Environment and Natural Resources Trust Fund
2017 Request for Proposals (RFP)

Project Title: **ENRTF ID:** **130-E**

Harnessing Natural Nitrogen Fixation to Replace Industrial Production

Category: E. Air Quality, Climate Change, and Renewable Energy

Total Project Budget: $ 847,000

Proposed Project Time Period for the Funding Requested: 3 years, July 2017 - June 2020

Summary:

This project will leverage recent success in optimizing a nitrogen-fixing bacterium to construct a sustainable route to cheap biofertilizers by utilizing agricultural residues and waste steams or direct microbial electrosynthesis.

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Sponsoring Organization: U of MN

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Web Address

Location

Region: Central, Metro

County Name: Hennepin, Ramsey, Stevens

City / Township: Morrie, Minneapolis, Saint Paul

Alternate Text for Visual:

Images depict the two routes to produce sustainable fertilizers that will be addressed in this project

_____ Funding Priorities _____ Multiple Benefits _____ Outcomes _____ Knowledge Base

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

_____ Capacity Readiness _____ Leverage _______ TOTAL _____%

PROJECT TITLE: Harnessing Natural Nitrogen Fixation to Replace Industrial Production

I. PROJECT STATEMENT

CONCEPT – The goal of this project is to leverage our recent success optimizing a nitrogen-fixing bacterium that delivers high yields of fixed atmospheric nitrogen to *construct a sustainable route to cheap biofertilizers*. The energy requirements for this approach will be met through either direct microbial electrosynthesis coupled to renewable energy platforms (wind/solar) or through the treatment of agricultural residues to provide cellulose derived sugars on-site to grow the bacterium. Replacing industrial nitrogen production with a biologically based alternative process would lower human-derived atmospheric carbon inputs, provide a sustainable and locally produced commodity product with commercial value, and establish Minnesota as a global leader in efforts to curb global warming.

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

- Excessive nitrogen application results in 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 carbon dioxide into the atmosphere due to the dependence of this process on petroleum derived fuels.
- Transportation of nitrogen from industrial production sites to geographically dispersed farms adds further costs, and storage of certain forms of nitrogen can be a danger to farmers and their communities.

Prior to the introduction of industrially derived nitrogen fertilizers, farmers knew that rotating crops such as soybeans, alfalfa and clover on alternating years results 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 the root systems of these plants. These relationships have several benefits;

- Nitrogen fixation is coupled to photosynthesis. The fuel required to fix nitrogen biologically by the bacterium is obtained through sugars provided by the plant.
- Nitrogen is produced on site, requiring no transportation costs or handling of dangerous chemicals.
- Nitrogen is applied directly to the plant in a biologically controlled "timed-release" manner, limiting issues associated with over-application and excess runoff of nitrogen to our waterways.

Our project goal is to overcome the final obstacle to the efficient production of cheap fixed-nitrogen through a biological process. Our recent research efforts have resulted in a nitrogen-fixing bacterium that produces high yields of fixed-nitrogen for use as a biofertilizer. To complete the cycle and make this process truly sustainable, we need to couple this biological process to a cheap energy source. We will pursue two parallel paths to this goal. The first path constitutes a minimal risk approach to convert agricultural residues (wheat straw, corn stover and additional crop residues or waste streams) into simple sugars. The second path is a higher risk project that will require a more long-term commitment, but also has a much greater potential reward, and would seek to produce fixed nitrogen through the application of an electrosynthesis strategy.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Conversion of Agricultural Residues into a Feedstock for Nitrogen Production Budget: $502,000

The first activity will focus on the conversion of agricultural and alternative biomass wastes into simple sugars to grow high-nitrogen yielding bacteria. Current waste materials will be recycled to produce an alternative to industrially-derived nitrogen. We will be evaluating only sustainable processes, including approaches to produce formic acid (derived from atmospheric carbon dioxide) to pretreat biomass and generate specific sugars as a fuel source for our bacteria. We will also pursue efforts to produce nitrogen in a form that is more recalcitrant, lowering the release of nitrogen back to the environment. Important to this activity, each of the proposed components are supported by previous studies in these areas, and we would be looking primarily at combining and applying these techniques in a complex process for demonstration purposes, with a goal of transferring this to a benchtop pilot program at the West Central Research and Outreach Center (WCROC).

Outcome	Completion Date
1. Determine optimal process parameters through the evaluation of various current techniques to convert specific agricultural biomass samples (corn or wheat derived) into simple sugars for use in biological nitrogen production.	Dec 1st, 2018
2. Collect and evaluate various additional agricultural and alternative biomass wastes for suitability as a feedstock of simple carbohydrates for use in biological nitrogen production.	June 1st, 2019
3. Determine yield potential of biological nitrogen with various feedstocks described in outcomes 1 and 2, and determine any compounds that inhibit the growth of our nitrogen-fixing bacterium for secondary removal through improved biological consortia.	August 15th, 2019
4. Demonstrate a fully functional integrated biomass conversion system for nitrogen production through bench-scale pilot plant system.	Jan 31st, 2020

Activity 2: Electrobiosynthesis Approaches to Biological Nitrogen Fixation **Budget: $345,000**

The second activity will aim to develop a pathway to microbial electrosynthesis of nitrogen using mediator based strategies. While this strategy represents a higher risk, recent studies provide a precedent for the potential application of this new technology to a broad range of bacteria, including strains similar to our model nitrogen-fixing model bacterium. The potential success of such an approach would be truly transformational, allowing us to link direct biological production of nitrogen from cells growing on the surface of an electrode. Success in this area would provide a direct route to biological nitrogen production, and eliminate the need to couple this process to fossil fuel production, which is the current primary route to industrial nitrogen fixation.

Outcome	Completion Date
1. Demonstrate mediated microbial electrosynthesis as a proof of concept with our nitrogen-fixing model bacterium.	June 1st, 2018
2. Evaluate parameters to increase electron transport between our model nitrogen-fixing bacterium and various electrode materials.	Jan 31st, 2019
3. Construct a functional microbial electrosynthetic apparatus for pilot scale testing in combination with solar cells or wind turbine derived energy sources.	Jan 31st, 2020

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, Professor Craig Sheaffer from the Department of Agronomy and Plant Genetics and Michael Reese, Renewable Energy Director, at the West Central Research and Outreach Center (WCROC). Brett is an expert in biological nitrogen fixation. Craig is an expert in sustainable cropping systems. Michael has expertise in renewable energy systems.

B. Project Impact and Long-Term Strategy

Minnesota is a major agricultural state and will require long-term solutions to environmental issues associated with farming. Sustainable production of commodity fertilizer components through a biologically derived process would build upon the local economy while also lowering the impact of farming on global warming. Success within this realm would be a true game changer, replacing an antiquated process that has been responsible for enormous quantities of carbon added to the atmosphere. 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 likely draw private support.

C. Timeline Requirements

The demonstration aspect of the project will be completed within 3 years.

2017 Detailed Project Budget

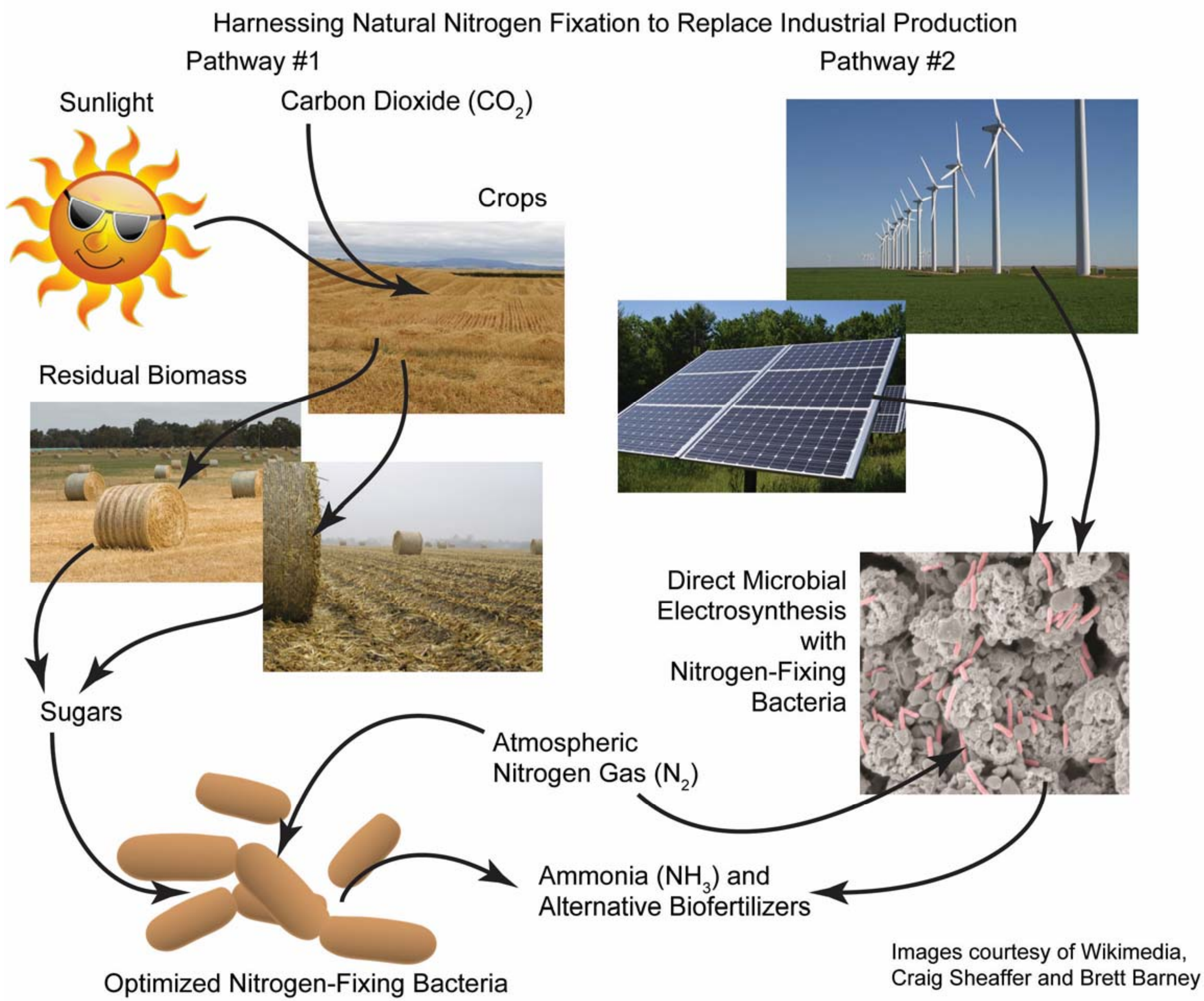
Project Title: Harnessing Natural Processes to Replace Industrial Nitrogen Production

IV. TOTAL ENRTF REQUEST BUDGET *3* years

BUDGET ITEM	AMOUNT	
Personnel:		$ 649,000
Brett Barney, Project Manager (75% salary, 25% benefits), Associate Professor, 9 Month Appointment, Summer Salary; 10% FTE for 3 years	$ 49,830	
Postdoctoral Microbiologist, Assistant Project Manager and Project Lead (82% salary, 18% benefits); 75% FTE for 3 years	$ 124,588	
Research Scientist, Greenhouse Studies and Maintenance (75% salary, 25% benefits); 50% FTE for 3 years	$ 66,219	
Researcher II, West Central Research and Outreach Center (WCROC); 100% FTE for years 2 and 3	$ 85,027	
Junior Scientist, Laboratory Experiment Data Analysis (79% Salary, 21% Benefits); 100% FTE for 3 years	$ 137,497	
1 Graduate Research Assistant, UMN (Twin Cities) Laboratory Experiment Data Analysis (57% salary, 43% benefits); 50% FTE for 3 years each	$ 135,598	
3 Undergraduate Technicians, Laboratory and Field Data Collection (100% salary, 0% benefits); 10% FTE for 3 years (generally rotating 1 year appointments)	$ 50,241	
Professional/Technical/Service Contracts:		$ 25,000
Electrical and Mechanical Consulting or Contractor Services to assist in installation and routine maintenance of bench top pilot scale systems (Estimated at $15K based on past experience).	$ 15,000	
DNA Sequencing Analysis, Sequencing of Microbes grown under batch process conditions for analysis and optimization, Locally sourced through either the University of Minnesota Sequencing Center or Local Companies (Costs based on historical values).	$ 10,000	
Equipment/Tools/Supplies:		$ 168,000
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 (Based on historical costs of approximately $1250 a month)	$ 45,000	
Biomass Processing Reactor design and components to convert corn stover and wheat straw biomass into simple sugars . Reactor costs are based on previous reactor design estimates for housings and reactor components purchased through various scientific and equipment suppliers. Costs are based on previous project experience.	$ 80,000	
Electrode construction and testing materials, including various electronics to measure voltage and current in relation to yield, as well as materials for the construction of various electrode surfaces to interact with the microbes. Costs are based on previous project experience.	$ 40,000	
Publication Charges: Costs associated with the broad dissemination of research findings in journals that are largely accessible to the broader public. Costs based on 3 publications total at $1000 per publication.	$ 3,000	
Acquisition (Fee Title or Permanent Easements):		$ -
Travel:		$ 5,000
Travel of West Central Research and Outreach Center (WCROC) participants from Morris to Twin Cities for quarterly meetings and technology exchange (4 trips per years at ~$160 per trip).	$ 2,000	
Travel by UMN (St. Paul) participants to WCROC for sample collection or to manage potential field studies at experiment stations or evaluate potential future studies.	$ 3,000	
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =		$ 847,000

V. OTHER FUNDS *(This entire section must be filled out. Do not delete rows. Indicate "N/A" if row is not applicable.)*

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*	$ 419,148	
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	In Progress
$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 Natural Nitrogen Fixation to Replace Industrial Production
Pathway #1
Pathway #2
Sunlight
Carbon Dioxide (CO_2)
Crops
Residual Biomass
Sugars
Direct Microbial Electrosynthesis with Nitrogen-Fixing Bacteria
Atmospheric Nitrogen Gas (N_2)
Ammonia (NH_3) and Alternative Biofertilizers
Optimized Nitrogen-Fixing Bacteria
Images courtesy of Wikimedia, Craig Sheaffer and Brett Barney

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	Student Research Technician, Utah Water Research Laboratory (USU)

Craig Sheaffer, co-Project Manager, Professor, Department of Agronomy and Plant Genetics (UMN)

Craig Sheaffer brings expertise in plant systems that are important components of agriculture in Minnesota

Michael Reese, co-Project Manager, Renewable Energy Director, West Central Research and Outreach Center

Craig Sheaffer brings expertise in renewable energy systems

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.