Environment and Natural Resources Trust Fund

2022 Request for Proposal

General Information

Proposal ID: 2022-208

Proposal Title: Characterizing Microbial Diversity of Ephemeral Minnesota Wetlands

Project Manager Information

Name: Christine Salomon

Organization: U of MN - College of Pharmacy

Office Telephone: (612) 626-3698

Email: csalomon@umn.edu

Project Basic Information

Project Summary: Collection of foundational information about unique microbial species associated with plants, animals, insects and sediments in vernal (ephemeral) wetland pools throughout Minnesota that serve as critical and threatened habitat

Funds Requested: $200,000

Proposed Project Completion: June 30 2024

LCCMR Funding Category: Small Projects (H)

Secondary Category: Foundational Natural Resource Data and Information (A)

Project Location

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

Region(s): NW, Central, Metro, SW,

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

Region(s): SW, NE, Central, Metro,

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.

Vernal (or "ephemeral") pools are unique, temporary, and fragile wetland ecosystems that occur throughout Minnesota. These pools are relatively small (puddle to football field size) and may only last for a few months. Because they are not connected to larger bodies of water, they do not contain fish predators which provides critical habitat for the development of many ecologically important species including rare plants, blue spotted salamanders, several frog species, the threatened Blanding's turtles, six species of fairy shrimp, and many insects. Although the animal, plant and insect fauna of vernal pools have been characterized in some locations, very little is known about the microbial diversity of these pools on both temporal (seasonal) and spatial (location) scales. The bacterial and fungal communities in these pools may provide essential functions in the sediments or through association with wetland plants, animals and insects. Due to the relatively small size and fleeting nature of vernal pools, they are often unnoticed, under-studied and generally unprotected, especially during their dry seasons. The characterization and cataloging of the microbes that are unique to these wetlands will provide foundational data for improved understanding, and ultimately, better protection of these critical wetland habitats

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 plan to collect foundational data about the microbes found in vernal pools in different locations and seasons. This will include taxonomic data (through DNA sequencing of both cultured and uncultured microbes), functional information about how microbes interact, and associated physical data of the collection site (pool size, depth, plants and animals observed, seasonal data). Because so little is currently known about bacteria and fungi associated with vernal pools, we aim to compile and compare basic microbial community information from distinctly different geographic locations ranging from quartzite bedrock pools in southwestern Minnesota to nutrient-rich ephemeral ponds in forests on the North Shore over several seasons. Despite their microscopic size, bacteria and fungi can have significant effects on the growth and development of macro-organisms like plants, animals and insects. This data collection and resulting database will provide a rich resource for developing hypotheses about how to support and conserve both the visible, and sometimes rare or endangered organisms, as well as invisible, but ecologically important, microbial communities. We hope to increase the visibility of these unique environments to mitigate the loss of wetlands across Minnesota and inspire conservation efforts for these relatively unseen and under-appreciated critical habitats.

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?

1. Taxonomic data for ~400 isolates of bacteria and fungi associated with at least 5 geographically separated vernal pools and distinct seasons.
2. Metagenomic sequence data that will provide a more complete picture of abundance and location of uncultured microbes, including associations with rare plants or animals
3. Database containing taxonomic and functional data for cultured isolates
4. Library of unique bacteria and fungi that will be available for functional and ecological studies

This foundational data should provide improved understanding of potential roles, functions and uniqueness of wetland microbes, justifying expansion of protection, study and conservation of vernal pools statewide.

Activities and Milestones

Activity 1: Characterization of cultivatable bacteria and fungi from vernal pools

Activity Budget: $158,557

Activity Description:
We will sample vernal pools that are well documented in Minnesota State and County Parks and Nature Preserves (March-August), emphasizing locations with endangered species including Split Rock Creek and Blue Mounds State parks in the southwest, Jay Cooke State Park and Hartley Park in the Duluth area, and Tettegouche State Park on the north shore. In cooperation with the DNR and with collection permits, we will collect small (< 1/2 teaspoon) samples of sediments, non-endangered plants and insects, and amphibian egg masses. Microorganisms (> 200 per year) will be isolated from these materials in the lab using selective media and antibiotics (for fungi) and antifungals (for bacteria). DNA will be isolated from each strain using standard methods and sequenced at the UMN Genomics Center (16s rRNA and ITS gene regions for bacteria and fungi, respectively) for taxonomic identification. Each strain will be cultured on solid media and tested for interactions with a panel of fungi and bacteria, and the most abundant strains will be cultured and extracted for additional antagonism testing. These data will be collectively used to develop a table of characteristics to begin assessing and comparing microbial communities associated with different seasons (wet vs dry), geography, and hosts.

Activity Milestones:

Description	Completion Date
Isolation of bacteria and fungi from vernal pools (sediment, plants, egg masses, insects)	June 30 2024
DNA sequencing of isolated microbial strains	June 30 2024
Measurement of biological activities of extracts from isolated microbes	June 30 2024

Activity 2: Metagenome community sequencing (bacteria and fungi)

Activity Budget: $30,231

Activity Description:
Subsamples of the sediments, plants, insects and egg masses collected for microbial cultivations (activity 1) will be subjected to DNA extraction and metagenome analysis. Microbial community DNA samples (minimally, 5 locations x 2 seasons x 10 samples = 100 samples per year) will be sequenced by the UMN Genomics Center using marker gene sequencing with dual-indexing on an Illumina MiSeq instrument. We will utilize the bioinformatics analysis platform provided by the genomics center (Qiime 1.9) to filter sequence data, organize into operational taxonomic units (OTUs), and determine alpha and beta diversity. These data will also be compared to the sequence data of the individual cultured strains obtained in activity 1. These data will provide a more complete taxonomic picture of what bacteria and fungi are present in each sample without the inherent bias associated with studying only cultured strains. The same analysis will be applied as for activity 1, to compare taxonomy and abundance of OTUs to the physical characteristics of the pools.

Activity Milestones:

Description	Completion Date
Isolate DNA from bulk samples collected from vernal pools	May 31 2024
Sequence microbial communities using Illumina next generation sequencing	May 31 2024
Determine alpha and beta diversity of microbial communities	June 30 2024

Activity 3: Development of vernal pool microbial database

Activity Budget: $11,212

Activity Description:

All of the vernal pool collection information (GPS coordinates, depth, size, observed plants and animals, weather) will be combined with the taxonomic data (for cultured strains and uncultured metagenome samples) and functional data (microbial interactions and antagonism data for cultured strains) into an online, searchable database. Because we want these data to be accessible to both the public and scientists, we will use a simple system like Google Sheets for public facing information, and an internal database (AirTable) to include any sensitive data (ie. detailed locations of rare or endangered plants or animals). This data table will allow us (and others) to make inferences and new hypotheses about seasonal variation, and identify which microbes are found in various locations and associated with different hosts or substrates (such as sediments). We anticipate finding high variation in species by location of the pools geographically as well as seasonally, and by nutrient loads. Future work can utilize these data to better understand possible relationships between microbes and plant or animal species which could provide compelling ecosystem information for justifying protection and documentation of vernal pools throughout Minnesota.

Activity Milestones:

Description	Completion Date
Compile sequence data from microbiome and cultures, temporal and spatial data and physical pool characteristics	June 30 2024
Convert data tables into online, accessible database	June 30 2024

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Robert Blanchette	University of Minnesota, Department of Plant Pathology	Dr. Blanchette will be responsible for the isolations and sequencing of fungi. His group will also analyze the DNA data from the community microbiome sequencing experiments and develop spatial and temporal comparisons.	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?

This project will provide foundational data on both cultured and uncultured bacteria and fungi associated with sediments, plants, animals and insects in vernal pools. We expect these results to lead to a better understanding about these complex and unique habitats, as well as additional hypotheses about relationships between microbes and associated organisms. Ultimately, we hope to increase both public and scientific awareness of these ecosystems to inspire continued research and protection. Additional research could be funded by the NSF, USFW and/or through a "phase II" request from the LCCMR

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Biological Control of White Nose Syndrome in Bats - Phase II	M.L. 2016, Chp. 186, Sec. 2, Subd. 06d	$452,000

Project Manager and Organization Qualifications

Project Manager Name: Christine Salomon

Job Title: Associate Professor

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

Dr. Salomon (PI) is an Associate Professor at the Center for Drug Design and a faculty member in the Biotechnology Institute at the University of Minnesota. Dr. Salomon earned her Ph.D. at the Scripps Institution of Oceanography, UCSD, in the area of natural products chemistry from invertebrates and microbes. She continued her training in the Department of Microbiology at the University of Minnesota where she worked on understanding how soil microbes biosynthesize chemical compounds. Dr. Salomon's current research program is focused on the discovery and utilization of novel microbes that can be used for biological control of agricultural and wildlife pathogens and production of unique compounds for biomedical and biotechnological applications. She has successfully secured both internal (Academic Health Center, Biotechnology Institute) and external (US Department of Agriculture and US Fish and Wildlife) support for her research program. Dr. Salomon was the Project Manager on a previous funded ENRTF project "Biological Control of White Nose Syndrome in Bats – Phase II", ML 2016 Chp 186, sec 2.

Dr. Robert Blanchette (Co-PI) is a professor in the Department of Plant Pathology. He has been involved with research and teaching of forest and landscape trees at the University for over 30 years. He currently teaches undergraduate and graduate classes at the University of Minnesota on forest and shade tree diseases. Research is in the area of forest pathology and wood microbiology with investigations underway on the biology and ecology of tree pathogens, tree

defense mechanisms and managing tree diseases using integrated control procedures. Dr. Blanchette has served as project leader on several past projects including 2015-084 Preventing a new disease of pines in Minnesota, 2013-19B Finding Disease Resistant Elm Trees in Minnesota and 2016-131-D Winning the Dutch elm disease battle Phase II.

Organization: U of MN - College of Pharmacy

Organization Description:
Dr. Salomon is in the Center for Drug Design at the University of Minnesota and a member of the Biotechnology Institute. These centers play a central role in providing training and coordinating research in biological, chemical and engineering sciences at the University of Minnesota. The University of Minnesota Biotechnology Institute was established to catalyze the development of a biotechnology industry in Minnesota. It also serves as an important resource for industry by providing connections with academic research partners.

The Department of Plant Pathology at the University of Minnesota is one of the oldest plant pathology departments in the country. Since 1907 the Department has had a strong impact on plant health, agricultural development, and ecosystem vitality on a local, national, and international scale. It is involved with solving today's complex plant health problems through cutting-edge research. The department provides sound plant health advice to stakeholders throughout Minnesota and around the globe and is educating the next generation of plant health professionals and change-makers through a modern and broad plant pathology curriculum.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount
Personnel								
Principle Investigator		Project mananager and bacterial diversity, chemistry and bioactivity testing lead.			36.5%	0.1		$18,473
Research associate		Responsible for microbial cultivations, extract production, and biological testing			36.5%	0.6		$47,335
Research Associate		Fungal isolations, sequencing and analysis of pure cultures and community microbiomes			36.5%	0.4		$44,463
Junior scientist		Bacterial isolations, biological assay testing, database management for sequence and bioactivity data, general lab support			0%	1.2		$32,252
undergraduate research assistant		media and sample prep, sample management, fungal cultivations, general lab support			0%	0.4		$10,750
Co-Principal Investigator		Manager for fungal isolations and community analysis			0%	0.1		-
							Sub Total	**$153,273**
Contracts and Services								
							Sub Total	**-**
Equipment, Tools, and Supplies								
	Tools and Supplies	Field collections and microbiology supplies: media, reagents, petri dishes, tubes, gloves, field sampling materials	field collections and microbiology supplies for bacterial and fungal isolations and characterizations					$10,000
	Tools and Supplies	Microscopy supplies and electron microscopy costs	microscopic characterization of microbial strains, years 1 and 2					$800
	Tools and Supplies	Molecular biology/sequencing costs: (DNA isolation kits, PCR supplies, enzymes, reagents, sequencing costs) x 300 samples/year	Sequencing of DNA from pure cultures and Illumina next generation metagenomic sequencing for community analysis) for activity 2, years 1 and 2					$16,000
	Tools and Supplies	Chemical supplies (solvents, chromatography materials, reagents, tubes, glassware, pipettes)	Supplies for conducting chemical extractions for biological assays and					$8,000

			fractionation of extracts for additional testing					
	Tools and Supplies	Supplies for biological assays (pipettes, pipette tips, epi tubes, culture tubes, petri dishes, media, 96 well plates, reagents, gloves), estimated 300 samples per year	Supplies for conducting antagonism studies, antimicrobial and immuno-modulation assays, 2 years					$4,800
							Sub Total	**$39,600**
Capital Expenditures								
							Sub Total	**-**
Acquisitions and Stewardship								
							Sub Total	**-**
Travel In Minnesota								
	Miles/ Meals/ Lodging	estimated 6 trips per year with 2-3 scientists, 1-2 days, distance from 120-450 miles round trip x 2 years, lodging for longer trips.	In-state round trip travel for sampling from vernal pools in spring and late summer each year for 2 years					$4,060
							Sub Total	**$4,060**
Travel Outside Minnesota								
							Sub Total	**-**
Printing and Publication								
	Publication	Scientific manuscript publication costs (open access) -at least one open access paper	Publication of scientific data and results obtained during this project					$1,067
							Sub Total	**$1,067**
Other Expenses								
		Repair of equipment and instrumentation (e.g. vacuum pumps, water baths, incubators, shakers, etc.) and calibration of instruments (pipettes, balances) estimated at $1000 per year for 2 years	Funds for inevitable breakage, repair of glassware and instrumentation and calibration of instrumentation					$2,000

							Sub Total	**$2,000**
							Grand Total	**$200,000**

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				
			Non State Sub Total	**-**
			Funds Total	**-**

Attachments

Required Attachments

Visual Component

File: 80accbd2-78b.pdf

Alternate Text for Visual Component

The visual component contains a map of Minnesota showing the location of the proposed study sites (Tettegouche, Jay Cooke, Blue Mound and Split Rock Creek State Parks and Richardson Nature Center). It also shows two images of a medium sized (~20 meter diameter), shallow vernal pool in a forested area that is wet in early spring but completely dry in late spring. There is a brief explanation of the proposal to isolate, sequence and characterize microbes from vernal pools from at least 5 locati...

Administrative Use

Does your project include restoration or acquisition of land rights?

No

Does your project have potential for royalties, copyrights, patents, or sale of products and assets?

Yes

Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?

Yes

Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF? If so, describe here:

Yes, Although we do not currently anticipate any immediate products or assets from this project in this two year time-frame, it is possible that we will discover microbes with useful activities that we may want to develop further.

Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

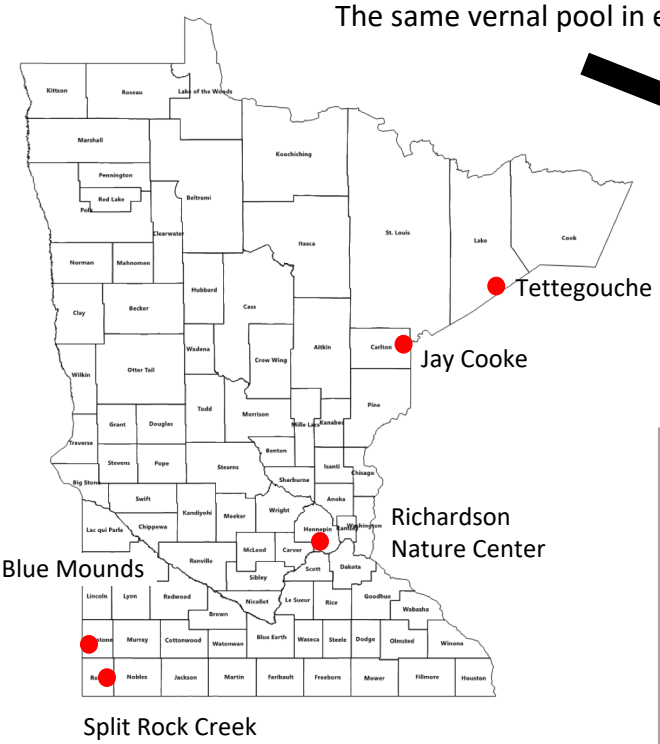
Yes, Sponsored Projects Administration

Characterizing Microbial Diversity of Ephemeral Minnesota Wetlands

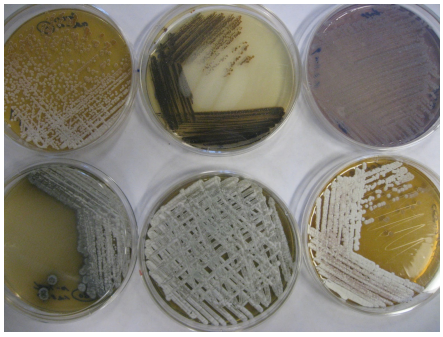
Project Manager: Christine Salomon, UMN, Proposal ID: 2022-208

Vernal pools are small, temporary wetland ecosystems that provide critical habitat for many species of plants, animals, and insects, but are relatively unnoticed and unprotected due to their ephemeral nature. (www.vernalpool.org). Little is known about associated microbes in these habitats.

The same vernal pool in early and late spring.



Study locations: State parks and nature centers with vernal pools



We propose to sequence and characterize cultured and uncultured microbial communities from vernal pools from at least 5 locations throughout the state and develop a microbial database of taxonomic and functional data.

The characterization and cataloging of the microbes that are unique to these wetlands will provide foundational data for improved understanding, and ultimately, better protection for these critical wetland habitat ecosystems.

Examples of endangered or threatened species associated with vernal pools: Water hyssop (*Bacopa rotundifolia)*, Hairy waterclover (*Marsilea mucronate),* Blanding's turtle (*Emys blandingii*), and Four-toed salamander (*Salamandra scutata*),