Environment and Natural Resources Trust Fund

M.L. 2022 Approved Work Plan

General Information

ID Number: 2022-188

Staff Lead: Corrie Layfield

Date this document submitted to LCCMR: June 9, 2022

Project Title: PFAS Fungal-Wood Chip Filtering System

Project Budget: $189,000

Project Manager Information

Name: Jiwei Zhang

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Project Reporting

Date Work Plan Approved by LCCMR: June 27, 2022

Reporting Schedule: March 1 / September 1 of each year.

Project Completion: June 30, 2024

Final Report Due Date: August 14, 2024

Legal Information

Legal Citation: M.L. 2022, Chp. 94, Art. , Sec. 2, Subd. 08f

Appropriation Language: $189,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to identify, develop, and field-test various types of waste wood chips and fungi to sequester and degrade PFAS leachate from contaminated waste sites. This appropriation is subject to Minnesota Statutes, section 116P.10.

Appropriation End Date: June 30, 2025

Narrative

Project Summary: Develop and implement a fungal filtering system that combines the benefits of both waste wood chips and soil fungi to sequester and degrade PFAS leachate from contaminated waste sites.

Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.

Per- and Polyfluoroalkyl Substances (PFAS) are a large group of emerging environmental contaminants that are globally ubiquitous. These "forever chemicals" are highly recalcitrant and possess the capability to persist in the environment and accumulate in living organisms. PFAS exposure is increasingly being linked to a variety of adverse health outcomes. Regarding this, strategies to remediate PFAS polluted environments are urgently needed.
Not only the water systems but also soils have been found to be large reservoirs of PFAS chemicals, even in locations distant from any known source. The ubiquitous presence of PFAS in soils around the world is concerning and will likely have long-term ramifications on attempts to remediate contaminated waters. In Minnesota, MPCA (Minnesota Pollution Control Agency) and MDH (Minnesota Department of Health) have discovered PFAS pollution throughout the state since the early 2000s. Among these sites, landfills represent a large repository for PFAS, with 98 out of 101 MPCA tested landfill sites found PFAS and alarmingly 59 sites had detectable levels exceeding health guidelines. A cost-effective means to intercept leachate from landfills and remove PFAS pollutants, before they can escape to groundwater, will be critical for conserving Minnesota's environment.

What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.

Using woodchips which have been proven to filter other pollutants such as nitrogen and metals from water combined with wood decay fungi that have demonstrated good results in removing contaminants.
We seek to develop a mycoremediation system as an alternative method that integrates fungi with low-cost forest residues to filter and degrade PFAS. Sequestration and degradation of PFAS by the "fungal-woodchips" filtering system will be estimated after fungal acclimation in cultures spiked with both wood substrate and PFAS compounds. Fungal pathways involved in PFAS degradation will be investigated through isotopic metabolites mapping, thus enabling us to dissect the fundamental bioprocesses and guide their industrial applications. PFAS that is sequestered by the filtering system will be sent to the microwave-assisted gasification system for further destruction ("pyrolysis"), eliminating any remaining pollutants. A potential biochar byproduct from pyrolysis can be recycled as the PFAS absorption media, further improving the values of the "fungal-woodchips" filtering system.

What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?

Development of a cost-effective method to intercept PFAS before it enters groundwater and degrades it in-situ using wood-decay fungi to begin controlling PFAS pollution from the array of contaminated sites throughout Minnesota. Establishing a platform for the further study of PFAS bioremediation to inform long-term efforts to remove PFAS from soil and water, and providing insights into fungal metabolic capabilities that can be applied to improve not just PFAS remediation but a variety of other applications.

Project Location

What is the best scale for describing where your work will take place?

Statewide

What is the best scale to describe the area impacted by your work?

Statewide

When will the work impact occur?

During the Project and In the Future

Activities and Milestones

Activity 1: Test fungal capacities in sequestering and degrading PFAS

Activity Budget: $77,200

Activity Description:
The goal of activity one is to identify fungal species able to degrade PFAS. Pure cultures of wood decay fungi and fungal consortia in landfill leachate and sewage sludge will be challenged by spiking PFAS substance in woodchips media, acclimating their capacities of PFAS sequestration and degradation monitored by GC/LC-MS and ion-selective electrode (for F-), respectively. Up to two dozen wood-decay fungi will be inoculated onto liquid or solid growth media containing the perfluorooctanoic acid (PFOA), a representative PFAS, and wood chips to test fungal capacities to degrade PFAS via monitoring F- liberation (i.e., defluorination). The best performance species will be further optimized for the cultural conditions to improve the defluorination rate, PFOA degradation will be confirmed by LC-MS/MS, and PFOA sequestration will be tested by measuring the acetonitrile extracted residuals in wood-mycelium. A lab-scale continuous microwave-assisted gasification (CMAG) system will be used to convert the woodchips/fungal biomass from the above cultures, and the composition of the syngas and biochar will be analyzed to confirm the complete removal of PFAS.

Activity Milestones:

Description	Approximate Completion Date
Screen Fungal Defluorination Capacities	December 31, 2022
Optimize Cultural Conditions for PFAS Defluorination	April 30, 2023
Confirm the PFAS Degradation and Sequestration by LC-MS	June 30, 2023
Test the Combined Use of CMAG for PFAS removal	August 31, 2023

Activity 2: Investigate the fungal degradative pathways of PFAS

Activity Budget: $54,400

Activity Description:
In this activity, the best-performing wood decay fungi obtained from the defluorination screening process will be used to further study the degradative mechanisms. Fungal strains will be cultured in liquid or solid Highley's media spiked with C13-labeled PFOA, facilitating metabolite identification. PFAS carbons incorporated into degradation metabolites will be identified during cultivation stages using HPLC in tandem with hybrid quadrupole orbitrap high-resolution MS, thus reconstructing the degradative path. Ultra-high mass accuracy of the mass spectrometer will be employed to enable us to reveal the exact molecular formula of molecular ions. Reported PFAS metabolites throughout the literature will be used to enhance the annotation confidence of PFAS degradation products. Enzyme activities will be measured to map the metabolite pathway to understand the degradative mechanisms. We envision this objective will be “high-reward” and will provide first-ever insights on fungal pathways of PFAS degradation. This fundamental understanding will enable us to optimize the remediation method by such as targeting culturing conditions or further investigating gene pathways for genetic engineering.

Activity Milestones:

Description	Approximate Completion Date
Identify the PFAS Degradation Pathway Metabolites	September 30, 2023
Measure the Enzyme Activities During PFAS Degradation	October 31, 2023
Reconstruct the Degradative Path of PFAS	December 31, 2023

Activity 3: Monitor the performance of the “fungal-woodchips” filtering system on landfill leachate

Activity Budget: $57,400

Activity Description:

Working with collaborators from Wenck Environmental Consulting suitable landfill field sites near the Twin Cities will be identified. Lab-scale tests followed by field setup will be performed to remediate PFAS using the fungal-woodchip filtering system. The lab-scale reactors to test PFAS removal from the contaminated field samples will be first designed and tested. Landfill leachates containing PFAS compounds will be added to fungal pre-colonized woodchips in flask reactors for monitoring PFAS degradation using LC-MS/MS, and key factors will be optimized to improve the remediation efficiency. For field setup, Wenck will support the Zhang lab by providing access to the site and equipment necessary for the installation of the pilot filtering system. Landfill leachate will filter through the fungi-woodchip application and PFAS concentrations will be monitored over the course of the experiment. Long-term monitoring of the field setup will be leveraged by other funds. Pyrolysis via CMAG will be performed in tandem with the filtration system to further remove PFAS and recycle wood chips to produce biochar. Pyrolysis will be performed using the equipment and expertise of Dr. Ruan who has agreed to allow us the use of the well-established pyrolysis lab to produce biochar.

Activity Milestones:

Description	Approximate Completion Date
Consult with Wenck-Stantec For Field Test	September 30, 2022
Lab-scale Test and Optimization with Landfill Leachate	February 28, 2024
Set Up Field Test for the Long-term Monitoring of PFAS Leachate	May 31, 2024
Final Data Compilation and Project Write-up	June 30, 2024

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Roger Ruan	University of Minnesota	Co-PI on the project, working on using "pyrolysis" to heat treat the sequestered PFAS for the complete removal.	Yes
Ed A. Matthiesen	Wenck Environmental Consulting	Industrial Partner	No

Dissemination

Describe your plans for dissemination, presentation, documentation, or sharing of data, results, samples, physical collections, and other products and how they will follow ENRTF Acknowledgement Requirements and Guidelines.
Our research findings will be widely disseminated through published journal articles, community outreach through the UMN Biotechnology Institute, and K-12 partners. Our work will also be shared with industry partners Wenck-Stantec and SKB environmental, where findings will be used to further the development of remediation methods for PFAS compounds. In addition to industry and community partners, the audience for our work will also include other researchers and institutions investigating the environmental effects of PFAS, PFAS degradation, and fungal metabolism. The insights into PFAS effects on fungi, fungal degradation pathways, and PFAS degradation byproducts will be useful to a wide range of fellow researchers and industry professionals.
In our dissemination efforts, we will acknowledge the financial support from the Minnesota environment and natural resources trust fund in project publications,
signage, and other public communications and outreach related to work completed using the appropriation. The acknowledgment will occur, as appropriate, through the use of the trust fund logo or the inclusion of language attributing support from the trust fund. We understand this is important to enable the public to know about the support of the Environment and Natural Resources Trust Fund and to make the funding uses transparent.

Long-Term Implementation and Funding

Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this work be funded?
The data gathered will be used to inform further research into the genes and metabolic pathways involved in PFAS degradation and to further improve the field application. Studies of the molecular mechanisms of PFAS degradation will be further investigated by applying for other related academic grants from such NSF.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount
Personnel								
Assistant Professor		PI			36.5%	0.3		$30,526
Research Assistant		Assist PI in carrying out project			19.9%	2		$124,543
Professor/Director		Co-PI			36.5%	0.04		$9,995
							Sub Total	**$165,064**
Contracts and Services								
Lab services	Sub award	The UofMinnesota Center for Mass Spectrometry and Proteomics or the Bridget Ulrich lab at Natural Resources Research Institute (Duluth, MN) will be performing LC/MS to test PFAS degradation				1		$2,936
							Sub Total	**$2,936**
Equipment, Tools, and Supplies								
	Tools and Supplies	Laboratory supplies and tools in year 1, including incubator or small equipment (<5,000$), chemicals, enzymes, dishes, flasks, and other lab consumables related to performing fungal tests on PFAS degradation	Materials to setup and perform laboratory procedures for culturing, screening, laboratory scale testing, and analysis					$10,000
	Tools and Supplies	Laboratory supplies in year 2, including chemicals, enzymes, dishes, flasks, and other lab consumables related to performing both lab- and field-scale PFAS degradation	Install lab and field applications and collect samples to facilitate monitoring of PFAS degradation					$8,000
							Sub Total	**$18,000**
Capital Expenditures								
							Sub Total	**-**
Acquisitions and Stewardship								

							Sub Total	**-**
Travel In Minnesota								
	Miles/ Meals/ Lodging	400 miles/yr, two people approx. 12 trips (up to six trips for installation and for monitoring)	Travel to and from field site for installation and monitoring (year 2)					$500
							Sub Total	**$500**
Travel Outside Minnesota								
	Conference Registration Miles/ Meals/ Lodging	Support 2 people to travel to take conferences.	Taking conferences to share the research.	X				$1,500
							Sub Total	**$1,500**
Printing and Publication								
	Publication	Publishing fee	Publish papers					$1,000
							Sub Total	**$1,000**
Other Expenses								
							Sub Total	**-**
							Grand Total	**$189,000**

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
Travel Outside Minnesota	Conference Registration Miles/Meals/Lodging	Support 2 people to travel to take conferences.	PFAS is an emerging nationwide environmental problem, and its remediation needs a lot of fundamental understanding which can be obtained by sharing research at national conferences. Taking the large group conferences will help us understand the most recent progress of the research on PFAS remediation at the national level and exchange ideas with peers, thus better serving the development of our proposed bioremediation method that can be used to solve the local PFAS problem in Minnesota.

Non ENRTF Funds

Category	Specific Source	Use	Status	$ Amount
State				
			State Sub Total	**-**
Non-State				
In-Kind	Waived UMN overhead	Waived UMN overhead	Secured	$231,000
			Non State Sub Total	**$231,000**
			Funds Total	**$231,000**

Attachments

Required Attachments

Visual Component

File: 145692a2-310.pdf

Alternate Text for Visual Component

The use of a combination of woodchips and wood-decay fungi to control PFAS leachate originating from contaminated waste sites. Rain or irrigation carries pollutants that can then contaminate areas outside the waste site eventually entering groundwater. This leachate is filtered through the fungal biofilter installed around the periphery...

Optional Attachments

Support Letter or Other

Title	File
Wenck Letter of Support	d0d00070-f03.pdf
CMSP Letter of Support	b9d916f7-fdb.pdf
LCCMR Proposal	27b16835-b8a.pdf
Institutional Approval to submit	966a5844-045.pdf
Approved Research Addendum	b475654b-ce4.pdf
Background Check Certification Form_2022-05-23	5f13f7f2-e7a.pdf

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

To whom it may concern:
The work plan has been revised and updated according to LCCMR comments.
Please help proceed with the research plan.
Thank you!
Regards,
Dr. Jiwei Zhang

Additional Acknowledgements and Conditions:

The following are acknowledgements and conditions beyond those already included in the above workplan:

Do you understand and acknowledge the ENRTF repayment requirements if the use of capital equipment changes?

N/A

Do you agree travel expenses must follow the "Commissioner's Plan" promulgated by the Commissioner of Management of Budget or, for University of Minnesota projects, the University of Minnesota plan?

Yes, I agree to the UMN Policy.

Does your project have potential for royalties, copyrights, patents, or sale of products and assets?

Yes

Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?

Yes

Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?

No

Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

No

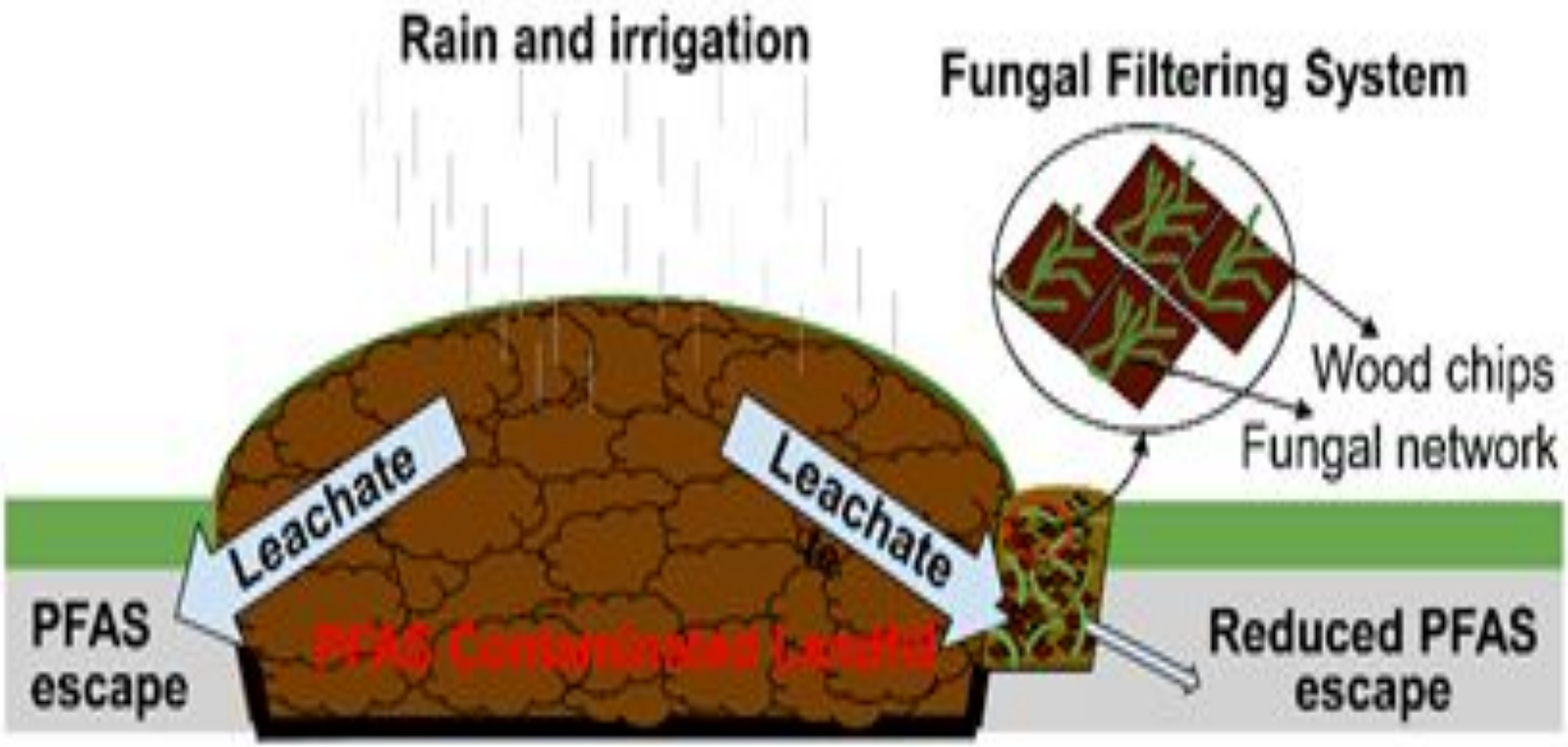

Figure 1: Using a fungal Filter to reduce PFAS leachate