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

Project Title: **ENRTF ID: 036-B**

Phosphorus Accumulating Fungi to Control Agricultural Runoff Pollution

Category: B. Water Resources

Total Project Budget: $ 361,000

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

Summary:

This project will utilize non-mycorrhizal fungi that can access insoluble soil phosphorus to significantly decrease needs for phosphorus fertilizers and reduce phosphorus pollution to our water and lakes.

Name: Kathryn Bushley

Sponsoring Organization: U of MN

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Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Fungi can reduce phosphorus pollution of water resources by helping plant access unavailable soil phosphorus (P) and reducing need for fertilizer input. a) Non-mycorrhizal fungi grow in and on the surface of root, b) Fungi solubilize unavailable phosphorus in soil and accumulate phosphorus (polyphosphate) in roots, c) soybean in greenhouse trial grown with fungus compared to d) soybean in greenhouse trial grown without fungus.

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

PROJECT TITLE: Phosphorus Accumulating Fungi to Control Agricultural Runoff Pollution

PROJECT STATEMENT: This project proposes to use novel fungi we isolated from soybean fields in Minnesota to control phosphorus (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. A large portion of phosphorus applied to agricultural fields rapidly binds to the surface of soil particles and becomes insoluble and inaccessible to plants. It is estimated that only 0.1% of the total phosphorus in soil can actually be accessed by plants.

- In Minnesota, agricultural lands are the primary source of nutrient runoff to rivers and lakes and are causing extensive problems such as eutrophication.
- Current agricultural practices add much more phosphorus than in needed, which can saturate the soil's holding capacity and lead to high phosphorus levels in runoff.
- The world's phosphorus reserves are limited with the possibility of global depletion in the next century. Alternate solutions to both reduce P input by more efficiently accessing insoluble P reserves in soil will soon become a necessity.

Our research group recently found a group of fungal strains in MN's soybean field that can convert insoluble phosphorus in soil reserves to polyphosphate, a phosphorus storage compound that 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, radish, camelina, mustards) cannot form mycorrhizal associations and mycorrhizae require plant roots to grow and growing inoculum for successful application can be a technical challenge. Preliminary results from a greenhouse test of the fungal strain, where we inoculated the fungus directly into the soil, showed significant improvement in soybean plant growth (ref. visual). These newly identified interactions between plants and –non-mycorrhizal could be as important for efficient P utilization from soil as mycorrhizal fungi. However, unlike mycorrhizal fungi, they can be easily cultured and simply amended to soil and thus represent an untapped resource for improved utilization of P resources by all crops. **The main aim of this project is to utilize fungi that can access insoluble P in soil to significantly decrease needs for phosphorus fertilizer input and to reduce phosphorus pollution to our water and lakes.**

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Study changes in soil phosphorus pools after introduction of fungal strains **Budget: $133,000**

The objective of this step is to study the changes in soil phosphorus composition due to the enrichment of non-mycorrhizal soil fungi. We will test these fungi in soils samples collected from different regions of Minnesota (Lamberton, Princeton, Waseca and Crookston field stations as well as from fields of farmers from diverse regions of the state interested in participating in the research) in order to understand whether these fungi will be effective in the different types of soil environments in Minnesota. Using chemical approaches well established in the Hu lab, the physical and chemical properties, specifically the amount and chemical forms of P in the soil before and after the fungal growth, will be characterized. We will initially focus on a strain of *Mucor* (UMN-B34) strain, which we have shown in a greenhouse trial to have positive effects on plant growth and P accumulation but will also screen other promising strains identified in Activity 2.

Outcome	Completion Date
1. Characterize the ability of fungi to grow on different soil types (soil type, pH, mineral content (e.g. major soil types in MN and different pH conditions)	Year 1 - 05/2019
2. Test the P soil reserves before and after fungal growth and detection of polyphosphate in fungal tissue	Year 1 - 05/2019

Activity 2: Testing of ability of fungi to improve P accumulation and transfer to plants in laboratory and greenhouse assays **Budget: $142,000**

We will investigate the interactions between our fungi and plant roots of two crop plants (corn, soybean). Tissue culture assays will be used to screen isolates (over 300 available) to test whether these fungi form interactions with or directly colonize plant roots as well as to access P from both accessible and inaccessible forms in the tissue culture media. Phosphorus content of shoot and root in different P treatments will be used to assess whether the fungi assist in P acquisition. Strains that show promise in in-vitro assays will then be tested in the greenhouse in different soil types. By comparing the response in soils with different phosphorus content and pH, we will be able to understand what changes in soil P these microbes may cause and whether they may accumulate P to help avoid P leaching. A suitable delivery system and method for the administration of the fungi will also be tested in the greenhouse.

Outcome	Completion Date
1. Investigate interaction of fungal hyphae with plant roots and ability to assist in P acquisition in in-vitro tissue culture assays	*Year 1 – 05/2019*
2. Greenhouse testing of promising strains with Corn and soybean	*Year1- 2 – 05/2020*
3. Evaluation of optimal soil conditions for fungal treatment	*Year 2 – 05/2020*
4. Test different types of fungal inoculum (spores versus tissue) and develop methods to deliver fungal strain to the plant root/spore seed treatment or/ encapsulation)	*Year 2 – 05/2020*

Activity 3: Conduct field trials with corns and soybeans **Budget: $86,000**

We will work with a farm at Lamberton, MN (supporting letter attached) to study the application of these fungi in the field. Corn and soybean will be tested and the amounts of phosphorus fertilizer and fungal inoculum will be correlated with plant yield performance, nutrient uptake by plants, and grain yield for both crops. The soil phosphorus level will also be evaluated at planting, midseason after the fungal application, and harvest.

Outcome	Completion Date
1. Field trials with Corn and soybean	*Year 3 - 10/2021*
2. Chemical analysis of P nutrients in soil, runoff water, and plants after harvest	*Year 3 - 06/2021*

III. PROJECT STRATEGY

A. Project Team/Partners: The team includes Professor Kathryn Bushley and her postdoc researcher Dr.Ruchika Bajaj from the Plant and Microbial Biology Department; Professor Bo Hu and his postdoc researcher Dr. Aravindan Rajendran, from the Department of Bioproducts and Biosystems Engineering Department, University of Minnesota. We are partnering with the Frame Farms at Lamberton, MN for our field study and supporting letter from the owner, Carmen Fernholz, is attached in the proposal.

B. Project Impact and Long-Term Strategy: The project will have a broad impact on both academia and industry. The results will provide fundamental knowledge on how these fungi function in P mobilization and utilization in soil. The possible applications derived from this research will lead to sustainable developments in nutrient management and agricultural practices, and alleviate the deteriorating conditions related to eutrophication caused by P accumulation and agricultural run-off. Both labs have worked on fungi from soybean fields in MN, with funding from Minnesota Soybean Growers and other sources which may provide synergistic and longer-term sustainability for the project.

C. Timeline Requirements: The project will be completed in 3 years, with the first two years for soil testing and lab-scale study and the remaining one-year for on-site field trials to evaluate success in the field and further develop implementation technologies.

2018 Detailed Project Budget

Project Title: Phosphorus Accumulating Fungi to Control Agricultural Runoff Pollution

IV. TOTAL ENRTF REQUEST BUDGET *3* years

BUDGET ITEM	AMOUNT
Personnel:	
Kathryn Bushley, Assistant Professor, Project Manager (68% Salary, 32% Benefits), 10% FTE ($10,000) for years 1-3	$ 30,000
Bo Hu, Associate Professor, Co-Project Manager(68% Salary, 30% Benefits), 8.3% FTE ($10,000) for years 1-3	$ 30,000
Ara Rajendran, Research Associate (68% Salary, 32% Benefits), 67% FTE ($37,000 salary plus benefits) for years 1-3. Soil and plant P analysis, field trials.	$ 146,000
Ruchika Bajaj, Postdoctoral Associate (78% Salary, 22% Benefits), 100% FTE ($47,000 plus benefits) for years 1-2, plant tissue culture and greenhouse studies	$ 115,000
Undergraduate student researcher for greenhouse and field trials ($5000 each for years 2-3)	$ 10,000
Professional/Technical/Service Contracts:	
N/A	
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). $4,000/year.	$ 12,000
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). $4,000/year.	$ 12,000
Acquisition (Fee Title or Permanent Easements): N/A	$ -
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 =	$ 361,000

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: University of MN F&A at rate of 54% of proposed budget as "in-Kind" services not allowed in proposed budget.	$195,000	*secured*
Past and Current ENRTF Appropriation:	N/A	
Other Funding History:	N/A	

Phosphorus Accumulating Fungi to Control Agricultural Runoff Pollution

Kathryn Bushley, Bo Hu and Aravindan Rajendran
University of Minnesota

(c)Plant growth with fungi

(d) Control with no fungi

Transfer to plants

Healthy Crops

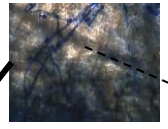
Case (1) Soybean Plant

Case (2) Corn

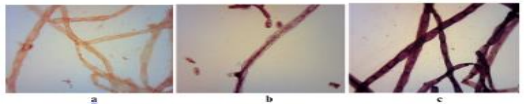
P accumulation by non-Mycorrhizal soil fungi

P solubilization / mineralization

Insoluble Soil P



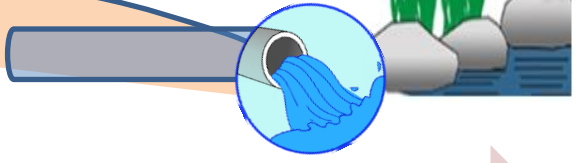
(a) Non-mycorrhizal fungi colonizing the roots for better nutrient transfer



(b) Polyphosphate staining of fungal colonized roots

Healthy Aquatic ecosystem

Source of P loading



Activity 1: Study changes in soil phosphorus pools after introduction of fungal strains

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

Activity 3: Conduct field trials with corn and soybeans

PROJECT TITLE: Phosphorus Accumulating Fungi to Control Agricultural Runoff Pollution

Qualifications of Project Directors:

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. Dr. Bushley will coordinate the overall project and lead research on laboratory and greenhouse assays on interactions of plants and fungi.

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, such as lichen biofilm co-cultivation of fungi and microalgae, pelletized fungal fermentation, and solid and hemi-SolidSF of filamentous fungi, to produce bioprducts and biofuel from agricultural waste and residue, and to remove nutrients and pollutant from contaminated water. As the PI of the project, Dr. Hu will design and coordinate the research; the Post-Doc researcher will assist in design and experimentation; and the graduate student will assist in data collection and dissertation.

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.