

Final Abstract

Final Report Approved on December 10, 2024

M.L. 2021 Project Abstract

For the Period Ending June 30, 2024

Project Title: Exploring Minnesota's Wetlands: Our Resource for Future Medicine

Project Manager: Brian Dingmann

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Website: <https://www.crk.umn.edu/>

Funding Source:

Fiscal Year:

Legal Citation: M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 03k

Appropriation Amount: \$210,000

Amount Spent: \$209,200

Amount Remaining: \$800

Sound bite of Project Outcomes and Results

Project outcomes include cataloging bog microbial diversity across Minnesota's ecoregions, creating DNA libraries, and advancing antimicrobial research. Over 150 students were trained in molecular techniques, and their findings were presented at major conferences. This work fosters student retention in STEM fields and contributes to public health, sustainability, and best management

Overall Project Outcome and Results

The project successfully cataloged the microbial functional diversity and community structure of bogs across Minnesota's ten ecoregions. DNA libraries were created from wetland samples to analyze community diversity, providing foundational insights into bog ecosystems. This data is being compiled into a manuscript for publication and shared with stakeholders to inform best management practices.

Significant progress was made in characterizing the Actinomycetes bacterial group, known for producing antimicrobial compounds. Preliminary protocols for isolating Actinomycetes were developed, leading to promising leads for new antimicrobials. While further research is needed to optimize these methods, this effort underscores the potential for

bog ecosystems to address public health challenges through novel antimicrobial discoveries.

The project served as a robust educational platform, training over 150 students in molecular and microbiological techniques, including 26.5% of the biology/health science students at the University of Minnesota Crookston (UMC). Nine undergraduate researchers participated directly in the lab, contributing to protocol development and manuscript co-authorship. Students presented findings at regional and national conferences, gaining valuable experience in scientific communication. One student, the lead author of a published manuscript, secured a research technician role at Columbia University. Northwest Technical College students have also contributed.

Students from UMC participated in field sampling over two summers. Advanced techniques, such as Nanopore sequencing and chemical characterization, were employed to assess inter-bog microbial diversity and identify antimicrobial compounds. These efforts yielded over 1,000 microbial isolates, with 176 showing activity against safe ESKAPE pathogen relatives and fewer than 20 demonstrating consistent antimicrobial activity.

This project exemplified the intersection of science, education, and public impact. It fostered STEM engagement among students, advanced research on microbial diversity and antimicrobial discovery, and provided practical data for the conservation and sustainable management of Minnesota's bog ecosystems. Dissemination efforts ensured broad scientific and public engagement with the findings.

Project Results Use and Dissemination

The project results were disseminated through student presentations at local, regional, and national conferences, including the American Society for Microbiology and the National Council of Undergraduate Research. Students and faculty published a literature review and are preparing additional manuscripts for peer-reviewed journals. Findings were incorporated into microbiology courses at UMC and NTC, introducing students to research methods. Additionally, the Science Museum of Minnesota was awarded an LCCMR grant to create an interactive online story map showcasing 100 funded projects across Minnesota's ecoregions. The lead PI was interviewed, and the "story" of this project will be part of it.



Environment and Natural Resources Trust Fund

M.L. 2021 Approved Final Report

General Information

Date: December 19, 2024

ID Number: 2021-278

Staff Lead: Noah Fribley

Project Title: Exploring Minnesota's Wetlands: Our Resource for Future Medicine

Project Budget: \$210,000

Project Manager Information

Name: Brian Dingmann

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Project Reporting

Final Report Approved: December 10, 2024

Reporting Status: Project Completed

Date of Last Action: December 10, 2024

Project Completion: June 30, 2024

Legal Information

Legal Citation: M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 03k

Appropriation Language: \$210,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota, Crookston, to work with White Earth Tribal and Community College to catalog bog microbe diversity in Minnesota's ecoregions, test for potential antibiotic-producing microorganisms, and establish methods to enhance any antibiotic cultures discovered.

Appropriation End Date: June 30, 2024

Narrative

Project Summary: Our Minnesota bogs are an essential resource. As we investigate inter-bog microbial diversity in these critical habitats, we could find the next antibacterial, antifungal, or antiviral medicinal product.

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

Our Minnesota bogs are unique and vital wetland resources because they are the most carbon-dense ecosystems on the planet. Bogs are an essential carbon sink since carbon is bound and sequestered in the peat layer. Microorganisms play an indispensable role in bog habitat geochemical cycling, and preparing sequencing libraries of the microbial communities from Minnesota's mid-latitude bogs would allow future studies to better analyze microbial structure and diversity. We will measure inter-bog functional community structure and diversity in this preliminary study.

Additionally, the University of Minnesota Crookston (UMC) and White Earth Tribal and Community College (WETCC) students participate in Tiny Earth. This antibiotic discovery initiative utilizes techniques to investigate various soils for potential antibiotic-producing microorganisms. Due to increased antibiotic resistance, there is a great need to discover and develop new antimicrobials. To our knowledge, the microbial communities of Minnesota's bogs have not been mined for potential antibiotic producers.

This project provides enriched educational opportunities for underrepresented students in Minnesota. Student learner outcomes will assess students' perceptions of science and gauge their understanding of the "nature of science." Through this process, this study would contribute to the overall understanding of Minnesota's peatlands.

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

Almost all of our new antibiotics are remakes or variants on existing antibiotic architecture. For pharmaceutical companies, it is easier and more profitable to use existing antibiotic frameworks to design drugs instead of searching for novel antibiotics in nature. The need for new antimicrobials is self-evident in this time of growing antibiotic resistance. The Tiny Earth Studentsourcing Antibiotic Discovery project has standardized the methodology to isolate and characterize soil bacteria. By training undergraduates from two institutions, we would have a small army of investigators searching for antibiotic-producing bacteria in bogs. We hypothesize our Minnesota bogs contain a potentially unexploited reservoir of these bacteria. Specifically, the bogs are inhabited by the Actinomycetes bacterial group that has shown to provide antibacterial, antifungal, and antiviral medicinal natural products. Faculty and undergraduate students working on this project will develop sustainable protocols which characterize the microbial community functionality and diversity within bogs. Additionally, bacteria capable of producing novel antimicrobials will be targeted.

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?

The project would catalog the bog microbial functional diversity and community structure in Minnesota's ten ecoregions. The database/protocols will be widely disseminated to stakeholders for best management practices.

Enhancement sampling for the Actinomycetes bacterial group would lead to potential new sources for antimicrobials. These types of efforts are crucial for the health and wellbeing of not only Minnesotans but society in general.

The University of Minnesota Crookston and White Earth Tribal and Community College undergraduates will be trained in molecular and microbiological techniques for future careers in health and conservation (e.g., DNR, USDA, MPCA, and graduate schools).

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

Activities and Milestones

Activity 1: Characterize the microbial community's ability to produce potential antimicrobials

Activity Budget: \$87,156

Activity Description:

The crisis of antibiotic resistance provides an immediate and critical need for new and novel antimicrobials. Natural products, such as secondary metabolites produced from microorganisms, constitute the majority of our antibiotics. The Tiny Earth, Studentsourcing Antibiotic Discovery project has standardized the methodology to isolate and characterize potential producers of antimicrobial bacteria. After isolation and purification of soil bacteria, we will conduct competition experiments against the so-called safe pathogens to identify likely antibiotic-producing cultures. Subsequently, chemical extraction/fractionation would lead to the identification of antimicrobial compounds. Faculty and undergraduate students at UMC and WETCC will employ both molecular techniques and microbiological methods to identify and selectively enhance the culture of Actinobacteria. This group of bacteria is known to exist in bogs and has demonstrated the ability to produce antibacterial, antifungal, and antiviral products. Molecular techniques will identify Actinobacteria genes that are responsible for the production of potential antibiotics. Additionally, we will establish empirically derived methods to enhance the cultures of these antibiotic-producing bacteria since most soil bacteria cannot usually be cultured. Aspects of this activity will be incorporated into suitable courses at UMC and WETCC. Ultimately, the result of this activity will produce "leads" for further antimicrobial processing.

Activity Milestones:

Description	Approximate Completion Date
Field sampling: 2 summers of bog sampling with 4-5 students from UMC and WETCC	August 31, 2022
Protocol development (e.g., sampling, safety, molecular and microbiology, etc.) in lab meetings and mentoring	October 31, 2022
Microbiological and molecular techniques: 4-5 students each summer from UMC and WETCC); UMC microbiology.	January 31, 2024
Chemical Analysis: summer (4-5 students from UMC and WETCC), microbiology class	January 31, 2024

Activity 2: Characterize the microbial community functionality and diversity in bogs

Activity Budget: \$122,844

Activity Description:

Bogs are found in all ten ecoregions of the state and are an essential carbon sink for global carbon cycling. Conservation and preservation of our Minnesota peatlands are critical as carbon dioxide levels continue to rise worldwide. The importance of wetlands in geochemical cycling and specifically the microbial community in these processes is well established. The proposed research investigates the microbial functional diversity and community structure within our Minnesota bogs. Using commercially available kits, faculty and undergraduates will analyze the diversity of the microbial community between bogs (inter-bog diversity). To facilitate a more efficient and effective understanding of our wetlands/bogs, we propose to establish a microbial diversity database that may be used in future assessment and best management practices by stakeholders. The databases and associated protocols would be widely disseminated to the various stakeholders (e.g., DNR, MPCA, etc.). Aspects of this activity will be incorporated into suitable courses at UMC and WETCC. Students engaged in this work will be well prepared for required internships and future employment. Ultimately, this activity will result in a sequencing library for further data "mining" regarding bog microbial diversity.

Activity Milestones:

Description	Approximate Completion Date
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Protocol development and review: summer undergrads (4-5 students from UMC and WETCC), microbiology class	December 31, 2022
Statistical Analysis: Summer (4-5 students from UMC and WETCC), descriptive and application statistics	August 31, 2023
Scientific writing: Summer (4-5 students from UMC and WETCC); LCCMR documentation and publication	June 30, 2024

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Lorna Lague	White Earth Tribal and Community College	President of White Earth Tribal and Community College	No
Karl Anderson	University of Minnesota Crookston	Co-PI: managing students; microbiological and molecular technique guidance.	Yes
Venugopal Mukku	University of Minnesota Crookston	Co-PI: managing students; chemical extraction and fractionation guidance.	Yes

Dissemination

Describe your plans for dissemination, presentation, documentation, or sharing of data, results, samples, physical collections, and other products and how they will follow ENRTF Acknowledgement Requirements and Guidelines.

The results of the work outlined in this proposal will be disseminated in a number of formats and venues. Since undergraduate students will be heavily involved in this work, they will be presenting their results via poster and oral presentations at any number of local, regional, and national conferences and symposia. UM Crookston (UMC) hosts an undergraduate research day each year in which students discuss the research projects they work on to their peers and other faculty. WETCC will also be holding student research presentations. Students from UMC have regularly presented their research findings at both regional and national meetings (e.g., American Society for Microbiology, American Chemical Society, National Council of Undergraduate Research). At White Earth Tribal and Community College (WETCC), we hope to encourage similar presentations, however, students specifically plan to present their findings at the Annual Biomedical Research Conference for Minority Students. Similarly, UMC and WETCC faculty have been presenting at many of these and other conferences (e.g., American Society for Microbiology, Society of Environmental Toxicology and Chemistry). We plan to continue our attendance at these conferences in order to disseminate our findings for this project.

We also anticipate disseminating our results to various state agencies (e.g., DNR, MPCA) that may be interested in our work. Besides dissemination of results, we also anticipate informing the public of our work on this project through various media outlets. The Mahnomen Pioneer and Anishinaabeg Today and KROXAM and Crookston Times will be will be great outlets for WETCC and UM Crookston, respectively. As land-grant institutions, UMC and WETCC are obligated to engage in public outreach and education, thus we expect that this project will be utilized by our institution to achieve its land-grant mission.

While the scientific merit of this project has been thoroughly outlined, the educational impact of this work is just as important to UMC, WETCC, and our students. Having students engaged in meaningful research is known to help in student retention at both the academic institution and in STEM fields. Thus, we plan on incorporating this project into a number of our biology and chemistry courses to give as many of our students as possible the opportunity to engage in original research. We have already started this process by incorporating some of the concepts/methods outlined in this project into our general microbiology courses, as a way to introduced students to research-based methods in microbial sciences. One of our larger goals is to expose all of our students majoring in biological sciences to some original research with the hopes that a few of them will engage in more involved research projects and potentially related academic and/or career paths.

As a requirement of funding, we will acknowledge the Environment and Natural Resources Trust Fund through use of

the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the ENTRF Acknowledgment Guidelines.

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 work be funded?

We have used most of the techniques for several years in our teaching laboratories at the University of Minnesota Crookston and have the existing infrastructure to train the undergraduates as mentioned in this proposal. The proposed database and baseline data sets will be widely disseminated to the appropriate entities (e.g., DNR, MPCA, etc.). Once sampling and data analysis protocols are established the biomonitoring can be incorporated into existing citizen scientist resource management programs. If we find some promising or at least interesting leads for antibacterial, antifungal, or antiviral medicinal natural products we will aggressively seek additional funding opportunities.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount	\$ Amount Spent	\$ Amount Remaining
Personnel										
Undergraduate Researchers		Undergraduate research; 4-5 students per summer from UMC and WETCC hired by UMC			8%	0.39		\$93,290	-	-
co-PI		Venugopal Mukku (UMC research collaborator)			36.5%	0.26		\$11,484	-	-
co-PI		Karl Anderson (UMC research collaborator)			36.5%	0.39		\$12,700	-	-
Project Manager/PI		Brian Dingmann (project manager)			36.5%	0.39		\$19,200	-	-
Research Professional		Research technician oversight of routine lab research			32%	1		\$46,000	-	-
							Sub Total	\$182,674	\$182,674	-
Contracts and Services										
							Sub Total	-	-	-
Equipment, Tools, and Supplies										
	Tools and Supplies	Chemical reagents	Various chemical reagents will be used that are considered general chemical consumables.					\$1,000	\$1,000	-
	Tools and Supplies	Chemical extraction	Extraction, and fractionation is necessary to identify potential antimicrobial products from the bog microbes.					\$1,000	\$1,000	-
	Tools and Supplies	DNA sequencing lab supplies	DNA sequencing is necessary to identify microbial strains in the bog environment.					\$13,200	\$13,200	-
	Tools and Supplies	Microbiological lab supplies	Microbiological culturing and manipulating soil microbes.					\$10,000	\$10,000	-
	Tools and Supplies	QPCR on 200 samples	To analyze and quantify the microbial community					-	-	-

			structure and function we will investigate 200 samples across the state.							
	Tools and Supplies	General use Polymerase Chain Reaction (PCR) reagents	To analyze microbial structure and function we will investigate 200 samples across the state. General PCR will allow us to gauge structure and funtion to focus research.					-	-	-
	Tools and Supplies	BIOLOG Lab supplies	activity one: microbial diversity and functional analysis					-	-	-
							Sub Total	\$25,200	\$25,200	-
Capital Expenditures										
							Sub Total	-	-	-
Acquisitions and Stewardship										
							Sub Total	-	-	-
Travel In Minnesota										
	Miles/ Meals/ Lodging	Travel to sample bogs with no overnight stays. Bog sampling will require 2 summers with the bog sampling "clustered" to reduce travel/trips.	Travel (\$0.575 per mile of travel, 14,989 miles sampling around state)					\$1,626	\$1,076	\$550
							Sub Total	\$1,626	\$1,076	\$550
Travel Outside Minnesota										
							Sub Total	-	-	-
Printing and Publication										
	Printing	Printing at UMC and WETCC for students and dissemination student posters to promote project.	There will be required printing for internal use by students and when we want					\$500	\$250	\$250

			to promote our project to the public (e.g., student posters, pamphlets,etc.)							
							Sub Total	\$500	\$250	\$250
Other Expenses										
							Sub Total	-	-	-
							Grand Total	\$210,000	\$209,200	\$800

Classified Staff or Generally Ineligible Expenses

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

Category	Specific Source	Use	Status	\$ Amount	\$ Amount Spent	\$ Amount Remaining
State						
			State Sub Total	-	-	-
Non-State						
			Non State Sub Total	-	-	-
			Funds Total	-	-	-

Attachments

Required Attachments

Visual Component

File: [c032089e-535.pdf](#)

Alternate Text for Visual Component

The attached picture provides an overview of the proposed research. Preserving Minnesota's Wetlands: Our Resource for Future Medicine....

Supplemental Attachments

Capital Project Questionnaire, Budget Supplements, Support Letter, Photos, Media, Other

Title	File
White Earth Tribal and Community Support Letter	675a24b5-44d.pdf
Exploring Minnesota's Wetlands: Our Resource for Future Medicine	1b61d04f-42c.pdf
Children's Services Background Check Form	9e5f854d-f6e.pdf

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

Faculty salaries were reduced by eliminating two weeks per summer per faculty member. We reduced the number of undergraduates from both UM Crookston and White Earth Tribal and Community College each summer of the grant to lower student salaries. And finally, we reduced the amount of supplies that would correspond to the reduced workload from the faculty and undergraduates. Overall, the budget was reduced by \$37,000 to reflect the recommended grant total of \$210,000.

Additional Acknowledgements and Conditions:

The following are acknowledgements and conditions beyond those already included in the above workplan:

Do you understand and acknowledge the ENRTF repayment requirements if the use of capital equipment changes?

N/A

Do you understand that travel expenses are only approved if they follow the "Commissioner's Plan" promulgated by the Commissioner of Management of Budget or, for University of Minnesota projects, the University of Minnesota plan?

Yes, I understand the UMN Policy on travel applies.

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

No

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

N/A

Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?

N/A

Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

Yes, Sponsored Projects Administration

Work Plan Amendments

Amendment ID	Request Type	Changes made on the following pages	Explanation & justification for Amendment Request (word limit 75)	Date Submitted	Approved	Date of LCCMR Action
1	Amendment Request	<ul style="list-style-type: none"> • Budget - Personnel 	We are requesting a re-budget from the undergraduate researcher's salary budget to a new position category (research professional). The amount would total \$46,000, which would cover salary and fringe for one year. A recent UMC graduate has done such great work I would like to have her take on more responsibilities and become a research liaison for other undergrad researchers. This outcome would promote our goal of educating the next generation of researchers.	February 16, 2023	Yes	February 17, 2023
2	Amendment Request	<ul style="list-style-type: none"> • Budget • Other • Budget - Personnel • Budget - Capital, Equipment, Tools, and Supplies • Budget - Travel and Conferences 	We want to reallocate more funds to salary from our lab supplies to finish our work and be able to disseminate our final reports and manuscripts. It is critical to have the students and faculty work on the final analysis and manuscripts.	May 7, 2024	Yes	May 8, 2024

Status Update Reporting

Final Status Update August 14, 2024

Date Submitted: August 14, 2024

Date Approved: December 2, 2024

Overall Update

1. The project cataloged the bog microbial functional diversity and community structure across Minnesota's ten ecoregions. DNA libraries were created from various wetland samples to analyze community structure and diversity. The final analysis is being compiled for manuscript dissemination, ensuring the resulting database and protocols are shared with stakeholders for best management practices.
2. Enhancement sampling for the Actinomycetes bacterial group led to potential new sources for antimicrobials. Initial protocols for isolating Actinomycetes were developed, but more research was needed to optimize these methods. This continued work is essential for advancing public health and societal well-being.
3. The project also served as an educational platform, training undergraduates from the University of Minnesota Crookston and White Earth Tribal and Community College in molecular and microbiological techniques. Nine students, representing 26.5% of UMC's biology/health science students, participated in the research lab. Over 150 students from general microbiology courses at UMC and Northwest Technical College were trained through this project. The lead author of a published manuscript, an undergraduate student, worked as a research technician at Columbia University after graduating from UMC.

Activity 1

The project involved a comprehensive approach to studying Minnesota's bogs, integrating microbiological, molecular, and chemical techniques to uncover new antimicrobial-producing microbes. Each summer, 4-5 students from UMC and WETCC participated in microbiological and molecular techniques, including isolation and antimicrobial susceptibility testing. In the chemical analysis phase, students utilized High-Performance Liquid Chromatography (HPLC) and Mass Spectrometry (Mass Spec) to characterize chemical extracts from microbial isolates, which provides critical insights into the potential antimicrobial compounds.

Field sampling was conducted over two summers, where students collected bog samples, which were then processed in the lab. The development of protocols, encompassing sampling methods, safety procedures, and both molecular and microbiological techniques, was a key focus during lab meetings and mentoring sessions.

In total, nine undergraduate researchers, representing 26.5% of UMC's biology program, contributed to the project. These students played a significant role in protocol development and were co-authors on a published manuscript. The project's primary outcome was to deliver foundational data on Minnesota's bogs, while simultaneously training the next generation of scientists. Notably, the chemical characterization efforts have led to promising leads for new antimicrobial agents.

(This activity marked as complete as of this status update)

Activity 2

The research project aimed to characterize the microbial community functionality and diversity within Minnesota's bogs. Over the summer, 4-5 students from UMC and WETCC participated in both microbiology and protocol development activities. Statistical analyses were conducted to assess descriptive and application statistics, while protocol development was integral to the microbiology classes, focusing on safe lab practices and method refinement.

We employed advanced Nanopore sequencing to analyze inter-bog diversity, leading to significant insights into microbial populations. Students and faculty showcased several project findings at professional academic/scientific conferences, which highlighted that over 1000 microbial isolates were initially selected, with 176 showing inhibition against ESKAPE pathogen safe relatives. Among these, fewer than 20 pure cultures consistently demonstrated antimicrobial activity.

Continued research focused on scaling up the cultures to 1L for chemical characterization and identification. Further chemical characterization using HPLC and Mass Spec was performed, revealing that the red pigment produced by one of the isolates, ApK#2, was not prodigiosin or carotenoid, as initially hypothesized. Instead, additional Electrospray Ionization LC-MS and genome sequencing are being employed to further characterize this pigment. The project has culminated in a manuscript, set to be published later this year.

(This activity marked as complete as of this status update)

Dissemination

The results of this project were disseminated through various prominent venues and events. Undergraduate students presented their findings via posters and oral presentations at local, regional, and national conferences, including those hosted by the American Society for Microbiology, the American Chemical Society, and the National Council of Undergraduate Research. These larger conferences provided an excellent platform for students to showcase their work to a broader audience.

Additionally, UM Crookston (UMC) hosted an annual undergraduate research day, where students had the opportunity to discuss their research projects with peers and faculty members. This event was a key component of the dissemination strategy, allowing students to engage with the academic community and gain valuable feedback on their work. Faculty from UMC and White Earth Tribal and Community College (WETCC) also participated in these conferences, contributing to the broader academic discourse.

These efforts ensured that the findings of the project were widely shared within the scientific community, highlighting the importance of student involvement in meaningful research.

Students and faculty have published a literature review manuscript and anticipate having at least one more manuscript in a quality peer-reviewed journal.

Status Update Reporting

Status Update May 1, 2024

Date Submitted: May 7, 2024

Date Approved: May 8, 2024

Overall Update

We have had nine undergraduate researchers working in the laboratory, 26.5% of the total students enrolled in the biology program at UMC. We have a manuscript that was published and co-authored by undergraduates. Our outcome is to provide foundational information about the bogs of Minnesota, and we have done this along with training the next generation of scientists. And finally, we have some exciting leads for new antimicrobial-producing microbes. The final part of the grant is to characterize and publish our findings on one very interesting microbe.

Activity 1

We have identified some interesting antimicrobial-producing microbes and will disseminate our findings over the next two months.

Activity 2

We have used sequencing to characterize the inter-bog diversity and will finish this work over the final two months of the grant period. Dissemination of our findings will follow.

Dissemination

We already have one literature review manuscript published and will probably have at least one more manuscript in a quality journal.

Status Update Reporting

Status Update November 1, 2023

Date Submitted: November 1, 2023

Date Approved: December 20, 2023

Overall Update

The project would catalog the bog microbial functional diversity and community structure in Minnesota's ten ecoregions. The database/protocols will be widely disseminated to stakeholders for best management practices.

The functional diversity and community structure have begun to be described using genomic DNA analysis sequencing. The protocols have been developed, and the analysis is ongoing.

Enhancement sampling for the Actinomycetes bacterial group would lead to potential new sources for antimicrobials. These types of efforts are crucial for the health and wellbeing of not only Minnesotans but society in general.

The enhancement sampling for the northern Minnesota wetlands has been conducted, with analysis ongoing.

The University of Minnesota Crookston and White Earth Tribal and Community College undergraduates will be trained in molecular and microbiological techniques for future careers in health and conservation (e.g., DNR, USDA, MPCA, and graduate schools).

We have trained 32 students this fall in the teaching microbiology lab and 52 students at the Northwest Technical College. Four students were trained and worked full-time over the summer in both microbiological and molecular techniques. In addition, we have submitted a manuscript that is now in press (Current Clinical Microbiology Reports) and has four undergraduates as authors.

Activity 1

Protocol development (e.g., sampling, safety, molecular and microbiology, etc.) in lab meetings and mentoring

Protocols continue to be revised under supervision, but basic protocols are now established and being used on a routine basis.

Field sampling: 2 summers of bog sampling with 4-5 students from UMC and WETCC

We have taken student on bog sampling for the last two summers. We were not able to include WETCC students this past summer, however, we continue to work with instructors from both WETCC and the Northwest Technical College to provide field sampling experiences for students.

Microbiological and molecular techniques: 4-5 students each summer from UMC and WETCC); UMC microbiology.

We have had 4-5 students being trained in molecular and microbiological techniques from UMC and from the Northwest Technical College.

Chemical Analysis: summer (4-5 students from UMC and WETCC), microbiology class

Chemical analysis is ongoing, and students are being trained. We hope to stay on track to finish most of the analysis by the stated deadlines for this milestone and the prior one.

Activity 2

Statistical Analysis: Summer (4-5 students from UMC and WETCC), descriptive and application statistics

Statistical analysis is ongoing, with the first functional diversity analysis nearing completion. We hope to stay on track to finish this work by the stated deadline.

Protocol development and review: summer undergrads (4-5 students from UMC and WETCC), microbiology class

Students have continued to be mentored, with several students now having been continuously working in the lab for over two years.

Scientific writing: Summer (4-5 students from UMC and WETCC); LCCMR documentation and publication

We have submitted a manuscript that is now in press (Current Clinical Microbiology Reports) and has four undergraduates as authors.

Dissemination

We have submitted a manuscript that is now in press (Current Clinical Microbiology Reports) and has four undergraduates as authors.

Status Update Reporting

Status Update May 1, 2023

Date Submitted: May 1, 2023

Date Approved: May 5, 2023

Overall Update

1. The project would catalog the bog microbial functional diversity and community structure in Minnesota's ten ecoregions. The database/protocols will be widely disseminated to stakeholders for best management practices.

Soil samples from northern Minnesota have been processed for sequencing data analysis. Samples from the remaining ecoregions will be processed as well this summer.

2. Enhancement sampling for the Actinomycetes bacterial group would lead to potential new sources for antimicrobials. These types of efforts are crucial for the health and wellbeing of not only Minnesotans but society in general.

Isolates are continually being purified that demonstrate inhibition against our safe-relative pathogens. There are roughly 50 isolates now being processed and characterized for potential sources of antimicrobials.

3. The University of Minnesota Crookston and White Earth Tribal and Community College undergraduates will be trained in molecular and microbiological techniques for future careers in health and conservation (e.g., DNR, USDA, MPCA, and graduate schools).

Three research posters have been presented at national conferences by students that include six current undergraduate researchers. Two posters were presented this April at the National Conference of Undergraduate Research (NCUR) and the third was presented at the Annual Biomedical Conference for Minoritized Scientist

Activity 1

1. Microbiological and molecular techniques: 4-5 students each summer from UMC and WETCC); UMC microbiology.
2. Field sampling: 2 summers of bog sampling with 4-5 students from UMC and WETCC
3. Protocol development (e.g., sampling, safety, molecular and microbiology, etc.) in lab meetings and mentoring

Progress is ongoing with four students now hired for the upcoming summer. A total of eighty students have been trained in the microbiology teaching and research lab. Along with students from UMC and WETCC we have a collaboration to train students from Northwest Technical College in Bemidji. WETCC students participate in the summer sampling through a summer class at WETCC. Protocols and procedures have been established, but we continue to evaluate to optimize results and model the nature of science with the undergraduates.

4. Chemical Analysis: summer (4-5 students from UMC and WETCC), microbiology class

Progress is ongoing regarding this last outcome, however, we are still working on some issues that are related to the chemical analysis and hope to work these issues out this summer.

Activity 2

1. Statistical Analysis: Summer (4-5 students from UMC and WETCC), descriptive and application statistics
2. Protocol development and review: summer undergrads (4-5 students from UMC and WETCC), microbiology class

Microbial diversity data will be available in early summer and will be analyzed with the students. We have created a workflow regarding this analysis and again have worked to engage the students in the nature of science.

3. Scientific writing: Summer (4-5 students from UMC and WETCC); LCCMR documentation and publication

Our research group was invited to submit a literature review to Current Clinical Microbiological Reports. The manuscript will be submitted by May 25, 2023. Several students are involved in this process with a recent graduate working with the PIs to complete the manuscript.

Dissemination

As mentioned earlier, we have had six students present three research posters at national conferences. We will be submitting a manuscript by May 25, 2023 that is a literature review that has involved several undergraduate researchers.

Status Update Reporting

Status Update November 1, 2022

Date Submitted: February 16, 2023

Date Approved: February 17, 2023

Overall Update

Outcome 1 (biodiversity): We are still in the process of collecting samples and processing them regarding outcome. Overall, we are still on schedule to complete this outcome by the end of August 2023 as stated in the proposal.

Outcome 2 (antimicrobial activity): We continue to make progress regarding Outcome 2. There are currently six undergraduate researchers working in the laboratory on this outcome in addition to the thirty students in the general microbiology teaching laboratory. Moreover, we have added six students at the Northwest Technical College that are also working on this outcome. In summary, we have over 50 microbial isolates that show some activity against our safe pathogen relatives.

Outcome 3 (undergraduate training): As stated above, we have made progress regarding isolation of microbes that demonstrate inhibitory activity against safe relative pathogens from the wetland samples. In doing so, we have trained over sixty students so far in microbiological and molecular techniques.

Activity 1

Milestone 1 (molecular and microbiological training): We have trained over sixty students at UM Crookston and another six students at Northwest Technical College. In addition, we have worked to build our collaboration with White Earth Tribal and Community College (WETCC) by taking the biology summer course out sampling.

Milestone 2 (chemical analysis): We are currently doing chemical analysis on five isolates that have shown antimicrobial inhibition.

Milestone 3 (field sampling): We are currently behind on our proposed field sampling due to getting starting so late in 2021. However, we are still on track to finish our field sampling and analysis by the end of August 2023.

Milestone 4 (protocol development): We continue to review our protocols for the proposed research, but have completed the work as proposed (deadline October 2022).

Activity 2

Milestone 1 (statistical analysis): We proposed to have this milestone completed by the August 2023. Despite a delay in our sampling schedule, we still hope to have most if not all of the statistical analysis complete by the stated deadline.

Milestone 2 (biodiversity protocol development): We continue to make progress on the overall strategies to collect and analyze the inter-bog diversity and have several collaborations underway that will aid this effort. Deadline is the end of this year, which is still very conceivable with most of the protocols completed as of this update.

Milestone 3 (documentation and dissemination): This milestone will mark the end of the project and demonstrate our overall progress. We continue to make progress toward this milestone.

Dissemination

We plan to provide information to various stakeholders that will be able to utilize our findings. However, we have already had two students present their research with several other students that will present their contributions by the end of May 2023. We propose that there will be ongoing student presentations and several publications that arise from this research project.

Status Update Reporting

Status Update May 1, 2022

Date Submitted: May 2, 2022

Date Approved: May 6, 2022

Overall Update

Outcome 1: The project would catalog the bog microbial functional diversity and community structure in Minnesota's ten ecoregions. The database/protocols will be widely disseminated to stakeholders for best management practices.

Work has begun to collect functional diversity and community structure, however, more emphasis on this outcome will occur over the next two summers.

Outcome 2: Enhancement sampling for the Actinomycetes bacterial group would lead to potential new sources for antimicrobials. These types of efforts are crucial for the health and well-being of not only Minnesotans but society in general.

We have used a general protocol for sampling, however, in May 2022 we will begin a comprehensive evaluation of protocols to enhance the Actinomycetes bacterial group.

Outcome 3: The University of Minnesota Crookston and White Earth Tribal and Community College undergraduates will be trained in molecular and microbiological techniques for future careers in health and conservation (e.g., DNR, USDA, MPCA, and graduate schools).

Students in the general microbiology class in fall 2021 were exposed to microbiological and molecular techniques. More training will occur in summer of 2022 with UMC and WETCC students regarding this outcome and continue as we advance.

Activity 1

Activity 1: Characterize the microbial community's ability to produce potential antimicrobials

Milestone #4: Protocol development (e.g., sampling, safety, molecular and microbiology, etc.) in lab meetings and mentoring

Soil samples were collected from several locations in northern Minnesota after receiving permission/permits from the Minnesota DNR. After receiving permission/permits, the following areas were sampled: Hayes Lake State Park, Lake Bemidji State Park, Luxemburg Peatland, Sprague Creek Peatland, and Lost River Peatland. These samples were frozen at -80 degrees celsius and used in the general microbiology laboratory course at the University of Minnesota in the fall semester (September to December 2021). The students in the general microbiology class were able to isolate and preliminarily characterize 56 isolates that demonstrated some activity against safe relative pathogens.

In conclusion, preliminary results indicated that over 60% of the chosen isolates demonstrated inhibitory activity. Additional follow-up will involve more characterization of the chemical substances using various nonpolar solvents to identify the chemical structure. Students used and modified protocols in the fall academic semester. One student continued this work through the spring semester to further modify and develop these techniques. In May, one student will evaluate other media that were not

Activity 2

Activity 2: Characterize the microbial community functionality and diversity in bogs

Milestone #2: Protocol development and review: summer undergrads (4-5 students from UMC and WETCC), microbiology class

Students have not participated in protocol development and review during the fall and spring semesters. We will continue to evaluate protocols, especially during the summer work. The characterization of the microbial community functionality and diversity will be emphasized over the summer. Samples from more sites will be collected to better understand the microbial community diversity during the summer season (2022).

Dissemination

A student presented a poster at the Annual Biomedical Research Conference for Minoritized Students. She began her research last the end of December and was able to accomplish most of the progress reported above. This is the first of several opportunities for the students involved in this research to disseminate their work.