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

Project Title: **ENRTF ID: 071-B**

Enriching Native Fungi to Control Aquatic Phosphorus Pollution

Category: B. Water Resources

Sub-Category:

Total Project Budget: $ 356,000

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

Summary:

This project proposes to use novel fungi from Minnesota agricultural fields that can uptake, store, and transfer P to plants to reduce P fertilizers and P pollution from agricultural runoff.

Name: Kathryn Bushley

Sponsoring Organization: U of MN

Title: Assistant Professor

Department: College of Biological Sciences

Address: PMB, 140 Gortner Lab, 1479 Gortner Ave

St. Paul MN 55108

Telephone Number: (612) 625-8213

Email kbushley@umn.edu

Web Address https://cbs.umn.edu/contacts/kathryn-bushley

Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Workflow to develop P-accumulating fungi to improve plant access to P and reduce P pollution in aquatic environments

_____ 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: Enriching Native Fungi to Control Aquatic Phosphorus Pollution

I. PROJECT STATEMENT

This project proposes to use novel phosphorus (P) accumulating fungi we isolated from agricultural fields in Minnesota to control P pollution from agricultural runoff. Phosphorus is a common component in fertilizers, but it can also be a vital pollutant when leached into aquatic environments. Agriculture contributes 38% of the global phosphorus load, and rest coming from domestic sewage (54%) and industry (8%). While domestic sewage and industry have been actively regulated, agricultural run-off still poses the greatest threat to our aquatic environment. In Minnesota, agricultural runoff is the primary source of excess nutrient input into rivers and lakes, causing eutrophication and concern among both regulators and industry about expensive mitigation approaches such as buffer zones. A large portion of P applied to agricultural fields rapidly binds to the surface of soil particles and becomes insoluble and inaccessible to plants. Thus, current agricultural practices apply more P fertilizer than is necessary, causing widespread P pollution in aquatic ecosystems.

Our research group recently identified a group of fungal strains from Minnesota soybean fields that can convert insoluble P forms in soil to polyphosphate, a P storage compound that some microbes can accumulate in their tissues and transfer to plants. Mycorrhizal fungi are well known to accumulate polyphosphate and transfer it to plants, thereby enhancing growth and yield. However, many crop plants (canola, buckwheat, camelina etc.) cannot form mycorrhizal associations. As mycorrhizae require plant roots to grow, successful application can also be a technical challenge. The newly identified species showed similar capability to stimulate plant P uptake, but unlike mycorrhizae they can be easily cultured and simply amended to soil, and thus represent an untapped resource for improved P utilization by all crops. The main aim of this project is to use fungi that can access plant unavailable P in soil to significantly reduce phosphorus fertilizer input and P pollution to our water and lakes.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Screen native Minnesota fungi for ability to transform and accumulate phosphorus

The objective of this activity is to identify key native fungi that are able to accumulate and/or transform plant unavailable P reserves in soil. We will test fungi isolated from soil samples and roots collected from corn, canola, and soybean from different regions of Minnesota (Lamberton, Princeton, Waseca and Crookston field stations as well as from farmer fields) for their ability to 1) uptake and store different forms of P and 2) transform plant unavailable P reserves in soil to plant available forms. We will utilize simple laboratory assays to measure ability of these fungi to grow and accumulate P as polyphosphate on different P sources, including plant available (H_2PO_4) and both plant unavailable inorganic (rock phosphate, and Ca and Fe-PO4) and organic (phytate) forms. Using chemical approaches well established in the Hu lab, the amount and chemical forms of P in soil before and after fungal growth will be characterized. We have already identified several promising candidates from the soybean rhizosphere, including a strain of *Mucor* (UMN-B34), which in a preliminary greenhouse trials had positive effects on plant growth and P nutrition. We will screen a collection of >150 fungi previously isolated from rhizosphere and roots of major crops in Minnesota (corn, soybean, and canola).

ENRTF BUDGET: $132,000

Outcome	Completion Date
1. Screen native fungi for ability to uptake and store P from different sources	*Year 1 – 05/2020*
2. Test changes in P soil reserves before and after fungal growth	*Year 1 - 05/2020*

Activity 2: Test ability of fungi to improve P transfer to plants in laboratory and greenhouse assays

For the top P accumulating strains identified in Activity 1, we will investigate the interactions between these fungi and two crop plants (corn and soybean). Tissue culture assays will be used to screen isolates to test whether these fungi can improve plant P nutrition when grown on plant unavailable forms of P. Phosphorus

content of shoot and root in different P treatments will be used to assess P uptake compared to controls. Strains that show promise in in-vitro assays will then be tested in the greenhouse in different major soil types present in Minnesota. By comparing the response in soils with different C: N ratio, phosphorus content, and pH, we will be able to understand optimal conditions for fungal growth, to characterize changes in soil P compounds in different soil types, and to determine whether they may reduce P leaching from soil. A suitable delivery system for the fungi will also be optimized and tested in greenhouse experiments.

ENRTF BUDGET: $140,000

Outcome	Completion Date
1. Testing of promising strains with corn and soybean seedlings in tissue culture	*Year 2 – 05/2021*
2. Greenhouse studies to test efficacy of fungal inocula in transferring P to plants and P accumulation and changes in P soil reserves in different soil types	*Year2 – 05/2021*
3. Development of optimal fungal inoculum formulations and methods to deliver fungal strain to the plant root/spore seed treatment or/ encapsulation)	*Year2 – 05/2021*

Activity 3: Conduct field trials with corn and soybeans to assess efficacy in reducing P in agricultural runoff

To test efficacy of these fungi in reducing P pollution, we will work with a farm at Lamberton, MN to study the application of the top-performing fungi in field trials. Corn and soybean will be tested with different levels of phosphorus fertilizer and fungal inoculum. Measurements of P in runoff, plant yield performance and P content of shoots of each crop will be recorded. The soil phosphorus level and major chemical forms of P present will be evaluated at planting, midseason after the fungal application, and harvest.

ENRTF BUDGET: $84,000

Outcome	Completion Date
1. Field trials with corn and soybean	*10/2021*
2. Chemical analysis of P in soil, runoff water, and plants after harvest	*06/2022*

III. PROJECT PARTNERS:

A. Partners receiving ENRTF funding

Name	Title	Affiliation	Role
Kathryn Bushley	Assistant Professor	PMB, UM	Project Director
Bo Hu	Professor	BBE, UM	Co-Project Director

B. Partners NOT receiving ENRTF funding

Name	Title	Affiliation	Role
Bruce Wilson	Professor	BBE, UM	Collaborator

IV. LONG-TERM-IMPLEMENTATION AND FUNDING: The project will have a broad impact on both academia and industry. Potential outcomes include more sustainable P nutrient management and reduced P pollution of aquatic ecosystems from agricultural runoff. Both Bushley and Hu labs have worked on fungi from corn, canola, and soybean fields in MN, with funding from Minnesota Soybean Growers and will seek future funding with USDA for longer-term sustainability for the project.

V. TIME LINE REQUIREMENTS: The project will be completed in 3 years. During the first year we will screen additional fungi for ability to accumulate P or to transform P in soil. The second year will focus on testing the top candidates in more realistic greenhouse assays for their ability to transfer P to plants using different soil types. Activities 1 and 2 will be completed by May of 2021 in order to conduct field trials in summer 2021.

2019 Proposal Budget Spreadsheet

Project Title: Enriching Native Fungi to Control Aquatic Phosphorus Pollution

IV. TOTAL ENRTF REQUEST BUDGET - 3 years

BUDGET ITEM (See "Guidance on Allowable Expenses")	**AMOUNT**
Personnel:	
Kathryn Bushley, Assistant Professor, Project Manager (68% Salary, 32% Benefits), 10% FTE	$ 30,000
Bo Hu, Associate Professor, Co-Project Manager(68% Salary, 30% Benefits), 8.3% FTE ($10,000) for years 1-3	$ 30,000
Postdoctoral researcher (100% Salary, 21.4% Benefits), 100% FTE ($47,500 annually for salary plus benefits) for years 1-2	$ 116,000
Graduate student 100% FTE ($27,000 salary, plus tuition ($15,000) and health benefits) for years 1-3, plant tissue culture and greenhouse studies	$ 134,000
Undergraduate student researcher for greenhouse and field trials ($5000 each for years 2-3)	$ 10,000
Professional/Technical/Service Contracts:	$ -
Equipment/Tools/Supplies:	$ -
Chemicals and media for soil P testing and media for growing fungi (including glucose, yeast extract, minerals, phosphorus, peptone, and other chemicals for extraction). $5,000/year.	$ 15,000
Petri plates and tissue culture flasks and media for culturing fungi and plants, chemicals for in-vitro assays and supplies for greenhouse trials (pots, soil, fertilizers). $5,000/year.	$ 15,000
Acquisition (Fee Title or Permanent Easements):	$ -
Travel:	
In-state travel (Mileage, lodging, and meals) for Research Associate, Undergraduate assistants and Project Directors for soil collection (year 1) and sample collection for field trials (year 3)	$ 3,000
Additional Budget Items:	
Rental of greenhouse and growth chambers at UM Facilities ($1,500 for years 1 and 2)	$ 3,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 356,000

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:	*$ 192.24*	
Past and Current ENRTF Appropriation:	$ -	N/A
Other Funding History:	$ -	*N/A*

Enriching Native Fungi to Control Aquatic Phosphorus Pollution

Kathryn Bushley, Bo Hu, and Bruce Wilson
University of Minnesota

Plant growth with fungi

Control with no fungi

Transfer to plants

Healthy Crops

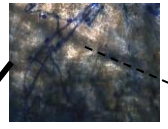
P accumulation by non-Mycorrhizal soil fungi

Case (1) Soybean Plant

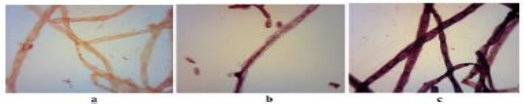
Case (2) Corn

P solubilization / mineralization

Insoluble Soil P



Non-mycorrhizal fungi colonizing the roots for better nutrient transfer



Polyphosphate staining of fungal colonized roots

Healthy Aquatic ecosystem

Source of P loading

Activity 1: Screen native Minnesota fungi for ability to transform and accumulate phosphorus

Activity 2:Test ability of fungi to improve P transfer to plants in laboratory and greenhouse assays

Activity 3: Conduct field trials with corn and soybeans to assess efficacy in reducing P in agricultural runoff

PROJECT TITLE: Enriching Native Fungi to Control Aquatic Phosphorus Pollution

Dr. Kathryn Bushley is an Assistant Professor in Plant and Microbial Biology at the University of Minnesota. She is a fungal biologist studying plant-microbe interactions. She has worked with fungi for over a decade with expertise in both plant pathogenic and insect pathogenic fungi. At the University of Minnesota, she has worked extensively over the last several years with plant growth promoting root endophytic fungi and on a project characterizing fungi isolated from corn and soybean fields in Minnesota for their potential to serve as biocontrol agents of nematode and insect agricultural pests. This work has involved collection of over 1000 isolates grouping into approximately 300 species groups. Her lab also investigates novel chemicals produced by fungi that may have use in agriculture or medicine. She has also worked with other root endophytic fungi (*Piriformospora indica*) that show promise for improving nutrient (including P) acquisition and stress tolerance in plants. Dr. Bushley will coordinate the overall project and lead research on in-vitro plant tissue culture laboratory tests and greenhouse assays on ability of fungal strains to transfer P to plants.

Dr. Bo Hu is a junior Associate Professor at Department of Bioproducts and Biosystems Engineering, University of Minnesota. With more than 10 years of active research experience specifically in biomass utilization, nutrient utilization, fermentative conversion, and waste management. He is leading projects to remove phosphorus from manure and from wastewater in the septic tank systems, projects to reveal projects on synthetic ecology in lichen biofilm formation by co-culturing mixotrophic microalgae and filamentous fungi. He has completed projects to develop a co-digestion system in Jer-Lindy dairy farm in Minnesota and a modified anaerobic digestion system for biohydrogen production. Dr. Hu's team at UMN has also isolated a large collection of fungi from soybean fields in Minnesota and developed DNA based microbial analysis by using high-throughput pyrosequencing methods to identify fungal species. His team is also developing several conversion platforms to produce bioproducts and biofuel from agricultural waste and residue and to remove nutrients and pollutant from contaminated water. Dr. Hu will lead chemical analyses of P species in soil and in plant tissue.

Dr. Bruce Wilson is a Professor in the Department of Bioproducts and Biosystems Engineering, University of Minnesota. He has extensive experience in hydrological modelling of both quantity and quality of surface waters, especially those in agricultural systems. He will lead monitoring and modelling of P in runoff for Activity 3 of the project.

Organizational Description: The University of Minnesota facilities for the project include Dr. Bushley's laboratory in 898 BioScience that is well equipped for both fungal growth and plant growth assays. Equipment includes two sterile hoods for culturing, Innova New Brunswick shakers, Nikon compound microscope, and centrifuges for preparing media and culturing fungi and plant tissue cultures. Dr. Hu's laboratory has all the necessary equipment and facilities for both culturing fungi and soil chemical analyses, including Bio-Rod electrophoresis, New Brunswick refrigerated incubation shakers, Beckman Allegra X-15R Refrigerated Centrifuge, VWR refrigerated water heater circulator, Bioreactor/fermentor, Agilent 7820 A GC-FID- TCD, Agilent Micro-GC, Agilent 1260 HPLC (Diode Array detector, Refractive Index Detector and autosampler), and Dionex ICS 2100/ ICS 1100 bundle ThermoFisher Scientific. Both PI's also have access to growth chambers and greenhouse space in the MN Plant Growth Facilities and have interactions with Minnesota soybean growers to facilitate field trials. Director Bushley also has active collaborations with researchers at the UM Southern Research and Outreach Center in Waseca.