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Using Mycofiltration Treatment for Stormwater Management

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16. Abstract

Federal and State environmental regulations require transportation construction and retrofit projects to manage stormwater and improve water quality. MassDOT has legal, financial, and ecological obligations to mitigate pollution from stormwater runoff entering water bodies. Existing green and gray infrastructure in place across the Commonwealth is not always able to address non-point-source stormwater pollution. Mycofiltration is a promising stormwater management technology that utilizes mycelium, or fungal webs, as biological filters to mitigate water contaminants passing through woodchips, straw, or soil. This low-cost and low-tech solution could be added to MassDOT's typical Stormwater Control Measures (SCMs) to mitigate stormwater containing nitrogen, phosphorus, and biological pollutants (e.g., *E. coli*). This project analyzed existing literature and case studies on mycofiltration, documented interviews with subject matter experts, and identified MassDOT SCMs most suited for mycofiltration. Conceptual details for mycofiltration SCMs are provided, as are lists of potential local fungal inoculant vendors and academic research partners for future studies. The research indicates that there is currently not enough scientific peer-reviewed literature to support deploying mycofiltration as an addition to MassDOT stormwater SCMs. However, with further testing and verification, there may be benefits of including fungi as SCMs in the future.

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Using Mycofiltration Treatment for Stormwater Management

Final Report

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Executive Summary

This study of Using Mycofiltration Treatment for Stormwater Management was undertaken as part of the Massachusetts Department of Transportation (MassDOT) Research Program. This program is funded with Federal Highway Administration (FHWA) State Planning and Research (SPR) funds. Through this program, applied research is conducted on topics of importance to the Commonwealth of Massachusetts transportation agencies.

Transportation construction and retrofit projects are regulated to manage stormwater and improve water quality. While obligated to mitigate stormwater pollution from entering water bodies, MassDOT's existing green and gray infrastructure solutions sometimes fall short, particularly for non-point-source pollution. Specifically, MassDOT is focused on reducing contaminants that have total maximum daily load (TDML) limitations for impaired water bodies from flowing into receiving water bodies. These contaminants of concern include nitrogen, phosphorus, total suspended solids (TSS), and biological contaminants (such as fecal coliform like *E. coli*). Mycofiltration and mycoremediation are low-cost and low-tech emerging stormwater solutions that utilize fungi's mycelium, or fungal webs, as biological and chemical filters within organic matter and soil substrates to improve water quality. As such, mycofiltration could easily be layered into existing MassDOT stormwater control measures (SCMs) such as sediment control barriers, bioretention cells, or compost blankets to assist in filtering out small particulates; destroying pathogens; mitigating phosphorus and nitrogen impacts; capturing heavy metals; and breaking down pesticide, herbicide, and hydrocarbon pollutants.

This project investigated the feasibility of mycofiltration through the analysis of existing literature and case studies on mycofiltration and interviews conducted with subject matter experts. Two types of fungi, saprophytic and mycorrhizal, were found to be most suited for and commonly used in mycofiltration systems. Saprophytic fungi are decomposers often found on dead wood and are the fungi most cultivated by people (*1,2*). Mycorrhizal fungi form mutualistic relationships with 80-95% of all terrestrial plants on earth; they provide water and nutrients to plant roots in exchange for carbohydrates (*1,2*). These fungi readily grow on materials employed in existing MassDOT SCMs, respectively cellulose material (woodchips or straw substrate), or within soil media and on plant roots.

A review of existing literature and case studies on mycofiltration, as well as interviews with subject matter experts, revealed encouraging results in support of mycofiltration's ability to treat stormwater and reduce this study's contaminants of concern. Saprophytic fungi, such as wine cap mushrooms (*Stropharia rugoso-annulata*) and oyster mushrooms (*Pleurotus ostreatus*) were consistently found to excel at reducing or eliminating fecal coliform concentrations in water. Comparative field studies of mycelium-inoculated bioretention cells against non-inoculated controls revealed favorable results in the removal of nutrients such as phosphorus and nitrogen when filtration media is inoculated, particularly when biofilters are planted. One case study employing both saprophytic and mycorrhizal fungi for the restoration of an abandoned logging road documented reduced erosion relative to two adjacent projects which did not utilize mycofiltration (*3*). Fungi have been studied in a wide range of extreme environments and have been found to remediate contaminants or to ameliorate their effects on plants. Furthermore, mycelium has also been shown to bolster plant health and growth, and also support beneficial soil microbes under an array of stressors.

The apparent successes of mycofiltration are tempered by an overall lack of field, lab, and replicable case studies exploring this new technology. Moreover, many documented studies suffer from limited data points, few in-study replicates, and short experimental durations. The lack of field applications of mycofiltration and methodical evaluation of its efficacy means there are no established design parameters or expected outcomes from implementation. Despite this dearth of directly executable information, there does exist some data on approaches to mycofiltration, potential substrates, and mycelial inoculate options which can inform conceptual mycofiltration SCM design.

While additional research into mycofiltration is needed before deploying mycofiltration systems across the Commonwealth, Offshoots and MassDOT collaborated to identify five existing MassDOT SCMs most suited for the addition of fungi to create enhanced mycofiltration. These SCMs include: (1) Compost Filter Tubes, (2) Compost Blankets, (3) Coir Logs or Wattles, (4) Bioretention Soils, and (5) Bioretention Soils with a Woodchip Overlay. Also, while not employed by MassDOT as an SCM, Compost Berms were considered as a sixth SCM candidate to consider for mycofiltration. Offshoots has provided conceptual CAD details for how mycofiltration could be integrated into these SCMs and provided recommendations and axonometric diagrams proposing their deployment for either permanent or temporary use during construction.

MassDOT will not be able to immediately implement mycofiltration as an SCM for stormwater management and water quality improvement without first undertaking critical laboratory and field trials to vet mycofiltration design and management parameters. Offshoots has provided recommended potential research questions to explore in lab and field tests and documented local fungal inoculant vendors and academic research partners for future studies. Although mycofiltration is not ready to be rolled out across the Commonwealth, many studies are underway nationally and internationally and additional data on mycofiltration will emerge as they are completed. However, MassDOT should undertake further research to define mycofiltration treatment design and operating parameters that meet the needs of transportation projects, particularly for Massachusetts-specific climate conditions.

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List of Acronyms

Acronym	Expansion
AASHTO	American Association of State Highway and Transportation Officials
AM	Arbuscular Mycorrhizal
BMPs	Best Management Practices
CASQA	California Stormwater Quality Association
DNA	Deoxyribonucleic Acid
FHWA	Federal Highway Administration
INVAM	International Collection of Vesicular Arbuscular Mycorrhizal Fungi
iSWM	integrated Stormwater Management
MassDOT	Massachusetts Department of Transportation
MOCZM	Massachusetts Office of Coastal Zone Management
ODOT	Oregon Department of Transportation
PAHs	Polycyclic Aromatic Hydrocarbons
SCMs	Stormwater Control Measures
SPR	State Planning and Research
TMDL	Total Maximum Daily Load
TSS	Total Suspended Solids
UNHSC	University of New Hampshire Stormwater Center
USDA	United States Department of Agriculture

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1.0 Introduction

This study of Using Mycofiltration Treatment for Stormwater Management was undertaken as part of the Massachusetts Department of Transportation (MassDOT) Research Program. This program is funded with Federal Highway Administration (FHWA) State Planning and Research (SPR) funds. Through this program, applied research is conducted on topics of importance to the Commonwealth of Massachusetts transportation agencies.

1.1 Project Background

Federal and State environmental regulations require transportation construction and retrofit projects to manage stormwater and improve water quality. MassDOT has legal, financial, and ecological obligations to mitigate pollution from stormwater runoff entering water bodies. While there is existing green and gray infrastructure in place across the Commonwealth to address stormwater runoff, non-point-source pollution is not always easily addressed by current technologies, and often cost or other factors can prohibit the installation of such infrastructure.

Mycofiltration is a nascent stormwater management technology that utilizes mycelium, or fungal webs, as biological filters within organic matter and soil substrates (growing media). These mycelial webs physically capture small particulates such as silt and pathogenic bacteria; some fungi species secrete enzymes and antibiotics that can stun, kill, or sterilize pathogens which they then consume (*1*). This low-cost and low-tech solution could be a beneficial addition to MassDOT's typical Stormwater Control Measures (SCMs) for stormwater management and to improve water quality within transportation projects. Adding fungi to sediment control barriers, bioswales, tree trenches, or compost slope blankets could improve the functioning of these systems. The research in this report helps to define mycofiltration treatment design and operating parameters and considers how mycofiltration systems could meet the needs of transportation projects.

1.2 Project Objective

The objective of this project is to investigate the feasibility of mycofiltration by conducting a literature review and expert interviews. The product of this project is this research synthesis report providing findings on the state of mycofiltration and recommendations for MassDOT toward integrating mycofiltration into SCMs for stormwater treatment design and operations and water quality improvement completed between September 2021 – December 2022. This project was specifically interested in researching contaminants that have Total Maximum Daily Load (TMDL) limitations for impaired water bodies set by the EPA including phosphorus, nitrogen, and Total Suspended Solids (TSS), and biological contaminants such as fecal coliform like *E. coli*.; the research is targeted toward the removal/remediation of these contaminants. The report also identifies research gaps that must be tested before the implementation of fungi in SCMs. Additional goals for this project include identifying research that could support water quality credit permitting and ascertaining the need for future research such as field trials in conditions typical of MassDOT transportation projects.

1.3 Project Tasks

The project included four primary research tasks.

1.3.1. Task 1: Initial Investigation

Task 1 is an initial investigation of mycofiltration through:

1. A comprehensive literature review
2. Case study investigations
3. Expert interviews with mycologists and scientists working in stormwater filtration

1.3.2. Task 2: Mycofiltration Best Management Practices

Task 2 identified potential mycofiltration system SCMs for MassDOT project types and recommends implementation applications and requirements for each.

1.3.3. Task 3: Conceptual Details and Next Step Recommendations

Task 3 included drafting conceptual details to implement the recommended mycofiltration systems, and recommendations of next steps for further research to test feasible methods for each identified SCM and potential pilot projects.

1.3.4. Task 4: Potential Implementation Partners

Task 4 developed two lists of potential implementation partners for MassDOT:

1. Potential local research partners and academics who could assist in future pilot project development
2. Potential vendors and local suppliers of fungal inoculant

2.0 Research Methodology

2.1 Task 1: Initial Investigation Methodology

Task 1 entailed conducting an initial investigation into mycofiltration via a literature review, case studies, and expert interviews. The methodologies used for conducting each are noted.

2.1.1. Literature Review

The initial literature review for this project was carried out using combinations of 19 search terms with 21 databases. The list of search terms and the databases can be found in Appendix A.

Initially, 113 peer reviewed articles and a handful of published theses and dissertations were collected. Of these articles, 42 or 37.2% were found to be relevant and were comprised of 17 articles or 15% on mycofiltration specifically with four field studies and nine lab studies; 24 articles or 21.2% on mycoremediation that were not mycofiltration specific; and 1 article or 0.9% related to phosphorus recovery by plants, but not specifically on mycofiltration.

Figure 2.1 illustrates the categories of articles described above. Of the 71 articles or 62.8% of the total that were found not to be relevant, 51 articles or 45% did not pertain to mycofiltration or mycoremediation; 7 articles or 6.2% were not accessible; and 13 articles or 11.5% were on mycelial treatment of industrial wastewater.

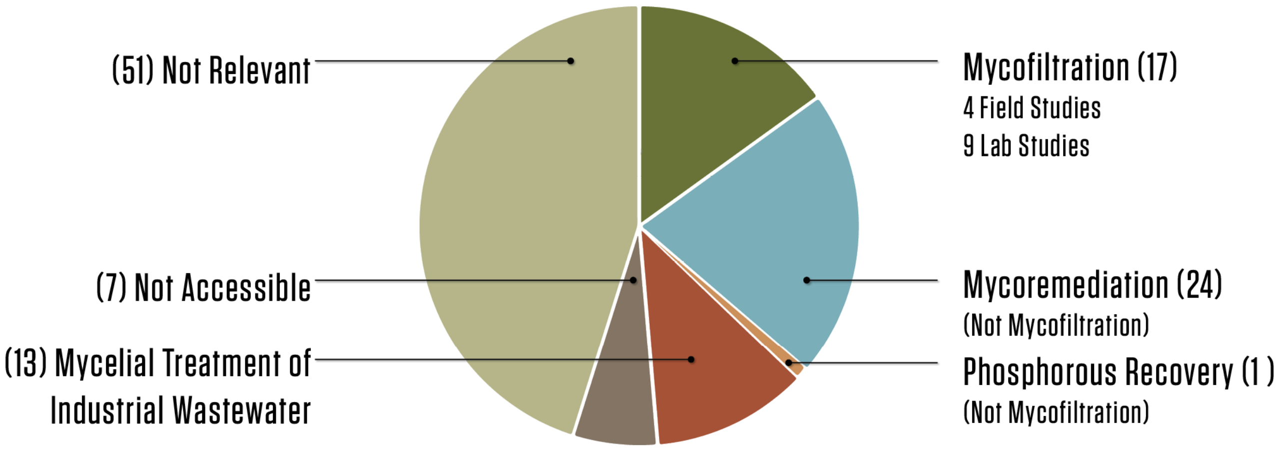

Figure 2.1: Results of literature review

Additional peer-reviewed articles which were shared by interviewees and from diving deeper into specific topic sources have been added to Appendix A. These topics include the role of mycelium in the phosphorus and nitrogen cycles, the process of lignin breakdown by mycelium, and the ability of mycelium to tolerate salt.

2.1.2. Case Studies

From the literature review and other non-peer-reviewed sources, a small catalog of mycofiltration case studies was collected. Case studies deemed relevant can be found in Appendix B.

2.1.3. Expert Interviews

Based primarily on our literature review findings 30 researchers and practitioners with subject matter expertise in or related to mycoremediation, mycofiltration, or mycology were contacted for interviews, and 12 interviews were conducted with 13 participants. These professionals came from the fields of mycology, civil engineering, ecology, soil science, and biology. Interview notes are located in Appendix C and video recordings have been provided to MassDOT.

2.2 Task 2: Mycofiltration Best Management Practices Selection Methodology

In Task 2, Offshoots had a series of virtual meetings and email conversations with the MassDOT project champions to discuss existing MassDOT SCMs and their suitability for mycofiltration. Six SCMs were agreed upon and recommendations for augmentation and enhancement of these SCMs with fungi were based on findings from the literature review, case studies, and expert interviews.

2.3 Task 3: Conceptual Details and Next Step Recommendations Methodology

For Task 3 conceptual details for mycofiltration SCMs were drafted based on reference materials from the California Stormwater Quality Association Stormwater BMP Handbook, Oregon Department of Transportation Standard Drawings, the State of Oregon Department of Environmental Quality Construction Stormwater Best Management Practices Manual, the North Central Texas Council of Governments integrated Stormwater Management (iSWM) Technical Manual, and the Massachusetts Department of Transportation Compost Blankets for Erosion Control and Vegetation Establishment report (*4,5,6,7,8*). Recommended next steps for further research into mycofiltration and feasibility testing were developed through analysis of findings from the literature review, case studies, and expert interviews. These recommended next steps and research questions can be found in Chapter 4.0 Implementation and Technology Transfer.

2.4 Task 4: Potential Implementation Partners Methodology

In Task 4, three lists of potential implementation partners for MassDOT were developed. The first list is of local research partners who could assist in future pilot projects to scientifically test research questions that still need to be answered. These individuals are interviewees who offered or expressed interest in providing their assistance in future MassDOT mycofiltration research. The second list is comprised of New England vendors who could supply fungal inoculants. These vendors were either revealed through interviews or were found through online research. The vendor list is not meant to be exhaustive, and other resources not identified in the report are likely available. Finally, a brief list of potential vendors of compost filter tube materials was developed using providers who have previously supplied these and other erosion control materials to MassDOT. These lists can be found in Chapter 4.0 Implementation and Technology Transfer.

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3.0 Results

3.1 Task 1 Initial Investigation Results

3.1.1. Contaminants of Concern

The first step of Task 1 was determining the primary contaminants of concern. Since the goal of this study is to better manage and treat stormwater to improve water quality, this research has focused on several contaminants of concern which are associated with roadways or can be conveyed to water bodies via roadway infrastructure.

Figure 3.1 illustrates potential sources of contaminants including road and car debris, deicing chemicals, corridor control or roadside maintenance, lawn and landscape care, vehicle emissions, illegal dumping, stormwater outfall pipe, and atmospheric deposition.

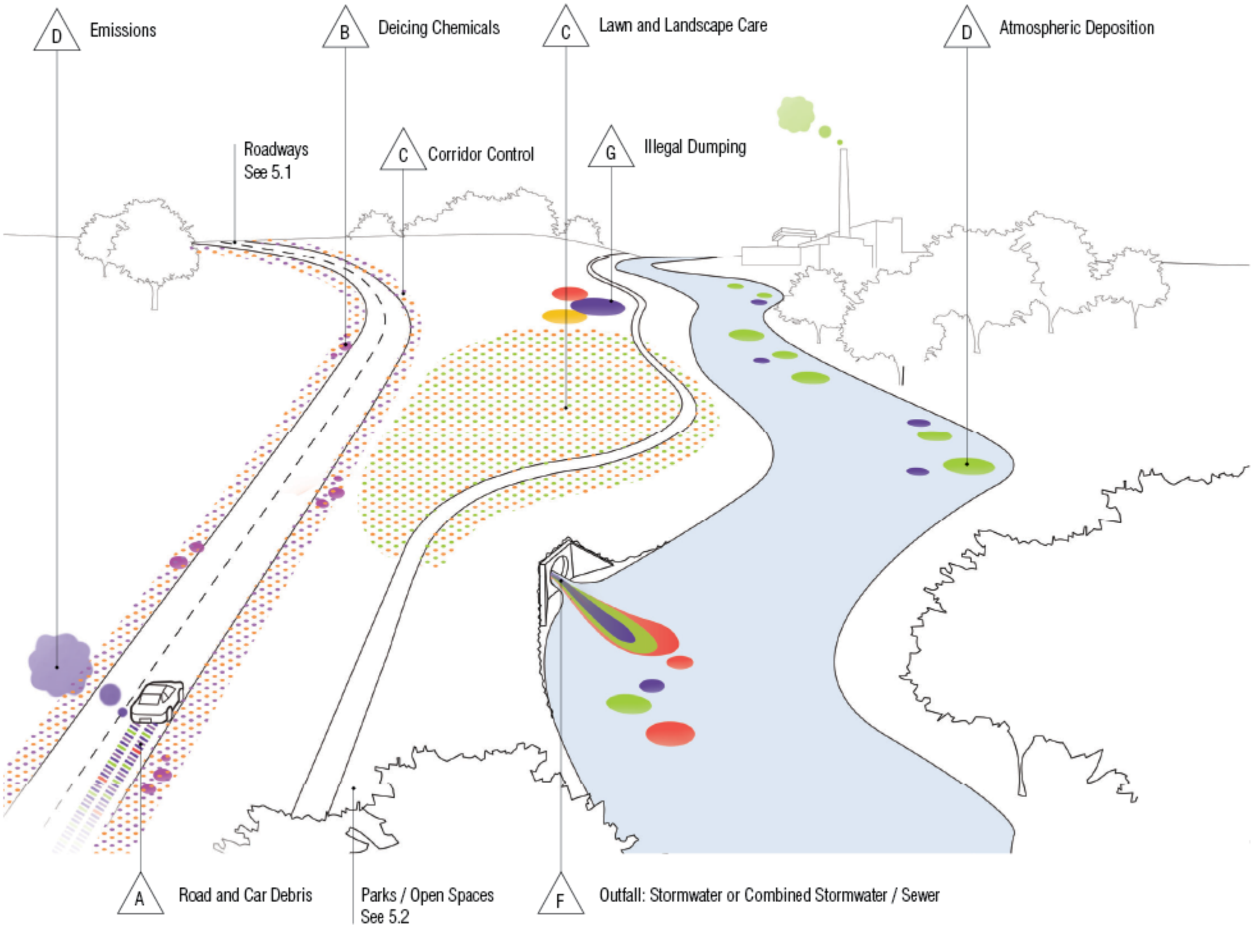

Figure 3.1: Roadway contaminants along river corridors

Source: Adapted from PHYTO (*9*).

Figure 3.1 shows a birds eye view of a roadway separated from a river corridor by open space. The potential sources of contaminants described above are illustrated.

The primary contaminants of concern include biologic contaminants which are primarily fecal coliform bacteria such as *E. coli*; nitrogen and phosphorus; petroleum and other hydrocarbons; metals from brake pads and other auto debris; pesticides and herbicides; and salts, particularly sodium chloride road salt. Massachusetts has established Total Maximum Daily Load (TMDL) requirements for nitrogen and phosphorus. TMDLs are calculations of the maximum amount of a pollutant that a waterbody can accept and still meet the state's Water Quality Standards for public health and healthy ecosystems.

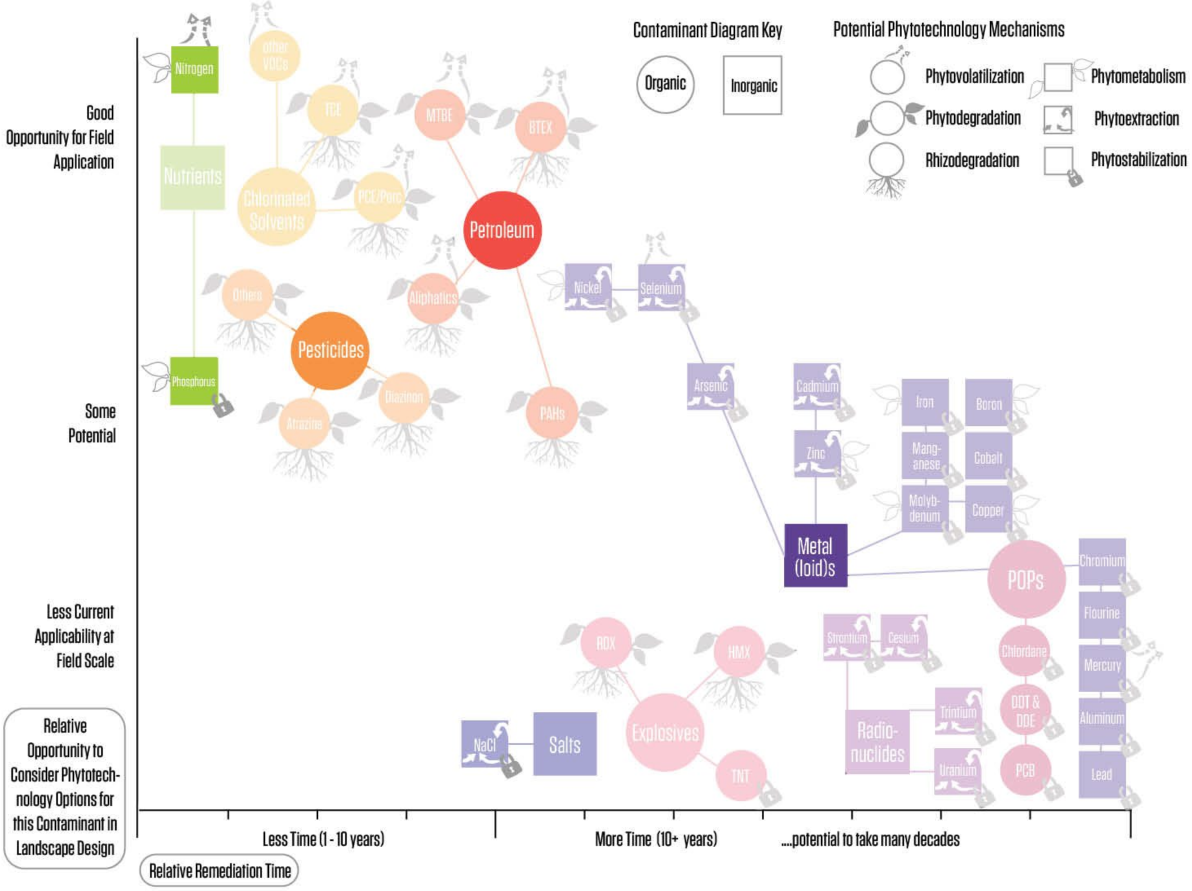

Figure 3.2: Contaminants of concern

Source: Adapted from PHYTO (*9*).

Figure 3.2 shows a scatter plot highlighting the contaminants of concern. The X axis represents relative remediation time from 0 to10 years and increasing to many decades. The Y axis represents relative opportunity to consider remediation from increasing from poor to good potential. Biologic contaminants and nutrients have a good potential within a relatively short period of time. Pesticides have some potential within 5-10 years. Petroleum has fairly good potential in 10 years or more. Metals have poor potential for remediation over many decades.

3.1.2. Four Types of Fungi

The second investigation of Task 1 researched fungi to determine which types and species are most suited for mycofiltration. Fungi are multicellular organisms with a single cell wall made of chitin, the same molecule that makes up insect exoskeletons and crustacean shells. Fungi are comprised of hyphae, one-cell thick strands that search for food and mates. Collectively many hyphal threads are called mycelium and when two genetically compatible hyphal threads meet, they fuse and a dikaryotic (cell with a maximum of two nuclei) mycelium forms. For some species, this is when the mycelium's fruiting body, the mushroom, forms. Fertilized spores are then released from which new hyphal threads will grow (*1,2*). Fungi can largely be broken into four different categories:

1. saprophytic
2. mycorrhizal
3. parasitic
4. endophytic

The mycofiltration research reviewed utilizes saprophytic and mycorrhizal fungi only.

Saprophytic fungi are decomposers. These fungi mostly feed on dead hosts and are the fungi most commonly cultivated by people. Oyster, shiitake, and reishi mushrooms are all saprophytic fungi. Many of these fungi can be found growing on fallen trees as they possess enzymes that can break down the lignin, the main structural fiber of wood, found in the cell walls of woody plants (*1,2*). Certain saprophytic fungi may favor specific tree species. Saprophytic fungi sometimes form more mutualistic relationships with plant roots similar to mycorrhizal fungi (*10*). Figure 3.3 (*2*) shows an oyster mushroom growing on a tree trunk.

Figure 3.3: Oyster mushroom

Mycorrhizal fungi are mutualistic, they form mutually dependent partnerships with the root systems of 80–95% of all terrestrial plants on earth. In these symbiotic relationships fungi exchange nutrients that they find with plants, and in return receive carbohydrates created by the plant's photosynthesis. While many of these fungi do not create fruiting bodies, some do such as chanterelles and truffles, as shown in Figure 3.4 (*1,2*). Nearly all mycorrhizal fungi fall into one of two categories: (1) endomycorrhizae or (2) ectomycorrhizae. Endomycorrhizae penetrate plant cells and form intraradical structures (structures within the plant's roots). Approximately 95% of the world's terrestrial plants are compatible with endomycorrhizae including vegetables, grasses, flowers, shrubs, and both fruiting and ornamental trees. The largest functioning group of endomycorrhizae is the arbuscular mycorrhizae, named for their arbuscules which are the sites of nutrient exchange. Ectomycorrhizae are compatible with 5% of the world's terrestrial plants, particularly woody plants, conifer trees, and some deciduous trees like oak and other nut trees (*2*). Ectomycorrhizae transfer nutrients into the plant roots via extensive nutrient-absorbing networks of hyphae called Hartig nets that cover the plant cell walls and grow between them (*11*). Figure 3.4 shows an illustration of a tree seedling and its roots below ground with a network of mycelium and clusters of black truffle fungi 3.5 shows the differences between endomycorrhizae and ectomycorrhizae relationships at the cellular level.

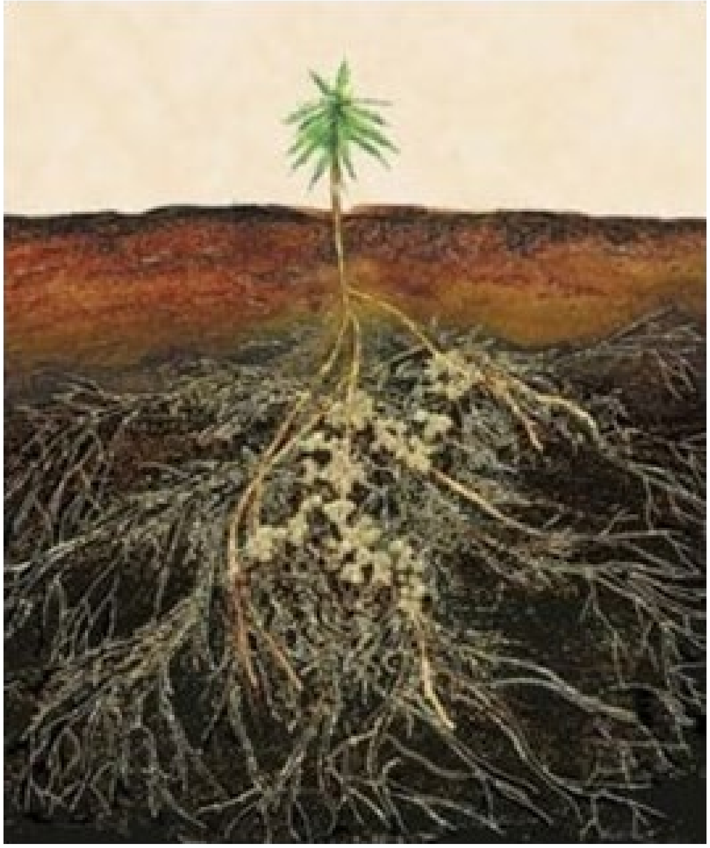

Figure 3.4: Black truffle

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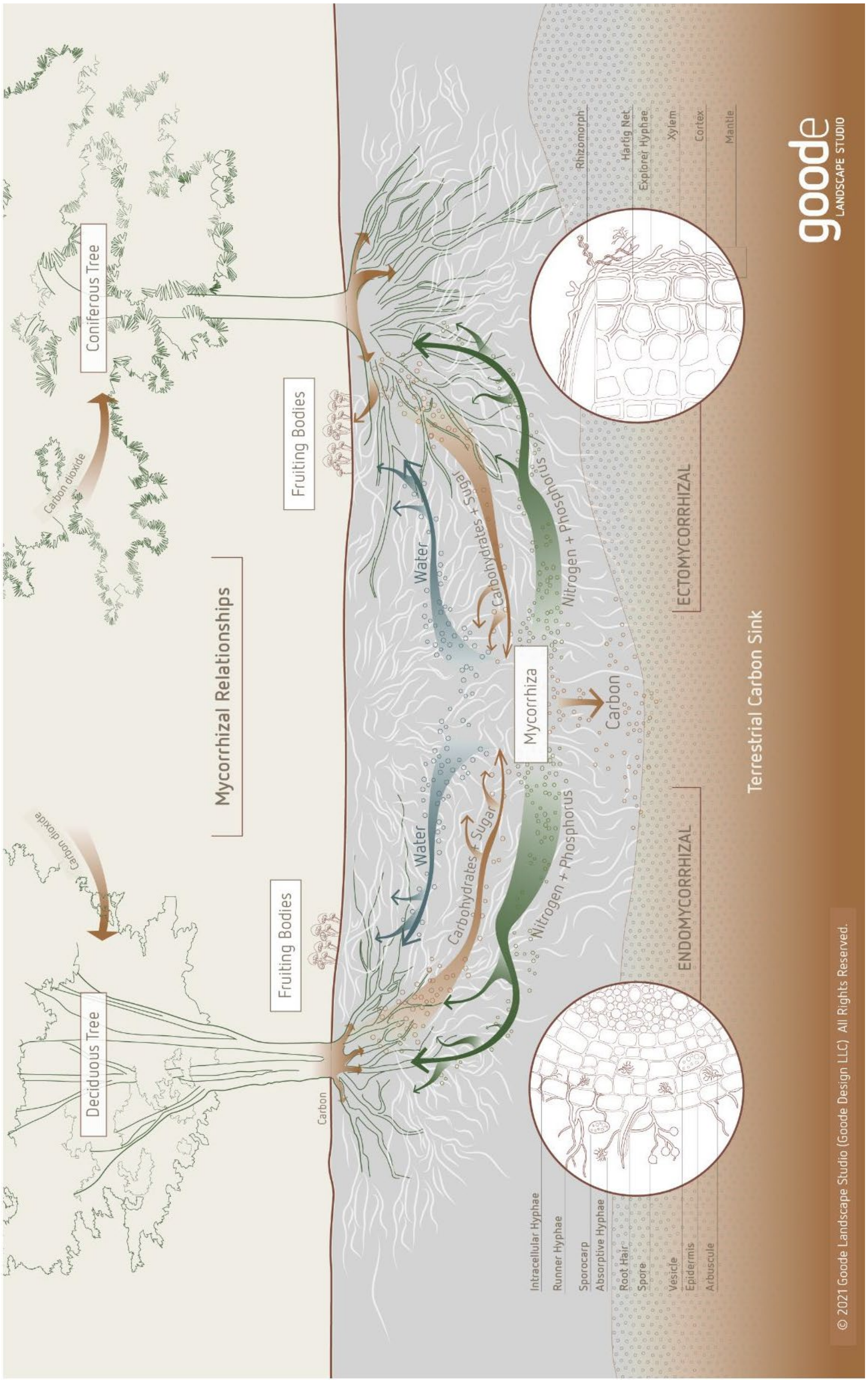

Figure 3.5: Endomycorrhizae and Ectomycorrhizae

Source: Goode Landscape Studio, 2021.

Figure 3.5 illustrates the Terrestrial Carbon Sink showing the relationship of endomycorrhizae and ectomycorrhizae to trees as described above. The tree absorbs carbon dioxide from the atmosphere and converts carbon to carbohydrates and sugars. The tree give carbohydrates and sugars to the mycorrhizae. In turn, the mycorrhizae transfer excess carbon into the soil which is a terrestrial carbon sink. Fungal fruiting bodies or mushrooms transfer carbon to the forest floor.

Parasitic fungi latch onto a host and unlike mycorrhizal fungi, they steal away nutrients without providing anything in return. Some parasitic fungi create visible fruiting bodies, a few are edible and desirable like chaga and lion's mane mushrooms, and others are edible yet undesirable such as the polypore mushroom (*Piptoporus betulinus*). Some parasitic fungi have had significant effects on tree populations in the United States. The Chestnut Blight introduced into the US in the early 1900s caused significant loss of the American Chestnut. Furthermore, some parasitic fungi do not produce fruiting bodies and have more toxic effects such as rye ergot (*Secale cornutum*). Figure 3.6 **(*12*)** shows a photograph of birch polypore mushrooms growing on a tree trunk.

Figure 3.6: Birch polypore mushroom

Endophytic fungi are the least well-understood of the four categories of fungi. They are believed to exist in almost all plants and could be considered mutualistic pathogens. While they can aid in plant survival by providing antibiotics and other benefits to the plant, they can also turn parasitic and cause the plant harm. *Penicillium chrysogenum* is an endophytic fungus famous for producing the antibiotic Penicillin. Figure 3.7 (13) shows endophytic fungus growing on a petri dish.

Figure 3.7: Endophytic fungi

3.1.3. Literature Review Findings Overview

The literature review revealed that much research has been done to show that mycelium, in coordination with plants and soil microbes, can be effective in removing pathogens and nutrients from soil and water. Additionally, studies show that certain fungal species can aid in the extraction, immobilization, or breakdown of certain pollutants from soil and water. Unfortunately, the literature also revealed a dearth of lab and field studies exploring mycofiltration. A summary of the findings is provided.

Substrate

Several lab and field studies showed that mycelium growing on woodchips substrate performs better than mycelium growing on straw at reducing *E. coli* concentrations. This is likely due to prior bacterial contamination of the straw which can cause a net export of bacteria and nutrients (*14,15,16,17*). If the growing substrates are sterilized, not only are pathogenic bacteria removed, but so are potentially beneficial microbes, and the effectiveness of mycofiltration can be dampened (*18*).

Fungi Species

All the lab and field studies for mycofiltration focused on inoculating media with saprophytic or mycorrhizal fungi. Most studies utilized saprophytic fungi, and wine cap mushrooms (*Stropharia rugoso-annulata*) and oyster mushrooms (*Pleurotus ostreatus*) were the species most frequently tested. It is unknown if these species were selected because of their effectiveness, or because they are easily grown, however, in one fairly rigorous study wine cap mushrooms (*Stropharia rugoso-annulata*) proved to recover the best out of a number of fungi species after undergoing saturation-heat-freezing resiliency testing.

Climate

Several papers investigated the adaptability of mycelium to withstand large swings in temperature or moisture. This is an especially important question in Massachusetts where periods of drought, cold or hot temperatures could potentially kill fungi. In general, more research into this topic is necessary as only a few papers were found on this topic. The adaptability of mycelium to extremely wet environments was shown in one study where mycelium grown in mycofilters floating on water developed a mucus-like mesh (basidiomycota) that enabled it to maintain its effectiveness in removing *E. coli* from its environment (*16*). In addition, seven of the 12 field and lab studies found were completed on the West Coast of the United States with a climate that is overall milder than Massachusetts but has more dramatic periods of precipitation and drought (*19,20,21,3,14,18,22*).

Nutrient Removal (Nitrogen and Phosphorus)
Field studies exploring mycofiltration have seen favorable results in removing nutrients such as phosphorus and nitrogen when filtration media is inoculated, particularly when biofilters are planted. Plants inoculated with arbuscular mycorrhizae accumulate significantly more phosphorus in their biomass than plants not inoculated and leach less nitrogen (*23*). While nutrient leaching was still an issue in these field studies due to the use of nutrient-rich compost, inoculated bioretention cells were found to leach less than their control counterparts (*3,21*). Mycelium has also been shown to bolster plant growth and support beneficial soil microbes under an array of stressors.

Biological Pollutants
In a fairly robust study performed with EPA funding, fungi were tested for effectiveness in removing *E. coli*, and oyster mushrooms (*Pleurotus ostreatus*) performed the best at removing 100% of the bacteria (*22*). Other column studies have verified the efficiency of oyster mushrooms (*Pleurotus ostreatus*) seeing them remove 99% of fecal coliform bacteria with a straw media and 98% with a straw media when in a hyper-wet environment (*16,17*). While not as successful as the column studies, a mesocosm study (controlled outdoor experimental system) utilizing wine cap mushrooms (*Stropharia rugoso-annulata*) found that inoculated woodchips performed 20% better at reducing *E. coli* and fecal coliform than the control (*14*).

Summary of Literature Reviewed
While the results of the lab and field studies are very informative, their limited number and other shortcomings make clear that our understanding of mycelium and its capabilities for mycofiltration are still developing. Only four papers documenting field studies were found, and one of these was a graduate research thesis. Nine papers documented lab studies, with most of these being column studies. Both the lab and field studies were of short duration, with the former lasting minutes to hours, and the latter lasting six to 15 months. Data sampling during most studies occurred infrequently, and several studies suffered further from human errors. Finally, the results of most studies are further constrained by their limited number of control and experimental groups.

3.1.4. Precedent Case Studies

Like lab and field studies, there is a scarcity of precedent case studies where mycofiltration has been applied in the field for stormwater management. Furthermore, many of the case studies found are anecdotal with little data to support their apparent success. Design parameters in these case studies, such as the type and quantity of materials used, are often left unspecified, and in some cases, even the fungi tested are left unmentioned. While their lack of detail makes these case studies non-replicable and unreliable, they do speak to an increased implementation of mycofiltration beyond the study of university academics, and with apparent success.

The six case studies evaluated a range of contaminants including Total Organic Carbon (TOC), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), Total Petroleum Hydrocarbons (TPH), fecal coliform, zinc, copper, phosphorus, coal, petrochemical, and sediment. Four case studies tested saprophytic fungi, and two of these also tested mycorrhizal fungi. The other two case studies did not reveal the fungi tested. Like the peer-reviewed lab and field studies, the mycorrhizal mycofiltration treatments were found to reduce phosphorus and nitrogen concentration, and saprophytic treatments led to a reduction in fecal coliform bacteria. Five of the six case studies occurred in either Washington state or Oregon, with Paul Stamets and his company Fungi Perfecti being responsible for three of those. One of the more relevant case studies of the six documented was conducted by Paul Stamets and David Sumerlin in Tahuya State Forest and appears to have reduced sediment erosion from an abandoned logging road into a stream supporting salmon habitat better than two adjacent restoration experiments that did not utilize mycofiltration (*24*). Three years after woodchips, bark, fir needles, and straw were inoculated with mycorrhizal fungi and oyster mushrooms (*Pleurotus ostreatus*), there was a nearly contiguous mycelial net binding everything together at the gravel/woodchip interface. The results of this study like all the case studies were not published in peer-reviewed journals; the case study was published on Stamets website. It was evidenced that a mycelial mat had formed, but a net reduction of neither nutrients nor TSS was specifically documented.

3.1.5. Expert Interviews

Interviews with subject matter experts further confirmed that there is a lack of field studies trialing mycofiltration and that there is not enough peer-reviewed data to start applying microfiltration in the field without further field-trialed research (*10*,*25*). Even so, there has been considerable discussion on biofilter design, namely plant, soil, and substrate material selection. In several interviews, the debate over the virtues and drawbacks of woodchips versus compost as growing mediums for fungi arose. In summary, most interviewees expressed concern that including compost in stormwater filtration systems can leach nutrients, contributing nitrogen and/or phosphorus to water rather than removing it. In addition, woodchips were often suggested as a superior substrate for supporting saprophytic fungi growth, although there exists concern over them floating during heavy rain events (*25,26,27*).

Multiple interviewees acknowledged that translating lab studies into field studies is difficult, and advocated for designing simple systems that mimic natural ecologies and can be easily constructed and maintained (*25,27*). Tied to these discussions is the role of both bacteria and mycelium on nutrient cycling. Several conversations discussed how anoxic zones (saturated zones with no oxygen in the soil) in bioretention are likely very important for bacteria to remove nitrogen through denitrification. This denitrification may or may not involve fungi, but it may be beneficial to start adding these anoxic zones to bioretention systems to maximize nitrogen removal (*10,25,26,27*). In addition, the use of drinking water treatment residuals can be used to aid in the uptake, or absorption, of phosphorus, and that this technology could be considered to be added to stormwater filters to maximize phosphorus removal (*10,25,26,27*).

Another subject that repeatedly came up in interviews was fungi nativeness. Many studies use commercially sourced saprophytic and mycorrhizal fungi, but there are concerns regarding the potential and unforeseen impacts of introducing non-local or non-native strains of fungi into the environment (*10*). The precautionary principle is a philosophical concept that emphasizes caution and review before implementing new innovations or species to vet out and avoid potentially disastrous outcomes. For example, many plants that are now considered invasive in the US were originally introduced for their ornamental qualities in gardens. Is it a problem to introduce non-native or generalist fungi species that could alter the ecosystem in unintended ways? This concern may be for naught though, as many plant nurseries already are using commercially available mycorrhizal packets to inoculate their plants.

One surprising discovery in our interviews with several mycologists is that studies have shown that commercially available packets are often dead with no-live spores. For this reason, commercially available spore packets are likely not advisable and should be tested for viability before utilization (*28,25*). MassDOT may already be planting inoculated plants in projects across the state if the commercial spore products are working. For example, Amherst Nursery uses Organic Plant Magic brand fertilizer with mycorrhizal fungi on their plants (*26,29*). Rather than bioaugmenting soils (introducing new fungi), another approach to increasing mycorrhizal populations is through biostimulation (environmental modification to stimulate the growth of existing microbial communities). Adding molasses to native soils would be one way to biostimulate existing fungal communities (*10*).

It was suggested in several interviews that utilizing native, locally sourced wood chips (to recruit native saprophytic fungi) and native, locally sourced reference soils (to recruit mycorrhizal fungi) might be better than trying to introduce inoculated fungi for mycofiltration since they are adapted to the environment and not a lab. Alternatively, fungi from native soils can be isolated, propagated in a lab, and then reintroduced for mycofiltration. However, before biostimulation or lab propagation, consideration should be given to testing which fungi are in the reference materials as they could be native or non-native fungi (*26*).

3.2 Task 1 Mycofiltration Research Synthesis

3.2.1. Mycofiltration Treatment Design

As evidenced by the literature review and precedent case studies analysis, and confirmed in interviews with subject matter experts, there are no established design parameters or expectable outcomes based on the previous implementation of mycofiltration. Despite this, there are two main approaches to mycofiltration treatment used in the case studies investigated. The first approach treats surface water flow by passing water through an inoculated substrate, and the second approach treats subsurface flow by passing water through a bioretention cell that includes fungi.

Inoculated Substrate

A common mycofiltration method for treating surface flow is through inoculated substrate contained in biodegradable fabric. These mycofiltration bags are typically fabric bags such as a burlap sacks that are stuffed with a cellulose-based substrate such as woodchips or straw. These bags are inoculated with saprophytic mycelium (decomposers), soaked, and left to grow for a period of time. Once the mycelium has colonized the substrate, the bags can be placed in the flow path of the water to filter particulate and biological contaminants such as *E. coli*.

Figure 3.8: Mycofiltration bags in watercourse

Figure 3.8 (*23*) shows a photograph of mycofiltration bags stacked across the top of a small stream bed riffle. The resulting dam slows the flow of water to allow filtration through the bags.

Mycofiltration Bioretention Cells

Bioretention cells direct and attenuate stormwater before it enters a water body, filtering out sediment and removing contaminants. Conventionally, bioretention cells consist of a sand bed, soil, and filtration media such as gravel or mulch. Additionally, many bioretention cells are planted. Australian guidelines for biofilter media suggest using filtration media with a low nutrient content to reduce nutrients from leaching out of the bioretention cell into the stormwater as it passes through and then out into the adjacent water body. In Australia, often pure sand is utilized so that added compost does not leach nutrients (*30,26*). To enhance the performance of bioretention cells, soils can be inoculated with mycorrhizal fungi, and woodchip overlayers for these systems can be inoculated with saprophytic fungi (*26*). In one of the precedent field studies for phosphorus removal which was completed in the Dungeness Watershed, WA, mycorrhizal fungi were incorporated into planted bioretention soil, and saprophytic fungi were added to alder mulch as a topdressing. Unfortunately, both the control bioretention cell that did not include fungi and the test bioretention cell leached phosphorus from the system, likely because the bioretention soil contained compost. However, it was documented that the cell inoculated with the fungi leached less phosphorus than the control without the fungi (*3*). Figure 3.9 shows a planted bioretention cell.

Figure 3.9: Bioretention cell

Figure 3.9 (*31*) shows a wide, shallow landscape swale planted to large masses of ferns, native grasses and trees. The swale is between a building and a road which are impervious surfaces contributing to stormwater run-off into bioretention cell.

3.2.2. Mycelium as Physical Actors

A fungi's mycelial web acts as a biological filter that physically captures small particulate such as silt and pathogenic bacteria. Some mycelia secrete enzymes and antibiotics to stun, kill, or sterilize pathogenic bacteria which they then consume as food (*1*). Figure 3.10 shows a mycelial web or mat growing on woodchips and intercepting fine sediment in flowing water. In their partnership with plants, mycorrhizal fungi physically extend the reach of plant roots to access nutrients and water; the single-cell hyphae of mycorrhizal fungi can extend up to 11cm beyond a plant's rhizosphere (*11*). Moreover, the single-cell diameters of mycorrhizal hyphae mean there is more surface area directly in contact with soil and so the diffusion distance nutrients and water must travel is reduced, further improving uptake by the mycelium. Arbuscular mycorrhizae play another important role in stabilizing soil through the production of the protein glomalin which binds soil aggregates together. Glomalin improved soil structure can lead to an increase in soil organic matter storage, and this in turn means increased phosphorus storage is made accessible to plants (*11*). The research indicates that mycelium extends the plant's root zone and ability to act as a physical filter.

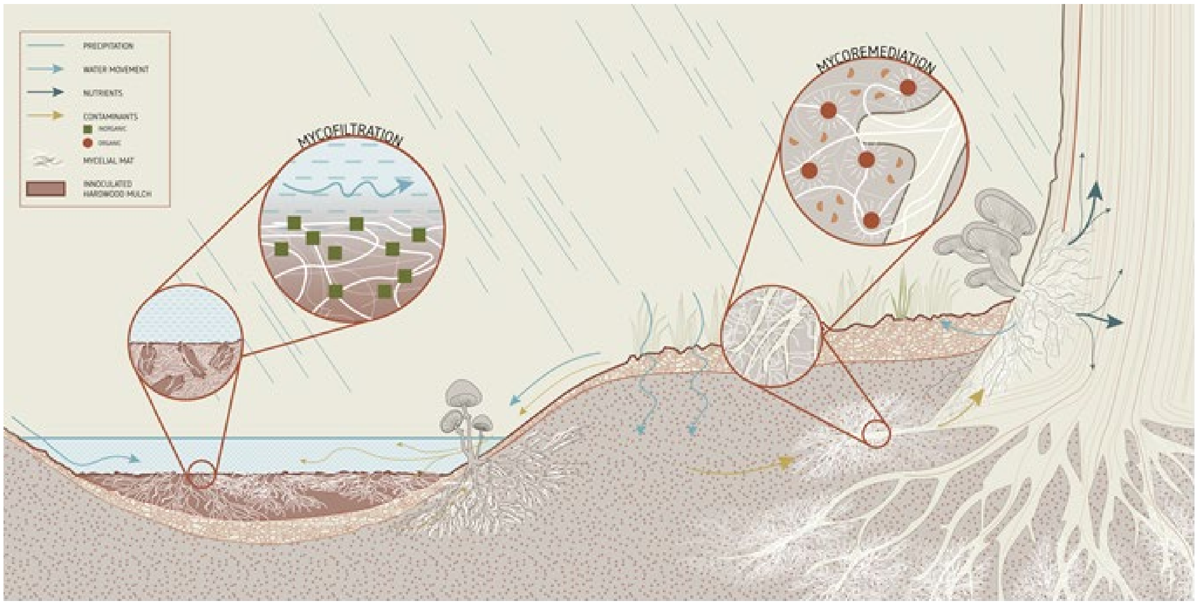

Figure 3.10: Mycofiltration and mycoremediation

Source: Goode Landscape Studio, 2021.

Figure 3.10 illustrates a cross section through a bioretention cell and an adjacent upland tree. Precipitation falls to the ground and stormwater flows downslope in a bioretention cell lined with a layer of inoculated wood fiber mulch. Saprophytic fungi in the mulch provide mycofiltration and capture of inorganic contaminants. On the right, arbuscular mycorrhizae in the trees roots provide mycoremediation by uptake of water absorbed by the soil and organic contaminants. Organic contaminants are made bioavailable by the j Saprophytic fungi in tree trunk transfer nutrients into the tree.

3.2.3. Mycelium and Phosphorus

In addition to physically accessing more nutrients for plants, mycorrhizae biochemically make more nutrients accessible, in particular phosphorus. Arbuscular mycorrhizae are highly efficient at taking up phosphorus (*11*). Phosphorus takes many forms in soil, and not all forms are accessible to plants to uptake. An example of an inaccessible form is "primary minerals" or naturally existing soil minerals like apatite. Another is "secondary minerals" or mineral phosphorus compounds where ions of iron (Fe^{3+}), aluminum (Al^{3+}), and calcium (Ca^{2+}) are bonded to precipitates (insoluble compounds) of phosphorus. Thirdly, adsorbed (adhered) phosphorus can be found bound to iron and aluminum oxides of soil particles and thus inaccessible to plants. Mycelium can secrete organic acids to dissolve these three inaccessible forms of phosphorus making them part of the soil solution, and therefore accessible to plants. Additionally, mycelium can secrete phospholytic enzymes to assist in the mineralization (decomposition) of inaccessible organic phosphorus. Hyphae uptake orthophosphate directly from the organic residues, this biochemical process bypassing the soil solution plant available phosphorus in the soil (*11*). When no additional fertilizer is added, increased plant uptake of phosphorus should translate into reduced soil phosphorus levels (*11*). When it comes to the soil in bioretention cells, soil with less phosphorus is desirable. Soil media with a low phosphorus index will enhance uptake rather than export of phosphorus which can occur if the soil is too rich in phosphorus (*3*). Soils with too high a concentration of phosphorus can reduce the ability of mycorrhizae to colonize plants (*11*).

While mycelium can help accumulate phosphorus in the plant, removing it from the site entirely requires phytoextraction. This means a plant can take up the phosphorus, but the plant must be harvested to remove the phosphorus from the system. Otherwise, when the plant dies and decays all its absorbed phosphorus will return to the soil or the soil's surface where it has a greater chance of being carried away in runoff (*11*). Not all plants mobilize and accumulate phosphorus equally. For the best results in removing phosphorus from the soil, it could be helpful to intercrop plants that excel at mobilizing phosphorus with those that can hyper-accumulate phosphorus (*11*).

3.2.4. Mycelium and Nitrogen

Just like for phosphorus, mycorrhizal inoculation has been found to enhance nitrogen uptake by plants, both directly and indirectly (*26,25*). Both endomycorrhizae and ectomycorrhizae (both types of mycorrhizal fungi) can increase the uptake of soluble inorganic nitrogen, but ectomycorrhizae can also increase the uptake of insoluble and soluble organic nitrogen (*32*). Arbuscular mycorrhizal hyphae uptake both nitrate and ammonia and then convert them into plant-accessible forms and translocate them inside the plant roots (*33*). In addition to transferring nitrogen to plants, mycorrhizae use nitrogen they collect to create their own mycelial enzymes (*10*).

As mycorrhizae contribute organic carbon to the soil, they indirectly contribute to soil denitrification as this drives changes in microbial communities which can lead to nitrogen removal (*26,25,33*). Some bacteria that are denitrifying bacteria are known to reside on mycelium in a state of suspension, and when the mycelium loses its vigor, this signals the bacterium to grow (*1*). Furthermore, biological denitrification via anaerobic bacteria can be encouraged by designing and constructing bioretention cells with anoxic zones that remain submerged (*3*). Plant selection is also important when dealing with nitrogen removal. Salt-tolerant plants are often superior at nitrogen removal, whereas using nitrogen-fixing plants would result in an increase in soil nitrogen levels and nutrient leaching (*26*).

3.2.5. Mycelium and Other Contaminants

Mycorrhizae confer several other benefits to plants in addition to providing plants with greater access to water and nutrients, namely the ability to tolerate contamination, and swings in environmental conditions such as temperature and drought (*34,35,33*). Certain fungi, primarily saprophytic fungi, have evolved to break down long chain hydrocarbons called lignin in wood. Unbreakable by most enzymes, these fungi have evolved lignolytic enzymes that can cut these long hydrocarbon chains into smaller molecules. These smaller molecules are then metabolized by microbes (*1*). Lignolytic enzymes also break down the long chain hydrocarbons of pollutants such as petroleum and Polycyclic Aromatic Hydrocarbons (PAHs) often found along roadways (*36*). Turkey Tail (*Trametes versicolor*), and oyster mushrooms (*Pleurotus ostreatus*) are both noted for their ability to degrade hydrocarbons and are present in the Eco-Machine at the Fisherville Mill Canal in Grafton, MA (*37*). Jurak et al. found that wheat straw compost did not see a change in lignins during the composting process until the compost was inoculated with mycelium (*38*). 16 days after inoculation 45% of lignins were completely metabolized and the rest were already modified (*38*). This preliminary research points to evidence that mycelium can help to break down organic contaminants in the soil such as petroleum compounds.

Heavy metals can be toxic for lignolytic fungi and impede degradation, but some fungi such as the oyster and king trumpet mushrooms (*Pleurotus ostreatus* and *Pleurotus eryngii*) have been able to degrade PAHs in environments contaminated with metals such as cadmium, manganese, and mercury (*39*). Additionally, arbuscular mycorrhizal fungi can play a role in enhancing phytostabilization and phytoextraction of metals in soils contaminated by trace elements such as copper, zinc, cadmium, and lead (*39,19*). While every fungal species has its own environmental constraints, some species can remediate soils and water in extreme conditions. The *Psathyrella* species can remediate soils in arctic conditions, and the tropical marine fungus *Cochliobolus lunatus* can degrade PAHs in high-salinity soil and water (*36*). Mycorrhizae contribute organic carbon to the soil, which can aid in the removal of certain contaminants, but this can also be done by amending soil media. In a study by Blecken et al., biofilters with added organic carbon in the filter media saw an increase in copper removed (*8*). While research indicates that fungi may enhance the breakdown (mycoremediation) of organic pollutants or stabilization or extraction of metals with plants, the specifics on which fungi and plant species to utilize are still developing.

3.2.6. Mycelium and Salt

Salt is a common roadway contaminant that can cause soil to be inhospitable to plant growth. In salt-stressed soils, phosphate (PO_4^{3-}) precipitates with ions of calcium (Ca2+), magnesium (Mg^{2+}), and zinc (Zn^{2+}) to become less available to plants (*40*). Fortunately, there are many salt-tolerant fungi found in saline soils that can facilitate plant growth (*41,42*). Arbuscular mycorrhizal fungi, in particular, have been shown to improve plant hormonal status, growth, and osmotic balance among other things in salt-stressed soils (*41*). Multiple studies have referenced that arbuscular mycorrhizae of the genus *Glomus* enhance the tolerance of plant species growing under salt stress; often conveying benefits to the plant such as increased nutrient uptake, decreased metal uptake, or improved water use efficiency (*35*). Fungal tolerance to salt is species-specific. For arbuscular mycorrhizal fungi in coastal vegetation on Japan's Okinawa Island, colonization rates were not reduced even when salinity levels were as high as 200mm (*43*). Not all fungi species are as infallible however; in other species, certain soil salinity concentrations have been shown to inhibit arbuscular mycorrhizal colonization capacity, spore germination, hyphal growth, and fungal metabolism (*44,45*). Even though arbuscular mycorrhizal fungi cannot completely remove salt from their environment, many can alleviate some of the effects on plants (*35*). The effects of salt on fungi introduced into the system must be carefully studied before deployment.

3.3 Task 2 Identification of Mycofiltration System Best Management Practices for MassDOT

3.3.1. Stormwater Control Measures (SCMs)

Through conversations with MassDOT, six existing stormwater SCMs that MassDOT currently utilizes were agreed upon that could potentially be augmented with fungi to enhance their water quality performance. As revealed in Task 1, these systems can be classified as either surface-level filtration systems (e.g., mycofiltration bags or compost filter tubes) or subsurface filtration systems (e.g., bioretention cells). Depending on the SCM, each system could then be augmented with saprophytic fungi, mycorrhizal fungi, or both to enhance water quality. The six SCM systems where the addition of fungi could be considered for water quality enhancement are as follows:

Surface-Level Filtration SCMs
1. Compost Filter Tube
2. Compost Blanket
3. Compost Berm
4. Coir Log or Wattle
5. Bioretention Soil with Woodchip Overlayer

Subsurface Filtration SCMs:
1. Bioretention Soil
2. Bioretention Soil with Woodchip Overlayer

These SCMs can be referenced in the MassDOT Sediment Control Barrier Design Matrix found in Appendix D, and a draft version of the MassDOT’s revised Sediment Control Barrier Spec can be found in Appendix E. The AASHTO material specifications are in Appendix H.

The word "compost" in this context refers to Filter Berm Media or Compost Blanket Media described in Table 3.1. In parallel to this project, MassDOT has a separate research effort with TetraTech and others to re-evaluate MassDOT compost specifications and other material descriptions. While not finalized, terms for materials will likely include the materials listed in Table 3.1. This table describes the two aforementioned materials as well as two other categories used by MassDOT.

Table 3.1: Compost and woodchip categories

Name	Description
Organic Soil Amendment	Fine, garden variety compost rich in nutrients
Filter Berm Media or Compost Blanket Media	Material used for compost filter tubes, filter berms or compost blanket as Stormwater Control Measures (SCMs)
Triple-Shredded Mulch/Wood Fiber	Typically, a mix of tree bark and wood that has been run through a tub grinder three times
Woodchips	Fresh woodchips typical of a commercial woodchipper
Landscape Mulch	Typically shredded bark or bark nuggets placed on soil surface

3.3.2. Temporary SCMs for Construction

The four surface-level SCMs can also be categorized as temporary, short-term stormwater technologies, as they are typically deployed for erosion control during and immediately following construction. Compost filter tubes and compost berms are considered sediment control barriers. Their purpose is to slow runoff velocity and filter suspended sediments from stormwater flow (*46*). Sediment control barriers are used to contain sediment stockpiles, to break slope length, and to retard or obstruct the flow of water into a work zone from an uphill slope or road surface (*46*).

Compost Filter Tube

Compost filter tubes are 12-inch or 9-inch tubes made of a biodegradable fabric (e.g., cotton, jute, or burlap) filled with compost. Compost filter tubes are tamped into place to ensure good contact with soil but are not trenched. Occasionally compost filter tubes may be stacked (*46)*. Due to their biodegradable nature, compost filter tubes can be left in place to decompose on-site in a naturalized area, however, if aesthetics are a concern, the fabric can be cut and removed, and the compost inside can be raked, and blended into the soil (*46*). Figure 3.11 shows two continuous rows of compost filter tubes installed on a grassy slope adjacent to a highway.

Figure 3.11: Compost filter tube

Compost Blanket

Compost blankets are a minimum ½-inch to 1-inch-deep layer of compost blanket media blown or pneumatically applied onto prepared soil to temporarily stabilize soil and provide organic matter for plant growth (*46*). When proposed with seeding, the seed is broadcast in conjunction with the compost blanket. When proposed with planting, the compost blanket is applied after planting (*46*). Combining compost blankets with seeding and/or planting can ensure long-term slope stabilization (*8*). Figure 3.12 below shows a worker applying compost filter media onto unvegetated soil of a slope. Compost is pneumatically blown through a flexible tube approximately 4 inches in diameter.

Figure 3.12: Compost blanket

Compost Berm

Like compost filter tubes, compost berms are linear sediment filters. The compost berm SCM is not currently utilized at MassDOT but is utilized at several other state DOTs in the United States. As with compost blankets, compost berms are comprised of blown or pneumatically applied filter berm media. The trapezoidal berm is blown in a row along the contour of a slope in a disturbed area to filter sediment-laden sheet flow runoff before it exits the site. Compost berms can be installed at any point along a slope as needed. During construction, compost berms should be inspected regularly for sediment buildup, undercutting, and other failures. Compost berms may be vegetated or unvegetated and are often left in place post-construction or tilled into the soil (*7*). Figure 3.13 below shows a compost berm. a compost berms along unvegetated steep slopes adjacent to a highway. There are parallel of compost berms evenly spaced 15–20 apart from top to bottom of slope. Filter media is placed on top of jute mesh covering the slope.

Figure 3.13: Compost berm

Coir Log or Wattles

Coir logs or wattles are similar to compost filter tubes in that they are tubes made from a biodegradable material such as coir netting, jute, or burlap. However, instead of being filled with compost, they are filled with densely packed coconut husk fiber known as coir. Coir logs are available in various sizes including 9, 12, 16 or 20-inches in diameter. Coir wattles are similar to coir logs but the fibers are more loosely packed resulting in a lighter more flexible material. Both logs and wattles can be staked in place and planted with vegetation, which over time can replace the erosion control provided by the degrading coir logs or wattles (*48*). For mycofiltration, it is imagined that a wedge of woodchips or other fungi-supporting substrates would be placed adjacent to the coir log or wattle on the uphill slope to allow fungi to grow into the log or wattle. It is hypothesized that mycelium could aid in extending the functional life of the log or wattle as it’s network grows.

Figure 3.14: Coir log or wattles

Figure 3.14 shows coir logs placed to stabilize an eroding river bank. Two logs are stacked to achieve the height needed to stabilize the bank. Logs are held in place by metal straps attached to wood stakes driven into ground. The coir log bank is backfilled with sand and planted to native grasses.

3.3.3. Permanent SCMs

Bioretention cells are a permanent SCM typically deployed in a bioretention system such as a rain garden, or swale that will have an enduring subsurface water filtration function (*49*). Bioretention soils are typically utilized in these systems and produced off-site to create a uniform soil mix free of weeds and other undesirable organic and inorganic material (*50,51*). By weight, the current MassDOT specification for bioretention soils is that they shall consist of 85% or more sand, 15% or less gravel, 10% or less silt, and 5% or less clay (*51*). While an organic content of 4–7% is desired, true compost is not recommended in bioretention soils due to the potential for nutrient leaching. Bioretention cells with a woodchip overlay are comprised of a standard bioretention soil design and application within a bioretention system, and then the application of a compost blanket over it. Figure 3.15 shows a bioretention cell, which is a wide, shallow swale seeded to a close cropped grass. The swale is lined with shade trees on both sides.

Figure 3.15: Bioretention cell

Figure 3.16: Bioretention cell with planting

Figure 3.16 shows a bioretention cell parallel to a roadway with a woodchip overlay. Curb cuts along the right side of the road allow stormwater to flow into the swale. The bottom of the swale is planted to a row of native shrubs and perennials. There is a maintained lawn between the road and the swale.

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4.0 Implementation and Technology Transfer

4.1 Task 2 Mycelial Application to SCMs

4.1.1. Inoculating Mycofiltration SCMs

The six selected SCM systems, except compost berms, are already employed by MassDOT across the Commonwealth. Through inoculating these SCMs with mycelium, additional filtration benefits may be conferred to these technologies to enable or enhance their ability to treat biological and chemical contamination and filter out fine sediments. Due to their inoculation with mycelium, and to avoid confusion over the meaning of the word "compost," the mycofiltration system SCMs will henceforth be referred to as:

Temporary SCMs (for use during construction)
1. Myco Filter Tube (Compost Filter Tube + Saprophytic Fungi)
2. Myco Blanket (Compost Blanket + Saprophytic Fungi)
3. Myco Berm (Compost Berm + Saprophytic Fungi)
4. Myco Coir Log or Wattle (Coir Long or Wattle, supporting woodchips upslope inoculated with Saprophytic Fungi)

Permanent SCMs
5. Myco Bioretention (Bioretention cell + Mycorrhizal Fungi)
6. Myco Bioretention+ (Bioretention cell and Compost Blanket + Saprophytic and Mycorrhizal Fungi)

Inoculation with mycelium can occur in one or more of three areas depending on the SCM, (1) the woodchip media, (2) the soil media, or (3) the plant material's roots. For the compost blanket, the compost filter tube, the compost berm, the woodchips overlaying the bioretention soil, and the woodchip wedge uphill of the coir log or wattle, the woodchip media could be inoculated with saprophytic fungi either in the field or before installation. Saprophytic fungi spores, spent mycelial spawn from a mushroom farm (i.e., wheat and sawdust media with active but not fruiting fungi), or mycelium-bearing wood or reference soil from sources local to the installation site could be used to inoculate the woodchips. In the bioretention systems, the soil can also be inoculated before installation if the soil is brought on-site from somewhere else, or it could be inoculated in the field. Commercially available mycorrhizal fungi spores have typically been found to not contain live spores therefore local soil or decomposing wood materials with native mycelial species are likely a better choice for inoculation (*25,28,52*). For plant material planted in these SCMs, indirect inoculation of the plant roots can occur through adding reference soil at the growing phase of the plants, contact with fungi in the woodchips and soil, or plant roots can be directly inoculated via the use of root tip cuttings from a mycelium colonized host plant. For seed material, soluble mycorrhizae could be applied prior to overseeding if future research determines this is worthwhile since recent research has shown that coating seeds with arbuscular mycorrhizae does not effectively colonize plants (*52*). The best methods found to inoculate existing plants to date with arbuscular mycorrhizae (AM) include directly adding native reference soils to new plantings, or inoculating the plants being installed with native mycorrhizae not typically available in commercial packets. A new kind of packet 'Mycobloom' is produced using a different manufacturing process than typical commercial packets and better colonization results of AM native to prairies in Kansas are occurring (*52*).

In-field mycelial inoculation establishment can be amplified through the addition of supplemental carbon sources such as wheat straw (*53*). This can be done whether introducing new fungi to the substrate (bioaugmentation) or if just stimulating the growth of existing native fungi communities (biostimulation). Providing additional nutrient sources to mycorrhizae during establishment should be done cautiously however, as overfeeding can cause the fungi to become parasitic to its plant partners (*54*). Resources for inoculation can be found through partnerships with mycologists at local Massachusetts universities as well as privately run mushroom farms and mycelium growing operations in Massachusetts and across New England (*55*). Additionally, the Rodale Institute has partnered with the United States Department of Agriculture (USDA) to develop a low-cost On-Farm Arbuscular Mycorrhizal (AM) Fungus Inoculum Production System, and the International Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM) have publicly available protocols for harvesting and propagating arbuscular mycorrhizal fungi from soil (*53*). Depending on the scale of mycofiltration implementation across MassDOT projects, the scale of the projects themselves, and the results of future MassDOT mycofiltration colonization testing, on-site inoculation may become infeasible. Instead, large volumes of soil and woodchip material will need to be inoculated before field application, but consideration for how long the fungi will live while being stored in larger piles or while transported must be considered.

4.2 Task 3 Deployment and Future Research Recommendations for Identified Best Management Practices

4.2.1. Recommendations for SCM Deployment: More Research is Necessary

The research in mycofiltration is in its early stages, and it is not yet known which fungi species or substrates would perform best, including long-term nutrient removal efficiencies and/or the long-term implications on impacts to the ecosystem surrounding these practices, especially if non-native fungi are introduced. **Additional research studies must be completed prior to any SCM deployment.** The need for future studies and research questions for evaluation are included in section 4.2.2

It is suggested that all of the MYCO SCMs be further tested in peer-reviewed field studies to see if they should be further considered by MassDOT for water quality enhancement. Myco Filter Tubes, Myco Blankets, and Myco Coir Logs or Wattles could be considered for temporary erosion control and filtration of surface flow during and immediately following construction projects, just as their non-inoculated versions are currently. Myco Berms, while not currently a MassDOT SCM, could be used in a comparable manner. Myco Bioretention and Myco Bioretention+ are permanent stormwater and silt filtration technologies that could be deployed as swales, rain gardens, or bioretention cells to deal with subsurface flow. These six SCMs could be augmented with either saprophytic fungi, mycorrhizal fungi, or a combination of both. Woodchip media can be inoculated before deployment at a facility, or in situ. **All of these parameters would need to be tested in an academic peer-reviewed setting prior to any deployment in the field.**

Appendix F shows conceptual CAD details created by Offshoots for each of these six SCMs with an added layer of mycelial inoculation. Details were created using standard MassDOT specifications and other relevant details, specifications, and reference material from California, Oregon, and Texas. AutoCAD files for the CAD details have been provided to MassDOT.

Axonometric diagrams showing these six SCMs implemented within a hypothetical site between a roadway and a waterbody can be found in Appendix G. The axons also show point and nonpoint pollution sources and how pollutants move across the site.

4.2.2. Questions to Evaluate in Future Studies

Offshoots' initial investigation into mycofiltration and subsequent interviews have revealed a lot about the promising technology, however many questions around materials, implementation, and performance remain. **To reiterate, it is not recommended that any of the MYCO SCMS be deployed at this time. More research into long-term viability and nutrient, biological, and TSS removal efficiencies is needed, with field studies and later pilot projects that track long-term results.** While future pilot studies verifying the efficacy of mycofiltration will explore many of these questions, some may require prior controlled research studies. Questions for future evaluation include:

Materials

1. Where can MassDOT source wood fiber material? Can it be regionally sourced where it will be applied? Can specific wood species be selected? Does the wood fiber source effect the fungal species that colonize over time?
2. Can fungal strains genetically local to the ecoregions of Massachusetts be sourced and used for inoculating wood fiber material? What is the effectiveness of using these fungi for Phosphorus Removal, Nitrogen Removal, TSS, and Biological pollutants? Do these native strains have any impact on reducing petroleum or heavy metals often typically found on roadways? Are they tolerant to salt and can they live in road-salted conditions?
3. Can MassDOT source triple-shredded wood fiber (mulch that has been run through a grinder three times)? Is this source material better than others to utilize in the MYCO SCMs for supporting saprophytic fungi growth? Currently, triple-shredded mulch is not utilized at MassDOT.
4. Should spent mycelium blocks from a mushroom farm be utilized to inoculate the MYCO SCMs? Are any of these species native? Are these species effective at removing the contaminants of concern? Can MassDOT secure reliable sources of spent mycelium blocks from a mushroom farm for inoculating woodchips?
5. Can MassDOT secure reliable sources of collected and propagated native mycelium spores and/or spawn?

Testing mycelial colonization of various substrates in the lab and the field with different sources of fungal inoculate (wild and cultivated, spores, spawn, and spent mycelial blocks) will be a critical next step to determine an economical and efficient means for mycofiltration system construction. To successfully implement mycofiltration, scalable and stable solutions will be needed (*26*). If regional wood sources and native wild-sourced fungi are to be used a catalog and map of Massachusetts fungi will be needed. Field guides such as *Mushrooms of NE America* by Tim Broney exist but may be best paired with online documented observations of native species such as those recorded on https://www.iNaturalist.org. Additionally, MassDOT could partner with local universities and/or local mycological associations – the Commonwealth is home to three according to the North American Mycological Association website – to conduct mushroom hunting walks to document species along MassDOT roads in various ecoregions in Massachusetts. For mycorrhizal fungi identification, MassDOT may want to support a soil testing project across the Commonwealth to detect and document mycorrhizal fungi DNA. Dr. Jenny Bhatnagar at Boston University is currently working with the Boston Parks Department to conduct similar work in the City of Boston (*28*).

Implementation

1. Can wood fiber stay in large piles pre-and or post-inoculation? What are the requirements to maintain mycelium health during storage?
2. Should wood fiber be bio-augmented (inoculated with mycelium), or should the existing fungal species present in wood fibers be allowed to colonize?
3. Should wood fiber be biostimulated with sawdust, molasses, or another food source so that fungal colonization progresses more rapidly?
4. Are commercially available mycorrhizal fungi (spore packets) viable? Do packets even contain spores? Do any of these spore packets include native fungi or just generalists?
5. How effective at inoculating woodchips are spent mycelium blocks from a mushroom farm?
6. How effective at inoculating woodchips are collected and propagated native mycelium spores and/or spawn?
7. Should inoculation of wood fiber or soil media happen in situ or prior to application at an off-site facility?
8. What are the optimal parameters around inoculation? How much inoculant is needed for success?

The major question for implementation is whether to introduce new fungal species to a site (bioaugmentation) or to amplify existing fungal species (biostimulation). The generalist fungal species likely are the most amenable to commercial propagation, and their use may supersede and replace existing native mycelial colonies (*56*). Across the global south, the *Amanita muscaria* fungus has become an invasive problem, as has an ectomycorrhizal fungus and the pine trees with which it has a symbiotic relationship (*28,54*). Additionally, introduced generalist fungal species may not be able to contribute worthwhile benefits such as the degradation of recalcitrant pollutants. One school of thought is that if new fungal species are to be introduced on-site, they should be able to do something special that native species cannot (*56*). Another potential issue with commercially available mycorrhizal fungi is their viability. Harvard Professor Donald Pfister had his mycology students test spores in commercially available mycorrhizal fungi packets to identify species and they found zero fungal spores, and this is substantiated in several peer-reviewed papers (*54,28,52*).

Performance

1. How do mycofiltration systems perform across seasons and under different environmental conditions, both in the field and through climate-threshold testing and resiliency testing? What happens in a drought? What happens when frozen for periods of time? What happens as the climate changes and our weather in the Massachusetts region becomes warmer and wetter?
2. What is the efficacy of each mycofiltration SCM in removing nitrogen, phosphorus, TSS, and biological contaminants such as *E. coli* from stormwater? How do the SCMs perform if they are left on-site or removed (if temporary)?
3. What is the durability of each mycofiltration SCM?
4. How does the efficacy of each mycofiltration SCM change over time? What are their effective lifetimes and maintenance needs?

Related to this final performance question is whether SCMs accumulated toxicity or pollutant buildup over time. Peter McCoy contends that when a burlap mycofiltration bag deteriorates, it releases the contaminants it had captured (*53*). Other studies have posited that mycelium may not eat or destroy all captured *E. coli* and that they can be released when the mycelial web is at capacity (*17,22*). These research questions as well as others that will come up during the field-testing process must be answered first before any deployment as pilot projects, or then to a larger scale.

4.3 Task 4 Potential Implementation Partners and Vendors

Before deployment or even testing of mycofiltration systems can begin, MassDOT must secure funding for future research projects, select research partners for studies, and source supplies of the substrate and inoculate materials.

4.3.1. Potential Local Research Partners

Four of the interviewees contacted as part of Task 1 expressed an interest in being future potential local research partners with MassDOT to assist in pilot projects testing the efficacy of mycofiltration in Massachusetts. These potential partners include:

1. Dr. Jenny Bhatnagar, Mycologist
    a. Title: Assistant Professor of Biology
    b. Institution: Boston University
    c. Area of Expertise: Mycology
    d. Phone: (617) 353-6957
    e. Email: jmbhat@bu.edu
2. Dr. Thomas Ballestro

    a. Title: Associate Professor of Civil and Environmental Engineering, Director of UNH Stormwater Center
    b. Institution: University of New Hampshire
    c. Area of Expertise: Stormwater management
    d. Phone: (603) 862-1405
    e. Email: Tom.Ballestero@unh.edu
3. Dr. David Hibbit, Clark University, Mycologist
    a. Title: Professor of Biology
    b. Institution: Clark University
    c. Area of Expertise: Mycology
    d. Phone: (508) 793-7332
    e. Email: dhibbett@clarku.edu
4. Jacquelyn Burmeister
    a. Title: Senior Environmental Analyst, Lakes and Ponds
    b. Institution: City of Worcester DPW&P Water Operations Division
    c. Area of Expertise: Water quality monitoring and improvement
    d. Phone: (508) 929-1300 Ext. 2126
    e. Email: BurmeisterJ@worcesterma.gov

4.3.2. Potential Vendors of Fungal Inoculant

Fungal inoculant can be purchased as spawn from places like North Spore in Maine, or as mycelium such as the spent blocks of substrate available from Massachusetts mushroom farms such as Fat Moon Farm. Three Northeast fungal inoculant vendors are:

1. North Spore
    a. Address: 90 Bridge St, Westbrook, ME 04094
    b. Phone: (207) 352-0264
    c. Email: info@northspore.com
    d. Website: https://northspore.com/
2. Fat Moon Farm
    a. Address: 41 West St, Westford, MA 01886
    b. Phone: (978) 496-9606
    c. Email: grow@fatmoonmushrooms.com
    d. Website: https://fatmoonmushrooms.com
3. Mycoterra Farm, South Deerfield, MA
    a. Address: 75 Stillwater Rd, South Deerfield, MA 01373
    b. Phone: (413) 397-3654
    c. Email: mycoterrafarm@gmail.com
    d. Website: http://mycoterrafarm.com/

4.3.3. Potential Vendors of Compost Filter Tube Materials

MassDOT works with a variety of vendors to provide compost filter tubes and woodchips for projects across the Commonwealth. Two such companies are:

1. Groundscapes Express, Inc.
    a. Address: P.O. Box 737, Wrentham, MA 02093
    b. Phone: (508) 384-7140
    c. Email: office@groundscapesexpress.com
    d. Website: https://www.groundscapesexpress.com/
2. Filtrexx Northeast Systems
    a. Address: 84 Daniel Plummer Rd, Goffstown, NH 03045
    b. Phone: (603) 621-9800
    c. Website: https://www.filtrexxns.com/

4.4 Technology Transfer

Technical drawing files and slide presentations summarizing the research were developed during the course of this project and have been passed on to MassDOT. These files include the excel file for the Appendix A literature review table, the video recordings with the subject matter interviews from which the notes in Appendix C were transcribed, and the AutoCAD details developed for the six mycofiltration SCMs which can be found in Appendix F. In addition, the final project research presentation slides were transmitted to MassDOT.

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5.0 Conclusions

MassDOT is required to manage and improve the water quality of stormwater before entering water bodies; however, existing green and gray infrastructure solutions are not always enough to manage non-point-source pollution from stormwater. The emerging stormwater management technology of mycofiltration holds promise as a low-cost and low-tech solution that can be incorporated into existing MassDOT SCMs to filter out small particulates, capture and break down chemical pollutants, and destroy pathogens. This project investigated the feasibility of mycofiltration through a literature review and interviews with subject matter experts.

The results of this research showed that mycofiltration holds potential for reducing the contaminants of concern of this study in treated stormwater. Despite promising results, there remains a clear dearth of mycofiltration field, lab, and replicable case studies. Moreover, many documented studies suffer from limited data points, few in-study replicates, and short experimental durations. Comparative studies of mycelium-inoculated woodchip and straw substrate versus sterile substrate have shown that mycelium is an important actor in phosphorus and nitrogen cycling. Mycelium in coordination with soil microbes can enhance the nutrient uptake of plants, although the mechanisms involving nitrogen are not well understood (*57*). Various species of fungi have proven to remediate contaminants or ameliorate their effects on plants in a wide range of environmental extremes, but specifics are still unknown.

Although there exists guiding information on which approaches, substrates and mycelial inoculates to use which were used to inform conceptual mycofiltration SCM design, there are very few instances where mycofiltration has been applied in the field and methodically evaluated to determine its efficacy. As such, there are no established design parameters or reliable outcomes from implementation. While some mycelia are self-reliant in filtering out and hunting pathogenic bacteria, mycofiltration and mycoremediation of chemical contaminants is largely a partnership between fungi, other soil microbes, plants, and abiotic factors such as soil chemistry. Finally, there are critical scientific questions that must be evaluated regarding mycelial nativeness and whether bioaugmentation with commercial saprophytic and mycorrhizal spores should be done or if locally occurring species should be biostimulated or collected, isolated, cultivated, and reintroduced. Commercial spores may already be ubiquitous in the environment due to plant nurseries inoculating plants with mycorrhizal fungi, and MassDOT's future mycofiltration research can influence change in native plant nursery inoculation practices (*26,29*).

Due to the nascent nature of mycofiltration and the existing knowledge gaps of the technology, particularly for the Massachusetts climate, MassDOT will not be able to immediately implement mycofiltration as an SCM for stormwater management and water quality improvement without first undertaking laboratory and field trials to vet mycofiltration design and management parameters. If successful, the results of field trials could support water quality credit permitting. With future mycofiltration research in mind, Offshoots has provided recommended mycofiltration implementation for each conceptual SCM, listed potential research questions to explore in lab and field tests, and documented local fungal inoculant vendors and academic research partners for future studies included in the report.

6.0 References

The papers analyzed as part of the literature review in addition to these references can be found in Appendix A. Notes from interviews with subject matter experts can be found in Appendix C.

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7.0 Appendixes

7.1 Appendix A: Literature Review

The initial literature review used combinations of the following 19 search terms on the following 21 databases. Additional sources have been added over time from interviews and from deeper dives into specific topics. An Excel spreadsheet of the literature review results has been provided to MassDOT.

7.1.1. Search Terms

Mycofiltration
Mycoremediation
Mycelium
Stormwater
Filtration
Fungal
Bioremediation
Phosphorous
Inorganic
Mushroom
Mycorrhizal
Water
Wastewater
Phosphate
Solubilizing
Pollutants
Biodegradation
Pluerotus ostreatus
Bacterial

7.1.2. Research Databases

Biochemistry
Biophysical Journal
Bioresource Technology
Ecology
Google Scholar
Journal of Ecology
JSTOR
Nature
Science
ScienceDirect
Web of Science
Advances in Ecological Research
Biodegradation (Journal)
Environmental Monitoring and Assessment
International Journal of Environmental Research
International Journal of Environmental Science and Technology
Journal of Ecological Engineering
Journal of Environmental Engineering
Journal of Molecular Biology
Journal of Soil and Water Conservation
Waste Management & Research

7.1.3. Literature Review Tables

1. Citations Table
2. Field Studies Summary Table
3. Lab Studies Table

Appendix A - Citations Table

Relevant	Author	Year	Title of Article/Book	Title of Journal/Newspaper	Full Chicago Citation	Field Study (X)	Lab Study (X)
Yes	Adams et al. Jonathan Todd.	2007	Fisherville Eco-Machine Pilot Final Report	John Todd Ecological Design, Inc Solutions for Water Planning and Treatment	Adams, Jeffery, Beam, Matt, Christy, Olin, Todd, John, and Todd, Johnathan. Fisherville Eco-Machine Pilot Final Report. John Todd Ecological Design, Inc Solutions for Water Planning and Treatment, 2007. http://baptheblackstone.org/whatwedoing/Water_Planning_Treatment/Fisherville_Final_Report.pdf.	X	
Yes	Azcón-Aguilar, C., and Barea, J.M.	1997	Applying mycorrhiza biotechnology to horticulture: significance and potentials	Scientia Horticulturae	Azcón-Aguilar, Concepción, and J. M. Barea. "Applying mycorrhiza biotechnology to horticulture: significance and potentials." Scientia Horticulturae 68, no. 1-4 (1997): 1-24.		
Yes	Benedict, T.	2011	Mycofiltration for the Puget Sound: Pleurotus ostreatus & fecal coliform bacteria	Thesis, The Evergreen State College	Benedict, Tim.a "Mycofiltration for the Puget Sound: Pleurotus ostreatus & fecal coliform bacteria." Evergreen State College, Thesis (2011): 1-10.		
Yes	Blecken et al.	2009	Impact of a submerged zone and a carbon source on heavy metal removal in stormwater biofilters	Ecological Engineering	Blecken, Godecke-Tobias, Yaron Zinger, Ana Deletić, Tim D. Fletcher, and Maria Viklander. "Impact of a submerged zone and a carbon source on heavy metal removal in stormwater biofilters." Ecological Engineering 35, no. 5 (2009): 769-778.		
Yes	FAWB	2009	Guidelines for Soil Filter Media in Bioretention Systems		FAWB. "Guidelines for Soil Filter Media in Bioretention Systems." (2009).		
Yes	George et al.	2002	Fecal coliform removal in wastewater treatment plants studied by plate counts and enzymatic methods	Water Research	George, Isabelle, Philippe Crop, and Pierre Servais. "Fecal coliform removal in wastewater treatment plants studied by plate counts and enzymatic methods." Water Research 36, no. 10 (2002): 2607-2617.		X
Yes	Harris, J.P.	2012	Degradation of Harmful Bacteria in Simulated Wastewater and Stormwater Runoff by the White Rot Fungus Pleurotus Ostreatus	Undergraduate Thesis, University of Delaware	Harris, John Paul. "Degradation of harmful bacteria in simulated wastewater and stormwater runoff by the white rot fungus Pleurotus ostreatus." PhD diss., 2012.		X
Yes	Juniper, S., and Abbott, J.K.	2006	Soil salinity delays germination and limits growth of hyphae from propagules of arbuscular mycorrhizal fungi	Mycorrhiza	Juniper, Sato, and L. K. Abbott. "Soil salinity delays germination and limits growth of hyphae from propagules of arbuscular mycorrhizal fungi." Mycorrhiza 16, no. 5 (2006): 371-379.		
Yes	Juniper, S., and Abbott, J.K.	1993	Vesicular-arbuscular mycorrhizas and soil salinity	Mycorrhiza	Juniper, S., and L. Abbott. "Vesicular-arbuscular mycorrhizas and soil salinity." Mycorrhiza 4, no. 2 (1993): 45-57.		
Yes	Jurak et al.	2015	Fate of carbohydrates and lignin during composting and mycelium growth of Agaricus bisporus on wheat straw based compost	PLoS One	Jurak, Edita, Arjen M. Punt, Wim Arts, Mirjam A. Kabel, and Harry Gruppen. "Fate of carbohydrates and lignin during composting and mycelium growth of Agaricus bisporus on wheat straw based compost." PLoS One 10, no. 10 (2015): e0138909.		
Yes	Kennen, K., and Kirkwood, N.	2015	Phyto: principles and resources for site remediation and landscape design		Kennen, Kate, and Niall Kirkwood. Phyto: principles and resources for site remediation and landscape design. Routledge, 2015.		
Yes	Kenny, S.	2008	Staff Report of Oakland Bay Activities 3/1/08 to 6/30/08		Kenny, Stephanie. "Staff Report of Oakland Bay Activities 3/1/08 to 6/30/08." Mason County. July 16, 2008. https://masoncountywa.gov/forms/Env_Health/MRA_2008_Qtr2.pdf.	X	
Yes	Köhl, L., and van der Heijden, M.G.	2016	Arbuscular mycorrhizal fungal species differ in their effect on nutrient leaching	Soil Biology and Biochemistry	Köhl, Luise, and Marcel GA van der Heijden. "Arbuscular mycorrhizal fungal species differ in their effect on nutrient leaching." Soil Biology and Biochemistry 94 (2016): 191-199.		X
Yes	Kohler et al.	2009	Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress	Environmental and Experimental Botany	Kohler, Josef, José Antonio Hernández, Fuensanta Caravaca, and Antonio Roldán. "Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress." Environmental and Experimental Botany 65, no. 2-3 (2009): 245-252.		
Yes	Kumar et al.	2019	Arbuscular Mycorrhizal fungi-mediated mycoremediation of saline soil: Current knowledge and future prospects	Recent Advancement in White Biotechnology Through Fungi	Kumar, Dileep, Priyanka Priyanka, Pramendra Yadav, Anurag Yadav, and Kusum Yadav. "Arbuscular Mycorrhizal fungi-mediated mycoremediation of saline soil: Current knowledge and future prospects." Recent Advancement in White Biotechnology Through Fungi (2019): 319-348.		
Yes	Martinez, S.E.	2016	E. Coli Removal by Pleurotus Ostreatus Mycofilter in Simulated Wet Environmental Pond	Masters Thesis, University of New Mexico	Martinez, Savannah E. "E. Coli Removal by Pleurotus Ostreatus Mycofilter in Simulated Wet Environmental Pond." (2016).		X

Yes	Mathur et al.	2018	Improved photosynthetic efficacy of maize (Zea mays) plants with arbuscular mycorrhizal fungi (AMF) under high temperature stress	Journal of Photochemistry and Photobiology B: Biology	Mathur, Sonal, Mahaveer P. Sharma, and Anjana Jajoo. “Improved photosynthetic efficacy of maize (Zea mays) plants with arbuscular mycorrhizal fungi (AMF) under high temperature stress.” Journal of Photochemistry and Photobiology B: Biology 180 (2018): 149-154.		
Yes	Melville, A.D.	2016	Assessment of a Mycorrhizal Fungi Application to Treat Stormwater in an Urban Bioswale	PhD Dissertation, Portland State University	Melville, Alaina Diane. "Assessment of a mycorrhizal fungi application to treat stormwater in an urban bioswale." PhD diss., Portland State University, 2016.	X	X
Yes	Palacios Y.M., and Winfrey, B.K.	2021	Three mechanisms of mycorrhizae that may improve stormwater biofilter performance	Ecological Engineering	Palacios, Yussi M., and Brandon K. Winfrey. "Three mechanisms of mycorrhizae that may improve stormwater biofilter performance." Ecological Engineering 159 (2021): 106085.		
Yes	Pini, A.K., and Geddes , P.	2020	Fungi Are Capable of Mycoremediation of River Water Contaminated by E. coli	Water, Air, and Soil Pollution	Pini, Andrea K., and Pamela Geddes. "Fungi Are Capable of Mycoremediation of River Water Contaminated by E. coli." Water, Air, & Soil Pollution 231, no. 2 (2020): 1-10.		X
Yes	Poor et al.	2018	The Role of Mycelium in Bioretention Systems: Evaluation of Nutrient Retention in Mycorrhizae-inoculated Mescocosms	Journal of Environmental Engineering	Poor, Cara J., Casey Balmes, Michael Freudenthaler, and Ashley Martinez. "The role of mycelium in bioretention systems: Evaluation of nutrient retention in mycorrhizae-inoculated mescocosms." (2018): 1.		X
Yes	Poor, C., and Kube, J.	2019	Variation in Nutrient and Metal Retention in Bioretention Systems with Mycorrhizae-Inoculated Soil	World Environmental and Water Resources Congress 2019: Water, Wastewater, and Stormwater; Ubran Water Resources; and Municipal Water Infrastructure	Poor, Cara, and Jenna Kube. "Variation in nutrient and metal retention in bioretention systems with mycorrhizae-inoculated soil." In World Environmental and Water Resources Congress 2019: Water, Wastewater, and Stormwater; Urban Water Resources; and Municipal Water Infrastructure, pp. 71-79. Reston, VA: American Society of Civil Engineers, 2019.		X
Yes	Rogers, T.	2012	Experimental Evaluation of Mycoremediation of Escherichia coli Bacteria in Solution using Pleurotus ostreatus	PhD Dissertation, The Evergreen State College	Rogers, Tim. "Experimental evaluation of mycoremediation of Escherichia coli bacteria in solution using Pleurotus ostreatus." PhD diss., Evergreen State College, 2012.		X
Yes	Rubin, J.A., and Görres, J.H.	2021	Potential for Mycorrhizae-Assisted Phytoremediation of Phosphorus for Improved Water Quality	International Journal of Environmental Research and Public Health	Rubin, Jessica A., and Josef H. Görres. "Potential for Mycorrhizae-Assisted Phytoremediation of Phosphorus for Improved Water Quality." International Journal of Environmental Research and Public Health 18, no. 1 (2021): 7.		
Yes	Sheng et al.	2008	Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress	Mycorrhiza	Sheng, Min, Ming Tang, Hui Chen, Baowei Yang, Fengfeng Zhang, and Yanhui Huang. “Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress.” Mycorrhiza 18, no. 6-7 (2008): 287-296.		
Yes	Smith, S. E., and Read, D.J.	2008	Mycorrhizal Symbiosis		Smith, S. E., and D. J. Read. “Mycorrhizal Symbiosis, 3rd editio Edn.” (2008).		
Yes	Stamets et al.	2013	Comprehensive Assessment of Mycofiltration Biotechnology to Remove Pathogens from Urban Stormwater	Fungi Perfecti's EPA SBIR Phase 1 Research Results. EPA Contract #: EP-D-12-010	Stamets, Paul, M. Beutel, Alex Taylor, Alicia Flatt, Morgan Wolff, and Katie Brownson. "Comprehensive assessment of mycofiltration biotechnology to remove pathogens from urban stormwater." Fungi Perfecti’s EPA SBIR Phase I Research Results. EPA Contract#: EP-D-12-010 (2013).		X
Yes	Stamets, L.D.C.	2012	Best Mycorestoration Practices for Habitat Restoration of Small Land Parcel	Masters Thesis, The Evergreen State College	Stamets, Le Dena Che. "Best Mycorestoration Practices for Habitat Restoration of Small Land Parcels." (2012).	X	
Yes	Stamets, P., and Sumerlin, D.	2003	MycoRestoration of Abandoned Logging Roads		Stamets, Paul, and David Sumerlin. MycoRestoration of Abandoned Logging Roads. 2003. Accessed on October 25, 2021 https://fungi.com/blogs/articles/mycorestoration-of-abandoned-logging-roads.	X	
Yes	Taylor et al.	2018	Engineering Analysis of Plant and Fungal Contributions to Bioretention Performance	Water	Taylor, Alex, Jill Wetzel, Emma Mudrock, Kennith King, James Cameron, Jay Davis, and Jenifer McIntyre. "Engineering analysis of plant and fungal contributions to bioretention performance." Water 10, no. 9 (2018): 1226.	X	
Yes	Taylor et al.	2015	Removal of Escherichia coli from syntheic stormwater using mycofiltration	Ecological Engineering	Taylor, Alex, Alicia Flatt, Marc Beutel, Morgan Wolff, Katherine Brownson, and Paul Stamets. "Removal of Escherichia coli from synthetic stormwater using mycofiltration." Ecological engineering 78 (2015): 79-86.		X
Yes	Taylor, A.W., and Stamets, P.E.	2014	Implementing Fungal Cultivation in biofiltration systems - the Past, Present, and Future of Mycofiltration	National Proceedings: Forest and Conservation Nursery Associations	Taylor, Alex W., and Paul E. Stamets. "Implementing fungal cultivation in biofiltration systems–the past, present, and future of mycofiltration." National Proceedings: Forest and Conservation Nursery Associations—2013 23 (2014).		
Yes	Thomas et al.	2009	Field Demonstrations of Mycoremediation for Removal of Fecal Coliform Bacteria and Nutrients in the Dungeness Watershed, Washington	Pacific Northwest National Laboratory	Thomas, S. A., L. M. Aston, D. L. Woodruff, and V. I. Cullinan. "Field demonstration of mycoremediation for removal of fecal coliform bacteria and nutrients in the Dungeness watershed, Washington. Final Report." Pacific Northwest National Laboratory. PNWD-4054-1 (2009).	X	

Yes	Wiesche et al.	2003	The Effect of Interaction Between White-rot Fungi and Indigenous Microorganisms on Degradation of Polycyclic Aromatic Hydrocarbons in Soil	Water, Air, and Soil Pollution	Wiesche, C., R. Martens, and F. Zadrazil. "The effect of interaction between white-rot fungi and indigenous microorganisms on degradation of polycyclic aromatic hydrocarbons in soil." Water, Air and Soil Pollution. Focus 3 (2003).		X
Yes	Winfrey et al.	2017	Arbuscular mycorrhizal fungi in Australian stormwater biofilters	Ecological Engineering	Winfrey, Brandon K., Belinda E. Hatt, and Richard F. Ambrose. "Arbuscular mycorrhizal fungi in Australian stormwater biofilters." Ecological Engineering 102 (2017): 483-489.	X	
Yes	Yamato et al.	2008	Community of arbuscular mycorrhizal fungi in a coastal vegetation on Okinawa island and effect of the isolated fungi on growth of sorghum under salt-treated conditions	Mycorrhiza	Yamato, Masahide, Shiho Ikeda, and Koji Iwase. “Community of arbuscular mycorrhizal fungi in a coastal vegetation on Okinawa island and effect of the isolated fungi on growth of sorghum under salt-treated conditions.” Mycorrhiza 18, no. 5 (2008): 241-249.		
Maybe - not mycofiltration	Amjad et al.	2017	Mycoremediation of Potentially Toxic Trace Elements—a Biological Tool for Soil Cleanup: A Review	Pedosphere	Amjad, A. L. I., G. U. O. Di, Amanullah Mahar, Wang Ping, S. H. E. N. Feng, L. I. Ronghua, and Zengqiang Zhang. "Mycoremediation of potentially toxic trace elements—a biological tool for soil cleanup: a review." Pedosphere 27, no. 2 (2017): 205-222.		
Maybe - not mycofiltration	Anasonye et al.	2018	Role of Biochar and Fungi on PAH Sorption to Soil Rich in Organic Matter	Water, Air, & Soil Pollution	Anasonye, Festus, Priit Tammeorg, Jevgeni Parshintsev, Marja-Liisa Riekkola, and Marja Tuomela. "Role of biochar and fungi on PAH sorption to soil rich in organic matter." Water, Air, & Soil Pollution 229, no. 2 (2018): 1-14.		X
Maybe - not mycofiltration	Anderson, C., and Juday, G.	2016	Mycoremediation of Petroleum: A Literature Review	Journal of Environmental Science and Engineering	Anderson, Christin, and Glenn Juday. "Mycoremediation of petroleum: a literature review." J. Environ. Sci. Eng. A 5 (2016): 397-405.		
Maybe - not mycofiltration	Ariste et al.	2020	Mycoremediation of phenols and polycyclic aromatic hydrocarbons from a biorefinery wastewater and concomitant production of lignin modifying enzymes	Journal of Cleaner Production	Ariste, Arielle Farida, Ramón Alberto Batista-García, Vinoth Kumar Vaidyanathan, Nikila Raman, Vasanth Kumar Vaithyanathan, Jorge Luis Folch-Mallol, Stephen A. Jackson, Alan DW Dobson, and Hubert Cabana. "Mycoremediation of phenols and polycyclic aromatic hydrocarbons from a biorefinery wastewater and concomitant production of lignin modifying enzymes." Journal of Cleaner Production 253 (2020): 119810.		X
Maybe - not mycofiltration	Battini et al.	2017	Facilitation of phosphorus uptake in maize plants by mycorrhizosphere bacteria	Scientific Reports	Battini, Fabio, Mette Grønlund, Monica Agnolucci, Manuela Giovannetti, and Iver Jakobsen. "Facilitation of phosphorus uptake in maize plants by mycorrhizosphere bacteria." Scientific reports 7, no. 1 (2017): 1-11.		
Maybe - not mycofiltration	Becquer et al.	2014	From soil to plant, the journey of P through trophic relationships and ectomycorrhizal association	Frontiers in Plant Sciences	Becquer, Adeline, Jean Trap, Usman Irshad, Muhammad A. Ali, and Plassard Claude. "From soil to plant, the journey of P through trophic relationships and ectomycorrhizal association." Frontiers in plant science 5 (2014): 548.		
Maybe - not mycofiltration	Bi et al.	2019	Response of arbuscular mycorrhizal fungi and phosphorus solubilizing bacteria to remediation abandoned solid waste of coal mine	International Journal of Coal Science and Technology	Bi, Yinli, Li Xiao, and Rongrong Liu. "Response of arbuscular mycorrhizal fungi and phosphorus solubilizing bacteria to remediation abandoned solid waste of coal mine." International Journal of Coal Science & Technology 6, no. 4 (2019): 603-610.		X
Maybe - not mycofiltration	Cabral et al.	2015	Arbuscular mycorrhizal fungi in phytoremediation of contaminated areas by trace elements: mechanisms and major benefits of their applications	World Journal of Microbiology and Biotechnology	Cabral, Lucélia, Claúdio Roberto Fonsêca Sousa Soares, Admir José Giachini, and José Oswaldo Siqueira. "Arbuscular mycorrhizal fungi in phytoremediation of contaminated areas by trace elements: mechanisms and major benefits of their applications." World Journal of Microbiology and Biotechnology 31, no. 11 (2015): 1655-1664.		
Maybe - not mycofiltration	Chibuike, G. U	2013	Use of mycorrhiza in soil remediation: A review	Scientific Research and Essays	Chibuike, G. U. "Use of mycorrhiza in soil remediation: a review." Scientific Research and Essays 8, no. 35 (2013): 679-1687.		
Maybe - not mycofiltration	Govarthanan et al.	2018	Myco-phytoremediation of arsenic- and lead-contaminated soils by Helianthus annuus and wood rot fungi, Trichoderma sp. isolated from decayed wood	Ecotoxicology and Environmental Safety	Govarthanan, M., R. Mythili, T. Selvankumar, S. Kamala-Kannan, and H. Kim. "Myco-phytoremediation of arsenic-and lead-contaminated soils by Helianthus annuus and wood rot fungi, Trichoderma sp. isolated from decayed wood." Ecotoxicology and environmental safety 151 (2018): 279-284.		X
Maybe - not mycofiltration	Harms et al.	2011	Untapped potential: exploiting fungi in bioremediation of hazardous chemicals	Nature Reviews Microbiology	Harms, Hauke, Dietmar Schlosser, and Lukas Y. Wick. "Untapped potential: exploiting fungi in bioremediation of hazardous chemicals." Nature Reviews Microbiology 9, no. 3 (2011): 177-192.		
Maybe - not mycofiltration	Jones et al.	1998	A comparison of arbuscular and ectomycorrhizal Eucalyptus coccifera: growth response, phosphorus uptake efficiency and external hyphal production	New Phytologist	Jones, Melanie D., D. M. Durall, and P. B. Tinker. "A comparison of arbuscular and ectomycorrhizal Eucalyptus coccifera: growth response, phosphorus uptake efficiency and external hyphal production." New Phytologist 140, no. 1 (1998): 125-134.		X
Maybe - not mycofiltration	Kubátová et al.	2001	PCB congener selective biodegradation by the white rot fungus Pleurotus ostreatus in contaminated soil	Chemosphere	Kubatova, A., P. Erbanova, I. Eichlerova, L. Homolka, F. Nerud, and V. Šašek. "PCB congener selective biodegradation by the white rot fungus Pleurotus ostreatus in contaminated soil." Chemosphere 43, no. 2 (2001): 207-215.		X
Maybe - not mycofiltration	Lambert, D.H. and Weidensaul, T.C.	1991	Element Uptake by Mycorrhizal Soybean from Sewage-Sludge-Treated Soil	Soil Science Society of America Journal	Lambert, D. H., and T. C. Weidensaul. "Element uptake by mycorrhizal soybean from sewage-sludge-treated soil." Soil Science Society of America Journal 55, no. 2 (1991): 393-398.		X

Maybe - not mycofiltration	Li et al.	1991	Extension of the phosphorus depletion zone in VA-mycorrhizal white clover in a calcareous soil	Plant and Soil	Li, Xiao-Lin, Eckhard George, and Horst Marschner. "Extension of the phosphorus depletion zone in VA-mycorrhizal white clover in a calcareous soil." Plant and Soil 136, no. 1 (1991): 41-48.		
Maybe - not mycofiltration	Michelot et al.	1998	Update on metal content profiles in mushrooms—toxicological implications and tentative approach to the mechanisms of bioaccumulation	Toxicon	Michelot, Didier, Eliane Siobud, Jean-Christophe Doré, Claude Viel, and Françoise Poirier. "Update on metal content profiles in mushrooms—toxicological implications and tentative approach to the mechanisms of bioaccumulation." Toxicon 36, no. 12 (1998): 1997-2012.		X
Maybe - not mycofiltration	Pointing, S.	2001	Feasibility of bioremediation by white-rot fungi	Applied Microbiology and Biotechnology	Pointing, S. "Feasibility of bioremediation by white-rot fungi." Applied microbiology and biotechnology 57, no. 1 (2001): 20-33.		
Maybe - not mycofiltration	Rhodes, C.J.	2014	Mycoremediation (bioremediation with fungi) - growing mushrooms to clean the earth	Chemical Speciation & Bioavailability	Rhodes, Christopher J. "Mycoremediation (bioremediation with fungi)–growing mushrooms to clean the earth." Chemical Speciation & Bioavailability 26, no. 3 (2014): 196-198.		
Maybe - not mycofiltration	Ruiz-Aguilar et al.	2002	Degradation by white-rot fungi of high concentrations of PCB extracted from a contaminated soil	Advances in Environmental Research	Ruiz-Aguilar, Graciela ML, José M. Fernández-Sánchez, Refugio Rodríguez-Vázquez, and Héctor Poggi-Varaldo. "Degradation by white-rot fungi of high concentrations of PCB extracted from a contaminated soil." Advances in Environmental Research 6, no. 4 (2002): 559-568.		
Maybe - not mycofiltration	Sadañoski et al.	2020	Evaluation of bioremediation strategies for treating recalcitrant halo-organic pollutants in soil environments	Ecotoxicology and Environmental Safety	Sadañoski, Marcela Alejandra, Ana Silvia Tatarin, Mónica Lucrecia Barchuk, Mariana Gonzalez, César Nicolás Pegoraro, María Isabel Fonseca, Laura Noemí Levin, and Laura Lidia Villalba. "Evaluation of bioremediation strategies for treating recalcitrant halo-organic pollutants in soil environments." Ecotoxicology and Environmental Safety 202 (2020): 110929.		X
Maybe - not mycofiltration	Smith et al.	2011	Roles of Arbuscular Mycorrhizas in Plant Phosphorus Nutrition: Interactions between Pathways of Phosphorus Uptake in Arbuscular Mycorrhizal Roots Have Important Implications for Understanding and Manipulating Plant Phosphorus Acquisition	Plant Physiology	Smith, Sally E., Iver Jakobsen, Mette Grønlund, and F. Andrew Smith. "Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition." Plant physiology 156, no. 3 (2011): 1050-1057.		
Maybe - not mycofiltration	Stella et al.	2017	Bioremediation of long-term PCB-contaminated soil by white-rot fungi	Journal of Hazardous Materials	Stella, Tatiana, Stefano Covino, Monika Čvančarová, Alena Filipová, Maurizio Petruccioli, Alessandro D'Annibale, and Tomáš Cajthaml. "Bioremediation of long-term PCB-contaminated soil by white-rot fungi." Journal of hazardous materials 324 (2017): 701-710.		X
Maybe - not mycofiltration	van der Heijden et al.	1998	Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity	Nature	Van Der Heijden, Marcel GA, John N. Klironomos, Margot Ursic, Peter Moutoglis, Ruth Streitwolf-Engel, Thomas Boller, Andres Wiemken, and Ian R. Sanders. "Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity." Nature 396, no. 6706 (1998): 69-72.		
Maybe - not mycofiltration	Winquista et al.	2014	Bioremediation of PAH-contaminated soil with fungi – From laboratory to field scale	International Biodeterioration & Biodegradation	Winquist, Erika, Katarina Björklöf, Eija Schultz, Markus Räsänen, Kalle Salonen, Festus Anasonye, Tomáš Cajthaml, Kari T. Steffen, Kirsten S. Jørgensen, and Marja Tuomela. "Bioremediation of PAH-contaminated soil with fungi–From laboratory to field scale." International Biodeterioration & Biodegradation 86 (2014): 238-247.	X	X
No	Abu-Elsaoud et al.	2017	Arbuscular mycorrhizal strategy for zinc mycoremediation and diminished translocation to shoots and grains in wheat	PLoS One	Abu-Elsaoud, Abdelghafar M., Nivien A. Nafady, and Ahmed M. Abdel-Azeem. "Arbuscular mycorrhizal strategy for zinc mycoremediation and diminished translocation to shoots and grains in wheat." PLoS One 12, no. 11 (2017): e0188220.		
No	Acharya et al.	2019	A comparative assessment of conventional and molecular methods, including MinION nanopore sequencing, for surveying water quality	Scientific Reports	Acharya, Kishor, Santosh Khanal, Kalyan Pantha, Niroj Amatya, Russell J. Davenport, and David Werner. "A comparative assessment of conventional and molecular methods, including MinION nanopore sequencing, for surveying water quality." Scientific reports 9, no. 1 (2019): 1-11.		
No	Adelaja et al.	2017	Mycoremediation of Pesticide Contaminated Soil Using Mushroom Pleurotus ostreatus	International Journal of Health and Economic Development	Adelaja, O. D., K. L. Njoku, and M. O. Akinola. "Mycoremediation of Pesticide Contaminated Soil Using Mushroom Pleurotus ostreatus." International Journal of Health and Economic Development 3, no. 2 (2017): 20-27.		
No	Adenipekun, C.O., and Lawal, R.	2012	Uses of mushrooms in bioremediation: A review	Biotechnology and Molecular Biology Reviews	Adenipekun, C. O., and Rasheedah Lawal. "Uses of mushrooms in bioremediation: A review." Biotechnology and Molecular Biology Reviews 7, no. 3 (2012): 62-68.		
No	Akhtar, N., and Mannan, M.A.U.	2020	Mycoremediation: Expunging environmental pollutants	Biotechnology Reports	Akhtar, Nahid, and M. Amin-ul Mannan. "Mycoremediation: Expunging environmental pollutants." Biotechnology Reports 26 (2020): e00452.		
No	Al-Dhabaan, F.A.	2021	Mycoremediation of crude oil contaminated soil by specific fungi isolated from Dhahran in Saudi Arabia	Saudi Journal of Biological Sciences	Al-Dhabaan, Fahad A. "Mycoremediation of crude oil contaminated soil by specific fungi isolated from Dhahran in Saudi Arabia." Saudi Journal of Biological Sciences 28, no. 1 (2021): 73-77.		

No	Arya et al.	2018	Arbuscular Mycorrhizal Fungi As Phosphate Fertilizer for Crop Plants and Their Role in Bioremediation of Heavy Metals	Fungi and their Role in Sustainable Development: Current Perspectives	Arya, Arun, Shalini Ojha, and Simranjeet Singh. "Arbuscular Mycorrhizal Fungi As Phosphate Fertilizer for Crop Plants and Their Role in Bioremediation of Heavy Metals." In Fungi and their Role in Sustainable Development: Current Perspectives, pp. 255-265. Springer, Singapore, 2018.		
No	Bender et al.	2016	Mycoremediation Applications for Stormwater Management	Undergraduate Project, University of Colorado, Boulder	Cooper, Melanie Dickison, Leopold Kisielinski, and Erica Wiener. "Mycoremediation Applications for Stormwater Management."		
No	Beutel, M.W., and Larson, L.	2015	Pathogen removal from urban pond outflow using rock biofilters	Ecological Engineering	Beutel, Marc W., and Laurie Larson. "Pathogen removal from urban pond outflow using rock biofilters." Ecological Engineering 78 (2015): 72-78.		
No	Bhatnagar et al.	2021	Wastewater treatment and Mycoremediation by P. ostreatus mycelium	IOP Conference Series: Earth and Environmental Science	Bhatnagar, A., E. Tamboli, and A. Mishra. "Wastewater treatment and Mycoremediation by P. ostreatus mycelium." In IOP Conference Series: Earth and Environmental Science, vol. 775, no. 1, p. 012003. IOP Publishing, 2021.		X
No	Bhatt et al.	2002	Mycoremediation of PAH-contaminated soil	Folia Microbiologica	Bhatt, M., T. Cajthaml, and V. Šašek. "Mycoremediation of PAH-contaminated soil." Folia Microbiologica 47, no. 3 (2002): 255-258.		
No	Binder et al.	2013	Phylogenetic and phylogenomic overview of the Polyporales	Mycologia	Binder, Manfred, Alfredo Justo, Robert Riley, Asaf Salamov, Francesc Lopez-Giraldez, Elisabet Sjökvist, Alex Copeland et al. "Phylogenetic and phylogenomic overview of the Polyporales." Mycologia 105, no. 6 (2013): 1350-1373.		
No	Blagodatsky et al.	2020	Myco-phytoremediation of mercury polluted soils in Ghana and Burkina Faso	EGU General Assembly Conference Abstracts	Blagodatsky, Sergey, Miriam Ehret, Frank Rasche, Imke Hutter, Regina Birner, Beloved Dzomeku, Oble Neya, Georg Cadisch, and Jens Wünsche. "Myco-phytoremediation of mercury polluted soils in Ghana and Burkina Faso." In EGU General Assembly Conference Abstracts, p. 19583. 2020.		X
No	Cecchi et al.	2019	From waste to resource: mycoremediation of contaminated marine sediments in the SEDITERRA Project	Journal of Soils and Sediments	Cecchi, Grazia, Laura Cutroneo, Simone Di Piazza, Greta Vagge, Marco Capello, and Mirca Zotti. "From waste to resource: mycoremediation of contaminated marine sediments in the SEDITERRA project." Journal of Soils and Sediments (2019): 1-11.		
No	Chatterjee et al.	2017	Mushrooms: from nutrition to mycoremediation	Environmental Science and Pollution Research	Chatterjee, Soumya, Mukul K. Sarma, Utsab Deb, Georg Steinhauser, Clemens Walther, and Dharmendra K. Gupta. "Mushrooms: from nutrition to mycoremediation." Environmental Science and Pollution Research 24, no. 24 (2017): 19480-19493.		
No	Clary et al.	2008	Can Stormwater BMPs Remove Bacteria?	Stormwater Magazine	Clary, Jane, Jonathan Jones, Ben Urbonas, Marcus Quigley, Eric Strecker, and Todd Wagner. "Can stormwater BMPs remove bacteria? New findings from the international stormwater BMP database." Stormwater Magazine May 5 (2008): 1-14.		
No	Cording, A.	2016	Evaluating stormwater pollutant removal mechanisms by bioretention in the context of climate change	PhD Dissertation, The University of Vermont	Cording, Amanda. Evaluating stormwater pollutant removal mechanisms by bioretention in the context of climate change. The University of Vermont and State Agricultural College, 2016.		
No	Daniels, R.J.	2012	Nitrous oxide emissions of higher fungal mycelium under various wastewater concentrations	Masters Thesis, State University of New York College of Environmental Science and Forestry	Daniels, Russell James. Nitrous oxide emissions of higher fungal mycelium under various wastewater concentrations. State University of New York College of Environmental Science and Forestry, 2012.		
No	Dickson et al.	2019	Mycoremediation of petroleum contaminated soils: progress, prospects and perspectives	Environmental Science: Processes & Impacts	Dickson, Udeme John, Michael Coffey, Robert John George Mortimer, Marcello Di Bonito, and Nicholas Ray. "Mycoremediation of petroleum contaminated soils: Progress, prospects and perspectives." Environmental Science: Processes & Impacts 21, no. 9 (2019): 1446-1458.		
No	Federici et al.	2012	Bioaugmentation of a historically contaminated soil by polychlorinated biphenyls with Lentinus tigrinus	Microbial Cell Factories	Federici, Ermanno, Mariangela Giubilei, Guglielmo Santi, Giulio Zanaroli, Andrea Negroni, Fabio Fava, Maurizio Petruccioli, and Alessandro D'Annibale. "Bioaugmentation of a historically contaminated soil by polychlorinated biphenyls with Lentinus tigrinus." Microbial cell factories 11, no. 1 (2012): 1-14.		
No	Foght et al.	2001	Bioremediation of DDT-Contaminated Soils: A Review	Bioremediation Journal	Foght, Julia, Trevor April, Kevin Biggar, and Jackie Aislabie. "Bioremediation of DDT-contaminated soils: a review." Biorernediation Journal 5, no. 3 (2001): 225-246.		
No	Gallagher et al.	1997	Biosorption of synthetic dye and metal ions from aqueous effluents using fungal biomass	Studies in Environmental Science	Gallagher, K. A., M. G. Healy, and S. J. Allen. "Biosorption of synthetic dye and metal ions from aqueous effluents using fungal biomass." In Studies in Environmental Science, vol. 66, pp. 27-50. Elsevier, 1997.		X
No	Gao et al.	2010	A critical review of the application of white rot fungus to environmental pollution control	Critical Reviews in Biotechnology	Gao, Dawen, Lina Du, Jiaoling Yang, Wei-Min Wu, and Hong Liang. "A critical review of the application of white rot fungus to environmental pollution control." Critical reviews in biotechnology 30, no. 1 (2010): 70-77.		

No	Gil-Martínez et al.	2020	Trace elements and C and N isotope composition in two mushroom species from a mine-spill contaminated site	Scientific Reports	Gil-Martínez, Marta, Carmen M. Navarro-Fernández, José M. Murillo, María T. Domínguez, and Teodoro Marañón. "Trace elements and C and N isotope composition in two mushroom species from a mine-spill contaminated site." Scientific reports 10, no. 1 (2020): 1-13.		
No	Haidukowski et al.	2017	Decontamination of Fumonisin B_1 in maize grain by Pleurotos eryngii and antioxidant enzymes	Phytopathologia Mediterranea	Haidukowski, Miriam, Giuseppe Cozzi, Nunzio Dipierro, Simona L. Bavaro, Antonio F. Logrieco, and Costantino Paciolla. "Decontamination of Fumonisin B_1 in maize grain by Pleurotos eryngii and antioxidant enzymes." Phytopathologia Mediterranea (2017): 134-145.		
No	Hanafiah et al.	2019	Performance of wild-Serbian Ganoderma lucidum mycelium in treating synthetic sewage loading using batch bioreactor	Scientific Reports	Hanafiah, Zarimah Mohd, Wan Hanna Melini Wan Mohtar, Hassimi Abu Hasan, Henriette Stokbro Jensen, Anita Klaus, and Wan Abd Al Qadr Imad Wan. "Performance of wild-Serbian Ganoderma lucidum mycelium in treating synthetic sewage loading using batch bioreactor." Scientific reports 9, no. 1 (2019): 1-12.		X
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No	Kumar et al.	2021	Genome sequence analysis of deep sea Aspergillus sydowii BOBA1 and effect of high pressure on biodegradation of spent engine oil	Scientific Reports	Kumar, A. Ganesh, D. Manisha, K. Sujitha, D. Magesh Peter, R. Kirubagaran, and G. Dharani. "Genome sequence analysis of deep sea Aspergillus sydowii BOBA1 and effect of high pressure on biodegradation of spent engine oil." Scientific reports 11, no. 1 (2021): 1-19.		
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No	Kumar, V., and Dwivedi, S.K.	2021	Mycoremediation of heavy metals: processes, mechanisms, and affecting factors	Environmental Science and Pollution Research	Kumar, Vinay, and Shiv Kumar Dwivedi. "Mycoremediation of heavy metals: processes, mechanisms, and affecting factors." Environmental Science and Pollution Research (2021): 1-38.		
No	Lalitha et al.	2019	Usage of Pleurotus ostreatus for Degradation of Oxytetracycline in Varying Water Salinities in Brackishwater Aquaculture System	Journal of Coastal Research	Lalitha, Natarajan, Prasanna Kumar Patil, Rameshbabu Rajesh, and Moturi Muralidhar. "Usage of Pleurotus ostreatus for Degradation of Oxytetracycline in Varying Water Salinities in Brackishwater Aquaculture System." Journal of Coastal Research 86, no. SI (2019): 138-141.		
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No	Molla, A.H., and Fakhru'l-Razi, A.	2012	Mycoremediation—a prospective environmental friendly technique of bioseparation and dewatering of domestic wastewater sludge	Environmental Science and Pollution Research	Molla, Abul Hossain, and Ahmadun Fakhru'l-Razi. "Mycoremediation—a prospective environmental friendly technique of bioseparation and dewatering of domestic wastewater sludge." Environmental Science and Pollution Research 19, no. 5 (2012): 1612-1619.		X
No	Novotný et al.	1999	Extracellular oxidative enzyme production and PAH removal in soil by exploratory mycelium of white rot fungi	Biodegradation	Novotný, Čeněk, Pavla Erbanová, Václav Šašek, Alena Kubátová, Tomáš Cajthaml, Elke Lang, Jürgen Krahl, and František Zadražil. "Extracellular oxidative enzyme production and PAH removal in soil by exploratory mycelium of white rot fungi." Biodegradation 10, no. 3 (1999): 159-168.		X
No	Olorunfemi et al.	2015	Toxicological Evaluation of Drinking Water Sources in Some Rural Communities in Southern Nigeria after Mycofiltration Treatment	Polish Journal of Environmental Studies	Olorunfemi, Daniel, Uruemu Efechuku, and Janice Esuana. "Toxicological Evaluation of Drinking Water Sources in Some Rural Communities in Southern Nigeria after Mycofiltration Treatment." Polish journal of environmental studies 24, no. 3 (2015).		X
No	Ormond et al.	2010	LID Meets Permaculture: Sustainable Stormwater Management in the Mountains of Western North Carolina	Low Impact Development 2010: Redefining Water in the City	Ormond, Timothy, Bailey Mundy, Mary Weber, and Zev Friedman. "LID Meets Permaculture: Sustainable Stormwater Management in the Mountains of Western North Carolina." In Low Impact Development 2010: Redefining Water in the City, pp. 935-948. 2010.		
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No	Prigionea et al.	2009	Chromium removal from a real tanning effluent by autochthonous and allochthonous fungi	Bioresource Technology	Prigione, Valeria, Mirco Zerlottin, Daniele Refosco, Valeria Tigini, Antonella Anastasi, and Giovanna Cristina Varese. "Chromium removal from a real tanning effluent by autochthonous and allochthonous fungi." Bioresource Technology 100, no. 11 (2009): 2770-2776.		
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No	Roshandel et al.	2021	Mycoremediation of oil contaminant by Pleurotus florida (P.Kumm) in liquid culture	Fungal Biology	Roshandel, Farzaneh, Sara Saadatmand, Alireza Iranbakhsh, and Zahra Oraghi Ardebili. "Mycoremediation of oil contaminant by Pleurotus florida (P. Kumm) in liquid culture." Fungal Biology (2021).		
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No	Singh et al.	2015	Soil fungi for mycoremediation of arsenic pollution in agriculture soils	Journal of Applied Microbiology	Singh, M., P. K. Srivastava, P. C. Verma, R. N. Kharwar, N. Singh, and R. D. Tripathi. "Soil fungi for mycoremediation of arsenic pollution in agriculture soils." Journal of applied microbiology 119, no. 5 (2015): 1278-1290.		
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No	Vijayaraghavan, K., and Balasubramanian, R.	2015	Is biosorption suitable for decontamination of metal-bearing wastewaters? A critical review on the state-of-the-art of biosorption processes and future directions	Journal of Environmental Management	Vijayaraghavan, K., and R. Balasubramanian. "Is biosorption suitable for decontamination of metal-bearing wastewaters? A critical review on the state-of-the-art of biosorption processes and future directions." Journal of environmental management 160 (2015): 283-296.		
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No	Young et al.	2015	Degradation of Bunker C Fuel Oil by White-Rot Fungi in Sawdust Cultures Suggests Potential Applications in Bioremediation	PLoS One	Young, Darcy, James Rice, Rachael Martin, Erika Lindquist, Anna Lipzen, Igor Grigoriev, and David Hibbett. "Degradation of bunker C fuel oil by white-rot fungi in sawdust cultures suggests potential applications in bioremediation." PloS one 10, no. 6 (2015): e0130381.		
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Not Accessible	Bolan, N.S.	1991	A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants	Plant and Soil	Bolan, N. S. "A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants." Plant and soil 134, no. 2 (1991): 189-207.		
Not Accessible	Pinedo-Rivilla et al.	2009	Pollutants Biodegradation by Fungi	Current Organic Chemistry	Pinedo-Rivilla, Cristina, J. Aleu, and I. G. Collado. "Pollutants biodegradation by fungi." Current organic chemistry 13, no. 12 (2009): 1194-1214.		
Not Accessible	Shivalkar et al.	2021	Bioremediation for Environmental Sustainability - Chapter 1: Bioremediation: a potential ecological tool for waste management	Bioremediation for Environmental Sustainability	Shivalkar, Saurabh, Vishal Singh, Amaresh Kumar Sahoo, Sintu Kumar Samanta, and Pavan Kumar Gautam. "Bioremediation: a potential ecological tool for waste management." In Bioremediation for Environmental Sustainability, pp. 1-21. Elsevier, 2021.		
Not Accessible	Taylor, A.	2014	The Effects of Fungal Cultivation on Bacteria Removal in Stormwater Biofiltration Systems	Presentation	Taylor, Alex. "The Effects of Fungal Cultivation on Bacteria Removal in Stormwater Biofiltration Systems." (2014).		X
Book	Anastasi et al.	2013	The Bioremediation Potential of Different Ecophysiological Groups of Fungi	Fungi as Bioremediatiors	Anastasi, Antonella, Valeria Tigini, and Giovanna Cristina Varese. "The bioremediation potential of different ecophysiological groups of fungi." In Fungi as bioremediators, pp. 29-49. Springer, Berlin, Heidelberg, 2013.		
Book	Bosco, F. and Mollea, C.	2019	Mycoremediation in Soil	Environmental Chemistry Recent Pollution Control Approaches	Bosco, Francesca, and Chiara Mollea. "Mycoremediation in soil." Environ. Chem. Recent Pollut. Control. Approaches (2019).		
Book	Gadd, G.M.	2001	Fungi in bioremedation	Fungi in Bioremediation	Gadd, Geoffrey M., and Geoffrey M. Gadd, eds. Fungi in bioremediation. No. 23. Cambridge University Press, 2001.		
Book	Mohapatra et al.	2018	Bioremediation of Insecticides by White-Rot Fungi and Its Environmental Relevance	Mycoremediation and Environmental Sustainability	Mohapatra, Debasish, Sakti Kanta Rath, and Pradipta Kumar Mohapatra. "Bioremediation of insecticides by white-rot fungi and its environmental relevance." In Mycoremediation and Environmental Sustainability, pp. 181-212. Springer, Cham, 2018.		

Book	Purchase, D.	2016	Fungal Applications in Sustainable Environmental Biotechnology		Purchase, Diane, ed. Fungal applications in sustainable environmental biotechnology. Springer, 2016.		
Book	Singh, H.	2006	Mycoremediation: Fungal Bioremediation		Singh, Harbhajan. Mycoremediation: fungal bioremediation. John Wiley & Sons, 2006.		
Book	Stamets, P.	2005	Mycelium running: how mushrooms can help save the world		Stamets, Paul. Mycelium running: how mushrooms can help save the world. Random House Digital, Inc., 2005.		

7.1.3 Appendix A:
Field Studies Table

Study Name, Year	**Location**	**Researchers/ Stakeholders**	**True Field or Quasi-Field/ Lab**	**Study Duration**	**Saprophytic Fungus Species Tested**	**Mycorrhizal Fungus Species Tested**	**Planted (Y/N)**	**Biofilter or Mycofilter Bag**	**Substrate(s) Tested**	**Contaminates Tested**	**Results**	**Shortcomings/ Design Takeaways**	**Replications**
Taylor et al., 2014	Seattle, WA	WSU, Fungi Perfecti, USFWS, Earth Resource Technologies (under contract from NOAA & NMFS)	Quasi-Field/Lab	15 months	*Stropharia rugoso-annulata*	N/A	Yes	Biofilter (Bioretention mesocosm)	Alder woodchips (3") over bioremediation on soil mix (40% compost, 60% sand)	fecal coliform, e. coli, pH, TSS, BOD, TOC, DOC, Alkalinity, Ammonia/Ammonium, Total Nitrogen, Nitrate, Nitrite, Ortho-Phosphate, Total Phosphorous, Total and Dissolved Metals, 18 PAH congeners	Wood mulch – slow release of total/ortho-phosphorous. Net loss of phosphorous and nitrogen (leaching from compost) – less over time. Bioretention cells with fungi and plants leached less. Total metals went from net export to net retention.	Need to account for at least a year of phosphorus and nitrogen leaching. Bioretention installations that endure extended dry periods might benefit from a more compacted soil	12 bioretention mesocosms (3 each: soil, plants, fungi, plants+fungi) | 5 quarterly sampling events, influent samples taken in triplicate, 20 min effluent collection | influent and effluent collection
Thomas et al., 2009	Lower Dungeness Watershed, WA	Battelle, Jamestown S'Klallam Tribe, for EPA/DOE programs/ agreements	Field	6 months	*Pleurotus ostreatus,* *Pleurotus ulmarius,* *Stropharia rugoso-annulata*	Down to Earth Soluble Mycorrhizae	Yes	Biofilter	Alder woodchips over sandy loam and compost	fecal coliform, total nitrogen, total phosphorous	TN was removed after soil was saturated with water below the drain creating an anaerobic zone – anoxic zones can be used for denitrification. TP leached the entire time – treatment cell did better in spike event	Soil media with low P-index will enhance P-uptake in soils rather than export. Design features should include soil media with high organic content and features that allow for submerged or anaerobic zones to allow for denitrification to take place	Two biofilters - one control, one experimental. A) samples collected on monthly basis | B) samples taken at regular hourly intervals and periodic weekly intervals | C) coliform samples
Winfrey et a., 2017	Perth, Melbourne and Sydney Australia	UCLA, Monash University	Field	N/A	N/A	N/A	Yes	Biofilter	N/A	N/A	Study determined 17% of existing biofilter plants had naturally occurring mycorrhizal associations	Look to existing mycorrhizal association of plants. Engineered soil may be devoid of natural soil microorganisms. Wetland plant species develop mycorrhizal associations in dry conditions to a greater extent than in wet conditions (Rickerl et al., 1994). Australian guidelines for biofilter media suggest using low nutrient content media (FAWB, 2008)	32 existing biofilters across three cities were evaluated.

7.1.3 Appendix A: Lab Studies Table

Study Name, Year	Location	Researchers/ Stakeholders	True Field or Quasi-Field/ Lab	Study Duration	Saprophytic Fungus Species Tested	Mycorrhizal Fungus Species Tested	Planted (Y/N)	Biofilter or Mycofilter Bag	Substrate(s) Tested	Results	Shortcomings/ Design Takeaways	Replications
Benedict, 2011	Olympia WA	Evergreen State College	Lab	Unknown	*Pleurotus ostreatus*	N/A	N	—	Woodchips	Woodchips alone - 12% reduction, Sterilized woodchips & Mycelium - 63% reduction, unsterilized woodchips & mycelium - 87% reduction	Sterilization removes some beneficial microbes which need to be preserved or replaced.	
Taylor, 2015	Pullman, WA	Fungi Perfecti, Washington State University	Lab	30 minutes	*Stropharia rugosoannulata*	N/A	N	—	Alder Woodchips vs. Woodchip/Straw Mix (75:25)	Woodchips and mycelium - 20% reduction over control. Woodchip/straw mix had a net export of bacteria w/ or w/o mycelium	Short test: Only 30min	5 groups of 3 (vigor tested mycelium (VTM) on woodchips, non-vigor tested mycelium (NVTM) on woodchips, VTM on woodchip/straw mix, NVTM on woodchip/straw mix, woodchips alone.)
Stamets et al., 2013	Pullman, WA	Fungi Perfecti, Washington State University, USEPA	Lab	30 minutes to 3.5 hours	*Laetiporus spp.* *Fomitopsis spp.* *Pleurotus spp.* *Pholiota spp.* *Stropharia spp.* *Irpex spp.*	N/A	N	—	woodchips: saw-dust: straw mixes (A=100:0:0), (B=50:50:0), (C=25:25:50), (D=50:25:25), (E=25:50:25)	*Stropharia spp*. Was most resilient to saturation, drying, heating, and freezing and removed freely suspended E. coli from water (~20% reduction with 50:50:0 media). Other fungi did not recover as well following the resiliency testing. Pleurotus removed sediment bound E. coli up to 100% compared to control average of ~40%.	Short test: Only 30min or 3.5 hours. Straw led to leaching of bacteria. *Stropharia* the most resilient fungi was tested for bacteria removal. But the fairly resilient Pholita wasn't, and then not very resilient *Pleurotus* sp. was for sediment? Resilience of *Irpex ssp.* not discussed. Testing mix up w/ *Pleurotus* - control w/ woodchips vs. experimental containing straw.	30 batches of mycofilters (6 diff. species and 5 diff. substrates), 17 mycofilters per batch (14 inoculated, 4 control) - 510 mycofilters total. 19 batches proceeded to resiliency testing. 8 other batches of mycofilters also cultivated. 10 mycofilters were tested for permeability. Bacteria removal tests were replicated 3 times.
Martinez, 2016	Albuquerque, NM	University of New Mexico	Lab	20 minutes	*Pleurotus ostreatus*	N/A	N	—	Burlap sack with barley straw	Mycofilters removed average of 98% of E. coli. Mucus like substance grew from mycelium (*Basidiomycota*) - like mesh/net in water, but solid when removed.	Mimicked floating mycofilter in permanently wet detention pond. Not fully applicable (25% of retention basin surface covered).	twelve reactors - 3 empty controls, three mycofilters w/o mycelium, three mycofilters w/ mycelium, three mycofilters w/ mycelium but not dosed with synthetic stormwater

Pini and Geddes, 2020	Chicago, IL	Northeastern Illinois University, Fungi Perfecti	Lab	96 hours	*Pleurotus ostreatus*	N/A	N	—	Wheat straw	Mycelia treatment removed over 99% of E. coli from synthetic and river water samples. Mycelium must inoculate media for a period of time prior to testing for effective results - 3 weeks best tested here.	Fungi Perfecti provided mycelium. Straw released bacteria and nutrients feeding E. coli and adding to thermotolerant coliforms bacteria count.	
Harris, 2012	Newark, DE	University of Delaware, Phillip's Mushroom Farms, Delaware Water Resource Center	Lab	N/A	*Pleurotus ostreatus*	N/A	—	—	Foam Cubes with malt broth	Biocell reactors containing live compost saw a reduction in E. coli levels after the first 12 hours. Biocell reactors with dead compost saw exponential increase in E. coli levels.	Mycelium not well established enough prior to testing. Stock solution unable to provide consistent concentration of E. coli into reactor over course of experiment.	Not 100% clear - this doesn't make sense: 6 reactors - 3 underwent Treatment 1 & 3 (T1 & T3), 3 underwent Treatment 2 & 4 (T2& T4). T1 - spend mushroom compost, T2 - autoclaved mushroom compost, T3- sterile water, T4 - simulated wastewater
Poor et al., 2018; Poor and Kube, 2019	Portland, OR	University of Portland	Lab	6 months	N/A	MycoApply Endo/Ecto and MycoApply Ultrafine Endo	Y	—	Bioremediation Soil Mix (40% compost), Earthlite Bioswale ES Soil (Compost, biochar, soil microbes)	Plant root networks inoculated with mycorrhizae more extensive, leaching initially halved in inoculated columns, but halfway through reversed and leached more than control. Proprietary soil leached only nitrogen. In first 5 tests: inoculated system had Copper lower than control but higher then proprietary, zinc about the same between all, less ammonia exported from proprietary and more nitrate (bacteria), Total Phosphorous and phosphate lower for proprietary much less than control and other.	Soil type matters and potentially so does other microbial populations. 4 month drying period halfway through sampling may have impacted efficacy of fungi to retain nutrients and metals. Samples taken every 9 days otherwise.	3 control City of Portland BSM, 3 control - inoculated, 3 Earthlite Bioswale ES Soil

Köhl et al., 2016	Zurich, CH	Agroscope, CH Utrecht University, NL University of Zurich, CH	Lab	3 hrs - leachate collection	N/A	*Claroideoglomus claroideum*, *Rhizoglomus irregulare,* and *Funneliformis mosseae*	Y	Mycofilter/ Microcosm	Sterilized Grassland soil from field	Nutrient leaching is influenced by AM fungal species and host plant/AM fungal species combination. AM fungal species differ in their effect on nutrient leaching. Both plant systems saw reduced nitrate leaching with AM fungal inoculation compared to non-mycorrhizal control. NO3- leached 292x higher in the *Trifolium* sp. control than the *Lolium* sp. control, and 14x higher when AM fungi were present. Clover abundance has been shown to positively correlate with nitrogen leaching, and grass systems usually have high N efficiency and lower nitrogen losses via leaching. Some AM species such as *Lolium multiflorum* and other grasses are known to be colonized by AM fungi even though their biomass doesn't respond strongly to the fungi.	Can't compare the two plant species results against each other due to different fertilizer values and soil volume. Was there enough time for fertilizer to be engaged by plant and rhizosphere prior to leach test with only 48hrs? Soil thought to have strong Phosphorus-fixing ability, and so reduced amount found in leachate. Benefits of AM fungi dependent on biotic and abiotic factors such as host plant identity, soil type, fertilization treatment, inoculum identity, and soil nitrogen and phosphorous	8 *Trifolium sp.* replicates and 10 *Lolium* sp. replicates (for each fungal inoculation and control?)

7.2 Appendix B: Case Studies Table

Book/Web Published

Source Type	Study Name, Year	Location	Researchers/ Stakeholders	True Field or Quasi-Field/ Lab	Study Duration	Saprophytic Fungus Species Tested	Mycorrhizal Fungus Species Tested	Planted (Y/N)	Biofilter or Mycofilter Bag	Substrate(s) Tested	Contaminates Tested	Results	Shortcomings/ Design Takeaways	Replications
Technical Report	Adams et al., 2007	Grafton, MA	John Todd Ecological Design, INC	Field	12 months	*Pleurotus ostreatus* and *Trametes versicolor*	N/A	No	Biofilter (Terrestrial Trickle-Filter System)	Unknown	TOC, COD, TSS, TPH	Very successful	Terrestrial system focused on petroleum and PAH decomposition	Two fungal strains. No control. Just measuring influent and effluent concentrations
Health Department Meeting Notes	Kenny, 2008	Ecler Road creek, Mason County, WA	Mason County Public Health Department with Paul Stamets, Fungi Perfecti LLC	Field	1 month	Unknown	N/A	No	Mycofilter Bag?	Unknown	fecal coliform	Potentially promising results showing reduction in fecal coliform bacteria	Short duration of 8 days of sampling over 1 month. Data and results presented are unclear. No mention of substrate and species used. Doesn't include map of experiment and sampling sites. Burlap sacks can break or blow out and block sediment. Hemp sacks or burlap sacks with thicker threading may ameliorate some issues.	Pilot project in creek with upstream and downstream monitoring.

Masters Thesis	Melville, 2016	Portland, OR	Portland State University, Portland Community College, Sunmark Environmental, PermaMatrix Inc.	Field	4 months	N/A	PermaMatrix BSP Foundation	Yes	Biofilter	PermaMatrix BSP Foundation and Earthlite stormwater filter media over existing soil	zinc, copper, phosphorous	Experimental biofilters had reductions in all contaminates by end of study while controls all saw increase in contaminates. Nitrates and ammonia appeared to be reduced as well by mycorrhizal treatment. Oyster mushrooms fruited between September and December, but mycellium grew from march to November in the field.	Only three days of samples taken over two-month period. Only one control and one experimental.	One experimental biofilter and one control
Case Study in Masters Thesis	Stamets, 2011	Tatoosh Island Neah Bay, WA	Paul Stamets, USDA, Makah Tribe	Field	N/A	*Maramiellus candidus*	N/A	No	N/A	Native Salmonberry canes	Coal, Petrochemicals	Discovered fungi native to island capable of remediation and local growing substrate	—	—
Case Study in Masters Thesis	Stamets, n.d.	Pat Labine's Farm	Paul Stamets/Fungi Perfecti, Washington State Department of Natural Resources,	Field	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Web Blog	Stamets, 2003	Tahuya State Forest Reclamation Site	Paul Stamets/Fungi Perfecti, Washington State Department of Natural Resources,	Field	3 years(?)	*Pleurotus ostreatus*	MycoGrow	Yes	Biofilter	"Hog Fuel" - 3"-12" crude mix of bark, woodchips, and fir needles. Covered with wheat straw.	N/A - Erosion control	No testing, but mycelium at woodchip/gravel interface found to hold things together	No measurement of system effectiveness in preventing erosion into stream below.	—

7.3 Appendix C: Expert Interview Notes

Below are notes taken during interviews with researchers and experts in mycology and related fields. All available video recordings of the interviews have been supplied to MassDOT.

7.3.1. Interview 1: Dr. Mia Maltz

Interviewee: Dr. Mia Maltz
Title: UC President's Postdoctoral Fellow in the Division of Biomedical Sciences + Cofounder of CoRenewal
Institution: University of California, Riverside + CoRenewal (The Amazon Mycorenewal Project)
Phone: (951) 827-2491 Ext. 2489
Email: mia.maltz@ucr.edu
Meeting Date: 12/14/21

- Research:
  - Mia has a Meta-Analysis paper from 2015 in Restoration Ecology that cites a study at the Elwha Dam that used woodchips
    - Cook KL, Wallender WW, Bledscoe CS, Pasternack G, Upadhyaya SK (2011). Effects of native plant species, mycorrhizal inoculum, and mulch on restoration of reservoir sediment following dam removal, elwha river, Olympic peninsula, Washington. Restoration Ecology 19:251-260.
  - She recently received funding for a 3-year study (2022-2025)
  - Have a straw wattle maker – will be using straw wattles – soil/soil slurries – fungi from native systems
  - Interested in microbial communities
  - Woodchips – need more moisture than straw
  - Primary decomposers need more substrate
- Fungi Databases (native ranges):
  - Mycocosm.com – historical
  - Macrofungi consortium collection
  - Micro fungi consortium collection North American
  - Funga – MycoDB – Peat response to mycorrhizae
  - Mycoflora project – fundis.org
  - FunFun
  - Funguild – Which papers put fungi into which functional group
- People to contact:
  - Cathy Aime – mycologist & Herbaria Director @ Purdue University
  - David Hibbit – mycologist @ Clark University
  - CO-Renewal (non-profit)
    - Brendan O'Brian
    - Jacquelyn Burmeister (City of Worchester)

            - Nitrogen and Phosphorous, stormwater & water quality monitoring
    - Dianne Stevenson
    - Serita Frey – UNH – Funfun -fungal functional traits
    - Amy Zanne – DC
    - Peter Kennedy (UMN) – Funguild
    - NH Nguyen – Funguild
    - Bjorn Lindhal – Upsalla, Sweden
    - Inge Bodecker – Upsalla, Sweden
    - Jenny Talbot Bhatnager (BU) – Decomposers in Disguise
- Issue of nativeness/Invasive Species/Soils:
    - Following the Precautionary Principal (a philosophical idea that emphasizes caution, pausing and review before leaping into new innovations that may prove disastrous) is probably good idea – be wary of introducing non-native myco- species and the unintended effects that may follow.
        - Using a reference soil is preferable to using a commercial inoculant
    - Native soil and native plants support native pollinators and other wildlife
        - Even inoculate with native soils carries potential hazards – spreading pathogens around (e.g., oak death)
    - Complex communities (and local ones at that) are safer and have more traits to help mycelium survive
    - Two approaches to improving microbial communities: Bioaugmentation (add microbes) vs. biostimulation (add molasses – help grow indigenous strains)
    - How do you scale up in the field – what are constraints – co-metabolism (labile vs. recalcitrant species) – agents of community assembly
- Inoculated vs non-Inoculated
    - Labile carbon users (more simple species)
    - Single species approach
    - Successional (primary, secondary, tertiary, mycorrhizal)
- Nutrients:
    - Nitrogen:
        - Nitrogen is backbone of mycelial enzymes
        - Nitrogen is a limiting factor in decomposition
    - Phosphorous:
        - Arbuscular mycorrhizae – really respond to phosphorous
    - Saprophytic and mycorrhizal fungi – produce similar/same enzymes
        - Some saprophytic fungi grow around roots

7.3.2. Interview 2: Dr. Brandon Winfrey

Interviewee: Dr. Brandon Winfrey
Title: Lecturer in Water Engineering Department of Civil Engineering
Institution: Monash University
Phone: +61 3 990 55549
Email: Brandon.Winfrey@monash.edu
Meeting Date: 12/15/21

- Biofilter design and its relation to climate is regionally specific.
    - Rainfall patterns help determine sizing.
    - In Australia biofilter sizes must be equal to 2% of the impervious area.
    - Plants, hydrology, filter media are all factors.
    - Issue with wood mulch is that it can float away in a big storm – many Australian biofilters use rocks or gravel, or biofilters are mulched after plant establishment.
    - Wood needs to be supplied naturally to be sustainable. Hydrology needs to be designed so the lower part of the system remains moist and so fungi persist throughout droughts.
- Plants in Biofilters
    - Typically used are herbaceous native species that can withstand periods of drought and inundation
    - Roots of plants – important for filtration
    - Strategy for plant selection: Identify plant traits associated with pollutant removal, among other factors
    - It is important to use non-nitrogen fixing plants! Otherwise, could lead to an increase in nutrient leaching!
- Nutrients
    - Salt tolerant plants tend to be better at Nitrogen removal
    - Carbon is used for denitrification in the submerged zone.
    - Mycorrhizae extends rootzone enable greater access to nutrients – exchange nutrients with plants for carbon (carbohydrates)
    - Brandon sent a review paper which looked at the mechanisms which might improve performance when inoculating biofilters.
- Built examples of mycofiltration systems
    - Brandon was only aware of a few purpose-built mycofiltration systems
    - Mycofiltration systems will uptake some Phosphorous and Nitrogen
    - Mycelium transforms the rhizosphere – allows microbes to better deal with Phosphorous and Nitrogen
- Fungi and Contaminates
    - Does not know about fungi's tolerance to salt
- Fungi Nativeness – Commercial vs. Local fungi
    - In one column study looked at pollutant removal
        - Isolated spores from field site
            - Separated out mycelium from native soil
        - Compared plants inoculated with native mycelium against others inoculated with proprietary mycorrhizal mix (these did very well)
    - Spores from field sites (even native soil) still need to be assessed as they may be invasive.
    - Nursery growers are using commercial mycorrhizae mixes already – plants will be bringing these mycorrhizae with them anyway. Brandon's study used a proprietary mix used by nurseries in every state and territory of Australia.
    - For mycorrhizal fungal inoculation to become a BMP, we need scalable solutions

        - Compost tea – not scalable, stable, consistent
- People to contact:
    - Pat Kangas – University of Maryland Ecologist
    - Gregory H. Lefevre – University of Iowa
    - Allen Davis – University of Maryland - Guidelines in Prince George County Maryland

7.3.3. Interview 3: Dr. Cara Poor

Interviewee: Dr. Cara Poor
Title: Associate Professor Shiley School of Engineering
Institution: University of Portland
Phone: (503) 943 8743
Email: poor@up.edu
Meeting Date: 01/03/22

- Lack of Field Studies
    - Confirmed there are not many field applications testing mycofiltration.
- Research
    - Bioremediation soil and MycoApply were used in her study because that is what would likely be implemented in a field application in Portland.
        - Bioremediation soil (40% compost) and MycoApply to inoculate the soil and plants (prior to planting)
        - Consulted the City of Portland's Bioretention plant list for plant selection.
    - Shortcoming of her study: Results from latter half of experiment cannot be relied on - test columns were left for about 6 months in a greenhouse in winter – likely dried out (typically winter is wet in the pacific northwest, and summer droughts only last about 2 months).
    - Mycorrhizal inoculation did seem to enhance nitrogen and phosphorous uptake by the plants.
- Design takeaways:
    - Think about what can be feasibly constructed and maintained
    - After column studies maybe scale up to do planter-scale studies, and then field studies.
- Many Mycofiltration Design Questions remain
    - What quantities of inoculants to use?
    - Which types of fungi, etc.
- People to Contact
    - Allen Davis at the University of Maryland –bioretention and nutrient research, not necessarily Myco.
    - Greg Lefevre – has a paper on bark chips and saturated zone.

7.3.4. Interview 4: Dr. Sally Brown

Interviewee: Dr. Sally Brown
Title: Research Associate Professor School of Forest Resources

Institution: University of Washington
Phone: (206) 616-1299
Email: slb@u.washington.edu
Note: Debra Darby (Tetratech & Working on composting project for MassDOT) also participated in this call
Meeting Date: 01/20/22

- Research
    - Has studies different blends of compost for Green Stormwater Infrastructure
- Biofiltration
    - Goal for biofiltration along highways: stop flooding, capture sediment, and remove metals, nutrients, pathogens, and organic compounds from the water.
        - Compost can do more than woodchips to deal with these although woodchips will provide sediment retention and flow rate reduction
        - Not sure about benefits of mycorrhizae for treating these things – not mycologist
        - Compost is a living tool – may get natural mycelial inoculation overtime
        - Woodchips alone are not going to give you a stable plant community over the long term
- People to Contact:
    - Curtis Hinman (sp.) – Lead on bioretention soil mixtures – looked at all sorts of special ingredients. Compost works pretty well without any special things added.
    - Allen Davis – leader in the field. Has papers on long term bioretention studies & research on using spent water treatment residuals
    - Kale Kurtz – Seattle public utilities
        - Has experience with Seattle Sea streets program – Old installations with no water coming out of them
        - Deep compost knowledge (worked at soil test labs)
    - Texas and Minnesota DOTs – long term compost users
    - Texas POCs – Scott McCoy and Barry Cogburn
- Design Considerations:
    - What do we want out of immediate inoculation?
        - Inoculation vs. survival is not the same – need to understand if it's worth it.
    - Big fan of the KISS principal (Keep It Simple Stupid) – don't overengineer
    - We are looking to perform biomimicry with mycelium
    - Excess phosphorous in soil is a big deal – but you don't want a sand pit (won't look nice and support plants).
        - Tools for designing bioretention – phosphorous saturation index and phosphorous saturation ratio. Based on standard soil test MALIC3 (?) – two measures can product phosphorous solubility.
            - It is possible to bring down phosphorous solubility by using spent water treatment residuals (WTR) - iron and alum based. Sand WTR mix and compost on top – acts as glue

        - Stop particulate movement – reduce phosphorous movement
        - Contact time – Sally has found that is not as big a deal.
    - Tough to predict in field study based on lab studies is tough
    - Think about what we want these systems to look like. Think about maintenance – will a gardener be necessary – likely not a budget for this
    - Our project is relatively large scale – local stuff is going to work better than highly specialized materials (e.g., coconut coir)
    - MassDOT doesn't have their own composting operation
        - Leaf litter is the primary compost material for large scale mulch composting in MA
    - Soils on side of road are usually very compacted – closer in hardness to concrete than typical soils
    - Complex systems can rebound, and the different pieces can help each other.

7.3.5. Interview 5: Peter McCoy

Interviewee: Peter McCoy
Title: Founder of Mycologos
Institution: Mycologos
Phone: (206) 616-1299
Email: info@mycologos.world
Meeting Date: 01/27/22

- Previous DOT research into mycofiltration/mycoremediation
    - Peter McCoy worked with Washington DOT
    - Nebraska DOT looked at Wine Cap mushrooms (Stropharia rugosa-annulata) for fecal coliform / filtration technique
    - The DOT studies mainly focused on immediately adjacent roadside swales, which is likely the cause of their failure. McCoy said that settling ponds or spaces where mycelium stayed in contact with pollutants for longer periods of time would be much more feasible environments.
    - Not clear what were the takeaways/deliverables
- Biostimulation / Augmentation
    - Strains can be preconditioned in the lab (even native strains), or they can be stimulated (Biostimulation – amplification of native fungi) or augmented in the field
    - Sometimes strains need or do better with additional food sources (wheat straw)
- Mycoremediation potential
    - Heavy metals – mycelium (dead or alive) has structures to bind metals (2+ ions – mercury, cadmium, cesium) but not eliminate them
        - Easy to do in water, hard to do in soil
        - Naturally forms complexes with heavy metals on the surface of mycelium tissue using little features called 'siderophores', which are naturally used by mycelium to absorb iron and other +2 metals.

        Siderophores are small ‘high-affinity’ compounds produced by microbes to chelate iron.
    - Alkaline wash can allow for reuse (can fill up in an hour)
        - You can use a basic solution of baking soda to wash those metals off after they have been captured on the siderophores and capture/recycle the rinsed off metals
    - A lot of papers for mercury, cadmium, arsenic, cesium removal using this property
- Molecular degradation
    - Molecular degradation is a misnomer of filtration.
    - Different fungi have unique capabilities (e.g., those that can break down lignin can breakdown complex hydrocarbons)
    - Again, with hydrocarbons, they’re not ‘filtering’ per se. It’s actually an enzyme producing process that involves chemical reactions; there needs to be enough time for the fungi to essentially ferment it.
    - Need time for enzymes to act
    - Easier to do in soil, harder to do in water
        - It needs time to perform other functions; it doesn’t work in a running water system because it doesn’t have enough time.
    - Tough in cold weather
        - In a cold, roadside scenario, they haven’t found anything conclusive. It just comes down to their growing environment + what the mycelium needs/ likes. Roadside runoff involves a colder fluid with low dissolved oxygen and very little time. Fungi can’t do anything in this condition.
- Bacteria
    - Mycelium is a microscopic sieve.
    - Wine cap mushrooms (Stropharia rugosa-annulata) filter out bacteria
    - Might not actually be eating bacteria as previously thought – might be holding onto it?
    - Have been examples of it being re-released over time if its absorbed and trapped, and then that mycelium naturally dies and degrades, the E. Coli can be re-released.
    - Also, a maintenance intensive system.
- Phosphorous
    - AMF (Arbuscular Mycorrhizal Fungi) are into solubilizing Phosphorus from phosphate rock; AMF can increase that or mitigate. AMF are the Phosphorous movers and shakers of the soil
        - AMF could increase phosphorus uptake / associate with plants
    - Other Benefits
        - Look at paper on increasing salt tolerance using arbuscular mycorrhiza
        - AMF can decrease plant sensitivity to heavy metals
- Mycofiltration Durability
    - Burlap mycofiltration bags only have about a one-month lifespan before breaking and releasing contaminants; avoid use
- Propagating mycelium/fungi

    - Information in Peter's book Radical Mycology
    - Rodale Institute USDA has a PDF on low-cost on-farm AMF production
    - INVAM – West Virginia
        - Protocols for harvesting from soil/propagating
        - Global collection of AMF
- Areas for future research
    - Something that should be researched and tested further is actually using mycelium inoculated straw or similar structures to BIOSORB the contaminants, then move them to a facility (BIOREACTOR) that has ideal, controlled conditions for mycelium to break down pollutants. Need to capture chemical substrates + then treat those with fungi. If you could capture the roadside wastewater. Then treat it in a secondary environment (like a bioreactor).
    - The reason bioreactor or similar controlled environment is best, is because it gives more time, correct temperatures, and you can introduce more food for the fungi (cosubstrate). Need to slow down the water so it has time to do its thing (even in non-bioreactor situations, like settling ponds).
- Social engagement tips
    - Know your audience, if you've got people already excited about certain aspects of fungi, engage with those aspects (cultivation, foraging, lab work, etc.)
    - For more hands-on workshops something like King Stropharia/Wine Cap/Garden Giant is a great mushroom for workshops. It grows fast, it's really easy to cultivate, it has tons of benefits for soils everywhere, and people are drawn to it. Installs, gardens, etc. with.

7.3.6. Interview 6: Dr. Gregory LeFevre

Interviewee: Dr. Gregory LeFevre
Title: Associate Professor, Civil and Environmental Engineering
Institution: University of Iowa
Phone: (319) 335-5655
Email: gregory-lefevre@uiowa.edu
Meeting Date: 01/31/22

- Lab focuses on recalcitrant pollutants (trace Organics – hydrocarbons, pesticides, etc.)
    - Most general microbes can break down most pollutants
    - If we're going to introduce something into the environment it needs to do something special
    - Lab studies products and pathways or organic contaminants and their breakdown
- Gregory's PhD research
    - Organic contaminates leaching out of pavement seals
        - Only banned state by state / county by county
    - PAHs – coal tar based
- Best Practices/Resources/Design Guidelines

  - Stormwater for Smart Growth – Book (~10 years old)
  - State Stormwater Centers
  - Iowa Department of Natural Resources
  - ASCE National BMP Database
    - Treatment efficacy white paper
    - Maybe not design guidelines
  - States have their own design language
- Common well known – oyster
  - Versicolor (invasive in US need a lab permit) – Useful "lab rat"
- Woodchip bioreactor
  - Create a bunch of surface area
  - Allows more time to biologically decompose
  - Temperature sensitivity (low temp = less effect)
- Contacts
  - Jordie Wolfand - Paper on white rot fungi degrading recalcitrant pollution

7.3.7. Interview 7: Dr. Jenny Bhatnagar

Interviewee: Dr. Jenny Bhatnagar
Title: Assistant Professor of Biology
Institution: Boston University
Phone: (617) 353-6957
Email: jmbhat@bu.edu
Meeting Date: 02/14/22

- Other mycologists in Area/Country – absorption of molecules by fungi
- Using mycofiltration/mycoremediation
- Filters out of cellulous – out of mycelium & fungal cells – stuff in some infrastructure
- Woodrots – understated their biogeochemistry better
- Johnathan Schilling – University of Minnesota – biochemistry of Woodrot Fungi (bioremediation)
- University of Wisconsin Madison – woodrots (paper fungi)
- Dan Linder – knows taxonomy
- Cornell person….?
- Mass Myco Community – New England to New Jersey
- David Hibbett
- Woodrots – great, control their location well
- Jenny – ectomycorrhiza in urban systems
  - Boston trees – almost no mycorrhizae and no root mass
  - Working with David Moreno Mateos at GSD and his PhD candidate Katie (bioinformatics) looking at how to reestablish ectomycorrhizal fungi in city – leverage ectomycorrhizal fungi – planted trees in
  - Ecological network
- Bring in Rural soil
  - Working with someone at the Parks Department
- Lucy Guitiera – street tree soil expert

    - Possibly NY person
- Reference soil – or use some root tips
- Swillis fungi – good for establishment of young oaks and pines
    - Are there more resources on what trees/plants/fungi pair well together?
- Ectomycorrhizae can turn pathogenic when a plant is under stress
- Invasion of pine trees in southern hemisphere was driven by an invasion of fungi
- Jenny's lab would be interested in potentially partnering with MassDOT in the future
- When testing for what's underground, does that disturb or kill plants?
    - 6-9 cores of soil are taken at different depths
    - DNA of fungi in soil is sequenced
    - Minirhizotron visualizes hyphal growth underground
    - In-growth core can be placed before a tree is planted – limit roots and or ectomycorrhizae – just clean soil to start – pull out later.

7.3.8. Interview 8: Dr. Donald Pfister

Interviewee: Dr. Donald Pfister
Title: Asa Gray Research Professor of Systematic Botany
Institution: Harvard University
Phone: (617) 495-2368
Email: dpfister@oeb.harvard.edu
Meeting Date: 02/28/22

- Grasses maybe for phosphorous
    - Endomycorrhizal fungi – mining for phosphorous
    - Scale of who takes up the best
- Wastewater fungi literature
    - Fungi found on leach fields
- People to contact
    - Colleen Hansleman – Might be at Woods Hole?
        - Previously did manganese accumulation research on fungi with Donald Pfister
    - Cathy Aimes at Purdue – done a lot of work on yeasts (yeast physiology)
        - Microbial communities – full of yeast
    - David Hibbett – Ask about mycorrhizal host/plant relationship
    - David Moreno Mateos – GSD Ecologist
        - His PhD student is working on an interesting project
- Bioaccumulation – concerns over what is released after death – some fungi are good at accumulating metals – so much so harvesting plants can be bad.
- Mycorrhizal fungi can be invasive
    - Amanita muscaria is invasive in the global south
- Mycoportal – herbarium specimens
    - Can search by location, collection site, year
    - Useful in figuring out particular fungi in an area
    - Sometimes there's information on the fungi's association with a primary tree
- Don't use spores that come in packets

    - Donald had his students test spores in packets to identify species and they found NO spores
- Types of strains – survival is based on temperature/moisture/climate regimes
- Carbon source must be provided for mycorrhizae for establishment, but overfeeding them could cause the fungi to become parasitic
- Saprobes – live 1-3years, like mulch in garden, needs to be replenished
    - Annuals, perennials, trees
- Harvesting grass that mycoremediated high nutrient soils – hay harvesting meadow – could be monetized?

7.3.9. Interview 9: Dr. Serita Frey + Dr. Thomas Ballestro

Interviewee 1: Serita Frey (University of New Hampshire)
Title: Professor of Natural Resources and the Environment
Institution: University of New Hampshire
Phone: (603) 862-3880
Email: Serita.Frey@unh.edu

Interviewee 2: Dr. Thomas Ballestro (University of New Hampshire Stormwater Center)
Title: Associate Professor of Civil and Environmental Engineering, Director of UNH Stormwater Center
Institution: University of New Hampshire
Phone: (603) 862-1405
Email: Tom.Ballestero@unh.edu

Meeting Date: 02/28/22

- Serita Frey
    - Studies fungi physiology and biology
    - Doesn’t recommend inoculating anything – doesn’t believe it adds any value.
        - Local mycorrhizae would be outcompeted, if left alone local native mycorrhizae will eventually establish.
    - Time to decompose depends on the context of the substrate
        - High nutrient water – happens faster (decomposition is often limited by nitrogen and maybe phosphorous)
        - Heavy saturation or drought conditions – slow decomposition
        - Cold weather – Slow decomposition
        - Warm weather – faster decomposition
    - Phosphorous – sorption
    - Hydrocarbons – breakdown
- Thomas Ballestro
    - Not aware of anyone looking at fungi in stormwater filter systems – plan to start an experiment looking for fungal E-DNA that in Fall 2022.
    - Without removing dead vegetation from bioretention cell system ever 2-3 years the system starts to release nitrogen and phosphorous
    - Bioretention – aerobically

        - Don't like loose woodchips – float away, create clogs and blowouts
        - Triple shredded bark/wood mulch mixed in – doesn't foul/clog system – retains moisture and grow plants – 5-15% wetland soils
    - Want dissolved organic carbon in organic systems – food source for mycelium
        - More wood – fungi dominant
        - Less wood – microbial dominant especially if its anaerobic
    - Immobilize nutrients
        - Nitrogen immobilization
        - Highest C:N ratio possible (woodchips are good)
    - Systems don't always have anaerobic zone
        - Depends on whether phosphorous or nitrogen is a bigger issue
    - Woodchips may not be sourced from one area
        - Use topsoil where microbial communities
    - Feeding native fungi in soil – encourage growth on woodchips?
        - Promote colonization – use native soil and keep moist
    - N2O is an issue
        - How to deal with it?
        - Longer flow time
    - Plug-Flow reactor
    - 12-hour residence time (24-36hrs is better)
- Number 1 Goal – Stormwater Management – Treat stormwater
    - Mow Grass, Don't Tend Gardens
        - MASSDOT areas – not maintained
        - Not even raked out (2hrs per year) vs. 20hrs of mowing that occurs per year
- Grasses maintained permeability better then plants
- UNH stormwater maintenance guidelines – find study or ask Tom – N+P didn't rerelease
- UNH specs on website
    - Subsurface gravel soil
    - Bioretention soil
    - Spreadsheet – length of time for active WTR (water treatment residuals) – 30 years
    - Sphagnum peat
    - Wood Derivatives
    - Water Treatment Residuals
        - Plants can survive in there except when Zero Valent Iron is used as resulting pH makes it hard to grow. But this is the gold standard for water treatment residual effectiveness (also very expensive)
    - Alum sludge is spec
- UNH Bioretention Soil Spec (ballpark)
    - 60-80% sand
    - 10% Native topsoil
    - 5% or less fines (clays)
    - 3-15% Wood/Organic Matter
- Tom is interested in being a potential collaborator

    - Real time sessions
    - UV vs cleaning monthly
- Landscape Metrics - ASLA

7.3.10. Interview 10: Dr. David Hibbett

Interviewee: Dr. David Hibbett
Title: Professor of Biology
Institution: Clark University
Phone: (508) 793-7332
Email: dhibbett@clarku.edu
Meeting Date: 03/1/22

- Fisherville Project – White Rot Fungi example
    - Sterilization is cost and energy intensive
    - Mycelium spawn too is an expensive commodity
    - Scalability is a question in terms of effectiveness as well as cost. Climate change will cause stormwater runoff volumes to increase regionally
- Mushroom Harvesters
    - Spawn leftovers have active fungi in wheat/sawdust media
        - Waste material with promise – could be used for ecological restoration
    - Oak Sawdust is a good substrate for growing mycelia
    - Far Moon Mushrooms – Elizabeth Westford
        - Uses sawdust brick with oat bran
        - MycoTerra – Julia Coffey (worked with Paul Stamets)
            - Experimented with using spent spawns as soil additive
            - Log grown shitake
    - NorthSpore in Portland, ME - sells spawn
    - Fungi Perfecti – sells spawn
- Inoculation can be done on a benchtop with an autoclave
    - Endo (AM) fungi can't be cultivated independently – need to use a potted plant (Sorghum)
- Online Databases
    - Funga Association
    - FUNGUILD – Nutritional
    - Native Ranges
        - iNaturalist –photos are georeferenced
        - Mushroom Observer – photos are georeferenced
        - Gbif – global bioinformatics facility – Natural History/herbarium reported species globally
- Field guides
    - Mushrooms of NE North America – Tim Broney
    - Mushrooms of North America – Alan Baset
- Other Mycology Groups
    - Boston Mycological Survey

    - NAMA – North American Mycological Associations – Clubs by State (artists, foodies, naturalists)
- People to Contact
    - Mushroom Harvesters
        - Jean Burnette – owner of Fisherville Site
        - Owns an industrial composting business
        - Experienced with regulatory things
    - Joe Morton – West Virginia University
        - INVAM – Sorghum inoculation with different plants
- David is possibly interested in participating in future studies and working as a research advisor
- Fungal mycelium – knit together
- Review articles – fungus host associations, and Smith and Reed Mycorrhizal text book
- Saprotrophic fungi – may not remove nitrates
    - White rot fungi
- Good Research Project for Students
    - Match fungi to wood types/feedstocks
    - Native grasses with fibrous roots – check local fungi species
- Mycorrhizal fungi mixes – exotic commercial species
    - Spores can be filtered out from soil
- It's unknown how long it takes to establish AM fungi
- Conferences
    - Mycologist Society of America
        - Applied / non-applied
    - Northeast mycological foray – non-professional costs
    - Radical Mycology Conference
    - Fungal Genetics
    - ESA Conference

7.3.11. Interview 11: Jacquelyn Burmeister

Interviewee: Jacquelyn Burmeister
Title: Senior Environmental Analyst, Lakes and Ponds
Institution: City of Worcester DPW&P Water Operations Division
Phone: (508) 929-1300 Ext. 2126
Email: BurmeisterJ@worcesterma.gov
Meeting Date: 03/3/22

- Jacquelyn agrees from a from preliminary look into Myco that there's not enough data to add a specific BMP into their current grouping of BMPs
- Maintenance is a challenge for regular BMPs (mostly bioretention), Jaquelyn's department mostly uses a company called FocalPoint that make biofiltration units with underdrains (glorified raingardens). FocalPoint designs systems and oversee contractors' construction.

    - One was installed next to a large patch of knotweed, so that's going to be a management battle
    - Keeping tabs on microbial communities of fungi would add another layer, a more technical one than picking trash and weeds (is the microbial community healthy, does it need to be reinoculated?)
- A pre-inoculated soil or inoculated plant roots could be an easy solution. Topsoil is not something that's currently spec'd.
- Jacquelyn's department haven't yet had to manage the plants in the FocalPoint systems as they're only a few years old but will need to create a management plant (gardening related) to deal with these systems over time and as more come online
- Jacquelyn's department has ability to oversee research projects unlike the larger sewer department
- Her concern is the fragility of the system though, they have a lot of grey infrastructure – storm events are flashy and scouring is an issue (climate change is causing flooding events to become flashier). BMPs need to be scour-resistant (the FocalPoint systems seem to be)
- When space doesn't exist for FocalPoint systems, Jacquelyn's department creates upstream solutions and grey infrastructure like deep sump catch basins and hydrodynamic separators (grey infrastructure doesn't deal with contaminates dissolved in water)
- Jacquelyn is interested in collaborating with MassDOT, and a lab that deals with microbial communities to figure out what's existing in the City of Worcester's current infrastructure and possibly inoculating what's already out there. She would be happy to help with sample testing, providing a location for a design to be tested. The department has collaborated with WPI, UNH, Holy Cross, Clark University, and Worcester State University in the past.

7.3.12. Interview 12: Dr. Allen Davis

Interviewee: Dr. Allen Davis
Title: Professor in Civil and Environmental Engineering, Affiliate Professor in Plant Science and Landscape Architecture
Institution: University of Maryland
Phone: (301) 405-1958
Email: apdavis@umd.edu
Meeting Date: 03/8/22

- Media / Media Selection
    - Site specific
        - Competing Criteria: Low leaching vs. fines (metal sorption)
    - Allen Davis's typical soil spec for bioretention media is roughly:
        - 50% sand
        - 30% sandy topsoil
        - 20% triple shredded wood mulch
- Compost is not good for phosphorous capture

  - Biosolids & compost – leach years' worth of nutrients in weeks (agronomic loads)
- Woodchips isn't bad (holds nutrients and water retention)
  - Triple Shredded Mulch is best
- Start with media low in phosphorous and nitrogen
- Iron, aluminum, calcium-based minerals – all help phosphorous bind
- Drinking water treatment residuals can be used but NOT wastewater treatment residuals
  - Hydrous Al and Fe (aluminum and iron)
  - Sometimes there can be issues with arsenic – need to test for this prior to using
  - John Gulliver and Andy (??) – Zero Valence Iron
- Nitrogen
  - There is so much we still don't understand on the nitrogen side of things
  - There are biological transformations occurring
  - Organic nitrogen impacts are underestimated
    - Grab what you can during rainfall events and do what you can in-between events
- Upturned elbow in bioretention cell design is/should be best practice – there are no negatives to it
- Infiltration is good
- Look at Bill Heinz's work for best details
- Hard to do a mass balance with nitrogen – you assume any missing nitrogen denitrified (assume plants, N2, or NO)
  - A tighter soil (more silt particles/fines) may possibly provide an opportunity for more denitrification
- Plants
  - Heterogenous Plant Communities have advantages
    - Cut in Fall and compost
  - Plants don't need annual fertilizer treatment
- Some northern states say that bioretention soil with compost works better with road salt impacts.
- Internal Water Storage – More recent development for bioretention cells than what's in 2007 Prince Georges County guidelines.
- Best Guidelines for Bioretention Cells
  - North Carolina (Bill Heinz) – Best BMPs for Stormwater / Bioretention Cells
    - Update Sections year-to-year
- Minnesota – Might not be in regulations yet

Appendix D: Decision Matrix Sediment Control Barrier

Barrier Component (Per order subsection 675)	Dimensions	Lengths	Density	Normalized Life Expectancy	Efficiency*[1]	Reaction to High Flow Rates (movement)	Removal Required	Use Locations	Notes
Sedimentation Fence	36-inch height	100-foot roll	n/a	6–8 months	82.7%[2]	Poor	Yes	For turtle barrier in locations where fence is required by permit	not for use in high flow areas, frequent maintenance
Compost Filter Tube	12-inch diameter	10–100 feet	29–32 lbs./ft.[3]	1–1.5 years	92%	Good	project dependent	perimeter control of sediment; slope check; check dam; ditch check; storm drain and curb storm inlet protection; slope interruption practice used to reduce sheet flow velocities and prevent rill and gully erosion	preferred SCB for general purposes, compost performs as soil amendment after degradation, multi-purpose
Coir Log	12, 16, or 20 inches diameter	10–20 feet	9 lbs./cubic foot	3 years	85%	Excellent	project dependent	wetlands; stream edge, flow diversion; slope interruption practice used to reduce sheet flow velocities and prevent rill and gully erosion	For use where robust response is needed; is difficult to move; use in wet area where longevity is required or desired
Coir Wattle	6, 9, or 12 inches diameter	10–20 feet	7 lbs./cubic foot	2 years	85%	Good	project dependent	perimeter control of sediment; slope check; check dam; ditch check; CFT breach repair	alternate SCB for general purposes
Straw Bale	14 inches × 18 inches	36 inches	3.4 lbs./cubic foot	3 months to 1 year	85%	Fair/Good (slope dependent)	Yes	toe of slope; minor swales; drain inlets;	prone to rot and degradation; installation is relatively labor intensive; better alternative sediment control components are often available

1) Minnesota DOT Sediment Control Log Performance, Design, and Decision Matrix for Field Applications, May 2019.

*Efficiency based on sediment capture during flume test matching for all samples.

2) Performance Evaluations of Three Silt Fence Practices Using a Full-Scale Testing Apparatus (AlDOT used) 2017.

3) Federal Specifications for Compost Filter Socks for Sediment & Erosion Control, 2007/Filtrexx Design Manual Ver. 11.1.

7.5 Appendix E: Draft MassDOT Sediment Control Barrier Specification

ITEM 751.72 COMPOST TOPDRESSING SQUARE YARD

The work under this Item shall conform to the relevant provisions of Section 751 of the Standard Specifications and the following.

Work shall consist of furnishing and pneumatically applying compost as specified below. Compost shall be applied as a thin mulch blanket over prepared loam or prepared soil and shall be used in conjunction with seeding or planting unless specified otherwise. The intent of compost topdressing is to provide temporary soil stabilization and organic matter for plant growth.

For areas where compost is proposed with seeding, seed shall be broadcast and seeding shall occur in conjunction with compost topdressing, as specified under the relevant seeding item.

SUBMITTALS

Contractor shall submit to the Engineer samples and certified test results 60 days prior to application of compost. Test will be for compost, not a soil test, as specified below. Vender certification that material delivered meets the test results shall be submitted if requested.

No materials shall be delivered until the required submittals have been approved by the Engineer. Delivered materials shall match the approved samples. Approval of test results does not constitute final acceptance. The Engineer will reject any material that does not meet the Specifications.

MATERIALS

Compost may be a blended product of compost and fine wood chips.

Compost testing shall be by a laboratory approved by the US Compost Council using the Testing Method for the Examination of Compost and Composting (TMECC) protocols.

- Organic matter content shall be minimum 30 percent (dry weight basis).
- Moisture content shall be 35-55 percent (wet weight basis).
- pH shall be 6.5-8,5
- Conductivity shall be a maximum of 5 mmhos.
- Where soil is intended for vegetation (plants or grass), compost shall be tested for stability by CO2 evolution method and shall produce a maximum of 4mg CO2-C per gram of organic material per day. The product shall also be mature, meeting a minimum of 80% seed emergence and 80% seedling vigor.

Particle size shall not exceed 3/8 or ½ inch.

No kiln-dried wood, construction debris or ground palette is allowed.

The Engineer shall approve the Contractor's equipment for application.

CONSTRUCTION METHODS

Application of compost material shall not begin until the Engineer has approved the site and soil conditions. The Contractor shall notify the Engineer when areas are ready for inspection and application of compost.

Prior to application of compost, all areas to be topdressed shall have been graded to an even surface, and all debris and stones 2 inches or larger shall be removed. Surface preparation shall be compensated under applicable item for placement of loam, sand, ordinary borrow, topsoil rehandled and spread, or other specified substrate.

Compost topdressing shall be pneumatically applied (blown on) to a depth of one half to one inch unless specified otherwise on the plans.

For areas where compost is proposed with seeding, seed shall be broadcast and shall occur in conjunction with compost topdressing, as specified under the relevant seeding item.

METHOD OF MEASUREMENT AND BASIS OF PAYMENT

Item 751.72 will be measured and paid for at the Contract unit price per Square Yard which price shall include all labor, materials, equipment, and all incidental costs required to complete the work of pneumatically applying compost.

Surface preparation of substrate receiving compost topdressing shall be compensated under applicable item for placement of loam, sand, ordinary borrow, topsoil rehandled and spread, or other specified substrate.

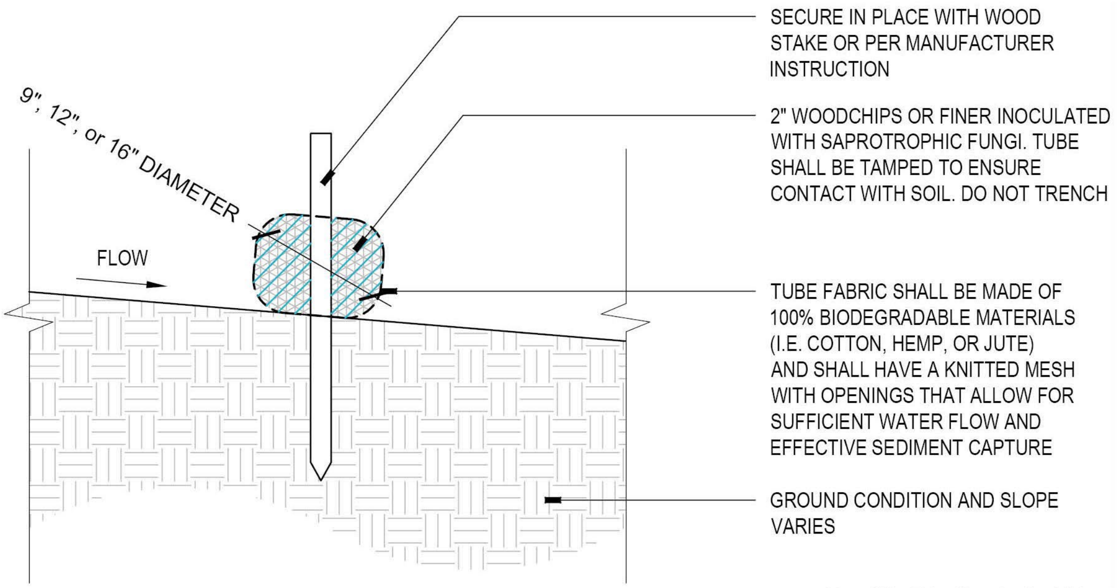
Seeding will be compensated for under the appropriate seeding item.

7.6 Appendix F: Conceptual Details

Conceptual CAD Details for each of the six Stormwater Control Measures (SCMs) with a mycofiltration layer included. These details are paired with images of their installation in the field. An AutoCAD file for these CAD details has been provided to MassDOT.

7.6.1. Temporary SCMs for Construction

MassDOT requires all sediment barriers to be made of biodegradable materials for a number of reasons, one being to minimize the release of microplastics into the environment.



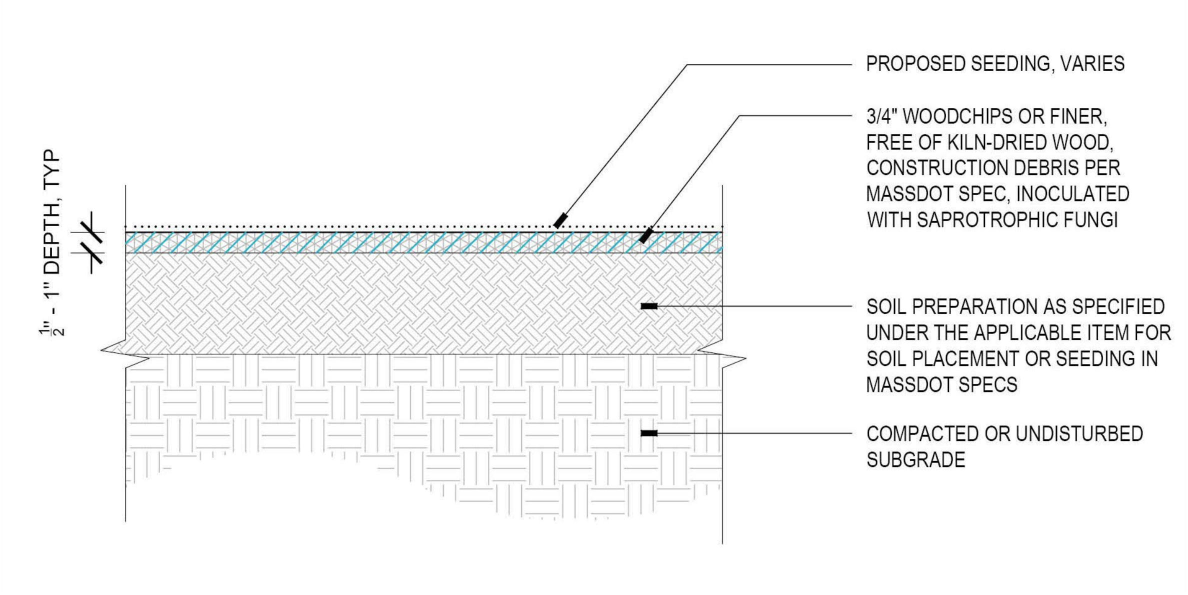
Myco Filter Tube (Drawing by Offshoots, 2022)

Note: MassDOT requires all sediment control barriers to be biodegradable.

Myco Filter Tube Application

Myco Filter Tube Application

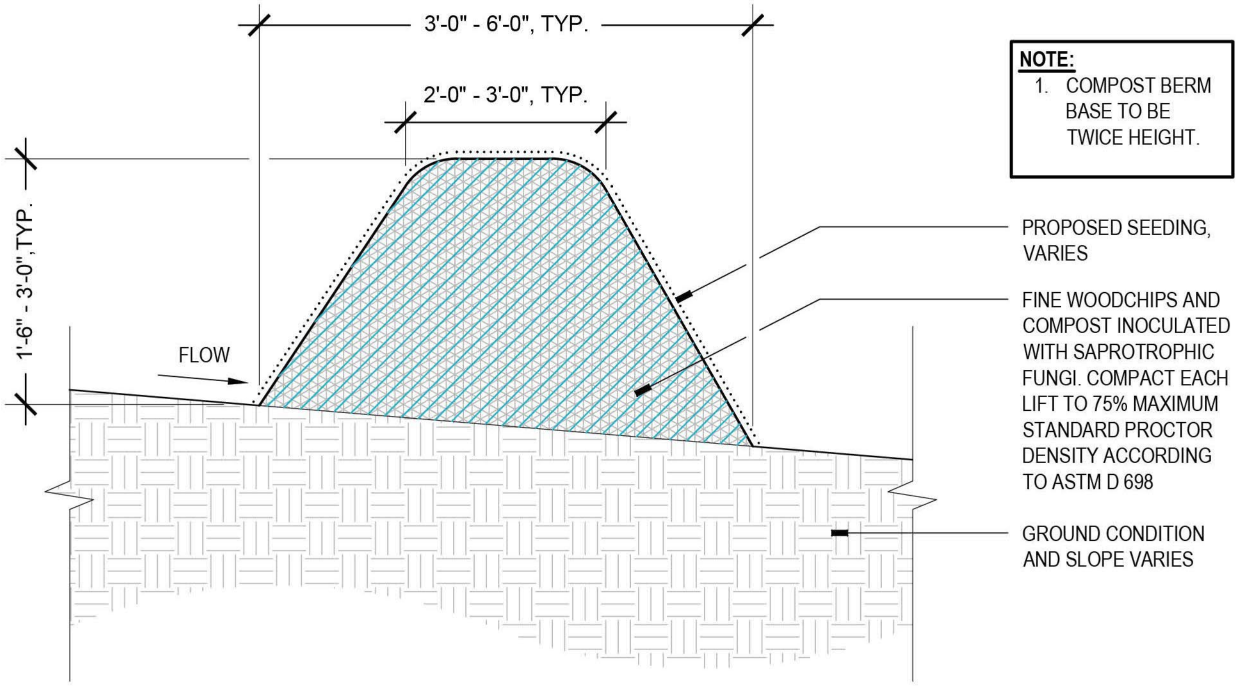
SCM 1: Myco Filter Tube



Myco Blanket (Drawing by Offshoots, 2022)

Myco Blanket Application

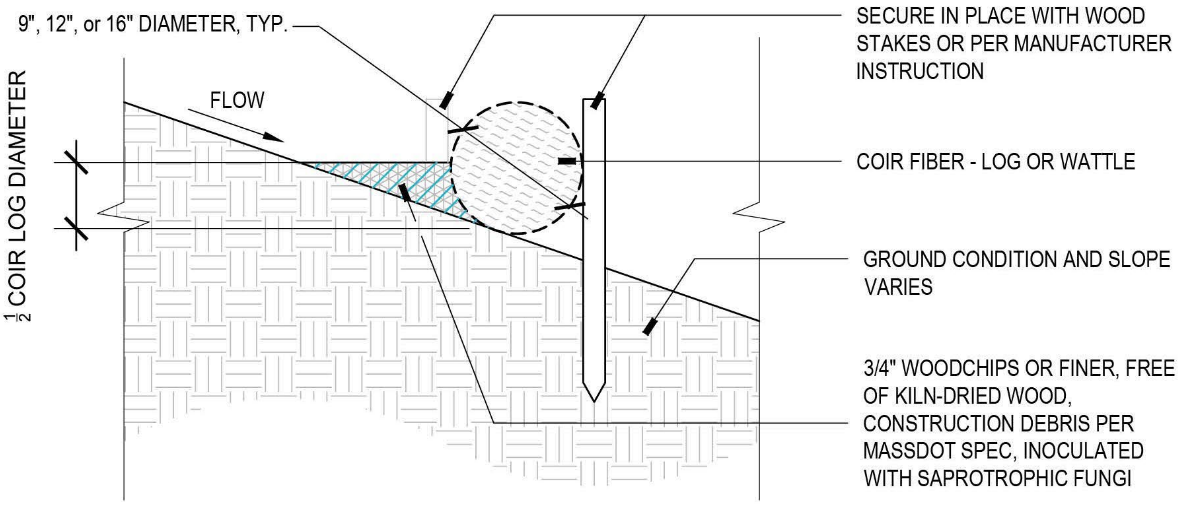
Myco Blanket Application



Myco Berm (Drawing by Offshoots, 2022)

Myco Berm Application

Myco Berm Application

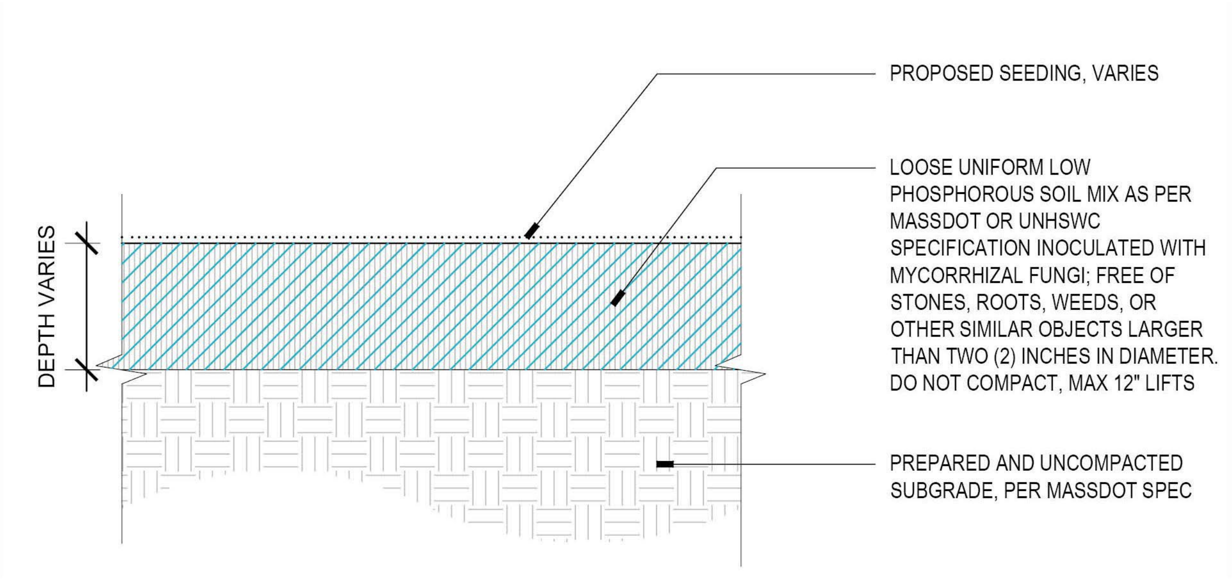


Myco Coir Log (Drawing by Offshoots, 2022)

Straw Wattles

Myco Coir Log Application

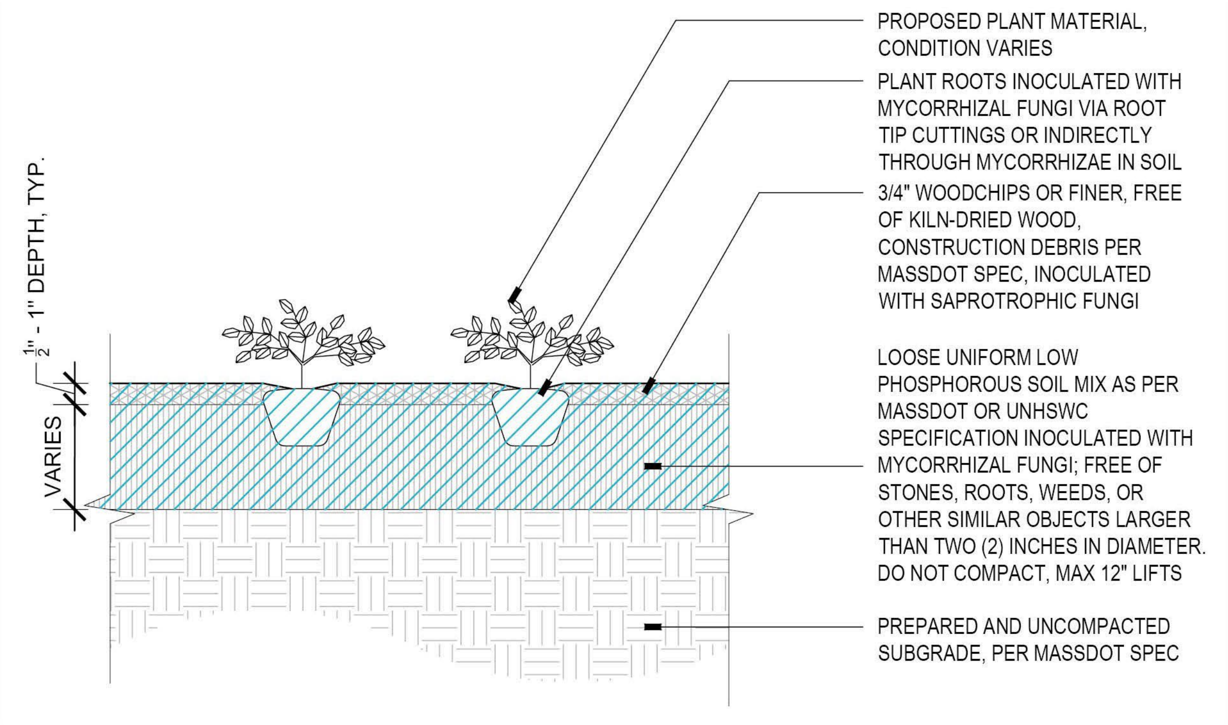
SCM 5: Myco Bioretention



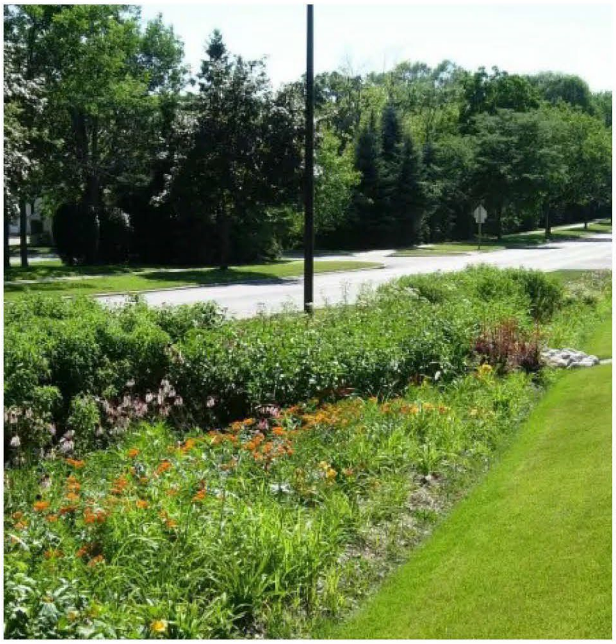
Myco Bioretention (Drawing by Offshoots, 2022)

Myco Bioretention Application

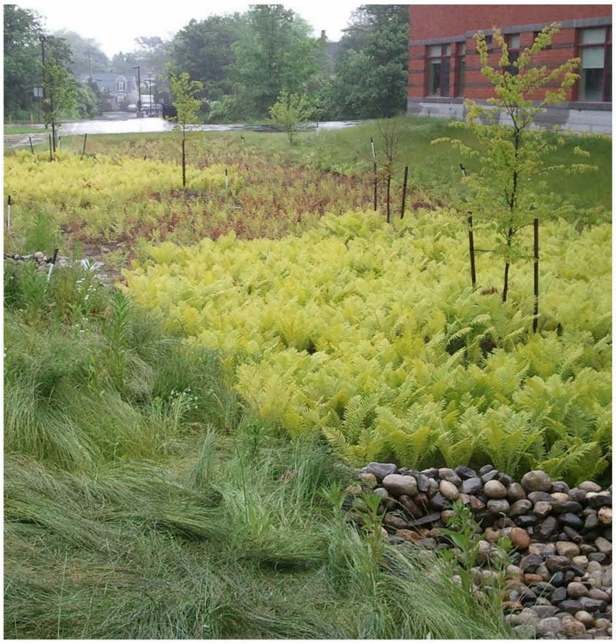
Myco Bioretention Application



Myco Bioretention+ (Drawing by Offshoots, 2022)



Myco Bioretention+ Application

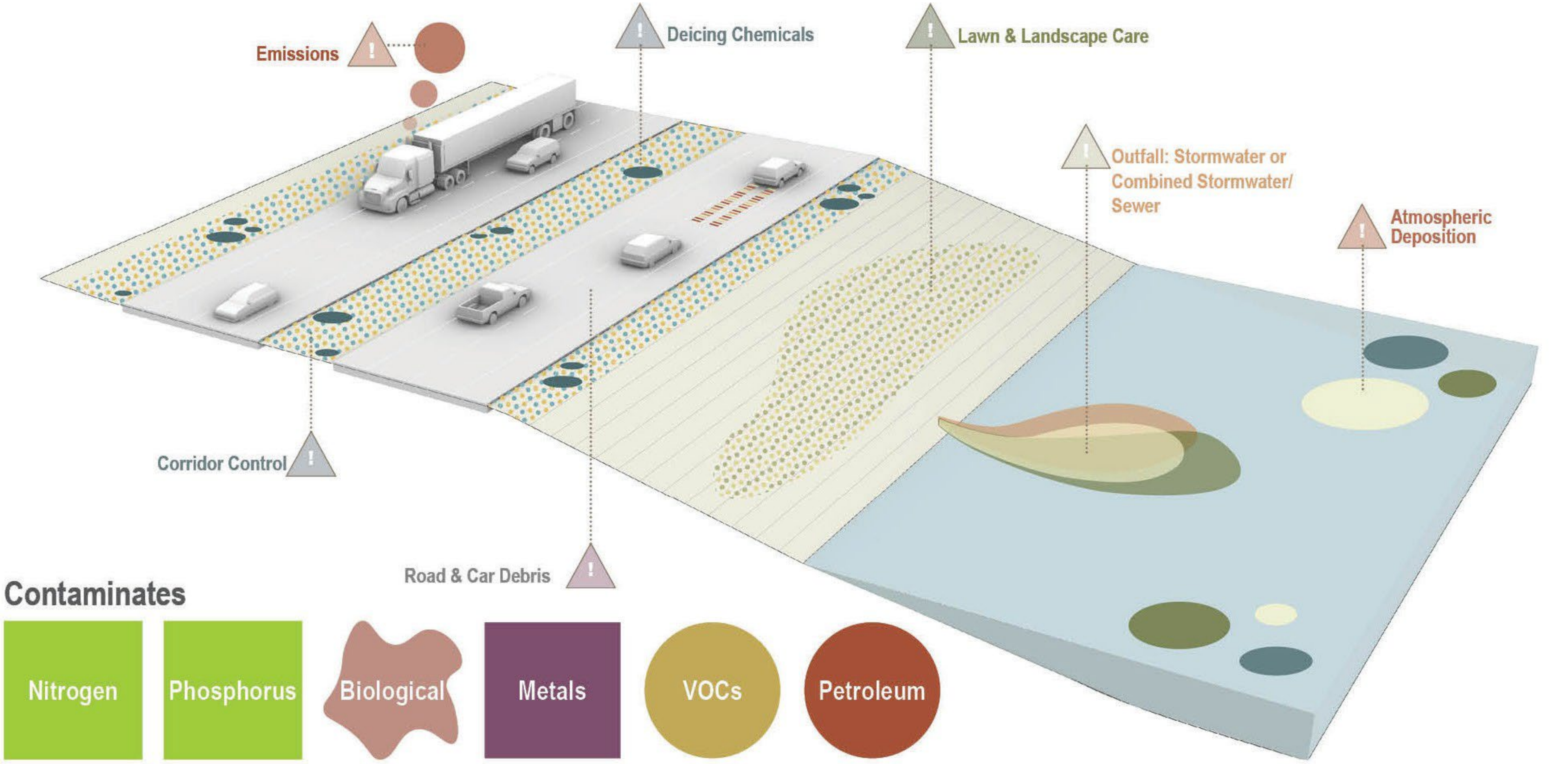


Myco Bioretention+ Application

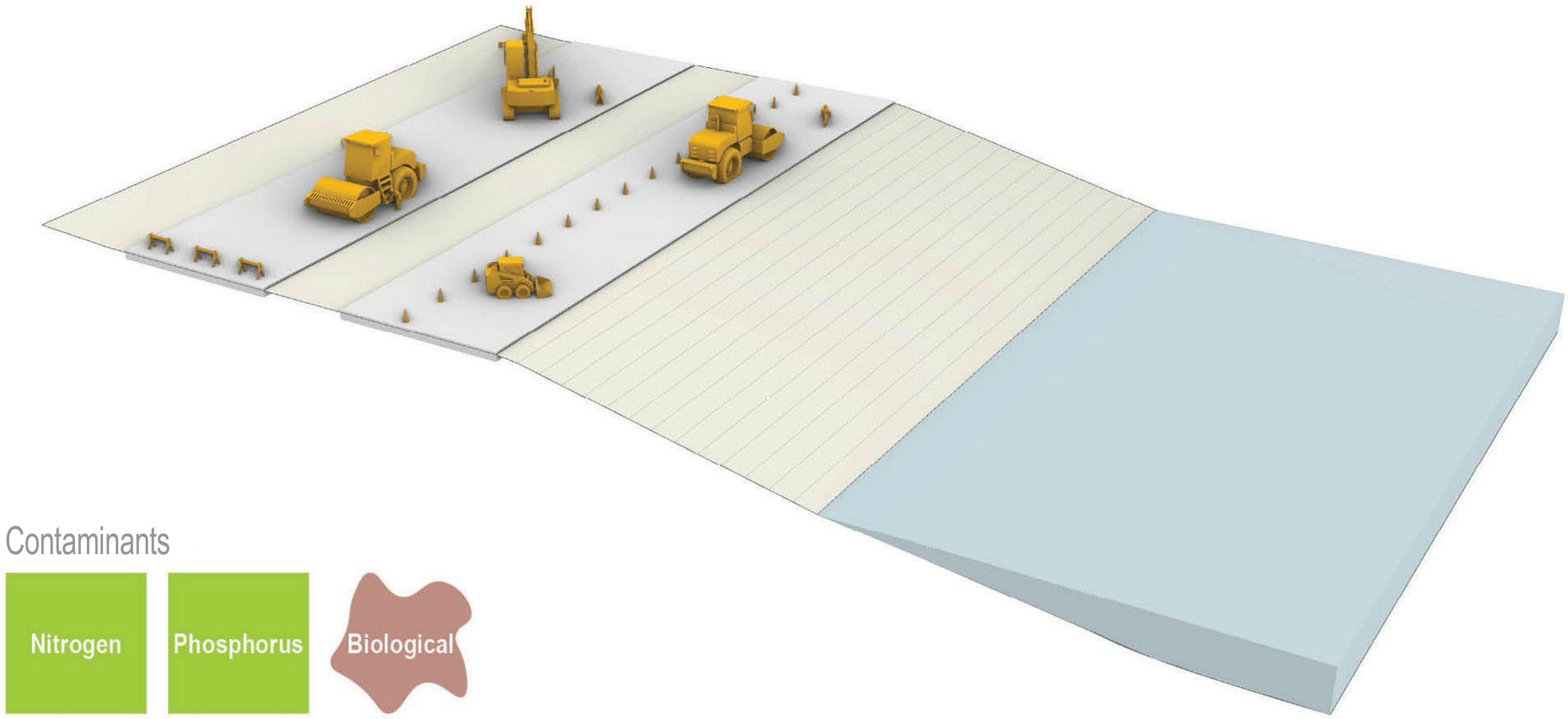
7.7 Appendix G: Stormwater Control Measure (SCM) Axonometric Diagrams

The following pages show each of the six SCMs implemented within a hypothetical site between a roadways and waterbody and the movement of pollutants across the site from point and non-point sources.

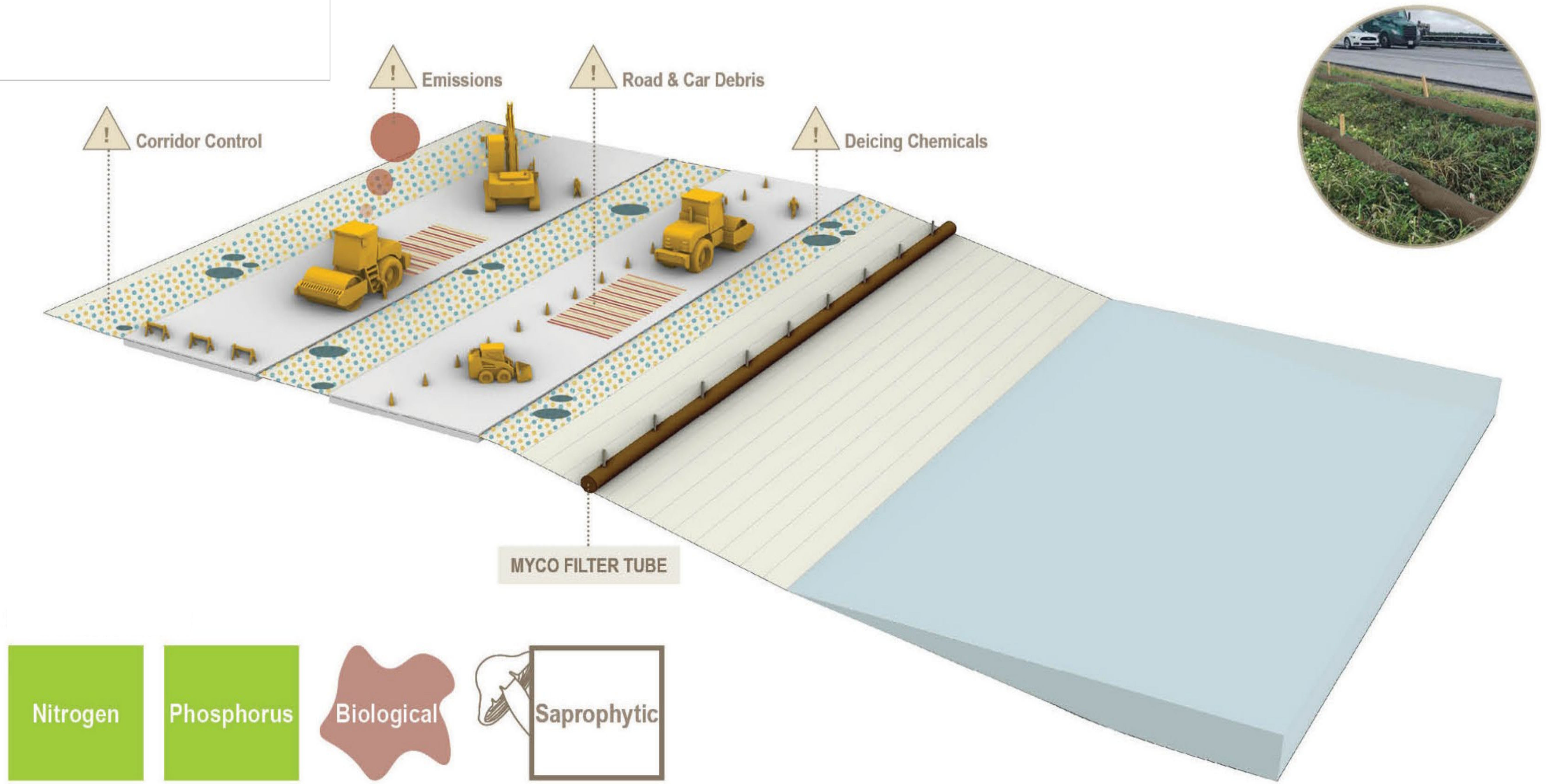
Base Roadway Condition: Contaminants



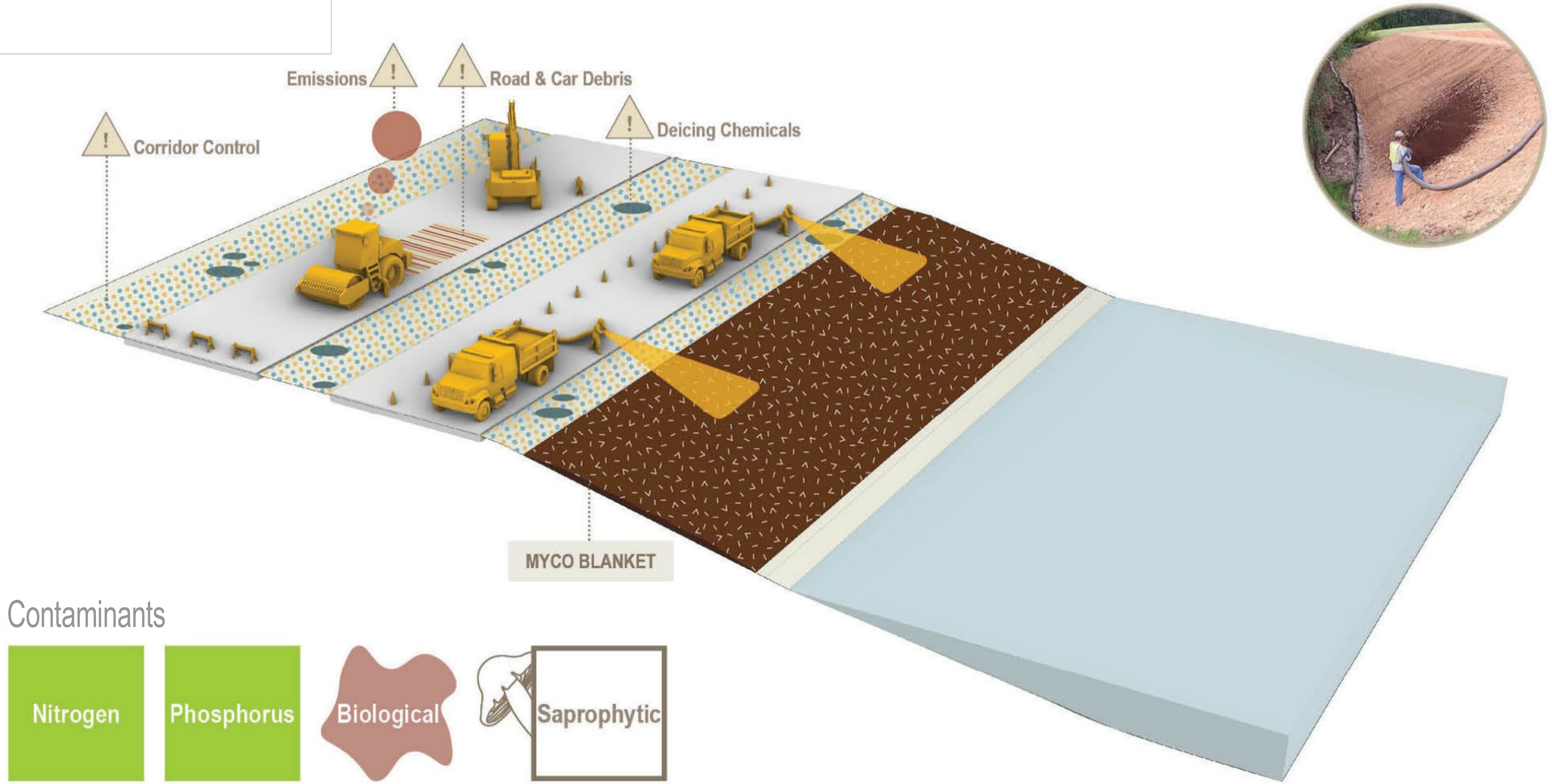
Base Roadway Condition Contaminates (Drawing by Offshoots, 2022)



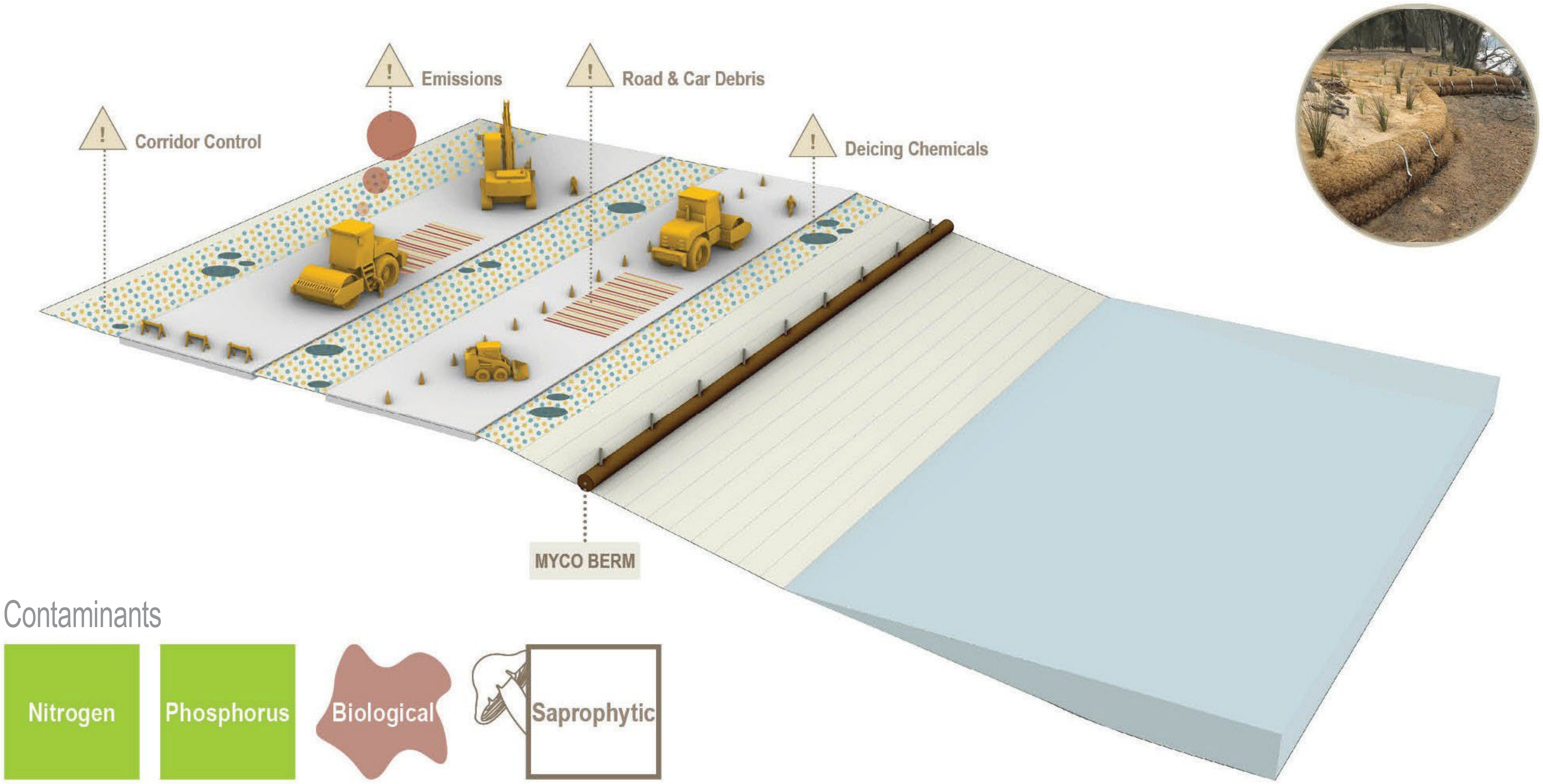
Base Roadway Condition (Drawing by Offshoots, 2022)



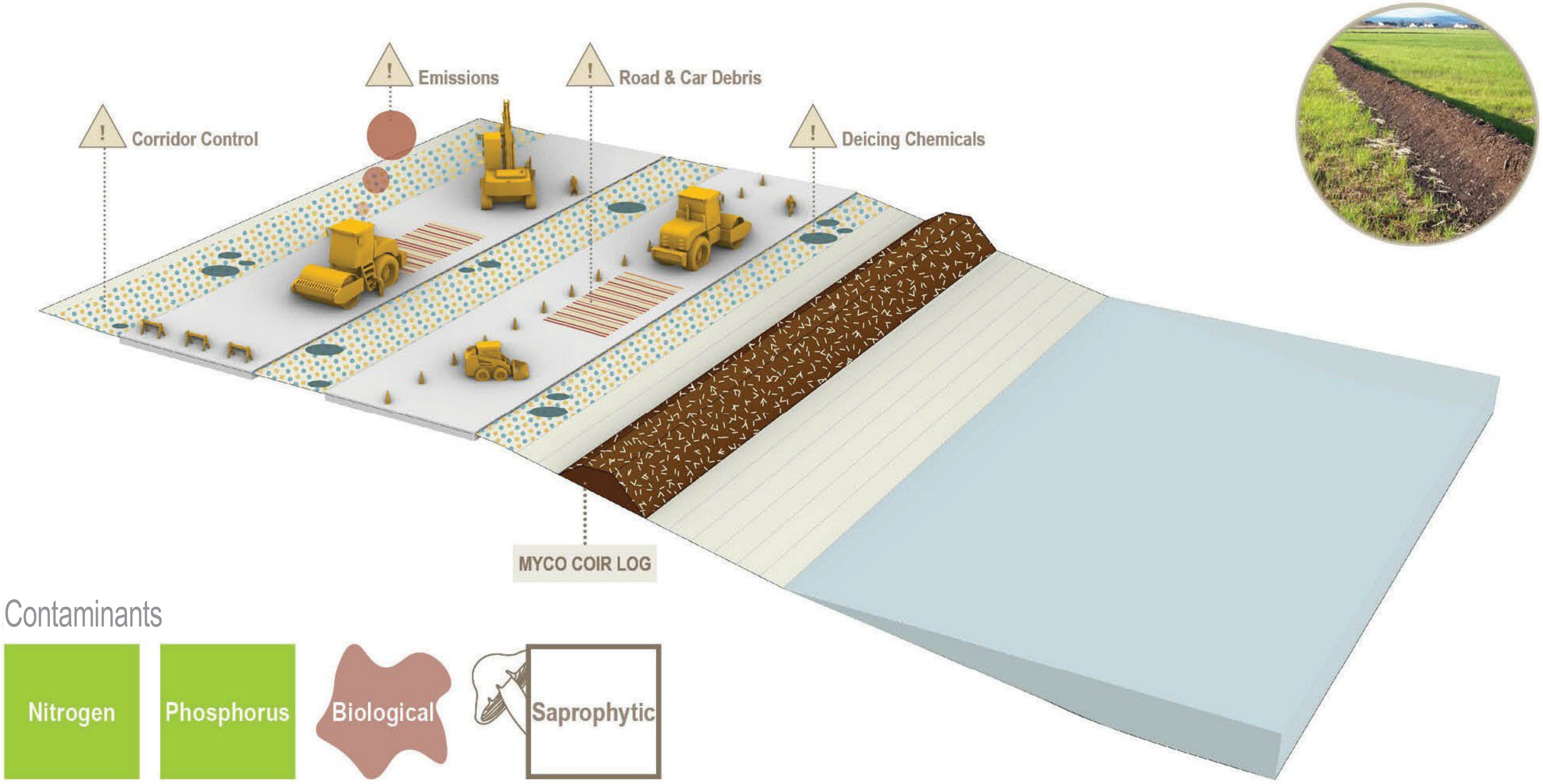
Myco Filter Tube (Drawing by Offshoots, 2022)



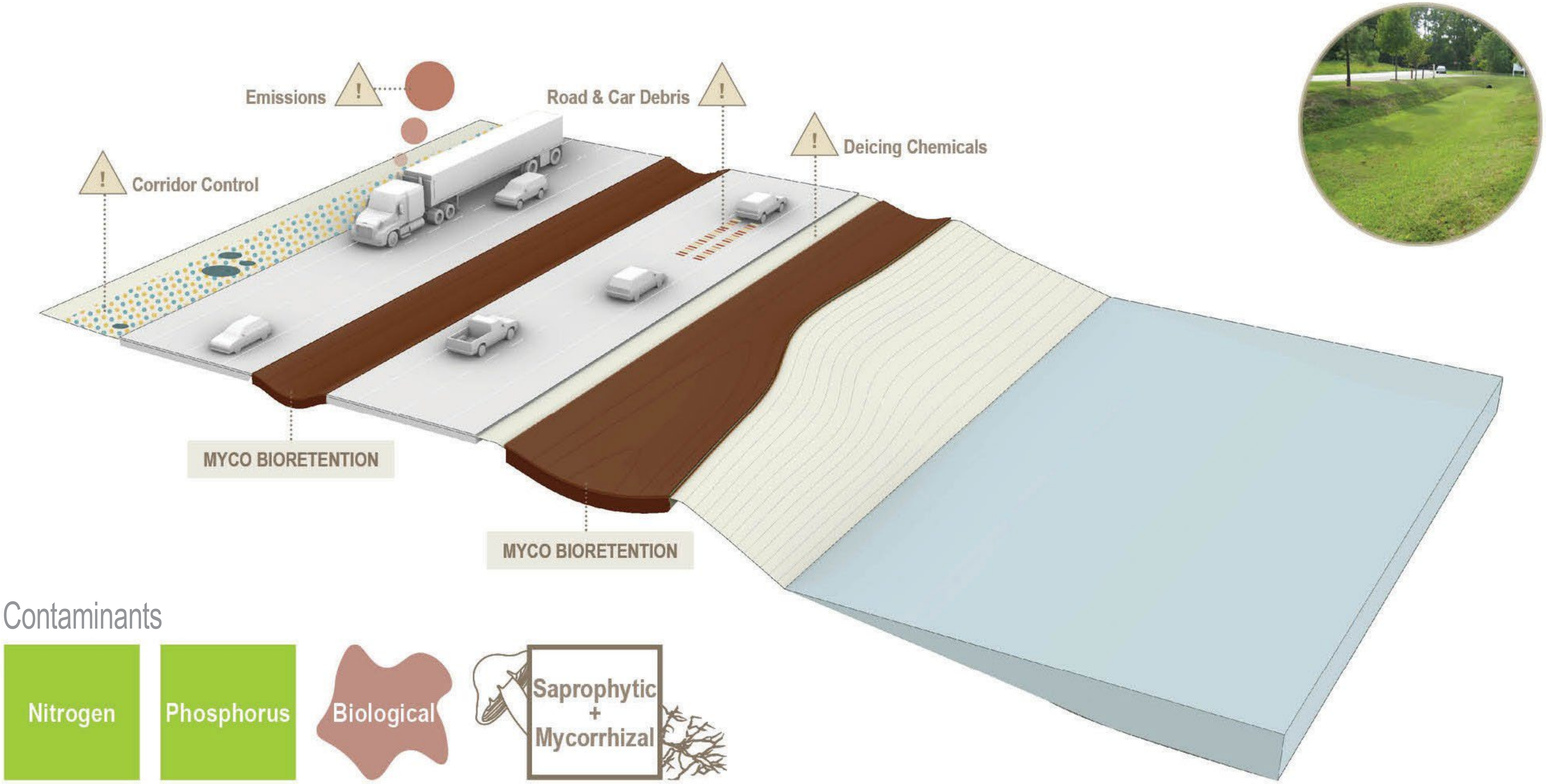
Myco Blanket (Drawing by Offshoots, 2022)



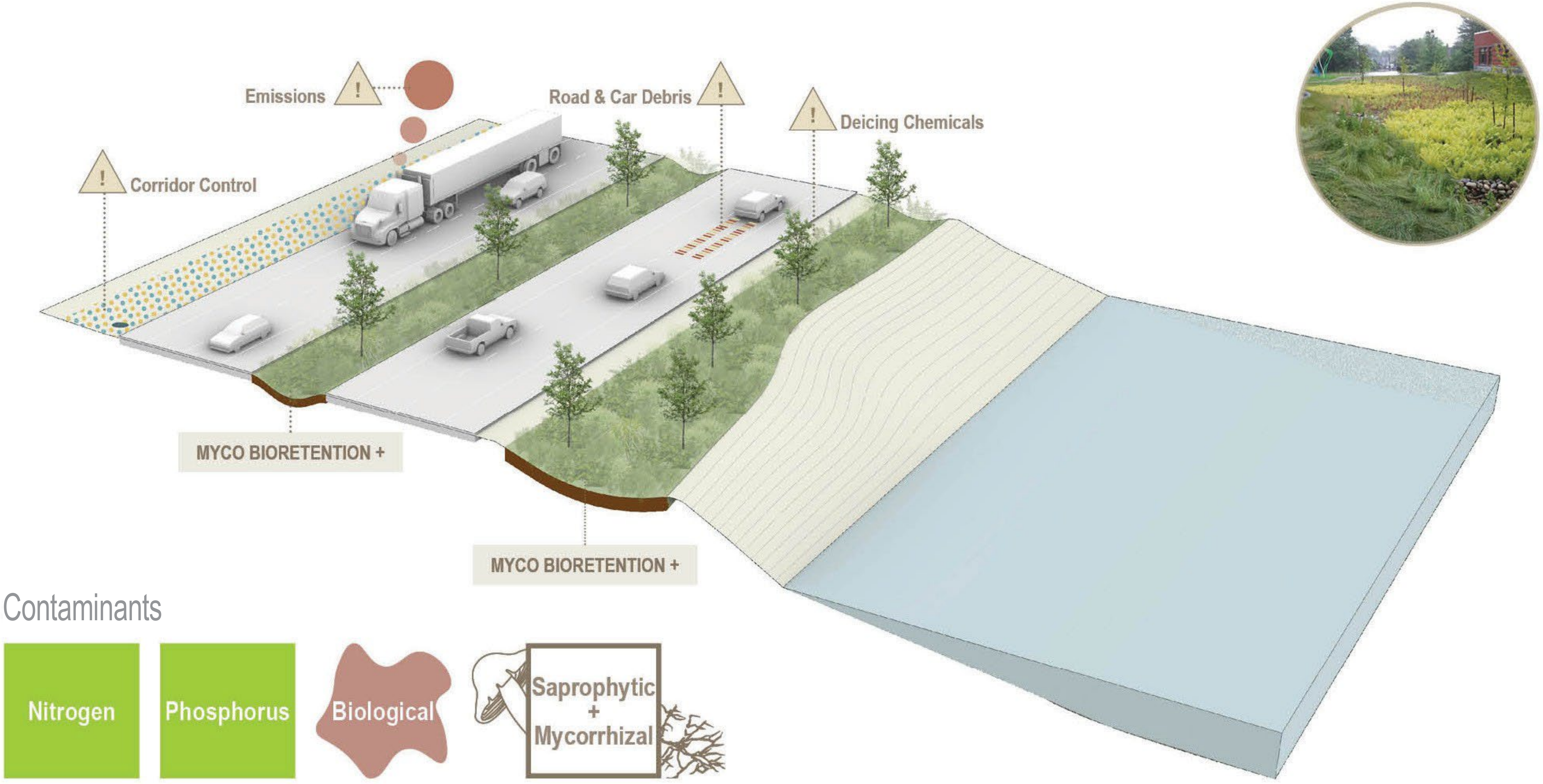
Myco Berm (Drawing by Offshoots, 2022)



Myco Coir Log (Drawing by Offshoots, 2022)



Myco Bioretention (Drawing by Offshoots, 2022)


Emissions
Road & Car Debris
Deicing Chemicals
Corridor Control
MYCO BIORETENTION +
MYCO BIORETENTION +
Contaminants
Nitrogen
Phosphorus
Biological
Saprophytic
+
Mycorrhizal

7.8 Appendix H: AASHTO Compost Material Specifications (revised 2022)

Filter Berm Media Parameters (R51-22)

Parameters[a,b]	Reported as (Units of Measure)	Filter Berm to Be Vegetated	Filter Berm to Be Left Unvegetated	Filter Tube Media
pH[c]	pH units	6.0–8.5	N/A	5.0–8.5
Soluble Salt Concentration[c] (Electrical Conductivity)	dS/m (mmhos/cm)	Max 5	Max 10	Max 10
Moisture Content	%, wet weight basis	30–60	30–60	< 60
Organic Matter Content	%, dry weight basis	25–65	25–100	25–100
Particle Size	% passing a selected mesh size, dry weight basis	3 in. (75 mm), 100% passing 1 in. (25 mm), 90% to 100% passing $^{3}/_{4}$ in. (19 mm), 70% to 100% passing $^{1}/_{4}$ in. (6.4 mm), 30% to 75% passing (no more than 60% passing $^{1}/_{4}$ in. (6.4 mm) in high rainfall/flow rate situations) Max particle length of 6 in. (152 mm)	3 in. (75 mm), 100% passing 1 in. (25 mm), 90% to 100% passing $^{3}/_{4}$ in. (19 mm), 70% to 100% passing $^{1}/_{4}$ in. (6.4 mm), 30% to 75% passing (no more than 50% passing $^{1}/_{4}$ in. (6.4 mm) in high rainfall/flow rate situations) Max particle length of 6 in. (152 mm)	2 in. (50 mm), 99% to 100% passing $^{3}/_{8}$ in. (10 mm), max of 50% passing Max particle length of 2 in. (50 mm)
Stability/Maturity[d]	—	—	—	—
Carbon Dioxide Evolution Rate	mg CO_2-C per g OM per day	< 4	< 8	< 8
Physical Contaminants (Man-made Inerts)	%, dry weight basis	< 0.5 (0.25 film plastic)	< 0.5 (0.25 film plastic)	< 0.5 (0.25) film plastic)

[a] Recommended test methodologies are provided in *Test Methods for the Examination of Composting and Compost* (TMECC, The U.S. Composting Council).

[b] Landscape architects and project (field) engineers may modify the allowable compost specification ranges based on specific field conditions and plant requirements.

[c] Each specific plant species requires a specific pH range. Each plant also has a salinity tolerance rating, and maximum tolerable quantities are known. When specifying the establishment of any plant or turf species, it is important to understand their pH and soluble salt requirements and how they relate to the compost in use.

[d] Stability/Maturity rating is an area of compost science that is still evolving, and as such, other various test methods could be considered. Also, never base compost quality conclusions on the result of a single stability/maturity test.

Compost Blanket Parameters (R52-22)

Parameters[a,b]	Reported as (Units of Measure)	Surface Mulch to Be Vegetated	Surface Mulch to Be Left Unvegetated
pH[c]	pH units	6.0–8.5	N/A
Soluble Salt Concentration[c] (Electrical Conductivity)	dS/m (mmhos/cm)	Max 5	Max 10
Moisture Content	%, wet weight basis	30–60	30–60
Organic Matter Content	%, dry weight basis	25–65	25–100
Particle Size	% passing a selected mesh size, dry weight basis	3 in. (75 mm), 100% passing 1 in. (25 mm), 90% to 100% passing $^3/_4$ in. (19 mm), 65% to 100% passing $^1/_4$ in. (6.4 mm), 0% to 75% passing Max particle length of 6 in. (152 mm)	3 in. (75 mm), 100% passing 1 in. (25 mm), 90% to 100% passing $^3/_4$ in. (19 mm), 65% to 100% passing $^1/_4$ in. (6.4 mm), 0% to 75% passing Max particle length of 6 in. (152 mm)
Stability[d]	—	—	—
Carbon Dioxide Evolution Rate	mg CO_2-C per g OM per day	< 4	< 8
Maturity[d] (plant bioassay)	%, germination and vigor	> 80 / 80	N/A
Physical Contaminants (Man-made Inerts)	%, dry weight basis	< 0.5 (0.25 film plastic)	< 0.5 (0.25 film plastic)

[a] Recommended test methodologies are provided in *Test Methods for the Examination of Composting and Compost* (TMECC, The U.S. Composting Council).

[b] Landscape architects and project (field) engineers may modify the allowable compost specification ranges based on specific field conditions and plant requirements.

[c] Each specific plant species requires a specific pH range. Each plant also has a salinity tolerance rating, and maximum tolerable quantities are known. When specifying the establishment of any plant or turf species, it is important to understand their pH and soluble salt requirements and how they relate to the compost in use.

[d] Stability/Maturity rating is an area of compost science that is still evolving, and as such, other various test methods could be considered. Also, never base compost quality conclusions on the result of a single stability/maturity test.