Environment and Natural Resources Trust Fund
2018 Request for Proposals (RFP)

Project Title: **ENRTF ID: 069-B**

Microbial Transformation of Plastics in Minnesota Waters

Category: B. Water Resources

Total Project Budget: $ 506,000

Proposed Project Time Period for the Funding Requested: 3 years, July 2018 to June 2021

Summary:

This project will study the ability of indigenous bacteria to biodegrade plastics found in contaminated waters across the state of Minnesota and determine their fates and potentially toxic by-products.

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Sponsoring Organization: U of MN

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Location

Region: Statewide, Metro, Northeast

County Name: Statewide, Hennepin, Ramsey, St. Louis

City / Township: Saint Paul, Duluth, Minneapolis

Alternate Text for Visual:

Image showing environments to be sampled and studied for plastic transformations in Minnesota water

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%

PROJECT TITLE: Microbial Transformation of Plastics in Minnesota Waters

I. PROJECT STATEMENT

CONCEPT – This project will study the ability of indigenous bacteria to biodegrade plastics found in contaminated waters across the state of Minnesota, and determine the fates of potentially toxic additives and by-products. Recent reports citing high levels of microplastics in freshwater lakes such as the Laurentian Great Lakes have confirmed concerns that the accumulation of microplastics in the environment is not an issue facing only terminal water bodies such as the Pacific Ocean, where this topic has been highlighted as a key element of the 'Great Pacific Garbage Patch'. Indeed, microplastics have infiltrated into many standing bodies of water throughout the world and across the state of Minnesota. Plastic waste within the environment contributes to the illness and deaths of countless fish, reptiles, marine mammals and bird species, and also diminishes the pristine nature of our public waters which are a valuable aspect of recreation in Minnesota. This unanticipated and detrimental result of our wide-scale adoption of plastics over the past century is an issue that will face generations to come. Importantly, very little is known related to the longevity of these plastics in our waters and the potential roles of microbial communities to alter the fate of these plastics. Steps should be taken now to both educate consumers and investigate the potential for natural and native populations of water-associated insects and bacteria to remove these materials from our waters, and better understand the persistence of this problem and the ability of nature to rectify or heighten this problem in the absence of human intervention.

Conventional plastics are widely believed to be non-biodegradable. Various reports of bacteria and fungi capable of degrading common plastics such as those found in beverage bottles (PETE), Styrofoam (polystyrene) and those used to store various liquids and chemicals (polyethylene; HDPE or LDPE) are now challenging this belief. These studies are important because they identify specific bacteria and fungi that degrade what has been thought to be a highly recalcitrant material, shattering the misconception that petroleum-derived commodity plastics are non-biodegradable. Our project will build upon the foundations of these reports, and the emerging realization that diverse microbial communities are better adapted than single organisms to degrading complex substrates such as you would find in many conventional plastics.

The goal of this project is to collect and characterize native cultures of environmental microbes from across Minnesota and enrich these cultures to determine rates of biodegradation and the composition and fates of the by-products of this degradation. These goals will be attained by first collecting seed cultures from locations proposed to harbor suitable plastic degrading microbes. These microbes will be enriched, and their genetic material will be characterized to understand both the diversity of the plastic degrading community and identification of genes and proteins potentially involved in plastic degradation. The by-products of plastic degradation by these communities will also be characterized to determine their environmental fate. Our work eventually may lead to treatment strategies to promote activities of plastic degrading microbes in contaminated areas. Finally, there will also be outreach to educate the public about the importance of this problem in an attempt to minimize the release of further plastics.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: *Collection of Microbial Communities Capable of Plastic Biodegradation* **Budget: $ 300,000**

In this activity, we will collect samples and enrich microbial communities with the highest ability to biodegrade targeted plastics. One aspect of this effort will be outreach with Junior High and High School teachers across the state to increase the number of potential sites sampled and tested and also educate students and their families about the environmental impacts of poor plastic waste management and the impacts on our waters. This effort will expand our sample size and geographical diversity to increase the potential for success, while also helping to educate future generations of Minnesotans.

Outcome	Completion Date
1. Construct simple laboratory bioreactor systems to enrich microbial communities for the biodegradation of target plastics such as polyethylene, polystyrene and PETE.	Dec 15, 2018

2. Prepare environmental sites to house test-site microcosms to enrich natural organisms capable of using different plastics as a growth substrate (insects, soil and water).	June 1, 2019
3. Collect microplastics from Lake Superior and various smaller water bodies across Minnesota that are either known or proposed to contain large quantities of microplastics.	Sept 30, 2020
4. Down-select specific enriched microbial communities and determine optimum parameters of growth to maximize biodegradation of select target plastics.	July 30, 2020

Activity 2: *Analysis of Plastic-Degrading Microbial Communities* **Budget: $ 206,000**

In the second activity, we will characterize microbial communities capable of biodegrading microplastics by using metagenomic sequencing, genetic and biochemical approaches. These experiments will identify both the types of microbes that are prominent in these communities and the different genes encoding enzymes involved in plastic degradation. These studies will assist in identifying specific microbes native to Minnesota, and also can be used to inform potential future biotechnological applications beyond this project, which could convert waste plastics into usable products versus simply degrading them into carbon dioxide.

Outcome	Completion Date
1. Determine the composition of enriched microbial communities from Outcome 4 of Activity 1 to identify the diversity and abundance of plastic degrading organisms.	Oct 15, 2020
2. Perform large-scale metagenomics sequencing to reconstruct genomes of plastic degrading bacteria.	Feb 15, 2021
3. Analyze genomes to identify candidate genes involved in plastic waste degradation and demonstrate their functionality using genetic and/or biochemical techniques.	June 1, 2022

III. PROJECT STRATEGY

A. Project Team/Partners

The research team includes Professor Brett Barney from the Department of Bioproducts and Biosystems Engineering and the Biotechnology Institute, who will oversee the project. Professor Barney's lab has been isolating natural communities of bacteria capable of biodegrading plastics for several years. Professor Jeff Gralnick from the Department of Plant and Microbial Biology will grow anaerobic communities and assist with metagenomics studies. Professor Ann Fallon from the Department of Entomology will be responsible for isolating microbial communities from the guts of various insect species from Minnesota and provide these to fellow team members. Professor Elizabeth Minor from the Large Lakes Observatory and Department of Chemistry and the University of Minnesota Duluth will be responsible for collecting plastics from Lake Superior and locations in Northern Minnesota and provide these samples to seed further enrichment cultures.

B. Project Impact and Long-Term Strategy

This is a long-term project, and the goals of the project are not the immediate cleanup of any specific site, as the shear volumes of material and expanse of areas across the state that are likely affected are too vast to consider one or more specific field sites at this time. We anticipate that these foundational studies will provide the first clear picture of the longevity of these plastics in the environment in Minnesota as they currently exist. A better understanding of these communities would aid in determining ways to take advantage of these natural communities to remove these contaminants from our water in the future.

C. Timeline Requirements

This project has a target for completion of 3 years. Certain proof-of-concept aspects have already been completed, and precedence for the success of other aspects of this project has been established through recent literature reports. Further support would be sought through additional funding sources based on the overall success of the project.

2018 Detailed Project Budget

Project Title: Microbial Transformation of Plastics in Minnesota Waters

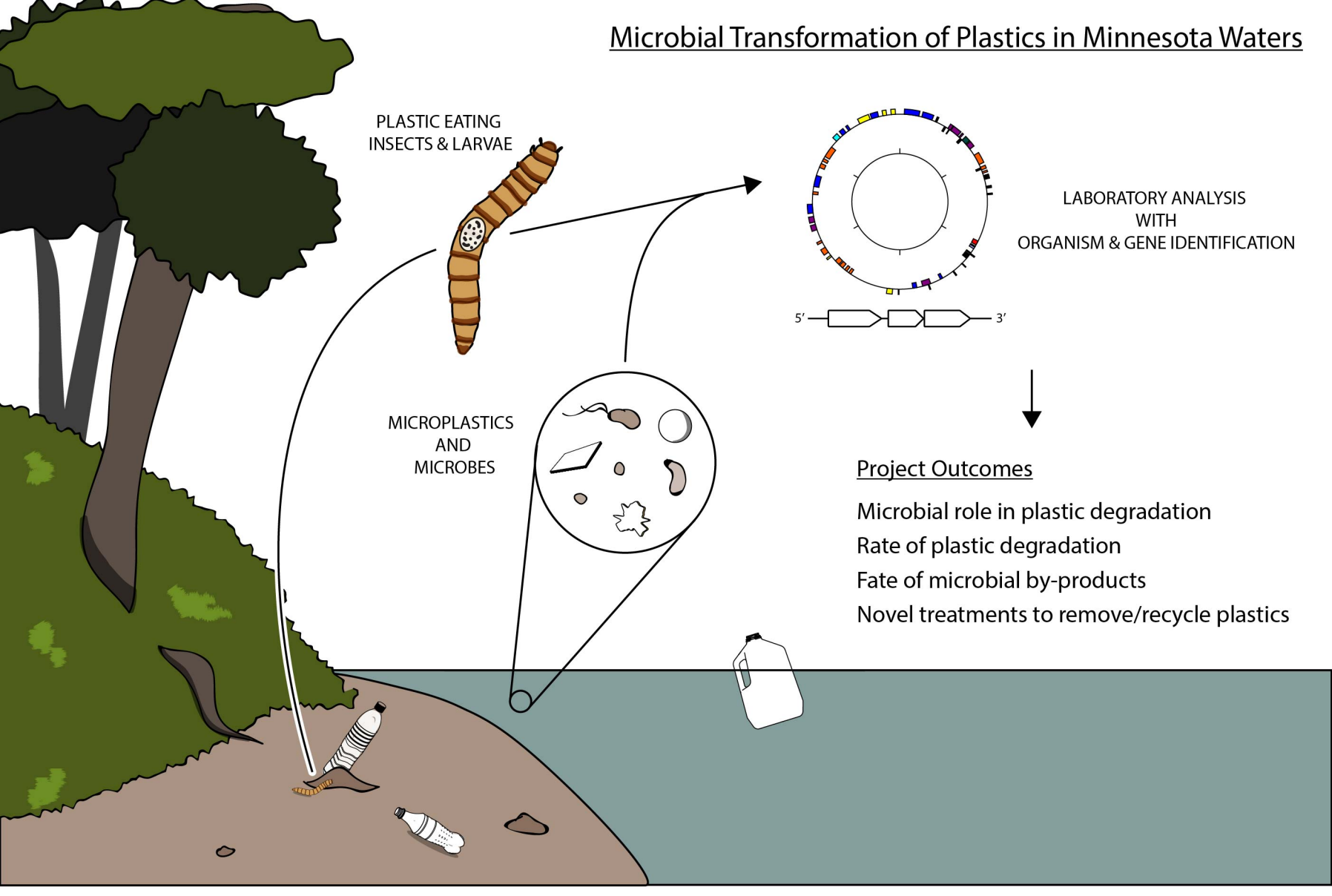
IV. TOTAL ENRTF REQUEST BUDGET 3 years

BUDGET ITEM	AMOUNT
Personnel:	
Brett Barney, Project Manager (75% salary, 25% benefits), Associate Professor, 9 Month Appointment, Summer Salary; 5% FTE for 3 years	$ 21,000
Jeff Gralnick, co-Project Manager (75% salary, 25% benefits), Associate Professor, 9 Month Appointment, Summer Salary; 5% FTE for 3 years	$ 25,000
Elizabeth Minor, co-Project Manager (75% salary/25% fringe), Professor, 9 Month Appointment, Summer Salary, 5% FTE for 1 year	$ 7,000
1 Graduate Research Assistant, UMN (Twin Cities), Laboratory Experiment Data Analysis, supervised by Barney (56% salary/44% fringe); 50% FTE for 3 years	$ 148,000
1 Graduate Research Assistant, UMN (Twin Cities), Laboratory Experiment Data Analysis, supervised by Gralnick (59% salary/41% fringe); 50% FTE for 2 years	$ 93,000
Graduate Research assistant, UMD (Duluth), Laboratory Experiment Data Collection and Field Study Data Collection and Analysis, (87% salary/13% fringe), Summer Salary for 1 year	$ 6,000
3 Undergraduate Research Assistants, UMN (Twin Cities), Laboratory Experiment and Field Study Data Collection, Supervised by Barney/Gralnick/Fallon (100% salary) 600 hours per year, 3 years	$ 87,000
1 Undergraduate Research Technician, UMD (Duluth), Laboratory Data Collection and Field Study Data Collection (78.5% salary/21.5% fringe) Summer Salary for 2 years	$ 10,000
Professional/Technical/Service Contracts:	
Equipment/Tools/Supplies:	
Laboratory Supplies: General Laboratory Chemicals, Media, Reagents and Kits for Performing Routine Molecular Biology, Analytical Reagents, DNA Synthesis of Primers, Liquid Nitrogen for Strain Storage. Combined laboratory supplies for the labs for all 4 PIs (Barney, Gralnick, Minor, Fallon).	$ 61,000
Lab services - DNA Sequencing for metagenomics work, performed at University of Minnesota Sequencing Facilities.	$ 33,000
Equipment rental - R/V Blue Heron/Kingfisher for plastic collection from Lake Superior (Elizabeth Minor, UMD, Duluth)	$ 11,000
Acquisition (Fee Title or Permanent Easements):	
Travel:	
Travel by Elizabeth Minor and students between Duluth and various field sites in Northern Minnesota.	$ 1,000
Travel by Brett Barney and students between the Twin Cities campus and various field site across Minnesota.	$ 3,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 506,000

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ To Be Applied To Project During Project Period:	$ -	*Not Applicable*
Other State $ To Be Applied To Project During Project Period:	$ -	*Not Applicable*
In-kind Services To Be Applied To Project During Project Period: Unpaid Indirect Costs	$ 247,000	
Funding History:	$ -	New Proposal
Remaining $ From Current ENRTF Appropriation:	$ -	Not Applicable

Microbial Transformation of Plastics in Minnesota Waters



Project Outcomes

Microbial role in plastic degradation

Rate of plastic degradation

Fate of microbial by-products

Novel treatments to remove/recycle plastics

Project Manager Qualifications

Brett Barney, Project Manager

Education:

Ph.D. Biochemistry, Arizona State University, 2003
B.S. Professional Chemistry, Utah State University 1993

Work and Research Experience:

2015 – Present Associate Professor, Bioproducts and Biosystems Engineering (UMN)
2010 – Present Faculty Member, BioTechnology Institute and Microbial and Plant Genomics Institute (UMN)
2009 – 2015 Assistant Professor, Bioproducts and Biosystems Engineering (UMN)
2003 – 2009 Research Assistant Professor and USDA Postdoctoral Fellow (USU)
1999 – 2003 Research Assistant and NSF Fellow, Department of Chemistry and Biochemistry (ASU)
1993 – 1999 Fiber Laboratory Manager, Research Chemist, Senior Laboratory Technician and Associate Chemist, Fresenius Medical Care, Ogden, Utah
1991 – 1993 Research Technician, Utah Water Research Laboratory (USU)

Jeffrey Gralnick, co-Project Manager, Associate Professor, Department of Plant and Microbial Biology (UMN)

Jeff brings expertise in the growth of anaerobic organisms and next generation sequencing

Ann Fallon, co-Project Manager, Distinguished McKnight Professor, Department of Entomology (UMN)

Ann brings expertise in the isolation of bacteria from the gut of various insects

Elizabeth Minor, co-Project Manager, Professor, Large Lakes Observatory and Head, Department of Chemistry (UMD)

Elizabeth brings expertise in the isolation of microplastics from Lake Superior and other regional water bodies

Dr. Barney's laboratory is focused on microbiology for sustainability. Dr. Barney has more than 25 years of experience in both basic and applied research in both academia and industry, including experience managing projects and laboratories in a range of settings. Previous research funding has come from the National Science Foundation (NSF), the United States Department of Agriculture (USDA), the United States Department of Energy (DOE), the Defense Advanced Research Projects Agency (DARPA), Minnesota's Discover, Research and InnoVation Economy (MnDRIVE) and the Initiative for Renewable Energy and the Environment (IREE).

The Barney laboratory is housed in the Cargill building for Microbial and Plant Genomics at the University of Minnesota. The laboratory contains the primary equipment to perform this research project, including facilities to cultivate various bacteria, autoclaves, analytical instrumentation for analysis (gas chromatography, spectrophotometers, and balances), thermocyclers for PCR reactions, centrifuges, electrophoresis equipment and various incubators. Additional facilities include the Biotechnology Resource Center, the Genomic Sequencing Center and a broad range of additional analytical laboratories which are available as pay services.

Organization Description

Dr. Brett Barney (PI) has been a professor with the Department of Bioproducts and Biosystems Engineering at the University of Minnesota since 2009. The Bioproducts and Biosystems Engineering Department serves as a core department combining Agricultural Engineering, Biological Engineering and Environmental and Ecological Engineering. The University of Minnesota provides a range of facilities and sufficient laboratory space to perform each of the activities described in this proposal. Additionally, controlled environments including greenhouse space sufficient for this work is conveniently located next door to Dr. Barney's laboratory space. UMN Sponsored Projects Administration (SPA) is the entity authorized by the Board of Regents to manage project agreements with the LCCMR program.