



Environment and Natural Resources Trust Fund

M.L. 2024 Approved Work Plan

General Information

ID Number: 2024-175

Staff Lead: Tom Dietrich

Date this document submitted to LCCMR: June 10, 2024

Project Title: Enabling Nature to Destroy Environmental PFAS Contaminants

Project Budget: \$378,000

Project Manager Information

Name: Romas Kazlauskas

Organization: U of MN - College of Biological Sciences

Office Telephone: (612) 624-5904

Email: rjk@umn.edu

Web Address: <https://cbs.umn.edu/>

Project Reporting

Date Work Plan Approved by LCCMR: June 20, 2024

Reporting Schedule: June 1 / December 1 of each year.

Project Completion: June 30, 2026

Final Report Due Date: August 14, 2026

Legal Information

Legal Citation: M.L. 2024, Chp. 83, Sec. 2, Subd. 08i

Appropriation Language: \$378,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to identify enzymes and microbes that can break down soil-based per- and polyfluoroalkyl substances (PFAS) into nontoxic elements. This appropriation is subject to Minnesota Statutes, section 116P.10.

Appropriation End Date: June 30, 2027

Narrative

Project Summary: Low-levels of perfluoroalkyl substances (PFAS) contaminate water and soil in Minnesota. We propose to identify enzymes and microbes that break down PFAS, making them non-toxic.

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

Perfluoroalkyl substances (PFAS) comprise nearly 5,000 different synthetic chemicals that are used widely in products and industrial processes. A few of the most studied PFAS are hazardous to human health. PFAS contamination has been found in water, sediment, and soil throughout Minnesota. The Environmental Protection Agency recently ruled that even low-level contamination with PFAS is harmful.

Most PFAS contamination is low-level contamination over wide areas. A mild, environmentally compatible, in-situ decontamination of PFAS is needed for these large areas in Minnesota.

In nature, microbes degrade fallen branches and trees and recycle the material. The recycling is done by enzymes - special proteins within these microbes that digest the material as food. We have used these natural digestive processes to degrade chemical contaminants, but not PFAS, which have sometimes been called forever chemicals. We hypothesize that PFAS are a poor food source for microbes. For this reason, PFAS-degrading microbes are not common in nature.

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.

We propose to accelerate the formation of PFAS-degrading microbes. First we propose to identify an enzyme that starts the degradation of PFAS and second to identify a microbe that produces such an enzyme and tolerates the fluoride produced.

PFAS have been shown to degrade using harsh chemical conditions that clip the end of the PFAS molecules and make non-toxic products. This cannot be done naturally in the environment but must be done in a factory environment. We hypothesize that enzymes that naturally carry out similar reactions can be used in natural settings. Identifying such an enzyme would eliminate the need for harsh chemical conditions and allow PFAS to degrade at the contamination site. One half of the project is to find such enzymes. Manufacturing and testing the enzyme on contaminated materials will come next.

To reduce the cost of treatment, we hypothesize that a microbe could make the enzyme at the contamination site, similar to the way natural degradation of trees works. We propose to identify microbes that grow well in the presence of PFAS. These microbes will be tested for their ability to grow and degrade PFAS contaminants to restore the environment to its original clean state.

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?

- Identification of five enzymes that clip the ends of PFAS at the 2-position to start their degradation.
- Identification of five enzymes that clip the ends of PFAS at the 3-position to start their degradation.
- Laboratory test of decontamination of PFAS-contaminated materials with enzymes.
- Laboratory test of decontamination of PFAS-contaminated soils with enzymes.
- Identification of five microbes that grow well in the presence of PFAS and fluoride.
- Identification of a PFAS and fluoride--tolerant microbe that produces the enzymes.
- Laboratory test of decontamination of PFAS-contaminated materials.
- Laboratory test of decontamination of PFAS-contaminated soils.

Project Location

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

Region(s): Metro

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

Statewide

When will the work impact occur?

In the Future

Activities and Milestones

Activity 1: Identifying enzymes for decontamination of PFAS-contaminated soil

Activity Budget: \$189,000

Activity Description:

First, we propose to identify enzymes that can clip the ends of the PFAS molecules. This step weakens the molecules so they fragment into non-toxic pieces. We will detect the clipping by adding a reagent that turns the solution purple when it encounters the non-toxic pieces. We previously developed this method with funding from a Fortune 50 company. We will start the current project by searching public databases of millions of enzymes to find those that clip the ends of molecules similar to PFAS. We will narrow the results to about forty candidates. We will experimentally test these candidates by first making the enzymes and then measuring if they can clip PFAS molecules. We expect that approximately ten of the candidates will clip the PFAS molecules. Second, we will make larger amounts of the most promising five enzymes and test their ability to defluorinate 3,3-difluorobutanoic acid, 2,2-difluorobutanoic acid, perfluorobutanoic acid and perfluorooctanoic acid. We will mix enzymes and the PFAS compounds, allow them to sit a room temperature, and then remove a sample, mix it with our reagent and look for the purple color. The intensity of the purple color will reveal how much PFAS has been decontaminated.

Activity Milestones:

Description	Approximate Completion Date
Identification of five enzymes that clip the PFAS at the 2-position to start degradation.	November 30, 2024
Identification of five enzymes that clip the PFAS at the 3-position to start degradation.	May 31, 2025
Laboratory test of decontamination of PFAS-contaminated materials with enzymes.	November 30, 2025
Submission of manuscript for publication in an open-access peer-reviewed journal	November 30, 2025
Laboratory test of decontamination of PFAS-contaminated soils with enzymes.	May 31, 2026

Activity 2: Decontamination of PFAS-contaminated soil with microbes

Activity Budget: \$189,000

Activity Description:

First, we propose to identify microbes that grow well the presence of PFAS. We will start by searching public databases of millions of microbes to identify those that may grow in the presence of PFAS. We will test the microbes by growing them with and without PFAS and fluoride and choosing the ones that show little or no difference. The cloudiness of the solutions reveals how well the microbes grow. We expect to focus on five microbes that are able to tolerate PFAS and their degradation product fluoride. Next, we propose to use five that contain an enzyme identified in Activity 1 to clip the PFAS molecules. Finally, we will test the enzyme-containing microbes for their ability to decontaminate PFAS-contaminated soil and water. The reagent from Activity 1 will turn purple as the non-toxic fragments form.

Activity Milestones:

Description	Approximate Completion Date
Identification of five microbes that grow well in the presence of PFAS and fluoride	November 30, 2024
Identification of a PFAS and fluoride-tolerant microbe that produces the enzymes	May 31, 2025
Laboratory test of decontamination of PFAS-contaminated materials	November 30, 2025
Laboratory test of decontamination of PFAS-contaminated soils	May 31, 2026
Submission of two manuscripts to an open-access, peer-reviewed journal	May 31, 2026

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Lawrence Wackett	U of MN - College of Biological Science	Co-Investigator. Lawrence Wackett is best known for the Biodegradation Database, which predicts how microbes degrade man-made chemicals in the environment. Prof. Wackett's role is to identify microbes suitable for the biodegradation of polyfluorinated substances (PFAS) in the environment.	Yes

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.

The results of the research will be published in open access, peer-reviewed journals and posted on publicly accessible sites such as BioRxiv.org. The peer review ensures that the logic and supporting data justify the conclusion made. The open access ensures that no journal subscription is required to read and view the results. Costs for open access publication fees are included in the budget.

The results will also be shared in public databases used by specialists. One database (RAPID; Reaction Activity Prediction IDentification) is maintained by us at the University of Minnesota contains information on important enzyme reactions, including those with fluorinated compounds. A second database is enviPath, which predicts microbial biotransformation of organic environmental contaminants. We will ensure that all new reactions discovered in our research are included in both of these databases.

To reach state agencies, Minnesota companies and concerned citizens, we will make direct contacts and also partner with the Institute on the Environment at the University of Minnesota. The Institute on the Environment has a broad reach to state agencies, Minnesota companies and concerned citizens, and can help us by hosting public sessions on PFAS biodegradation. We will directly contact researchers at the Minnesota Pollution Control Agency, 3M, Cargill, and BIOMADE to discuss next steps for applications including the scale-up of the manufacture of microbes or enzymes. In year two of the project, we will make and post a YouTube video on the biodegradation of PFAS intended for non-specialists. This video will also be shown at the Minnesota State Fair.

All written communications will include the following sentence at a minimum "Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR)." If possible and relevant, the following sentences will be included "The Trust Fund is a permanent fund constitutionally established by the citizens of Minnesota to assist in the protection, conservation, preservation, and enhancement of the state's air, water, land, fish, wildlife, and other natural resources." - and - "Currently 40% of net Minnesota State Lottery proceeds are dedicated to growing the Trust Fund and ensuring future benefits for Minnesota's environment and natural resources." The video presentation planned for display at the State Fair will include a verbal acknowledgement ("Funding provided by the Minnesota Environment and Natural Resources Trust Fund.") and display an ENRTF logo, which is available at the URL:

https://www.lccmr.mn.gov/pm_info/manager_info_index.html#acknowledgement.

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?

This proposal will identify a path by which the forever PFAS chemicals will no longer last forever. The next stage of the project would be a scaling up from the laboratory to field trials to estimate costs and demonstrate effectiveness. We will expand the team to include engineers and environmental experts, including those at the Minnesota Pollution Control Agency, and community participants for field testing. We are exploring additional funding from companies and from BioMADE, a national center for furthering bio-manufacturing that involves companies such as Cargill and is headquartered at the University of Minnesota.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount
Personnel								
Postdoctoral Research Fellow		Identification of enzymes that degrade PFAS			21%	2		\$151,000
Postdoctoral Research Fellow		Identification of microbes that support PFAS degradation			21%	2		\$151,000
Undergraduate research student		Measurement of enzyme properties			0%	0.2		\$8,000
Undergraduate research student		Measurement of microbe properties			0%	0.2		\$8,000
							Sub Total	\$318,000
Contracts and Services								
Genwiz	Professional or Technical Service Contract	DNA sequencing				0.02		\$1,000
							Sub Total	\$1,000
Equipment, Tools, and Supplies								
	Tools and Supplies	Supplies for enzyme and microbe discovery	Chemicals and supplies for measurement of PFAS fragmentation and for the growth of microbes and preparation and characterization of enzymes and enzyme variants. Typical suppliers are Sigma-Aldrich, Fisher Scientific, Integrated DNA Technologies and Twist Biosciences.					\$53,000
							Sub Total	\$53,000

Capital Expenditures								
							Sub Total	-
Acquisitions and Stewardship								
							Sub Total	-
Travel In Minnesota								
							Sub Total	-
Travel Outside Minnesota								
							Sub Total	-
Printing and Publication								
	Publication	Open access fees for three peer-reviewed publications	Permit everyone to access the publications without the need for a subscription to the journal.					\$6,000
							Sub Total	\$6,000
Other Expenses								
							Sub Total	-
							Grand Total	\$378,000

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
---------------	---------------------	-------------	--

Non ENRTF Funds

Category	Specific Source	Use	Status	\$ Amount
State				
In-Kind	University of Minnesota	Indirect costs of research	Secured	\$208,000
			State Sub Total	\$208,000
Non-State				
			Non State Sub Total	-
			Funds Total	\$208,000

Attachments

Required Attachments

Visual Component

File: [1ba96cb5-f78.pdf](#)

Alternate Text for Visual Component

Map of PFAS contamination in Minnesota and structure of PFAS showing the weak link...

Supplemental Attachments

Capital Project Questionnaire, Budget Supplements, Support Letter, Photos, Media, Other

Title	File
UMN Sponsored Projects Authorization to submit	83b11c76-14f.pdf
Research Addendum revised 2024-279_final	cea2df8c-af9.pdf

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

4 05/09/2024 Activities and Milestones We did not see obvious changes needed to your work plan following peer review. If we missed something, please make those changes now.

Most of the changes were to the research addendum as detailed in the response to the reviewers. The changes made to the works plan were:

We have expanded the project outcomes from five to eight to match the proposed milestones.

We have expanded the milestones to eight and adjusted the completion dates to align with the reporting dates.

Added some more specific details to activities 1 and 2.

5 05/10/2024 General Information Please indicate that there is potential for royalties, patents, or intellectual property.

completed

6 05/10/2024 Activities and Milestones Activities missing deliverable and synthesizing milestones e.g. agency report, publications, white paper. How will this information be captured in a manner that others can use and implement, especially in context of your dissemination plan?

Two more milestones have been added submission of one manuscript to an open-access, peer-reviewed journal (Nov 2025) and two more manuscripts in May 2026. A patent application is also possible, but since it is uncertain, it has not been added to the milestones.

7 05/10/2024 Dissemination Please include in the Dissemination section a statement about how Environment and Natural Resources Trust Fund will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the ENTRF Acknowledgment Guidelines.

The following text has been added:

All written communications will include the following sentence at a minimum "Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR)." If possible and relevant, the following sentences will be included "The Trust Fund is a permanent fund constitutionally established by the citizens of Minnesota to assist in the protection, conservation, preservation, and enhancement of the state's air, water, land, fish, wildlife, and other natural resources." - and - "Currently 40% of net Minnesota State Lottery proceeds are dedicated to growing the Trust Fund and ensuring future benefits for Minnesota's environment and natural resources."

The video presentation planned for display at the State Fair will include a verbal acknowledgement ("Funding provided by the Minnesota Environment and Natural Resources Trust Fund.") and display an ENRTF logo, which is available at the URL: https://www.lccmr.mn.gov/pm_info/manager_info_index.html#acknowledgement.

8 05/10/2024 Budget Please clarify the services mentioned in your "tools and supplies" budget line. Will you be contracting for lab services? If so, please recategorize those expenses as professional/technical contracts, with each service on its own budget line.

A separate entry for DNA sequencing services with Genwiz has been added. There is no contract involved; when we send them a sample, they return the DNA sequence and bill us \$10. The estimated total cost is \$1,000 (100 samples). The description of tools and supplies has been revised.

OLD

Chemicals for measurement of PFAS fragmentation and for the growth of microbes and preparation of enzymes. Services include samples of microbial strains, synthesis of DNA, sequencing of DNA.

NEW

Chemicals and supplies for measurement of PFAS fragmentation and for the growth of microbes and preparation and characterization of enzymes and enzyme variants. Typical suppliers are Sigma-Aldrich, Fisher Scientific, Integrated DNA Technologies and Twist Biosciences.

June 10, 2024 response to e-mail:

1. Review and correct any errors in the project manager contact information on the Project Collaborators page (Tab 2).

The project manager contact information is correct. No changes were needed.

2. Review your appropriation language, shown at the bottom of Tab 4 (Narrative), to confirm the scope of your project as provided in law.

The appropriation language accurately defines the scope of the project. No changes were needed.

3. Respond to any remaining staff requests for revisions found on Tab 10 (Comments and Revisions). This may include updating the title of your project, which may have been changed to follow appropriation customs or other reasons.

The staff has not made any requests for revisions.

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?

N/A

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

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

Does your project include the pre-design, design, construction, or renovation of a building, trail, campground, or other fixed capital asset costing \$10,000 or more or large-scale stream or wetland restoration?

No

Do you propose using an appropriation from the Environment and Natural Resources Trust Fund to conduct a project that provides children's services (as defined in Minnesota Statutes section 299C.61 Subd.7 as "the provision of care, treatment, education, training, instruction, or recreation to children")?

No