Final Abstract

Final Report Approved on December 5, 2025

M.L. 2022 Project Abstract

For the Period Ending June 30, 2025

Project Title: Chronic Wasting Disease Prion Soil Research

Project Manager: Tiffany Wolf

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Funding Source:

Fiscal Year:

Legal Citation: M.L. 2022, Chp. 94, Sec. 2, Subd. 03n

Appropriation Amount: $732,000

Amount Spent: $708,936

Amount Remaining: $23,064

Sound bite of Project Outcomes and Results

With LCCMR support, MNPRO has united teams of multidisciplinary scientific researchers that have advanced methods for environmental prion detection and revealed new insights into prion persistence, transport and degradation. The discoveries from this project lay an important foundation that ensures continued progress in understanding and mitigating CWD transmission.

Overall Project Outcome and Results

Chronic Wasting Disease (CWD) is a contagious,100% fatal neurological disease affecting deer. Infected deer shed CWD-causing prions into the environment throughout infection and even after death. Interactions between prions and soil are complex, necessitating better tools and methods to understand how the interactions influence the potential risk for CWD transmission to deer. The Minnesota Center for Prion Research and Outreach (MNPRO) advanced environmental research of CWD prion persistence, degradation, and remediation in Minnesota through three primary project objectives: 1) optimization of prion detection in soils; 2) spatial modeling of CWD in association with Minnesota's diverse environmental characteristics; and 3) formation of a scientific consortium to advance environmental CWD research. Key outcomes included the validation and optimization of RT-QuIC for detection of prions in Minnesota soils, including

identification of factors contributing to false positivity and development of a framework for validating RT-QuIC across other soil types. Additionally, MNPRO developed a targeted mass spectrometry assay to confirm prion detections by RT-QuIC, showing promise for use with soil as well. Spatial modeling revealed new insights between specific soil components (e.g., organic matter) and water flow characteristics with CWD detection in deer. This project resulted in three new scientific publications, 30 conference presentations, publicly available datasets, several new grant proposals, and enhanced public outreach materials on environmental prions. The establishment of multidisciplinary scientist teams has fostered novel lines of research into environmental prion control, ensuring continued progress in understanding and mitigating CWD transmission.

Project Results Use and Dissemination

This project resulted in three new scientific publications, with four additional draft manuscripts at various stages of submission. MNPRO's students and scientific teams contributed 30 conference presentations at local, regional and national levels. Translating the science behind the environmental dimension of prions and how new knowledge gleaned from this project can be leveraged for CWD mitigation has been a top priority. As such, MNPRO has enhanced public outreach materials with key information on environmental prions. Scientific products also included new datasets made publicly available and several grant proposals intended to advance environmental prion research toward new insights and discoveries.

Environment and Natural Resources Trust Fund

M.L. 2022 Approved Final Report

General Information

Date: December 5, 2025

ID Number: 2022-294

Staff Lead: Michael Varien

Project Title: Chronic Wasting Disease Prion Soil Research

Project Budget: $732,000

Project Manager Information

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Project Reporting

Final Report Approved: December 5, 2025

Reporting Status: Project Completed

Date of Last Action: December 5, 2025

Project Completion: June 30, 2025

Legal Information

Legal Citation: M.L. 2022, Chp. 94, Sec. 2, Subd. 03n

Appropriation Language: $732,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to study chronic wasting disease prions in soils, including the assessment of sites where carcasses with chronic wasting disease have been disposed.

Appropriation End Date: June 30, 2025

Narrative

Project Summary: The goal of this project is to advance research related to the contamination, persistence, detection, and risk of transmission related to CWD prions in soil.

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

Chronic Wasting Disease is a contagious, 100% fatal neurological disease affecting deer. Infected deer shed CWD-causing prions into the environment throughout the duration of illness and through decomposition after death. Research demonstrates that the interactions between prions and soil is a complex system affected by the variable organic and inorganic microenvironment of soil types. Minnesota's diverse ecosystems and associated soils, along with the reality that CWD continues to be detected across the state, demonstrate an opportunity to learn more about the prion/soil interface and how that influences the potential risk for CWD transmission to deer. The variability of soil matrices also complicates consistent and accurate prion detection with current testing schemes. Our Minnesota Center for Prion Research and Outreach (MNPRO) laboratory has RT-QuIC testing functionality, a highly advanced and sensitive prion detection assay that has capability for environmental samples such as soil. Although capability is apparent, further investigation is necessary to optimize RT-QuIC testing of soil for CWD prions.

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.

CWD prions are resistant to degradation and can remain infectious in the environment for years, expanding the risk of CWD spread. Therefore, it is critical that research specific to the interaction of soils and prions continues to make progress. Our team proposes to: 1) develop a Minnesota soils map that will both inform and be updated by ongoing experiments to model and characterize prion risk across the state; 2) form a consortium of prion/soil scientists across UMN and the U.S. to collaboratively strategize, design, and perform prion ecological research; and 3) conduct ongoing research to optimize RT-QuIC for prion detection in soil under different conditions, building on existing LCCMR soil funding, with direct application to the soils of Minnesota. To accomplish the proposed work plan, we will leverage knowledge gleaned from ongoing experiments conducted with previous LCCMR support (2020 CWD Prion Research in Soils) and the growing capacity of MNPRO as a multidisciplinary, collaborative, research center, resulting from 2022 Establishing a Center for Prion Research and Outreach.

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?

Specific outcomes of this project that are critical to advancing future environmental research around CWD prion persistence, degradation, and remediation in Minnesota include: 1) the optimization of methods for prion detection in soils, under different conditions and of different compositions; 2) spatial modeling associated with the soil diversity in Minnesota and the risk of CWD transmission to native cervids; and 3) formation of a scientific prion/soil consortium to develop new avenues of research on CWD ecology in the environment.

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

Activities and Milestones

Activity 1: Soil-based spatial modeling of CWD risk in Minnesota

Activity Budget: $171,286

Activity Description:
Recent research demonstrates that prions bind efficiently to clay particles in soil, which could either promote or inhibit transmission due to conformational changes in prion structure that affects infectivity to cervids. Yet binding of prions to clay also has been suggested as a potential method of environmental mediation, as clay could sequester prions and reduce exposure. Ongoing studies also demonstrate that relationships between clay and CWD incidence in deer in the field are mixed. For example, higher clay content was associated with CWD in Colorado but not Wisconsin. Relevance of other soil properties, such as heavy metal contamination or organic content, are less well understood. As Minnesota is characterized by a diversity of soil types and matrices across the state, we can leverage the knowledge gleaned from these previous studies and utilize the draft map to prioritize soil sampling and testing for Activity 3. Further, over time the draft map will be updated by MNPRO's discoveries and ongoing experimental and field studies emerging from Activity 4. Future efforts would integrate soil, hydrology, and deer movement risk maps to determine what factors best explain the spatial variation of CWD in Minnesota.

Activity Milestones:

Description	Approximate Completion Date
Recruit a new graduate student researcher to the project.	January 31, 2023
Up-to-date comprehensive literature review of science behind prion variation by soil type.	August 31, 2023
A draft Minnesota soil-based prion risk map to model prion risk across the state	June 30, 2024

Activity 2: Formation of a soil/prion research consortium

Activity Budget: $52,158

Activity Description:
A complex problem such as prion interaction with various environmental compounds is best undertaken by a team of dedicated, experienced, multi-disciplinary scientists. Leveraging MNPRO's capacity as a center for cultivating multi-disciplinary prion research, we will create a structure for successful team science. We will work with UMN's Strategic Partnerships and Research Collaborative (SPARC) to design and host a collaborative workshop, outline a strategy for participant recruitment, and develop a transparent and inclusive approach to research prioritization and support. The workshop will focus on needs, opportunities, and methods to advance research on the prion/soil interface. Participants will include UMN researchers, Minnesota scientists (e.g. from MPCA) and national leaders (joining virtually if needed) in environmental prion research. The workshop will be a launchpad for new ideas and a research consortium that will develop and conduct new avenues of soil/prion research (Subprojects 1-3 that make up Activities 4-6). The consortium will continue with regular, remote meetings at a frequency that facilitates necessary communications for research development and advancement - biweekly to monthly - with an annual summit.

Activity Milestones:

Description	Approximate Completion Date
Design and planning of workshop and process for research prioritization.	January 31, 2023
Host the prion/soil science workshop with formation of prion/soil science consortium.	February 28, 2023
Prioritization and design of new prion/soil studies (to feed into Activity 4).	June 30, 2023
Host regular consortium meetings (biweekly to monthly) to advance soil prion science.	June 30, 2025

Activity 3: RT-QuIC detection of CWD prions in soil

Activity Budget: $198,556

Activity Description:

RT-QuIC is a CWD screening test that is capable of detecting prions in live and dead animals (including carcass remains), as well as the environment - samples such as plants, soil, and water. Yet, environmental detection of prions using RT-QuIC technology is still early in its development. Given soil matrix complexity, more work is needed to optimize methods for prion detection across soils with different mineral and organic content. MNPRO began such efforts with LCCMR funding in 2021, and these efforts will continue with this Activity. A variety of RT-QuIC protocols developed with previous funding will continue to be investigated and utilized to optimize detection methods on known CWD positive and negative soils. Based on the detection methods developed across experimental soil conditions, we will screen soil samples collected from areas across Minnesota, as informed by the spatial analysis of Activity 1, including those with known CWD exposures.

Activity Milestones:

Description	Approximate Completion Date
Recruit and hire a new soil/protein scientist to MNPRO.	January 31, 2023
RT-QuIC optimization for the detection of CWD prions in soil.	May 31, 2023
Experimental study of CWD prion binding and detection across different soil types and compositions.	January 31, 2024
Screen soil samples from multiple Minnesota locations for CWD prions using RT-QuIC.	June 30, 2025

Activity 4: Subproject 1: Identification and Enhancement of Molecules with Anti-Prion Activity

Activity Budget: $100,000

Activity Description:

Subproject 1: Prions are unique infectious agents that are comprised only of protein and are unlike viruses and bacteria. CWD has been detected in 30 states in the United States, including Minnesota. CWD prions are shed from deer where they interact with the environment. Binding of prions to soil can result in prions surviving conventional disinfection methods. Current best practices for prion decontamination, such as incineration, or harsh chemical treatments, are not feasible for most environmental applications. A practical method for environmental prion decontamination is critically needed to prevent the spread of CWD among deer and to other species. We have shown that digestion of PrPSc with subtilisin, an enzyme that degrades proteins, reduces prion infectivity when prions are bound to soil. This treatment has the potential to lower or eliminate infectivity at identified or presumed CWD-contaminated surfaces in captive and wild settings. The overall objective of this study is to engineer subtilisin and screen extracts from environmental bacterial and fungi to identify novel compounds with anti-prion activity to expand the toolbox of potential prion mitigation tools.

Activity Milestones:

Description	Approximate Completion Date
Graduate student recruitment	August 31, 2023
Identification of novel natural compounds from Minnesota	June 30, 2024
In silico protease engineering	July 31, 2024
Screening of existing library of natural compounds for anti-prion activity	July 31, 2024
In silico protease evaluation for anti-prion activity	May 31, 2025
Screening of newly identified natural compounds for anti-prion activity	May 31, 2025

Activity 5: Subproject 2: Method Development for RT-QuIC Assay to Detect Chronic Wasting Disease (CWD) in Soil and Surface Water Samples

Activity Budget: $100,000

Activity Description:
Subproject 2: CWD is neurodegenerative disease of deer and other cervids, caused by a misfolded prion protein. Affected deer have been found in Minnesota both on deer farms and in the wild. The misfolded prion protein can attach to materials in the environment, such as soils, organic material, and sediments, where it stays for long periods of time. Detection of CWD on environmental materials presents multiple challenges due to the differences between environmental materials and the animal tissues on which the detection methods were developed. Real-time quaking induced conversion (RT-QuIC), a method for CWD detection, offers many advantages over other CWD prion detection techniques. Because RT-QuIC relies on the fluorescence of an indicator dye, it is possible that the natural fluorescence of some some soil components may interfere with CWD detection. We will systematically test the fluorescence properties of environmental materials from different regions in Minnesota according to known properties and the mechanisms underlying RT-QuIC. We will also evaluate environmental sampling and sub-sampling protocols to develop a robust and scientifically-sound procedure for environmental sampling and CWD detection.

Activity Milestones:

Description	Approximate Completion Date
Complete Field Collections for Soils & Surface Waters	November 30, 2023
Environmental characterization of soils and waters for possible interferences	February 28, 2024
Revised RT-QuIC protocol for environmental materials	September 30, 2024
Final Report and draft of Peer-Review Publication	December 31, 2024

Activity 6: Subproject 3: Microwave assisted pyrolysis for prion remediation

Activity Budget: $100,000

Activity Description:
Subproject 3: Prions, the causative agent of transmissible spongiform encephalopathies (TSEs), are notoriously resistant to chemical and physical degradation. This extraordinary resilience has led to challenges in decontamination of prion-contaminated materials and prion-bearing biowaste. Among the effective means of prion destruction is incineration at extreme (>600°C) temperatures. Here, we propose the use of a new technology for prion remediation, microwave assisted pyrolysis (MAP). MAP technology offers several unique advantages over conventional incineration, with possible improvements to process time, throughput, portability, and cost. In this project, we will assess the viability of MAP for prion remediation in soils and tissues using a benchtop MAP instrument. There are a number of variables that will likely play into the efficacy of a MAP system for prion decontamination, including parameters of the MAP process and characteristics of the contaminated samples. A select set of these variables will be systematically optimized to ascertain ideal conditions for prion decontamination in each sample type.

Activity Milestones:

Description	Approximate Completion Date
Conduct initial RT-QuIC assays with negative, unspiked materials to identify interferences and mitigation	January 31, 2024
Pyrolysis and RT-QuIC experiments are conducted	October 31, 2024
Preparation of first manuscript (tissue decontamination)	November 30, 2024
Characterization of pyrolysis products	January 31, 2025
Preparation of second manuscript (soils and biosolids decontamination)	April 30, 2025

Activity 7: The ecology of prions in soil.

Activity Budget: $10,000

Activity Description:
CWD prions are resistant to degradation and bind to various compounds in soil, allowing them to remain infectious in the environment for years. The goal of this Activity is to leverage the knowledge gained in the 2021 LCCMR soil grant and Activities1and 3 of this work plan, as well as the strategy derived by the newly formed soil/prion consortium of Activity 2. We envision that through this Activity, using new protocols for the extraction and detection of prions in soil with RT-QuIC, the consortium will begin new studies that will serve as initial phases of research into prion binding, detection, persistence and degradation under different environmental conditions. In the final 1.5 years of this grant, initial studies will generate new, preliminary data and information that will direct and leverage future funding opportunities for ongoing environmental CWD research. This work will enhance our understanding of the persistence and potential transmission of CWD in the soil environment and add to an overall assessment of ecological risk.

Activity Milestones:

Description	Approximate Completion Date
Peer-reviewed Research Addendums for new research projects identified by prion/soil consortium in Activity 2.	December 31, 2023
Amend work plan budget as needed to support new soil/prion research projects.	January 31, 2024
Complete initial studies of prion-soil ecological projects.	April 30, 2025
New funding opportunities identified by consortium for continuation of prion-soil ecological research.	June 30, 2025

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Stuart Lichtenberg	University of Minnesota	Investigator on Activity 6 (Subproject 3) and overall effort in environmental prion detection and remediation.	Yes
Jason Bartz	Creighton University	Investigator on Activity 4 (subproject 1) and overall efforts in environmental prion detection and remediation.	Yes
Rodrigo Morales	University of Texas Houston	Collaborator	No
Mikael Elias	University of Minnesota	Investigator on Activity 4 (Subproject 1)	Yes
Christine Salomon	University of Minnesota	Investigator on Activity 4 (Subproject 1)	No
Roger Ruan	University of Minnesota	Investigator on Activity 6 (Subproject 3)	No
Paul Chen	University of Minnesota	Investigator on Activity 6 (Subproject 3)	No
Cara Santelli	University of Minnesota	Investigator on Activity 5 (Subproject 2)	No
Diana Karwan	University of Minnesota	Investigator on Activity 5 (Subproject 2)	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 environmental dimension of CWD transmission is a critical area for CWD research. This unique project, uniting expertise across disciplines and combining spatial and environmental assessments, is a first of its kind. The methods and approach taken will be of broad interest to those tasked with characterizing the potential risks associated with CWD-positive prions in soils, and the findings critical to the work of our own state and tribal agencies in protecting our wild deer herds and the surrounding ecosystem. Findings will be shared directly with state and tribal agencies through project reports. We will also use the following opportunities to share methods and findings more broadly:

1. dissemination via the MNPRO website: https://mnpro.umn.edu/
2. presentation at local, regional, and national scientific, management, and public/stakeholder meetings
3. publication of findings in peer-reviewed scientific (e.g. Science of the Total Environment) and professional journals (e.g. The Wildlife Professional)
4. dissemination to the media via press releases and UMN Research Briefs
5. testimonials to LCCMR and other policy platforms

The Minnesota Environment and Natural Resources Trust Fund (ENRTF) will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the ENRTF Acknowledgement 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?
The environmental dimension of CWD transmission is a critical area for CWD research. The methods and results of this

study will be of broad interest and lay a strong foundation for future hypothesis-driven research related to prion contamination, persistence, degradation and remediation. The Beltrami Co. dump site, depending on results produced from this project, has the potential to become a long-term environmental prion research demonstration site. Thus, the results from this project will be leveraged for funding from federal agencies, including USDA, USFWS, and NSF.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount	$ Amount Spent	$ Amount Remaining
Personnel										
2 Assistant Professors		Two assistant professors will serve in the role of Primary Investigator (Project manager) and Co-PI to manage the project and guide research.			33.5%	0.45		$77,394	-	-
Assistant Professor		An assistant professor specializing in water and soil science will advise a graduate student and guide/partake in field and laboratory research			33.5%	0.15		$28,302	-	-
Researchers		A researcher will help integrate the research plan into field and laboratory investigations			33.5%	0.03		$3,422	-	-
Lab technician		A laboratory technician will perform field sampling and lab analyses of soil for prion detection.			28.7%	1.5		$103,157	-	-
Graduate students		Two graduate student researchers will facilitate data collection and analysis and focus on prion/soil research			87.9%	2.25		$233,203	-	-
							Sub Total	**$445,478**	**$445,478**	**-**
Contracts and Services										
UMN-SPARC	Service Contract	Professional design, planning and facilitation of collaborative workshop and research prioritization process.				0.05		$6,930	$6,930	-
Bartz Lab of Creighton University	Subaward	The Bartz Lab will be conducting studies to support Activity 4 (by assessing enzymatic potential for prion degradation in soils).				0		$10,000	$10,000	-
Subproject 1 Identification and enhancement of molecules with antiprion	Subaward	The Elias Lab at UMN will lead Activity 4.				1		$90,000	$89,538	$462

Subproject 2 Method Development for RT-QuIC Assay to Detect Chronic Wasting Disease (CWD) in Soil and Surface Water Samples	Subaward	The Karwan Lab will be leading the research in Activity 5.				0.32		$60,113	$37,511	$22,602
Subproject 3 Method Development for RT-QuIC Assay to Detect Chronic Wasting Disease (CWD) in Soil and Surface Water Samples	Subaward	This team, led by Stuart Lichtenberg, will lead research of Activity 6.				0.6		$103,374	$103,374	-
							Sub Total	**$270,417**	**$247,353**	**$23,064**
Equipment, Tools, and Supplies										
	Tools and Supplies	Prion detection	Lab R/D and analytical testing by RT-QuIC and Mass Spectrometry					$8,413	$8,413	-
							Sub Total	**$8,413**	**$8,413**	**-**
Capital Expenditures										
							Sub Total	**-**	**-**	**-**

Acquisitions and Stewardship										
							Sub Total	**-**	**-**	**-**
Travel In Minnesota										
	Miles/ Meals/ Lodging	Lodging and per diem support for 4-5 people x 2-days in Minneapolis/St. Paul.	Support for external participants to travel to the Twin Cities for a 2-day collaborative workshop.					$892	$892	-
							Sub Total	**$892**	**$892**	**-**
Travel Outside Minnesota										
	Conference Registration Miles/ Meals/ Lodging	Travel for 1-2 MNPRO staff and students to participate in 1 national scientific conference.	To disseminate project findings, establish new collaborative partners, gain new knowledge in prion and environmental research.	X				$1,876	$1,876	-
							Sub Total	**$1,876**	**$1,876**	**-**
Printing and Publication										
	Publication	Publication costs range $2-3,000 per manuscript; we anticipate the publication of 2-3 manuscripts from this original research.	Publication will allow broad dissemination of research results to the scientific community to build on our findings.					$4,924	$4,924	-
							Sub Total	**$4,924**	**$4,924**	**-**
Other Expenses										
							Sub Total	**-**	**-**	**-**
							Grand Total	**$732,000**	**$708,936**	**$23,064**

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
Travel Outside Minnesota	Conference Registration Miles/Meals/Lodging	Travel for 1-2 MNPRO staff and students to participate in 1 national scientific conference.	MNPRO is at the forefront of scientific advancement for environmental prion research, which necessitates continuous dialogue with the scientific community to progress using cutting-edge knowledge and techniques.

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: 7375fe64-d51.pdf

Alternate Text for Visual Component

Visual summary of project goals, approach, and outcomes....

Supplemental Attachments

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

Title	File
UMN SPA Support	0e1159ac-c42.pdf
Background Check Certification Form	1d8ad658-03b.pdf
Subproject 1 Research Addendum	406beee5-703.pdf
Subproject 2 Research Addendum	12c6f2ba-6e8.pdf
Subproject 3 Research Addendum	4453b135-e57.pdf
Overall and Subprojects Budget	bed67160-011.xlsx
Amended_Overall and Subprojects Budget	94647055-ce9.xlsx
Amended_Subprojects Budget	6252f3ef-000.xlsx
Amended Subprojects Budget_March 2025	bf212f0e-8ae.xlsx
Final Report of Dissemination Activities	af309355-fba.pdf
Final Visual Component	f90aba9b-435.pdf
Grunklee et al. 2025_MethodsX	5134ae06-7f9.pdf
Grunklee et al. 2025_Prion	cb68b93f-27e.pdf
Li et al. 2025	772ff2b4-487.pdf
2024 Beltrami Local Landowner Report	6dfc26ed-ab0.pdf

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

NA

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?

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?

No

Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

Yes, Sponsored Projects Administration

Do you understand that a named service contract does not constitute a funder-designated subrecipient or approval of a sole-source contract? In other words, a service contract entity is only approved if it has been selected according to the contracting rules identified in state law and policy for organizations that receive ENRTF funds through direct appropriations, or in the DNR's reimbursement manual for non-state organizations. These rules may include competitive bidding and prevailing wage requirements

Not acknowledged

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	• Budget - Capital, Equipment, Tools, and Supplies • Activities and Milestones • Budget - Personnel • Budget - Professional / Technical Contracts • Budget - Travel and Conferences • Budget - Printing and Publication	Budget revised and new Activities 4-6 added to accommodate new subprojects developed through Activity 2. Research Addendums for subprojects were reviewed by external reviewers and approved. Minor revisions to Research Addendums have been completed by subproject leads.	June 2, 2023	Yes	July 20, 2023
2	Amendment Request	• Budget • Budget - Personnel • Budget - Professional / Technical Contracts • Budget - Capital, Equipment, Tools, and Supplies • Project Collaborators - Project Partner Info • Activities and Milestones • Budget - Travel and Conferences • Attachments	Needed to add collaborators that will be receiving funding to accomplish the work added in last Amendment (Activities 4-6). All new collaborators are University of Minnesota faculty from different colleges and disciplines.	September 19, 2023	Yes	September 22, 2023
3	Amendment Request	• Other • Attachments	Subproject 1 (Activity 4) is requesting a budget amendment to meet objectives. We request a rebudgeting of $5,000 for supplies to cover reagents, substrates, media and other consumables needed to produce, purify and characterizes protease for prion degradation. We also request a rebudgeting of $5,000 to cover protein production, gene synthesis of novel protease candidates and sequencing services to verify gene mutation engineering. An amended subproject budget (Elias tab) is attached.	September 3, 2024	Yes	October 22, 2024

4	Amendment Request	• Budget - Professional / Technical Contracts • Attachments	Subproject 3 requires machine shop services for equipment modifications necessary to achieve project objectives. There was no line item for such services in the budget previously, and this has been added. Funds were redistributed from the MAP reactor supply line to accommodate the needed work.	November 13, 2024	Yes	December 13, 2024
5	Amendment Request	• Budget - Capital, Equipment, Tools, and Supplies • Budget - Travel and Conferences • Budget - Printing and Publication • Attachments	We are requesting to move funding from "Travel outside of Minnesota" and "Printing and Publication" to support travel in Minnesota to complete project activities and tools and supplies to complete planned analytical testing. Subproject teams (Elias and Lichtenberg) would also like to shift funds within their budget to align better with remaining activities. A new spreadsheet with the proposed subproject amendments has been attached.	February 24, 2025	Yes	March 26, 2025
6	Amendment Request	• Budget - Travel and Conferences • Budget - Printing and Publication	We are requesting to shift funds from conference travel to publication costs to accommodate costs associated with poster printing and publication charges for a manuscript of this project that was recently accepted for publication.	May 23, 2025	Yes	May 27, 2025
7	Completion Date	Previous Completion Date: 06/30/2025 New Completion Date: 12/31/2031	Staff changing date to process amendment request. This is just a work around for rigid system logic.	May 27, 2025	Yes	May 27, 2025
8	Completion Date	Previous Completion Date: 12/31/2031 New Completion Date: 06/30/2025	Staff changing date back to original date after processing the amendment request. This is just a work around for rigid system logic.	May 27, 2025	Yes	May 27, 2025
9	Amendment Request	• Budget - Capital, Equipment, Tools, and Supplies • Budget - Travel and Conferences • Budget - Printing and Publication	We are requesting to shift unspent funds from lab supplies and travel to printing/publications to utilize unspent funds related to lab testing and travel to cover new publication fees associated with	July 2, 2025	Yes	July 9, 2025

			a project-related manuscript accepted for publication just prior to project end. We're also requesting to move $181 from remaining in-state travel to conference travel to cover conference expenses incurred in the previous reporting period.			
10	Completion Date	Previous Completion Date: 06/30/2025 New Completion Date: 12/31/2031	Staff changing date to process amendment request. This is just a work around for rigid system logic.	July 9, 2025	Yes	July 9, 2025
11	Completion Date	Previous Completion Date: 12/31/2031 New Completion Date: 06/30/2025	Staff changing date back to original date after processing the amendment request. This is just a work around for rigid system logic.	July 9, 2025	Yes	July 9, 2025

Status Update Reporting

Final Status Update August 14, 2025

Date Submitted: October 8, 2025

Date Approved: December 1, 2025

Overall Update

Our scientific teams supported by this grant have made exciting achievements. First, advances in spatial modeling revealed new insights into prion persistence in soil, including specific soil components as well as water flow (Activity 1). These findings will facilitate future work to understand why CWD outbreaks occur in some areas and not in others. Our teams have now validated and optimized RT-QuIC for detection of prions in Minnesota soils, including identifying steps of the process likely to introduce false positivity (Activity 3). These efforts provide a framework for validation and optimization of RT-QuIC across other soil types into the future. Additionally, we have developed a targeted mass spectrometry assay to confirm prion detections in RT-QuIC, which we also confirmed can be used for prions in soil extractions. Finally, we've amassed new, multidisciplinary scientist teams (Activities 2 and 7) that have initiated novel lines of study related to the detection and remediation of prions in soils and other environmental matrices (Activities 4-6). To date, these activities have resulted in 3 new scientific publications (with at least 4 others drafted), 30 conference presentations, new publicly available data sets, 3 new grant proposals, and new material for public outreach on environmental prions.

Activity 1

Using recent advances in software designed for large-scale spatial data analysis, we developed advanced statistical models to investigate the spatial epidemiology of CWD in white-tailed deer across Minnesota and neighboring states (Wisconsin, Michigan, and Iowa). Using surveillance data collected from 2001 to 2021, the models explore how CWD occurrence relates to habitat and soil characteristics. We found that CWD presence was more associated with soil chemistry and hydrology than with soil texture (e.g., clay content, as previously reported). Specifically, higher CWD detection in deer was associated with soils having lower organic matter, reduced movement of water through the soil), lower copper concentrations, lower elevation, and steeper slopes. These results suggest that prion persistence in soil may be influenced by how readily prions bind to components such as organic matter and minerals, as well as by water movement and microbial activity in the soil. This can guide the development of landscape-level predictive models that inform prion management and surveillance strategies. Through this effort, we developed new methods for spatially relating key soil data from across the US in future analyses; we are making those methods and soil layers freely available and two manuscripts detailing this work are in development.
(This activity marked as complete as of this status update)

Activity 2

The UMN CWD environmental consortium met monthly throughout the life of the grant after it inception. These monthly meetings served as a platform for researchers to share project updates and advances and obtain input from others. In early May, MNPRO hosted a consortium event, titled "Break it Down", where students and research scientists had an opportunity to share their work through presentation of scientific posters, and a panel of experts was convened to discuss the scientific needs related to CWD prions in the environment. The event also served as a venue to share new scientific discoveries with the broader community. Given the positive feedback, MNPRO will continue to host UMN CWD environmental consortium meetings as well as an annual science fair like "Break it Down."
(This activity marked as complete as of this status update)

Activity 3

Over the past reporting period we've determined whether CWD can be extracted from soils and observed using mass spectrometry. We did this by spiking soils two soil types found at the Beltrami Country dumpsite (histosols and alfisol)

with CWD. We initially used recombinant PrP for spiking, however, this proved difficult to generate and this substrate was not protease-resistant, a requirement for the targeted mass spectrometry assay. We instead used a tissue extract containing bona fide CWD as the spiking agent and targeted mass spec was performed on soil extracts (spiked and not spiked) as well as an equivalent amount of CWD extract. We mapped amino acid sequences within the samples to four target peptides we'd identified from earlier experiments. Relative recovery for the histosols was ~40% whereas the alfisol exhibited a recovery of ~1%, in comparison to the CWD extract. Additionally, through a discovery run we identified an additional peptide that we will add to our targeted assay library. These mass spectrometry experiments lay important groundwork for ongoing experimentation to establish the targeted assay as a confirmatory test for prion detection by RT-QuIC. Several manuscripts from the collective body of work under this activity are in development. *(This activity marked as complete as of this status update)*

Activity 4

We have identified natural compounds with anti-prion activity, and are working to optimize their properties. Our screening efforts have yielded five compounds that demonstrate significant prion degradation potential, with a reduction in prion protein levels of around 70% compared to untreated controls. In addition to compound/extract screening, we are also investigating the potential of proteases to degrade prions. We have developed new variants of the subtilisin protease, an enzyme with known antiprion activity, which exhibit higher proteolytic activity than the parental enzyme. Notably, one of these variants displays increased stability, with a melting temperature 14.1°C higher than the wild-type enzyme. This enhanced stability is crucial for potential field applications, where enzymes may be exposed to varying environmental conditions. We have evaluated the activity and stability of these protease variants, using assays such as the 96-well plate immunoassay and a protease activity assay that accounts for the structural specificity of prions. Next steps will be to leverage these new data to confirm these results and continue optimization with a goal of scaling these experiments to assess prion remediation under real-world environmental conditions. *(This activity marked as complete as of this status update)*

Activity 5

Based on early project findings and our broader laboratory work, we have determined that CWD prions in streams and rivers tend to associate with suspended sediment (Li et al 2025). At all but one site, filtered waters were negative for CWD prions based on RT QuIC analysis. At that one site, in Winona County, both analytical runs of the water returned positive results for CWD. Future samplings and analysis are planned under LCCMR Project 2025-323. Water samples from Winona County have also been further analyzed. Workflows have been initiated for water samples from around the state to iteratively:

- Undergo evaluation for CWD via RT-QuIC
- Test for environmental background presence of fluorescent organic matter

This work remains iterative in terms of testing for background fluorescence and RT-QuIC behavior in different environmental surface waters around Minnesota. Tests performed under this task show differences between dyes, such as Thioflavin T (the standard dye used in RT-QuIC) and Thioflavin X (ThX), are promising for determining false seeding behavior, which could make samples falsely appear positive. These protocols, and notably which dye to use with RT-QuIC, will be used and refined as we sample more waters around Minnesota under LCCMR 2025-323. *(This activity marked as complete as of this status update)*

Activity 6

Over the past four months, the microwave-assisted treatment system has been used to treat various prion-contaminated samples. Experimental results demonstrated its effectiveness in both soil and tissue treatment. For prion-contaminated soil, complete inactivation of prions in soil can be achieved by using the MAP system and maintaining the temperature above 500°C for 20 minutes, with an activated carbon-to-soil mass ratio of 1:0.75. In the case of prion-contaminated tissue (deer brain), complete prion inactivation using the MAP system required maintaining the temperature, i.e., 500°C, for 60 minutes. The longer treatment time is due to higher moisture content and prion protein concentration compared to soil samples. Notably, the microwave technology significantly reduces processing time compared to conventional prion inactivation methods, offering a more efficient solution for both soil and tissue

decontamination. The preliminary process and system developed could be further improved, e.g., by better mixing and addition of catalysts, etc.
Potential applications of the microwave technology could extend to other prion-contaminated samples, such as in contaminated water. A mobile continuous-microwave-system could be used for on-site treatments as well.
(This activity marked as complete as of this status update)

Activity 7
Three new projects were developed through new collaborations established under the umbrella of this grant, which became and are described in Activities 4-6. These project ideas were generated through a grant-sponsored workshop that brought new researchers from multiple disciplines together. The outcome were Activities 4-6, which were new projects that focused on the prion detection and remediation, each led by newly established, multidisciplinary scientific teams. Each were successfully funded and completed during the grant period. As of this final grant report, one team had already received additional funding support to continue their prion detection work, with an eye toward applications to further understand prion transport by sediments in water. Another team has already begun competing for funding, and the final team has a robust, preliminary data set to leverage as they seek new funding support. Each of these subprojects has resulted in new avenues of research that hold strong promise for solutions to environmental prion remediation.
(This activity marked as complete as of this status update)

Dissemination
Our dissemination efforts targeted both the scientific and public communities, with intention to reach multiple stakeholder groups. We leveraged opportunities where MNPRO could exhibit a booth on CWD to include information on what we've learned about prions in the environment. These efforts also resulted in the publication of 3 manuscripts produced from the data generated by this grant, 30 scientific presentations on environmental prions, integration of new knowledge in classroom lectures, participation in 26 outreach events across university, Tribal community and public venues, and 23 media mentions. Please see Final Dissemination Activities, attached.

Additional Status Update Reporting

Additional Status Update August 14, 2025

Date Submitted: July 2, 2025

Date Approved: July 9, 2025

Overall Update
Placeholder text in order to submit amendment request.

Activity 1
Placeholder text in order to submit amendment request.

Activity 2
Placeholder text in order to submit amendment request.

Activity 3
Placeholder text in order to submit amendment request.

Activity 4
Placeholder text in order to submit amendment request.

Activity 5
Placeholder text in order to submit amendment request.

Activity 6
Placeholder text in order to submit amendment request.

Activity 7
Placeholder text in order to submit amendment request.

Dissemination
Placeholder text in order to submit amendment request.

Additional Status Update Reporting

Additional Status Update August 14, 2025

Date Submitted: May 23, 2025

Date Approved: May 27, 2025

Overall Update
Placeholder text in order to submit amendment request

Activity 1
Placeholder text in order to submit amendment request

Activity 2
Placeholder text in order to submit amendment request

Activity 3
Placeholder text in order to submit amendment request

Activity 4
Placeholder text in order to submit amendment request

Activity 5
Placeholder text in order to submit amendment request

Activity 6
Placeholder text in order to submit amendment request

Activity 7
Placeholder text in order to submit amendment request

Dissemination
Placeholder text in order to submit amendment request

Status Update Reporting

Status Update March 1, 2025

Date Submitted: February 24, 2025

Date Approved: March 26, 2025

Overall Update

Each scientific team supported through this grant has made exciting progress toward the overall outcomes of this grant. First, several of our scientific experiments to optimize the RT-QuIC protocol for improved prion detection in soils have been effective in reducing false seeding associated with the soil types found in northern Minnesota (Activity 2). We have also explored the use of mass spectrometry as a confirmatory test of what is detected in RT-QuIC. A targeted assay for the prion peptide has now been developed, and we will next evaluate its efficacy for prion detection using Minnesota soil types. In Activity 1, we have completed an initial analysis of CWD detection patterns across four states, including Minnesota, using a highly specialized spatial model. In focusing on areas where CWD has been detected, the model allows us to identify key environmental characteristics that may be important both for the persistence and transmission of CWD in our region. Following additional analyses, we will use the model to build a CWD risk map for the entire state of Minnesota. Finally, the consortium of scientific teams established through this grant (Activities 4-6) continue to make progress related to the detection and remediation of environmental prions.

Activity 1

Using highly specialized spatial models, we have analyzed CWD detection patterns from 20 years of white-tailed deer surveillance data in Minnesota, Wisconsin, Michigan, and Iowa in relation to soil chemical and physical properties, topography, and land-use. This is the largest spatial extent of these environmental variables modeled in relation to CWD to date, and necessary to overcome limitations using Minnesota data alone. We found that several soil variables (e.g., organic matter, cation exchange capacity, and minerals) are associated with CWD detection. Thus, soil properties may influence CWD persistence and transmission, providing valuable insights for wildlife management and further understanding the epidemiology of CWD. We are currently developing a risk map of the modeled region, which includes areas where CWD has been detected. We are also working on applying these model results to generate a CWD environmental risk map in regions of MN where the disease has not yet been detected or has been detected but has not persisted (e.g., northern MN). These results will enhance our ability to identify high-risk areas for CWD and may support the development of more targeted management strategies.

Activity 2

No additional updates to this activity except to report that the consortium continues to meet on a monthly schedule, with discussions focused on project activities and preliminary results obtained by research teams.

Activity 3

All experiments related to the removal of humic acid are now complete, and modifications to the extraction protocol for soil testing by RT-QuIC have resulted in an overall reduction in false seeding activity. We are currently in the process of writing these experiments up for peer-reviewed publication in the scientific journal, Prion. We have also completed experiments related to the development of a targeted peptide assay for prion detection using mass spectrometry. Over the course of these experiments, we identified peptide sequences specific to the prion protein, had custom peptides made for use in mass spectrometry, and were able to show that in using these peptides, we were able to confirm prion detection in mass spectrometry using a CWD-positive tissue sample and the synthetic prion protein used in RT-QuIC. We are currently in the process of writing these experiments up for scientific publication. These foundational experiments enable new opportunities for mass spectrometry confirmation of prion detection in soils by RT-QuIC. Over the final project period, we will continue experimentation of mass spectrometry confirmation using spiked soil samples, followed by Beltrami dump site field samples.

Activity 4
We have identified natural compounds with anti-prion activity, and are working to optimize their properties. Our screening efforts have yielded five compounds that demonstrate significant prion degradation potential, with a reduction in prion protein levels of around 70% compared to untreated controls. In addition to compound screening, we are also investigating the potential of proteases to degrade prions. We have developed new variants of the subtilisin protease, which exhibit higher proteolytic activity than the parental enzyme. Notably, one of these variants displays increased stability, with a melting temperature 14.1°C higher than the wild-type enzyme. This enhanced stability is crucial for potential field applications, where enzymes may be exposed to varying environmental conditions. We are continuing to evaluate the activity and stability of these protease variants, using assays such as the 96-well plate immunoassay and a protease activity assay that accounts for the structural specificity of prions.

Activity 5
Following prion detections from previous environmental samplings in 2024, the team has identified and sampled from rivers in the Southeast Minnesota area that are not downstream of captive cervid farms. These samples are currently awaiting characterization and testing for CWD. A new student has begun on the project this academic year and is currently in the process of getting trained on lab work. Additionally, we are designing a small lab test or sensitivity experiment to test for interferences of RT-QuIC with known organic matter. This will be another way to assess CWD test interferences on environmental materials. Work in the next quarter will focus on (1) characterizing water samples collected from SE Minnesota, (2) laboratory analysis, and (3) data analysis and report writing.

Activity 6
Over the past six months, significant progress has been made in modifying and upgrading a lab microwave system for prion sample treatment. The system was integrated into the BSL-2 prion lab within MNPRO, and preliminary experiments were conducted, where prion-contaminated soil samples were processed at a stable and uniform treatment temperature of 500°C over various treatment times. These advancements lay a strong foundation for the scalable application of microwave pyrolysis technology for prion inactivation. Throughout the project, several technical challenges were encountered and mitigated, including sparking during microwave operation, minor leakage causing inefficient heating, unstable temperature readings, and difficulty in consistently reaching the target treatment temperature. These issues have been resolved, resulting in improved efficiency and reliability of microwave pyrolysis technology in processing prion samples. As such, the project is progressing on schedule. Processed samples are currently under analysis, with results expected by the end of this month. In addition, a treatment plan for prion-contaminated brain tissue has been developed, and experiments are ongoing as planned. This progress ensures that the project is on track to meet its objectives and lays the groundwork for the potential application of the technology in the decontamination of prion-contaminated soil and animal tissues.

Activity 7
All subprojects (activities 4-6) are progressing and on track to be completed by the end of the grant period. One project has already received additional funding support to continue the study of sediment-bound prions transported through water, with the recruitment of a new graduate student to continue this important work. The other two projects are showing promising results for the remediation of prions, though additional seed funding may be needed to produce additional data for new competitive funding opportunities. Overall, these new scientific teams are producing exciting early results, and ongoing collaborations through MNPRO have contributed to their early successes.

Dissemination
Wille, E. A., Karwan, D. L., Lichtenberg, S. S., Grunklee, M., Schwabenlander, M. D., Wolf, T. M., Larsen, P. A. Characterizing Hydrological Transport Pathways of Chronic Wasting Disease in the Environment. WaterSciCon2024, St. Paul, Minnesota, June 24-27, 2024.
*scientific conference presentation

Status Update Reporting

Status Update September 1, 2024

Date Submitted: September 3, 2024

Date Approved: October 22, 2024

Overall Update
MNPRO's soil and environmental research teams continue to make progress in advancing the science around environmental prions. Our team working to use spatial models to understand environmental risks associated with CWD infections are working closely with Minnesota and Wisconsin DNRs to align project and state agency goals, as well as to obtain the additional data to meet those goals. Our RT-QuIC team continues to experiment with the soil extraction process to optimize assay specificity, while also validating mass spectrometry as another tool to confirm prion detection in samples. All subproject teams are also incrementally working toward milestones and are on track to meet project goals, generating new data that may be used to leverage additional funding for new lines of prion research. This consortium of researchers has been meeting monthly to share updates and new findings, cultivating a local community of environmental-prion research.

Activity 1
Progress toward this milestone since the last update has involved communications between the MNPRO team and Minnesota and Wisconsin departments of natural resource staff scientists to obtain the requisite CWD surveillance data to accomplish spatial modeling goals. Conversations with state partners has focused on opportunities to align LCCMR project and agency goals and objectives, identification of optimal models for meeting project and agency goals, as well as to ascertain additional data needs and sources of those data to meet shared goals. The project will require use of CWD surveillance data, as well as other variables considered important for CWD transmission (e.g., deer density and demographics, presence of deer farms, etc), which may be available as well through state agency partners. These discussions are ongoing with an eye toward establishing data sharing agreements in the near future. Simultaneously, we have assembled and processed publicly available environmental and soil data that will be tested in our models for associations with CWD risk. We expect that once the necessary data is transferred over to MNPRO, we will be able to advance the spatial model of risk to meet grant goals.

Activity 2
No additional updates to this activity except to report that the consortium continues to meet on a monthly schedule, with discussions focused on project activities and preliminary results obtained by research teams.

Activity 3
Soil extraction studies continue to investigate optimize prion recovery. We identified potential peptides for use of mass spectrometry to confirm prion detection; these peptides have been obtained and will be verified shortly. Proteomic studies of negative samples seeking other proteins causing interference has progressed; a workflow has been developed to explore a soil database of ~40,000 protein sequences. In RT-QuIC, the addition of a humic acid removal step resulted in dramatic improvements with reductions in false positivity. While proof of concept has been demonstrated, streamlining efforts continue to ensure a reproducible non-soil-specific extraction method for use in the assay.Dr Stuart Lichtenberg has led remediation work using chemical and physical methods. While some chemical agents have been shown to be ineffective against prions in soil samples, others appear to be consistently (50/100% bleach) or inconsistently (Virkon S) effective. Due to the complexities of soil, on-going studies continue.

Activity 4
This exploratory study investigated the potential of CWD prion degradation with natural compounds extracted from plants, bacteria, and fungi. We conducted a quick screening test for 196 compounds and extracts and evaluated the

degradation potential as the reduction in the amount of CWD prions using a 96-well plate immunoassay. The observed greatest reduction of CWD prions of approximately 80% was from two compounds isolated from fungi. Additional technical replicates will be applied to compounds showing a promising reduction in CWD prions. Different incubation conditions with more compounds will also be investigated. Another aspect of our investigation is the characterization and identification of proteases with prion-degrading ability. We have identified and produced several proteases that are promising candidates for prion inactivation. To evaluate the activity levels of these enzymes, we established a general protease activity assay. Hen egg white lysozyme (HEWL) fibrils are safe to use, large amounts of fibril can be made, and they are good models for prion research as they are structurally similar to prions. Fibril degradation can be examined using a dye (Thioflavin T) and recorded in real time. These assays will be used to characterize the newly identified proteases.

Activity 5

We have sampled additional field sites, in Southeast Minnesota in late March 2024. Subsequent RT-QuIC analysis of these waters indicated positive for CWD on multiple occasions. The team is currently looking for additional sites to which we will have access such that the water originates from a similar aquifer geology, but does not flow past a depopulated positive cervid farm. Our additional chemical characterization of the water for possible organic matter interferences depends on having non-CWD reference water to be analyzed in campus laboratories outside of MN PRO. Work in the next quarter will focus on (1) finding and collecting water from addition sites that is not positive for CWD, and (2) data analysis and report writing.

Activity 6

We have continued testing the benchtop microwave reactor manufactured for prion remediation. In our previous studies, we successfully tested our microwave system at high temperatures using soil samples. A key challenge we encountered was that when a large amount of soil was loaded, the temperature distribution became uneven due to the limited microwave penetration depth. To address this issue and improve both the temperature uniformity and efficiency of our current microwave system, we have designed a new approach for mixing the soil sample with a microwave absorber to enhance heat transfer and ensure uniform temperature distribution. We are currently also preparing an alternative design as a contingency in case the initial design does not perform as expected. This alternative involves incorporating a mixing shaft driven by a motor mounted at the top of the microwave reactor. Based on our prior experience with similar approaches, we are prepared to implement this design if the simpler initial version proves less effective. In spite of this, we are still on track for completion of our proposed work.

Activity 7

As noted in this report, all subproject teams are making progress toward meeting milestones, while MNPRO continues to provide support to each team as needed.

Dissemination

Conferences

Wolf TM, Larsen PA, Schwabenlander MD, Lichtenberg S, Oh S-H, Karwan D, Fulton D. MNPRO: A Multidisciplinary Think-Tank to Meet the Challenges of Chronic Wasting Disease. Native American FIsh and Wildlife Society Annual Meeting. Welch, Minnesota, May 15, 2024.

Grunklee, M., Wille, E., Ferguson-Kramer, V., Schwabenlander, M., Lichtenberg, S., Karwan, D., Larsen, P., Wolf, T. Detection of Chronic Wasting Disease in Soil at a White-Tailed Deer Carcass Dumpsite [Poster Presentation]. Conservation Sciences Spring Spotlight, St. Paul, MN, May 2, 2024.

Grunklee, M., Wille, E., Ferguson-Kramer, V., Schwabenlander, M., Lichtenberg, S., Karwan, D., Larsen, P., Wolf, T. Detection of Chronic Wasting Disease in Soil at a White-Tailed Deer Carcass Dumpsite [Poster Presentation]. College of Veterinary Medicine Office Graduate Programs Research Event, St. Paul, MN, February 16, 2024.

Teaching

Research activities and findings associated with this grant integrated into lectures:

Wolf T. Introduction to CWD. Guest Lecture, The Raptor Center's Future STEM Leaders Summer Intern program. Class

size: 6 high school summer interns. July 24, 2024.

Outreach

Leech Lake Band of Ojibwe's Wild Game Feast. Hosted by Leech Lake Tribal College. March 19, 2024. Presented to a group of 60+ Tribal hunters on MNPROs research, including related to this grant.

Status Update Reporting

Status Update March 1, 2024

Date Submitted: February 21, 2024

Date Approved: June 18, 2024

Overall Update

MNPRO continues to make progress toward milestones, and the research activity of the teams supported by this grant is exciting. As is often the case with science, progress has involved surmounting unexpected hurdles, troubleshooting challenges, and revising experimental procedures based on what we've learned. Our teams are leveraging resources in new ways to advance our ability to detect prions in soil, enhance understanding of prion-soil binding, and identify potential research avenues for remediation. In mapping CWD transmission risk based on soil characteristics, we have devised an approach that will allow us to build on previous scientific findings, while also exploring other soil characteristics that may be important for CWD transmission in Minnesota. Our efforts to employ mass spectrometry to identify interfering substances in RT-QuIC will lay the ground work for using mass spectrometry as an alternate method for confirming prion detection in soils (and other samples). The new subproject teams are also up and running, and while early in their own experiments, we are excited to see their progress and new insights. We are hopeful that the monthly meetings of the newly formed consortium will grow into a new think-tank for advancing the environmental science of prions.

Activity 1

Since the last update, we continue to make progress toward this milestone. The literature review of soil-prion interactions is complete and our team is in the process of drafting a manuscript of findings. We have also been assembling spatial data sets of soil characteristics and CWD case data (requested from MN Department of Natural Resources) across the Minnesota landscape to begin modeling relationships between soil characteristics and CWD presence. While the goal is to develop a predictive map of CWD for Minnesota, we are also recognizing that that limited distribution of CWD cases in the state (predominately in the southeastern part of the state), may limit the predictive power for the remainder of the state. Thus, we are exploring opportunities to obtain CWD data from Wisconsin to enhance our models. We anticipate the completion of this milestone as scheduled.

Activity 2

A research consortium of teams studying prions in soils and other environmental samples has been formed over the previous reporting period. It is composed of project investigators contributing to each of the newly formed subprojects associated with this grant as well as MNPRO researchers working on environmental detection of CWD. Since the fall of 2023, the research consortium has met monthly via Zoom to share updates on ongoing activities, new insights, challenges in the laboratory, or new information from related research. We are scheduled to continue with monthly meetings through the grant period and expect as work progresses, the Consortium may be leveraged as needed by project investigators for outside perspectives, requests for resources or other help.

Activity 3

The MNPRO team has continued to work with our in-house samples from Beltrami and other locations. Additionally, a panel of well characterized samples from multiple soil orders encompassing all soil structures have been obtained. We continue mass spectrometry studies; having failed to confirm any prion proteins in our initial study samples, we have refined our sample preparation to reduce interferences in the technology. As a result of this refinement, the development of a confirmatory assay using mass spectrometry may be used to evaluate samples with indeterminate RT-QuIC results. In addition, spiking experiments using both negative samples from Beltrami and the new panel are both ongoing and planned to further define the roles of soil texture and structure and remediation potential. We are also working to optimize prion-binding-driven extraction techniques, coupling our current method with methods from

proteomics. The goal is to by-pass soil-type-determined extraction methods in favor of a universal method of extracting prion from soil for optimal detection.

Activity 4
Graduate student Renata Widelak was recently recruited to help with this project in the Elias Lab. Renata is currently setting up different assays that are needed to produce, purify, and assay different protease mutants to degrade prions. In particular, assays for monitoring and measuring protease activity and kinetics are being evaluated. Most importantly, we are looking into assays that can best reflect the activity of proteases on prions. One assay option is the use of lysozyme, a protein that, under certain conditions, can form fibrils that are excellent models for prions. Other efforts were implemented to predict mutations on two proteases, proteinase K and subtilisin, that would increase their thermal stability. In achieving this objective, we could increase the conditions under which protease activity would be effective against prions. Through this effort, we have identified several mutations that are candidates to increase the enzymes' stability. In concert, the Salomon Lab is nearly finished with preparing two plates of approximately 200 microbial extracts and pure natural product compounds that will be turned over to the Bartz lab. In the Bartz lab, an undergraduate student, Emma Ziegler, will evaluate the compounds for anti-prion activity under a range of conditions.

Activity 5
Over the past reporting period our team has made progress toward Milestones 1 and 3 (M1 and M3). Our team has demonstrated the strong binding of CWD prions to waterborne sediments, therefore proving the effectiveness on some of the methods under M3 (e.g. filtration and centrifugation) for stream and runoff samples from two Minnesota regions in certain seasons. This result shows researchers and managers that CWD prions can be found on sediments in waters, when present, and that sediment separation methods can work to prepare samples for further analysis. We will expand on this finding and test its robustness in different surface water types found in Minnesota near CWD hotspots, such as north central and southeastern Minnesota.
Broader field sampling has been set back due to scheduling and timing of final approvals of the project. This will now take place in the first half of 2024, exact dates depending on weather. This will not effect the overall project timeline as the successes on laboratory methods will allow for faster processing of samples upon their collection.

Activity 6
The pilot microwave pyrolysis (MAP) instrument, which will enable the experiments needed for this activity, has been installed in the Soils Lab within MNPRO. A small setback was encountered when the apparatus that rotates the combustion chamber of the MAP instrument would not fully fit in a fume hood. We have subsequently modified the placement of the instrument in the hood to accommodate this part of the instrument to meet safety standards. Once initialization tests are complete, the instrument will be ready for all experiments we have planned for this activity. In addition, candidate experimental tissues have been characterized, and are ready for pyrolysis. Tissues that will be used to spike soil and biosolid samples have also been characterized, and are sufficiently rich in prions to provide excellent dynamic range for the decontamination experiments. Work is proceeding on schedule, and we do not foresee any major obstacles at this time. Next steps will include initial tissue pyrolysis in the microwave, using retropharyngeal lymph node. Depending on initial results, we will move to either tissues or soils next, followed finally by biosolids.

Activity 7
We are ahead of schedule for the milestones of this activity, which means that the subproject teams for Activities 4-6 have more time to devote to their individual projects. All teams have commenced their research and are making early progress toward project deliverables.

Dissemination
Teaching:
Guest lecture by TWolf, Case study: Chronic Wasting Disease. FW 4103, Principles of Wildlife Management, University of Minnesota. Saint Paul, Minnesota. October 26, 2023.
Scientific presentations:

Lichtenberg S.S., S. Thomas, D. Storm, D. Walsh, M. Milstein, S. Gretsch, M. Schwabenlander, T. Wolf. 2023. Recent advances in environmental prion detection and remediation. 266th American Chemical Society National Meeting, San Francisco, CA. Oral. August 13 – 17, 2023.

Wille, E. A., Karwan, D. L., Lichtenberg, S. S., Grunklee, M., Rowden, G., Ferguson-Kramer, V., Schwabenlander, M. D., Wolf, T. M., Larsen, P. A. Hydrological Transport and Persistence of Chronic Wasting Disease in Minnesota. Minnesota Water Resources Conference, St. Paul, Minnesota, October 17-18, 2023.

Status Update Reporting

Status Update September 1, 2023

Date Submitted: September 19, 2023

Date Approved: September 22, 2023

Overall Update

MNPRO continues to progress toward milestones. We are excited to have recently hired a new epidemiologist with a strong background in spatial analysis and risk modeling, including a recently developed model of spatial risk based on deer characteristics. This person will help us advance our efforts in Activity 1. We have also been working with the teams leading the subprojects added to the work plan with our last amendment to launch their individual projects. This requires some additional paperwork processing, which has experienced some recent delays. We are hoping to resolve this soon so these subprojects can begin in early September. Then, we will initiate a monthly consortium meeting to share information and updates. Finally, within MNPRO we continue to work toward optimizing RT-QuIC detection of prions in different soil types, with the goal of enhancing repeatability and reproducibility. Over the last reporting period, that has included the use of other methods and techniques to better understand which proteinaceous materials are present in the clean extracts from soil samples of interest—the components from the soil tested by RT-QuIC. This work is ongoing, but may yield additional information to help us better understand our RT-QuIC results related to soil screening.

Activity 1

Our team continues to make progress toward this milestone. Since the last update, our team met with a UMN librarian to broaden the scope of our literature search to ensure that we are able to assemble and synthesize as much information as possible. Students leading this endeavor are currently in the process of developing a written summary of this scoping review of the literature. Simultaneously, MNPRO has recruited and hired a new spatial epidemiologist, Kristin Bondo, who will join the team in November 2023. Dr. Bondo has recently created a spatial map of CWD risk based on known risk factors associated with deer in Pennsylvania and is excited to extend this tool to Minnesota, as well as add to it with soil layers determined to be most important for CWD risk from our ongoing literature review.

Activity 2

MNPRO has been working with the three new soil project teams since our last update in March to go through all necessary reviews and approvals to allow transfer of funds to begin their work. Notably, each project team developed a research addendum that was reviewed by external prion experts, who provided valuable feedback and support for each project. Subsequently, each project lead made requested revisions to the addendum and/or budget and drafted work plans that were turned into amended Activities 4-6 of this work plan. Following LCCMR approval of the new activities, we began the internal process of approvals to disseminate project funds to the new teams. Unfortunately, there has been some delay due to oversights in paperwork, which we are working through currently to finalize the transfer of funds. Our goal is to have funds transferred by early Sept such that teams may begin their work. At that stage, we will host monthly meetings for progress updates and information sharing as a research consortium.

Activity 3

The MNPRO team has collected and tested Beltrami county soil samples that are physically and chemically similar to those found at the Beltrami dumpsite to serve as negative controls. We have observed that results are not always repeatable/reproducible and tend to produce high false-positive rates in RT-QuIC. Due to these confounding factors, our team has begun a detailed characterization of the soil extract that is used in the RT-QuIC soil assay. Aside from routine chemical analyses, we are also using mass spectrometry to characterize the amino acid sequences that are present in the extracts. Preliminary results suggest that cervid prion protein is present in several of these samples, though more work is needed to confirm this finding. Additionally, several experiments were performed to attempt a ‘generalized’ depletion of interfering substances that coextract from soil using a suite of mild oxidants. The rationale behind these

experiments was that any interfering substances would likely be far more labile than highly recalcitrant prion aggregates. Initial results suggest that these so-called 'mild' oxidants have far greater capacity for prion degradation than previously reported. Though too potent for our purposes here, this does introduce the opportunity to investigate these substances as chemical remediation agents.

Activity 4
This new subproject is pending until final approvals are obtained to transfer project funds. Refer to Activity 2 for milestones reached since March 2023 detailing the process of getting this project developed, approved and initiated.

Activity 5
This new subproject is pending until final approvals are obtained to transfer project funds. Refer to Activity 2 for milestones reached since March 2023 detailing the process of getting this project developed, approved and initiated.

Activity 6
This new subproject is pending until final approvals are obtained to transfer project funds. Refer to Activity 2 for milestones reached since March 2023 detailing the process of getting this project developed, approved and initiated.

Dissemination
Grunklee, M., Wille, E., Ferguson-Kramer, V., Schwabenlander, M., Lichtenberg, S., Karwan, D., Larsen, P., Wolf, T. (2023, May 30 – June 2). Detection of Chronic Wasting Disease in Soil at a White-Tailed Deer Carcass Dumpsite [Poster Presentation]. 4th International Chronic Wasting Disease Symposium, Denver, CO, United States.

Schwabenlander, M., Bartz, J., Carstensen, M., Fameli, A., Glaser, L., Larsen, R., Li, M., Lindsey, L., Oliver, J., Shoemaker, R., Rowden, G., Stone, S., Walter, W., Wolf, T., Larsen, P. (2023, May 30 – June 2). Implementing a veterinary forensics approach to investigate chronic wasting disease at a deer carcass disposal site [Conference Presentation]. 4th International Chronic Wasting Disease Symposium, Denver, CO, United States.

Wille, E. A., Karwan, D. L., Lichtenberg, S. S., Grunklee, M., Rowden, G., Ferguson-Kramer, V., Schwabenlander, M., Wolf, T. M., Larsen, P. A. "Hydrological Transport and Persistence of Chronic Wasting Disease," Gordon Research Conference on Catchment Science: Interactions of Hydrology, Biology and Geochemistry, Andover, New Hampshire. (June 18, 2023).

Status Update Reporting

Status Update March 1, 2023

Date Submitted: February 22, 2023

Date Approved: March 17, 2023

Overall Update

MNPRO has gotten a great start to achieving the projected outcomes: 1) the optimization of methods for prion detection in soils; 2) spatial modeling of CWD risk associated with the soil diversity in Minnesota ; and 3) formation of a scientific prion/soil consortium to develop new avenues of research on CWD in the environment. Critically, since the start of the grant, we have grown our team to include two new soil scientists, one of which having experience in research of prions in soils, and a graduate student. These new personnel have been integrated into a growing scientific team that is dedicated to the optimization of our detection assays for prions in soil. This team has also begun a comprehensive review of the literature that will inform future efforts to spatially model and map CWD risk across Minnesota. We are also ahead of schedule in organizing a new consortium to explore new avenues of prion research within the soil environment, having hosted a 1-day workshop in early February to bring together scientists across various disciplines to consider the issues of prion persistence, degradation and remediation. We are excited to see how the synergy of these activities will lead to new successes.

Activity 1

Over the first 6 months of the grant, we have assembled a small, multidisciplinary team that meets regularly to make progress on this and other activities. The team includes a new soil scientist with background in studying prions in soils, a hydrologist that focuses on the transport of soil-bound contaminants through water, and two graduate students who will be focusing their studies on the detection of prions in these various environmental matrices. Accordingly, this team has been working toward assembling and synthesizing the literature related to prion binding, persistence and degradation in soils, with an eye toward creating a spatial model of risk. As this team advances efforts around a comprehensive literature review that will inform a spatial model, MNPRO is also working toward hiring a new research scientist in epidemiology to lead model development. Through these collective efforts we anticipate meeting our projected milestones.

Activity 2

On February 7, 2023, MNPRO partnered with the Strategic Partnerships and Research Collaborative to host an in-person workshop on the University of Minnesota St. Paul campus. The purpose of this effort was to catalyze collaborative research that advances the ability to detect, monitor and better understand the ecology of prions in soil through the development of new research ideas and proposals for innovative, team-science. The workshop convened a total of 13 attendees that included scientists with a broad range of targeted competencies—including environmental remediation, environmental microbiology, nanotechnology, hydrology, plant ecology and more—together with prion and animal health experts. Workshop participants brainstormed 14 distinct research questions and prioritized two areas of research: 1) prion degradation, and 2) development and optimization of assays (new and existing) for prion detection in environmental samples. Within these areas of research, three new projects were identified for further development by newly formed scientific teams. Over the next several months MNPRO will work with project team leaders in proposal development and external review.

Activity 3

MNPRO leadership has been aligning existing resources with the current grant to create a “think-tank” environment around RT-QuIC optimization for enhanced detection of prions across different soil types. With this grant, we were able to hire a new soil scientist who joined MNPRO in October, coming to the lab with a strong background in laboratory assay quality assurance. Since joining the team she has been trained in RT-QuIC and has begun conducting experiments

around quality control and sample preparation and handling for optimization. MNPRO also recruited and hired Stuart Lichtenberg, a leading scientist on CWD in soils, who joined the lab in January to facilitate these research efforts. A key hurdle in this area of research is the interference of unknown compounds in some soils (including those from the Beltrami carcass dump site) with reliable RT-QuIC performance. We are excited to see new research ideas to identify and mitigate those interfering compounds emerging from the workshop in Activity 2, while Dr. Lichtenberg pursues other avenues in parallel to circumvent RT-QuIC interference through this activity. MNPRO is optimistic that with this collective of experts working together, we have the best opportunity to make important scientific breakthroughs for prion detection in soils.

Activity 4

Since the prions in soil workshop was held in early February 2023 (see Activity 2), three project ideas emerged with potential for development into Research Addenda to be conducted under this or new work plan activities. MNPRO has outlined a plan for the newly formed research teams to work together to develop the Research Addenda and will work with project leaders to identify potential external reviewers. Given the enthusiasm generated by the workshop, MNPRO is hoping to have addenda in hand for circulation to external reviewers by the end of March. This accelerated schedule (from projected milestones) allows MNPRO ample time to manage the review process and work plan amendments while also giving the new research teams maximum time to conduct their studies within the timeframe of this grant.

Dissemination

Nothing to report at this time.