LABORATORY TO LANDSCAPE: MYCORESTORATION


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ABSTRACT


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Mycorestoration is the use of saprophytic fungi to remediate pollutants from land and water. To address contaminant presence in non-point source pollution primarily from agricultural runoff, we worked towards identifying the community-scale implementation process of mycofiltration, a type of mycorestoration that filters contaminants from water, in Arcata, California. This place-based study used Participatory Action Research (PAR) to collaboratively conduct Transdisciplinary Research (TR) to collect baseline qualitative and quantitative data that did not exist for Arcata's context. These data have been compiled into a written implementation plan which is intended to exist as a living document that is referenced and transformed by additional projects pursued by community members. It includes background information for Arcata and mycofiltration, designs, baseline evidence for effectiveness, and identification of potential sites for mycofilters, and recommended subsequent projects. The methods used to compose this implementation plan provided insight into distinct disciplinary and epistemological divides that affect access to knowledge about mycofiltration. We found that environmental restoration focused on longevity and intrinsic value must be pursued through cooperative community collaboration.

ACKNOWLEDGEMENTS

COLLABORATORS

This project was conducted by a team of researchers from differing positionalities. I, Riley Allen, was the primary researcher, author, and coordinator of the study. Sandrine Thompson, Levon Durr, and Hannah Hartmann were my research partners who were major contributors to the research process. Because I was not solely responsible for the development of the knowledge produced in the project, this document is written using the pronoun "we" as an acknowledgement of their work and commitment.

Sandrine Thompson made it possible to conduct the experiments at the Arcata Marsh Research Institute (AMRI). She was a biology graduate student who worked at the lab and helped us determine and perform the best available methods for testing our water samples. She dedicated her time and knowledge to accommodate our project.

Levon Durr, mycologist and owner of Fungaia Farm, was a significant contributor to this research. He served on my thesis committee, was responsible for my initiation into the world of mycology, and has helped shape the project from its inception as our local expert. He provided many materials for conducting the experiments, allowed us to use his facilities at Fungaia Farm to chip wood and complete the inoculation processes, and used his personal community relations to guide us. Levon was also a primary point of reference when determining knowledge gaps for implementing mycofiltration and how to fill them.

Hannah Hartmann worked most closely with me throughout the research. As an HSU undergraduate majoring in environmental science and management with an emphasis in environmental restoration and minors in geographic information systems (GIS), fire ecology, and soil science, she provided insight from several related western scientific disciplines. I referred to her nearly every time I needed insight or clarification about topics that were related to the natural sciences. Our juxtaposed disciplinary positionalities were incredibly useful for understanding and investigating mycorestoration. In addition, she authored the maps and methods section included in Appendix C, though we developed our parameters and inputs together, helped source alder, and was thoroughly present for nearly all of the field work.

TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF APPENDICES

INTRODUCTION

In September 2019, Humboldt State University's (HSU) student-led Mycology
Club sent out a newsletter informing my student research partner on this project, Hannah
Hartmann, an undergraduate student in the Environmental Science and Management
department at HSU, that Levon Durr, owner of Fungaia Farm and our community partner
on this project, would be presenting about a successful mycorestoration project he
collaborated on with the Mid Klamath Watershed Council (MKWC) in Orleans,
California, unceded traditional territory of the Karuk people. The presentation was to be
held at a local community center just outside of Arcata, California, unceded traditional
territory of the Wiyot people, at a regular meeting hosted by the Humboldt Bay
Mycological Society. We arrived at this presentation, coffee mugs in hand, expecting to
learn a thing or two about fungi. We left the presentation as student research partners
with a community partner and a goal of meeting the identified community need to
address local environmental pollution through mycorestoration.

Durr spoke about the aggressive white rot oyster mushroom (*Pleurotos ostreatus*)
used to remediate diesel fuel which leaked from an above-ground storage tank into the
surrounding soil at a site located approximately 60 yards from the Klamath River. Durr
presented in a way that personified the fungi instead of objectifying them[1]. He

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[1] Durr's personification of fungi is not a wholly original perspective. I fully acknowledge that many
Indigenous epistemologies consider more-than-human organisms to be equal to human relatives, and have
since time immemorial (Risling Baldy 2020). In contrast, western epistemologies, more often than not,
blatantly objectify all living organisms that are not human as well as a great many humans (Whitt 2010).

characteristically described their mycelium's ability to transform petroleum-based hydrocarbons into carbohydrates that simply become snacks for the organism, remediating the pollution from the site. Through his enthusiastic presentation, we were able to understand mycorestoration as the strategic cultivation and installation of saprophytic fungi, one of the primary decomposers in forest ecosystems found along the coast in the Pacific Northwest (PNW), to address pollutants ranging from petroleum-based substances in soil to *E. coli* in agricultural runoff (Durr 2016). The positive results of the Orleans project demonstrated mycorestoration's potential for becoming a Best Management Practice (BMP) at a community scale in Humboldt County through collaborative cooperation. That said, Durr also identified the following prerequisites to successfully scaling mycorestoration in a local context:

1. Criteria for identifying sites ideal for installation

2. A written implementation plan that can be presented to potential funding sources and entities with the power to grant permission to implement the biotechnology

3. Baseline data providing evidence that the biotechnology is effective and financially feasible

4. Overcoming preconceived dispositions about fungi through dissemination of accessible knowledge about the biotechnology

Available literature supports Durr's assertions regarding gaps in knowledge that need to be filled in order to proceed with expanded community-based implementation of

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This has been demonstrated by the ongoing genocide and ecocide experienced by Indigenous peoples and lands in North America (Reed 2020, Norton 1979, Pellow 2018, and Gilio-Whitaker 2020).

mycorestoration. Much of the research done on this biotechnology provides evidence confirming general effectiveness, but is written using a western scientific framework that is generally not accessible to academics from other disciplines nor those outside of the academic institution. For example, Singh 2006 offers a 592 page manuscript that describes the biological features of fungi using jargon that can hardly be translated to English. This is one of the few studies that does not cite Paul Stamets or his LLC, Fungi Perfecti.

Stamets is arguably a primary source for mycologists around the world because he is among the first in the field to support his findings in ways that meet the standards of western science. These have been adapted from his dense, western scientific studies for incorporation into more recent publications that are more easily understood. He also patented mycorestoration and its subcategories as a biotechnology in 2001, however, the terminology and select methodologies were released to the public domain in 2015. This has blocked others (institutions, corporations, and individuals alike) from claiming subsequent control over the associated knowledge (Fungi Perfecti 2015), but confirmed Stamets as a principal authority on mycorestoration. The remaining studies not written by Stamets on mycorestoration were conducted in collaboration with Stamets and/or Fungi Perfecti or cites his research. All of those, aside from Durr 2016 and Darwish 2013, in addition to his more recent publications, are based in Washington state, traditional territory of at least 29 distinctive groups of Indigenous peoples. This holds significance in the context of this project because Arcata is also located along the coastal forest ecosystems of the PNW. While the documented data from these studies may be

applicable to our context, Arcata presents a new location with new biophysical, cultural, and social contexts.

Studies demonstrating effectiveness seem to be the focus of published mycofiltration research, with the exception of Darwish who articulates that in terms of implementing mycorestoration, "the answer is clear: we need more community-scale mycological remediation work and experimentation and, most importantly, transparent communication and collaboration between experimenters" (Darwish 2013, 131). Stamets et al. 2013, SA Thomas et al. 2009, and Benedict 2011 show results that confirm fungi's ability to remediate toxins from the environment, but are generally inconsistent. They all identify the need for additional research showing a more definitive positive correlation between fungi and decreased levels of toxins before implementation can be further pursued. They also show inconsistent results in their western scientific experiments indicating that more studies with more refined methods need to be conducted as well. Benedict 2011 is the only other study found with an intention of implementing mycofiltration in a specific location, Synder Creek, in a specific community, but the extent of the project, conducted alongside Fungi Perfecti (Stamets, Le Dena Che' 2012), ended up as benchmark data demonstrating effectiveness. When presented with Benedict's data, Evergreen State College's Office of Sustainability deemed it to be intriguing, but incomplete. The author and interested parties concluded that additional research was needed before implementation could be considered (Benedict 2011). In the context of Arcata, there are no currently existing studies on mycofiltration nor the implementation process for doing it in this place. This project has been designed, in

response to the identified gaps in the literature and perceived need, to establish the

groundwork for future research geared towards presenting mycofiltration as a strategic,

effective option for addressing water quality.

The objectives of our work are to contribute additional baseline data measuring

effectiveness of mycofiltration, establish an implementation plan as a living document

that can be expanded and improved by future research, and present this research in ways

that are accessible to interested community members. These are specifically applicable to

Arcata's context.

Arcata, or Goudi'ni[2], is a small town located adjacent to Humboldt Bay in

Northern California.

Wiyot people have lived in the Humboldt Bay region for thousands of years. The North
Coast of California is rich with abundant terrestrial, riverine, estuarine, and marine
resources. Wiyot people lived in permanent villages along the waterways which also served
as travel and trade routes. Seasonal camps were made on the tribal lands and prairies, and
mountainous regions provided berries, acorns, pine nuts, wild game, and basketry
materials. Wiyot people actively managed their resources, burning for open grasslands,
cultivating edible bulbs, and following strict hunting and fishing protocols (wiyot.us
2020).

The Wiyot people were violently displaced from their territory beginning around 1850 by

swarms of European settlers who had initially arrived to steal gold from the land and its

peoples as well as the land itself (Norton 1979). They justified their genocidal actions

with racist ideologies like Manifest Destiny (Reed 2020), then evaded persecution using

legal frameworks that were and continue to be both racist and speciesist (Pellow 2018).

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[2] Goudi'ni is the Wiyot place name for Arcata.

Perhaps the most gruesome example of this is evidenced by the Tuluwat massacre on

Indian Island, located just south of Arcata, on February 26, 1860. Over 200 sleeping

elders, men, women, and children were murdered under the cover of darkness during the

Wiyot World Renewal Ceremony at this culturally significant site. The morally bankrupt

white men who carried out these acts of genocide were never prosecuted nor revealed by

their settler community (Norton 1979). Subsequent settler occupation of the Wiyot's

center of the world has led to severe ecological degradation.

Hazardous materials used at the site included paints, solvents, metals, petroleum products and other chemicals related to ship maintenance and repair. Improper material handling of the waste disposal practices resulted in extensive contamination of groundwater and soil as well as intertidal and subtidal habitat in the harbor (EPA 2018, 2).

This site-specific example shows just one local account of a repetitive history

indicating that settler colonial development is destructive to land and its Indigenous

peoples. Arcata is unceded Wiyot territory. The current community makeup of the city, as

it exists today, is dominated by settlers whose historically capitalist, individualistic ways

of living have resulted in severe degradation of local ecosystems in a manner parallel to

the contamination of Tuluwat.

By combining extensive conversations with Durr, mycological information

gleaned from the literature, and Arcata's historical and ecological context, we developed

the following research questions which frame the extent of this project.

1. What skillsets are needed to navigate the implementation process of

   mycorestoration in Arcata, California?

2.  How does the requirement of productive interdisciplinary cooperation affect the implementation process?

3.  What is the process of implementing community-scale mycorestoration from the ground up?

4.  What are the implications of conducting research with a focus on environmental longevity?

Using Participatory Action Research (PAR) as the primary method of addressing our research questions, we produced a report of the western scientific experiments conducted in collaboration with the Arcata Marsh Research Institute (AMRI), an initial version of a localized implementation plan, and a rich discussion section that addresses each research question to provide a nuanced understanding of implementing mycofiltration in Arcata.

## METHODOLOGY

Environmental degradation caused by the unsustainable, capitalist structure of this settler colonial state is the overarching problem this project seeks to address in the localized context of Arcata, California. We do this by investigating the specific processes of implementing mycofiltration at a community scale to address water pollution. The knowledge gaps associated with those processes include: accessible and existing knowledge related to mycorestoration, applicable mycofiltration baseline data for Arcata's social, cultural, and ecological context, and a comprehensive understanding of the necessary contents of an implementation plan. We use a mixed-methods approach to

conduct this research focused on context specific applicability that overcomes

disciplinary boundaries through Participatory Action Research (PAR), Transdisciplinary

Research (TD), Applied Research (AR), western Scientific Method, and Geographic

Information System (GIS) research. These frameworks allowed us to fill the identified

knowledge gaps by actively pursuing the implementation of mycofiltration in Arcata.

Participatory Action Research (PAR)

PAR takes lived experience as the starting point for investigation, places emphasis upon
the research process, values the knowledge produced through collaboration and in action,
and reconsiders the value of research as a vehicle for social change (Kindon 2005, Cahill
2004, Pain 2004, Fine et al. 2001, and Cahill 2007, 4)

In this project, PAR was used as the overarching method in which the subsequent

methods were embedded. I, as the primary researcher, positioned myself in fields outside

of my social science disciplinary training by coordinating a series of physical

experiments following western Scientific Method principles and a GIS mapping project.

We collaboratively conducted physical experiments using facilities lended by the Arcata

Marsh Research Institute (AMRI), and expertise lended by Sandrine Thompson (HSU

Biology graduate student), Hannah Hartmann (HSU Environmental Science and

Management undergraduate student), and Levon Durr (mycologist and owner of Fungaia

Farm). These experiments were conducted chronologically so that the first could inform

the second. The first, Experiment 1A, determined the minimum ratio of spawn to

substrate that should be used to successfully inoculate a mycofilter by testing different

ratios and observing if the colonization was successful and how long it took to reach peak

colonization. Using the ratio determined by Experiment 1A, we inoculated multiple

sample filters in 5-gallon buckets to test their effectiveness in reducing *E. coli* counts in

effluent water in Experiment 1B.2. The GIS mapping project was conducted with

Hartmann who served as the lead expert, map maker, and co-author for this section of the

research. We developed original parameters based on available literature that were used

in ArcGIS Pro with available local data to identify potential sites of mycofilter

installation. I was able to collect data by observing, reacting to, and documenting those

processes through the collaborative projects. These data, along with the reports generated

from the western scientific methods, were compiled to produce the implementation plan.

### Transdisciplinary Research (TD)

TD research strives for (a) grasping the relevant complexity of a problem, (b) accounting for multiple and diverse values that underpin diverse perceptions of that problem, (c) linking abstract and case-specific insights to build on understanding of the problem and (d) elucidating options for change based on common interest (Pohl and Hirsch 2007 and Wiesmann and Hurni 2011, and Adler et al. 2018, 181).

We chose to employ the identified methods based on the understanding that

community-scale implementation of an environmental restoration project is inherently

transdisciplinary because it "integrates the natural, social, and health sciences in a

humanities context, and transcends their traditional boundaries" (Choi and Pak 2020, ).

Successful integration of disciplines requires qualitative and quantitative modes of

inquiry that acknowledges and respects other epistemologies (Zhang 2020) because

environmental degradation exists as a social issue. We, therefore, addressed local water

contamination, and its associated implications, as a complex societal problem requiring

interdisciplinary collaboration that produced multifaceted, transferable knowledge

(Dennis 2020, Newel 2001, Callahan 2020, Bolman and Deal 2017, Adler et al. 2018, and

Raadschelders 2011).

## Applied Research (AR)

Transfer of knowledge from a source to a target 'is more complex than ascertaining whether a given practice is effective in source sites, as evaluation researchers might have it; it requires theoretical insight into how observed practices actually mobilize human action and bring about substantively significant effects' (Barzaley 2007, 522 and Adler et al. 2018, 187).

We focused on applying the known biological functions and effectiveness of

mycofiltration to our local context as well as producing original baseline data using

available resources. These quantitative data inform much of the content of the

implementation plan, while the process of producing those data resulted in the qualitative

data informing the social and political factors associated with implementation. We came

to understand the process and implications of implementing mycofiltration by actively

working to do it through collaborative research spanning multiple disciplines.

The ultimate goal of this project is to facilitate the implementation of

mycorestoration in Arcata to address water pollution with a focus on long-term

community health. We have found that this must be done by bridging epistemologies,

navigating existing social structures, and addressing issues of environmental justice

which requires a mixed-methods approach.

## Obstacles and Knowledge Gaps

In the context of mycorestoration, resilience can have both qualitative and quantitative meanings. Mycofilters themselves are incredibly resilient in that they can withstand tremendous amounts of stress in the form of fluctuating temperatures, contaminant levels, and flow rates (Stamets et. al. 2013). This is due to the strength, intelligence, and physical resilience of mycelium itself, and exemplifies our quantitative model. The research processes employed in this project revealed an understanding of qualitative resilience by overcoming the various obstacles and knowledge gaps we encountered along the way.

Our first major obstacle was conducting this research during the COVID-19 pandemic. We were organizing fieldwork when the first lockdown began in March 2020. Our campus closed, social distancing began, and we all watched people begin to die from the disease. At this stage in the research, we were supposed to be meeting with community members who might have been interested in contributing their knowledge about Arcata, environmental restoration, mycology, public policy, water quality, etc. It quickly became clear, though, that absolutely everyone's lives had been affected by the pandemic, and an up-and-coming project about a biotechnology that not many people were aware of simply was not a priority. In many cases, we deemed asking for help with this to be practically unethical. We reached out to who we could via email and phone calls, and tried to explain why implementing mycorestoration was important for our community. We did not receive many responses. As a result, this project was formed in part by who and what we were ethically able to access. For example, both Darwish and Stamets give the written impression that sourcing hardwood wood chips can be as easy as

visiting local arborists, wood mills, and garden stores. We called around for several weeks with nothing to show for it. They either did not call us back or were unable to assist us. We were nearly unable to conduct our experiments, as the wood chips were an absolute requirement, but were fortunate enough to happen across our desired species of tree on a friend's property. We had already begun redesigning our studies accordingly for if we could not get the wood chips, and, given these circumstances, showed resilience by adapting as needed to complete the project while being respectful to our preoccupied community.

Beyond the pandemic, community-scale implementation of mycofiltration as a topic had its own, predetermined set of challenges in the form of knowledge gaps. Few experts in the field of mycorestoration currently exist. Those who have, namely Paul Stamets, present their knowledge in a way that seems to co opt fungi for their personal success, not an active attempt to build this information for the health of the environment and the health of the community. It does not give any clear instruction on how to do this without limitless resources, perfect conditions, and the clout of a PhD, however, this is where the implementation process lives. At several points in time, we referred to *Mycelium Running* hoping to find the answer to a question we had happened upon. An example of this occurred when we were developing the parameters for identifying potential sites for installation using GIS methods. Specifically, he says that "a gently sloped area below a feeding lot or manure pond, where effluent from the lot or pond continually seeps through, is an ideal site to install a mycofilter" (Stamets 2005, 68). We know that mycofilters can be used in numerous applications other than what is specified

by Stamets in the quote, but there is no further mention of *how* to do apply them. This was a major obstacle to our pursuit. When we had questions that were not satisfactorily answered by available information, we pooled our resources and developed original transdisciplinary, applicable methods of understanding these specifications of mycofiltration.

The physical implementation of mycorestoration, as it currently exists, is not accessible (though an understanding of the biotechnology itself can be). In an effort to make it accessible and meaningful to the community, and making many mistakes along the way, we pushed the boundaries of disciplinary research. This is what we mean by qualitative resilience.

DISCUSSION

In this section, we present our responses to the research questions specified in the Introduction section. The responses were composed through reference of available literature, original data from our studies, and knowledge gained from the experience of conducting our studies.

What skillsets are needed to navigate the implementation process of mycorestoration in Arcata, California?

The Mycofilter section of Appendix A includes a chronological list of the implementation process of mycofiltration. To answer this research question, we discuss the skills needed for each step of that process.

Select a site and complete an initial inspection of the land

Site identification for selection can happen in several ways. It could be organic, like when we drove past a particular pasture land just outside of Arcata on a rainy day, and saw cows up to their ankles in soggy soil and feces while the runoff moved in steady streams towards the bay. Or it could be the result of intentional methodologies using specific parameters to objectively determine sites that meet the criteria, like the GIS methods included in Appendix C. This path requires expertise in GIS either as an individual or through collaboration with an expert. Site identification could also be the result of discussions within the community. Throughout our research processes, we often

found that our most fruitful advances resulted from informal conversations with community members who were interested in finding solutions to local environmental degradation. They would engage in these conversations by contributing their specialized knowledge about areas they were particularly concerned about. While the interactions were not formally noted and used as data for the purpose of this project, they represent one of several ways sites may be identified as a candidate for mycorestoration.

Once a site is established as a potential location for mycofilter installation, the next step is gathering baseline information about it. Key information includes but is not limited to: property ownership, physical land characteristics, native ecology, and present contaminants. The person currently in charge of the land will most likely know this information, so a designated meeting with the project coordinator, mycologist, and owner would be the most efficient step forward in most cases. A trip to the site in which this information is discussed in detail amongst the participants lends itself to initiating an implementation plan.

Choose a mycofilter design based on site features

Land characteristics inform optimal mycofilter design. These include slope (runoff intensity and direction), use (livestock, diverse fruits and vegetables, cash crops), treatment (pesticide and herbicide use, tilling, waste), topography, and hydrology. For example, if an agricultural site's runoff is determined to have significant *E. coli* counts at particular points in the area, layered bioswale mycofilter(s) are a good option to consider because they can be installed at the lowest slope where the *E. coli* counts are highest.

This allows them to be efficient in addressing that contamination in accumulated surface and flowing water where it is most needed at the site, maximizing effectiveness.

Determine required materials, find sources

Site features also influence substrate and spawn selection as part of the mycofilter design decisions when ecosystem health is prioritized. A distinct possibility exists that the fungi could further damage the land instead of healing it if we were to introduce invasive, non-native fungi to the local ecosystem by cultivating it as a mycofilter, and encourage it to compete with that place's existing organisms by combining spawn with its ideal substrate in ideal conditions. The fungi could disrupt the existing balance in that ecosystem, making matters worse. Our solution to this possibility calls for collaboration across disciplines and epistemologies. Western environmental scientists often specialize in one or a few factions of the discipline (eg. soils, botany, mycology, forestry, etc). They are established and can be an excellent source for knowledge about ecosystem balance requirements and species prescriptions for specific ecological contexts. We argue, though, that the best source for knowledge about populations of native species are the native peoples of that place. Their knowledge, often called Traditional Ecological Knowledge (TEK), spans back to time immemorial. Settler knowledge, even the western scientific variety, simply cannot compete in terms of wisdom and accuracy[3]. Therefore, we believe that respectfully consulting the Indigenous peoples of a place for the specified purpose of healing through mycofiltration is a kindness to that ecosystem, but

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[3] Indigenous knowledge has been targeted through continuous settler colonial genocide of Indigenous peoples and subsequently co-opted as "discoveries" by western scientists (Reed 2020 and Norton 1979).

acknowledge that it must be done without being forceful or extractive to the Indigenous knowers[4]. Consultation of expert knowledge provides the best method for determining the most appropriate species of fungi to constitute the mycofilter.

In Arcata, sourcing spawn is very easy. Levon Durr, community partner and mycologist on this project, and owner of Fungaia Farm, is the primary local source for spawn and mycological knowledge around Arcata. We purchased the spawn for our experiments in Appendix B from Fungaia Farm which ensured access to the quantity and quality of spawn needed without outsourcing. Durr is established near Arcata, and should be the first point of contact when deciding to pursue local, community-scale implementation of mycorestoration.

Sourcing the hardwood wood chips that saprophytic fungi, like *Stropharia rugosoannulata* and *Pleurotos ostreatus,* eagerly grow on, can be more difficult. We spent the better part of several weeks calling local arborists, wood mills, and garden stores with nothing to show for it[5]. The alder wood chips we sourced came from a friend's property and Jonsteen Plant Nursery (Hartmann's employer). In both cases, Hartmann felled and bucked two small trees, then we rented a chipper from Don's Rent All, and chipped the alder at Fungaia Farm. Our method of sourcing alder was only successful because of community connections. Where and how hardwood wood chips are locally sourced in subsequent projects will also depend on the community itself.

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[4] We also acknowledge that true healing of the land cannot be accomplished without giving the land back to its rightful relations.
[5] This outreach was conducted through late spring and summer 2020 when the COVID-19 lockdowns began. Under these unprecedented circumstances, we were unable to connect well with these sources.

Create a budget estimating cost

A budget for installing a mycofilter should include consideration of labor, materials, and maintenance. All are informed by the design and size of the mycofilter. Labor costs will depend on the method of doing the labor. A group of community volunteers with shovels would cost little to nothing, and is a very possible method of getting the job done using the available community resources such as the HSU Mycology Club and local sports teams looking for volunteer opportunities. We consider this to be the best option because it drastically reduces overall cost and footprint compared to renting heavy equipment and/or hiring a crew, and furthers the community collaboration needed to make mycofiltration successful at a community-scale.

Compiling the scaled amounts of selected materials with the prices of sourcing them results in the bulk of the budget because initial installation is the most expensive part of this process. Maintenance, on the other hand, should only have to be done every 2-3 years depending on the site and success of the installation. It includes adding more layers of organic material (e.g. wood chips, straw, cardboard, etc) and either bulk spawn or sawdust spawn as the existing fungi decomposes the available organic material turning it into soil. Only a layer or two is needed when performing maintenance, so materials would need to be re-sourced at that time, but at much lower quantities. The term maintenance refers to upkeep of both the mycofilter itself as well as the community relations that allowed that filter to be initially installed. In this way, materials can be re-sourced as needed without going through the whole process again, saving time and money.

Write a site-specific implementation plan

The next step in the process is writing a site-specific implementation plan. We encourage the use of Appendix A as a starting point that is built upon by subsequent projects and further individualized for its intended purpose. The implementation plan should include the gathered information from the previous steps in a clearly written format that can convince land owners, permissive entities, and funders of the value and potential of mycofiltration. This requires writing skills that summarize mycofiltration and its associated details in a way that can be understood by a wide range of people from a wide range of specialties.

Inoculate substrate via the predetermined method for specified filter design

The inoculation process starts with the spawn. It takes several weeks for sawdust spawn to be ready for mixing with additional substrate to facilitate mycelial growth. When working with a mycologist, they will need to know how much is needed, then time to prepare the spawn. If the spawn is simply going to be layered between wood chips, cardboard, and other materials, the ready bags of sawdust spawn can be applied directly for inoculation. If the spawn is to be bulked, it should be mixed with substrate, placed inside a burlap bag, and allowed to colonize that before inoculation of the mycofilter. This bulking method can take a few weeks longer to complete, but can be more cost effective because less spawn will need to be purchased at the front end of the process. Either method is effective.

A few days before inoculation, substrate can be pasteurized using cold water, hot water, or lime. This will give the fungi a higher chance of colonizing the substrate before

having to compete with other aerobic organisms, but is not a necessity. In our experiments, we simply soaked the wood chips in water so that they were hydrated, then mixed the spawn and substrate accordingly. We did not pasteurize our substrate because we wanted to mimic likely field conditions as closely as possible, and the mycelium was successful in colonizing the wood chips through several trials. Substrate pasteurization is an option, but not a necessity for the inoculation process.

Install the mycofilter according to the site-specific design

Everything should be in order for installation at this point. In short, the determined area should be dug out to the desired depth, materials layered, and then the whole bed watered to give the fungi the best possible environment for colonization of the substrate. Each step of the process until this point requires a wide array of skillsets ranging from writing cohesively using compiled knowledge, networking with community members, mathematically calculating the budget and amount of materials, and arguably the resilience to continue forward on a path of implementing mycofiltration which has not yet been established.

Monitor the filter for effectiveness

Additional data demonstrating effectiveness and paths for improvement is needed in order to grow the implementation of mycofiltration. Monitoring the effectiveness of *in situ* mycofilters is one of the ways to collect and document these data, and is an essential step in any environmental restoration project. To do this in our context, regular water quality tests should be taken above and below the filters, and the results documented. Our studies, and others, indicate that contaminant levels should be significantly reduced

below the filter. The results, compiled over several years, can then be used to inform

additional installations and reinforce available literature.

<u>Learning and adapting</u>

       Mycofilters are effective because of fungi, a living organism with its own agenda.

Developing biotechnologies like these, particularly ones with such little accessible

information about it, is an inevitably imperfect process. We do not have control over the

environmental conditions outside of the laboratory nor can we precisely predict how the

fungi will act. Successfully installing a single effective mycofilter, as well as on a

community scale, requires learning and adaptation on our part. We can learn what tends

to work, like strategically layering substrate and spawn, then adapt those processes to a

new site-specific context. Each new application will result in new challenges, successes,

and failures which we can and must learn from. This is simply the nature of collaborating

with biological organisms who are our guide to healing the damage that all settlers have

contributed to. With that in mind, we must repeat these steps, each time learning from the

fungi, to reach community-scale mycofiltration.

       How does the requirement of productive interdisciplinary cooperation affect the

implementation process?

       We have shown that community-scale mycofiltration implementation requires

interdisciplinary collaboration to be successful. Implementation of environmental

restoration projects in a community context implies merging scientific and social

knowledge in a cohesive process. We initially endeavored to orchestrate this merge of

knowledge, but found that it is both a path forward for and a distinct barrier to implementing mycofiltration. Disciplinary and epistemological divides block actionable progress towards environmental and social justice because of a distinct incapability to understand one another.

Mycofiltration has effectively provided a lens into this dichotomy because mycology is typically nested in western science, yet the implementation process requires social considerations and expertise outside of academia. Aside from Durr, who has been our main knowledge source for anything relating to mycology, all of the environmental scientists we approached to discuss this research abruptly cut communication soon after we explained that we are working out the implementation of the biotechnology, not necessarily searching for scientific revelations relating to it. More often than not, they struggled to comprehend how they could contribute to the research in a meaningful way while knowing little to nothing about mycorestoration (even other mycologists). We assert that this is due to a severe communication barrier. They failed to understand the project as it exists, in part, because I, as the primary researcher, was unable to speak their language and make this project meaningful to them. Until we can bridge disciplinary language barriers and prejudices, interdisciplinary research will continue to be a major block to implementing mycorestoration.

What is the process of implementing community-scale mycorestoration from the ground up?

We have already discussed the process of installing a mycofilter from a community perspective, but the process of implementing mycofiltration on a community-scale from a community perspective requires a broader analysis. Installing a single mycofilter is a tangible step towards community implementation, provides frameworks for contextual replication, and teaches community collaboration through necessity. Community-scale implementation relies on cooperation between community members, municipal authorities, and academics from a wide array of disciplines. Before local implementation can be accomplished, two things must happen. (1) Some or all of the Recommended Next Steps in Appendix A must be pursued, and (2) a common understanding of why mycofiltration can be a meaningful method of addressing local contamination must be established.

Much of the available literature articulates that there are concerns about inconsistent data, flawed experiment designs, false positives skewing efficiency testing, and a general lack of credible research on the topic (Stamets, La Dena Che' 2012, Stamets et. al. 2013, and Darwish 2013). The Recommended Next Steps in Appendix A compile research projects that would address the existing body of literature that is lacking robust, widely comprehensible evidence for community-scale implementation of mycorestoration. Completion of each project brings us a considerable step closer to implementation, all of them requiring a committed research team in some form, by adding further credibility (and identifying faults) from a wide-array of perspectives. Additionally, persistent coordination between new and existing collaborators with diverse positionalities nurtures further acceptance for the biotechnology across ways of knowing.

Successful dissemination of fortified knowledge about mycorestoration is an important

move towards implementing mycorestoration from the ground up.

There is so much yet to be understood about the remediative properties of fungi that the world cannot simply wait for a small number of professional researchers to figure it out - and then patent the information. Without concerted (while somewhat playful) experimentation and research by people just like you and me, progress will only continue at a snail's pace… we must no longer look to the pedagogues of mycology for assistance, but toward each other for our collective understanding of the subject (Darwish 2013, 151).

What are the implications of conducting research with a focus on environmental

longevity?

While mycofiltration serves as the lens through which we understand the

implications of conducting research focused on environmental longevity, it, like most

environmental restoration projects, is simply a biodegradable bandage treating symptoms

of the actual problem. Scholars across disciplines and epistemologies have identified

anthropogenic activity as the primary cause of global environmental degradation,

however, this does not sufficiently recognize settler colonialism as the root of today's

societal structure. Dr. Dina Gilio-Whitaker, Dr. Kari Norgaard, Dr. Jack Norton, Dr.

David Pellow, Dr. Kaitlin Reed, Ron Reed, Dr. Cutcha Risling Baldy, Dr. Eve Tuck, and

Dr. K. Wayne Yang are just a few BIPOC (Black, Indigenous, and people of color)

scholars who overtly and unapologetically identify settler colonialism achieved through

ongoing genocide and land theft as the true source of environmental degradation.

Globally and locally, it must be addressed as a product of a racist, speciesist system

rooted in western imperialism.

Settlers giving land back and learning to live equitably is the most effective way to bridge environmental and community health leading to environmental longevity. Once that happens, we will actually be capable of grappling with restoration of more than human ecosystems that have been degraded by capitalist, settler colonial development. It requires a certain amount of societal dismantling with Indigenous peoples at the center seat of the table. This includes but is not limited to: learning accurate history from non-dominant sources, settler activism for land return, legal recognition of Indigenous tribes as sovereign nations, and settlers actively working with and for the land's Indigenous peoples to heal it for its intrinsic values, not monetary.

Ultimately, environmental longevity implies that ecosystems are balanced and can continue to facilitate life. Settler colonial capitalism is the antithesis to environmental longevity. Therefore, the most important implication of conducting research with this focus is that it must, inevitably, include Indigenous environmental justice.

REFERENCES

Adler, C.; Hadorn, G.H.; Breu, T.; Wiesmann, U. & Pohl, C. (2018). Conceptualizing the Transfer of Knowledge Across Cases in Transdisciplinary Research. *Sustainability Science*, 13: 179–190.

Assouline S. & Ben-Hur M. (2006). Effects of rainfall intensity and slope gradient on the dynamics of interrill erosion during soil surface sealing. *Catena,* 66: 211–220.

Barzelay, M. (2007). Learning from second-hand experience: methodology for extrapolation-oriented case research. *Governance,* 20(3): 521–54.

Benedict, T. (2011). Mycofiltration for the Puget Sound: Pleurotus ostreatus & fecal coliform bacteria. Evergreen State College. Thesis.

Bolman, L & Deal, T. (2017). *Reframing Organizations: Artistry, Choice, and Leadership*. 6th Edition. Hoboken, NJ: John Wiley & Sons, Inc.

Burkholder, J.; Bibra, B.; Weyer, P.; Heathcote, S.; Koplin, D.; Thorne, S. P. & Wichman, M.  (2007). Impacts of Waste from Concentrated Animal Feeding Operations on Water Quality. *Environmental Health perspectives,* 115(2): 308-312.

Callahan, Richard. (2020). Addressing Complex, Societal Challenges through Interdisciplinary Research. *Journal of Interdisciplinary Sciences*, 4(2): vi-x.

Cahill, C. (2007). The personal is political: Developing new subjectivities through participatory action research. *Gender, place and culture*, 14(3): 267-292.

Cahill, Caitlin; Arenas, Erica; Contreras, Jennifer; Jiang, NA; Rios-Moore, Indra; & Threatts, Tiffany. (2004). Speaking back: Voices of young urban womyn of color using participatory action research to challenge and complicate representations of young women. *Anita Harris (Ed) All About the Girl: Power, Culture and Identity New York: Routledge*, 231-242.

Choi, B. C. K., & Pak, A. W. P. (2006). Multidisciplinarity, interdisciplinarity and transdisciplinarity in health research, services, education and policy: 1. Definitions, objectives, and evidence of effectiveness. *Clinical & Investigative Medicine*, 29(6): 351–364.

Darwish, Leila. (2013). *A Grassroots Guide to Healing Toxic and Damaged Landscapes: Earth Repair.* New Society Publishers.

Dennis, Jeremy K. (2020). The Kantian Effect: Receiving the Integration of Knowledge in Interdisciplinary Theory. *Journal of Interdisciplinary Studies*, 4(2): 1-14.

Durr, Levon. (2016). *Mycoremediation Project: Using Mycelium to Clean Up Diesel-Contaminated Soil in Orleans, California.* Fungaia Farm.

EPA. (2018). Environmental Stewardship and Cultural Preservation on California's Coast: The Tulutwat Village Site on Indian Island in Humboldt County, California. https://semspub.epa.gov/work/HQ/100001200.pdf

Fine, Michelle; Torre, Maria Elena, Boudin, Kathy; Bowen, Iris; Clark, Judith; Hylton, Donna; Martinez, Migdalia; Missy; Rivera, Melissa; Roberts, Rosemarie A,; Smart, Pamela & Upegui, Debora. (2001). Participatory action research: from within and beyond prison bars. *Paul Mark Camic, Jean E. Rhodes & Lucy Yardley (Eds) Qualitative research in psychology: Expanding perspective in methodology and design.*

Fungi Perfecti. (2015). *Mycofiltration Enters the Commons*. https://fungi.com/blogs/articles/mycofiltration-enters-the-commons.

Gilio-Whitaker, Dina. (2020). *As Long as Grass Grows: The Indigenous Fight for Environmental Justice, from Colonization to Standing Rock.* Penguin Random House.

Haiyan, F.; Liying, S. & Zhenghong, T. (2015). Effects of rainfall and slope on runoff, soil erosion and rill development: an experimental study using two loess soils. *Hydrological Processes,* 29: 2649–2658.

Humboldt Baykeeper. (2013). *Fecal Coliform Loads Measured in Four Arcata Creeks*: *Water  Quality Program*. https://www.humboldtbaykeeper.org/programs/water-quality/901-fecal-coliform-loads-measured-in-four-arcata-creeks

Humboldt County Department of Health and Human Services. (2021). *Water Quality Test Results.* https://humboldtgov.org/1696/Water-Quality-Test-Results

Kindon, Sara. (2005). Participatory action research. Ian Hay (Ed) Qualitative Methods in Human Geography. 2nd Edition. *Oxford University Press*.

Moore, D. Alexopoulos; John Constantine and Vernon Ahmadjian. (2020). Fungus. *Encyclopedia Britannica*. https://www.britannica.com/science/fungus

Newell, W. H. (2001). A theory of interdisciplinary studies. *Issues in Integrative Studies,* 19: 1-25.

Norton, Jack. (1979). *Genocide in Northwestern California: When Our Worlds Cried.* Indian Historian Press.

Pain, Rachel. (2004). Social geography: Participatory research. *Progress in Human Geography*, 27(5): 667-85.

Pellow, David. (2018). *What is Critical Environmental Studies?* Polity Press.

Pohl C, Hirsch & G. Hadorn. (2007). Principles for designing transdisciplinary research. *Munich: Swiss Academies of Arts and Sciences.*

Raadschelders. J. C. N. (2011). *Public Administration: The Interdisciplinary Study of Government.* NewYork: Oxford University Press.

Reed, Ron & Kari Marie Norgaard. (2010). Salmon Feeds Our People: Challenging Dams on the Klamath River. *Human Rights and Conservation.*

Reed, Kaitlin. (2020). We Are a Part of the Land and the Land Is Us: Settler Colonialism, Genocide & Healing in California. *Humboldt Journal of Social Relations,* 1(42): 27-49.

Risling Baldy, Cutcha. (2020). Indigenous Voices for Decolonized Futures. *Bioneers Conference.* https://bioneers.org/cutcha-risling-baldy-indigenous-voices-decolonized-futures-zstf2101/

Risling Baldy, Cutcha & Carrie Tully. (2019). *Working for and toward Land Return of Goukdi'n (Jacoby Creek Forest).*

Scott, E. E.; Leh, M. D.; & Haggard, B. E. (2017). Spatiotemporal variation of bacterial water quality and the relationship with pasture land cover. *Journal of Water and Health,* 15(6): 839-848. doi:10.2166/wh.2017.101

Singh, Harbhajan. (2006). *Mycoremediation: Fungal Bioremediation.* New York: Wiley Interscience.

Stamets, Le Dena Che'. (2012). *Best Mycorestoration Practices for Habitat Restoration of Small Land Parcels.* Evergreen State College. Thesis.

Stamets, Paul. (2005). *Mycelium Running: How Mushrooms Can Help Save the World.* Ten Speed Press.

Stamets, Paul; Marc Beutel PhD; Alex Taylor; Alicia Flatt; Morgan Wolff; & Katie Brownson. (2013). *Comprehensive Assessment of Mycofiltration Biotechnology to Remove Pathogens From Urban Stormwater.* Mycofiltration Biotechnology for Pathogen Management. Fungi Perfecti, LLC.

Thomas, SA; LM Aston; DL Woodruff; & VI Cullinan. (2009). *Field Demonstrations of Mycoremediation for Removal of Fecal Coliform Bacteria and Nutrients in the Dungeness Watershed, Washington.* Battelle Memorial Institute.

Tuck, Eve & K. Wayne Yang. (2012). Decolonization Is Not a Metaphor. *Decolonization: Indigeneity, Education & Society,* 1(1): 40.

Wiesmann, U & H. Hurni. (2011). Research for sustainable development: foundations, experiences, and perspectives. *Perspectives of the Swiss National Centre of Competence in Research (NCCR) North-South*. Geographica Bernensia, Bern.

Whitt, Laurelyn. (2010). *Science, Colonialism, and Indigenous Peoples: The Cultural Politics of Law and Knowledge.* Cambridge University Press.

Wiyot.us. (2020). *Tribe History*. https://www.wiyot.us/.

Zhang, Wanqing. (2020). Practical Applications of Qualitative and Quantitative Approaches in Interdisciplinary Sciences: A New Perspective. *Journal of Interdisciplinary Sciences*, 4(2): 43-51.

APPENDIX A


Implementation Plan


The purpose of this document is to initiate the implementation of mycofiltration

on a community scale in Goudi'ni[6] or Arcata, California, unceded Wiyot ancestral

territory. We intend this to be the foundation of a living document[7] that can be built upon

by additional projects incorporating diverse methods of knowledge production. The

content presented in this preliminary version of an implementation plan has been

developed through collaborative, transdisciplinary research conducted within the project:

*Laboratory to Landscape: Mycorestoration.* The project compiles existing and original,

place specific data to investigate the implementation process of mycofiltration. These

data are organized within the subsequent sections to:

1. familiarize our readers with mycorestoration as an accessible biotechnology for

   addressing *E. coli* contamination,

2. contextualize its applicability in Arcata,

3. offer western scientific evidence showing its potential effectiveness,

4. detail characteristics of mycofilters,

5. map potential sites for installation, and

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[6] Wiyot place name for the Arcata
[7] We define "living document" as one that continues to be edited and transformed by subsequent
community researchers so that it is a reflection of that community as it currently exists.

6. identify additional projects that would advance the aforementioned initiatives of

this document.

## Background

[Mycorestoration is] the use of fungi to repair or restore the weakened immune systems of environments… [it] involves using fungi to filter water (mycofiltration), to enact ecoforestry policy (mycoforestry) or co-cultivation with food crops (mycogardening), to denature toxic wastes (mycoremediation), and to control insect pests (mycopesticides). Mycorestoration recognizes the primary role fungi play in determining the balance of biological problems (Stamets 2005, 55).

To facilitate a deeper understanding of mycofiltration as a subsection of

mycorestoration, we offer an overview of fungi from western scientific ways of knowing

as this tends to be the most relatable epistemology when communicating with settler land

owners, funding agencies, and municipalities.

The kingdom of fungi is composed of organisms including yeasts, rusts, smuts,

mildews, molds, and mushrooms. While they cannot be distinctly categorized as plant or

animal, fungi are more closely related to animals based on their heterotrophic mode of

obtaining nutrients. Their cellular structure does not allow them to prepare food for

themselves; they must get their food from external sources. Western scientists assert that

the fungi split from animals approximately 9 million years after plants making their

characteristics more animal-like than plant-like (Moore et al. 2020). Their intelligence

(ability to acquire what they need to survive, reproduce, and adapt), relationships with

other organisms, and abundance makes them ideal allies for addressing environmental

degradation (Stamets 2005).

Western science currently knows of approximately 144,000 species of fungi with countless more still undocumented. In fact, many mycologists label themselves primarily as taxonomists because of their continual documentation of new species which they categorize as: saprophytic, parasitic, or mycorrhizal. Saprophytic mycelium secretes lignocellulose degrading enzymes to decompose dead organic matter, then absorbs the newly accessible nutrients through the cell membrane. They are found all over the world requiring only an appropriate amount of available organic material to sustain themselves. Parasitic fungi get their nutrients by attacking living organisms. Generally, they enter a host through a weak point in the exterior, then absorb food from the interior tissue. Mycorrhizal fungi nourish themselves by invading the roots of plants and absorbing food from there. These associations are typically beneficial for both the plants and the fungi. In addition, approximately 90% of land plants depend on mycorrhizal associations because the fungi bring in mineral nutrients that would not have otherwise been accessible to the plant through mycelial networks that transfer nutrients as well as chemical messages (Moore et al 2020).

Mushrooms seen above ground are the fruiting bodies of the fungi. The underside of these mushrooms' caps have gills, teeth, or pores, depending on the species, that hold millions of spores. Each spore contains half of the genetic material required to generate a new individual. If the released spores land on suitable habitat, they germinate and produce hyphae. Hyphae are the white, threadlike filaments made of one or more cells surrounded by a cylindrical cell wall. Once a spore encounters a genetic mate, their hyphae fuse to create a complicated, tight-knit network designed to find and consume

nutrients. The vegetative body or thallus is known as mycelium. Mycelium constitutes the

vast majority of the fungi's mass and has the ability to grow many miles in their pursuit of

nutrients (Darwish 2013). Primarily limited by substrate quality and quantity, access to

food drives mycelial growth (Marshall 2021). Saprophytic fungi's indefinite, often

aggressive and effective, quest for nutrients, as well as its method of acquisition, makes

them ideal for strategic placement as biofilters to address contaminants.

Mycofiltration in Arcata

Mycofiltration can capture and metabolize the flow of toxins such as fecal coliform
bacteria (found in wastewater from farming practices or failing septic systems),
organophosphates (found in pesticides, detergents, and fertilizers), and [polychlorinated
biphenyls or] PCBs (e.g., those found in insulating fluids within electrical equipment in
power plants, industries, and large buildings) (Stamets, La Dena Che' 2012).


This document focuses on mycofiltration as the specific type of mycorestoration

that, we argue, is most widely applicable to our context in Arcata. Le Dena Che' Stamets,

biological relation of Paul Stamets, identifies the following list of ideal applications for

mycofiltration[8].

• Native Tribal lands

• Agricultural runoff (cow, pig, etc.)

• Marijuana grows

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[8] We reordered the list to reflect the most pressing applications for Arcata based on an environmental
justice framework. See (Stamets, Le Dena Che' 2012, 80) for the original list. In addition, Stamets defines
the listed applications as "potential target markets or consumers of vested interest." This language denotes a
focus on growth through capitalist structures as they currently exist. We identify the settler colonial,
capitalist structure as the primary source of environmental degradation in this place, and therefore, are
working towards absolving those structures through this work to create viable solutions for environmental
degradation.

• Life stock farms in violation

• Municipalities

• Watershed buffers

• "Buffers" or "riparian buffers" in National Forests where endangered or key-

stone species live

• Community garden buffers

• Conservation organizations

• Restoration organizations

• Commercial Fishing Industries

• State Agencies

• Land-use developers

• Individual landowners of leaking septic tank violations

• Individual landowners with adjacent neighbors who create pollution that travels

onto their properties

• Housing developments with stormwater regulations or violations

• Landscape companies

• Horse boarders, horse pastures


This list seems to have been compiled through research conducted mostly in the vicinity

of coastal Washington which encompasses riverine, estuarine, marine, and terrestrial

ecosystems, and settler colonial community development established through the

genocide of Indigenous peoples, comparable to those found in this area. Every entity

listed is present in Arcata.

        A 2013 study conducted by Humboldt Baykeeper indicates that Janes Creek

carries 3,890 MPN/100ml of *E. coli* into Arcata Bay, the northern section of Humboldt

Bay, affecting local shellfish quality and overall health of those aquatic ecosystems

(Humboldt Baykeeper 2013). California's standard for water quality specifies that *E. coli*

counts may not exceed 400 MPN/100ml in recreational waters (Humboldt County

Department of Health and Human Services 2021). Additionally, water quality data from

the National Water Quality Monitoring Council includes data for bacteria and viruses,

however, neither general coliform nor *E. coli* were specifically included which indicates a

need for more research on local presence of the bacteria. In response, a study conducted

by the North Coast Regional Water Quality Control Board took samples at several

locations suspected to be significantly contributing to *E. coli* levels in water sources. The

report seems to be the most up-to-date source for this localized information that informs

community action to limit *E. coli* counts, however, the results are not included in the

report because the scientific peer review process is not yet complete on that project.

Again, in order to move forward with the implementation of mycorestoration in and

immediately around Arcata, studies must specify levels of *E. coli* within the community.

Then, these data can be used to inform the implementation process of mycofiltration,

however, it is clear that *E. coli* presence has been acknowledged as a notable issue in this

place as it directly affects community health.

Mycofilters

At this point in time, available literature identifies two designs for mycofilters that could be selected for implementation: submerged and layered bioswale[9]. We recommend that remediators select a mycofilter design on a site-specific basis[10] using the subsequent steps for implementing a mycofilter[11].
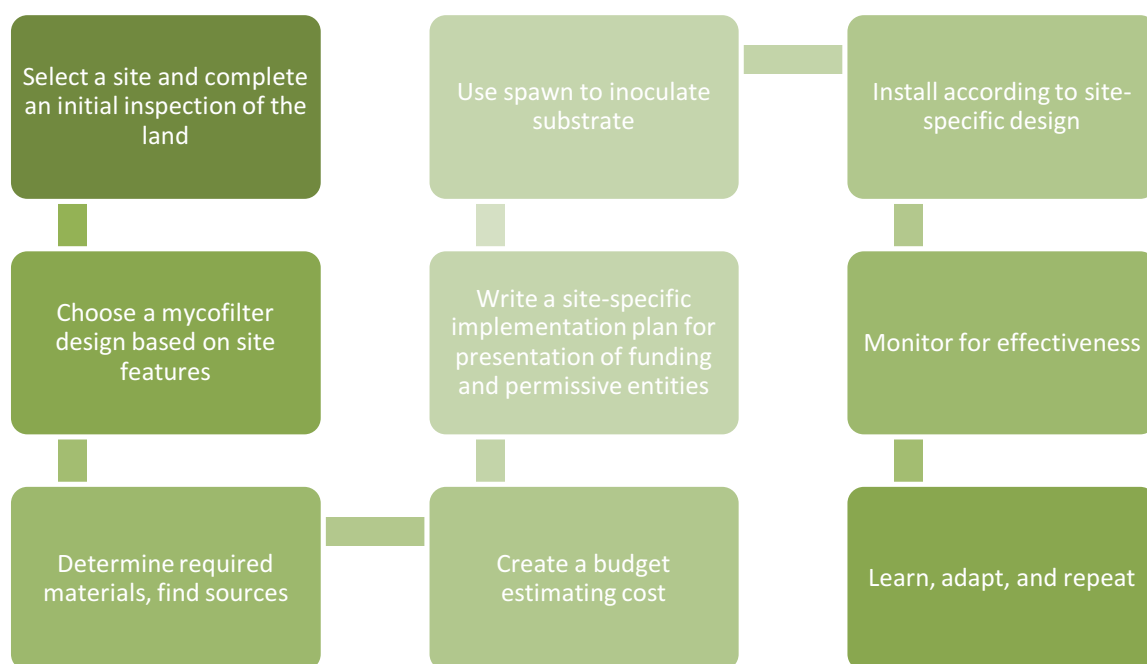


Figure 1: Visual representation of the process of installing a mycofilter.

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[9] These terms are not explicitly used to differentiate the types of mycofilter designs in other available literature. To the best of our knowledge, they are original terms that were developed based on the characteristics of their respective design.

[10] Consideration of site-specific characteristics includes but is not limited to: climate, native fauna and flora, topography, hydrology, land use, and property ownership.

[11] In the Pacific Northwest (PNW), early spring to late summer is the best time to inoculate substrate.

Mycofilter Designs

Submerged. This type of mycofilter is essentially composed of burlap bags filled with inoculated wood chips that are placed into flowing water channeled through human or natural structures. As the water flows through the filter, sediment-bound and free-flowing bacteria is caught, their hydrocarbon bonds degraded, and compounds returned to their elemental form resulting in available nutrients for fungal consumption. Straw can also be used as an effective substrate for encouraging mycelial growth (Darwish 2013 and Stamets 2005), and therefore, could fill the burlap bags, but there is evidence of straw's presence correlating with false positives of *E. coli* counts when testing using The Coliscan® Membrane Filter Method of Micrology Laboratories LLC for *Escherichia coli* and Total Coliforms (Coliscan® MF) (Stamets et al. 2013). This does not rule out using straw, but it would be difficult to evidence efficiency at this stage of the research process.

In theory, the submerged design would be ideal for use in streams that are known to contain unsafe levels of *E. coli* as well as other harmful bacteria and chemicals. In practice, placing several inoculated burlap bags in a stream where organic matter and organisms could be blocked from their migratory path would likely cause a decline in health of the ecosystem, and is, in many cases, illegal. This mycofilter design would be best suited for human-made water channels that simply direct runoff to its next destination, likely on private property. More research is needed to determine the best installation practice for this design because the inoculated burlap bags have the potential to burst during decomposition and flow rate fluctuation. It seems that these bags would

need to be replaced regularly as it would be difficult to reinforce this type of filter with additional substrate for the mycelium. That said, it has the potential to be a better, still more financially feasible, method of addressing contaminant presence in channeled flowing water.

      <u>Layered bioswale.</u> Ideal for addressing bacteria and chemicals from agricultural runoff, a layered bioswale, in the form of a mushroom bed, should be installed on a declining slope below the source of contaminants. It can take the form of a standing filter that addresses accumulated surface water or one that filters water out of a pond at a drainage point. Slopes, depths, and flows should be taken into consideration when selecting an installation location (Stamets 2005). *E. coli* is found in warm-blooded animal guts, so the contaminant source for that bacteria is often livestock (Humboldt Baykeeper 2013). In this way, land below manure ponds or feed lots can be an optimal candidate for a layered bioswale.

      Stamets recommends that the surface area of the bioswale be "at least several times larger than the surface area of manure ponds or feeding lots" (Stamets 2005, 68) Once the required dimensions for an effective bioswale are calculated, the amount of spawn and substrate can be determined as well. The ditches or furrows can be dug through manual labor[12] or using heavy machinery[13], then the internal materials can be layered as needed/selected, see table 1.

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[12] Perhaps enthusiastic volunteers with shovels!

[13] We feel that using heavy machinery is intrinsically contradictory to addressing environmental degradation given its extractive and destructive origins.

Table 1. Bottom-up visual of the potential elements within a layered bioswale.

| Stamets 2005: Mushroom Bed | Darwish 2013: Mushroom Bed |
|---|---|
| N/A | 7) 6 in. straw |
| 6) 4-6 in. straw | 6) 2-3 in. wood chips |
| 5) plain cardboard | 5) bulk or bunker spawn |
| 4) sawdust spawn | 4) 2-3 in. wood chips |
| 3) 4 in. corn cobs | 3) bulk or bunker spawn |
| 2) ¼ lb inoculated sawdust per sq. ft. | 2) 2-3 in. wood chips |
| 1) 3-4 in. sawdust or wood chips | 1) plain cardboard |

Juxtaposing the two designs offers a simple comparison between the available methods

for building a layered bioswale. Stamets recommends using corn cobs and sawdust spawn

in the layers to give the mycelium something to really grip on as it grows from the

inoculated sawdust spawn. Darwish, on the other hand, recommends alternatively

layering wood chips and bulk or bunker spawn. This is a potential design option because

the bulked spawn usually starts with sawdust or grain spawn that already colonized a

substrate (wood chips and/or straw), so the mycelium has already formed and is actively

searching for new substrate. The mycelium should, at this point, be able to jump onto the

newly introduced wood chips. Both bioswale designs dictate to thoroughly water the

layers and incorporate several inches of straw as the final layer to keep the moistened

materials in that state. If the filter dries out, it is unlikely that the mycelium will be able to

grow into an effective mycofilter. Besides the field demonstration conducted by Stamets

on his property in Washington (Stamets 2005), we were unable to locate another study that actually installs and monitors a scaled version of a layered bioswale. However, it seems to be the best long-term, accessible, financially feasible design for addressing chemical and bacterial contamination in the context of agricultural runoff.

<u>Baseline Evidence</u>

We conducted two experiments with the intention of contributing baseline evidence for implementing mycofiltration in Arcata to the existing body of literature. Our attempts at doing so are significant because they were conducted with the intention of presenting the background knowledge, methodology, and results so that it is available for use by other interested community members. One of our goals has been to do the preliminary work needed to familiarize budding practitioners with mycorestoration. We pursued this by working to facilitate an understanding of how mycofiltration works *in practice*. Our experiments are the result of transforming the available written and verbal knowledge about mycofiltration into a tangible form through an embodiment of transdisciplinary research. These forms are fully presented in the Experiment Report found in the appendix of *Laboratory to Landscape: Mycorestoration*, so, here, we offer a summarized version of the data and implications that are most relevant to the purpose of this document: implementation of mycofiltration.

Experiment 1A: spawn to wood chip ratio. We conducted this experiment to determine the minimum amount of sawdust spawn that can comfortably be used to inoculate an amount of substrate. In our case, the substrate was alder wood chips and the spawn was Stropharia rugosoannulata (Garden Giant). We compared mycelial colonization of the substrate by chronologically observing samples through the stages of initial growth to collapse. The three tested ratios of wood chips to spawn were 5:1, 10:1, and 20:1 respectively. After several weeks, all three ratios demonstrated the ability to reach peak colonization, but the higher ratios collapsed sooner than the lower ratios. This is because when more mycelium is produced, the substrate is consumed more quickly. We concluded that the 10:1 ratio would be ideal for scaling a mycofilter because it minimizes the amount of spawn that would need to be purchased and/or the time spent bulking spawn14, but is still effective in sufficiently colonizing the substrate.

Preliminary steps in the planning process of installing an effective mycofilter involves budgeting the amount of required materials. In most cases, the minimum material list for the interior of the mycofilter will include spawn, wood chips, straw, cardboard, and burlap. Knowing the most cost and functionally-effective ratio of wood chips to spawn (10:1) allows for a scalable estimation of cost. Fungaia farm was our source for *S. rugosoannulata* sawdust spawn. The spawn was available in 5 lb bags which can inoculate approximately 20 ft² of substrate. By incorporating the 10:1 ratio, we conclude that for each 5 lb bag of spawn, 50 lb of substrate is needed. Budgeting for

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[14] See Darwish 2013, page 138 for a comprehensive method for bulking spawn.

substrate and spawn can be determined using this scaling method (multiplying the amount of spawn by a factor of 10 to come up with the amount of substrate) once site specific dimensions and characteristics are determined, and sources are secured.

Experiment 1B.2: testing mycofilters' ability to remove E. coli from water. This study demonstrates the mycofilters' potential for effectively addressing E. coli contamination. We inoculated alder wood chips with *S. rugosoannulata* sawdust spawn at the 10:1 ratio identified by Experiment 1A[15], then ran effluent water, diluted at a 10:1 ratio and retrieved from the Arcata Wastewater Treatment Plant and Wildlife Sanctuary, through the mycofilter twice. Our results showed an 18% and 14% reduction in E. coli while other available studies[16] average a reduction of about 27%.

These data are relevant to the implementation process in that they add to other data indicating that mycofiltration is effective. All these other data were published by projects that are affiliated with Paul Stamets in some way. As the self-proclaimed inventor[17] of mycorestoration and mycofiltration, he determines access to almost all information known by western science regarding these biotechnologies. We based our experiment designs on what has already been published by working to fill some of the

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[15] This project, including both experiments, has been designed to produce results that can be built upon by additional studies. Using the 10:1 ratio from Experiment 1A to inform building the mycofilters in Experiment 1B.2 is an in-text example of our intentioned use of data.

[16] Further details can be found in the Discussion section of the Experiment Report in the appendix of *Laboratory to Landscape: Mycorestoration*.

[17] Fungi are predisposed as biological filters in their natural ecosystems whether western educated humans publish that information in western literature or not. We understand Stamets' claim as "inventor" of the biotechnology to mean that he was the first to claim the purposeful engineering of mycelium for addressing excessive contamination produced by the capitalist, settler colonial state. His rhetoric on the subject reflects the rhetoric used by violent, white colonial explorers, like Christopher Columbus, claiming to "discover" lands occupied by Indigenous peoples.

perceived need in this subject area while utilizing only local materials and primarily local intellect. Our process was intentionally local to (1) demonstrate the *possibility* of doing mycofiltration in Arcata without outsourcing and (2) conduct research that aligns, as much as possible, with our commitment to this community's longevity[18]. The cumulation of these data can be used as supporting evidence to justify the implementation of mycofiltration at community scales through strategic inclusion in a site-specific implementation plan.

<u>Identifying Potential Sites</u>

Development of the Geographic Information System (GIS) parameters for identifying potential sites via GIS methods was collaboratively completed with Hannah Hartmann as the lead expert in this section. We produced a map that can be used and referenced by authors of local site-specific implementation plans to provide pre-established identification of potential sites using the following parameters found in the Identification of Potential Sites section of *Laboratory to Landscape: Mycorestoration*:

1. Current land use includes livestock grazing and pastureland designation

2. Proximity to local waterways

3. Within 5 miles of Arcata city boundary

4. Slope (2-5 degrees)

As can be seen in figure 2, Bayside Park Farm, Cypress Grove's detention basin, and private property along Jackson Ranch Road have been selected by our criteria as a

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[18] We acknowledge that effective solutions to our interconnected environmental and societal problems require place-based focus.

potential site for installing a mycofilter. The researchers will need to go to the site with at

least the folks who "own[19]" the land and a local mycologist[20], and analyze the

characteristics of that site to determine which type of mycofilter is ideal for that context.

One of the ways we recommend our community successors build out this research is by

doing a long-term study, 3-5 years, to demonstrate possible methodologies for

implementation, collect data showing effectiveness *in situ*, and provide a more in-depth

description of financial feasibility given a scaled mycofilter.

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[19] We acknowledge that ownership of this land, unceded Wiyot ancestral territory, are legal in this settler colonial state's frameworks. However, Arcata and its surrounding areas intrinsically belong to the Wiyot people.
[20] Levon Durr, owner of Fungaia Farm and community partner on this project, has already expressed interest in serving as the mycologist in these local settings.

# Potential Sites for Mycofilter Installation in Arcata, California
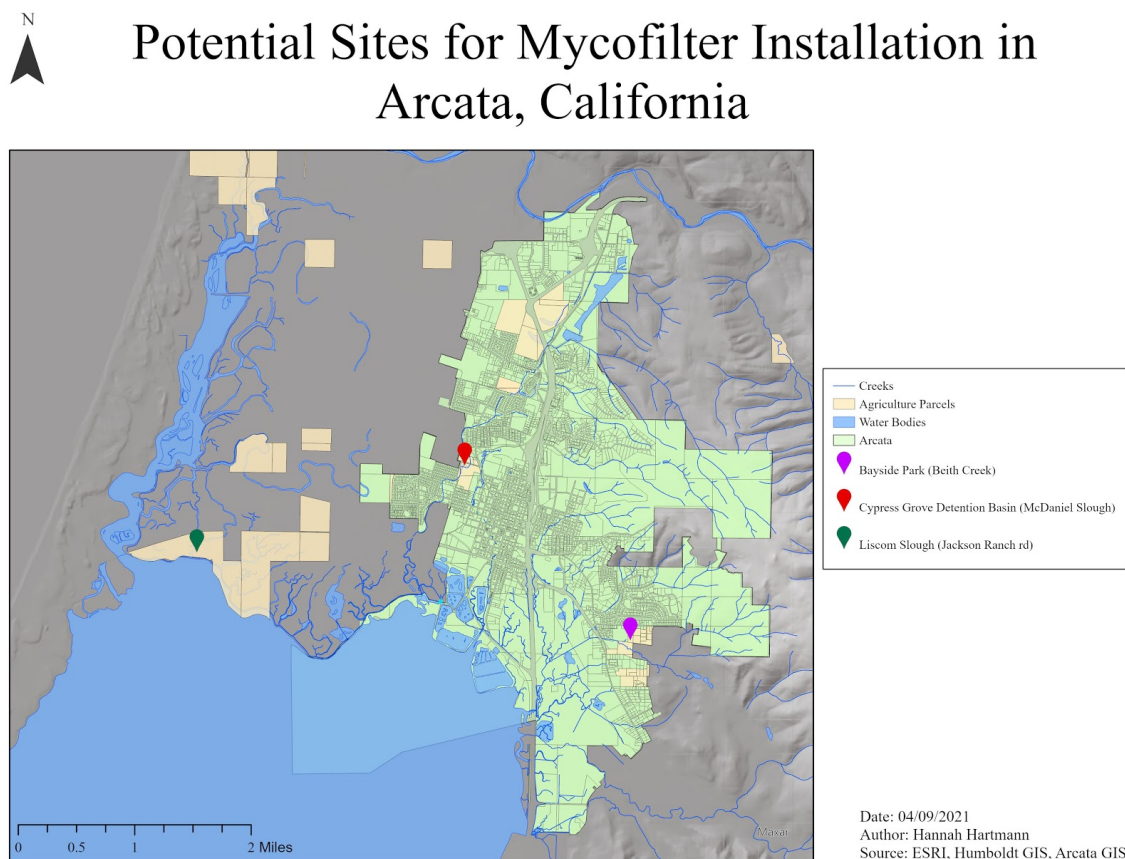


Figure 2. Identified sites within a 5 mile radius of Arcata city boundaries.

## Recommended Next Steps

The entirety of this project, as it exists in the context of the larger thesis, represents the first steps of implementing mycofiltration on a community scale. Its purpose is to compile the currently available knowledge about mycofiltration with a focus on our local context to provide a platform for additional projects to build upon. Our hope is that community members view this work as an opportunity to prioritize environmental and social justice for intrinsic reasons, not individualistic. Mycofiltration is a young study area without an abundance of knowledge and knowers in western science, and especially

without *accessible* knowledge to the communities that can benefit from strategic applications of the biotechnology. Expansion of this knowledge, on community scales done by committed community members from all specialties, is the best method for ensuring mycofiltration is developed for use by anybody for the benefit of everybody[21]. Through our research, we have identified several projects that could be subsequently conducted to appropriately grow and apply mycofiltration.

1. Conversations with Dr. Kaitlin Reed, assistant professor of Native American Studies at Humboldt State University, identified need for mycofiltration in local Indigenous territory downstream from illegal marijuana grows. Settlers making temporary camps for these grows are known to be significant sources of pollution, including but not limited to chemical pesticides and herbicides and fecal coliform bacteria. That pollution runs off into the local waterways and degrades the health of the local ecosystems leading to the degradation of Indigenous peoples' health. Respectful, ethical collaboration with the Indigenous peoples of the region and a local mycologist would be needed to identify specific sites for installation and subsequent steps for implementing mycofiltration in this context.

2. Further research on financial feasibility through experimentation with different methods of substrate pasteurization for optimized use of materials at a minimal cost

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[21] We define "everybody" as humans and more-than-humans (plants, rivers, other animals, etc). Our research is not intended to be owned or co-opted by corporations, governments, universities, or any other institution representative of the capitalist, settler colonial state.

3. More studies on mycofilter effectiveness to further develop a body of literature and hone in on best methods for conducting these experiments

4. Further dissemination of accessible knowledge about mycofiltration's potential for addressing water quality on a community scale in Arcata

   o A plan is in place to pursue this by using footage taken during the experiment process to produce a film to be posted on Youtube and the Fungaia Farm website that documents this thesis project as it exists in this original form.

5. A robust review of local regulations for permitting, permissions, and legality of implementing mycofiltration at community scales

6. Mapping historic differences of the land via settler colonial development that clearly shows the environmental degradation resulting from this version of community structure

7. More studies quantifying *E. coli* counts in water sources in and around Arcata

8. A robust review of the implications of patenting biotechnologies

9. More studies testing the effectiveness of mycofilters at different flow rates

APPENDIX B

Experiment Report

Mycofiltration is the intentional and judicious use of cultivated networks of fungal mycelium to facilitate water quality improvements in engineered ecosystems. This ecologically rational biotechnology is a promising technique for enhancing management of stormwater, graywater, and agricultural runoff (Fungi Perfecti 2015).

Saprophytic mycelium, decomposers in their natural ecosystems, already act as filters for excess nutrients and anthropogenic contaminants. By manually engineering mycofilters to mimic fungal colonization of a substrate and intentionally installing them in a strategic, predetermined location, practitioners co-opt the natural abilities of the fungi to address pollution in water and soil. Mycofiltration is considered a relatively low-cost, ecologically responsible method of addressing pollutants that can remediate an area without the use of harmful chemicals or relocation of contaminated materials (Stamets 2005, La Dena Che' Stamets 2012, and Darwish 2013). Mycofiltration, as an emerging study area with almost all its published studies dating from the early 2000s, has little consistent data verifying its effectiveness in human applications, and even less that is accessible to non-mycologists. However, a broad western scientific understanding of saprophytic fungi's biological functions and existing field and laboratory studies have led us to believe that a *Stropharia rugosoannulata* (Garden Giant)-alder wood chip mycofilter can be a feasible, perhaps preferable option for addressing *E. coli* contamination in Arcata, California. This report details several experiments related to

mycofiltration that are designed to build upon one another with the intention of providing high quality, accessible data that further demonstrates mycofiltration's potential for implementation on a community-scale.

Experiment 1A: Spawn to Wood Chip Ratio

　　　　The purpose of this experiment is to determine the minimum amount of sawdust spawn that can be required to initially inoculate and colonize hardwood wood chips. The ratios that were tested are (wood chip:spawn): 5:1, 10:1, and 20:1. By knowing the minimum amount of spawn needed to sufficiently colonize a quantity of substrate, we can estimate the cost of installing a mycofilter. The measurements are presented as ratios to allow for scalability. This informs part of determining financial feasibility for scaling mycofilters to meet the needs of a site-specific project. These results will be applied to the "Baseline Evidence" section of the implementation plan as well as used to determine the ratios used in Experiment 1B.2.

　　　　Methods. The alder logs used as substrate for this experiment were chipped on-site at Fungaia Farm the same day they were inoculated with sawdust spawn consisting of *S. rugosoannulata*. We quantified the varying ratios through volumetric measurements (US cups). Volumetric measurements allow for a simpler way of scaling the amount of wood chips and spawn to site specific projects. The freshly chipped wood chips were soaked in water in a large plastic bin for approximately 20 minutes prior to inoculation because the alder used was not felled and bucked within a timeframe that allowed for enough moisture to be present.

Throughout this process, no special care was taken to sanitize any of the equipment because one of our objectives is to demonstrate that inaccessible laboratory-based methods using specialized, often expensive equipment is not necessarily needed to implement mycofiltration. When applied to community-scale projects, perfectly sanitary tools are not a typically feasible option. That said, all of the instruments used for this project were checked for general cleanliness prior to use.

Each autoclavable plastic bag with a 0.5 micron filter was labelled according to the substrate and ratio used in the correlating test. For each test, the appropriate amount of wood chips and sawdust spawn was measured out, put into the large mixing bowl, and mixed thoroughly by hand. Once mixed, the material was transferred into the bag labelled with the corresponding substrate and ratio. Then, the bags were sealed using a heat sealer. The containers were left in a room with the temperature at approximately 60-70°F with one air exchange per day for between one to two months.
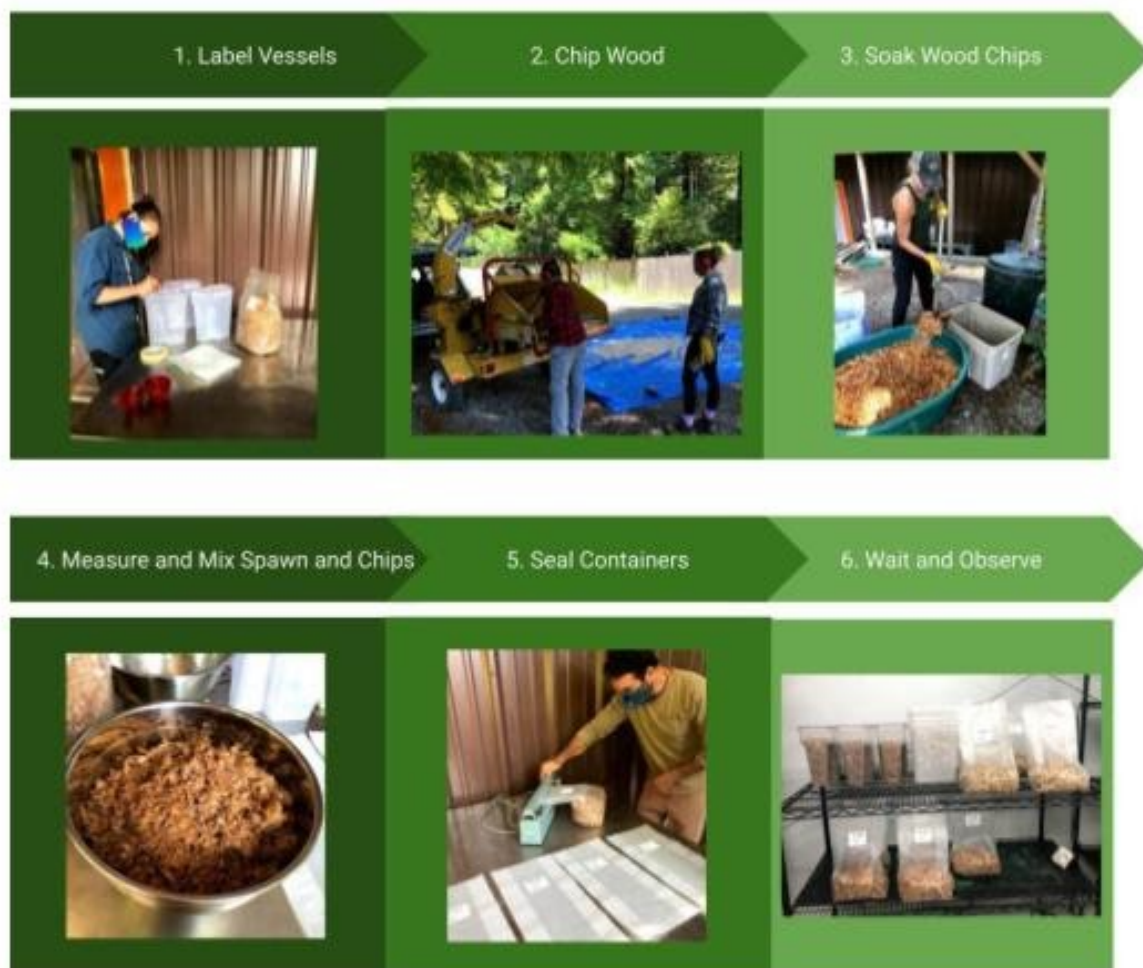
Figure 3. Graphic of methods used to conduct Experiment 1A.

Results. The mycelium in bags with a higher ratio of spawn to wood chips colonized faster than the others, however, all of them showed signs of colonization at levels that would render them usable for installation of a mycofilter. We define peak colonization as the white, weblike structure of mycelium visibly present over at least 80% of the substrate. Once molds and other alternate organisms appear, the filter is deemed to have begun the process of mycelial collapse.



Figure 4. X2 colonization at 28 days after inoculation

At a 5:1 ratio, X2 took approximately 32 days to reach peak colonization then molded and collapsed around 42 days. In slight contrast, Y2 (10:1) and Z2 (20:1) took about 34 days to reach peak colonization then molded and collapsed around 40 days. These data

indicate that, at the lower ratio, in the summer months in Arcata, the window for bulking

the spawn with more substrate for installation is about 10 days. The higher ratios have a

slightly smaller window of about 6 days.

Table 2. Results of Experiment 1A.

| Trial | Ratio (Wood Chip:Spawn) | Sawdust Spawn Amount (US Cups) | Wood Chip Amount (US Cups) | Colonization Achieved |
|---|---|---|---|---|
| X2 | 5:1 | 2.00 | 10 | Yes |
| Y2 | 10:1 | 1.00 | 10 | Yes |
| Z2 | 20:1 | 0.50 | 10 | Yes |

Conclusion. While we noted that the results from Experiment 1A show that we

could use any of the ratios tested, and using ratios like 20:1 and 50:1 would seem better

in terms of scalability and financial feasibility, it is also riskier in terms of mycelial

growth. The more spawn used, the more likely it is to outcompete other organisms and

colonize the substrate, however, using higher spawn ratios is more expensive because (a)

more spawn will need to be initially purchased or (b) more time will need to be spent

bulking the spawn to obtain the desired amount (Darwish 2013). In addition, the higher

ratios were shown to have a slightly smaller window of time for moving the colonized

substrate into the site-specific mycofilter design before the individual units begin to

collapse due to the mycelium running out of available sustenance. We ultimately decided

to use the 10:1 ratio in Experiment 1B.2 because, at that ratio, the spawn is more likely to

satisfactorily colonize the substrate while making this technology financially feasible for

community implementation.

Experiment 1B.2: Testing Mycofilters' Ability to Remove *E. coli* from Water

The purpose of this experiment is to determine if the filters colonized with *S. rugosoannulata* can reduce the amount of *E. coli* in water flowing through them. This demonstrative experiment uses resources entirely local to the area of interest. The samples from the trials were taken from the Arcata Wastewater Treatment Plant and used on-site during the procedure at the Arcata Marsh Research Institute (AMRI).

Methods. The mycofilters were formed in 5-gallon buckets for the purpose of this experiment. The first step in the process was preparing the buckets. We labelled the buckets accordingly: MycoFilter (MF)1, MF2, Wood chip/Sawdust (WS), and Top. MF1 and MF2 were marked with lines to measure 23 volumetric units. These filters have the same ratio and amount of substrate and sawdust spawn. We used the bucket with a higher colonization of mycelium when running the experiment. The remaining buckets were labelled: MF1- Water (W), WS-W, and Collection. MF1-W and WS-W were marked with lines to indicate 11 volumetric units. Then, we drilled several holes in MF1, MF2, WS, and the lids. These were covered with micropore tape to prevent air-borne bacteria from contaminating the filters while the mycelium grew in the wood chips.

A stand made of PVC pipe and plastic connectors was constructed to hold two buckets above another so the water travels via gravity through the first into the second, and through the second into the third on the ground.

Figure 5. Experiment 1B.2 mycofilter design.

The second bucket in the sequence were the filters. Holes in the bottom of the first and

second bucket provide scattered water dispersal that was designed to mimic field

conditions as closely as possible. The next step in the process was inoculating the wood

chips. A small alder was felled, bucked, chipped, and soaked on the morning of inoculation. At Fungaia Farm, the inside of the bottom of MF1 and MF2 were lined with untreated burlap to mimic a field mycofilter. The sawdust spawn was mixed into fresh alder wood chips at the 10:1 ratio determined by Experiment 1A. To do this, buckets were filled to the "9" line with alder chips then filled to the "10" line with sawdust spawn. The contents were hand-mixed until the sawdust spawn was evenly distributed. Then, alder chips were added to the "19" line and the sawdust spawn to the "20" line. The contents were thoroughly mixed again. MF1 and MF2 were fitted with the prepared lids then left in a room with the temperature at approximately 60-70°F with one air exchange per day until they were deemed to be fully colonized.

On the day of the experiment, a little over month after inoculation, the wood chip/sawdust (WS) bucket was prepared at Fungaia Farm using alder chips from the same batch that was chipped for MF1 and MF2. The bucket bottoms were lined with burlap before soaked alder chips were mixed in the bucket with plain sawdust at a 10:1 ratio. A lid was placed on WS while it was transported with MF1 and MF2 to AMRI that day where the water samples would come from the Arcata Wastewater Treatment Plant, trials would be conducted, and samples tested for results.

The wastewater was retrieved from point 3 of pond 1 by tying a string to the handle of a bucket, wading slightly into the water, tossing the bucket, allowing it to fill to at least a quarter of the way up, then hauling the bucket by the string and climbing back out. The water was taken from this location because at this point in the wastewater treatment, the solid chunks have been filtered out, but the water has not been treated for

contaminates. The *E. coli* count in this water is substantial enough to require dilution

before going through the filters in the trials. MF1-W and WS-W were filled to the "1"

line with pond water and then to the "11" line with freshwater from a hose to constitute a

10:1 dilution. The control sample was collected from the water in MF1-W and WS-W.

The bottom of top bucket (Top) was lined with burlap in the same manner as was

done for the filters, then placed at the highest point of the PVC stand. MF1 was placed in

the center position of the stand once the lid and tape were removed. The collection

bucket, from which samples would be taken, was placed on the ground below MF1. The

trial was conducted using the following method. The contents of MF1-W were poured

into Top which then flowed into and through MF1 and into Collection. Sample MF1-1

was secured from Collection using the corresponding, sterilized sample container. Now

empty, MF1-W and Top were rinsed with hose water. MF1-W was placed on the ground

below MF1. Top was replaced at the top of the PVC stand after being relined with fresh

burlap. The contents of Collection were poured into Top allowing the water to move all

the way through the filter for a second time. Sample MF1-2 was collected using the

corresponding, sterilized sample container. Top and Collection were rinsed with hose

water, and MF1 was removed from the stand. The same procedure was repeated with the

materials for WS. Samples collected from the WS trials were labelled WS-1 and WS-2.

Once the samples were collected, we utilized The Coliscan®    Membrane Filter

Method of Micrology Laboratories LLC for *Escherichia coli* and Total Coliforms

(Coliscan®    MF) to detect the amount of *E. coli* in each sample. We ran the tests using

dilution factors of 100 and 1000 in an effort to accurately gage the amount of *E. coli* and

other coliform in each sample. The following procedure was used to conduct the

Coliscan® MF method. 1.750 ml of Coliscan® broth was added to each of the 10

petri dishes using an air displacement micropipette. 90 ml of deionized water (DI) was

poured into a graduated cylinder then transferred into a mixing flask. The micropipette

was used to measure 10 ml of the sample and put into the DI in the mixing flask. This

constituted a 100 dilution factor. The contents of the mixing flask were swirled. A

gridded pad was added to the filter and the pump was turned on. The pipette was used to

draw 1ml of the contents in the mixing flask out then pour it over the filter. The mixing

flask was rinsed with fresh water then also poured over the filter. The inner walls of the

filter were rinsed, and the gridded pad was moved into its respective petri dish with the

broth. For the 1000 dilution, the same procedure was followed except the contents of the

mixing flask received an additional 100 ml of DI and was swirled before 10 ml of the

contents were poured through the filter.

Lids were placed on all of the prepared petri dishes, then put into an incubator at 34.6°C

for 24 hours. After 24 hours, they were removed from the incubator. The results in table 4

were collected by manually counting the colored growths on each filter. Blue growths

represent *E. coli*. Pink, red, and yellow growths represent other coliform

Results. It is known that there is a significant amount of *E. coli* present in the

effluent water found in point 1 of pond 3 according to data regularly collected by the

researchers at AMRI. Each sample began the same as the control samples which were

composed of one part effluent to ten parts fresh water. The results of the Coliscan® MF

method indicate that there were no units of *E. coli* present in the control samples,

however, they are present in all samples that went through a filter.

Each sample was tested using a 100 dilution factor as well as a 1000 dilution

factor because we were unsure which would be more effective for displaying an accurate

description of the amount of *E. coli*. The data in table 3 show that the amount of *E. coli*

decreased by approximately 18% after going through the mycofilter a second time. The

data in table 3 show that the amount of *E. coli* decreased by approximately 14% after

going through the mycofilter a second time. The samples that went through the

mycofilters were the only ones that consistently indicate a decrease in *E. coli* across

dilutions. The samples that went through the filters with only wood chips and sawdust

showed a decrease in *E. coli* only in the 1000 dilution. We measured a decrease from 1

unit to 0 units.

Table 3. Experiment 1B.2 trial results at a 100 dilution factor.

| Location | Coliscan MF (mL) | Dilution Factor | Filter Sample (mL) | True Sample (mL) | *E. coli* CFU | Gen. CFU | Tot. CFU | *E. coli* CFU/100 mL | Tot. CFU/100 mL |
|---|---|---|---|---|---|---|---|---|---|
| MF 1.1 | 1.75 | 100 | 1 | 0.01 | 11 | 159 | 170 | 110000 | 1700000 |
| MF 2.1 | 1.75 | 100 | 1 | 0.01 | 2 | 73 | 75 | 20000 | 750000 |
| TWC 1.1 | 1.75 | 100 | 1 | 0.01 | 3 | 90 | 93 | 30000 | 930000 |
| TWC 2.1 | 1.75 | 100 | 1 | 0.01 | 3 | 105 | 108 | 30000 | 1080000 |

| Location | Coliscan MF (mL) | Dilution Factor | Filter Sample (mL) | True Sample (mL) | *E. coli* CFU | Gen. CFU | Tot. CFU | *E. coli* CFU/100 mL | Tot. CFU/100 mL |
|---|---|---|---|---|---|---|---|---|---|
| C | 1.75 | 100 | 1 | 0.01 | 0 | 265 | 265 | 0 | 2650000 |

Table 4. Experiment 1B.2 trial results at a 1000 dilution factor.

| Location | Coliscan MF (mL) | Dilution Factor | Filter Sample (mL) | True Sample (mL) | *E. coli* CFU | Gen. CFU | Tot. CFU | *E. coli* CFU/100 mL | Tot. CFU/100 mL |
|---|---|---|---|---|---|---|---|---|---|
| MF 1.2 | 1.75 | 1000 | 1 | 0.001 | 7 | 143 | 150 | 700000 | 15000000 |
| MF 2.2 | 1.75 | 1000 | 1 | 0.001 | 1 | 15 | 16 | 100000 | 1600000 |
| TWC 1.2 | 1.75 | 1000 | 1 | 0.001 | 1 | 57 | 58 | 100000 | 5800000 |
| TWC 2.2 | 1.75 | 1000 | 1 | 0.001 | 0 | 50 | 50 | 0 | 5000000 |
| C | 1.75 | 1000 | 1 | 0.001 | 0 | 5 | 5 | 0 | 500000 |

Conclusion. Given the results that were collected, it appears that the mycofilters were able to decrease the amount of E. coli when comparing the unit count between the first cycle through the mycofilter and the second. The mycofilters were able to decrease amounts with only partial colonization and the water only staying in the filter for about a minute. Implementation of mycofilters can take the form of furrows filled with wood chips, and often pasteurized straw, layered with spawn (Stamets 2005) and cardboard (Darwish 2013). With sufficient water flow through the filter and sufficient weather conditions, the mycelium should be able to colonize the substrate making the biofilter viable for filtering "pathogens including protozoa, bacteria, and viruses, silt, and chemical toxins" (Stamets 2005, page 58). The field design allows for much slower, more natural movement of water through the filter meaning the abundance of mycelium and time allotted for it to conduct the process of enzymatic degradation should result in a sizable reduction of contaminants.

Discussion

The primary purpose of this report is to build on existing data that provides evidence, through western scientific methods, of mycofilters' ability to address *E. coli* presence in local water sources. We set out to do this in a way that allows for replication and improvement by any community member. Given my positionality as a student trained in social science, I found myself testing the limits of time and budget bound transdisciplinary research while hoping to test and build upon the scientific data that identify mycofiltration as a responsible, financially feasible, and biologically effective method of addressing *E. coli* in our community. The process of designing and conducting

these experiments, and the data collected from them, fill the need designated by Durr in three of the four instances identified in the Introduction section of the project write up. For reference, these are:

1. Baseline data providing evidence that mycofiltration is effective and financially feasible

2. A written implementation plan that can be presented to potential funding sources and entities with the power to grant permission to implement the biotechnology

3. Overcoming preconceived dispositions about fungi through dissemination of accessible knowledge about mycofiltration

Experiment 1A produced results that were reinforced by Experiment 1B.2. The 10:1 ratio of *S. rugosoannulata* sawdust spawn to alder wood chips allowed for mycelial growth in the subsequent trials. This duplication under different conditions (temperature, time of year, substrate source, vessel, and quantity) makes us confident that this ratio can be used to effectively inoculate scaled up versions of mycofilters in site specific designs. Our mycofilters in Experiment 1B.2 were inoculated in November and allowed to grow through December, compared to the filters in Experiment 1A which were inoculated in June and allowed to grow through July. At the end of the 37 days, the buckets were not considered to be completely colonized, however, we were forced to run the trial on that date due to time and funding constraints. It is highly probable that the colder temperature caused slower growth in Experiment 1B.2. This, in addition to unforeseen delays caused by the COVID-19 pandemic and needing to redesign and reconduct Experiment 1B.2, led to a less demonstrative experiment than we had anticipated.

While these experiments did not overwhelmingly demonstrate fungi's full potential for removing *E. coli* from effluent waste water, they offered additional evidence of mycelium inoculated wood chips having the ability to do so within experiment designs that were far from perfect. Several existing studies collectively indicate percentages of *E. coli* reduction in water passed through mycofilters that are only slightly higher than the percentages achieved by our study, 18% at a 100 dilution and 14% at a 1000 dilution. One study used *Pleurotus ostreatus* (Oyster) mycelium to produce a 26% reduction in *E. coli* (Benedict 2011). Another added mycorrhizae mycelium to a native vegetation bioretention cell resulting in a 29% reduction in *E. coli* in addition to the 66% reduction achieved by the native vegetation alone (Thomas et al. 2009). Perhaps most influentially, the following EPA funded project aims to present bench-test data evidencing the ability of *S. rugosoannulata* to reduce *E. coli* counts at low flow rates (.05 L/min) and high flow rates (2.2 L/min). At the low flow rates, reductions are noted as 27%, 20%, and 18%. At the high flow rates, they are 14%, 11%, and -8%. These percentages are achieved by a 3.96 gallon mycofilter which is slightly smaller than our 5 gallon mycofilters, but contained a comparatively higher percentage of mycelial colonization. Their effluent water is also diluted by a factor of 100 contrasting the factor of 10 used in our study (Stamets et al. 2013). The numbers presented in these examples are within reaching distance of the numbers we produced even with, in terms of western scientific standards, less than perfect methodologies. We argue that this is a significant indication of the mycelium's strength and resilience, and agree with the assertion that mycofiltration,

especially using *S. rugosoannulata*, has the potential for 100% removal of freely suspended *E. coli* in flowing water and sediment bound bacteria.

All of these data are incorporated into our implementation plan as evidence for potential funding sources and entities with the power to grant permission to implement the biotechnology that mycofiltration is indeed effective in addressing *E. coli* contamination. Effectiveness was demonstrated by the reduction percentages in Experiment 1B.2 and outside studies, all of which are either associated or conducted collaboratively with Paul Stamets and heavily funded. The results of Experiment 1A are applicable to calculating the cost of installing and maintaining a site-specific mycofilter. We have inferred that a 10:1 ratio of sawdust spawn to wood chips is relatively cost-effective and successful in promoting mycelial colonization of the substrate at a level that allows the fungi to break down contaminates. When applied to the site-specific volume of a mycofilter, a remediator can calculate the amount of substrate and spawn that needs to be purchased using the method described in the implementation plan. The cumulation of these data, researched in Arcata, serve their purpose of being place based, transdisciplinary western scientific evidence advocating for mycofiltration.

These experiments were designed with the understanding that they would produce some baseline data to work towards understanding the process of implementing mycofiltration on a community scale in Arcata. We have been successful in that endeavor given this project's potential for growth, accessibility, and comparative results. The community now has access to the knowledge displayed in this work which was specifically produced with community applicability and collaboration in mind. We knew

this project would serve as a start to the implementation process and now it is available

for progression as the community sees fit.

APPENDIX C

Identification of Potential Sites for Mycofiltration

This land belongs to the Wiyot people, whose name for this place is Goudi'ni. The current city of focus residing in this area is Arcata, located in California, and the surrounding area, see figure 5.
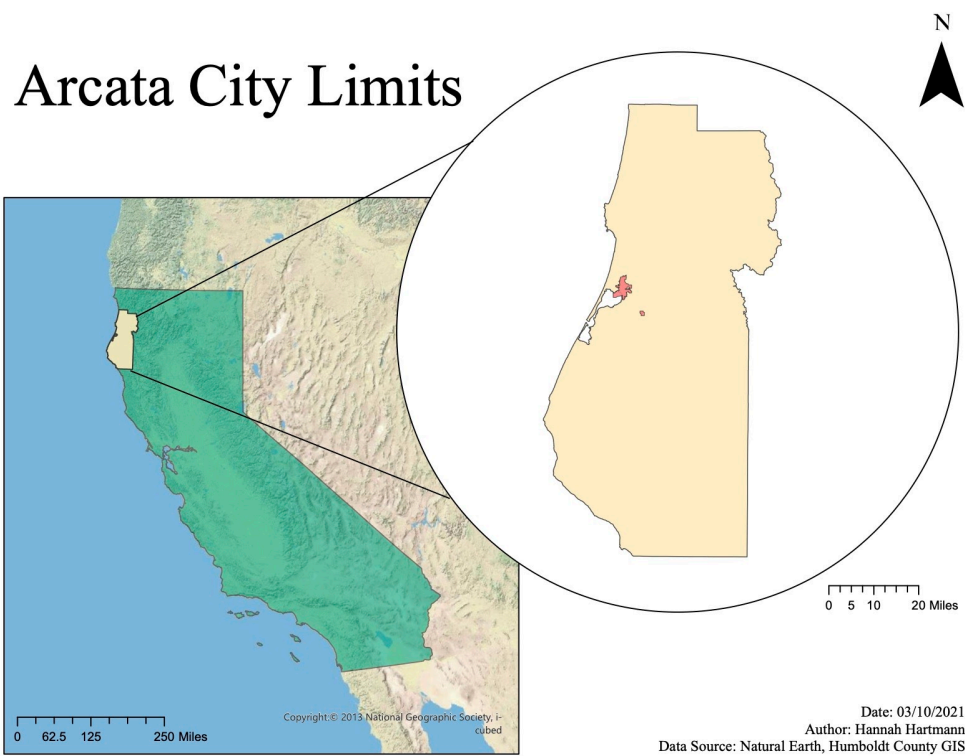


Figure 6. Locator map of Arcata, California.

A map was produced to create a graphical representation that is both aesthetically pleasing and informative for the possible installation locations for microfilters in Arcata, California. A set of parameters were developed to narrow down which sites were optimal

locations and had the highest possibility for mycofilter success. The parameters are as follows:

1. Current land use includes livestock grazing and pastureland designation

2. Proximity to local waterways

3. Within a 5 mile radius of the Arcata city boundary

4. Slope (2-5 degrees)

<u>Parameters</u>

<u>Current land use</u>. The first parameter created to select potential mycofilter installation sites is proximity to pasturelands. Pasturelands are defined as enclosed tracts of farmland designated for the grazing of domesticated livestock. The vegetation in these types of ecosystems largely include members of the family Poaceae (true grasses), Fabaceae (legumes), and a variety of forbs species. This criterion was selected because the concentration of Escherichia coli (E. coli) in waterways neighboring pasturelands has been shown to linearly increase with an increasing amount of pasture in the drainage area (Scott et. al. 2017). Although pastureland was the main designation, Humboldt County Web GIS and the city of Arcata's GIS download parcel jurisdictions classified these lands as agricultural. Therefore, agricultural lands were also included in this criterion.

Proximity to waterways. Another parameter developed is the proximity of these identified pasturelands to nearby waterways. It is known that agricultural lands used for the grazing of livestock pose a serious risk to the ecosystem and human health through the addition of harmful contaminants such as E. coli and other bacteria to local waterways (Burkholder et. al.  2007). These biological pollutants can enter water resources through pathways such as poorly constructed manure lagoons, through atmospheric deposition following wet or dry fallout, or as runoff from nearby farm fields consisting of high amounts of manure (through either intentional application or as livestock bi-product) (Burkholder et. al.  2007). For the purpose of this study, the focus will be on the entrance of E. coli into nearby waterways directly through surface runoff. This means a stream, creek, or other waterbody had to directly intersect the selected parcels.

Arcata city boundary. This parameter indicates that the site selection must occur within our area of interest (AOI), which is the city limit of Arcata, California. In addition to this, the area surrounding Arcata (5 mile radius) was included.
Slope between 2 and 5 degrees. Finally, the steepness of the slope was evaluated. Although there are other factors that impact runoff rates, such as physical alterations of soil (eg. soil crusting), soil texture, and rainfall intensity, slope gradients play an important role in the ability of discharge to either infiltrate or runoff the soil surface (Assouline S. and Ben-Hur M. 2006). Some studies suggest that there is an increase in runoff discharge with increasing slope gradient under varying rainfall intensities (Haiyan et. al. 2015). Although we know that increasing slope gradients lead to higher rates of

runoff, Paul Stamets in his book *Mycelium Running* discusses how mycofilters ideally should be installed on "gently sloped areas below a feeding lot or manure pond, where effluent from the lot or pond continually seeps through" (Stamets 2005, 68). For this reason, and due to the topographic variation Arcata experiences, slopes between 2 and 5 degrees were set as the parameter, as to make sure they were steep enough for sufficient runoff but mild enough for practical installation purposes.

Mapping Methods

A map was created using ArcGIS Pro software in an attempt to give a visual representation of locations in Arcata and in the surrounding area for possible mycofilter application. A series of analytical techniques were used. To begin, the full map was projected into NAD 1983 UTM Zone 10 N to ensure all uploaded data would be in this projection. Digital Elevation Models (DEMs) for Humboldt County were downloaded through USGS GIS data download. It required two files to cover the full extent of Arcata, so the two were combined into one file using the mosaic tool. Arcata city boundary shapefile was acquired through the city of Arcata's GIS download website (CoA GIS), and the DEM was then clipped to only include this city boundary. A hillshade was then created to make sure the new DEM does not have any artifacts, or lines running through the DEM that affect the map both visually and in calculations. This is done by using the spatial analyst tool and selecting hillshade, with the input raster being the newly clipped and mosaiced DEM. Azimuth and altitude were left to the default settings as they are already set for most western maps.

Additional information for parcel and land use designation was acquired through both Humboldt County (HumCo GIS) and the city of Arcata's GIS websites. A 5-mile buffer was set around the city of Arcata shapefile, to be sure parcels designated as agricultural lands slightly out of the city's jurisdiction were included. From CoA GIS, water bodies and waterways (including creeks) were also downloaded and uploaded. With all data uploaded, correctly projected, and overlaid, analysis began. The final parameter was slope angle, and to accomplish this a slope layer was created to indicate areas that included slopes between 2 and 5 degrees.

Results

Three specific sites were identified to fit all set parameters: Beith Creek which runs through Bayside Park, McDaniel Slough that borders Cypress Grove's detention basin, and Liscom Slough at a location along Jackson Ranch Rd, see figure 7.
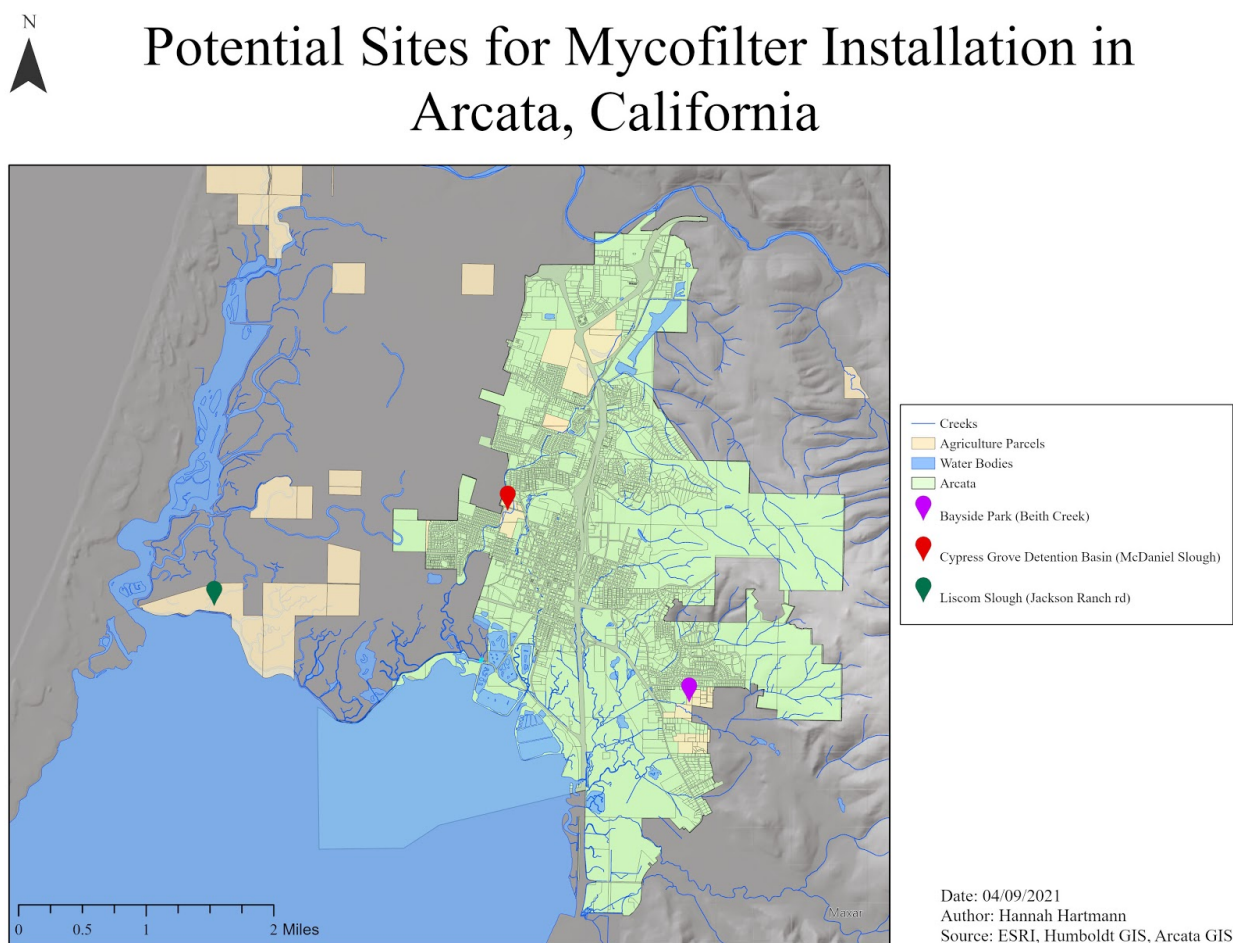
Figure 7. Sites selected within Arcata and the surrounding area to be candidates for

mycofilter application.

Figure 8. Aerial footage of Beith Creek parallel to Bayside Park (Google Maps).



Figure 9. Aerial footage of McDaniel Slough located at Cypress Grove's detention basin

(Google Maps).

Figure 10. Aerial footage of Liscom Slough off of Jackson Ranch Rd (Google Maps).

Conclusion

Mycofiltration is a potential solution for environmental degradation as a site-specific

remediative biotechnology that is plausible for implementation in Arcata, CA. The

parameters identified above were used as criteria for location selection, and resulted in

three sites that are of particular concern based on current land use, proximity to local

waterways, within a 5 mile radius of the Arcata city boundary, and slope. These are Beith

Creek (Bayside Park), McDaniel Slough (Cypress Grove's detention basin), and Liscom

Slough (along Jackson Ranch Rd).

Discussion

        The purpose of this project is to identify ideal sites for mycofilter installation

using GIS methods for inclusion in the *Laboratory to Landscape: Mycorestoration*

project. It adds to the local baseline data identified as missing from existing literature.

        The parcels categorized as "agricultural" by the city and county parcel data were

selected as potential sites for installing mycofilters, however, pasture lands and livestock

grazing have been identified through field observation as locations with more significant levels of general degradation as well as E. coli contamination compared to small scale fruit and vegetable farms. Therefore, these parcels are prioritized in this study as ideal sites. Site-specific data collection at the sites identified in this project is the next step of the implementation process.

While ownership of property is important to consider once a site has been identified, it has not been included in the site identification process itself. Ownership as a factor of site selection does not negate the need to address *E. coli* contamination. Private property owners are not necessarily obliged to prioritize the ecological health of the land they occupy. They generally can and actively do as they please with their property without consideration to community health as a whole (human and more-than-human). In many cases, it can be difficult to convince private property owners that they should invest their time, energy, and money into ecological repair for the benefit of the wider community. Public property that is owned by the city, on the other hand, can be a more accessible option for installing mycofilters because the city is obliged to prioritize community health and well-being. City officials may be more amicable towards mycofiltration because it is their inherent job as civil servants to invest in the community at large. In addition, the available GIS data does not provide specific information regarding who owns which properties thus making these criteria more applicable to site selection instead of potential site identification.

Paul Stamets is the widely accepted "inventor" of mycofiltration and identifies slope as one of the primary criteria to be considered when attempting to install a

mycofilter. This study offers a more critically accurate description of sites that should be prioritized by incorporating slope into the decision-making process. We consider slope on multiple scales. The scale of slope using these GIS methods still only skims the surface of potential installation sites because it is limited by the available data. Our parameters resulted in the identification of three sites that met our input criteria. However, on-the-ground observations combined with local knowledge indicate numerous sites that could also be potential installation sites demonstrating that GIS methods of identification are more effective at broader scales.

The western scientific methods employed make it difficult to sufficiently contextualize the root of local ecological degradation: the genocide of the Wiyot tribe which began in 1848 and continues today through continued settler-occupation in this place (Reed 2020). Since ecocide is directly correlated with genocide in this place, it is essential to acknowledge how this land has been changed since it was stolen by European settlers. GIS data containing traditional Wiyot territory is currently not accessible for our purpose of land acknowledgment in the context of this project. This has been identified as important future research for any project, particularly western scientific research focused on addressing environmental degradation, that is place-based in unceded Wiyot territory.