DRAFT
TECHNICAL MEMORANDUM

SUBJECT: Task 3.1.7 – Fungi in Bioretention Soil Media Literature Review

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To: Shanti Colwell, Seattle Public Utilities

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INTRODUCTION

Purpose

The purpose of this white paper is to summarize research findings and evaluate the effectiveness and potential consequences of using fungi as a component in bioretention soil media (BSM) for improved nutrient and metals removal.

Background Information

Bioretention systems are shallow earthen depressions, composed of BSM and plants, that provide water quality treatment and flow attenuation for stormwater runoff. These systems remove target pollutants from stormwater runoff through filtration and infiltration. Typical targeted pollutants for bioretention include:

- Sand, silt, and other suspended solids
- Heavy metals such as copper, lead, and zinc
- Nutrients such as nitrogen and phosphorus
- Bacteria and viruses
- Organic contaminants such as hydrocarbons, pesticides, and herbicides

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The current City of Seattle specification for BSM consists of 60 percent mineral aggregate and 40 percent fine compost by volume (Seattle 2017, Volume 3, Section 5.4.4.5). Compost consists of organic matter that is rich in nutrients such as nitrogen and phosphorus, and metals such as copper (Lopes et. al. 2011). Because of this, sand/compost filter media has been shown to export variable amounts of phosphorus, copper, and nitrogen to downstream receiving waters (Mullane et al. 2015; Herrera 2016). Many studies have been conducted to refine the specification for typical BSM to reduce export of these pollutants (Herrera 2016; Taylor et al. 2018; Xu et al. 2020).

For recent Seattle capital projects, the BSM blend has been adjusted to 70 percent mineral aggregate and 30 percent fine compost with an intent to reduce phosphorus export by reducing the compost fraction. Facilities constructed with the modified blend have not yet been monitored to evaluate performance.

A 2020 report entitled *Bioretention Media Blends to Improve Stormwater Treatment: Final Phase of Study to Develop New Specifications* (Herrera 2020) summarized the selection and leaching potential of a broad range of BSM components as well as pollutant export and capture characteristics of new BSM blends for water quality treatment. The study resulted in an improved BSM that supports plant growth and does not export nutrients or copper. The new media, deemed High Performance Bioretention Soil Media (HPBSM), consists of the following components:

- 2-inch mulch layer
- 18-inch primary layer consisting of:
  - 60 percent sand
  - 20 percent coconut coir
  - 10 percent high-carbon wood ash (biochar)
- An optional 12-inch polishing layer consisting of:
  - 90 percent sand
  - 7.5 percent activated alumina
  - 2.5 percent iron filings

While this improved BSM offers a higher performance than typical bioretention media, the report acknowledged the anticipated challenges of sustainably sourcing coconut coir, iron filings, and activated alumina. Fungi are one option to assess as an additive to the existing 70 percent sand/30 percent compost blend to mitigate the export of nutrients and obviate the need for less environmentally sustainable BSM components such as coir, iron, and activated alumina.

REVIEW OF FINDINGS

This review begins with a brief history of mycoremediation as well as an overview of the types of fungi involved, and their biogeochemistry and ecology as they pertain to potential use as BSM additives. Subsequently, we present a summary of target contaminant removal results from the studies included in this literature review. The review focuses on contaminants of concern in local receiving waters, specifically, nutrients, metals, and fecal coliform. The use of fungi in soil remediation is a mature science, however application in bioretention has not been well studied. We review here the available literature and subsequently present recommendations and next steps.

Mycoremediation

History

Mycoremediation, or fungal bioremediation, is a relatively new field of study that has increasingly come into practice over the last two decades as research into the potential for certain fungi to break down or assimilate toxic compounds shows promising results (Bumpus et al. 1985; Gianinazzi-Pearson 1996; Reddy 1995; Sing and Yu 1998; Singh 2006). To date, mycoremediation has primarily been related to the treatment of contaminated soils (Bosco and Mollea 2019; Goltapeh et al. 2013) and various agro-industrial wastewater streams (Dalecka et al. 2020; Espinosa-Ortiz et al. 2016; Kumar et al. 2018; Zahmatkesh et al. 2017), with very little research into the use of fungi in bioretention systems or stormwater treatment generally. Owing to this dearth of research results, this review includes results from other mycoremediation fields where inferences from their findings may reasonably be applicable.

There are two primary forms of fungi used as soil amendments to improve pollutant uptake white rot fungi (WRF) and mycorrhizal fungi. These two types of fungus are discussed below.

White Rot Fungi

WRF have historically been the most heavily researched and applied type of fungus in mycoremediation. They are unique in their production of various enzymes that can break down lignin, a complex plant polymer similar in structure to a myriad of inorganic pollutants. The potential of this was recognized early in the evolution of mycoremediation and is still actively studied (Bumpus et al. 1985; Chandra and Enespa 2019; Gao et al. 2010; Harms et al. 2011; Harvey and Scheer 2007; Reddy 1995). Various species of WRF have been demonstrated to also biodegrade polycyclic aromatic hydrocarbons (PAHs) and other simpler hydrocarbons (Ariste et al. 2020; Dickson et al. 2019; Kumar et al. 2019; Treu and Falandysz 2017), pesticides and herbicides (Maloney 2001; Mohapatra et al. 2018; Oliveira et al. 2015; Spina et al. 2018; Wolfand et al. 2016), *E. coli* (Pini and Geddes 2020; Taylor et al. 2015), fecal coliform (Thomas et al. 2009) as well as various contaminants in landfill leachates (Collado et al. 2019;

Islam et al. 2019; Islam and Yuan 2020), and agro-industrial wastewaters (Dalecka et al. 2020; Hultberg and Bodin 2017; Mir-Tutusaus et al. 2018; Ryan et al. 2008; Taheran et al. 2017). Various WRF have also been demonstrated to adsorb and/or bio transform heavy metals (Dixit et al. 2015; Kapahi and Sachdeva 2017; Wu et al. 2014). This is all promising research that may indicate that when WRF is applied in bioretention, some of these same benefits may be realized.

Mycorrhizal Fungi

Mycorrhizal fungi are those that form mutually beneficial relationships with plants. Unlike WRF, these fungi do not derive their energy from breaking down organic matter; instead, they form symbiotic relationships with living plants. From trees to grasses, most plants form mycorrhizal relationships; and, in many cases, they are essential to the survival of the plant. The hyphal network of fungal mycelium serves as an extension of the host plant's roots, aiding in the uptake of water, nutrients, minerals, and in the prevention of disease. The fungi benefit in the form of plant-produced sugars, which they are unable to produce themselves (Gianinazzi-Pearson 1996; Smith and Read 2008; Tedersoo et al. 2020). Nutrient exchange occurs between the mycelium, or hyphal network of the fungus, and the roots of the plant. Through this process mycorrhizal fungi can accumulate metals and other pollutants in the hyphal network (Singh 2006). Plant roots with a rich hyphal network have been found to absorb phosphorus and zinc more efficiently than plant roots alone (Smith and Read 2008).

Due to the complex nature of these underground relationships the study of mycorrhizae in the remediation of environmental contaminants is still in early stages, and is primarily within the field of phytoremediation, or the use of plants for pollutant sequestration and degradation (Cabello 2001; Chagnon and Brisson 2017; Coninx et al. 2017; Sharma et al. 2015). Mycorrhizal fungi have been shown to increases in plant vigor, resistance to heavy metals toxicity, and uptake of phosphorus and nitrogen, as well as improved soil biophysical properties (Asghari et al. 2005; Berruti et al. 2016; Elhindi et al. 2018;; Hewitt et al. 2020; Hildebrandt et al. 2007; Rice et al. 2002; Rillig and Mummey 2006; Sarkar et al. 2017; van der Heijden 2010; Vogelsang et al. 2004). These characteristics, when applied to planted bioretention cells, could potentially improve pollutant removal performance and increase facility longevity.

Summary of BSM-Related Results

Table 1 presents target contaminant removal findings from four studies found to be most applicable to the potential beneficial use of fungi-inoculated media and/or plants in bioretention systems based upon a thorough literature review.

Table 1. Summary of Fungi-Enhanced Bioretention Nutrient Removal Results.

Contaminant	Fungal Type(s)	Media	Influent Concentration	Effluent Concentration	Percent Reduction	Percent Reduction Versus Control[a]	Source
Total Phosphorus	Mycorrhizal	Column Study with 60/40 sand/compost and saturated zone. MycoApply[b] fungi.	Not available	Not available	-430 to 465	13 to 48	Poor et al. (2018)
Total Nitrogen			Not available	Not available	-400	0	
Total Phosphorus	Mycorrhizal and WRF	Full-scale bioretention with 60/40 sand/compost. Down to Earth® fungi and inoculated alder mulch.[c]	87 µg/L	558 µg/L	-906	-226	Thomas et al. (2009)
Total Nitrogen			1,977 µg/L	2,287 µg/L	-76	23	
Total Phosphorus	Mycorrhizal	Established swales. PermaMatrix® BSP Foundation® and Earthlite™ stormwater filter media.[d]	0.12 mg/L	0.06 mg/L	34	NA[e]	Melville (2016)
Total Phosphorus	WRF	Planted mesocosms with 60/40 BSM. Fungi Perfecti WRF inoculated alder mulch.[f]	0.097 mg/L	0.443 mg/L	-464	131	Taylor et al. (2018)
NO3+NO2			0.663 mg/L	4.377 mg/L	-1,213	-438	

[a] All studies had a control consisting of the same BSM without fungi inoculation, this column is the percent improvement over the control.

[b] MycoApply® composed of endo and ectomycorrhizal fungi from Mycorrhizal Applications (Grants Pass, Oregon).

[c] Down to Earth® is a soluble powdered blend of endomycorrhizae, ectomycorrhizae, and humic acid

[d] PermaMatrix® composed of endo and ectomycorrhizal fungi, bacteria, basalt, biochar, and reed sedge peat from PermaMatrix, Inc. (Portland, Oregon). Earthlite™ composed of rinsed Douglas fir biochar, reed sedge peat pellets, and shale.

[e] Treated swale exhibited a 33 percent increase over the control but this was due to a longer residence time in the swale, not fungi inoculation.

[f] Wine cap mushroom (*Stropharia rugoso-annulata*) from Fungi Perfecti, LLC (Olympia, Washington, USA).

Poor et al. (2018) conducted a column study at the University of Portland using nine 8-inch-diameter columns each containing 24 inches of BSM. Three BSMs were examined with triplicate replication: a control consisting of a City of Portland specificed mix with 60 to 70 percent sand and 30 to 40 percent compost; this same mix innoculated with MycoApply® Endo/Ecto and MycoApply® Ultrafine Endo from Mycorrhizal Applications (Grants Pass, Oregon); and a proprietary mix consisting of 33 percent compost, 60 percent sandy clay loam, 6 percent biochar, and 1 percent of a propritary mix of beneficial bactiera and mychorrhizae. For the purpises of this review we only analyze the results from the control and the innoculated control; the proprietary mix is not a comparable BSM so isolating the affect of just the mychorrhizae is not possible. Each column was planted with *Carex stipata*, a type of sedge; but the authors did not indicate how long the plants were allowed to establish before testing began. Stormwater was applied to each column in volumes equivalent to the 6-month 24-hour storm for Portland, Oregon, assuming a 15:1 drainage basin to facility area. Four tests were conducted each spaced 1 week apart.

Because compost was present in the BSMs used in the Poor et al. (2018) study, both the control and innoculated columns exported TP and TN (Table 1). However, the addition of fungi appeared to decrease TP export by between 13 and 48 percent, while having no apparent effect on TN export. Poor et al. (2018) also analyzed metals and found both the control and the inncoluated columns reduced dissolved copper and dissolved zinc by 45 and 81 percent, respectively. However, it did not appear that the addition of fungi resulted in an improvement over the control.

In Thomas et al. (2009), the researchers constructed and tested two full-scale bioretention systems near Olympia, Washington. The unlined cells were approximately 15 feet wide and 20 feet long and contained 18 inches of 60 percent sand/40 percent compost BSM underlain by a drain rock layer. The cells were planted with a wide range of native trees, shrubs, and grasses. The BSM and plants in one cell were innoculated with a mycorrhizal fungi (Down to Earth®, a commercial blend of mycorrhizal fungi). This cell also was overlain with a 2-inch layer of WRF inoculated alder mulch. The second cell was a control and was built identically, but not innoculated with fungi. The cells recieved intermittent runoff from an agricultural ditch and were online for 1 year prior to the collection of the first sample; consequently, any flushing from the media during that first year was not captured in the analysis. Starting in August 2007, seven monthly sampling events were conducted.

Figures 1 and 2 indicate that both cells were still flushing nutirents through the duration of the study. This, despite the fact that the systems had already been online for 1 year prior to sampling. The result was an export of TP and TN from both of the cells. The inoculated cell exported an average of 226 percent more TP and 23 percent less TN than the control cell (Table 1). This mixed result indciates that innoculation with fungi alone is not enough to prevent bioretention systems with compost from flushing nutrients. Thomas et al. (2009) also analyzed the influent and effluent for fecal coliform bacteria. They found that the innoculated cell reduced fecal coliform bacteria by 24 percent more than the control cell for an overall 90 percent removal (Table 2).

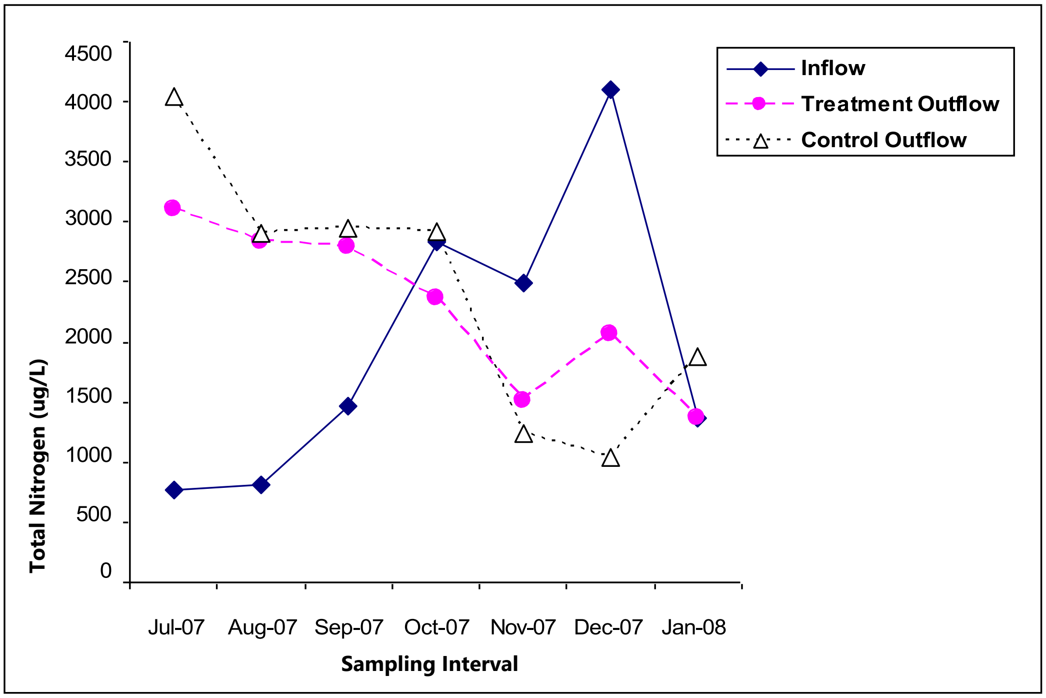

Figure 1. Total Nitrogen Concentrations Between July 2007 and January 2008 for Inflow, Treatment Outflow, and Control Outflow (Thomas et al. 2009).

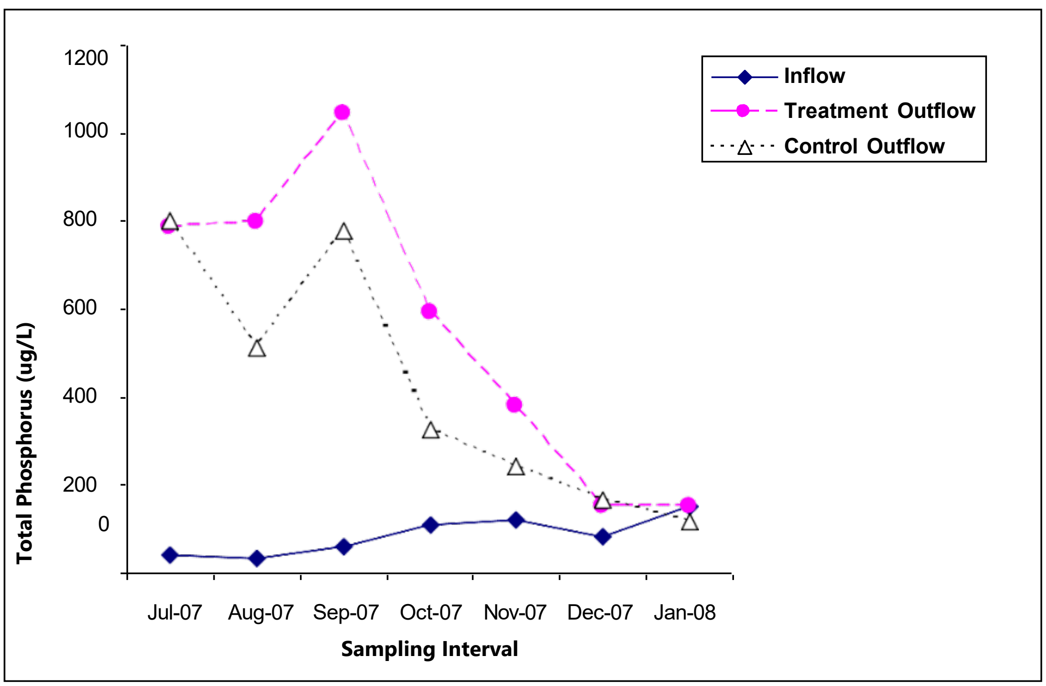

Figure 2. Total Phosphorus Concentrations Between July 2007 and January 2008 for Inflow, Treatment Outflow, and Control Outflow (Thomas et al. 2009).

Table 2. Summary of Fungi-Enhanced Bioretention Bacteria Removal Results.

Contaminant	Fungal Type(s)	Media	Influent Concentration	Effluent Concentration	Percent Reduction	Percent Reduction Versus Control[a]	Source
Fecal Coliform	Mycorrhizal and WRF	Full-scale bioretention with 60/40 sand/compost. Down to Earth® fungi and inoculated alder mulch.[b]	30 CFU/100 ml	3 CFU/100 ml	90	24	Thomas et al. (2009)

[a] All studies have a control consisting of the same BSM without fungi inoculation; this column is the percent improvement over the control.

[b] Down to Earth® is a soluble powdered blend of endomycorrhizae, ectomycorrhizae, and humic acid.

In Melville (2016), two 20-year-old bioretention swales that receive parking lot runoff on Portland Community College's campus were selected for study. This was a swale study so samples were not collected from an underdrain; instead, an influent sample was collected at both the treatment and control swale inlets; and an effluent sample was collected approximately 30 feet downslope. Both samples were collected from pooled water at the sampling locations for three events. The control swale received an uninnoculated amendment of the proprietary stormwater filter media, Earthlite™, composed of rinsed Douglas fir biochar, reed sedge peat pellets, and shale. The treatment swale received Earthlite™, as well as PermaMatrix® BSP Foundation, a locally produced biotic soil amendment containing endo and ectomycorrhizal fungi, bacteria, basalt, biochar, and reed sedge peat.

The results from this study are suspect as the sampled surface waters had little contact with the inoculated media. A 33 percent increase in TP removal and a 125 percent increase in dissolved zinc removal was observed in the treatment swale, but this swale had a residence time that exceeded the control swale by a factor of 4. Residence time is one of the primary drivers for treatment in bioswale surface waters, so it is not accurate to attribute the removal to the fungi inoculation.

A recent biorientation mesocosm study conducted at the Washington State Department of Transportation Ship Canal Test Facility in Seattle, Washington, provided a much higher level of rigor (Taylor et al. 2018). The study consisted of 12 mesocosms: three with un-inoculated 60 percent sand/40 percent compost BSM; three with the same BSM but planted with Pacific ninebark (*Physocarpus capitatus*) and un-inoculated alder mulch; three with BSM and planted with Pacific ninebark with alder mulch inoculated with mycelium of the WRF Wine Cap mushroom (*Stropharia rugoso-annulata*) from Fungi Perfecti, LLC; and three with BSM and only inoculated alder mulch and no plants. For this analysis we used the planted but un-inoculated mesocosms as the control, and the planted and inoculated as the treatment to represent the typical planted bioretention cell versus one receiving an a fungal amendment. Each mesocosm consisted of a 22-inch-wide stainless-steel drum with 18 inches of BSM underlain by a 9-inch drainage layer. The mesocosms were online for 400 days. During that period there were five sampling events spread over the duration of the study.

Both the treatment and control exported high levels of nutrients throughout the course of the study; however, both phosphorus and nitrogen export trended downward over time as the media flushed. The treatment mesocosms on average exported 131 percent less TP than the control mesocosms, yet exported 438 percent more nitrate + nitrite than the control. The treatment mesocosms achieved an average of 48 and 83 percent dissolved copper and zinc removal, respectively; however, these were slightly less than the control (57 and 85 percent). These data corroborate the Poor et al. (2018) result that the addition of mycelium does not improve metals removal from bioretention systems. Results from this study also concur with Poor et al. (2018) and Thomas et al. (2009) in that the addition of fungi to bioretention can improve nutrient removal, but not so much as to prevent export of nutrients from compost-based BSMs.

Table 3. Summary of Fungi-Enhanced Bioretention Metals Removal Results.

Contaminant	Fungal Type(s)	Media	Influent Concentration	Effluent Concentration	Percent Reduction	Percent Reduction Versus Control[a]	Source
Dissolved Copper	Mycorrhizal	Column Study with 60/40 sand/compost and saturated zone. MycoApply[a] fungi.	12.5 ug/L	Not available	45	0	Poor et al. (2018)
Dissolved Zinc			68 ug/L	Not available	81	0	
Zinc	Mycorrhizal	20-year-old swales amended with PermaMatrix® BSP Foundation and Earthlite™ stormwater filter media.[b]	0.03 mg/L	0.01 mg/L	72	NA[c]	Melville (2016)
Copper	Mycorrhizal		0.01 mg/L	ND	100	NA	
Dissolved Copper	WRF	Planted mesocosms with 60/40 BSM. Fungi Perfecti WRF inoculated alder mulch.[d]	16.73 mg/L	7.40 mg/L	48	-9	Taylor et al. 2018
Dissolved Zinc			43.65 mg/L	5.74 mg/L	83	-2	

[a] All studies had a control consisting of the same BSM without fungi inoculation, this column is the percent improvement over the control

[b] PermaMatrix® composed of endo and ectomycorrhizal fungi, bacteria, basalt, biochar, and reed sedge peat from PermaMatrix, Inc. (Portland, Oregon). Earthlite™ composed of rinsed Douglas fir biochar, reed sedge peat pellets, and shale.

[c] Treated swale exhibited a 125 percent increase over the control but this was likely due to a longer residence time in the swale.

[d] Wine cap mushroom (*Stropharia rugoso-annulata*) from Fungi Perfecti, LLC (Olympia, Washington, USA).

Summary and and Next Steps

The use of fungi for soil remediation is a well-studied topic, and the science has shown that it can be an effective remedial measure to reduce toxicity in contaminated soils (Bosco and Mollea 2019; Goltapeh et al. 2013). However, the application of fungi in stormwater filters is not a strong analog to their application in soils. The kinetics of pollutant removal are vastly different when pollutants are introduced in the aqueous phase, with residence times on the order of hours for bioretention systems, instead of years in the case of soil remediation. Consequently, if we hope to use fungi to enhance pollutant removal in stormwater filtration, it is important to study their application to BSM in the lab and in the field. This memorandum synthesizes the few studies that have analyzed the effect of fungi on stormwater filters, specifically.

The SPU specification for BSM is a 70 percent sand/30 percent compost mixture, which has been shown to export nutrients and copper (Chahal et al. 2016; Herrera 2016; Hurley et al. 2017; King County 2014; Mullane et al. 2015). The research presented herein indicates that the addition of fungi may reduce the severity of nutrient export but will not prevent the systems from acting as nutrient sources during the first few years of operation. In addition, the fungi does not appear to increase the metals removal capacity of standard compost-based media. The one study that examined the effect of fungi inoculated BSM treatment of bacteria reduction found a 24 percent improvement in bacteria removal over typical BSM reductions.

It is apparent from this research that inoculating the 70 percent sand/30 percent compost Seattle BSM with fungi will not prevent nutrient export nor improve metals removal during the first few years of operation. Research on the long-term impact of inoculation on BSM performance has not been conducted, but since mycorrhizal communities develop naturally with time in planted media, it can be inferred that the performance of un-inoculated BSM and inoculated BSMs will converge with time.

Future research should focus on using fungi to inoculate systems that do not contain compost. The improved nutrient and bacteria removal performance in such systems could result in a net reduction in influent stormwater.

With increasing availability of data as well as affordable, easily applied, locally produced commercial WRF and mycorrhizal fungi-containing soil amendments, it may be prudent to monitor developments in this field of study for potential beneficial use in future and existing bioretention systems.

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