Environment and Natural Resources Trust Fund

2021 Request for Proposal

General Information

Proposal ID: 2021-057

Proposal Title: Reducing Nutrients and Methane Emissions Using Wetland Microbes

Project Manager Information

Name: Chan Lan Chun

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Project Basic Information

Project Summary: The proposal aims to use recently discovered microbes from Minnesota wetlands to inexpensively remove nitrogen nutrients from water while consuming methane, a potent greenhouse gas, from urban and agricultural wastes.

Funds Requested: $334,000

Proposed Project Completion: 2024-06-30

LCCMR Funding Category: Water Resources (B)

Project Location

What is the best scale for describing where your work will take place?

Statewide

What is the best scale to describe the area impacted by your work?

Statewide

When will the work impact occur?

During the Project and In the Future

Narrative

Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.

Nutrient pollution from urban and agricultural sources is a widespread challenge impacting stream and lake health throughout Minnesota, as well as the health of surrounding communities. For example, elevated nitrate nitrogen levels in drinking water can cause "blue baby syndrome." Effective nutrient removal from wastewater streams is a critical component of strategies to remove water quality impairments in streams and lakes. Conventional biological nitrogen removal technologies (treatments that use bacteria to remove nitrogen) are often inefficient and require expensive, energy-intensive aeration along with an outside carbon source. At the same time, several urban and agricultural processes produce large amounts of methane, a carbon-rich, potent greenhouse gas, as a waste product. Since methane is approximately 30 times more potent than carbon dioxide as a greenhouse gas, treatment technologies that could stimulate bacteria to remove nutrients by using methane would help solve the two biggest environmental challenges facing society: nutrient enrichment of freshwater resources and global climate change.

What is your proposed solution to the problem or opportunity discussed above? i.e. What are you seeking funding to do? You will be asked to expand on this in Activities and Milestones.

We recently identified microbes in Minnesota's wild rice wetlands that can convert dissolved nitrogen nutrients into inert nitrogen gas (subsequently released to air) using natural sources of methane. In fact, freshwater wetlands contribute significantly to global methane emissions through natural biological processes and have diverse microbes producing and utilizing methane. The project aims to discover different types of methane-consuming microbes and examine their potential to remove nutrients from water while simultaneously reducing methane emissions from urban and agricultural processes. This process has not been adapted for engineered treatment systems (e.g. wastewater, landfill leachate, and agricultural waste) yet, and it may be a promising solution to inexpensively remove nitrogen if the ideal communities and treatment conditions are understood. This work will study a number of wetlands and peatlands in Minnesota and focus specifically on nutrient removing abilities of microbes under different environmental conditions (e.g. substrate, temperature, and other chemicals). Because most wastewater treatment systems generate methane as a byproduct, the process may be a cost-effective solution for removing nitrogen from wastewater using an inexpensive (free) carbon source. We envision that these native bacteria are able to address treatment applications specific to the chemistry and climate of Minnesota.

What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?

Findings gained from the project will be a basis to develop water treatment technologies that remove nitrogen and methane concurrently. Such applications ultimately will help mitigate water quality impairments and greenhouse gas emissions in Minnesota. The project outcomes include 1) a foundational database of microbial communities capable of methane-nitrogen processes in wetlands and built environments in Minnesota, 2) necessary information to develop a start-up phase of biological nutrient removal process using methane, and 3) identifying limitations to implementation of water treatment strategies. This work will put Minnesota at the forefront of nutrient reduction strategies and curbing greenhouse gas emissions.

Activities and Milestones

Activity 1: Identify methane-oxidizing microbes capable of nitrogen removal in various habitats and treatment systems in Minnesota

Activity Budget: $191,050

Activity Description:
The novel process of methane-nitrogen cycling is still in the early stages of development and limited information is available on the different bacteria capable of this process and the environmental conditions under which it occurs. We will use a systematic approach to identify and discover known and novel organisms using a combination of molecular biomarkers (genes) and nutrient enrichment experiments. A variety of environmental samples (e.g. sediment, sludge, aquatic plant root, feces) will be collected from natural habitats (e.g. wetlands/peatlands) and engineered systems (e.g. landfill, wastewater treatment, biogas facility). During the sample collection, we will measure methane emissions at the location. The samples will be divided into three for sample characterization (e.g. water chemistry, nitrogen forms, and methane), genomic DNA isolation and nutrient enrichment experiments. The enrichment experiments will include different levels of temperature, biofilm carriers (biofilm carriers or well-characterized biochar), and nitrogen species (nitrate/nitrite, ammonia, and urea). Microbial methane oxidation will be measured by determining the production of isotope-labeled carbon dioxide along with nitrogen speciation. The populations and functional dynamics of methane oxidizers coupled with nitrogen cycling will be analyzed using high-throughput DNA sequencing and quantitative polymerase chain reactions.

Activity Milestones:

Description	Completion Date
Identify and enrich methane-oxidizing microbes coupled with nitrogen cycling in diverse environments	2022-06-30
Examine microbial communities responsible for methane-nitrogen cycling using DNA-SIP microcosm	2022-12-31

Activity 2: Examine nutrient removal efficiencies of the microbes under different environmental conditions

Activity Budget: $142,950

Activity Description:
Based on identification and enrichment of the microbial methane-nitrogen process in Activity 1, we will test nitrogen nutrient removal efficiencies, particularly for ammonia and nitrate/nitrite, using synthetic wastewater in the laboratory. Particularly, nitrogen utilizing efficiency and stability of the selected bacteria communities from Activity 1 will be examined under various environmental conditions. The lab-scale bioreactors will be operated as a fed-batch and continuous culture mode to optimize environmental conditions of microbial communities in regard to their growth phase and age of the culture. During operation, methane and nitrogen removal rates will be monitored with other environmental parameters to obtain maximum removal capacities and the influence of adaptation of the bacteria to the substrate. A continuous culture mode can provide more precise and reproducible data than those obtained from batch cultures including the influence of substrate loading rate on the removal efficiency of nitrogen and the stability of the bacteria communities under different culture conditions (temperature change, variable nitrogen loading, and addition of other contaminants; sulfide, organics, and phosphate). Activity 2 will provide key physiological, kinetic, and ecological traits of the methane-nitrogen process, which are necessary information forward to design the start-up phase of treatment strategies.

Activity Milestones:

Description	Completion Date
Bioreactor operation and determining efficiency of methane and nitrogen removal	2023-06-30
Examine nitrogen-utilizing efficiency and stability of the selected microbial consortia in various environmental factors	2024-06-30

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Christopher Filstrup	University of Minnesota Duluth	Filstrup will be responsible for co-supervise a postdoc and students and activities associated with total and dissolved nitrogen analyses and isotope analysis.	Yes

Long-Term Implementation and Funding

Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this be funded?

Our findings will allow us to demonstrate that certain methane-utilizing microbes can be harnessed to treat nutrients in industrial and environmental settings. This funding is essential as a proof the concept of the nutrient treatment process, and will be used to leverage federal funding like National Science Foundation, Water Environment Federation, and United States Department of Agriculture to further develop this technology.

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Assessment of Microbes for Improving Wild Rice Restoration	M.L. 2017, Chp. 96, Sec. 2, Subd. 03f	$334,000
Evaluating Locally Sourced Materials for Road Salt Reduction	M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04o	$162,000

Project Manager and Organization Qualifications

Project Manager Name: Chan Lan Chun

Job Title: Assistant Professor

Provide description of the project manager's qualifications to manage the proposed project.

Dr. Chun will have chief management responsibilities for overseeing the proposed project. She will be responsible for working with Dr. Christopher Filstrup to ensure that project goals, results and timelines are met. Dr. Chun is an environmental engineer in the area of environmental biotechnology with research experiences in the analysis and use of microorganisms in natural and engineered environments. Dr. Chun have studied the distribution and diversity of microorganisms in aquatic and soil environments to understand role microbes play in water quality and human health using a combination of cultivation-dependent and cultivation-independent techniques (e.g. quantitative PCR and DNA sequencing approach). In addition, her work focuses on the development of treatment technologies and mitigation strategies to improve and restore ecosystem structure and function. She has published over scientific journal articles and book chapters. The collective research and organizational experiences of the project team members and the resources available to this project from the University of Minnesota should ensure the successful completion of the proposed project goals. Dr. Filstrup specializes in applied limnology, cultural eutrophication, harmful algal blooms, and freshwater resources management, and has nearly two decades of experience studying these issues and developing management strategies for freshwater systems.

Organization: U of MN - Duluth - NRRI

Organization Description:

The Natural Resources Research Institute (NRRI) is an applied research and economic development engine for the

University of Minnesota research enterprise. NRRI employs over 130 scientists, engineers and technicians to deliver on its mission to deliver research solutions to balance our economy, resources and environment for resilient communities. NRRI collaborates broadly across the University system, the state and the region to address the challenges of a natural resource based economy. NRRI researchers have extensive experience in managing large, interdisciplinary projects. NRRI's role is as an impartial, science-based resource that develops and translates knowledge. Projects include characterizing and defining resource opportunities, minimizing waste and environmental impact, maximizing value from natural resources and maintaining/restoring ecosystem function. Environmental Microbiology and Biotechnology Laboratory is equipped for research in the areas of microbial ecology, geochemistry, and molecular biology and includes computers and special software for genetic and phylogenetic analyses. Additionally, the lab has worked on various types of bioreactor system. This project have access to DNA sequencing facilities at the University of Minnesota Biomedical Genomics Center and the Minnesota Supercomputing Institute for analysis of DNA sequence data generated by this project.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount
Personnel								
Chan Lan Chun, Assistant Professor		PI, Chun will be responsible for project administration and management, supervising the post doc and students with Filstrup, and activities with microbial ecology and bioreactor experiments.			26.7%	0.24		$40,333
Christopher Filstrup, Researcher 7		co-PI, Filstrup will be responsible for co-supervise a postdoc and students and activities associated with total and dissolved nitrogen analyses and isotope analysis.			26.7%	0.3		$32,040
Postdoctoral Associate		A postdoc will be responsible for day-to-day research tasks and mentor a graduate student.			20.3%	1.5		$102,797
Graduate student		A graduate student will conduct field sampling and microbial ecological analysis for methane-utilizing microbes.			46.5%	0.81		$68,779
Undergraduate Assistant		An undergrad will assist field sampling and general lab analysis			0%	0.9		$24,553
							Sub Total	**$268,502**
Contracts and Services								
Natural Resources Research Institute	Internal services or fees (uncommon)	Nutrient analyses for characterization of N and P fractions: TN + TP $28 per sample x 100 samples/yr x 3yr Ammonia, nitrate/nitrite, soluble reactive phosphorus $36 per sample x 100 samples/yr x 3yr				0		$19,200
University of Minnesota Genomic Center	Internal services or fees (uncommon)	University of Genomic Center MiSeq run, library prep, and supercomputer usage fee:.~ $1,969/lane x 2 lanes + $10.95 library prep/sample x ~ 435 samples per project = $8700 Microfludic qPCR for nitrogen cycling genes: $14/samples x 400 samples = $5,600				0		$14,400
							Sub Total	**$33,600**
Equipment, Tools, and Supplies								

	Tools and Supplies	General operation and lab supplies	Chemical, thermal control, molecular biological assay agents, isotope standards, expendable lab supplies (e.g. plasticware, bottles, columns, disposable labware), and gas cylinders					$16,000
	Tools and Supplies	Field sampling and test supplies	Field sampling gears, methane analyzer, and its expendable supplies					$9,348
							Sub Total	**$25,348**
Capital Expenditures								
							Sub Total	**-**
Acquisitions and Stewardship								
							Sub Total	**-**
Travel In Minnesota								
	Miles/ Meals/ Lodging	Field sample collection	Sample collection, meeting with state agency, and field testing : ~2000 miles x $0.575/mi = $1,150 + vehicle rental use $10/day x 20 days= $200					$1,350
	Conference Registration Miles/ Meals/ Lodging	Conference and workshop	Project finding dissemination, $1,200 is budgeted for in-state conference attendance (e.g. Minnesota Water Resources Conference): registration for 2 people= $299, lodging $89/night x 2 nights x 2 rooms= $356; per diem/meals for 3 days $38.25 + $51 + $38.25= $127.50 x 2 people = $255; ~400 miles x $0.575/mi = $230 + vehicle rental use $10/day x 6 days= $60					$1,200
							Sub Total	**$2,550**
Travel Outside Minnesota								
							Sub Total	**-**

Printing and Publication								
	Publication	Publication cost	To cover publication costs (page charges, open access) for dissemination of research. Estimated as 2 papers @ $2000 each = $4000					$4,000
							Sub Total	**$4,000**
Other Expenses								
							Sub Total	**-**
							Grand Total	**$334,000**

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request

Non ENRTF Funds

Category	Specific Source	Use	Status	Amount
State				
			State Sub Total	**-**
Non-State				
In-Kind	UMN unrecovered indirect costs are calculated at the UMN negotiated rate for research of 55% modified total direct costs.	Indirect costs are those costs incurred for common or joint objectives that cannot be readily identified with a specific sponsored program or institutional activity. Examples include utilities, building maintenance, clerical salaries, and general supplies. (https://research.umn.edu/units/oca/fa-costs/direct-indirect-costs)	Secured	$170,149
			Non State Sub Total	**$170,149**
			Funds Total	**$170,149**

Attachments

Required Attachments

Visual Component

File: 0ec353a3-50c.pdf

Alternate Text for Visual Component

The visual art summarizes novel microbial carbon-nitrogen processes that can be a promising solution to remove nitrogen nutrients from water using methane and describes activities of the proposed project.

Optional Attachments

Support Letter or Other

Title	File
Sponsored Projects Authorization	108e817d-9f0.pdf

Administrative Use

Does your project include restoration or acquisition of land rights?

No

Does your project have patent, royalties, or revenue potential?

No

Does your project include research?

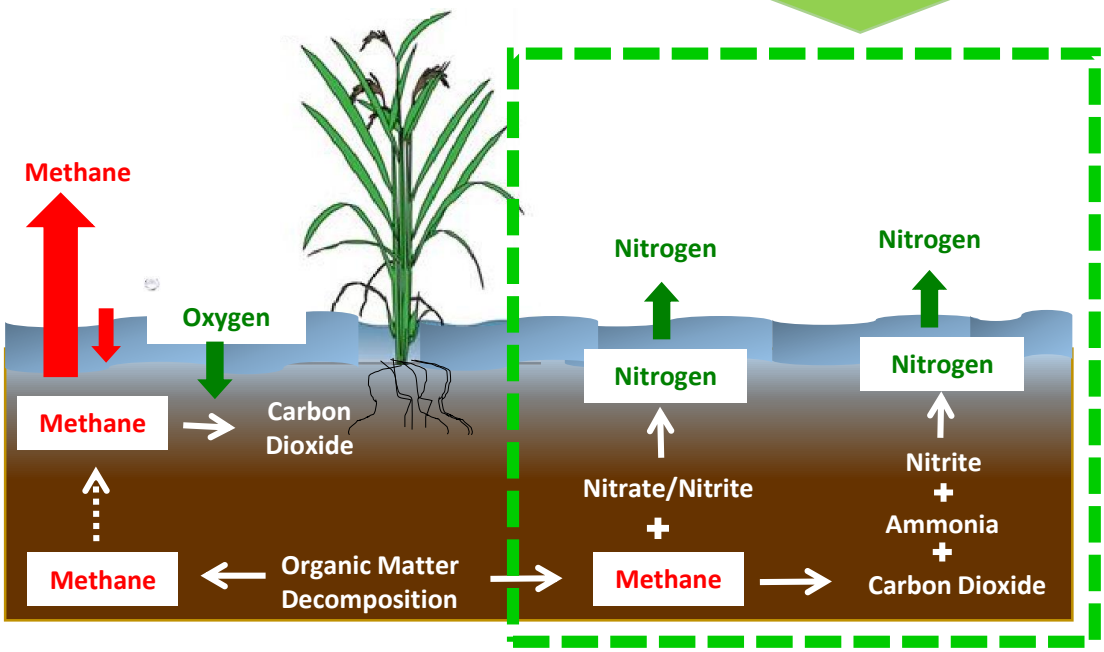
Yes

Does the organization have a fiscal agent for this project?

Yes, Sponsored Projects Administration

Reducing Nutrients and Methane Emissions Using Wetland Microbes

Novel microbial carbon-nitrogen processes are a promising solution to remove nitrogen nutrients using methane.



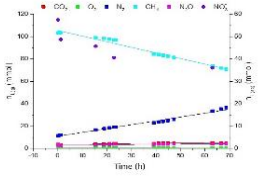
Identifying Organisms in Diverse Environments

Understanding Who, How, and What Conditions Favor Removal Processes

Microbial Ecology

Microcosms & Bioreactors

CH_4/N Removal



Project Outcome

A basis to develop treatment technologies that remove methane and nitrogen concurrently.