

**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

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## **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. Ineli gible** | **% Bene fits** | **# FTE** | **Class ified 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 fractionation of extracts for additional testing |  |  |  |  | $8,000 |
|  | 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](https://lccmrprojectmgmt.leg.mn/media/map/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