase of *E. coli* was shorter in the presence of wildfire residues compared with unburned soil: in the presence of wildfire residues, the *E. coli* concentration started to decrease after 4–7 days, compared to 11 days in the presence of unburned soil particles. Within day 4 and 11 of incubation with particles, *E. coli* concentration in the presence of wildfire residues were similar ($p = 0.578$) to that in the presence of black carbon particles and significantly different ($p \lll 0.05$) to *E. coli* concentration in the presence of unburned soil particles. After 11 days, the concentration of *E. coli* was drastically lowered in the presence of wildfire residues (1–10 times its initial concentration), but the *E. coli* concentration remained high in the presence of unburned soil particles: the concentration remained 147 times the initial concentration. Furthermore, the survival rate of *E. coli* was lower in the presence of wildfire residues than in unburned soil particles after 15 days of incubation. After 15 days, the *E. coli* concentration was below the detection limit when wildfire residues and biochar

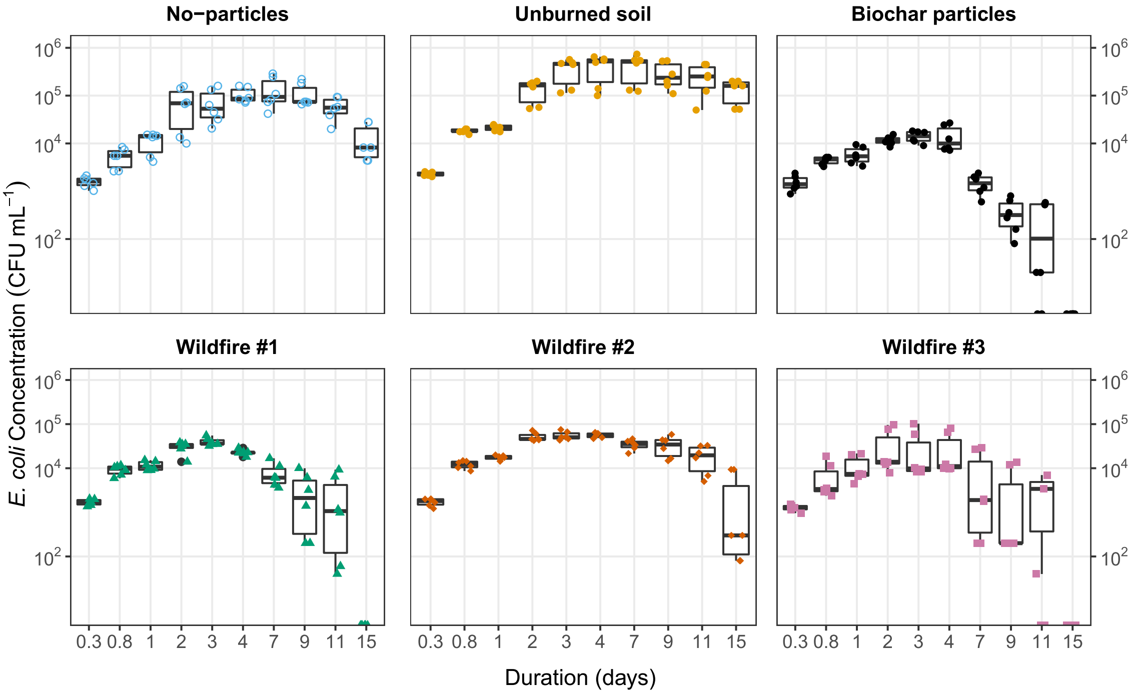

Fig. 2. Growth and die-off of *E. coli* in the stormwater without and with soil particles from wildfire affected areas, unburned soil, and biochar particles. Initial *E. coli* concentration was ~10^3 CFU mL^{-1}. Box-plot represents the concentration of *E. coli* in triplicated batches, with duplicate measurements per time point (n = 6). The detection limit is 20 CFU mL^{-1}, and the limit of statistically significant quantification on agar plate was 30 CFU on plate, which corresponds to 600 CFU mL^{-1}.

particles were present, but the concentration of *E. coli* remained high (77 times the initial concentration) in the presence of unburned soil particles after 15 days.

3.2. Removal of suspended solids depends on the subsurface depth

Suspended particles from unburned soil were removed during infiltration through sand filters, but the removal decreased with a decrease in the filter media depth (Fig. 3). The depth of filter media was negatively correlated (Pearson correlation coefficient, $r = -0.940$) to effluent peak concentration, but positively correlated (Spearman correlation $\rho = 1$) to the removal of suspended particles. The trend is similar for *E. coli* without the presence of particles (Fig. 3b). The removal of *E. coli* (also a type of particle) was consistently higher than soil particles, indicating greater adsorption or filtration of *E. coli* compared with soil particles. A three times increase in filter media depth decreased the peak height by half. An addition of a 1 cm sand layer increased the removal of suspended solids by 1.9%. Increases in influent particle concentration did not change the total concentration of effluent particles transported but shifted the distribution of concentrations of each size faction (Fig. S6, Supplementary Material): when the influent concentration was high, less small particles and a greater number of larger particles were passed through the sand filters.

3.3. Particle type affects the removal of suspended particles

Removal of suspended particles depended on particle type and concentration, but colloid-facilitated transport of *E. coli* was not observed in this study despite the transport of particles through sand filters (Fig. 4). Removal of suspended particles was 100% when particles concentration was below 0.7 g L^{-1} irrespective of the particle origin. However, particle removal decreased with increases in influent particle concentration above 0.7 g L^{-1}, and the removal rate depended on particle origin. For instance, while unburned soil was completely removed when solids concentration was 0.5 g L^{-1}, removal decreased to 62% when the suspended solids concentration increased to 3.0 g L^{-1}. Similarly, 100% of biochar particles were removed with suspended particles concentration of 0.5 g L^{-1}, but the removal slightly decreased to 96% when particles concentration was 2.9 g L^{-1}. When the suspended particle concentration was above 2.0 g L^{-1}, the sand filter removed biochar particles 10 times more efficiently than unburned soil. Removal of wildfire residues was closer to the removal of unburned soil rather than biochar particles. Although particle removal decreased with increases in particle concentration in the influent, *E. coli* were completely removed in the presence of wildfire residues, suggesting their effect on facilitated transport of *E. coli* in subsurface soil is unlikely.

Sand columns removed most of the suspended particles irrespective of the particle origin, leaving only fine particles (diameter < 3 μm) to pass through the sand filter (Fig. 5). For all particle type analyzed, the mode of particle size in the influent was higher than that of effluent samples, indicating removal of particles by sand filter. For influent solutions, the mode of the particle size distribution varied from 6.53 μm for wildfire particle #1 to 15.08 μm for biochar particles, whereas the mode of the particle size distribution of effluent was smaller, ranging from 0.55 μm for biochar particles to 1.35 μm for natural soil and wildfire residues. An increase in particle concentration in the influent solution did not significantly ($p = 0.14$) affect the particle distribution in the effluent (Fig. S6, Supplementary Material).

4. Discussion

4.1. Presence of wildfire residues in surface water suppresses bacterial growth

Our results showed that the fate of fecal indicator bacteria in

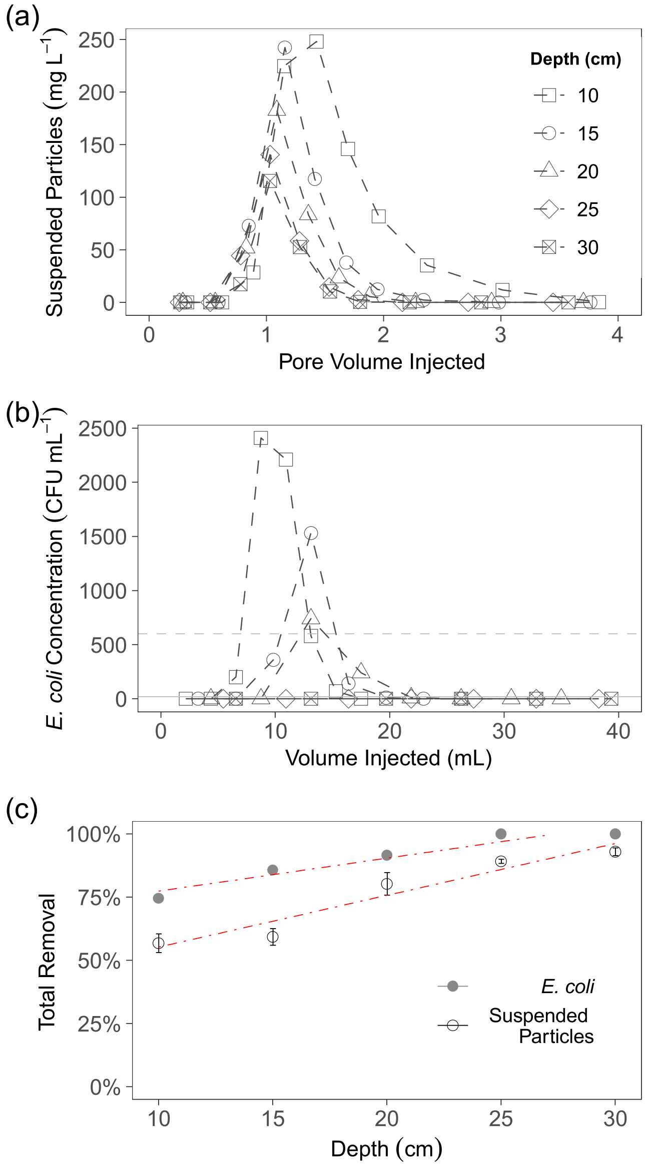

Fig. 3. Effect of sand filter depth on the removal of suspended soil particles when 1.0 mL of particle suspension (4.0 g L^{-1}) was spiked on sand column receiving particle-free water at 9.0 mL min^{-1}. (a) Suspended solids concentration peak decreased, and the centroid of peak appeared earlier with an increase in sand filter depth. (b) Decrease in bacterial transport with increase in sand filter depth. The gray solid line indicates detection limit (1 CFU on plate or 20 CFU mL^{-1}), whereas gray dashed line indicates quantification limit with statistical certainty (30 CFU on plate or 600 CFU mL^{-1}). (c) The removal (n = 10) of suspended particles and bacteria increased with increases in sand filter depth (Spearman correlation $\rho = 1$).

surface waters depended on not only the presence of suspended particles but also the source of particles or more particularly whether the soil contained wildfire residues. Wildfire residues suppressed bacteria growth and accelerated their die-off compared with unburned or unaffected soil. Natural soil particles typically contain organic matter and soil minerals, which can serve as a source of dissolved nutrients for bacteria (Friedrich et al., 1999). An alteration in nutrient concentration in water due to the presence of wildfire residue could be attributed to the observed change in microbial growth and persistence in this study. Similar results were observed in other studies with natural soil particles. For instance, increases in suspended particles content had been shown to increase nitrifying bacteria population in rivers (Xia et al., 2004) and phytoplankton growth in marine waters (Garzon-Garcia et al., 2018). However, wildfire residues are mostly composed of burned organic matter like ash and char that have less nutrients such as carbon and nitrogen than soil (Homann et al., 2011; Ilstedt et al., 2003). Thus, the decrease in nutrient leaching could suppress *E. coli* growth in our experiment. Wildfire residues including ash could also leach chemicals such as heavy metals that could be toxic to bacteria (Mitic et al., 2015). Mixing nitrate and phosphate to suspension containing similar concentration of wildfire residues, we observed negligible difference in nutrient concentration after mixing, indicating adsorption of nutrients on wildfire residues from stormwater had negligible effect on the result. One other possibility is that a wildfire residue or soil particle might attach multiple *E. coli* and make one colony on agar plate, thereby underpredicting the actual concentration of *E. coli*. This is particularly possible for biochar particles, which has higher adsorption capacity for *E. coli* than soil particles (Abit et al., 2012). In contrast to biochar, wildfire residues contain soil, ash, and a small quantity of black carbon, and the resulting mixture would have lower affinity to *E. coli* than biochar. The result could vary with properties of burnt materials, and mixing of other particles during their transport to surface waters. Overall, the result indicates that the export of wildfire residues to surface water would not increase pathogen concentration more than it would due to the deposition of unburnt soil.

4.2. Particle removal improves with increases in subsurface depth and lowers suspended particle concentration

Subsurface soil depth could vary from less than a meter to hundreds of meters. Thus, it is important to understand whether subsurface depth can influence potential groundwater contamination from wildfire residues. Injecting biochar or unburned soil residues (two extreme cases), we showed that increases in subsurface depth increased the removal of wildfire residues, but the removal decreased with increases in particle concentration. Increases in removal with increases in subsurface depth can be attributed to longer hydraulic retention time and increase in adsorption sites (Li and Davis, 2008; Mitchell et al., 2011). However, the removal decreased with increases in particle concentration, potentially due to the exhaustion of attachment sites. When the influent particle concentration was 2.0 g L^{-1}, only 62% of unburned soil particles were removed, which is significantly lower than the removal of biochar particles (96%) under the same condition. The result indicates that burnt residues have a stronger interaction with sand particles and are easier to be removed in subsurface soils.

4.3. Subsurface removal of wildfire residues are similar to unburned soil rather than biochar

The removal of wildfire residues by sand filters was similar to that of unburned soil particles than biochar particles. At particle concentration higher than 0.7 g L^{-1}, the removal of biochar particles was around 96%, which is significantly higher than the removal of unburned soil and wildfire particles (44%–68%). Biochar particles can serve as the nucleus of aggregation (Lehmann et al., 2011), forming larger colloids that are more likely to be removed than fine wildfire residues or soil particles. Furthermore, a change in surface properties of soil during wildfire could affect their removal. NMR analysis of wildfire residues confirmed the changes, consistent with a previous study (Otto et al., 2006). The result suggests that a significant portion of wildfire residues contain black carbon and ash in addition to soil. Wildfire residues have been shown to have a high content of aromatic carbon, which decreases their polarity and increases their water repellency properties (Knicker et al., 2006).

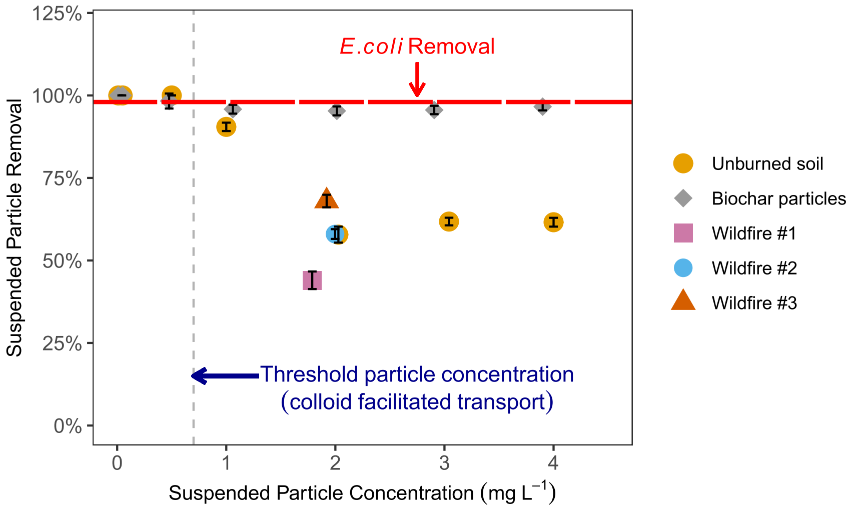

Fig. 4. Removal of particles of different origin and *E. coli* in 20-cm sand columns. The vertical dashed line represents the threshold particle concentration above which colloid-facilitated transport of bacteria is possible. The mean removal (n = 10) of particles varied with particle types and concentrations. Error bars represent standard deviation over mean. The horizontal dashed line indicates maximum removal (98%) of *E. coli*, owing to concentration of *E. coli* in the effluent below detection limit (20 CFU mL^{-1}).

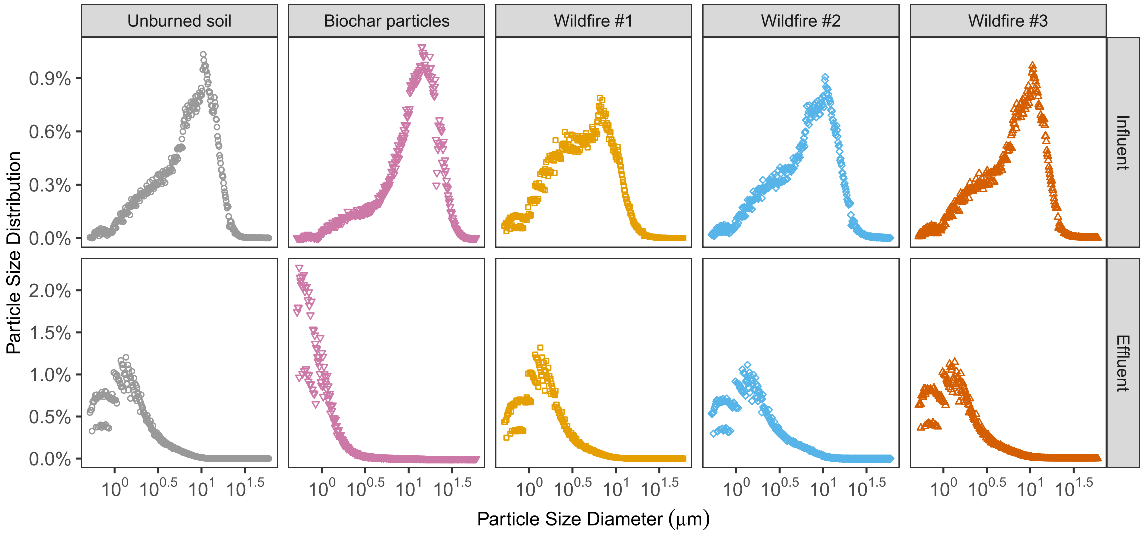

Fig. 5. Size distribution of suspended particles in influent (top) and effluent (bottom) samples varied with particle origin. Particle concentration in influent samples was 2.0 g L^{-1}.

An increase in water repellency was previously attributed to increased removal of wildfire residues in sands (Goebel et al., 2013).

Removal of biochar particles was higher than that of wildfire residues suggesting biochar may not be a good surrogate to predict the transport of wildfire residues in subsurface soil. A difference is attributed to how both are formed under intense heat. Although wildfire residues and biochar are formed under similar temperature conditions (~800 °C), biochar is produced in the absence of oxygen while wildfire residues are formed in the presence of oxygen, resulting in higher ash content. This key difference in production conditions appears to affect their removal during infiltration through the subsurface.

4.4. Colloidal particles (<3 μm) are poorly removed through subsurface infiltration

During subsurface infiltration, most particles regardless of their origin or types with size greater than 3 μm were removed. The particle size distribution of effluents indicates that finer particles were present in larger quantity when biochar was injected compared with unburned soil and wildfire residues. The effluent particle size range is similar to that of bacteria, which indicates that bacteria could move through the sand filter under the same conditions unless the interaction of bacteria with sand is stronger than the interaction of wildfire residues with sand. However, *E. coli* concentration in the effluent was below the detection limit in the effluent, indicating bacterial interaction with the sand surface was high. The presence of fine colloids in the effluent did not increase bacteria transport, suggesting colloid-facilitated transport of *E. coli* in the presence of wildfire residues is unlikely. In fact, colloid retarded transport of bacteria was observed in our study. Without soil colloids, bacteria removal in 10-cm columns was around 75%, which increased to near 100% with an increase in depth by 10 cm (Fig. S6, Supplementary Material). In the presence of suspended particles and bacteria, the removal of bacteria in 20-cm sand columns remained at 100% irrespective of nature of particles. The results contradicted the result in some previous studies (Muirhead et al., 2006; Walters et al., 2013), which showed that *E. coli* predominantly attached to suspended solids with particle diameter lower than 12 or 20 μm. In our study, particles larger than 3 μm were filtered out on top of the filter layer, and the deposited particles could block pores or flow paths in sand layer, thereby increasing removal of suspended bacteria and colloid-associated bacteria. The particle size distribution analysis showed that biochar particles were more mobile than wildfire residues and unburned soil, and the relative size in the effluent for biochar particles was smaller than wildfire residues and unburned soil. Soils from affected and unaffected regions have similar particle size distribution, which suggests that the effluent might be dominated by soil minerals rather than burned black carbon that might be a small fraction of total mass. But the presence of these particles did not affect *E. coli* concentration in the effluent, suggesting their deposition in subsurface soil would not increase microbial risk.

We used one strain of *E. coli.* However, the fate and transport behavior of *E. coli* could vary based on type of strains within species (Bolster et al., 2009) or by different types of pathogen species (Haznedaroglu et al., 2009). Thus, the result presented in this study could vary based on strain types. It should be noted that the transport of virus in the presence of wildfire could be much higher than the transport of bacteria, as unlike bacteria, removal of virus is minimal due to its small size (Sasidharan et al., 2016). Thus, future studies should include virus and actual pathogens, instead of indicator bacteria used in this study.

5. Conclusion and environmental implications

The study answered the question of whether a rainfall event following a wildfire, which help transport wildfire residues to surface waters or through subsurface soil, could increase the risk of microbial contamination of surface waters, subsurface soil, and consequently groundwater. Specific conclusions are:

- The presence of wildfire residues in surface water reduces the growth of indicator bacteria and accelerates their die-off when compared to unburned soil, suggesting microbial risk post-wildfire is minimal.
- Wildfire residues have a limited effect on the transport of pathogen through subsurface soil, although transport of these particles increased when their concentration exceeded 0.7 g L^{-1}.
- Transport of biochar particles in subsurface soil was less than wildfire residues, indicating biochar may not be a good surrogate to study the transport of wildfire residues in subsurface soils.

This study is the first study to examine potential implication of wildfire residues on microbial water quality of receiving water bodies. The result shows that wildfire residues may not have a measurable negative impact on microbial water quality because of the decrease in subsurface transport and the low viability of indicator bacteria on wildfire residues relative to natural soil particles. The results prove that wildfire residues can impair the growth of bacteria and may have wide implications on other natural processes. Naturally, soil and water contain billions of non-pathogenic bacteria, which serve many ecosystem functions such as biodegradation of chemical pollutants and nutrient cycling. Thus, the presence of wildfire residues could also have detrimental effect on these processes. Wildfire residues and their surface chemistry can also vary based on the condition and sources. Future studies should examine the effect of wildfire on the basis of different components such as ash type, black carbon, and soil mineralogy.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2020.115672.

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