Plant and Fungi Amendments to Bioretention for Pollutant Reduction over Time

Final Report to Washington State Department of Ecology
Stormwater Action Monitoring

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1. Introduction

The Western Washington Phase I and Phase II Municipal Stormwater General Permit requires the use of Low Impact Development (LID) where feasible to reduce the volume of stormwater runoff entering receiving waters and to improve water quality. The Stormwater Management Manual for Western Washington (SWMMWW) specifies design criteria for LID systems. Bioretention is a very commonly used LID approach composed of a layer of engineered soil media of mixed 60% sand and 40% compost by volume between a layer of mulch (surface) and drainage gravel (bottom).

Regional bench and field scale bioretention studies have indicated significant export of nutrients and dissolved copper from bioretention systems (Herrera 2014), which could be hazardous for some receiving waters. Despite this, bioretention treatment prevented acute lethal and sublethal toxicity in a variety of aquatic organisms exposed to roadway runoff (McIntyre et al. 2015, McIntyre et al. 2014, Spromberg et al. 2016). Only a small number of storm events were treated in these studies, raising the question of how bioretention performs in terms of biological effectiveness during constant stormwater loading conditions as bioretention systems age.

Plants are added to bioretention systems to improve aesthetics, requiring regular maintenance, particularly during establishment, and increasing costs if plants need to be replaced (EPA 2019). Treatment benefits of plants, in theory, include improved water quality by taking up contaminants into plant tissues, and maintenance of hydraulic conductivity by creating preferential flow paths, thereby delaying the clogging effect of fines that build up over time. However, the ability of plants to generate these benefits in bioretention systems is equivocal in the scientific literature (Skorobogatov et al. 2020), raising the question of whether plants are necessary in bioretention systems.

Fungi may also provide benefits for treatment of stormwater in bioretention systems. For example, the mushroom-forming fungus *Stropharia rugoso-annulata* grown on alder (*Alnus rubra*) wood chips yielded a 20% improvement in *E. coli* removal relative to wood chips alone ($p<0.05$) under laboratory conditions (Taylor et al. 2015).The research also indicated that *S. rugoso-annulata* is resilient to the year-round environmental conditions of a Puget Sound stormwater bioretention setting such as alternating wet and dry intervals and temperature extremes from 0 to 40 °C. Earlier work on *S. rugoso-annulata* indicated that this species will degrade polycyclic aromatic hydrocarbons (PAHs) in contaminated soil (Steffen et al. 2007). Fungal biomass has also been studied as an effective sorptive agent with the ability to take up nutrients (Poor et al. 2018) and bind significant amounts of copper from aqueous solutions (Simonescu & Ferdes 2012). Replicated field data is needed to determine whether field performance justifies incorporating this fungus as a part of the wood mulch in bioretention installations.

1.1. Study Objectives

The objectives of this study were to assess whether biological elements such as plants and fungal-inoculated mulch incorporated in bioretention systems provide meaningful long-term benefits to water quality. An additional objective was to monitor treatment performance (hydraulic and water quality) of bioretention installed in an urban watershed over two years.

2. Study Design

2.1 Treatments

Twelve under-drained bioretention mesocosms were constructed for the study. Six of the twelve bioretention mesocosms were planted with Pacific Ninebark (*Physocarpus capitatus*), receiving 3 plants each. In six of the mesocosms the mulch layer was inoculated with mycelium of the wine cap mushroom (*Stropharia rugoso-annulata*). These two amendments of the bioretention system, incorporated in a full factorial approach, resulted in four treatments with three replicates each (Table 1).

Table 1. Bioretention treatments used in study

Treatment Label	*n*	Explanation
BSM	3	Bioretention soil medium with mulch
BSM + F	3	Bioretention soil medium with fungi-amended mulch
BSM + P	3	Bioretention soil medium with mulch and plants
BSM + F + P	3	Bioretention soil medium with fungi-amended mulch and plants

2.2 Endpoints and Frequency of Measurement

Bioretention effectiveness for treating stormwater was determined by comparing chemistry and toxicity of influent stormwater to treated effluent water. Water chemistry was monitored approximately quarterly resulting in data on water chemistry and toxicity for 8 storm events across the 2-year study (Table 2). Chemistry of the bioretention soil media and/or its components was measured to assist with interpretation of water chemistry results. Nutrient and metal concentrations were measured in the compost and sand of the BSM prior to mixing. Metals and PAHs were measured in BSM after experimental cells were conditioned with clean water, and again at the end of the study. One opportunistic sampling of PAHs in BSM was additionally conducted five months after stormwater treatment began.

Additional measurements included soil temperature, soil moisture content, saturated hydraulic conductivity, and the growth of plants and fungi. Soil temperature and moisture content were recorded throughout the study by probes installed in the BSM. Saturated hydraulic conductivity was measured after compaction during installation and during the last three quarters of the study. Plant mass was measured at the start and end of the study, but only qualitative results are presented as explained below. Fungal growth was measured indirectly at the end of Year 1 and again at the end of the study (Year 2).

Results for hydrology, chemistry, and toxicology for both years of the study are presented below and detailed results are provided in Appendix 1 (summary tables of water chemistry), Appendix 2 (summary of sublethal impacts on zebrafish morphometrics), Appendix 3 (copies of analytical laboratory reports for soil chemistry), and Appendix 4 (copies of analytical laboratory reports for water chemistry).

2.3 Field Site Location and Preparation

The experimental bioretention cells were placed in an urban watershed in Seattle, WA; under the ship canal bridge of I-5 (N47°39'22.2"; W122°19'19.4") at a site owned by Washington State Department of Transportation and operated by the Washington Stormwater Center (Figure 1). Cells were placed in two offset rows oriented approximately north-south at the eastern edge of the site, partially covered by the northbound off-ramp at exit 169 (Figure 2). Cells were surrounded by 10 yards of fill to provide some thermal inertia as an approximation for in-ground conditions. Fill was covered with black plastic.

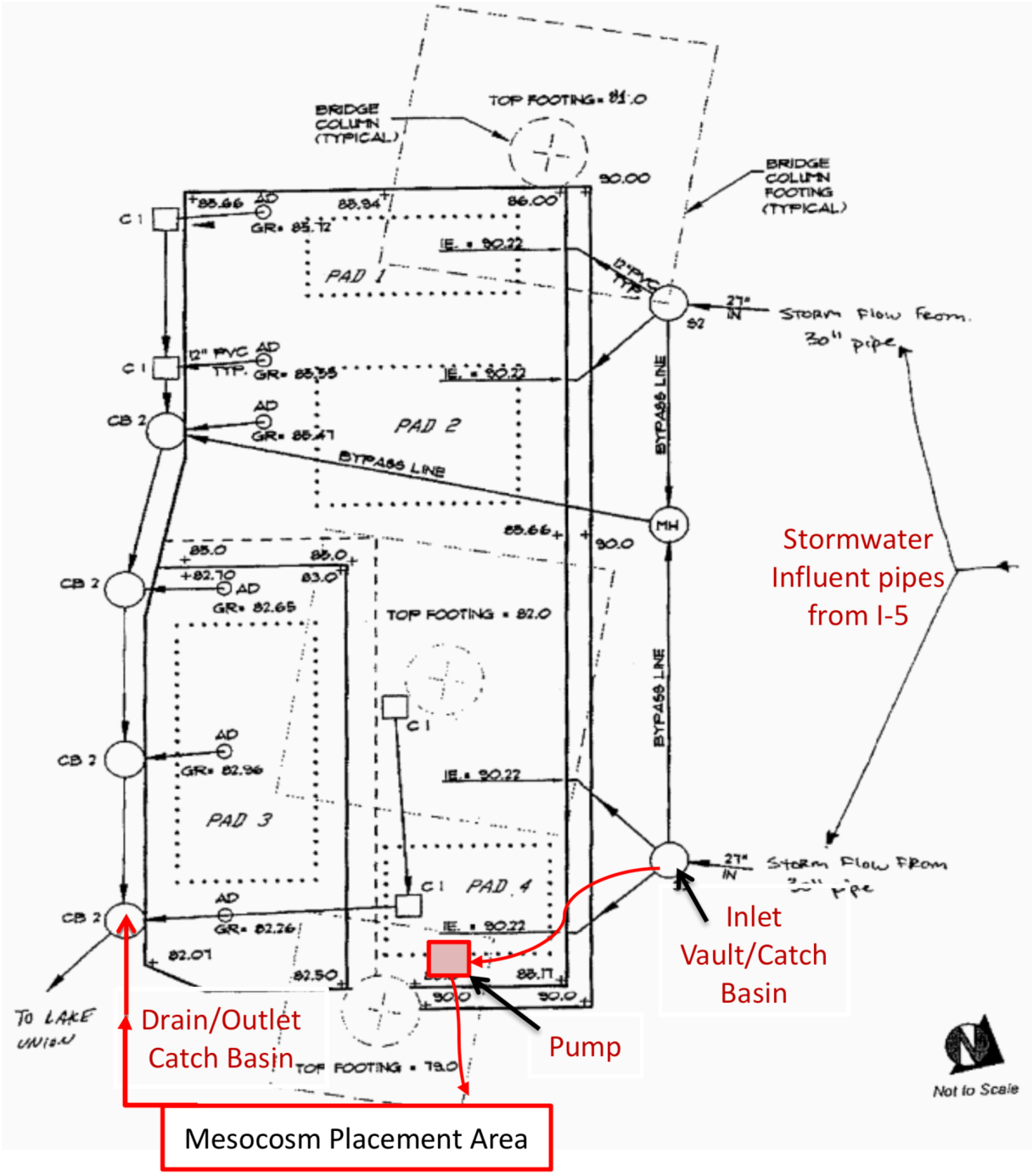

Figure 1. Placement of the bioretention cells (mesocosms in diagram) at the WSDOT site under the Ship Canal Bridge of I-5 in Seattle, WA, showing locations of vault from which stormwater was pumped and drain to which effluent was directed.

Figure 2. A) Location of study site under northbound I-5 in Seattle (small white rectangle). B) Bioretention cells surrounded by fill for thermal inertia, showing white standpipes and underdrains on right.

2.4. Bioretention Soil Mix (BSM)

The default Washington State bioretention soil mix (BSM) was prepared according to the Stormwater Management Manual for Western Washington (SMMWW). Two cubic meters of compost and two cubic meters of sand were donated by Cedar Grove Composting, Inc. in August 2016. The product specification sheets provided by Cedar Grove documented that the material met specifications set forth in the SMMWW (see Deliverable 2: Report on bioretention soil preparation).

To achieve a well-mixed BSM of 60% sand : 40% compost by volume, small batches of BSM were made from un-compacted sand (15 L) and compost (10 L). Compost was sifted through a 0.5-inch screen (100% passing) to break up clods and achieve a relatively even compost density prior to volumetric proportioning. Sand and compost were proportioned by volume in 5-L increments and the wet weight for each increment was recorded. Each volume of sand or compost was randomly collected from the sand or compost pile. A composite sample was collected from each 25-L batch for moisture analysis. Samples for moisture content assessment were collected from the sand and compost fractions between every fifth batch. These measurements provided the data necessary to calculate the dry mass of sand and dry mass of compost in each 25-L batch. In total, 89 individual batches (25 L and 29.0 ± 1.2 kg

(wet)) were prepared. This data was used later to proportion BSM in each of twelve field bioretention cells by total dry mass.

2.4.1 Installation of Bioretention Cells

Each of the twelve bioretention cells were constructed in 55-gallon (217 L) stainless steel drums (57-cm diameter, 84.8-cm height), each containing a 5-cm diameter slotted underdrain (commercial well casing pipe). The drain pipe was installed with the trough of the pipe 3.8 cm above the bottom of the drum. The drain pipe was imbedded in City of Seattle type 26 drain aggregate such that the gravel drainage layer extended 15.2 cm above the crown of the pipe. The total drainage layer depth was 24 cm. Bioretention soil media (BSM) was added to each drum based on total dry mass (145 ± 2.8 kg) and was tamped down to achieve a compaction roughly equivalent to 85% of the modified maximum dry density (as defined by ASTM D1557) per SMMWW specifications. This compaction resulted in a BSM bulk density of 1.41 ± 0.04 g/cc and soil depth of 40.1 ± 1.0 cm.

Six of the twelve bioretention cells were planted with Pacific Ninebark (*Physocarpus capitatus*). Each replicate of the BSM+P and BSM+P+F treatments received three small (130 ± 90 g each) bareroot transplants. Plants were divided among the drums by plant bareroot biomass such that each planted drum received a total of 400 ± 50 g of Pacific Ninebark. All twelve bioretention cells received a 7.6-cm top layer of alder (*Alnus rubra*) mulch (60% wood chips, 40% coarse sawdust by volume). Mulch was distributed by wet mass (10 ± 0.4 kg per bioretention cell). Mulch used in six of the cells (3 BSM+F and 3 BSM+P+F) was inoculated by Fungi Perfecti (LLC, Olympia, WA) with mycelium of the wine cap mushroom (*Stropharia rugoso-annulata*). The alder mulch and fungal inoculum were donated by Fungi Perfecti. Inoculated cells received 6.6 ± 0.3 kg of mycelium-infused alder mulch plus 3.4 ± 0.1 kg of alder mulch without added fungal mycelium. Every bioretention cell was equipped with a probe to measure soil moisture and temperature at 5-min intervals (Decagon Devices 5TE) and corresponding digital data loggers (Decagon Devices EM50). Soil probes were placed in the center area of each drum after 50% of the soil mass had been added (approximately 20 cm below the soil surface).

2.5. Stormwater Distribution System

A peristaltic pumping system was installed by collaborating King County staff to distribute runoff and log total runoff flow from the stormwater vault to each of the bioretention cells. This system provided urban stormwater runoff at a loading rate of 0.12 L/min whenever it rained approximately 2.4 mm (0.1”) or more within a 2-hour period in the watershed. This loading rate was chosen to ensure a realistic volume of runoff was treated across each year of the study (i.e. corresponding to approximately the annual rainfall expected on the contributing area).

A float switch located inside the vault triggered the pumps to turn on. A flow meter and totalizer on the pump inlet measured the rate and total volume of stormwater dosed to the

cells. Flow at the pump inlet was monitored continuously throughout the deployment period, except for maintenance when the pumps were turned off (i.e. clogged tubing, power outages, and vandalism). We verified the flow rate measurements with a graduated cylinder approximately monthly. We also observed occasional periods between rain events when sufficient flow was present in the vault to trigger the pumps, which complicates understanding the rainfall-to-dosing relationship. Any increased flow that triggered the pumps was applied to the mesocosms; however, its source remains unknown. To irrigate the plantings during the summer months, we rerouted the pumps to draw clean municipal drinking water from a 250-gallon truck-mounted polyethylene tote.

Semi-rigid Teflon was used for the inlet and distribution tubing, while the peristaltic pump tubing was flexible platinum cured silicon (Figure 3). Distribution manifold blocks were made from high density polyethylene (HDPE), while connector fittings were nylon. Summer irrigation water was withdrawn from the polyethylene tote using HDPE tubing and distributed by the same pump using the stormwater distribution lines.

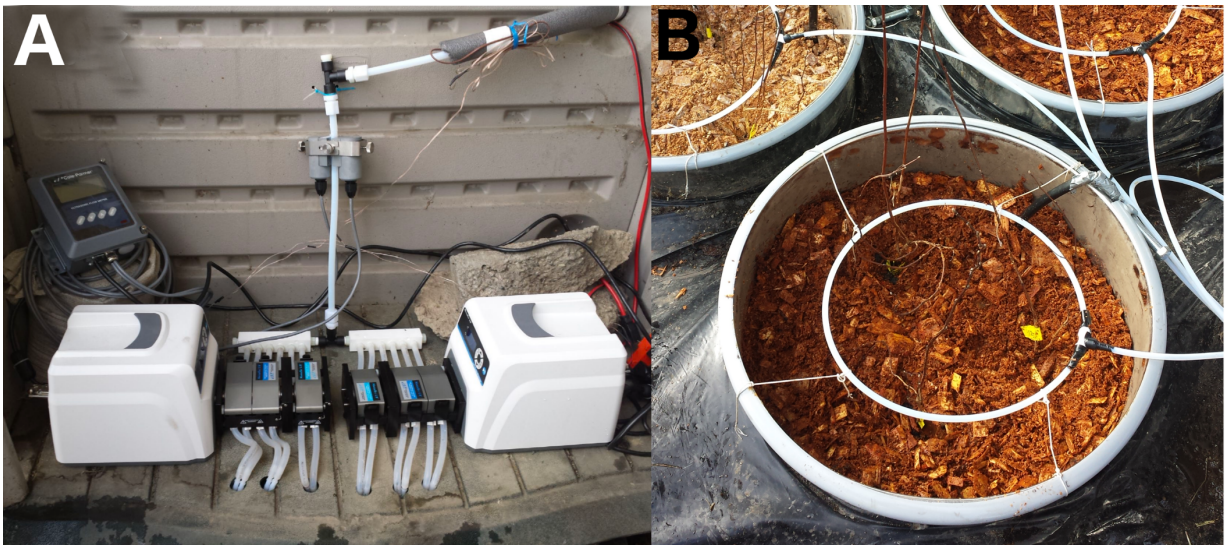

Figure 3. Dosing system for stormwater from the vault at the I-5 ship canal site to each of the 12 bioretention cells. A) A flow meter measured volume of incoming stormwater to the pumps. Each peristaltic pump fed six cells. B) Tygon tubing distributed stormwater to each cell.

2.6 Stormwater Sampling

The bioretention cells received runoff from any rainfall event that produced enough water to float the switch that turned on the peristaltic pumps located in the storage vault. Larger storm events on an approximately quarterly schedule were sampled. For each sampled storm a total of 15 composite water samples were collected — three influent samples and 12 effluent samples (one for each cell). These composite water samples (along with field blanks

and duplicates) were then analyzed for water quality parameters including nutrients, metals, bacteria, and PAHs, and were tested on zebrafish embryos for toxicity.

A total of 8 storm events were sampled; five during Year 1 and three during Year 2 (Table 2). More precipitation fell than expected during spring 2018 (Year 2) such that by Event 6 51% more runoff from the contributing area had been treated than expected based on an average year. Vandalism of equipment at the site during autumn 2018 (Year 2) prevented stormwater treatment from November 19, 2018 to March 9, 2019, resulting in cumulative runoff across the study of only 14% above expected based on average precipitation patterns (Table 2).

Table 2. Dates and timing of sampling events during the study.

Sampling Event	Date	Days Since Installation	Cumulative Stormwater Treated per Mesocosm (m^3)	Equivalent Cumulative Precipitation (1:20) (cm)	% of Expected Precipitation Treated
1	April 5, 2017	49	1.2	25	73%
2	June 8, 2017	113	2.0	40	84%
3	Oct 18, 2017	245	2.4	47	73%
4	Dec 19, 2017	307	3.9	76	83%
5	Mar 22, 2018	400	6.5	126	98%
6	Oct 25, 2018	617	12.6	248	151%
7	Jan 23, 2019	706	*	*	121%
8	Mar 12, 2019	754	13.0	254	114%

* Pumps not operating Nov 2018 – Mar 2019 (manually operated for Jan 2019 sampling event)

3. Study Results

3.1. Bioretention Amendments

3.1.1 Plants

The bareroot ninebark plants added to half of the bioretention cells in February 2017 (Figure 4A) grew well during their first spring (Figure 4B), but 50% died during the record-breaking summer 2017 drought (Table 3), despite weekly supplemental watering. Dead plants were replaced in February 2018, one year following installation, in the same manner as the original planting (bare root after over-wintering outdoors in pots). Dead plants were therefore present for 2 of the 8 sampled events (Oct & Dec 2017). During this time, one replicate of BSM+F+P had no live plants, whereas the other treatments with plants contained between 1 and 3 live plants. The new plants became established during Spring 2018.

Table 3. Plant survival across the first year of installation (Feb 2017-Jan 2018).

			Number of Plants in 2017	
Treatment	Replicate	Mesocosm #	Start	End
BSM	1	1	0	0
	2	5	0	0
	3	10	0	0
BSM+F	1	4	0	0
	2	7	0	0
	3	12	0	0
BSM+P	1	2	3	1
	2	6	3	2
	3	9	3	2
BSM+P+F	1	3	3	1
	2	8	3	3
	3	11	3	0

When the experiment was dismantled at the end of Year 2 (May 2019), plant masses were measured from each replicate cell, but the data was unfortunately lost. Qualitatively, plants replaced at the beginning of Year 1 (Feb 2018) were very successful (Figure 4C), with lush foliage and roots that grew down into the gravel layer. Most root growth was along the edges of the drum following the path of least resistance. The roots of plants from the original 2017 planting were not as well developed. No plants died during the remainder of the study.

Figure 4. Progression of plants in bioretention cells. A) Bareroot ninebark plants appear as twigs; February 16, 2017. Paler mulch was inoculated with fungi. B) Leaf growth on plants during their first spring; May 5, 2017. C) Five months after plants that died during Year 1 were replaced; July 24, 2018.

3.1.2 Fungi

3.1.2.1 Fungi at the end of Year 1

As a litter-decomposing fungus, *S. annulata-rugosa* decomposed the alder wood mulch, decreasing its mass over time. In half of the bioretention cells, alder mulch was inoculated with *S. annulata-rugosa*. Before the end of 2017 (Year 1) fungi were observed growing in all of the treatment cells, not just those inoculated as BSM+F and BSM+F+P. In Jan 2018, approximately 10 months after installation, we quantified the relative abundance of fungi across treatments by 1) measuring the mass of mulch remaining in each cell and 2) respiration of the mulch layer in fungi vs no-fungi treatments (change in mass across 5 days in the laboratory). All mulch was gently collected from the top of each cell and placed in a ventilation-controlled plastic bag used by the mushroom industry to monitor fungal respiration. These bags were placed in an air-conditioned room at the WSU-Puyallup Aquatic Toxicology Laboratory and the mass of mulch measured over five days. Decreases in mulch mass from installation values (Feb 2017) indicated microbial respiration since the beginning of the experiment. There was significantly less mulch in the fungal-inoculated treatments (BSM+F, BSM+P+F) than in treatments with mulch that had not been inoculated (BSM, BSM+P) (Figure 5A), indicating that the inoculated fungi had decomposed more mulch than had decomposed in treatments that did not originally contain fungi. During the laboratory respiration test, respiration rate increased as the mulch increased to room temperature and then remained steady. Significantly more mass was lost (up to 2-fold) from inoculated treatments than uninoculated treatments (Repeated Measures ANOVA: $F(1,10)=11.101$, $p < 0.008$)(Figure 5B). Visually, much more fungal mycelia were evident in the inoculated mulch. Together, these data support that although fungi did colonize all treatments, they were still more abundant after 10 months in the fungi-inoculated treatments than in the no-fungi treatments.

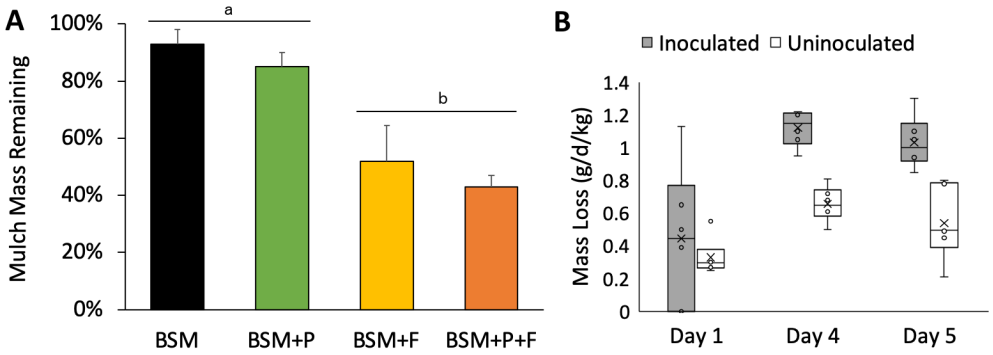

Figure 5. Assessment of fungal contamination of uninoculated treatments (BSM, BSM+P) compared with inoculated treatments (BSM+F, BSM+P+F) 10 months after installation. A) Average mass of mulch remaining in each treatment group in January 2018. Error bars are one standard deviation. Treatments sharing a letter are not statistically different. B) Microbial respiration of inoculated and uninoculated mulch in laboratory experiment during January 2018. Respiration rate was significantly higher in the inoculated treatments ($p = 0.008$).

3.1.2.2. Fungi at the end of Year 2

At the end of Year 2, the remaining mulch from each bioretention cell was collected and wet and dry masses determined (Figure 6). Moisture content (100 – %Solids) and dry mass were assessed by multivariate general linear model (GLM) with treatment as a fixed factor and post-hoc differences among treatments assessed using a Tukey test. Moisture content of mulch remaining at the end of the experiment (Figure 6A) was significantly different among treatments ($F(3,12) = 9.090$, $p = 0.006$), with significantly less moisture relative to BSM in the two treatments with plants (BSM+P, Tukey: $p = 0.005$; BSM+P+F, Tukey: $p = 0.020$). Average mulch moisture was also lower in the treatment with fungi (BSM+F; 23%) relative to BSM (33%) but the difference was not statistically significant (Tukey: $p = 0.117$). The mass of remaining mulch was significantly greater in the treatments without fungi ($\bar{x} = 0.62$ kg) than the treatments with fungi ($\bar{x} = 0.23$ kg), indicating that significantly more decomposition had occurred with fungi present ($t(10) = -2.461$, $p = 0.034$).

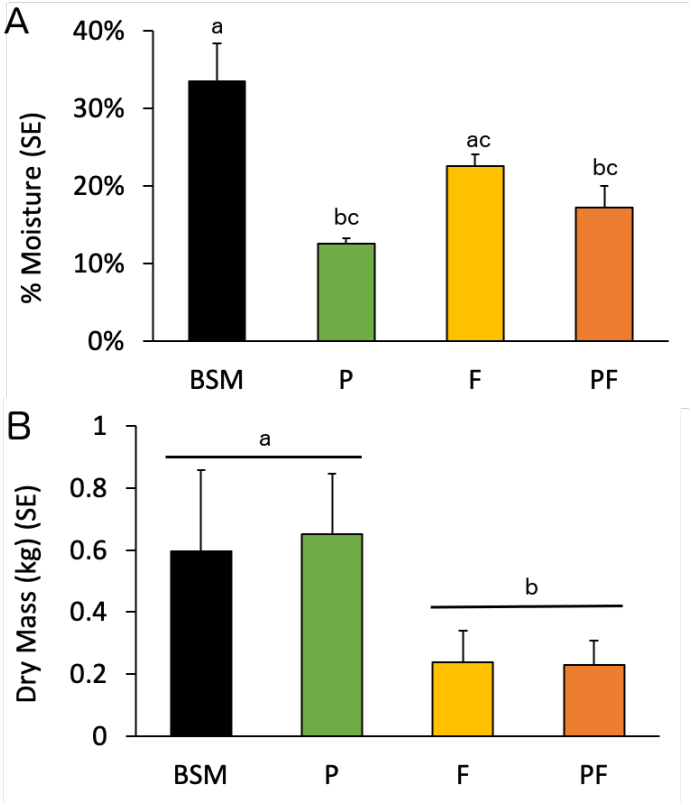

Figure 6. Mulch remaining at the end of the experiment was A) significantly drier for treatments with plants than in the unamended BSM, whereas B) BSM amended with fungi (with or without plants) showed significantly more decomposition. Treatment designations correspond to the descriptions in Table 1. Values are averages of the three replicates with error bars ± one standard error of the mean. Treatments sharing the same annotated letter are not statistically different. Statistics shown for panel B were by grouping treatments with and without fungi (F).

3.1.2.3 Fungi assessment across 2-year study

Mulch was not replenished during the study. As described above (Figure 5A), approximately half of the mulch in the treatments with fungi decomposed during Year 1 compared with 15% or less in the treatments without fungi. However, differences among treatments had largely disappeared by the end of Year 2. On average 80-93% of the original 3.5 kg (dry mass) had decomposed across all of the treatments by the end of the study (Figure 7). The similar final mass of mulch across all treatments shows that the rate of decomposition in the treatments inoculated with fungi slowed during Year 2, while the rate of decomposition of the treatments without fungi accelerated during Year 2. The reduced decomposition in the inoculated treatments was likely because of reduced substrate (food) availability as the mass of mulch declined beyond 50%. Meanwhile, the increased rate in the treatments that were not inoculated was likely a combined result of the greater availability of substrate in these treatments and the increased presence of microorganisms as all treatments continued to be colonized by ambient microorganisms, including the fungi noted during Year 1.

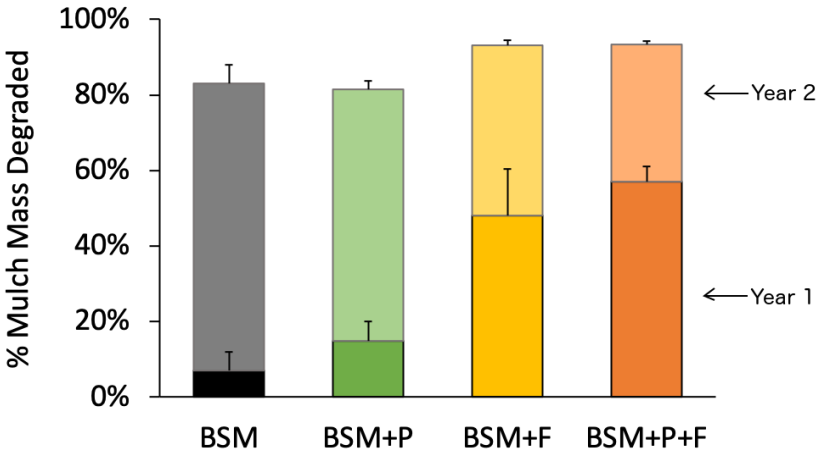

Figure 7. Average degradation of mulch across the two years of the study (dry weight basis), assessed at the end of Year 1 and at the end of Year 2. Error bars are one standard error of the mean.

3.2. BSM Physical Performance

3.2.1 Soil Temperature

The bioretention cells were surrounded with soil and covered with plastic to provide some thermal inertia similar to in-ground bioretention installations. Soil temperature recorded in 5-min intervals 20 cm below the surface of the BSM ranged from 3.7°C to 24.1°C. Concurrent air temperatures were -4.4°C to 32.2°C, showing the ability of the bioretention systems to moderate ambient temperatures by at least 8°C. Soil temperature varied diurnally and seasonally (Figure 8A). The time series were modeled on a quarterly basis as an autoregressive random effect of order 3 in a Bayesian model using integrated nested Laplace approximations (Rue et al. 2009). Estimated temperature values for each quarter were very

similar across bioretention treatment type; varying less than 1°C across the bioretention treatment type and remaining within 6% of the minimum estimated temperature value for each quarter. Unsurprisingly, there no statistically significant differences between the four treatment types on a quarterly basis. This would partially be due to the intermixing of treatments allowing, for example, shading of cells without plants by those with plants.

3.2.2 Soil Water Content

Volumetric water content was measured at approximately 20 cm depth in the BSM. Water content was higher overall during winter than during summer and predictably spiked during rain events (or weekly summer watering) (Figure 8B). Temporal dependency of the volumetric water content data was modeled as a random walk in a Bayesian model using integrated nested Laplace approximations (Rue et al. 2009) on a quarterly basis. This model showed that the four treatments produced statistically different trends over time. On a quarterly basis (Figure 9), water content was significantly higher in the bioretention treatment with fungi (BSM+F) than the others (Repeated measures GLM: $F(3,7)=10.168$, $p=0.006$, Tukey post-hoc $p=0.002$-0.014). The moist fungal mycelia in the inoculated fungi treatment may have reduced evaporation from the pore spaces in that treatment. This effect would likely have decreased over time as the other treatments were colonized with fungi. Comparing VWC over time is complicated by seasonal shifts and differences in precipitation among years. However, there was a trend towards higher water content during Year 2 compared with Year 1 for the treatments without plants (BSM, BSM+F), and a trend towards lower water content during Year 2 for treatments with plants (BSM+P, BSM+P+F). This was statistically significant ($\alpha<0.05$) during Quarter 1 (Feb-May) for BSM ($p = 0.011$), during Quarter 2 (May-Aug) for BSM+F ($p = 0.019$), and during Quarter 4 (Nov-Feb) for BSM+P ($p = 0.025$). Lower VWC for Year 2 for treatments with plants – especially the last 3 quarters when the new plants were beginning to thrive – likely reflects the greater water demand in these treatments compared to those without plants. In contrast, an increase in VWC in the treatments without plants could reflect an increasing presence of moist mycelia from the volunteer fungi, which would have increased over time in all treatments but perhaps have been overwhelmed by the presence of plants in the BSM+P+F treatment.

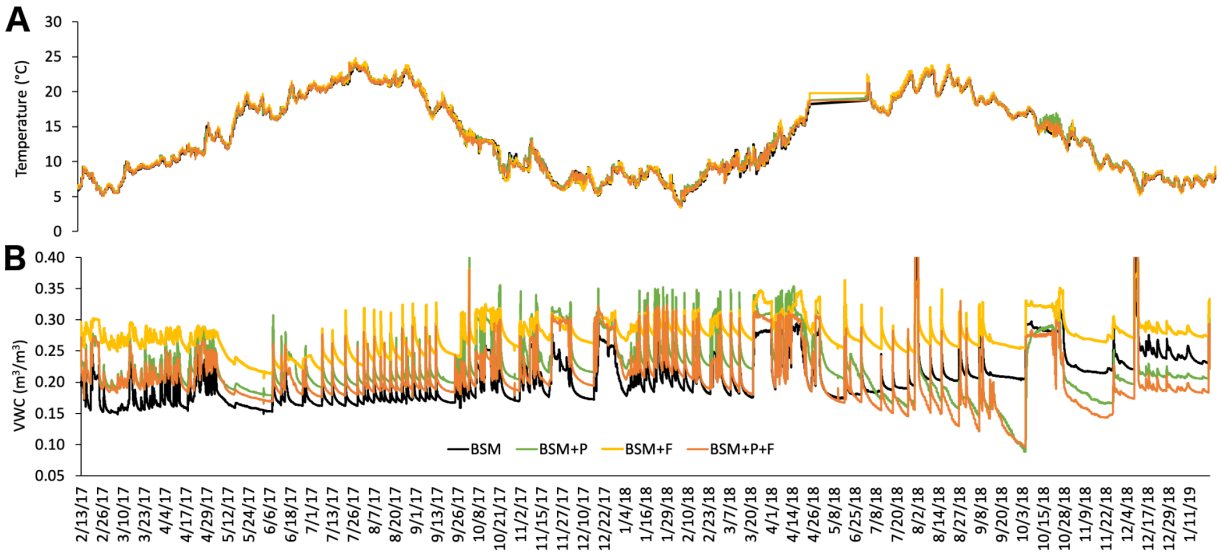

Figure 8. Average values of the three replicates of each bioretention treatment type from the soil probes measuring A) temperature and B) volumetric water content at 20 cm depth.

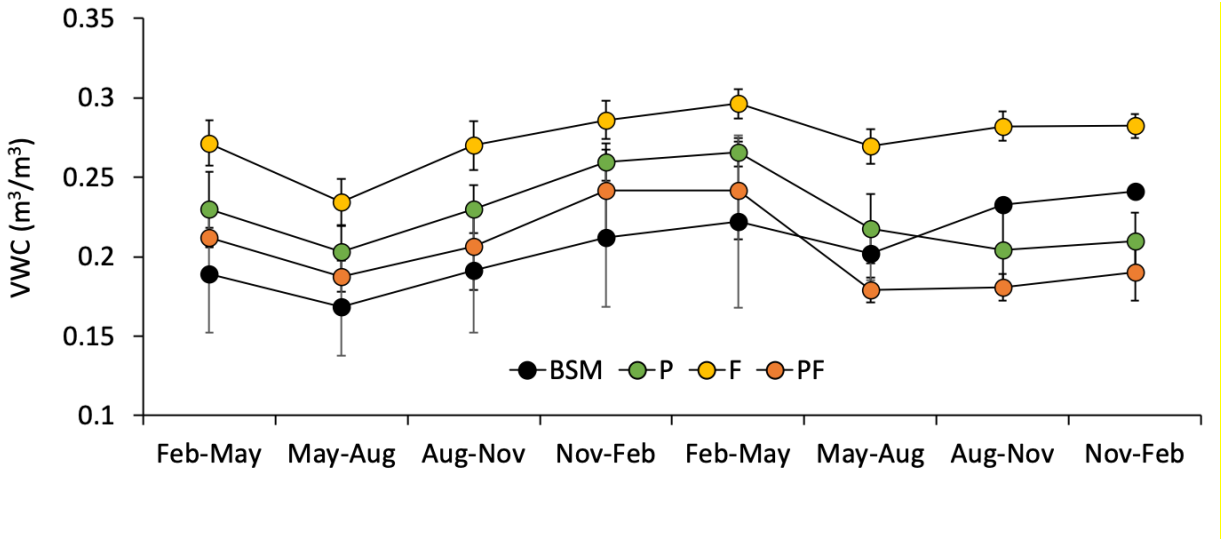

Figure 9. Volumetric water content across each quarter of the study for the four bioretention treatment types. Values are averages of three replicates ± standard deviation.

3.2.3 BSM Moisture

After 2 years, moisture content (100-%Solids) in the surface layer of BSM (0-15 cm) was significantly different among treatments ($F(3,16) = 49.166$, $p < 0.001$; Figure 10), with significantly lower moisture relative to BSM in the treatments with plants (BSM+P, BSM+P+F) (Tukey: $p < 0.001$). This pattern was similar to that for the mulch layer overlying the bioretention soil (Figure 6A), suggesting that plant demand resulted in lower available moisture in both the surface BSM and the overlying mulch.

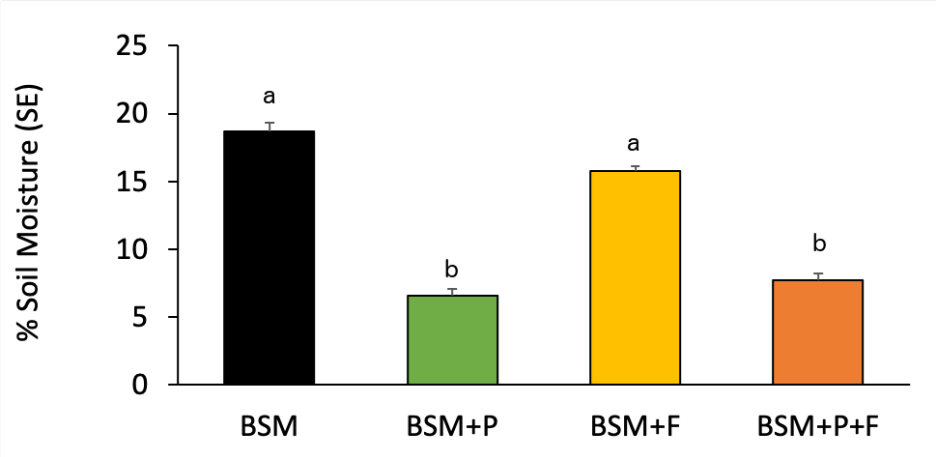

Figure 10. Average moisture content of the surface layer (0-15 cm) of the BSM in each bioretention cell (n=3 per treatment) at the end of the experiment. Error bars are ± one standard error of the mean. Values are statistically distinct for treatments with different letters.

3.2.4 Hydraulic Conductivity

Saturated hydraulic conductivity (K_{sat}) was determined by the falling head test:

$$Ksat = \frac{Ls}{h} \times \ln\left(\frac{H1}{H2}\right),$$

where *Ls* is the depth of the bioretention soil media in cm, *h* is the time in hours to drain from *H1* to *H2* in cm. Briefly, the underdrain of each replicate was closed and each replicate was filled with water to the brim (*H1* = height from brim of the drum to bottom of underdrain = 70.6 cm). After saturating for 24 h, each replicate was topped off if necessary and time to drain was recorded (*H2* = height from end water level to bottom of underdrain). The K_{sat} procedure was repeated three times in a row to obtain an average value for each replicate. Replicate values were used in statistical analysis to determine whether there were impacts on K_{sat} over time and among treatments. For tests conducted prior to initiating stormwater treatment, cells were filled with water from the bottom using an external standpipe. However, it was discovered that a layer of fines mobilized to the surface of each cell during standpipe filling (described below). As a result, cells were filled with water gently from the top for the three final testing events.

3.2.4.1 Hydraulic Conductivity Before Stormwater Treatment

Saturated hydraulic conductivity was assessed during installation after an initial compaction to 1.25 g/cm^3 and again after compaction to the target bulk density of 1.41 g/cm^3 (SD = 0.02). The average K_{sat} was approximately 100 cm/h after compaction to 1.25 g/cm^3 and 60 cm/h (SD = 20) after compaction to 1.41 g/cm^3 (Figure 11A). This was similar to the value of 48 cm/h predicted from the regression of K_{sat} on bulk density derived from the laboratory experiment using the same media (Deliverable 3.1: Report on water retention curves of bioretention soil mix). In order to average three K_{sat} measurements per cell, additional tests were conducted. However, K_{sat} rapidly decreased with each additional measurement as a layer of fines accumulated on the surface of the BSM (Figure 11B). When the average K_{sat} across all cells had reached 20 cm/h, the ~5-mm layer of fines (1.49 ± 0.29 kg dry mass) was removed from all cells (Figure 11C) and resuspended in water. Fines were reincorporated into the top 20 cm of BSM by removing the BSM and adding the water in increments alternating with layers of BSM. Following reincorporation of the fines, BSM was again compacted to 1.41 g/cm^3. After the systems began treating stormwater in Feb 2017, hydraulic conductivity testing was not conducted again until Year 2 in order to not disturb the bioretention soils as plants and fungi became established.

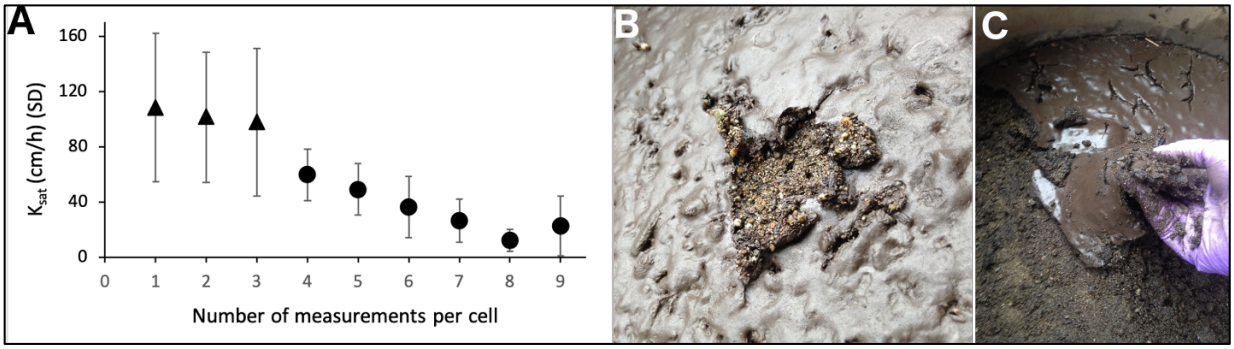

Figure 11. A) Saturated hydraulic conductivity (average of all 12 cells) over 9 repeated measurements during January 2017. Measurements 1-3 were conducted while BSM was at a bulk density of 1.25 g/cm^3 whereas measurements 4-9 were at the target bulk density of 1.41 g/cm^3. B) The layer of fine material that had settled on top of the BSM. C) Peeling the layer of fine material prior to reincorporation back in to the BSM.

3.2.4.2 Hydraulic Conductivity During Stormwater Treatment

Hydraulic conductivity testing reinitiated during Year 2 of the study was conducted in July and Dec 2018 and in April 2019, corresponding to quarters 6, 7, and 8 of the study, respectively (Table 4). Results were compared across time using a repeated measures general linear model in SPSS (version 26; IBM Corp) with treatment as a factor. The effect of time depended on treatment (Treatment × Time: $F(9,96) = 2.815$, $p = 0.006$). Simple main effects identified the dates for which K_{sat} differed from the post-installation values (Table 4). After two years of stormwater treatment, hydraulic conductivity had increased 47% for the treatment with plants (BSM+P) and had decreased 37% for the treatment with fungi (BSM+F), but was not significantly different for BSM-only or the treatment with both plants and fungi (BSM+P+F).

Table 4. Average (standard deviation) saturated hydraulic conductivity (K_{sat}) for each treatment (3 measurements for each replicate) for each test date in cm/h, showing statistical results for the effect of time within each treatment. Values sharing a letter within a treatment are not statistically different.

Treatment	Feb 2017	Jul 2018	Dec 2018	Apr 2019
BSM	47 (14)	83 (34) *	60 (38)	54 (29)
BSM+P	48 (10)	65 (12)	89 (50) *	68 (47) *
BSM+F	63 (15)	78 (24)	62 (14)	46 (16) *
BSM+P+F	70 (19)	54 (24)	63 (25)	49 (15)

3.3. Bioretention Soil Chemistry

3.3.1 Sand and Compost

Prior to mixing the sand and compost into the BSM, composite samples of compost and sand were collected separately by subsampling 10 locations at various depths throughout each two-yard pile. Subsamples were homogenized and three representative samples isolated for analysis. Sand and compost samples were submitted in triplicate to AmTest Laboratories (Kirkland, WA) for quantification of metals by ICP-MS according to method SW-846 6020A. Compost was additionally sent to SoilTest Farm Consultants, Inc. (Moses Lake, WA) for analysis of total nitrogen (ASTM D5373), total carbon (ASTM D5373), nitrate (S-3.10 b), ammonia (S-3.50), total phosphorus (EPA 3050A/6010B), Olsen phosphorus (S-4.20 b), cation exchange capacity (S-10.10 b), and pH (Table 5). Metal concentrations in the compost were many times higher than in sand except for Cr and Ni, which were similarly concentrated in compost and sand (Table 6). Ultimately, the leachability of these two components of BSM is what determines the contribution of each metal to effluents treated by the bioretention system.

Table 5. Nutrient and conventional parameters for compost used in BSM

	Total N	Total C	NO3-N	NH4-N	Total P	OLSEN P	CEC	pH
	%	%	mg/kg	mg/kg	mg/kg	mg/kg	meq/100g	
D.L.	0.01%	0.02%	0.8	0.7	4.3	0.9	0.1	0.1
Mean	1.5	18	233	38	2559	135	40	7.4
St. Dev.	0.1	2	58	11	51	24	2	0.1
RSD (%)	6.9%	8.6%	25%	28%	2%	17%	6%	1%

D.L. = detection limit; RSD = relative standard deviation

Table 6. Metal concentrations (mg/kg dry weight) of compost and sand used in BSM

	As	Cd	Cr	Cu	Ni	Pb	Zn
Compost							
D.L.	0.052	0.043	0.086	0.086	0.086	0.086	0.172
Mean	**7.95**	**0.459**	**16.6**	**39.9**	**11.3**	**35.7**	**148**
St. Dev.	4.64	0.045	1.69	8.21	1.08	7.06	34.9
RSD (%)	58.3%	9.88%	10.1%	20.6%	9.52%	19.8%	23.6%
Sand							
D.L.	0.025	0.021	0.043	0.043	0.043	0.043	0.085
Mean	**0.535**	**0.051**	**14.2**	**16.0**	**17.4**	**1.18**	**17.4**
St. Dev.	0.161	0.021	4.730	1.332	3.292	0.300	0.907
RSD (%)	30.0%	41.2%	33.2%	8.34%	18.9%	25.4%	5.20%

D.L. = detection limit; RSD = relative standard deviation

3.3.2 Contaminants in BSM prior to stormwater treatment

To determine whether conditioning the bioretention cells with clean water stratified chemical parameters, core samples of the BSM were collected and analyzed from a surrogate system at the Washington State University Puyallup Research and Extension Center. Three eight-inch surrogate columns were constructed with the same soil materials and following the same installation and conditioning protocol used during the installation of the field bioretention columns. Each of the three small soil columns received 18.1 ± 0.004 dry kg of BSM compacted to a depth of 40.9 ± 1.2 cm resulting in a soil bulk density of 1.36 ± 0.04 g/cm^3. Each cell received a volume of water proportionate to that the field cells received during conditioning and testing for saturated hydraulic conductivity.

After conditioning, the small surrogate cells were destructively sampled with a 1-cm diameter corer through the entire depth of the soil (40 cm) by randomly coring 15-20 times per column to acquire the necessary soil mass for chemical analysis. Each 40-cm soil core was divided into three separate samples by depth: 0-15 cm, 15-30 cm, 30-40 cm. Metals and other parameters were analyzed in all three depths. To reduce analytical costs, PAH concentrations were measured in the top layer only, where most PAHs were expected to be retained. Each column was sampled separately so that each depth was measured in triplicate. Samples were analyzed by ARI (Tukwila, WA) for a suite of PAHs and by AmTest laboratories (Kirkland, WA) for nutrients and metals.

Visual inspection of the data allowed us to conclude that most parameters did not vary with depth (standard errors of means overlap). One parameter was tested by ANOVA for possible depth stratification. Total nitrogen (TKN) appeared higher in the top layer of the BSM (Table 7), but the difference was not statistically significant at $\alpha = 0.05$ ($F_{2,8} = 4.90$, $p = 0.055$). These results assure that the soil chemical parameters of interest in the field bioretention cells were generally unstratified at the beginning of stormwater treatment.

Table 7. Soil parameters for depth strata of conditioned BSM before stormwater treatment. Error bars are one standard error of the mean of the 3 replicates. PAHs are the sum of low molecular weight (LMW; <4 rings), high molecular weight (HMW; 4 or more rings), and the sum of 20 individual PAHs.

Parameter	Units	DL	0-15 cm	15-30 cm	30-45 cm
Total Solids	%	0.1	77.9 (1.8)	81.4 (2.1)	82.3 (0.9)
Organic Mater	%	0.1	7.9 (1.1)	6.4 (1.0)	6.9 (0.4)
Total Organic Carbon	%	0.05	5.7 (3.8)	2.1 (1.0)	2.4 (2.4)
Ammonia	µg/g	6.4	145.3 (38.4)	105.7 (6.7)	105.0 (11.4)
Total Nitrogen (TKN)	µg/g	6.4	2833.3 (814.5)	1733.3 (378.6)	1566.7 (251.7)
Nitrate+Nitrite	µg/g	0.64	5.60 (1.84)	5.77 (1.42)	5.20 (2.62)
Total Phosphorus	µg/g	0.64	560.00 (147.31)	480.00 (121.24)	466.67 (83.27)
As	µg/g	0.186	2.553 (0.349)	2.053 (0.323)	2.530 (0.461)
Cd	µg/g	0.186	0.528 (0.161)	0.458 (0.200)	0.517 (0.243)
Cr	µg/g	0.186	45.700 (12.469)	29.400 (7.843)	28.533 (11.789)
Cu	µg/g	0.373	36.167 (7.206)	31.267 (6.282)	38.033 (15.782)
Pb	µg/g	0.186	6.273 (2.501)	6.433 (2.290)	7.200 (2.407)
Ni	µg/g	0.186	36.900 (8.516)	26.567 (6.550)	37.733 (12.484)
Zn	µg/g	1.86	98.00 (30.45)	103.17 (23.33)	79.43 (12.69)
LMW PAHs	µg/kg	various	102.1 (6.9)	nm	nm
HMW PAHs	µg/kg	various	146.2 (15.0)	nm	nm
Sum PAHs	µg/kg	various	248.3 (21.7)	nm	nm

3.3.3 Contaminants in BSM After Stormwater Treatment

3.3.3.1 Metals in BSM

Metal concentrations (As, Cd, Cr, Cu, Ni, Pb, Zn) were measured in the bioretention soil media at the end of the study at depths 15-30 cm and 30-45 cm. Metals were unfortunately not measured in the surface layer (0-15 cm) where the majority of accumulation was expected. Metal concentrations were assessed as a function of depth and treatment by multivariate general linear model, followed by analysis of simple main effects. Depth had a significant effect on copper concentrations across bioretention treatments ($F(1,16) = 7.289$, $p = 0.016$). This effect was driven by lower values in the treatments with plants (Simple main effects: BSM+P, $p = 0.044$; BSM+P+F, $p = 0.024$) relative to the BSM alone in the shallower layer

(Figure 12). These differences were not present at the deeper layer (p = 0.169-0.929). Neither depth nor treatment significantly affected the other metals (p = 0.102-0.834), nor was there a significant interaction between depth and treatment for any metal (p = 0.076-0.393).

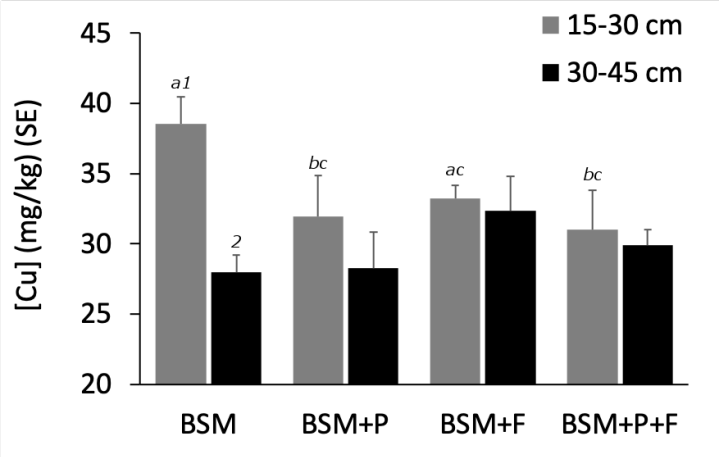

Figure 12. Average copper concentrations in two layers of bioretention soils from each cell (n=3 per treatment) at the end of the experiment. Error bars are one standard error of the mean. Within the shallower stratum, treatments with different letters are statistically distinct. There were no differences among treatments for the deeper stratum. Significant differences among depths within a treatment are indicated by different number labels (1 vs 2).

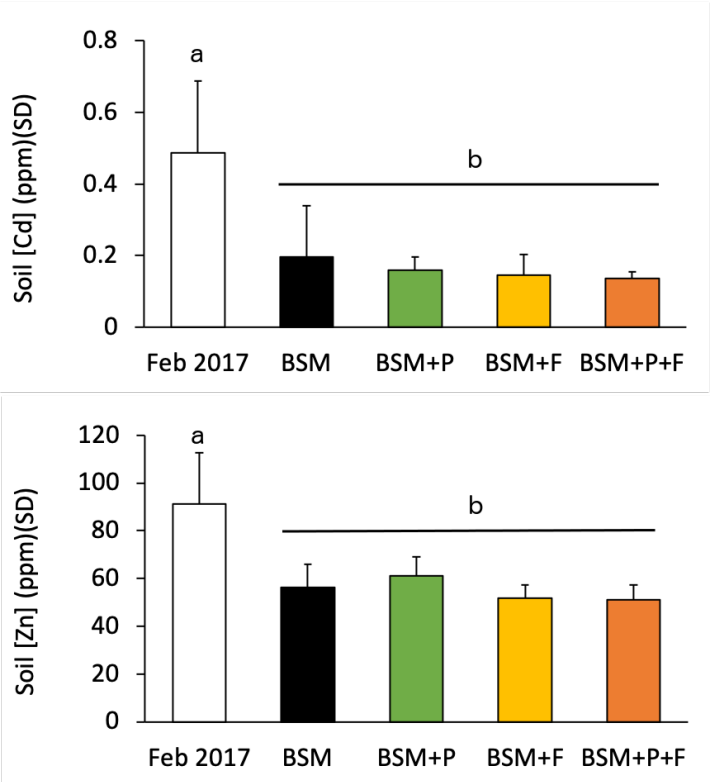

Figure 13. The two metals for which concentrations were changed between installation in Feb 2017 and the end of the experiment in May 2019. Bioretention types were bioretention soil media alone (BSM) with or without plants (P) and fungi (F). End concentrations were lower for all bioretention types than at installation. There were no significant differences compared with installation for As, Cu, Cr, Ni, Pb.

At the end of the study, concentrations of metals for the two lower depths sampled (15-30 cm; 30-45 cm) were compared with initial concentrations in a multivariate GLM with treatment and depth as factors. Significantly lower concentrations than at installation were found for all treatments for two metals (Figure 13); Cd (Dunnett post-hoc, $p \leq 0.004$) and Zn ($p \leq 0.001$). Concentrations were not different by depth ($F(1,20)=0.076, 3.626$, $p=0.071, 0.785$), nor did the treatment effects depend on depth (treatment × depth; $F(4,20)=0.496, 1.626$, $p=0.739, 0.207$). Concentrations in the different treatments at the end of the study were not significantly different from each other (all $p>0.08$). Average loss of metal concentration across the study was 67% for Cd and 40% for Zn. None of the other metals (As, Cu, Cr, Ni, Pb) at the two lower depths were significantly changed across the two-year study period ($F=0.598$-1.513, $p = 0.085$-0.872) (Table 8).

Table 8. Average concentration of metals (and % solids) on a dry weight basis (standard deviation) pooled across the two depths sampled at the end of the study (15-30 cm; 30-45 cm) for each bioretention treatment and at installation (START). BSM = bioretention soil medium; P = plants; F = fungi.

		Feb 2017	May 2019			
	units	START	BSM	BSM+P	BSM+F	BSM+P+F
Solids	%	81.8 (1.5)	81.3 (1.5)	93.5 (1.3)	84.3 (0.9)	92.3 (1.3)
As	mg/kg	2.3 (0.4)	2.1 (0.4)	2.2 (0.5)	2.2 (0.3)	1.9 (0.3)
Cd	mg/kg	0.49 (0.2)*	0.20 (0.1)	0.16 (0.04)	0.15 (0.06)	0.14 (0.02)
Cr	mg/kg	29.0 (9)	31.9 (8)	27 (4)	31.5 (7.3)	27.9 (7.1)
Cu	mg/kg	34.7 (11.4)	33.3 (6.3)	30.1 (4.7)	32.8 (3)	30.5 (3.4)
Ni	mg/kg	32.2 (10.8)	29.3 (3.7)	31.8 (0.8)	31.6 (1.9)	29.8 (5.0)
Pb	mg/kg	6.8 (2.1)	5.5 (1.7)	5.6 (0.8)	4.6 (0.6)	4.8 (0.8)
Zn	mg/kg	91.3 (21.2)*	56.2 (8.2)	61.2 (7.9)	51.9 (5.3)	51.2 (6.2)

* Significantly higher than in May 2019 for all treatments

3.3.3.2 PAHs in BSM

PAH concentrations were determined in the surface layer (0-15 cm) of each bioretention cell in July 2017 (five months after the start of stormwater treatment) and in May 2019 (after the end of the study). Treatment effects were determined by multivariate general linear model with treatment and congener as fixed factors. After five months of stormwater treatment, PAH concentrations were not significantly different among bioretention treatments ($F(3,168)=0.398$, $p = 0.755$), and did not depend on congener (congener × treatment $F(51,168)=0.288$, $p = 1.000$). At the end of the 2-year study, differences among treatments were detected ($F(3,168)=3.656$, $p = 0.014$). This effect did not depend on congener (congener × treatment: $F(51,168)=0.529$, $p = 1.000$). Tukey post-hoc test was used to determine which treatment(s) were different in PAH concentrations at the end of the study (May 2019). Concentrations were significantly smaller in the surface soil of the treatment with plants (BSM+P) than the treatment with fungi (BSM+F) (Tukey post-hoc, $p = 0.009$). Values for BSM alone and BSM+P+F were not different from either BSM+P or BSM+F (Tukey post-hoc, $p = 0.116$-0.999; Figure 14). The average (SD) sum of PAHs was 131 (8) µg/kg for BSM, 115 (27) µg/kg for BSM+P, 139 (26) µg/kg for BSM+F, and 132 (49) µg/kg for BSM+P+F. The difference between BSM+P and BSM+F was 17%.

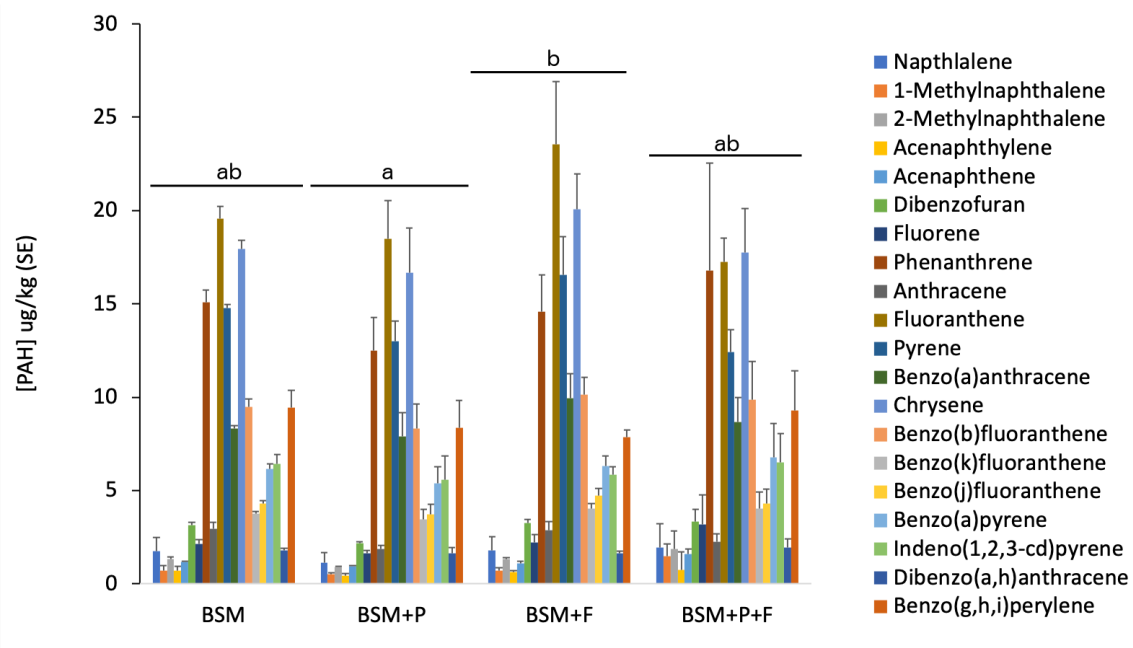

Figure 14. Average concentrations of PAH congeners in the surface layer of bioretention after two years of treating stormwater. Error bars are ± one standard error of the mean. Treatments sharing a letter were not significantly different.

To test for trends in PAHs in BSM over the entire study, we compared total PAHs at installation with values from May 2019 in each type of bioretention. Total PAHs in the surface layer of BSM (0-15 cm) decreased significantly over the study ($F(4,15)=8.324$, $p=0.003$), for all treatments (Dunnett post-hoc; $p=0.002$-0.007), with means 44-54% lower than initial concentrations. High molecular weight PAHs (>4 rings) were more abundant than low molecular weight PAHs (≤4 rings) in all soil samples (average ratio 1.4-1.7 across treatments and years). There appeared to be preferential reduction of the LMW PAHs, with decreases of 49-60% across treatments compared with 40-49% across treatments for HMW PAHs (Figure 15), although the difference was not statistically significant at $\alpha=0.05$ ($F(4,15)=2.939$, $p=0.076$).

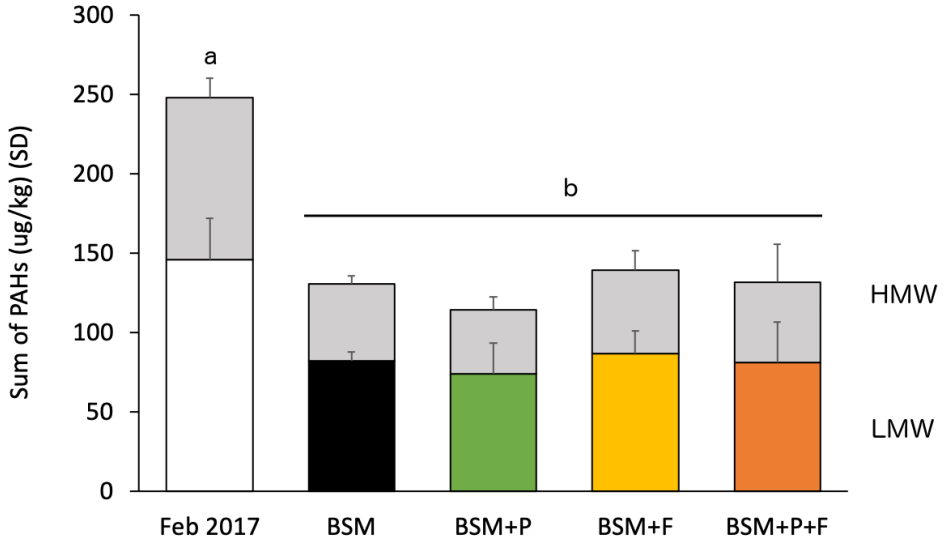

Figure 15. Polycyclic aromatic hydrocarbons (PAHs) in surface layer (0-15 cm) of bioretention soil prior to stormwater treatment (Feb 2017) and at the end of the study (May 2019). PAHs shown by the average sum of low molecular weight (LMW) and high molecular weight (HMW). Error bars are one standard deviation. Final PAHs were significantly lower than installation values for all bioretention types, for both LMW and HMW.

3.3.4. Contaminants in BSM relative to soil screening criteria

The U.S. EPA provides risk-based soil screening levels (Eco-SSLs) to assess ecological risks of certain contaminants in soils to various terrestrial organisms (plants, invertebrates, birds, mammals). We compared the concentrations in BSM to the most sensitive Eco-SSLs (EPA 2018) for metals and PAHs measured at the beginning of the study and at the end. As noted in Table 8 for metals and section 3.3.3.2 for PAHs, concentrations were significantly different only for Cd, Zn, and PAHs. Concentrations of As and Pb were below the most sensitive Eco-SSL, and also far below concentrations of these metals typical of western soils (<5th percentile). Concentrations of Cr, Cu, and Ni were similar to the Eco-SSLs, but also similar to concentrations typical of western soils (5th to 75th percentile). Concentrations of Cd and Zn decreased across the study. At the beginning of the study, Cd was above the Eco-SSL, but similar to the median concentration of western soils, whereas by the end of the study, concentrations were below the Eco-SSL and also far below concentrations typical of western soils (<5th percentile). For Zn, concentrations at the beginning of the study were above the Eco-SSL and high relative to western soils (75th to 95th percentile). By the end of the study, the concentration was still slightly above the Eco-SSL, but low relative to western soils (5th to 25th percentile). Ecological risks from PAHs in soils are often considered by the sum PAHs that are of low molecular weight or high molecular weight. Values in the BSM in this study were more than an order of magnitude lower than the most sensitive Eco-SSL for PAHs in soil (1.1 mg/kg dry weight; EPA 2018).

3.4. Water quality

Contaminants in stormwater were measured for influent and effluent waters including chemical and biological oxygen demand (COD, BOD), total and dissolved organic carbon (TOC, DOC), total suspended solids (TSS), total and dissolved metals (As, Cd, Cr, Cu, Ni, Pb, Zn), bacteria (fecal coliforms and *Escherichia coli*), nutrients (including ammonia, nitrite+nitrate, total nitrogen, ortho-phosphate, total phosphorus), and a suite of polycyclic aromatic hydrocarbons (PAHs). Although included in the Quality Assurance Project Plan (Deliverable 1), the parameters suspended sediment concentration (SSC) and calcium were not measured.

3.4.1 Export of contaminants following clean water conditioning

Newly installed 60:40 BSM exported suspended sediment (TSS), organic matter (BOD, COD, TOC, DOC), and phosphorus (ortho-P and TP) following conditioning with clean municipal water (Table 1). Some metals were detectable in the influent municipal water, and these concentrations increased in effluent for As, Cr, Cu, and Ni. In contrast, concentrations of metals in effluent were reduced from influent for dissolved Cd, Pb, and Zn. It is important to note that the influent municipal water was elevated in dissolved Zn (>200 g/L). This was an artefact of legacy plumbing to the fish lab building on the WSU campus as has been documented in other studies using this water source. A reverse osmosis (RO) system was recently installed in this building but was not yet available at the time the conditioning was conducted. The BSM effluent concentrations of PAHs (Table 10) and bacteria remained near or below detection limits.

Table 9. Average microbiology, conventional, nutrient, and metal concentrations in influent and effluent waters following BSM conditioning with clean water.

Type		Units	D.L.[a]	Influent (SD)[b]	Effluent (SD)
Microbiology	Fecal Coliform	MPN/100 mL	2	<[c]	<
	E. coli	CFU/100 mL	2	1 (0)	5 (0)
Conventional	pH	n.a.	0.1	6.9 (<0.1)	7.4 (0.1)
	TSS	mg/L	1	1 (0)	10 (5)
	BOD	mg/L	2	<	6 (<2)
	COD	mg/L	10	<	50 (34)
	TOC	mg/L	0.5	1.1 (0)	34.7 (13.3)
	DOC	mg/L	0.5	1.0 (0.1)	28.3 (6.7)
	Alkalinity	mg/L as $CaCO_3$	1	97 (4)	243 (12)
Nutrients	TAN	mg/L	0.005	0.054 (0.068)	0.059 (0.020)
	TKN	mg/L	0.1	<	1.9 (1.6)
	Nitrate + Nitrite	mg/L	0.01	2.55 (0.21)	2.30 (0.53)
	ortho-P	mg/L	0.005	0.037 (0)	0.447 (0.129)
	TP	mg/L	0.005	0.044 (0.006)	0.539 (0.108)
Total Metal	Arsenic	µg/L	0.5	1.2 (0.2)	2.6 (0.4)
	Cadmium	µg/L	0.5	<	<
	Chromium	µg/L	0.5	<	<
	Copper	µg/L	1	23 (30)	17 (4)
	Lead	µg/L	0.5	1.8 (0.2)	1.8 (1.0)
	Nickel	µg/L	0.5	23.6 (33.1)	6.6 (1.8)
	Zinc	µg/L	5	282 (55)	60 (16)
Dissolved Metal	Arsenic	µg/L	0.05	1.01 (0.02)	2.30 (0.26)
	Cadmium	µg/L	0.05	0.14 (0)	0.07 (0.01)
	Chromium	µg/L	0.1	0.4 (<0.1)	1.0 (0.2)
	Copper	µg/L	0.1	1.4 (0.2)	13.6 (3.3)
	Lead	µg/L	0.1	0.3 (<0.1)	0.1 (<0.1)
	Nickel	µg/L	0.05	0.67 (0.04)	5.32 (0.94)
	Zinc	µg/L	0.5	221.5 (0.7)	30.3 (11.6)

[a] Detection Limit

[b] Standard deviation

[c] '<' indicates all values were below the detection limit

Table 10. Average polycyclic aromatic hydrocarbon (PAH) concentrations in influent and effluent waters following conditioning of BSM with clean water.

PAH	Unit	D.L.[a]	Influent (SD[b])	Effluent (SD)
Naphthalene	µg/L	0.011	<[c]	<
1-Methylnaphthalene	µg/L	0.011	<	<
2-Methylnaphthalene	µg/L	0.011	<	<
Acenaphthylene	µg/L	0.011	<	<
Acenaphthene	µg/L	0.011	<	<
Dibenzofuran	µg/L	0.011	<	<
Fluorene	µg/L	0.011	<	<
Phenanthrene	µg/L	0.011	<	<
Anthracene	µg/L	0.011	<	<
Fluoranthene	µg/L	0.011	<	<
Pyrene	µg/L	0.011	<	0.017 (<0.011)
Benzo(a)anthracene	µg/L	0.011	<	<
Chrysene	µg/L	0.011	<	<
Benzo(a)pyrene	µg/L	0.011	<	<
Indeno(1,2,3-cd)pyrene	µg/L	0.011	<	<
Dibenzo(a,h)anthracene	µg/L	0.011	<	<
Benzo(g,h,i)perylene	µg/L	0.011	<	<
Perylene	µg/L	0.011	<	<

[a] Detection Limit
[b] Standard Deviation
[c] '<' indicates all values below detection limit

3.4.2 Water quality of treated stormwater

During stormwater treatment, net increases in concentration in the effluent from the bioretention cells were evident for DOC, nitrates, ortho-P, As, Cd, Cr, Ni (Net concentration; Table 11), indicating their continued release from the bioretention columns after conditioning. Biological and chemical oxygen demand were also exported from the bioretention columns during stormwater treatment. Average negative net concentrations for Cd and total Ni (Table 11) indicate that initial export of these contaminants led eventually to net retention. In contrast, reductions from influent were evident from the beginning of stormwater treatment for total suspended sediment (TSS), Cr, Cu, Pb, and Zn (Percent reduction; Table 12), despite bioretention columns initially being a source of these parameters to clean water (Table 9). Reductions from influent were also evident for FC, *E. coli*, and PAHs which did not leach appreciably from bioretention during conditioning (Table 12). Concentrations of fecal coliform and *E. coli* were highly correlated (Pearson $r^2 = 0.984$, $p<.001$). Average values for metals indicate that retention was lower for dissolved metals compared with total metals.

Table 11. Net concentration (effluent minus influent) for parameters with initially higher concentrations in effluent than influent across all eight events and all four treatments.

Parameter	Unit	Average Net Concentration	Standard Deviation
Nitrates	mg/L	1.6	2.1
ortho-P	mg/L	0.26	0.12
Total P	mg/L	0.30	0.23
DOC	mg/L	19	39
BOD	mg/L	6	16
COD	mg/L	198	368
Dissolved As	µg/L	1.3	1.1
Total As	µg/L	0.68	0.92
Dissolved Cd	µg/L	-0.04	0.05
Total Cd	µg/L	-0.04	0.08
Dissolved Ni	µg/L	0.09	2.10
Total Ni	µg/L	-0.52	3.2

Table 12. Percent reduction in concentration for conventional parameters with initially higher concentration in influent than effluent across all eight events and all four treatments.

Parameter	Average Reduction	Standard Deviation
Fecal coliform	92%	5%
E. coli	92%	2%
Total suspended sediment	72%	30%
Dissolved Cr	45%	15%
Total Cr	60%	22%
Dissolved Cu	58%	14%
Total Cu	75%	10%
Dissolved Pb	64%	11%
Total Pb	86%	16%
Dissolved Zn	89%	7%
Total Zn	91%	8%
Total PAHs	85%	12%

Changes in concentration over time and as a result of treatment were explored with a multivariate general linear model (GLM; SPSS v. 26, IBM Corp) with net concentration or percent removal as dependent variables, and treatment (BSM, BSM+F, BSM+P, BSM+F+P) and event as factors. Tukey's post-hoc was used to test for differences among treatments or events. Significance level was set at α=0.05. All of the contaminants showed significant changes among Events, but for nitrates, total- and ortho-P, DOC, dissolved Cu, total Pb and total Zn the difference among events was affected by bioretention treatment type (Treatment x Event interaction; Table 13). Differences among treatments and events were examined using simple main effects.

Table 13. Results of the multivariate general linear model of water quality for the four bioretention treatments across all eight sampling events. Parameters with higher effluent than influent concentrations were analysed as net concentration (nitrates, ortho-phosphate, dissolved organic carbon (DOC), biological oxygen demand (BOD), chemical oxygen demand (COD), As, Cd, Ni). Parameters with higher influent than effluent concentrations were analysed as % removal (fecal coliform bacteria (FC), *E. coli*, total suspended solids (TSS), Cu, Cr, Pb, Zn). Bold *p* values were statistically significant factors. Metals are designated as dissolved (d) or total (T).

Variable	Factor	df	*F*	*p*
Nitrates	Treatment	3, 95	2.609	0.086
	Event	7, 95	27.054	**<0.001**
	Treatment x Event	21, 95	1.338	**0.003**
oP	Treatment	3, 95	6.100	**0.001**
	Event	7, 95	66.013	**<0.001**
	Treatment x Event	21, 95	1.789	**0.040**
T P	Treatment	3, 95	6.122	**0.001**
	Event	7, 95	68.802	**<0.001**
	Treatment x Event	21, 95	3.150	**<0.001**
DOC	Treatment	3, 95	1.717	0.173
	Event	7, 95	13.328	**<0.001**
	Treatment x Event	21, 95	1.877	**0.029**
BOD	Treatment	3, 72	1.369	0.154
	Event	5, 72	9.708	**<0.001**
	Treatment x Event	15, 72	1.706	0.082
COD	Treatment	3, 48	0.785	0.511
	Event	3, 48	3.733	**0.021**
	Treatment x Event	9, 48	0.793	0.625
d As	Treatment	3, 95	0.196	0.898
	Event	7, 95	28.597	**<0.001**
	Treatment x Event	21, 95	1.609	0.076
d Cd	Treatment	3, 84	1.089	0.361
	Event	6, 84	128.125	**<0.001**
	Treatment x Event	18, 84	0.838	0.649
d Ni	Treatment	3, 95	1.813	0.154
	Event	7, 95	39.170	**<0.001**
	Treatment x Event	21, 95	0.507	0.958
T As	Treatment	3, 96	1.056	0.374
	Event	7, 96	38.603	**<0.001**
	Treatment x Event	21, 96	1.116	0.356
T Cd	Treatment	3, 84	1.140	0.341
	Event	6, 84	128.158	**<0.001**
	Treatment x Event	18, 84	1.275	0.240
T Ni	Treatment	3, 96	0.906	0.443
	Event	7, 96	138.756	**<0.001**
	Treatment x Event	21, 96	0.611	0.896
FC	Treatment	3, 95	0.401	0.753
	Event	7, 95	6.630	**<0.001**
	Treatment x Event	21, 95	0.776	0.736
E. coli	Treatment	3, 83	0.858	0.468
	Event	6, 83	3.603	**0.004**
	Treatment x Event	18, 83	0.904	0.577
TSS	Treatment	3, 95	2.592	0.060
	Event	7, 95	28.115	**<0.001**
	Treatment x Event	21, 95	1.529	0.100
d Cr	Treatment	3, 95	2.531	0.065
	Event	7, 95	78.677	**<0.001**
	Treatment x Event	21, 95	1.182	0.297

Table 13 continued

Variable	Factor	df	*F*	*p*
d Cu	Treatment	3, 95	1.651	0.154
	Event	7, 95	31.576	**<0.001**
	Treatment x Event	21, 95	2.062	**0.014**
d Pb	Treatment	3, 70	2.539	0.068
	Event	5, 70	19.425	**<0.001**
	Treatment x Event	15, 70	1.602	0.111
d Zn	Treatment	3, 95	2.509	0.074
	Event	7, 95	34.430	**<0.001**
	Treatment x Event	21, 95	0.832	0.672
T Cr	Treatment	3, 96	1.637	0.187
	Event	7, 96	70.175	**<0.001**
	Treatment x Event	21, 96	0.738	0.787
T Cu	Treatment	3, 96	0.293	0.830
	Event	7, 96	33.464	**<0.001**
	Treatment x Event	21, 96	1.443	0.132
T Pb	Treatment	3, 96	5.287	**0.003**
	Event	7, 96	60.624	**<0.001**
	Treatment x Event	21, 96	3.534	**<0.001**
T Zn	Treatment	3, 96	2.897	0.042
	Event	7, 96	92.751	**<0.001**
	Treatment x Event	21, 96	1.662	0.062

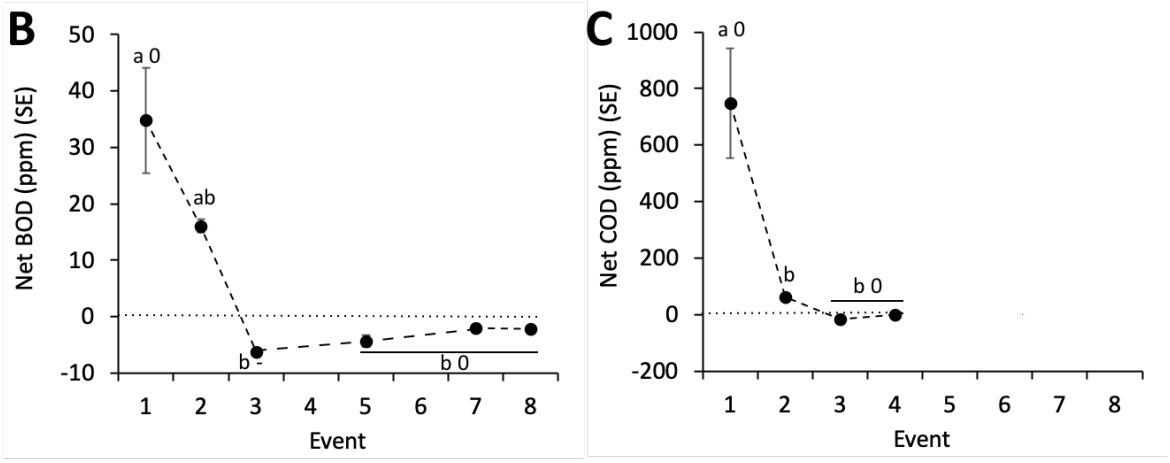

Figure 16. Average net concentrations of A) biological oxygen demand (BOD) and B) chemical oxygen demand (COD) for each sampling event across all treatments are shown with ± one standard error of the mean. Horizontal dotted lines show zero net export. Events with different letters are statistically different from each other. The '0' indicates no net export and '-' indicates significant removal.

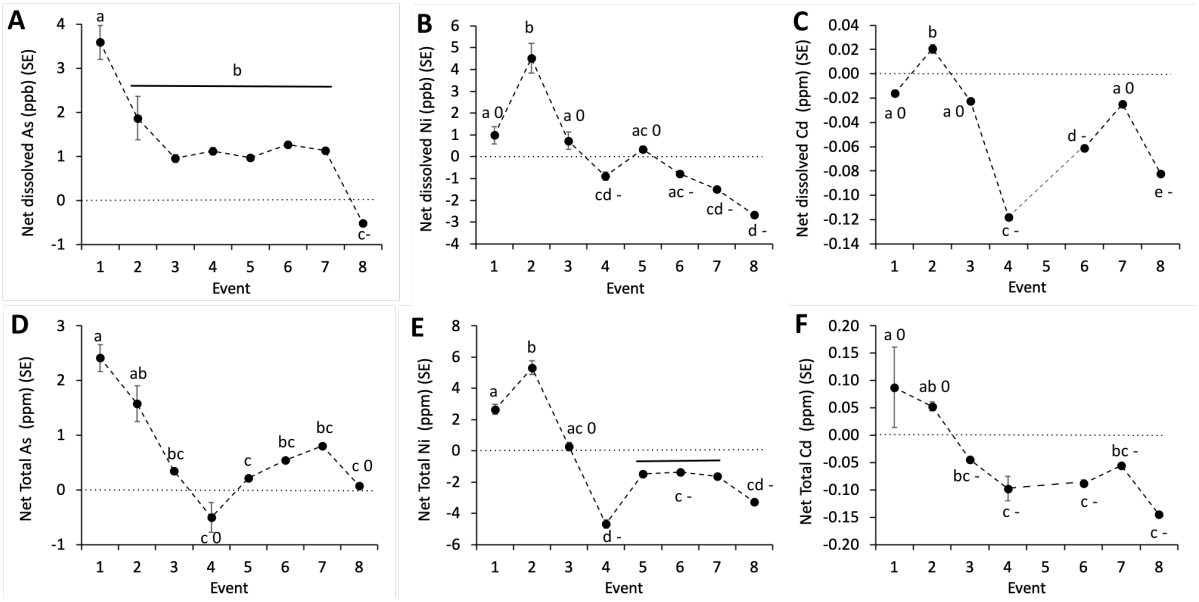

Figure 17. Average net concentrations of A) dissolved arsenic, B) dissolved nickel, C) dissolved cadmium, D) total arsenic, E) total nickel, F) total cadmium for each sampling event across all treatments are shown with ± one standard error of the mean. Horizontal dotted lines show zero net export. Events with different letters are statistically different from each other. The '0' indicates no net export and '-' indicates significant removal.

Among the contaminants that initially leached from the bioretention treatments, most had stopped leaching by Event 3, or were even retained relative to influent concentrations. These included biological and chemical oxygen demand (Fig 16A,B), dissolved and total nickel (Figure 17B,E), dissolved and total Cd (Figure 17C,F), and dissolved organic carbon (Figure 18D). More recalcitrant were arsenic, nitrates, total- and ortho-P; there was a net export of dissolved arsenic for all but the final event (Figure 17A), total arsenic for all but two events (Figure 17D), nitrates for five of the eight events (Figure 17A), and total- and ortho-P for all events (Figure 18A,B).

Bioretention treatment type affected nutrient and DOC concentrations. Net concentrations of total P and ortho-P in effluent from the bioretention treatments declined significantly over the 2-year study but did not stop leaching altogether (Figure 18A,B). In terms of treatment type, there was less export of P from treatments containing fungi initially; significantly less total P leached from treatments with fungi during Events 1 and 2 (Figure 18A), whereas the effect was more persistent for ortho-P (Figure 18B). The benefit of fungi appeared to decrease over the first year until there was no statistical difference in ortho-P export among bioretention types by Event 5 (Figure 18B). Nitrate export was high during Event 2 but significantly less was exported for the treatment with fungi only (BSM+F). There were no differences in nitrate export among treatment types for any other events (Figure 18C), and net nitrates were less than or equal to zero for Events 6-8 (Figure 18C). The bioretention treatments only leached DOC into effluent waters for the first two Events, with significantly

less DOC leached from treatments containing fungi (BSM+F, BSM+P+F) during Event 1 (Figure 18D).

Among the contaminants that did not initially leach from the BSM treatments, percent removal increased for TSS and metals, reaching removals of >90% by Event 4 for TSS (Figure 19A) and by Event 3 for Zn (Figure 20G,H) and total Pb (Figure 20F). Average removal of fecal coliforms (Figure 19B) and *E. coli* (Figure 19C) was generally high (84-100%) and varied as a logarithmic function of influent concentration (Figure 21). Significant correlations between influent concentration and percent removal were not evident for the other parameters.

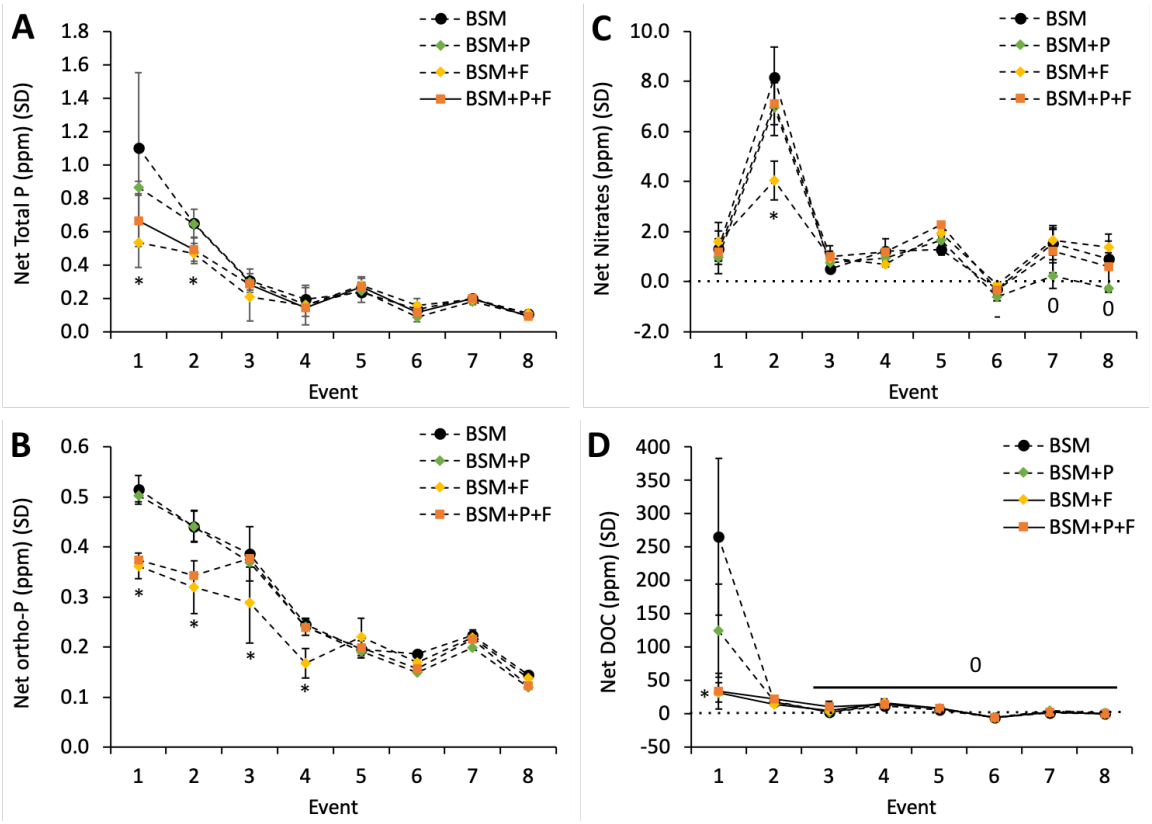

Figure 18. Net concentrations of A) total P, B) ortho-P, C) nitrates, and D) dissolved organic carbon (DOC) in effluent waters from the four bioretention treatment types for each sampling event (average ± standard deviation). A) Asterisk indicates that treatments with fungi (BSM+F, BSM+P+F) exported significantly less total P during Event 1 and BSM+F during Event 2. B) Asterisks indicate that treatments with fungi (BSM+F, BSM+P+F) leached significantly less ortho-P for Events 1 and 2 whereas BSM+F continued to leach less for Events 3 and 4. C) Asterisk indicates that BSM+F leached less nitrates during Event 2. D) Asterisk indicates that treatments with fungi (BSM+F, BSM+P+F) leached less DOC for Event 1. For events with no difference among treatments, '-' indicates significant retention by bioretention and '0' indicates that there was no net export.

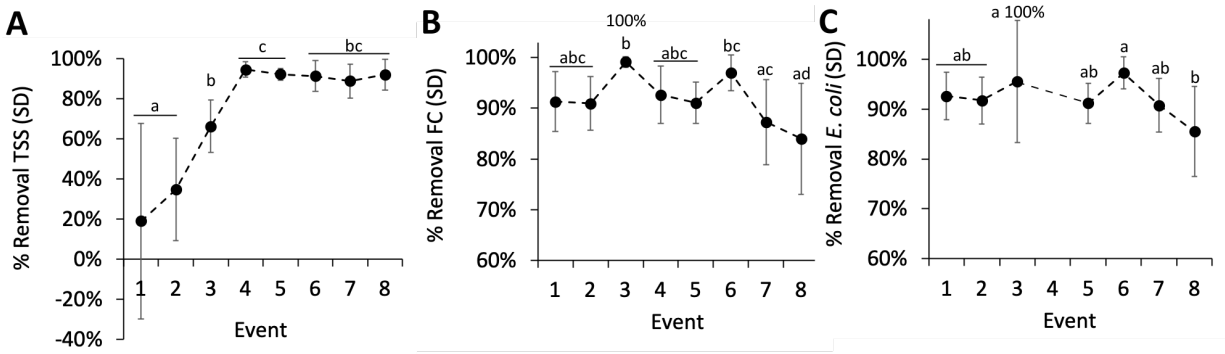

Figure 19. Average percent reduction in concentration of A) total suspended solids, B) dissolved zinc, C) dissolved cadmium, and D) dissolved chromium, E) dissolved lead, F) biological oxygen demand, G) fecal coliforms, H) E. coli, and I) chemical oxygen demand, for each of the eight sampling events across bioretention treatment type. Symbols sharing letters within a panel are not statistically different. '100%' indicates average was not different from 100% removal. Error bars are ± one standard deviation.

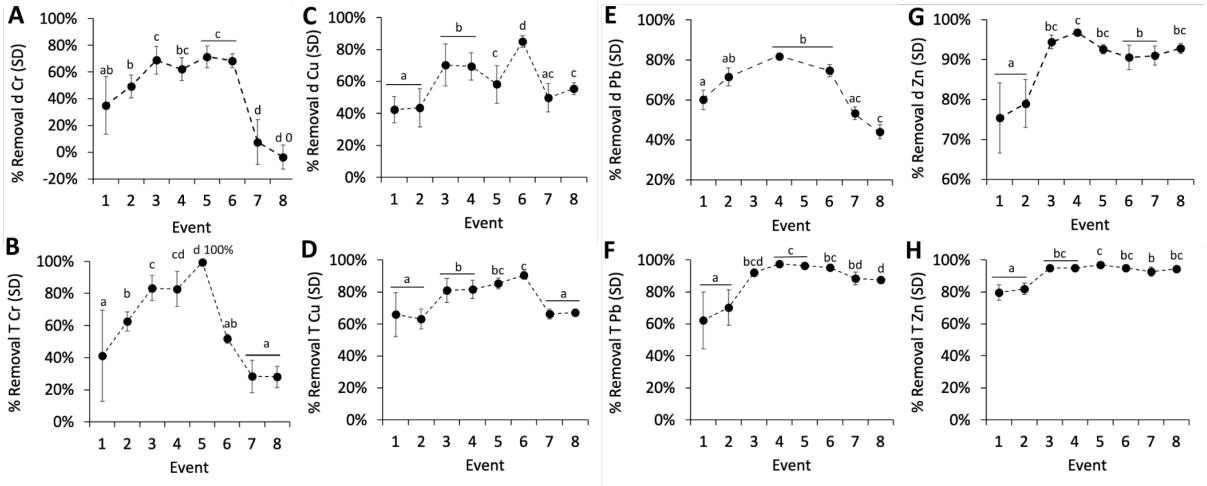

Figure 20. Average percent reduction in concentration of A) dissolved chromium, B) dissolved copper, C) dissolved lead, D) dissolved zinc, E) total chromium, F) total copper, G) total lead, H) total zinc for each of the eight sampling events across bioretention treatment type. Symbols sharing letters within a panel are not statistically different. '100%' indicates average was not different from 100% removal. Error bars are ± one standard deviation.

Removal of chromium and dissolved Pb showed a distinct loss of effectiveness after the initial increase (Figure 20A,B,E). This apparent loss of treatment ability was not directly related to influent concentration as there were no significant correlations between influent concentration and percent removal for any metal. Rather, lower removal rates may have followed a buildup of metal during prior storm events (Figure 22). Slight but significantly lower removal rates were also noted for later events for Cu, total Pb, and Zn (Figure 20C,D,F-H). In addition to occurring later in the study, these reductions in removal rates followed the dry period of Nov 2018 – Mar 2019 when the cells were not receiving runoff due to vandalism of the pumps.

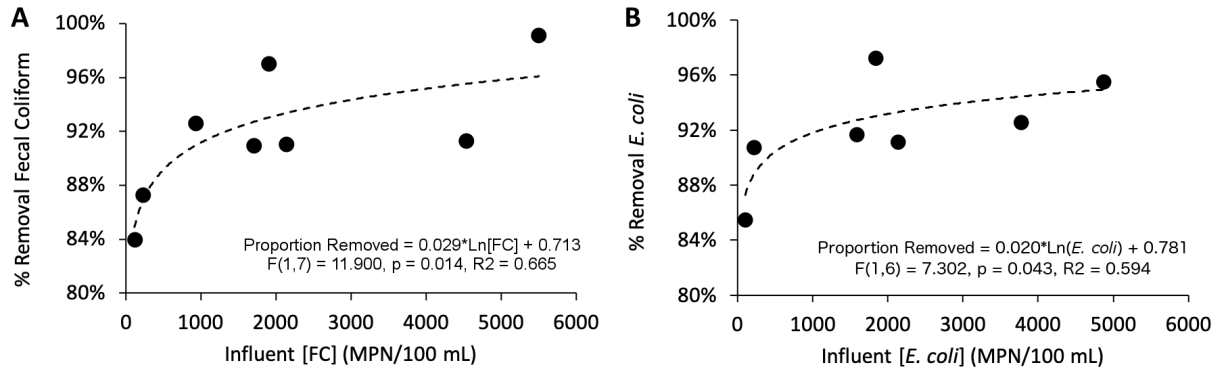

Figure 21. Logarithmic relationship between average influent concentrations of A) fecal coliforms and B) *E. coli* for each event and the percent removal achieved for each event across treatments.

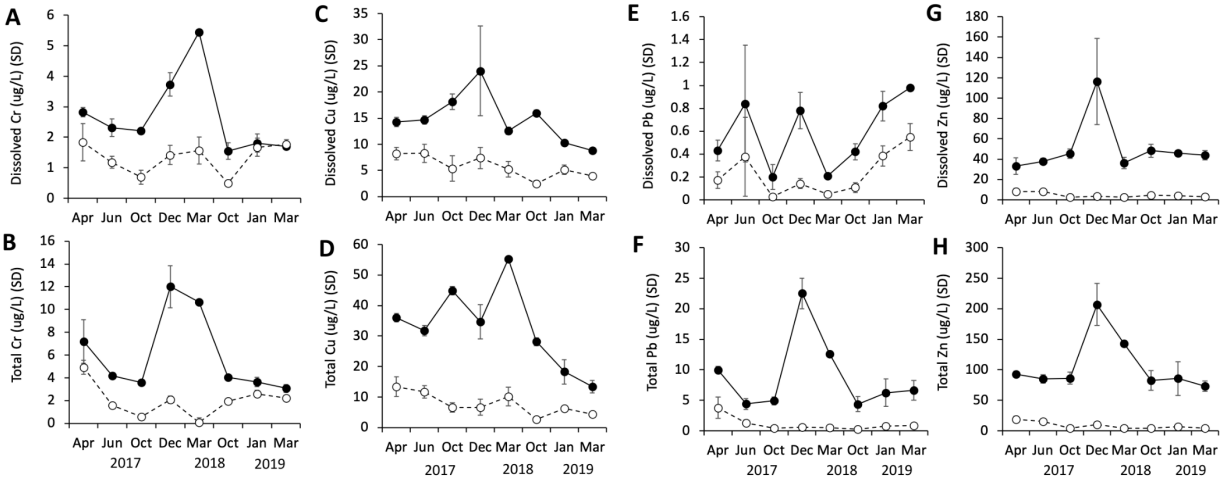

Figure 22. Concentrations of A) total suspended solids, B) fecal coliform, and C) *E. coli* in influent stormwater (filled black) and effluent (filled white) with ± standard deviation.

Percent removal of dissolved copper, total lead, and total zinc depended on treatment and event (Table 13). Prior to Event 3 there was significantly more removal for treatments with fungi (BSM+F, BSM+P+F) than treatments without (BSM, BSM+P) (Figure 23). For copper, the trend reversed for Events 3-5, with more removal for treatments without fungi (Figure 23A). For copper there were no differences among treatments after Event 5, or after Event 2 for total Pb and total Zn (Figure 23B,C).

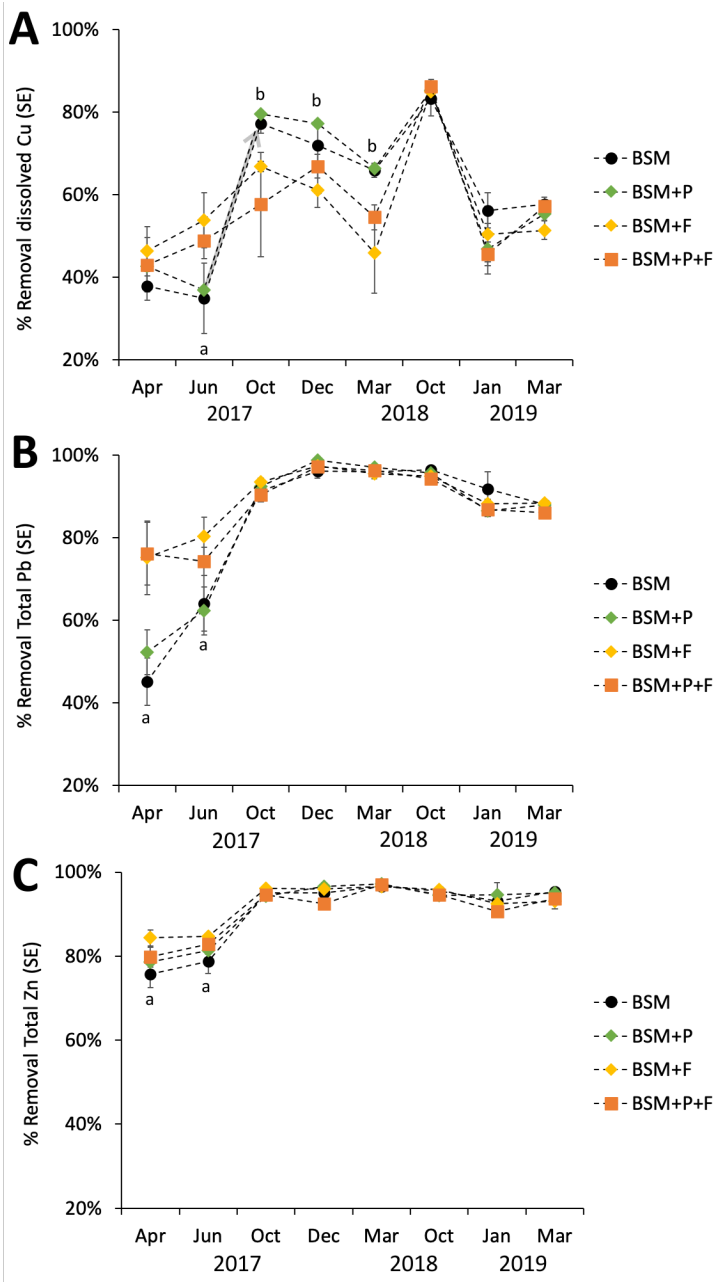

Figure 23. Average percent removal of A) dissolved copper, B) total lead, C) total Zn for each treatment and each event. The grey arrow indicates a large increase in removal of dissolved copper for treatments without fungi (BSM and BSM+P) between Events 2 and 3. 'a' indicates more removal for treatments without fungi (BSM, BSM+P) than treatments with fungi (BSM+F, BSM+P+F). 'b' indicates that treatments without fungi removed more dissolved copper than treatments with fungi during Events 3-5.

3.5. Mass balance of contaminants in BSM

We conducted a mass balance analysis of metals and PAHs in the bioretention cells. For each sampling Event, we used the average influent concentration of metal or PAH and the average effluent concentration across all bioretention treatments. These concentrations were assumed to be constant for the volume of stormwater treated during the interval between sampling Events (Table 2). The mass of each contaminant estimated for each interval was summed to arrive at a total inputs and exports. Net mass of contaminant was then calculated as a percent of the initial mass in the BSM (concentration x 145 kg BSM per cell).

Table 14. Mass of contaminant (mg) measured in BSM following conditioning (Initial), estimated mass contributed by stormwater across all 8 Events (Input), estimated mass exported from the bioretention cell (Export), net mass exported (Net), and net mass as a percent of the initial mass in BSM.

Contaminant	Initial[a]	Input	Export	Net	% Initial
As	332.2	43.6	51.2	-7.6	-0.7%
Cd	70.7	2.2	1.3	0.9	0.4%
Cr	4200.2	85.2	23.6	61.6	0.4%
Cu	5024.3	460.7	79.1	381.6	2.3%
Pb	988.4	112.9	9.3	103.6	3.1%
Ni	4661.8	52.8	40.4	12.4	0.1%
Zn	13238.5	1432.7	91.5	1341.2	3.0%
TPAH	36.0	5.8	0.9	4.9	13.7%

[a] Mass = initial concentration (Table 6) 145 kg of BSM

The analysis suggests that metal concentrations in the BSM as a whole would have changed by less than 5%, ranging from a net loss of <1% of the initial As to a net gain of 3.1% of the initial Pb (Table 14). It was expected that most contaminants would be retained in the top layer of the BSM. There were no significant increases in the concentration of contaminants in the two bottom layers (15-45 cm) of BSM (Table 8), and significant reductions in Cd and Zn (Figure 13). Unfortunately, because the top layer (0-15 cm) was not analyzed at the end of the study, we cannot confirm whether the masses estimated here were in fact retained in the BSM. In contrast to the metals, total PAH concentration was predicted to increase by approximately 14% (Table 14). Rather than a substantially increase, PAH concentrations in the BSM decreased by approximately half. Unlike the metals, PAHs can be lost to metabolism by microorganisms, although there were no significant differences in the treatments with vs without fungi added (Figure 15).

3.4.2.1 Potential for neurotoxicity

Dissolved copper is a known toxicant to the peripheral olfactory system of fish at low ppb concentrations (Baldwin et al. 2013). However, other water constituents – notably dissolved organic matter– can modify the bioavailability of copper to the olfactory system (McIntyre et al. 2008). At ratios of DOC:dCu (ppm:ppb) greater than approximately 1:3, copper is not bioavailable to induce neurotoxicity. In influent stormwater samples, the ratio of DOC to dCu was 0.16-0.81, with a value in the expected neurotoxic range for only one of the eight sampling events (Figure 24). The DOC in effluent water was at least that of the influent water whereas dCu was reduced by 42-85% from influent across sampling Events. As a result, the DOC:dCu was even higher (1.8-15.3) than in the influent water, ensuring that copper in the effluent from bioretention would not be neurotoxic. Cadmium is another metal that can be neurotoxic to fish olfactory systems in the range of some influent concentrations (i.e. >0.05 ppb). As such, it's bioavailable fraction may be added to that of copper, resulting in a higher risk of neurotoxicity than based on either metal alone. However, olfactory neurotoxicity studies with mixtures of Cd and Cu with Ni and Zn were antagonistic, with reduced instead of increased toxicity (Dew et al. 2016). Only influent stormwater, and only for one Event, would be expected to produce olfactory neurotoxicity in fish due to the metals present.

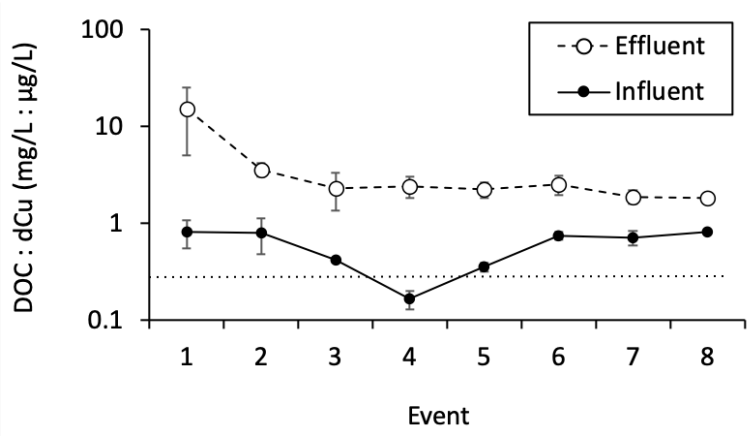

Figure 24. The ratio of average (±SE) dissolved organic carbon to dissolved copper in influent stormwater and effluent from the bioretention systems. The dotted line at 0.3 delineates samples expected to be toxic (<0.3) from those expected to be nontoxic (>0.3) to olfactory neurons.

3.6. Toxicity of influent and effluent waters

Previous studies showed that zebrafish embryos were sensitive to stormwater and that impacts were reduced or eliminated by bioretention treatment (McIntyre et al. 2014). This suggested that zebrafish embryos could be used as a screening tool to monitor bioretention treatment effectiveness between different treatments and across time in the current study. Composite samples of influent and effluent water samples were stored in amber glass jars at -20°C until bioassays were performed. Zebrafish embryos (*Danio rerio*) aged 2-4 hours post fertilization (hpf) were exposed at 28.5°C to the influent, the effluents, or freshly made fish

system water as a laboratory control (32 embryos per treatment). After 48 h of exposure, embryos were checked for mortality and photographs were taken using a digital camera mounted on a Nikon SMZ 800 stereomicroscope for analysis of morphometrics. Embryo length, eye area, periventral and pericardial areas were measured using Image J, an open source image processing program (Rueden et al. 2017).

3.6.1 Toxicity of bioretention effluent following clean water conditioning

The clean water effluent from the bioretention columns was not acutely toxic to zebrafish embryos. The endpoints measured (survival, eye area, pericardial area, periventral area and length) after 48-h exposure to the effluent were not statically different from the laboratory control and from the embryos exposed to the influent water (MANOVA, $p > 0.05$; Table 15). Therefore, bioretention materials themselves did not contribute any chemicals of concern for normal embryo-larval development.

Table 15. Summary of sublethal effects of runoff on zebrafish development at 48 hpf (hpf = hours post fertilization). Values presented are mean ± SE from each endpoint. Each treatment was tested using 32 embryos.

Treatment	Hatched	survival	Pericardial area (mm^2)	Periventral area (mm^2)	Eye area (mm^2)	Length (mm)
Control	2	100%	0.023 ± 0.001	0.018 ± 0.001	0.043 ± 0.001	2.839 ± 0.011
Effluent	0	100%	0.023 ± 0.001	0.019 ± 0.001	0.042 ± 0.002	2.852 ± 0.025
Influent	0	100%	0.025 ± 0.002	0.019 ± 0.001	0.044 ± 0.001	2.834 ± 0.010

3.6.2 Toxicity during stormwater treatment

Five of the eight influent stormwater samples affected endpoints measured in the 48-h zebrafish embryo exposures. Embryos exposed to influent stormwater from sampling Event 5 were significantly larger than embryos exposed to control water. This stimulation of growth was not ameliorated by bioretention treatment (Figure 25A). A decreased eye area was observed in embryos exposed to influent from sampling Events 2, 6, and 8 (Figure 25B). For Event 6, all four BSM treatments prevented impact to eye development, whereas no treatment prevented the impact for Event 2, and only BSM and BSM+P prevented the impact for Event 8 (Figure 25B). Cardiotoxicity, reflected by an enlargement in the pericardial area, was observed for influent stormwater from sampling Events 3, 5, and 6 (Figure 25C). This is the sublethal impact most commonly seen in embryos affected by acute exposure to road runoff (McIntyre et al. 2014). These effects were completely eliminated by all four BSM treatments for sampling Event 6, but for the other two Events the various treatments inconsistently and incompletely prevented toxicity. Additionally, sublethal impacts were observed in the absence of toxic influent for effluents from the first sampled event (April 2017). No sublethal impairments were evident in embryos exposed to influent or effluent waters from sampling Events 4 and 7 (Appendix 2).

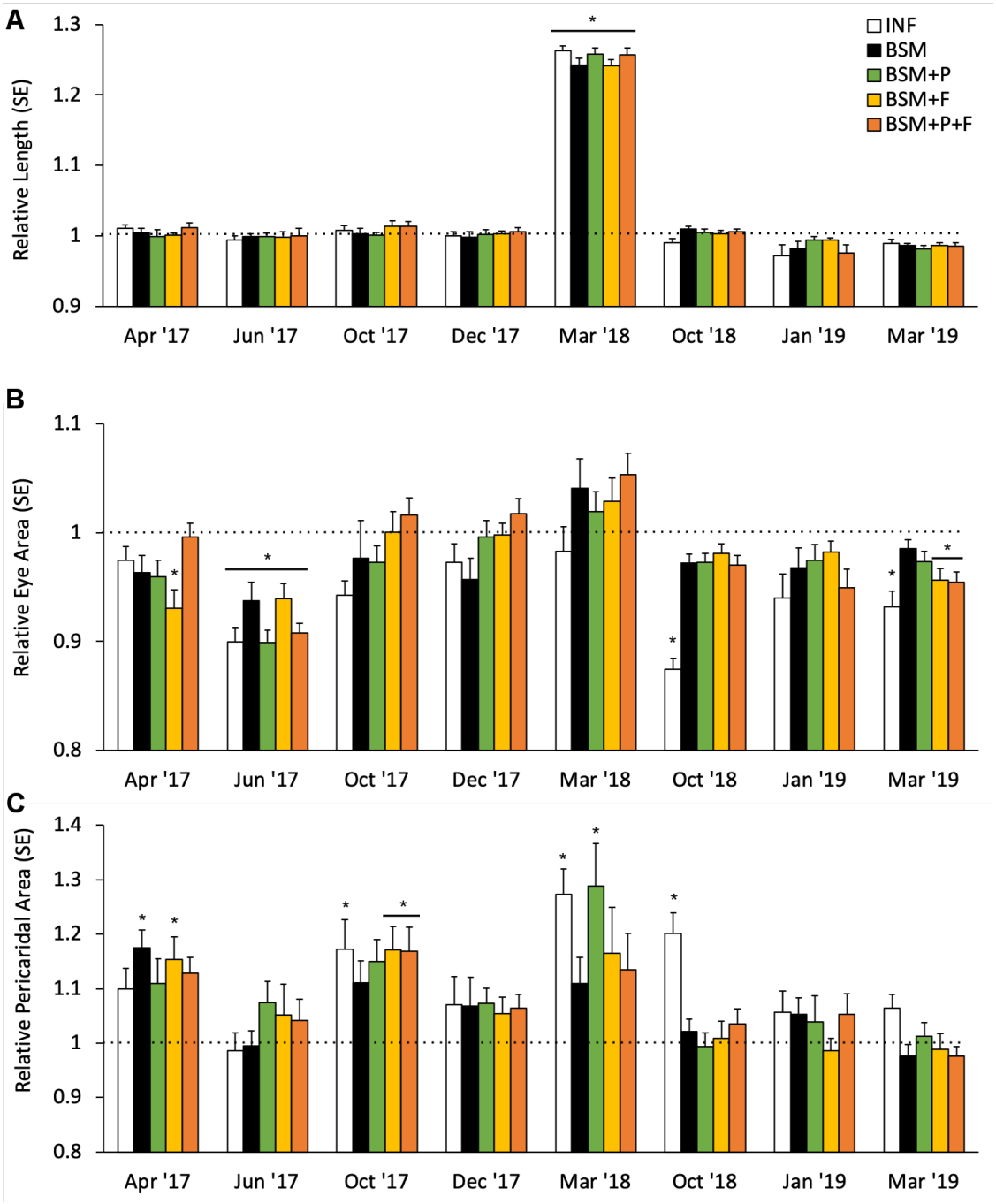

Figure 25. Zebrafish embryo morphometrics following 48 h exposure to influent stormwater (INF) or effluent from one of four bioretention treatments; bioretention soil media (BSM) with and without plants (P) and fungi (F). Values are expressed relative to the average of controls exposed to laboratory rearing water, shown by the dotted horizontal line at a value of '1'. Error bars are one standard error of the mean. Asterisks indicate significant difference from the control values.

4. Discussion

4.1 Water Quality

Bioretention treatment of stormwater, regardless of fungal and/or plant amendments, significantly improved water quality by removing metals, bacteria, solids, and aromatics from influent stormwater. Notably, average removal of bacteria was 92% across the study, and removal of particulate-associated pollutants, including TSS, PAHs, and total Cr, Pb, and Zn, increased dramatically to more than 90% within the first year of the study. Treatment of dissolved Zn also increased to >90% within the first 8 months while DOC changed from net export to 100% removal. As observed in other bioretention studies, nutrients and some metals were initially exported into the effluent from the bioretention media. Within the 2-year study period there was a 71% reduction in export of ortho-P from BSM, and export of total phosphorus and nitrates was reduced by more than 90%. There was often no net leaching of nitrates after the first year of treatment. Net leaching of As, Ni, and Cd into effluent from the BSM was eliminated during the study; in fact, Ni and Cd began to be sequestered by the bioretention media within the first year of the study.

4.2 Growth of Fungal and Plant Amendments

Fungi of the winecap mushroom (*Stropharia rugoso-annulata*) in the inoculated mulch (BSM+F, BSM+P+F) appeared to thrive during the first year of the study; degrading approximately 50% of the mulch compared with approximately 10% in the treatments not inoculated with fungi (BSM, BSM+P). Mycelia were observed growing down from the inoculated mulch into the BSM. The respiration experiment using collected mulch in January 2018 allowed us to determine that microbial activity was still higher in the inoculated treatments at the end of the year than in the uninoculated treatments, despite fungi being observed in all treatments before the end of the year. By the end of the second year, 93% of the mulch in the inoculated treatments had been degraded compared with 82% in the treatments that were not inoculated. This represents a significant increase in 'volunteer' microbial activity during Year 2 for the BSM and BSM+P treatments and a necessary slowing down of activity in the BSM+F and BSM+P+F treatments as their primary food source was used up. The mulch layer was not replenished during the study, which likely throttled the productivity of the fungi – inoculated or 'volunteer'.

Plants added as bareroot seedlings of ninebark (*Physocarpus capitatus*) in February 2017 grew during spring of Year 1 only to suffer from drought during summer 2017. Despite supplemental weekly watering (all treatments, not just those with plants), 50% of the plants died. Through consultation with the project's advisory committee, dead plants were replaced in February 2018 in the same manner as the original planting. However, three of the eight sampling events would have been affected; Event 3 (Oct 2017) and Event 4 (Dec 2017) while the plants were dead, and Event 5 (Mar 2018) while the plants were becoming established. At the time of take down (May 2019), it was noted that the second set of plants appeared better

established than the first set, with more well-developed root systems. One indication that plants were having a physical impact on the bioretention systems by the end of Year 2 was the observation that moisture content of the remaining mulch and of the surface layer of soil (0-15 cm) were significantly lower in the treatments with plants than the treatments without plants. Additionally, soil probes at 20 cm in the BSM recorded lower volumetric water (VWC) content during the last three quarters of the study for treatments with plants compared with the same period during Year 1. In contrast, the treatments without plants trended towards higher VWC during the same period. This data provides evidence that plants were finally becoming established during the latter part of the study. An effect of plants on water quality might therefore only be expected for the last 3 sampling events (6-8).

4.3 Effect of Fungal and Plant Amendments on Water Quality

Among the 23 water quality parameters analyzed, fungi improved water quality for seven parameters over the first 2-10 months: total- and ortho-P, nitrates, dissolved organic carbon, dissolved Cu, total Pb, and total Zn. The greatest impact was reduced leaching of phosphorus from treatments inoculated with fungi (BSM+F and BSM+P+F). The effect on ortho-P persisted for 10 months in the BSM+F group. The ability of fungi to translocate phosphorus is a well-known phenomenon for mycorrhizal fungi (Deacon 2006), and even for saprophytic fungi like the wine cap mushroom used here (Dighton 2016). Our results showing reduced export of P from bioretention with fungi agree with those of a recent study using a similar BSM to which a mixture of endo- and ectomycorrhizal fungi were added directly to the BSM (Poor et al. 2018).

Fungal inoculation also had a transient effect on treatment of several metals, with more removal of dissolved Cu, total Pb, and total Zn over the first 4 months for treatments with fungi than those without. A similar BSM containing a mixture of mycorrhizal fungi also removed copper at a higher rate than the BSM alone but showed no effect on removal of Zn (Poor et al. 2018). The lack of effect on Zn removal was proposed to be due to very low Zn concentrations in the BSM (7 mg/kg vs 100 mg/kg in the current study), which may have allowed high sorption even in the BSM alone. No differences were detected after 4 months for Pb and Zn, however for dissolved Cu there was more removal for treatments without fungi – a clear and distinct trend from that during the first two sampling events. Importantly, this shift was not associated with a decrease in removal by the treatments with fungi, but rather a very large increase (nearly 2-fold) in the removal of dissolved copper by the treatments without fungi. As described earlier, fungi were discovered in the BSM and BSM+P treatments before the end of Year 1. Perhaps the increase in effectiveness for these treatments was associated with a surge in microbial activity.

The most transient effect of fungi was on DOC and nitrate concentrations, which were affected for one event only. DOC was exported at high concentrations from the treatments without fungi for Event 1 only. In contrast, DOC export was 4-8 times lower for the treatments with fungi during Event 1. Subsequent sampling events showed low-to-no

significant export of DOC and no differences among treatment types. For nitrates, less leached from BSM+F for Event 2 when there was an elevated concentration in the influent stormwater.

Importantly, the benefits of fungi noted during the first year were evident despite the presence of at least some fungi in all bioretention replicates, which would have decreased our ability over time to measure a benefit of fungal inoculation. The clear decline in the benefit of fungi over time for phosphorus and metals in effluent water was likely a combination of reduced export generally from BSM over time, and the increasing presence of fungi in all treatments. No benefits of fungi were present during the second year of the study. This is not surprising given the necessary decrease in fungal activity in the inoculated treatments that would have occurred as the mulch was degraded, and the concurrent increase in fungal activity in the non-inoculated treatments as the mulch in those treatments was colonized by fungi and other microbiota.

There was no benefit of plants on water chemistry parameters throughout most of the study. In fact, the presence of plants tended to negate the benefit of fungi on water chemistry parameters; when there was an effect of fungi (BSM+F), the additional presence of plants (BSM+P+F) tended to reduce that effect. However, due to the poor initial establishment of plants, this confounding effect may be related to the loss of plants rather than the presence of plants. Changes in volumetric water content measured over time showed that plants became well established during the last three quarters of the study, encompassing the final three water quality sampling events. For these three events there was significantly less nitrates released into effluent waters from the treatment with plants (BSM+P), although this benefit was not evident in the treatment with both plants and fungi (BSM+P+F).

4.4 Toxicity of Influent and Effluent Waters

Zebrafish embryos exposed to influent stormwater in this study developed smaller eyes and/or pericardial edema in five of the eight sampling events. These effects are consistent with previous studies on stormwater (McIntyre et al. 2014). Overall, the influent stormwater sampled during the quarterly storm events tended to not be acutely toxic to zebrafish embryos for the endpoints and exposure duration tested. Among the 24 possible cases of toxicity (8 influent samples x 3 sublethal endpoints), influent samples produced only 7 cases of toxicity (1 endpoint for Event 2; 1 endpoint for Event 3; 2 endpoints for Event 5; 2 endpoints for Event 6; 1 endpoint for Event 8). Embryo length was previously recognized as a less sensitive endpoint, but we expected both eye area and pericardial edema would be affected for the sampled influent stormwater whereas each was affected in 3 out of 8 events. This was likely due to lower concentrations of contaminants in the influent runoff samples compared with stormwater samples that have been shown to cause acute sublethal toxicity using this model in the past (McIntyre et al. 2014). For example, cardiotoxicity observed in zebrafish embryos exposed to road runoff is associated with total PAH > 1 µg/L (McIntyre et al. 2016). In contrast, total PAHs measured in influent stormwater during sampling events for

the current study were typically <1 µg/L (Appendix 1). Although influent stormwater included runoff from I-5, the land area contributing runoff (12.8 hectares) also includes an unknown contribution area that is roadside landscaping and non-highway pavement. Furthermore, runoff is captured via a set of underground catch basins with an unknown residence time and once collected at the site is routed through an underground storage tank to the test mesocosms. It is likely that organic contaminants such as PAHs that tend to be associated with fine particles are being settled out and/or degraded biologically upstream from where the influent stormwater was collected for this study. Influent TSS to the bioretention cells supports that there was generally a low influx of fine particles in this system (Appendix 1, 3); only influent during Event 4 appeared to contain mean concentrations of TSS more typical of urban runoff from Phase I municipal discharges (Hobbs et al. 2015).

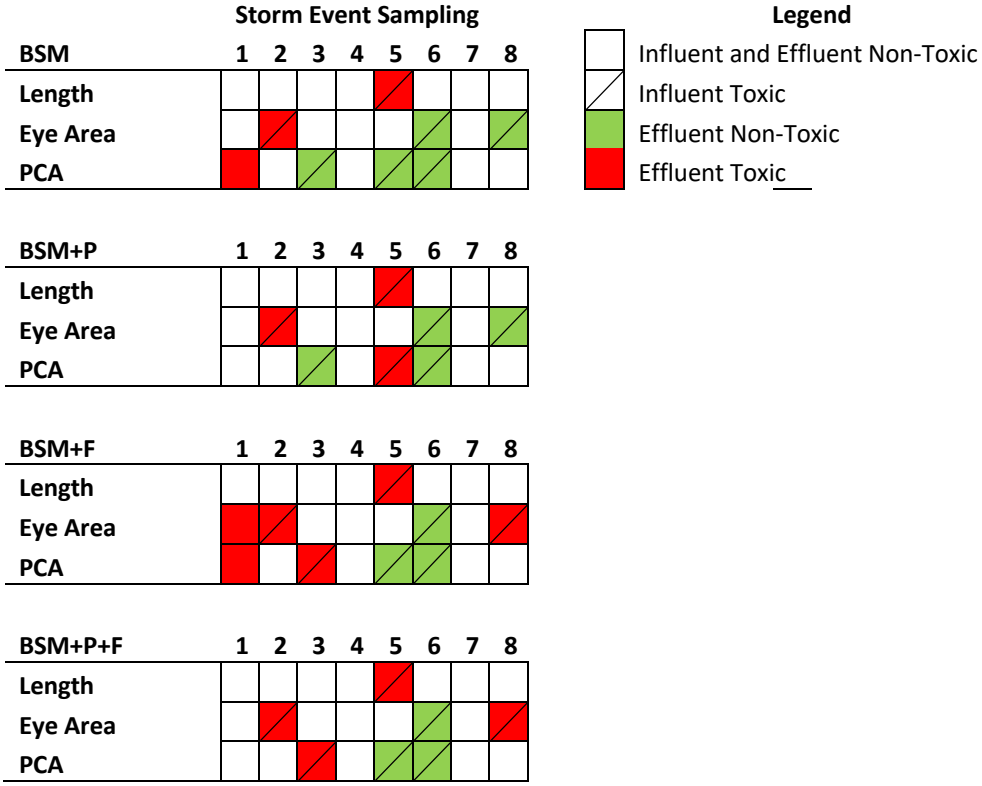

Figure 26. Summary of toxicity status of influent and effluent waters from each treatment for each sampling event.

Bioretention treatment did not always prevent the sublethal cases of toxicity caused by influent stormwater (boxes with diagonal slash colored red in Figure 26) and there was no clear additional benefit of plants and/or fungi. In fact, BSM alone appeared to perform better overall than the treatments with plants and/or fungi (lower ratio of red to green boxes for BSM alone), although there was not enough statistical power to test this hypothesis.

Neurotoxicity was not assessed in test organisms during the current study. The most potent neurotoxicant in stormwater is expected to be dissolved copper. The bioavailability of metals

including dissolved copper is strongly controlled by the DOC content of waters (diToro et al. 2002). Based on previous research (McIntyre et al. 2008, Linbo et al. 2009), a ratio of DOC: dCu >0.3 is sufficient to protect against acute neurotoxicity in developed sensory systems. In the current study, this ratio was exceeded for all but one influent stormwater sample and was greatly exceeded for all bioretention effluent samples. More recently, we reported that roadway runoff can cause neurotoxicity in the developing mechanosensory system of fish (Young et al. 2018). As this endpoint was not discovered until the current study was well underway, it was not assessed in the current study.

4.5 Contaminant levels in BSM

Despite good retention of metals during stormwater treatment, the mass balance analysis suggested that the mass of metals in the BSM as a whole would have increased very little (<3%) across the study period; i.e. the mass of metals added by stormwater treatment was very small relative to the mass of metals in BSM at installation. This was similar to the study on PCB accumulation in these same bioretention systems (Jack 2020).
The significant reduction measured in Cd (67%) and Zn (40%) from the lower layers of BSM is not easily explained, and cannot be interpreted in the context of the distribution of metals between the surface and deeper layers because metals were not measured in the surface layer at the end of the study.

In contrast, we calculated that PAHs were added to the BSM at a mass equivalent to 14% of the amount present at installation. Rather than measuring an increase between the beginning and end of the study, there was a 47% loss of PAHs from the surface layer of the BSM. This loss was likely caused by microbial degradation. In contrast to the expectation that inoculated fungi might increase degradation of PAHs in bioretention media, there was a slight but significantly higher concentration of PAHs in the treatment with fungi at the end of the study (May 2019) that was not present when the replicates were sampled opportunistically in July 2017. This result is not easily interpreted however because of loss of distinction between treatments with and without fungi that was occurring during the first year of the study. As described earlier, fungi had invaded all treatment media by the end of the first year of the study, and the higher microbial activity in the intentionally inoculated treatments during Year 1 would have been countered by lower rates during Year 2 as volunteer microbes in the remaining treatments became active degrading their more abundant food source (more mulch remaining in the treatments without added fungi).

An additional consideration for contaminant trends in BSM is the volume and composition of water administered to the bioretention cells from the baseflow noted in the description of the stormwater distribution system. By matching the timing of rain events with flow data we could estimate the relative amount of the treated volume of water that was composed of this baseflow, but we would still have no idea of the chemical composition of the baseflow for consideration of PAH or metal accumulation in BSM.

Overall, metal and PAH concentrations in the BSM were not of ecological concern as they were either below ecological screening levels and/or within concentrations typical for soils.

4.6 Physical Parameters

Bioretention systems tend to exhibit a decrease in hydraulic conductivity over time from the combined impact of hydraulic compaction and sediment loading. Whereas clogging due to sediment loading is focused at the surface of the BSM (e.g. 7 mm/year; (Gonzalez-Merchan et al. 2012), movement of fines from within the BSM itself can also cause clogging. A laboratory study investigating possible mechanisms responsible for hydraulic failure of bioretention systems in Australia demonstrated that fines from engineered bioretention soil can form an impermeable layer where the BSM interfaces with the underlying native soil (Siriwardene et al. 2007). In the current study, bioretention cells were saturated for the falling head test by filling from the bottom using an external standpipe. As described in section 3.2.4.1, 1.4 kg of fines (1% of dry BSM mass) accumulated on the surface of the BSM over repeated attempts to get three consistent K_{sat} values for averaging, resulting in a drastic reduction in K_{sat} (60 cm/h to 20 cm/h). This observation suggests that clogging due to migration of fines from the BSM might be relevant for the 60:40 bioretention mixture used in Washington State. Of note, this mass of mobile fines is several times the mass of suspended solids estimated to have been trapped by the bioretention systems from stormwater across the 2-year study (329 g).

Despite reincorporation of the mobile fines into the BSM and despite the large net retention of suspended solids from stormwater, we saw no net loss of hydraulic conductivity for the standard BSM across the 2-year study. Many factors are known to influence the amount and timing of clogging within bioretention systems (Le Coustumer et al. 2012), including lower hydraulic loading rates and incorporation of compost - both used in the Washington State 60:40 BSM as compared with many of the systems studied in the literature. For the BSM that included plants, we measured a significant net increase in hydraulic conductivity (42%). Plants may slow or even prevent the loss of hydraulic conductivity in bioretention systems over time (Dagenais et al. 2018, Virahsawmy et al. 2014), particularly for plants with shallower, thicker roots (Le Coustumer et al. 2012, Hart 2017) such as those of the Pacific ninebark in this study. In contrast, the presence of fungi resulted in an average reduction from initial K_{sat} values, whereas the treatment with both plants and fungi saw no significant net change in hydraulic conductivity.

5. Recommendations for future research

Several factors would have improved our ability to detect differences among treatments:

Study Design: The use of only three replicates per treatment group allowed insufficient statistical power for some endpoints with higher variability – most notably hydraulic conductivity. We recommend that at least 4 replicates be used in future studies.

Fungi: The mulch in the fungi-inoculated treatments was reduced by 50% after Year 1 of the study, and by 93% at the end of Year 2. In the treatments not inoculated with fungi only 15% of mulch was degraded after Year 1, and 83% after Year 2. In all treatments, therefore, the mass of mulch became a limiting factor for the productivity of microorganisms. We recommend that future studies intending to study fungal amendments renew the mulch on at least an annual basis.

Plants: Two factors affected the ability to detect an effect of plants on bioretention performance. 1) The loss of 50% of plants within the first 6 months meant that treatments with plants may have behaved more like BSM alone or BSM+F. Plants were not replaced until the end of Year 1 at which time another cycle of establishment was required for plants to become robust. More time might have allowed us to better distinguish a benefit of plants. 2) The plant used in the current study was deciduous, and therefore would have been dormant during the bulk of stormwater treatment. Although a benefit to hydraulic conductivity was evident, for studies geared towards detecting a benefit to treatment of chemical constituents we recommend use a grass or sedge.

Toxicity Testing: Lack of a consistent toxic response to influent stormwater using the zebrafish model with morphometric endpoints made it difficult to determine whether there were consistent differences in treatment ability among the bioretention treatment types. Within only 7 impacted endpoints across 8 events and 3 types of endpoint, we could not determine any trends among the treatments. We recommend that future studies use a more sensitive bioassay such as longer exposure durations, molecular indicators of exposure and harm, and/or more sensitive species such as coho salmon or *Baetis* spp. nymphs (McIntyre et al. 2015, 2016).

6. Recommendations for stormwater managers

In this study of the performance of the Washington State BSM (60:40 sand:compost), fungi provided multiple benefits. Most notably fungi reduced leaching of dissolved phosphorus from the BSM, but also improved treatment of metals such as Pb and Zn and increased the ability of the BSM to retain soil moisture needed for all microorganisms to thrive. In order to maintain the positive benefits of fungi for treatment of water pollutants sourced or treated by bioretention systems, we recommend that saprophytic fungi such as *S. rugoso-annulata* be resupplied with mulch substrate on an annual basis.

A detrimental outcome for the presence of fungi was decreased hydraulic conductivity by the end of the study. However, hydraulic conductivity increased in the presence of the plant, *P. capitatus*, used in the study. Together, fungi with plants showed no change in hydraulic conductivity across the study period – similar to the treatment without fungi or plants added. We therefore recommend that plants (especially those with thicker, shorter roots) be used to counter the detrimental effect of fungi on hydraulic conductivity.

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Appendix 1. Summary tables of water quality parameters for all sampling events

Table A1.1. Average water chemistry values (± standard deviation) for triplicate influent and effluent waters from each treatment for the 1st monitored storm event (April 5, 2017). BSM = bioretention soil medium, P = plants, F = fungi.

Type	Units	**Influent**	**BSM**	**BSM + P**	**BSM + F**	**BSM + P + F**
Conventional						
pH	n.a.	6.63 ± 0.02	6.84 ± 0.05	6.83 ± 0.11	6.86 ± 0.23	6.89 ± 0.10
Alkalinity	mg/L as $CaCO_3$	33 ± 12	97 ± 59	64 ± 55	123 ± 80	173 ± 166
Biological oxygen demand	mg/L	21 ± 20	110 ± 53	97 ± 67	57 ± 42	18 ± 16
Chemical oxygen demand	mg/L	46 ± 18	872 ± 740	1808 ± 2360	424 ± 586	472 ± 717
Dissolved organic carbon	mg/L	12 ± 6	277 ± 204	136 ± 121	43 ± 26	45 ± 47
Total organic carbon	mg/L	21 ± 5	417 ± 365	148 ± 99	57 ± 28	57 ± 54
Total suspended solids	mg/L	22 ± 7	23 ± 8	25 ± 10	7 ± 7	15 ± 10
Microbiology						
E. coli	CFU/100 mL	3767 ± 651	287 ± 199	310 ± 173	274 ± 171	247 ± 271
Fecal Coliform	MPN/100 mL	4533 ± 551	403 ± 206	433 ± 261	354 ± 236	383 ± 48
Nutrients						
Nitrate + Nitrite	mg/L	0.33 ± 0.02	1.62 ± 0.72	1.28 ± 0.47	1.91 ± 1.34	4.05 ± 4.25
ortho-Phosphate	mg/L	<0.005	<0.005	0.51 ± 0.02	0.37 ± 0.04	0.38 ± 0.05
Total Ammonia N	mg/L	0.31 ± 0.08	0.04 ± 0.01	0.04 ± 0.00	0.23 ± 0.27	0.23 ± 0.27
Total Kjeldahl N	mg/L	1.3 ± 0.1	8.9 ± 4.9	4.6 ± 1.3	4.9 ± 3.1	6.0 ± 4.5
Total Phosphorus	mg/L	0.08 ± 0.01	1.18 ± 0.45	0.97 ± 0.04	0.61 ± 0.15	0.62 ± 0.06

Table A1.2. Average concentrations of dissolved and total metals in μg/L (± standard deviation) for triplicate influent and effluent waters from each treatment for the 1st monitored storm event (April 5, 2017). BSM = bioretention soil medium, P = plants, F = fungi.

Metal	**DL (μg/L)**	**Influent**	**BSM**	**BSM + P**	**BSM + F**	**BSM + P + F**
Dissolved As	0.02	1.0 ± 0.03	6.0 ± 1.6	5.0 ± 0.6	3.6 ± 0.3	3.6 ± 0.7
Dissolved Cd	0.025	0.04 ± 0.01	0.06 ± 0.07	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.03
Dissolved Cr	0.05	2.8 ± 0.2	2.3 ± 0.1	2.3 ± 0.8	1.4 ± 0.4	1.5 ± 1.6
Dissolved Cu	0.1	14.3 ± 0.1	8.9 ± 0.9	8.2 ± 1.0	7.7 ± 1.5	8.1 ± 1.6
Dissolved Pb	0.05	0.43 ± 0.09	0.17 ± 0.07	0.24 ± 0.08	0.13 ± 0.03	0.58 ± 0.77
Dissolved Ni	0.05	2.2 ± 0.1	2.8 ± 0.4	2.5 ± 0.1	3.7 ± 2.7	3.8 ± 1.0
Dissolved Zn	0.5	33 ± 8	7 ± 1	8 ± 3	7 ± 1	11 ± 4
Total As	0.02	1.8 ± 0.1	5.2 ± 0.6	3.3 ± 2.9	3.9 ± 0.5	3.9 ± 0.4
Total Cd	0.025	n.m.	n.m.	n.m.	n.m.	n.m.
Total Cr	0.05	n.m.	n.m.	n.m.	n.m.	n.m.
Total Cu	0.1	36.0 ± 1.3	16.3 ± 3.0	16.2 ± 2.3	11.4 ± 2.1	10.7 ± 1.3
Total Pb	0.05	9.98 ± 0.53	5.30 ± 1.02	15.92 ± 18.42	2.48 ± 1.55	2.38 ± 1.32
Total Ni	0.05	4.6 ± 0.8	9.1 ± 1.7	6.1 ± 4.1	6.6 ± 1.8	7.5 ± 2.7
Total Zn	0.5	93 ± 5	23 ± 5	20 ± 4	15 ± 3	19 ± 4

Table A1.3. Average polycyclic aromatic hydrocarbon (PAH) concentrations in μg/L (± standard deviation) for triplicate influent and effluent waters from each treatment for the 1st monitored storm event (April 5, 2017). BSM = bioretention soil medium, P = plants, F = fungi. Standard deviations of zero indicate the value is ½ the detection limit; used when the PAH was detected in at least one replicate for one treatment. Values following '<' are equal to the detection limit. Values in **bold** have at least one detected replicate. n.m. = not measured for this event.

PAHs	Influent	BSM	BSM + P	BSM + F	BSM + P +F
Naphthalene	**0.056 ± 0.001**	**0.024 ± 0.025**	0.010 ± 0	0.010 ± 0	0.010 ± 0
1-Methylnaphthalene	<0.020	<0.020	<0.020	<0.020	<0.020
2-Methylnaphthalene	<0.025	<0.025	<0.025	<0.025	<0.025
Acenaphthylene	<0.030	<0.030	<0.030	<0.030	<0.030
Acenaphthene	<0.019	<0.019	<0.019	<0.019	<0.019
Dibenzofuran	<0.020	<0.020	<0.020	<0.020	<0.020
Fluorene	<0.020	<0.020	<0.020	<0.020	<0.020
Carbazole	n.m.	n.m.	n.m.	n.m.	n.m.
Phenanthrene	0.013 ± 0	**0.028 ± 0.027**	0.013 ± 0	0.013 ± 0	0.013 ± 0
Anthracene	<0.031	<0.031	<0.031	<0.031	<0.031
Fluoranthene	**0.058 ± 0.003**	**0.034 ± 0.041**	**0.018 ± 0.014**	0.011 ± 0	0.011 ± 0
Pyrene	**0.093 ± 0.001**	**0.055 ± 0.071**	0.014 ± 0	0.014 ± 0	0.014 ± 0
Benzo(a)anthracene	<0.029	<0.029	<0.029	<0.029	<0.029
Chrysene	<0.033	<0.033	<0.033	<0.033	<0.033
Benzofluoranthenes	<0.033	<0.033	<0.033	<0.033	<0.033
Benzo(a)pyrene	<0.074	<0.074	<0.074	<0.074	<0.074
Dibenzo(a,h)anthracene	<0.053	<0.053	<0.053	<0.053	<0.053
Perylene	<0.076	<0.076	<0.076	<0.076	<0.076
Indeno(1,2,3-cd)pyrene	<0.036	<0.036	<0.036	<0.036	<0.036
Benzo(g,h,i)perylene	<0.038	<0.038	<0.038	<0.038	<0.038
Total PAHs	**0.275 ± 0.004**	**0.179 ± 0.202**	**0.070 ± 0.014**	0.063 ± 0	0.063 ± 0

Table A1.4. Average water chemistry values (± standard deviation) for triplicate influent and effluent waters from each treatment for the 2nd monitored storm event (June 8, 2017). BSM = bioretention soil medium, P = plants, F = fungi.

Type	Units	**Influent**	**BSM**	**BSM + P**	**BSM + F**	**BSM + P + F**
Conventional						
pH	n.a.	7.57 ± 0.09	6.86 ± 0.14	6.89 ± 0.11	6.83 ± 0.09	6.78 ± 0.03
Alkalinity	mg/L as $CaCO_3$	61 ± 1	200 ± 20	197 ± 15	173 ± 23	197 ± 15
Biological oxygen demand	mg/L	21 ± 16	35 ± 2	33 ± 1	40 ± 3	41 ± 5
Chemical oxygen demand	mg/L	21 ± 6	69 ± 50	92 ± 10	91 ± 4	109 ± 32
Dissolved organic carbon	mg/L	12 ± 8	29 ± 4	30 ± 3	26 ± 3	34 ± 8
Total organic carbon	mg/L	26 ± 18	83 ± 7	51 ± 27	38 ± 4	53 ± 25
Total suspended solids	mg/L	18 ± 6	11± 4	15 ± 6	12± 5.5	9 ± 3
Microbiology						
E. coli	CFU/100 mL	1583 ± 475	85 ± 32	113 ± 64	177 ± 130	151 ± 28
Fecal Coliform	MPN/100 mL	1700 ± 656	105 ± 57	122 ± 73	209 ± 159	180 ± 20
Nutrients						
Nitrate + Nitrite	mg/L	1.17 ± 0.06	9.33 ± 2.08	8.13 ± 1.95	5.20 ± 1.35	11.60 ± 6.54
ortho-Phosphate	mg/L	0.05 ± 0.02	0.49 ± 0.06	0.49 ± 0.05	0.37 ± 0.09	0.39 ± 0.04
Total Ammonia N	mg/L	0.12 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.13 ± 0.08	0.13 ± 0.08
Total Kjeldahl N	mg/L	1.33 ± 0.32	6.13 ± 0.64	6.67 ± 0.72	4.33 ± 0.38	5.37 ± 0.68
Total Phosphorus	mg/L	0.09 ± 0.01	0.74 ± 0.08	0.74 ± 0.01	0.56 ± 0.06	0.58 ± 0.07

Table A1.5. Average concentrations of dissolved and total metals in μg/L (± standard deviation) for triplicate influent and effluent waters from each treatment for the 2nd monitored storm event (June 8, 2017). BSM = bioretention soil medium, P = plants, F = fungi.

Metal	**DL (μg/L)**	**Influent**	**BSM**	**BSM + P**	**BSM + F**	**BSM + P + F**
Dissolved As	0.02	2.1 ± 0.2	3.6 ± 0.2	3.3 ± 0.2	5.5 ± 3.3	3.7 ± 0.7
Dissolved Cd	0.025	0.05 ± 0.003	0.08 ± 0.03	0.09 ± 0.02	0.06 ± 0.01	0.07 ± 0.02
Dissolved Cr	0.05	2.3 ± 0.3	1.2 ± 0.3	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.3
Dissolved Cu	0.1	14.6 ± 0.8	9.5 ± 2.2	9.2 ± 0.2	6.8 ± 1.7	7.5 ± 1.1
Dissolved Pb	0.05	0.84 ± 0.51	0.39 ± 0.06	0.92 ± 0.61	0.13 ± 0.06	0.51 ± 0.55
Dissolved Ni	0.05	1.7 ± 0.02	5.6 ± 0.5	5.6 ± 0.1	7.6 ± 5.1	6.0 ± 0.9
Dissolved Zn	0.5	38 ± 3	8 ± 2	9 ± 2	6 ± 1	8 ± 3
Total As	0.02	2.8 ± 0.1	3.9 ± 0.1	3.6 ± 0.1	6.1 ± 3.7	4.0 ± 0.8
Total Cd	0.025	n.m.	n.m.	n.m.	n.m.	n.m.
Total Cr	0.05	n.m.	n.m.	n.m.	n.m.	n.m.
Total Cu	0.1	31.7 ± 1.8	13.3 ± 2.8	12.4 ± 0.7	10.1 ± 1.8	10.9 ± 1.1
Total Pb	0.05	4.4 ± 0.9	1.6 ± 0.5	1.6 ± 0.4	0.9 ± 0.4	1.1 ± 0.3
Total Ni	0.05	2.9 ± 0.2	7.4 ± 0.6	7.1 ± 0.2	10.4 ± 6.8	8.0 ± 1.8
Total Zn	0.5	85 ± 7	18 ± 4	16 ± 1	13 ± 1	15 ± 3

Table A1.6. Average polycyclic aromatic hydrocarbon (PAH) concentrations in μg/L (± standard deviation) for triplicate influent and effluent waters from each treatment for the 2nd monitored storm event (June 8, 2017). BSM = bioretention soil medium, P = plants, F = fungi. Standard deviations of zero indicate the value is ½ the detection limit; used when the PAH was detected in at least one replicate for one treatment. Values following '<' are equal to the detection limit. Values in **bold** have at least one detected replicate. n.m. = not measured for this event.

PAHs	Influent	BSM	BSM + P	BSM + F	BSM + P +F
Naphthalene	0.011 ± 0	**0.009 ± 0.002**	**0.010 ± 0.002**	0.007 ± 0	**0.008 ± 0.006**
1-Methylnaphthalene	<0.018	<0.001	<0.001	<0.001	<0.001
2-Methylnaphthalene	<0.022	<0.001	<0.001	<0.001	<0.001
Acenaphthylene	<0.027	<0.002	<0.002	<0.002	<0.002
Acenaphthene	**0.020 ± 0.021**	0.002 ± 0	0.002 ± 0	0.002 ± 0	0.002 ± 0
Dibenzofuran	<0.018	<0.002	<0.002	<0.002	<0.002
Fluorene	<0.018	<0.002	<0.002	<0.002	<0.002
Carbazole	n.m.	<0.001	<0.001	<0.001	<0.001
Phenanthrene	0.015 ± 0	**0.001 ± 0.001**	**0.002 ± 0.001**	**0.002 ± 0.001**	**0.001 ± 0.001**
Anthracene	<0.028	<0.001	<0.001	<0.001	<0.001
Fluoranthene	0.012 ± 0	**0.009 ± 0.003**	**0.012 ± 0.001**	**0.008 ± 0.003**	**0.010 ± 0.002**
Pyrene	**0.041 ± 0.006**	**0.016 ± 0.006**	**0.018 ± 0.003**	**0.010 ± 0.004**	**0.014 ± 0.003**
Benzo(a)anthracene	**0.025 ± 0.023**	**0.002 ± 0.002**	**0.002 ± 0.002**	0.0004 ± 0	0.0004 ± 0
Chrysene	**0.035 ± 0.039**	**0.005 ± 0.002**	**0.006 ± 0.001**	**0.003 ± 0.002**	**0.004 ± 0.001**
Benzofluoranthenes	<0.111[a]	<0.004	<0.004	<0.004	<0.004
Benzo(a)pyrene	<0.066	<0.003	<0.003	<0.003	<0.003
Dibenzo(a,h)anthracene	**0.074 ± 0.091**	0.0005 ± 0	0.0005 ± 0	0.0005 ± 0	0.0005 ± 0
Perylene	<0.068	<0.007	<0.007	<0.007	<0.007
Indeno(1,2,3-cd)pyrene	**0.047 ± 0.056**	0.0005 ± 0	0.0005 ± 0	0.0005 ± 0	0.0005 ± 0
Benzo(g,h,i)perylene	**0.043 ± 0.048**	0.0005 ± 0	0.0005 ± 0	0.0005 ± 0	0.0005 ± 0
Total PAHs	**0.321 ± 0.253**	**0.042 ± 0.040**	**0.035 ± 0.027**	**0.057 ± 0.043**	**0.061 ± 0.053**

[a] Reporting Limit (no Detection Limit provided)

Table A1.7. Average water chemistry values (± standard deviation) for triplicate influent and effluent waters from each treatment for the 3rd monitored storm event (October 18, 2017). BSM = bioretention soil medium, P = plants, F = fungi.

Type	Units	**Influent**	**BSM**	**BSM + P**	**BSM + F**	**BSM + P + F**
Conventional						
pH	n.a.	6.62 ± 0.12	6.78 ± 0.12	6.87 ± 0.07	6.82 ± 0.08	6.86 ± 0.19
Alkalinity	mg/L as $CaCO_3$	47 ± 1	140 ± 10	143 ± 6	133 ± 32	153 ± 12
Biological oxygen demand	mg/L	13 ± 1	6 ± 1	6 ± 1	7 ± 1	8 ± 2
Chemical oxygen demand	mg/L	33 ± 4	13 ± 2	9 ± 4	14 ± 3	34 ± 36
Dissolved organic carbon	mg/L	8 ± 0.06	10 ± 1	10 ± 1	12 ± 2	18 ± 14
Total organic carbon	mg/L	12 ± 1	12 ± 2	14 ± 6	13 ± 3	21 ± 17
Total suspended solids	mg/L	22 ± 5	8 ± 3	8± 5	5 ± 2	8 ± 2
Microbiology						
E. coli	CFU/100 mL	4867 ± 2003	1428 ± 2316	5 ± 4	47 ± 25	686 ± 1138
Fecal Coliform	MPN/100 mL	5500 ± 2427	103 ± 93	3 ± 6	47 ± 25	752 ± 1255
Nutrients						
Nitrate + Nitrite	mg/L	0.88 ± 0.02	1.37 ± 0.06	1.63 ± 0.25	1.80 ± 0.52	1.87 ± 0.76
ortho-Phosphate	mg/L	0.01 ± 0.00	0.40 ± 0.09	0.38 ± 0.01	0.30 ± 0.14	0.39 ± 0.06
Total Ammonia N	mg/L	0.35 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.01
Total Kjeldahl N	mg/L	1.37 ± 0.06	1.01 ± 0.08	0.97 ± 0.11	1.15 ± 0.33	2.00 ± 1.56
Total Phosphorus	mg/L	0.09 ± 0.02	0.39 ± 0.07	0.39 ± 0.01	0.30 ± 0.14	0.37 ± 0.03

Table A1.8. Average concentrations of dissolved and total metals in μg/L (± standard deviation) for triplicate influent and effluent waters from each treatment for the 3rd monitored storm event (October 18, 2017). BSM = bioretention soil medium, P = plants, F = fungi. '<' indicates values were below detection limit (DL).

Metal	**DL (μg/L)**	**Influent**	**BSM**	**BSM + P**	**BSM + F**	**BSM + P + F**
Dissolved As	0.02	1.7 ± 0.01	2.7 ± 0.2	2.5 ± 0.2	2.4 ± 0.3	2.8 ± 0.4
Dissolved Cd	0.025	0.05 ± 0	0.019 ± 0.011	0.025 ± 0.011	0.032 ± 0.008	0.034 ± 0.005
Dissolved Cr	0.05	2.21 ± 0.10	0.69 ± 0.19	0.60 ± 0.09	0.64 ± 0.08	0.82 ± 0.44
Dissolved Cu	0.1	18.1 ± 1.5	4.1 ± 0.7	3.7 ± 0.2	6.0 ± 0.4	7.7 ± 4.0
Dissolved Pb	0.05	0.20 ± 0.11	<	<	<	<
Dissolved Ni	0.05	1.7 ± 0.1	1.8 ± 0.2	1.9 ± 0.2	2.3 ± 0.1	3.7 ± 2.7
Dissolved Zn	0.5	45.4 ± 4.5	2.7 ± 0.5	2.4 ± 0.2	2.3 ± 0.5	2.9 ± 1.6
Total As	0.02	2.5 ± 0.1	2.8 ± 0.1	3.0 ± 0.1	2.7 ± 0.3	2.9 ± 0.5
Total Cd	0.025	n.m.	n.m.	n.m.	n.m.	n.m.
Total Cr	0.05	n.m.	n.m.	n.m.	n.m.	n.m.
Total Cu	0.1	34.7 ± 1.4	5.4 ± 1.1	4.7 ± 0.4	7.2 ± 0.3	9.2 ± 4.2
Total Pb	0.05	4.91 ± 0.64	0.42 ± 0.18	0.37 ± 0.04	0.31 ± 0.08	0.47 ± 0.15
Total Ni	0.05	2.47 ± 0.04	2.20 ± 0.33	2.14 ± 0.25	2.59 ± 0.02	4.01 ± 2.75
Total Zn	0.5	86 ± 10	4 ± 1	5 ± 1	3 ± 1	5 ± 2

Table A1.9. Average polycyclic aromatic hydrocarbon (PAH) concentrations in μg/L (± standard deviation) for triplicate influent and effluent waters from each treatment for the 3rd monitored storm event (October 18, 2017). BSM = bioretention soil medium, P = plants, F = fungi. Standard deviations of zero indicate the value is ½ the detection limit; used when the PAH was detected in at least one replicate for one treatment. Values in **bold** have at least one detected replicate. '<' indicates all replicates less than the detection limit of 0.011. n.m. = not measured for this event.

PAHs	Influent	BSM	BSM + P	BSM + F	BSM + P +F
Naphthalene	**0.023 ± 0.001**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
1-Methylnaphthalene	<	<	<	<	<
2-Methylnaphthalene	**0.007 ± 0.003**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Acenaphthylene	<	<	<	<	<
Acenaphthene	<	<	<	<	<
Dibenzofuran	<	<	<	<	<
Fluorene	<	<	<	<	<
Carbazole	<	<	<	<	<
Phenanthrene	**0.029 ± 0.002**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Anthracene	<	<	<	<	<
Fluoranthene	**0.040 ± 0.003**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Pyrene	**0.059 ± 0.003**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Benzo(a)anthracene	<	<	<	<	<
Chrysene	**0.024 ± 0.002**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Benzofluoranthenes	**0.022 ± 0.004**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Benzo(a)pyrene	<	<	<	<	<
Dibenzo(a,h)anthracene	<	<	<	<	<
Perylene	<	<	<	<	<
Indeno(1,2,3-cd)pyrene	<	<	<	<	<
Benzo(g,h,i)perylene	**0.038 ± 0.002**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Total PAHs	**0.242 ± 0.010**	0.044 ± 0	0.044 ± 0	0.044 ± 0	0.044 ± 0

Table A1.10. Average water chemistry values (± standard deviation) for triplicate influent and effluent waters from each treatment for the 4th monitored storm event (December 19, 2017). BSM = bioretention soil medium, P = plants, F = fungi. n.m. = not measured

Type	Units	**Influent**	**BSM**	**BSM + P**	**BSM + F**	**BSM + P + F**
Conventional						
pH	n.a.	6.11 ± 0.29	6.64 ± 0.10	6.68 ± 0.06	6.66 ± 0.06	6.68 ± 0.03
Alkalinity	mg/L as $CaCO_3$	20 ± 2	85 ± 2	92 ± 3.5	85 ± 10	87 ± 2
Biological oxygen demand	mg/L	n.m.	n.m.	n.m.	n.m.	n.m.
Chemical oxygen demand	mg/L	67 ± 6	47 ± 21	59 ± 8	58 ± 31	98 ± 80
Dissolved organic carbon	mg/L	4 ± 0.2	15 ± 6	17 ± 2	20 ± 9	18 ± 7
Total organic carbon	mg/L	8 ± 0.3	18 ± 7	21 ± 3	24 ± 12	33 ± 24
Total suspended solids	mg/L	66 ± 8	6 ± 4	3 ± 0.6	3 ± 2	2 ± 1
Microbiology						
E. coli	CFU/100 mL	< 1	< 1	< 1	< 1	< 1
Fecal Coliform	MPN/100 mL	923 ± 180	65 ± 59	37 ± 31	82 ± 78	88 ± 48
Nutrients						
Nitrate + Nitrite	mg/L	0.60 ± 0.03	1.80 ± 0.89	1.53 ± 0.61	1.3 ± 0.17	1.77 ± 0.25
ortho-Phosphate	mg/L	< 0.005	0.25 ± 0.02	0.24 ± 0.03	0.17 ± 0.05	0.24 ± 0.04
Total Ammonia N	mg/L	0.18 ± 0	0.01 ± 0.01	0.01 ± 0.001	0.02 ± 0.003	0.01 ± 0.004
Total Kjeldahl N	mg/L	1.27 ± 0.12	1.35 ± 0.61	1.23 ± 0.25	2.33 ± 1.21	1.47 ± 0.32
Total Phosphorus	mg/L	0.13 ± 0.02	0.32 ± 0.07	0.29 ± 0.02	0.29 ± 0.12	0.27 ± 0.05

Table A1.11. Average concentrations of dissolved and total metals in μg/L (± standard deviation) for triplicate influent and effluent waters from each treatment for the 4th monitored storm event (December 19, 2017). BSM = bioretention soil medium, P = plants, F = fungi. '<' indicates values were below detection limit (DL).

Metal	**DL (μg/L)**	**Influent**	**BSM**	**BSM + P**	**BSM + F**	**BSM + P + F**
Dissolved As	0.02	1.0 ± 0.0.2	2.2 ± 0.2	2.1 ± 0.2	1.9 ± 0.4	2.2 ± 0.2
Dissolved Cd	0.025	0.15 ± 0.06	<	<	0.04 ± 0.02	0.04 ± 0.03
Dissolved Cr	0.05	3.73 ± 0.38	1.46 ± 0.47	1.17 ± 0.16	1.60 ± 0.29	1.43 ± 0.25
Dissolved Cu	0.1	13.1 ± 1.6	6.7 ± 2.5	5.5 ± 0.3	9.3 ± 1.8	8.0 ± 1.2
Dissolved Pb	0.05	0.78 ± 0.16	0.12 ± 0.05	0.13 ± 0.01	0.13 ± 0.03	0.19 ± 0.04
Dissolved Ni	0.05	3.3 ± 0.7	2.2 ± 0.6	1.9 ± 0.1	3.1 ± 0.9	2.4 ± 0.4
Dissolved Zn	0.5	5.1 ± 21.4	3.9 ± 0.9	3.6 ± 0.2	3.3 ± 1.3	4.1 ± 1.1
Total As	0.02	7.3 ± 5.6	3.2 ± 0.8	2.8 ± 0.3	3.4 ± 0.9	5.0 ± 2.1
Total Cd	0.025	n.m.	n.m.	n.m.	n.m.	n.m.
Total Cr	0.05	n.m.	n.m.	n.m.	n.m.	n.m.
Total Cu	0.1	54.7 ± 6.3	9.2 ± 4.3	7.0 ± 0.8	12.6 ± 1.8	11.7 ± 1.6
Total Pb	0.05	22.5 ± 2.5	0.9 ± 0.7	0.3 ± 0	0.6 ± 0.3	0.6 ± 0.1
Total Ni	0.05	18.9 ± 18.7	3.6 ± 0.3	2.2 ± 0.3	3.7 ± 2.0	4.3 ± 1.8
Total Zn	0.5	207 ± 35	10 ± 3	7 ± 1	8 ± 3	16 ± 2

Table A1.12. Average polycyclic aromatic hydrocarbon (PAH) concentrations in μg/L (± standard deviations) for triplicate influent and effluent waters from each treatment for the 4th monitored storm event (December 19, 2017). BSM = bioretention soil medium, P = plants, F = fungi. Standard deviations of zero indicate the value is ½ the detection limit; used when the PAH was detected in at least one replicate for one treatment. Values in **bold** have at least one detected replicate. '<' indicates all replicates less than the reporting limit of 0.012. n.m. = not measured for this event.

PAHs	Influent	BSM	BSM + P	BSM + F	BSM + P +F
Naphthalene	**0.038 ± 0.005**	0.006 ± 0	0.006 ± 0	0.009 ± 0.005	0.006 ± 0
1-Methylnaphthalene	**0.014 ± 0**[a]	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
2-Methylnaphthalene	**0.024 ± 0.003**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Acenaphthylene	<	<	<	<	<
Acenaphthene	<	<	<	<	<
Dibenzofuran	<	<	<	<	<
Fluorene	<	<	<	<	<
Carbazole	<	<	<	<	<
Phenanthrene	**0.101 ± 0.010**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Anthracene	**0.014 ± 0.001**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Fluoranthene	**0.136 ± 0.016**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Pyrene	**0.233 ± 0.034**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Benzo(a)anthracene	**0.040 ± 0.009**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Chrysene	**0.089 ± 0.013**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Benzofluoranthenes	**0.098 ± 0.017**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Benzo(a)pyrene	**0.054 ± 0.011**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Dibenzo(a,h)anthracene	**0.007 ± 0.004**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Perylene	**0.018 ± 0.003**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Indeno(1,2,3-cd)pyrene	**0.041 ± 0.009**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Benzo(g,h,i)perylene	**0.137 ± 0.019**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Total PAHs	**1.056 ± 0.151**	0.096 ± 0	0.096 ± 0	**0.099 ± 0.005**	0.096 ± 0

[a] Standard deviation zero because each replicate had same detected value

Table A1.13. Average water chemistry values (± standard deviation) for triplicate influent and effluent waters from each treatment for the 5th monitored storm event (March 22, 2018). BSM = bioretention soil medium, P = plants, F = fungi. n.m. = not measured.

Type	Units	**Influent**	**BSM**	**BSM + P**	**BSM + F**	**BSM + P + F**
Conventional						
pH	n.a.	6.87 ± 0.04	6.91 ± 0.24	6.87 ± 0.16	6.64 ± 0.28	6.87 ± 0.03
Alkalinity	mg/L as $CaCO_3$	31 ± 1	67 ± 7	77 ± 10	75 ± 3	79 ± 19
Biological oxygen demand	mg/L	16 ± 2	11 ± 1	10 ± 3	17 ± 10	10 ± 1
Chemical oxygen demand	mg/L	n.m.	n.m.	n.m.	n.m.	n.m.
Dissolved organic carbon	mg/L	4 ± 1	10 ± 1	12 ± 1	13 ± 1	12 ± 1
Total organic carbon	mg/L	7 ± 1	11 ± 2	13 ± 2	16 ± 6	13 ± 1
Total suspended solids	mg/L	44 ± 3	4 ± 1	3 ± 0	4 ± 3	3 ± 1
Microbiology						
E. coli	CFU/100 mL	2133 ± 115	200 ± 72	111 ± 34	280 ± 92	163 ± 57
Fecal Coliform	MPN/100 mL	2133 ± 115	200 ± 72	111 ± 34	280 ± 92	170 ± 66
Nutrients						
Nitrate + Nitrite	mg/L	0.34 ± 0.02	1.60 ± 0.35	2.00 ± 0.17	2.27 ± 0.15	2.60 ± 0.10
ortho-Phosphate	mg/L	0.07 ± 0.01	0.27 ± 0.03	0.26 ± 0.02	0.29 ± 0.06	0.27 ± 0.03
Total Ammonia N	mg/L	0.32 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
Total Kjeldahl N	mg/L	1.07 ± 0.06	0.87 ± 0.03	0.94 ± 0.19	1.00 ± 0.09	1.10 ± 0.00
Total Phosphorus	mg/L	0.10 ± 0.01	0.34 ± 0.06	0.34 ± 0.03	0.38 ± 0.05	0.37 ± 0.05

Table A1.14. Average concentrations of dissolved and total metals in μg/L (± standard deviation) for triplicate influent and effluent waters from each treatment for the 5th monitored storm event (March 22, 2019). BSM = bioretention soil medium, P = plants, F = fungi. '<' indicates values were below detection limit (DL).

Metal	**DL (μg/L)**	**Influent**	**BSM**	**BSM + P**	**BSM + F**	**BSM + P + F**
Dissolved As	0.02	0.89 ± 0.03	1.80 ± 0.07	1.79 ± 0.07	1.95 ± 0.14	1.92 ± 0.07
Dissolved Cd	0.025	n.m.	n.m.	n.m.	n.m.	n.m.
Dissolved Cr	0.05	5.44 ± 0.06	2.00 ± 0.55	1.33 ± 0.31	1.58 ± 0.46	1.33 ± 0.19
Dissolved Cu	0.1	12.6 ± 0.7	4.3 ± 0.4	4.2 ± 0.2	6.8 ± 2.2	5.7 ± 0.7
Dissolved Pb	0.05	0.21 ± 0	<	<	<	<
Dissolved Ni	0.05	1.3 ± 0.1	1.2 ± 0.1	1.5 ± 0.3	2.0 ± 0.6	1.8 ± 0.2
Dissolved Zn	0.5	36.1 ± 5.7	2.5 ± 0.3	2.5 ± 0.4	2.8 ± 0.5	2.8 ± 0.5
Total As	0.02	1.8 ± 0.1	2.0 ± 0.1	2.0 ± 0.2	2.2 ± 0.3	2.0 ± 0.1
Total Cd	0.025	n.m.	n.m.	n.m.	n.m.	n.m.
Total Cr	0.05	n.m.	n.m.	n.m.	n.m.	n.m.
Total Cu	0.1	44.8 ± 0.5	5.8 ± 0.4	5.4 ± 0.1	8.3 ± 2.1	6.8 ± 0.8
Total Pb	0.05	12.6 ± 0.3	0.5 ± 0.2	0.4 ± 0	0.6 ± 0.3	0.5 ± 0.1
Total Ni	0.05	4.2 ± 0.1	2.2 ± 0.1	3.2 ± 2.1	2.9 ± 0.7	2.4 ± 0.3
Total Zn	0.5	142.7 ± 5.0	4.9 ± 1.0	4.1 ± 0.6	4.9 ± 1.7	4.3 ± 0.1

Table A1.15. Average polycyclic aromatic hydrocarbon (PAH) concentrations in μg/L (± standard deviation) for triplicate influent and effluent waters from each treatment for the 5th monitored storm event (March 22, 2018). BSM = bioretention soil medium, P = plants, F = fungi. Standard deviations of zero indicate the value is ½ the detection limit; used when the PAH was detected in at least one replicate for one treatment. Values in **bold** have at least one detected replicate. '<' indicates all replicates less than the reporting limit of 0.012. n.m. = not measured for this event.

PAHs	Influent	BSM	BSM + P	BSM + F	BSM + P +F
Naphthalene	**0.027 ± 0.002**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
1-Methylnaphthalene	<				
2-Methylnaphthalene	**0.019 ± 0.002**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Acenaphthylene	<	<	<	<	<
Acenaphthene	<	<	<	<	<
Dibenzofuran	<	<	<	<	<
Fluorene	<	<	<	<	<
Carbazole	**0.018 ± 0.003**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Phenanthrene	**0.070 ± 0.002**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Anthracene	<	<	<	<	<
Fluoranthene	**0.091 ± 0.002**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Pyrene	**0.135 ± 0.003**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Benzo(a)anthracene	**0.017 ± 0.001**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Chrysene	**0.051 ± 0.002**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Benzofluoranthenes	**0.053 ± 0.004**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Benzo(a)pyrene	**0.025 ± 0.006**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Dibenzo(a,h)anthracene	<	<	<	<	<
Perylene	<	<	<	<	<
Indeno(1,2,3-cd)pyrene	**0.017 ± 0.001**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Benzo(g,h,i)perylene	**0.071 ± 0.001**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Total PAHs	**0.594 ± 0.009**	0.072 ± 0	0.072 ± 0	0.072 ± 0	0.072 ± 0

Table A1.16. Average water chemistry values (± standard deviation) for triplicate influent and effluent waters from each treatment for the 6th monitored storm event (October 25, 2018). BSM = bioretention soil medium, P = plants, F = fungi.

Type	Units	**Influent**	**BSM**	**BSM + P**	**BSM + F**	**BSM + P + F**
Conventional						
pH	n.a.	6.97 ± 0.14	7.21 ± 0.20	7.23 ± 0.11	7.19 ± 0.10	7.21 ± 0.05
Alkalinity	mg/L as $CaCO_3$	82 ± 5	147 ± 15	145 ± 7	153 ± 6	150 ± 10
Biological oxygen demand	mg/L	<2	<2	<2	<2	<2
Chemical oxygen demand	mg/L	n.m.	n.m.	n.m.	n.m.	n.m.
Dissolved organic carbon	mg/L	12 ± 2	7 ± 4	7 ± 1	6 ± 0.2	6 ± 0.6
Total organic carbon	mg/L	15 ± 4	8 ± 4	7 ± 1	6 ± 0.6	7 ± 0.4
Total suspended solids	mg/L	22 ± 6	1.5 ± 1.3	2.2 ± 2.5	1.7 ± 2.0	2.2 ± 1.8
Microbiology						
E. coli	CFU/100 mL	1833 ± 351	53 ± 84	66 ± 68	14 ± 9	71 ± 65
Fecal Coliform	MPN/100 mL	1900 ± 265	53 ± 84	79 ± 90	15 ± 9	79 ± 70
Nutrients						
Nitrate + Nitrite	mg/L	1.07 ± 0.06	0.76 ± 0.21	0.44 ± 0.15	0.94 ± 0.04	0.74 ± 0.21
ortho-Phosphate	mg/L	0.06 ± 0.00	0.25 ± 0.00	0.21 ± 0.01	0.23 ± 0.03	0.22 ± 0.02
Total Ammonia N	mg/L	0.370 ± 0.020	0.012 ± 0.003	0.015 ± 0.006	0.012 ± 0.003	0.012 ± 0.001
Total Kjeldahl N	mg/L	1.66 ± 0.04	0.59 ± 0.57	0.37 ± 0.13	0.34 ± 0.10	0.37 ± 0.04
Total Phosphorus	mg/L	0.12 ± 0.01	0.26 ± 0.04	0.21 ± 0.03	0.28 ± 0.04	0.24 ± 0.02

Table A1.17. Average concentrations of dissolved and total metals in μg/L (± standard deviation) for triplicate influent and effluent waters from each treatment for the 6th monitored storm event (October 25, 2018). BSM = bioretention soil medium, P = plants, F = fungi.

Metal	DL (μg/L)	Influent	BSM	BSM + P	BSM + F	BSM + P + F
Dissolved As	0.05	1.89 ± 0.25	3.07 ± 0.60	3.02 ± 0.20	3.33 ± 0.0.20	3.22 ± 0.14
Dissolved Cd	0.05	0.09 ± 0.03	0.04 ± 0.03	<	<	<
Dissolved Cr	0.1	1.55 ± 0.27	0.55 ± 0.11	0.50 ± 0.08	0.46 ± 0.04	0.45 ± 0.09
Dissolved Cu	0.1	16.0 ± 0.6	2.7 ± 1.2	2.3 ± 0.3	2.4 ± 0.3	2.2 ± 0.5
Dissolved Pb	0.1	0.42 ± 0.07	0.11 ± 0.05	0.12 ± 0.02	0.08 ± 0.05	0.12 ± 0.06
Dissolved Ni	0.05	2.3 ± 0.1	1.8 ± 0.4	1.5 ± 0.2	1.5 ± 0.1	1.4 ± 0.1
Dissolved Zn	0.5	48.3 ± 6.3	4.6 ± 2.0	4.9 ± 0.9	3.5 ± 1.0	5.3 ± 2.0
Total As	0.05	4.3 ± 0.2	4.81 ± 0.73	4.83 ± 0.36	4.94 ± 0.20	4.85 ± 0.08
Total Cd	0.05	0.16 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.09 ± 0.04
Total Cr	0.1	4.05 ± 0.08	1.98 ± 0.13	2.00 ± 0.05	1.85 ± 0.17	1.95 ± 0.04
Total Cu	0.1	28.1 ± 1.3	3.0 ± 1.2	2.7 ± 0.4	2.6 ± 0.4	2.5 ± 0.3
Total Pb	0.1	4.34 ± 1.23	0.15 ± 0.06	0.18 ± 0.04	0.22 ± 0.08	0.25 ± 0.09
Total Ni	0.05	3.2 ± 0.3	1.7 ± 0.2	1.6 ± 0.1	2.4 ± 1.5	1.5 ± 0.9
Total Zn	0.5	82.6 ± 16.0	3.7 ± 0.8	4.6 ± 1.3	3.5 ± 1.0	4.5 ± 0.9

Table A1.18. Average polycyclic aromatic hydrocarbon (PAH) concentrations in μg/L (± standard deviation) for triplicate influent and effluent waters from each treatment for the 6th monitored storm event (October 25, 2018). BSM = bioretention soil medium, P = plants, F = fungi. Standard deviations of zero indicate the value is ½ the detection limit; used when the PAH was detected in at least one replicate for one treatment. Values in **bold** have at least one detected replicate. '<' indicates all replicates less than the detection limit of 0.011. n.m. = not measured for this event.

PAHs	Influent	BSM	BSM + P	BSM + F	BSM + P +F
Naphthalene	**0.016 ± 0.005**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
1-Methylnaphthalene	**0.012 ± 0.007**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
2-Methylnaphthalene	**0.008 ± 0.005**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Acenaphthylene	**0.008 ± 0.004**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Acenaphthene	**0.008 ± 0.005**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Dibenzofuran	<	<	<	<	<
Fluorene	**0.011 ± 0.009**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Carbazole	<	<	<	<	<
Phenanthrene	**0.022 ± 0.006**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Anthracene	**0.010 ± 0.007**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Fluoranthene	**0.035 ± 0.007**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Pyrene	**0.046 ± 0.003**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Benzo(a)anthracene	**0.010 ± 0.007**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Chrysene	**0.026 ± 0.005**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Benzofluoranthenes	**0.028 ± 0.009**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Benzo(a)pyrene	**0.016 ± 0.007**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Dibenzo(a,h)anthracene	**0.007 ± 0.003**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Perylene	<	<	<	<	<
Indeno(1,2,3-cd)pyrene	**0.011 ± 0.009**	0.006 ± 0	0.006 ± 0	0.006 ± 0	0.006 ± 0
Benzo(g,h,i)perylene	**0.032 ± 0.006**	0.006 ± 0	0.006 ± 0	0.006 ± 0	**0.008 ± 0.004**
Total PAHs	**0.306**	0.094	0.094	0.094	**0.096**

Table A1.19. Average water chemistry values (± standard deviation) for triplicate influent and effluent waters from each treatment for the 7th monitored storm event (January 23, 2019). BSM = bioretention soil medium, P = plants, F = fungi.

Type	Units	**Influent**	**BSM**	**BSM + P**	**BSM + F**	**BSM + P + F**
Conventional						
pH	n.a.	7.31 ± 0.06	7.09 ± 0.17	7.06 ± 0.09	7.09 ± 0.17	7.08 ± 0.13
Alkalinity	mg/L as $CaCO_3$	56 ± 2	51 ± 8	64 ± 12	55 ± 1	59 ± 18
Biological oxygen demand	mg/L	3.2 ± 0.3	<2	1.4 ± 0.6	<2	1.3 ± 0.6
Chemical oxygen demand	mg/L	n.m.	n.m.	n.m.	n.m.	n.m.
Dissolved organic carbon	mg/L	7.3 ± 2.2	8.4 ± 1.0	11.4 ± 3.1	9.4 ± 0.7	9.8 ± 1.1
Total organic carbon	mg/L	7.6 ± 1.3	9.4 ± 1.4	12.7 ± 4.6	9.6 ± 0.4	11 ± 1.0
Total suspended solids	mg/L	18.0 ± 13.9	1.7 ± 0.6	3.0 ± 2.6	0.7 ± 0.3	2.7 ± 0.6
Microbiology						
E. coli	CFU/100 mL	213 ± 121	14 ± 6	24 ± 11	21 ± 16	20 ± 15
Fecal Coliform	MPN/100 mL	220 ± 111	18 ± 10	38 ± 21	26 ± 16	31 ± 27
Nutrients						
Nitrate + Nitrite	mg/L	0.79 ± 0.04	2.30 ± 0.62	1.02 ± 0.51	2.43 ± 0.59	2.00 ± 0.87
ortho-Phosphate	mg/L	0.02 ± 0.00	0.24 ± 0.02	0.22 ± 0.01	0.24 ± 0.03	0.24 ± 0.02
Total Ammonia N	mg/L	0.13 ± 0	0.014 ± 0.007	0.024 ± 0.012	0.024 ± 0.002	0.020 ± 0.009
Total Kjeldahl N	mg/L	0.65 ± 0.07	0.99 ± 0.03	0.95 ± 0.19	0.89 ± 0.09	0.95 ± 0.08
Total Phosphorus	mg/L	0.07 ± 0.03	0.27 ± 0.02	0.25 ± 0.02	0.27 ± 0.03	0.27 ± 0.02

Table A1.20. Average concentrations of dissolved and total metals in μg/L (± standard deviation) for triplicate influent and effluent waters from each treatment for the 7th monitored storm event (January 23, 2019). BSM = bioretention soil medium, P = plants, F = fungi.

Metal	**DL (μg/L)**	**Influent**	**BSM**	**BSM + P**	**BSM + F**	**BSM + P + F**
Dissolved As	0.05	1.23 ± 0.12	2.34 ± 0.58	2.38 ± 0.06	2.17 ± 0.43	2.55 ± 0.22
Dissolved Cd	0.05	0.05 ± 0	<	<	<	<
Dissolved Cr	0.1	1.81 ± 0.29	1.67 ± 0.34	1.88 ± 0.46	1.50 ± 0.27	1.65 ± 0.07
Dissolved Cu	0.1	10.3 ± 0.6	4.5 ± 0.7	5.5 ± 1.1	5.1 ± 1.2	5.6 ± 0.5
Dissolved Pb	0.1	0.82 ± 0.13	0.29 ± 0.07	0.42 ± 0.04	0.38 ± 0.10	0.43 ± 0.11
Dissolved Ni	0.05	3.2 ± 0.3	1.5 ± 0.2	1.9 ± 0.6	1.6 ± 0.4	1.8 ± 0.1
Dissolved Zn	0.5	45.8 ± 2.9	3.4 ± 1.1	4.6 ± 0.5	3.8 ± 1.8	4.9 ± 0.4
Total As	0.05	2.2 ± 0.2	3.07 ± 0.37	2.91 ± 0.05	2.95 ± 0.17	3.20 ± 0.25
Total Cd	0.05	0.08 ± 0.02	<	<	<	<
Total Cr	0.1	3.63 ± 0.40	2.84 ± 0.42	2.60 ± 0.42	2.38 ± 0.41	2.59 ± 0.20
Total Cu	0.1	18.3 ± 4.0	5.9 ± 0.8	6.2 ± 0.8	6.3 ± 0.3	6.3 ± 0.5
Total Pb	0.1	6.24 ± 2.29	0.76 ± 0.01	0.84 ± 0.15	0.73 ± 0.12	0.82 ± 0.11
Total Ni	0.05	4.2 ± 0.4	2.4 ± 0.5	2.5 ± 0.2	2.4 ± 0.4	2.7 ± 0.7
Total Zn	0.5	85.4 ± 27.4	5.9 ± 1.3	6.9 ± 2.0	6.4 ± 2.2	8.0 ± 0.6

Table A1.21. Average polycyclic aromatic hydrocarbon (PAH) concentrations in μg/L (± standard deviation) for triplicate influent and effluent waters from each treatment for the 7th monitored storm event (January 23, 2019). BSM = bioretention soil medium, P = plants, F = fungi. Standard deviations of zero indicate the value is ½ the detection limit; used when the PAH was detected in at least one replicate for one treatment. Values in **bold** have at least one detected replicate. '<' indicates all replicates less than the detection limit of 0.010. n.m. = not measured for this event.

PAHs	Influent	BSM	BSM + P	BSM + F	BSM + P +F
Naphthalene	**0.018 ± 0.004**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
1-Methylnaphthalene	<	<	<	<	<
2-Methylnaphthalene	**0.007 ± 0.003**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Acenaphthylene	<	<	<	<	<
Acenaphthene	<	<	<	<	<
Dibenzofuran	<	<	<	<	<
Fluorene	<	<	<	<	<
Carbazole	<	<	<	<	<
Phenanthrene	**0.026 ± 0.007**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Anthracene	<	<	<	<	<
Fluoranthene	**0.032 ± 0.014**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Pyrene	**0.052 ± 0.024**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Benzo(a)anthracene	**0.009 ± 0.005**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Chrysene	**0.024 ± 0.013**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Benzofluoranthenes	**0.021 ± 0.013**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Benzo(a)pyrene	**0.009 ± 0.005**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Dibenzo(a,h)anthracene	<	<	<	<	<
Perylene	<	<	<	<	<
Indeno(1,2,3-cd)pyrene	**0.010 ± 0.008**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Benzo(g,h,i)perylene	**0.044 ± 0.029**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Total PAHs	**0.258**	0.055	0.055	0.055	0.055

Table A1.22. Average water chemistry values (± standard deviation) for triplicate influent and effluent waters from each treatment for the 8th monitored storm event (March 12, 2019). BSM = bioretention soil medium, P = plants, F = fungi.

Type	Units	**Influent**	**BSM**	**BSM + P**	**BSM + F**	**BSM + P + F**
Conventional						
pH	n.a.	7.09 ± 0.04	6.63 ± 0.11	6.69 ± 0.08	6.67 ± 0.14	6.75 ± 0.16
Alkalinity	mg/L as $CaCO_3$	75 ± 4	59 ± 27	55 ± 1	57 ± 10	53 ± 2
Biological oxygen demand	mg/L	5.4 ± 1.3	2.5 ± 0.3	5.1 ± 2.5	2.8 ± 0.5	2.8 ± 0.7
Chemical oxygen demand	mg/L	n.m.	n.m.	n.m.	n.m.	n.m.
Dissolved organic carbon	mg/L	7 ± 0.1	10 ± 1	12 ± 1	13 ± 1	12 ± 1
Total organic carbon	mg/L	11 ± 2	11 ± 2	13 ± 2	16 ± 6	13 ± 1
Total suspended solids	mg/L	18 ± 10	4 ± 1	3 ± 0	4 ± 3	3 ± 1
Microbiology						
E. coli	CFU/100 mL	103 ± 21	11 ± 2	22 ± 12	9 ± 5	17 ± 12
Fecal Coliform	MPN/100 mL	113 ± 15	15 ± 5	26 ± 15	10 ± 5	22 ± 18
Nutrients						
Nitrate + Nitrite	mg/L	0.94 ± 0.06	1.83 ± 0.25	0.65 ± 0.08	2.30 ± 0.53	1.55 ± 1.02
ortho-Phosphate	mg/L	0.03 ± 0.00	0.18 ± 0.01	0.15 ± 0.00	0.17 ± 0.02	0.16 ± 0.03
Total Ammonia N	mg/L	0.02 ± 0.00	<0.02	<0.02	<0.02	<0.02
Total Kjeldahl N	mg/L	0.70 ± 0.08	0.87 ± 0.07	0.74 ± 0.14	0.93 ± 0.08	0.90 ± 0.04
Total Phosphorus	mg/L	0.09 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.19 ± 0.05	0.18 ± 0.05

Table A1.23. Average concentrations of dissolved and total metals in μg/L (± standard deviation) for triplicate influent and effluent waters from each treatment for the 8th monitored storm event (March 12, 2019). BSM = bioretention soil medium, P = plants, F = fungi.

Metal	**DL (μg/L)**	**Influent**	**BSM**	**BSM + P**	**BSM + F**	**BSM + P + F**
Dissolved As	0.05	2.53 ± 0.11	2.00 ± 0.08	1.99 ± 0.12	2.08 ± 0.20	2.01 ± 0.30
Dissolved Cd	0.05	0.11 ± 0.01	<	<	<	0.04 ± 0.02
Dissolved Cr	0.1	1.70 ± 0.03	1.90 ± 0.23	1.75 ± 0.15	1.69 ± 0.06	1.72 ± 0.10
Dissolved Cu	0.1	8.8 ± 0.4	3.7 ± 0.3	3.9 ± 0.2	4.3 ± 0.3	3.8 ± 0.2
Dissolved Pb	0.1	0.98 ± 0.02	0.49 ± 0.01	0.65 ± 0.21	0.53 ± 0.03	0.52 ± 0.11
Dissolved Ni	0.05	4.41 ± 0.03	1.7 ± 0.2	1.8 ± 0.1	1.8 ± 0.2	1.7 ± 0.2
Dissolved Zn	0.5	43.9 ± 4.6	2.7 ± 0.4	3.2 ± 0.2	3.5 ± 0.9	3.2 ± 0.4
Total As	0.05	2.09 ± 0.07	2.22 ± 0.14	2.12 ± 0.12	2.18 ± 0.19	2.16 ± 0.21
Total Cd	0.05	0.17 ± 0.03	<	<	<	<
Total Cr	0.1	3.08 ± 0.34	2.31 ± 0.33	2.15 ± 0.09	2.05 ± 0.09	2.35 ± 0.14
Total Cu	0.1	13.4 ± 2.0	4.2 ± 0.3	4.3 ± 0.3	4.5 ± 0.2	4.5 ± 0.4
Total Pb	0.1	6.62 ± 1.58	0.79 ± 0.08	0.80 ± 0.08	0.77 ± 0.08	0.92 ± 0.09
Total Ni	0.05	5.0 ± 0.2	2.0 ± 1.0	1.7 ± 0.1	1.7 ± 0.2	1.6 ± 0.2
Total Zn	0.5	73.3 ± 8.7	3.4 ± 0.2	3.6 ± 0.1	5.1 ± 2.3	4.6 ± 0.9

Table A1.24. Average polycyclic aromatic hydrocarbon (PAH) concentrations in μg/L (± standard deviation) for triplicate influent and effluent waters from each treatment for the 8th monitored storm event (March 12, 2019). BSM = bioretention soil medium, P = plants, F = fungi. Standard deviations of zero indicate the value is ½ the detection limit; used when the PAH was detected in at least one replicate for one treatment. Values in **bold** have at least one detected replicate. '<' indicates all replicates less than the detection limit of 0.011. n.m. = not measured for this event.

PAHs	Influent	BSM	BSM + P	BSM + F	BSM + P +F
Naphthalene	**0.017 ± 0.004**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
1-Methylnaphthalene	<	<	<	<	<
2-Methylnaphthalene	<	<	<	<	<
Acenaphthylene	<	<	<	<	<
Acenaphthene	<	<	<	<	<
Dibenzofuran	<	<	<	<	<
Fluorene	<	<	<	<	<
Carbazole	<	<	<	<	<
Phenanthrene	**0.019 ± 0.010**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Anthracene	<	<	<	<	<
Fluoranthene	**0.025 ± 0.018**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Pyrene	**0.033 ± 0.022**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Benzo(a)anthracene	<	<	<	<	<
Chrysene	**0.012 ± 0.011**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Benzofluoranthenes	**0.015 ± 0.017**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Benzo(a)pyrene	**0.008 ± 0.004**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Dibenzo(a,h)anthracene	<	<	<	<	<
Perylene	<	<	<	<	<
Indeno(1,2,3-cd)pyrene	**0.008 ± 0.004**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Benzo(g,h,i)perylene	**0.016 ± 0.013**	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Total PAHs	**0.153**	0.055	0.055	0.055	0.055

Appendix 2. Summary of sublethal impacts of influent and effluent for all sampling events

Table A2.1. Sublethal effects of treated and untreated runoff on zebrafish development at 48 hpf (hours post fertilization). Values are means ± standard error. Significant differences compared to the control are marked by asterisks (Dunnett's post hoc test following multivariate generalized linear model, $p < 0.05$).

Event	Treatment	Pericardial area (mm^2)	Eye area (mm^2)	Length (mm)
April	Influent	0.017±0.003	0.049±0.003	2.98±0.06
April	BSM	0.018±0.002*	0.048±0.004	2.96±0.07
April	BSM + F	0.017±0.003	0.048±0.003	2.95±0.13
April	BSM + P	0.017±0.002*	0.050±0.003	2.99±0.09
April	BSM+P+F	0.017±0.003*	0.047±0.003*	2.95±0.04
April	Control	0.015±0.002	0.050±0.003	2.97±0.06
June	Influent	0.021±0.003	0.045±0.003*	2.90±0.08
June	BSM	0.021±0.003	0.046±0.005*	2.91±0.06
June	BSM + F	0.022±0.003	0.045±0.003*	2.92±0.07
June	BSM + P	0.022±0.004	0.045±0.002*	2.92±0.14
June	BSM+P+F	0.021±0.003	0.047±0.003*	2.94±0.07
June	Control	0.021±0.005	0.050±0.004	2.91±0.10
Oct	Influent	0.022±0.005*	0.043±0.003	2.93±0.09
Oct	BSM	0.022±0.005	0.044±0.008	2.91±0.11
Oct	BSM + F	0.021±0.003	0.044±0.003	2.90±0.06
Oct	BSM + P	0.022±0.004*	0.046±0.004	2.94±0.10
Oct	BSM+P+F	0.022±0.004*	0.046±0.004	2.94±0.10
Oct	Control	0.019±0.003	0.046±0.004	2.89±0.07
Dec	Influent	0.025±0.005	0.046±0.004	2.97±0.08
Dec	BSM	0.024±0.004	0.045±0.004	2.97±0.11
Dec	BSM + F	0.024±0.005	0.048±0.003	2.99±0.09
Dec	BSM + P	0.024±0.003	0.048±0.003	2.99±0.08
Dec	BSM+P+F	0.024±0.003	0.047±0.002	2.98±0.06

Dec	Control	0.022±0.005	0.048±0.004	2.98±0.08
March	Influent	0.022±0.004*	0.047±0.005	3.69±0.10*
March	BSM	0.019±0.003	0.051±0.005	3.67±0.09*
March	BSM+F	0.022±0.006*	0.048±0.004	3.68±0.11*
March	BSM+P	0.020±0.006	0.050±0.005	3.67±0.13*
March	BSM+P+F	0.020±0.007	0.049±0.005	3.62±0.12*
March	Control	0.017±0.005	0.048±0.005	2.92±0.19
Oct	Influent	0.022±0.0007*	0.037±0.0004*	2.68±0.015
Oct	BSM	0.018±0.0004	0.041±0.0003	2.73±0.010
Oct	BSM+F	0.018±0.0004	0.041±0.0003	2.72±0.012
Oct	BSM+P	0.018±0.0006	0.042±0.0004	2.72±0.013
Oct	BSM+P+F	0.019±0.0005	0.041±0.0004	2.73±0.010
Oct	Control	0.018±0.0005	0.042±0.0003	2.71±0.015
Jan	Influent	0.021±0.004	0.048±0.0011	2.88±0.05
Jan	BSM	0.021±0.003	0.049±0.0009	2.92±0.03
Jan	BSM+F	0.020±0.006	0.050±0.0007	2.95±0.01
Jan	BSM+P	0.019±0.006	0.050±0.0005	2.95±0.01
Jan	BSM+P+F	0.021±0.007	0.048±0.0009	2.90±0.03
Jan	Control	0.020±0.005	0.051±0.0004	2.97±0.01
Mar	Influent	0.024±0.0006	0.046±0.0007*	2.97±0.02
Mar	BSM	0.022±0.0005	0.048±0.0004	2.96±0.01
Mar	BSM+F	0.023±0.0006	0.048±0.0005	2.95±0.01
Mar	BSM+P	0.022±0.0006	0.047±0.0005*	2.96±0.01
Mar	BSM+P+F	0.022±0.0004	0.047±0.0005*	2.96±0.01
Mar	Control	0.022±0.0005	0.049±0.0004	3.00±0.01

Appendix 3. Laboratory reports for soil chemistry

Appendix 4: Laboratory reports for water chemistry