Environment and Natural Resources Trust Fund
2019 Request for Proposals (RFP)

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

Harnessing What's Within: Minimizing Nitrogen Pollution through Localization

Category: B. Water Resources

Sub-Category:

Total Project Budget: $ 290,000

Proposed Project Time Period for the Funding Requested: June 30, 2022 (3 yrs)

Summary:

The goal of this project is to construct sustainable biofertilizers with minimal-runoff potential by utilizing natural strains of nitrogen-fixing microbes found living inside the leaves and stems of plants.

Name: Brett Barney

Sponsoring Organization: U of MN

Title: Associate Professor

Department: CFANS/Bioproducts and Biosystems Engineering

Address: 1390 Eckles Ave

St. Paul MN 55108

Telephone Number: (612) 626-8751

Email bbarney@umn.edu

Web Address http://barneybioproductslab.cfans.umn.edu/brett-barney

Location

Region: Statewide, Metro

County Name: Statewide, Hennepin, Ramsey

City / Township: St. Paul

Alternate Text for Visual:

Image illustrating the primary concept behind this proposal

_____ Funding Priorities _____ Multiple Benefits _____ Outcomes _____ Knowledge Base

_____ Extent of Impact _____ Innovation _____ Scientific/Tech Basis _____ Urgency

_____ Capacity Readiness _____ Leverage _______ TOTAL ______%

____ If under $200,000, waive presentation?

PROJECT TITLE: Harnessing what's within: Minimizing Nitrogen Pollution through Localization

I. PROJECT STATEMENT

CONCEPT – The goal of this project is to construct inexpensive and sustainable biofertilizers with minimal-runoff potential by utilizing natural strains of nitrogen-fixing microbes found living inside the leaves and stems of plants. To achieve this goal, we will isolate and analyze nitrogen-fixing microbes residing within plants to catalog and characterize these strains for successful application as a localized and continuous source of nitrogen fertilizer. Localization of these biofertilizer strains within the above-ground tissues of the plant achieves our goal of limiting the losses of surface-applied nitrogen to the surrounding soils and water systems.

BACKGROUND. Prior to the introduction of industrially derived nitrogen fertilizers, farmers understood that rotating crops such as soybeans, alfalfa and clover on alternating years resulted in improved yields of crops such as wheat or corn the following year. Decades of research have taught us that the reason why certain crops improve soils is due to a symbiotic relationship between these plants and specific bacteria that live within or around the root systems of these plants. These symbiotic relationships have several benefits. The plant is fed a continuous supply of nitrogen from the bacterium as long as it supplies food (such as sugar) to the bacterium, which minimizes or eliminates the need for direct human intervention via nitrogen fertilizer application. However, this root-nodule based symbiosis only occurs within specific crops, while other key crops (corn and wheat) and native plants important to Minnesota still require external application of nitrogen to achieve desired yields.

RELATED ISSUES. Agriculture requires substantial resources to produce the crops that meet the needs of our modern society. Nitrogen is a primary component of fertilizers, and while industrial processes have enabled decades of increased agricultural production, this comes at both an environmental and an economic cost;

- ***Excessive nitrogen application results in high runoff and downstream water contamination*** leading to eutrophication, as has been highlighted in reports by the Minnesota Pollution Control Agency.
- Industrial nitrogen fixation is the main route to the production of ammonia. It consumes 3-5% of natural gas production and ***requires about 1-2% of all worldwide energy consumption***, releasing massive amounts of CO_2 into the atmosphere due to the dependence of this process on petroleum derived fuels.
- Transportation of nitrogen from industrial nitrogen production sites to geographically dispersed farms adds costs, and ***storage of ammonia can be a danger to farmers, their communities and the environment***, as illustrated by several recent accidents resulting in evacuations and hospitalizations in rural Minnesota.

BENEFITS. Complementing or replacing industrial nitrogen production with the natural nitrogen-fixation process would lower human-derived atmospheric carbon inputs, providing a sustainable and locally produced commodity product with commercial value. This would establish Minnesota as a global leader in efforts to combat climate change. In addition, because the nitrogen that is produced is released directly within the plant, there would be a minimal potential for runoff of excess nitrogen into lakes and streams. Localization of the bacterium within the plant also provides the bacterium with a direct route to receive the sugars produced by the plant to fuel this biologically driven process. ***Successful examples of these above-ground endophyte relationships can be found in commodity crops such as sugarcane and rice.***

GOAL. Our project goal is to overcome barriers to the efficient production of inexpensive nitrogen through the natural biological process of nitrogen-fixation. Our recent research efforts have already resulted in nitrogen-fixing bacteria that produce high yields of fixed-nitrogen for use as a biofertilizer. The next step to our long-term goals is to better understand how natural bacteria that reside within the plant tissues (endophytes) are able to accomplish this feat and serve as a valuable symbiont without eliciting a disease response (pathogenicity). ***Once this task is accomplished and merged without previous efforts, we should be able to pursue longer-term goals of replacing industrial fertilizers and requirements for nitrogen application altogether.*** This current project allows us to approach this initial task by identifying natural strains native to Minnesota that are able to function as nitrogen-fixing endophytes for the development of optimal biofertilizer seed cultures.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Identification of Nitrogen Fixation Potential from Minnesota Endophytes Budget: $290,000

This activity will focus on determining methods to enhance the natural processes associated with beneficial endophyte bacteria that colonize various plants, and in many cases, enhance the general health of the plant through these associations. With a focus on symbiotic bacteria native to Minnesota, we aim to obtain a large catalog of bacteria, with the goal of studying the potential to expand their distribution, developing methods and practices that supplement or replace the requirement for industrially-provided fertilizers. By producing the nitrogen directly within the plant, we will eliminate potential migration of this nitrogen into our soils and groundwater, lakes and streams.

Outcome	Completion Date
1. Develop a large collection (~100 strains) of natural nitrogen-fixing bacterial endophytes from various native plants and commodity crops across Minnesota.	Dec 1st, 2020
2. Confirm the potential to reintroduce natural nitrogen-fixing bacteria as endophytes into selected target plants through simple or novel delivery techniques.	June 1st, 2020
3. Down-select several of the target nitrogen-fixing bacteria with the highest potential to displace the need for externally provided industrial fertilizers and develop natural biofertilizers for application by farmers and organic gardeners.	August 15th, 2020
4. Sequence strains of several down-selected target nitrogen-fixing bacteria (~10 strains) to better understand optimal features of these strains, and assure that wide-scale introduction would not result in any detrimental effects (harm to the plants or the environment).	Jan 31st, 2021

III. PROJECT PARTNERS:

The research team includes Professor Brett Barney from the Department of Bioproducts and Biosystems Engineering and the Biotechnology Institute at the University of Minnesota, who will oversee the project. Professor Barney is an expert in the field of biological nitrogen fixation and has studied this process for 20 years, while pursuing the goal of developing improved biofertilizers for the past eight years. Neil Olszewski from the Department of Plant and Microbial Biology is a plant expert with more than 30 years of research experience in the field, and has also recently developed a plant line that can serve as an important screen for nitrogen stress, which would also be ideal for identifying optimal nitrogen-fixing bacteria associated with this project.

IV. LONG-TERM IMPLEMENTATION AND FUNDING:

Minnesota is a major agricultural state and requires long-term solutions to environmental issues associated with farming. ***Sustainable production of internally-produced biofertilizer with minimal runoff potential through a biologically derived process would build the local economy and save farmers money while also lowering the impact of farming on climate change*.** Success from this project would be truly transformative, replacing an antiquated process that has been responsible for enormous quantities of carbon added to the atmosphere, and damage to our lakes and streams related to over-application or severe weather events. Previous funding of this project through the MnDRIVE program at the UMN has already overcome a major hurdle toward the production of biologically derived nitrogen. Successful demonstration of the goals set here would draw private support.

V. TIME LINE REQUIREMENTS:

This project has a target for completion of 3 years. As preliminary work, a small number of bacteria that behave as model endophytes have been collected along with additional bacteria from Minnesota. Preliminary studies are underway, but would be substantially expanded once the project is funded. Further support would be sought through additional funding sources based on the overall success of the project.

2019 Detailed Project Budget

Project Title: Harnessing what's within: minimizing nitrogen pollution through localization

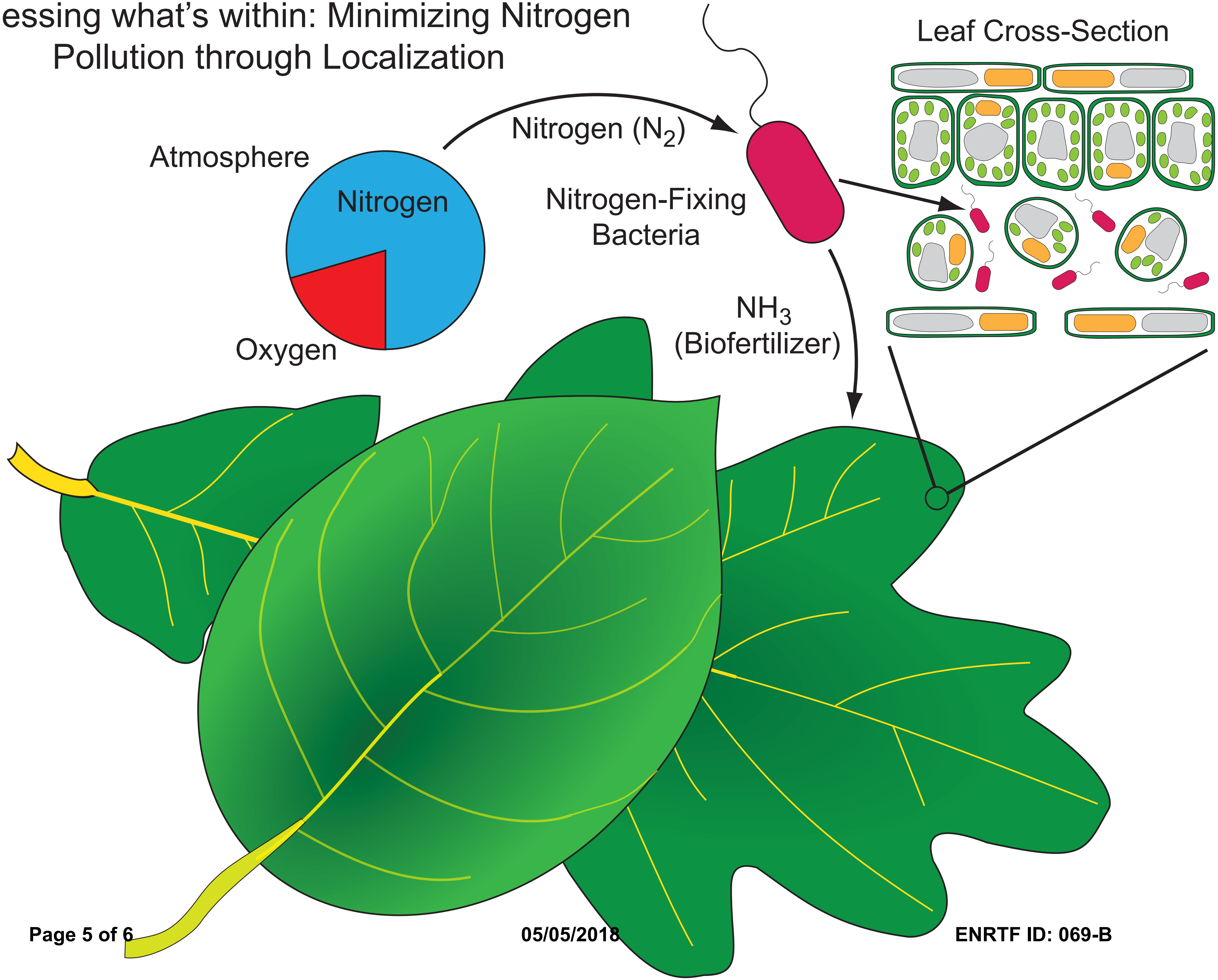
IV. TOTAL ENRTF REQUEST BUDGET 3 years

BUDGET ITEM	AMOUNT
Personnel:	$ 242,000
Brett Barney, Project Manager (75% salary, 25% benefits), Associate Professor, 9 Month Appointment, Summer Salary; 10% FTE for 3 years, $42,000	
1 Graduate Research Assistant, UMN (Twin Cities), Laboratory Experiment Data Analysis, supervised by Barney and Olszewski (56% salary/44% fringe); 50% FTE for 3 years, $149,000	
2 Undergraduate Technicians, Laboratory and Field Data Collection (100% salary, 0% benefits); 10% FTE for 3 years (generally rotating 1 year appointments), $51,000	
Professional/Technical/Service Contracts:	
DNA Sequencing Analysis, Sequencing of Endophyte Microbes for analysis and Characterization, Locally sourced through either the University of Minnesota Sequencing Center or Local Companies. (10 Straings at ~$1,000 per strain)	$ 10,000
Equipment/Tools/Supplies:	
Laboratory Supplies: General Laboratory Chemicals for Media and Reagents ($200 per month) and Kits for Performing Routine Molecular Biology ($400 per kit), Analytical Reagents, DNA Synthesis of Primers ($200 per month), Liquid Nitrogen for Strain Storage	$ 36,000
Acquisition (Fee Title or Permanent Easements):	$ -
Travel:	
Travel across state of Minnesota for bacterial strain collection by project participants (4 trips per year at ~$160 per trip), to be reimbursed by the University Compensation Plan.	$ 2,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 290,000

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ To Be Applied To Project During Project Period:	N/A	
Other State $ To Be Applied To Project During Project Period:	N/A	
In-kind Services To Be Applied To Project During Project Period: Unpaid Indirect Costs	$ 130,000	
Funding History:	$ -	
$500,000 - MnDRIVE Transdisciplinary Research Program: Enhancement of Microbial Biofertilizers for Sustainable Food Systems. This grant funded advanced studies to complete laboratory demonstration projects, showing that developed strains could provide sufficient nitrogen to support plant cells (algae based system).	$ 500,000	Completed
$150,000 - IREE Career Award: Microbial Communities for Enhanced Biofuel Feedstock Production; This proposal funded initial studies into beneficial nitrogen-fixing bacteria and their application as a biofertilizer.	$ 150,000	Completed
Remaining $ From Current ENRTF Appropriation:	$ -	Not Applicable

Harnessing what's within: Minimizing Nitrogen Pollution through Localization



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)

Neil Olszewski, co-Project Manager, Professor, Department of Plant and Microbial Biology (UMN)

Neil brings expertise in plant management that are important components of agriculture in Minnesota.

Dr. Barney's laboratory is focused on biological fertilizers (biofertilizers) for minimizing costs associated with biofuels and agriculture. 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 Cargill building was designed with the intention to promote interdisciplinary collaborations and provide a shared lab space for each floor, which facilitates flexible group sizes. This large laboratory space is designed around a shared communal format, with various rooms available for utilization for specific experiments. 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.