REQUEST FOR PROPOSALS FOR
Scientific Peer Review Services

LEGISLATIVE-CITIZEN COMMISSION ON MINNESOTA RESOURCES
MINNESOTA STATE LEGISLATURE

REQUEST FOR PROPOSALS

Scientific Peer Review Services

Solicitor:	Legislative-Citizen Commission on Minnesota Resources ("LCCMR")
Deadline for Questions Regarding the RFP:	Questions regarding the RFP must be received by the LCCMR by **4:30 p.m. (CDT) on Wednesday, August 14, 2024**.
Deadline for Receipt of Proposals:	Proposals must be received by the LCCMR by **4:30 p.m. (CDT) on Monday, August 26, 2024**. Late applications may not be accepted.
Proposal method:	Proposals may be submitted in writing or electronically by email. Email submissions are preferred.
Deliver Proposals to:	Attn: Scientific Peer Review Services Legislative-Citizen Commission on Minnesota Resources Centennial Office Building, 1st Floor 658 Cedar Street Saint Paul, Minnesota 55155 Email: lccmr@lccmr.mn.gov Emailed proposals are encouraged.
Contact Person:	Diana Griffith Legislative-Citizen Commission on Minnesota Resources Centennial Office Building, 1st Floor 658 Cedar Street Saint Paul, Minnesota 55155 (651) 296-2406 (voice) Email: lccmr@lccmr.mn.gov
Contents of this Packet:	Cover Page This Page Summary, Page 3 Contents of Proposal, Page 4 Consideration of Proposals, Page 4 Questions regarding the Proposal, Page 5 Proposals Due, Page 5 General Terms and Conditions, Pages 5-8 Exhibits (attached) • Exhibit A: Sample Research Addendum • Exhibit B: Peer Reviewer Comment Sheet Exhibit C: Sample Request for Response to Review

SUMMARY

The Legislative-Citizen Commission on Minnesota Resources (LCCMR) is requesting proposals to coordinate scientific peer review of research proposals recommended by the LCCMR for funding by the Legislature from the state's Environment and Natural Resources Trust Fund.

Minnesota Statute 116P.08 subdivisions 6 and 7 require that research proposals be peer reviewed before receiving an appropriation. The statutes also specify what information must be included in the research proposal, what questions the peer reviewers must address, and how much peer reviewers may be compensated for their work. The LCCMR has created template proposal and peer reviewer comment forms to reflect these requirements.

Assistance is needed to perform the following:

- Identify and recruit all subject matter expert reviewers. Reviewers must be knowledgeable in general research methods in the areas of environment and natural resources, be located outside the state of Minnesota, and have no conflict of interest with the proposal.
- Assign and secure remote/mail reviews for approximately 40 research proposals to be provided by LCCMR. Each proposal (also referred to as a "research addendum," see example in Exhibit A) will ideally receive three reviews. Reviews will include comments on questions provided by LCCMR to comply with state statute (Exhibit B). No LCCMR approval of individual assignments is needed.
- Provide anonymized reviews to research proposers with a request to address reviewer comments and to revise the research addendum as appropriate (Exhibit C).
- Collect appropriate documentation and provide payment to each reviewer upon satisfactory completion of reviews. Payment is set in state statute at $55.00 per day; LCCMR authorizes payment for up to three days per review.
- Provide copies of each peer review, a list of all reviewers, proposers' responses to reviews, and revised final research addenda as final outputs to LCCMR at completion.

The work is anticipated to start in early September 2024 upon execution of a contract for services. The entire review process must be complete and the revised research addenda submitted to LCCMR by December 6, 2024. The contract may be extended if agreed to by both parties in writing. The initial contract and any amendments may not exceed a total of five years.

Payments to the contractor will be the sole compensation for services. Payment of federal income tax, FICA payments, and state income tax for any of the contractor's employees would be the responsibility of the contractor as well as payments to reviewers and any associated tax. Any issuance of federal tax forms to reviewers will be the responsibility of the contractor. The contractor will invoice for services provided at periodic intervals and in an agreed format as negotiated. An itemization of peer review payments made must be included on invoices.

The contractor's books, records, documents, and accounting procedures and practices relevant to the contract would be subject to examination by the LCCMR, the State Auditor or the Legislative Auditor, as appropriate, for a minimum of six years from the term year end of the

contract.

The services would be performed in accordance with the specifications and the general terms and conditions enclosed.

CONTENTS OF PROPOSAL

Proposals must include:

a. A transmittal cover letter that includes an indication of intent to respond to this RFP, a summary of the prospective submitter's understanding of the project, and the contractor's contact information.
b. A description of the contractor's knowledge and experience providing scientific peer review services.
c. A description of how the contractor identifies and recruits subject matter experts that will perform the peer reviews.
d. The contractor's professional credentials, including past experience on providing such services.
e. A not-to-exceed amount for total consulting costs that is either broken out by an hourly rate or deliverable schedule.
f. Any other terms or conditions required by the contract.
g. No less than two professional references. The LCCMR may contact the references to verify the extent and the quality of work provided.
h. Accessibility Standards. The State of Minnesota requires all information and communication technology (ICT) to conform to the State of Minnesota Digital Accessibility Standards, which complies with Section 508 of the federal Rehabilitation Act of 1973 and Web Content Accessibility Guidelines 2.0. Your answers to the following questions attest to your ability to ensure that the State fulfills its legal and operational responsibilities as they relate to the final outputs delivered to LCCMR as described in this solicitation:
    i. Describe how you ensure that your staff and any relevant contractors have the knowledge and skills to ensure that all electronic documents (and other materials, as applicable) delivered to LCCMR are accessible.
    ii. Provide examples of electronic documents (and other materials, as applicable) your organization has produced that are accessible. The documents and materials must be relevant to the services called for in this solicitation.

CONSIDERATION OF PROPOSALS

The LCCMR reserves the right to reject any or all proposals, the right to waive any irregularity, the right to enter into a contract that varies from the specifications or general conditions, and the right to negotiate at any time with those that submit proposals or with any other party. The LCCMR will not necessarily select the proposals that offer the lowest price; the LCCMR reserves the right to consider price, quality, experience, reliability, convenience, and any other factors deemed relevant.

QUESTIONS REGARDING THE PROPOSAL

Please submit all questions regarding the Request for Proposals by email by **4:30 p.m. (CDT) on**

Wednesday, August 14, 2024 to: lccmr@lccmr.mn.gov

A compiled list of questions received and responses provided will be posted to https://www.lccmr.mn.gov/about/rfp_for_scientific_peer_review_services_2024.html by 4:30 p.m. (CDT) on Friday, August 16, 2024.

PROPOSALS DUE

Proposals must be received by the LCCMR by **4:30 p.m. (CDT) on Monday, August 26, 2024**. Electronic submissions are encouraged and can be emailed to the address below. Proposals are to be submitted to:

Attn: Scientific Peer Review Services
Legislative-Citizen Commission on Minnesota Resources
Centennial Office Building, 1st Floor
658 Cedar Street
Saint Paul, Minnesota 55155
Email: lccmr@lccmr.mn.gov

GENERAL TERMS AND CONDITIONS

A. "LCCMR" means the Minnesota Legislative Coordinating Commission. In matters arising out of this proposal or out of any resulting contract, the authorized agent for the LCCMR is the Chair of the Legislative Coordinating Commission or the Executive Director of the Legislative Coordinating Commission.

B. The LCCMR reserves the right to reject any and all consultant proposals received as a result of this Request for Proposals, or to negotiate separately with any consultant in any manner necessary.

C. The LCCMR reserves the right to require a consultant to make an oral presentation of its proposal to the LCCMR to permit and develop the specifics of a consultant's proposal.

D. The LCCMR is not responsible for any cost incurred by the consultant in responding to this Request for Proposals.

E. Payment for any contract entered into as a result of the Request for Proposals will be made on a negotiated periodic basis after receipt of billings accompanied by the appropriate verification of work time and satisfactory completion of tasks to billing date. In accordance with Minnesota Statutes 3.225, subdivision 6, paragraph (b), no more than 90 percent of the amount due under the contract may be paid until the LCCMR 's authorized agents have certified that the consultant has satisfactorily fulfilled the terms of the contract.

F. All contractors and subcontractors must conform to the labor laws of the State of Minnesota, and to all other laws, ordinances and legal requirements affecting the work in this state. The consultant must conform with and agree to the provisions of Minnesota

Statutes, Section 181.59, which prohibits discrimination in the hiring of labor by reason of race, creed, or color. That statute reads as follows:

> *181.59 DISCRIMINATION ON ACCOUNT OF RACE, CREED OR COLOR PROHIBITED IN CONTRACT.*
>
> *Every contract for or on behalf of the State of Minnesota, or any county, city, town, township, school, school district, or any other district in the state, for materials, supplies, or construction shall contain provisions by which the contractor agrees:*
>
> *That, in the hiring of common or skilled labor for the performance of any work under any contract, or any subcontract, no contractor, material supplier, or Vendor, shall, by reason of race, creed, or color, discriminate against the person or persons who are citizens of the United States or resident aliens who are qualified and available to perform the work to which the employment relates;*
>
> *That no contractor, material supplier, or Contractor, shall, in any manner, discriminate against, or intimidate, or present the employment of any person or persons identified in clause (1) of this section, or on being hired, prevent, or conspire to prevent, the person or persons from the performance of work under any contract on account of race, creed or color;*
>
> *That a violation of this section is a misdemeanor; and*
>
> *That this contract may be cancelled or terminated by the state, county, city, town, school board, or any other person authorized to grant the contracts for employment, and all money due, or to become due under the contract, may be forfeited for a second or any subsequent violation of the terms or conditions of this contract.*

G. In accordance with Minnesota Statutes, section 176.182, the consultant must provide to the LCCMR acceptable evidence of compliance with the worker's compensation insurance coverage requirement of Minnesota Statutes, section 176.181, subdivision 2.

H. If the amount of any contract entered into as a result of the Request for Proposals exceeds $100,000 and the consultant has employed more than 40 full-time employees in this state or in the state in which the consultant has its primary place of business on a single working day in the 12 months immediately preceding the due date for the proposal, the consultant must comply with the affirmative action plan requirements of Minnesota Statutes, section 363A.36, as follows:

1) If the 40 full-time employees were employed in Minnesota, consultant must, prior to submission of the proposal, either have a certificate of compliance issued by

Minnesota Commissioner of Human Rights or that commissioner must have received from the consultant an application for such a certificate. Prior to signing a contract resulting from a successful proposal, the consultant must have the certificate of compliance.

2) If the consultant did not have more than 40 full-time employees in Minnesota but did have that number in another state in which the consultant has its primary place of business, the consultant must, prior to signing a contract resulting from a successful proposal, either have a certificate of compliance issued by the Minnesota Commissioner of Human Rights or certify that the consultant is in compliance with federal affirmative action requirements.

I. As required under Minnesota Rules, part 5000.3600, subpart 9: It is hereby agreed between the parties that Minnesota Statutes, section 363A.36, and Minnesota Rules, parts 5000.3400 to 5000.3600, are incorporated into any contract between these parties based upon this specification or any modification of it. A copy of Minnesota Statutes, section 363A.36 and Minnesota Rules, parts 5000.3400 to 5000.3600 is available from the LCCMR upon request.

J. As required by Minnesota Statutes, section 270C.65 subdivision 3, a consultant must provide to the LCCMR either its federal taxpayer identification number, its Social Security number, or its Minnesota tax identification number (if applicable). This information may be used in the enforcement of federal and state tax laws. Supplying these numbers could result in action to require consultant to file state tax returns and pay delinquent state tax liabilities. A contract will not be approved unless these numbers are provided. These numbers will be available to federal and state tax authorities and state personnel involved in approving the contract and the payment and audit of state obligations. These numbers will not be made available to any other person without the express written permission of the consultant.

K. As required under Minnesota Statutes 3.227, the consultant must certify its compliance with Minnesota Statutes chapter 3, including the non-discrimination provisions of Minnesota Statutes, section 3.226, in the execution and performance of any contract entered into as a result of the Request for Proposals.

L. All data and information supplied to the consultant by the LCCMR under any contract entered into as a result of the Request for Proposals, and all work products and interim and final reports prepared by the consultant in the performance of its obligations under any contract entered into as a result of the Request for Proposals, are the property of the LCCMR and must be remitted to the LCCMR upon completion or termination of this agreement. The consultant must not use, willingly allow the use of, or cause to have the materials used for any purpose other than performance of the obligations under this agreement without the prior written consent of the LCCMR.

M. Work must begin within the timeframe set in the signed contract between the LCCMR and the consultant which may be extended upon mutual agreement of both parties. The signed contract will terminate upon full performance by all parties of the contract agreement.

N. Any contract entered into as a result of the Request for Proposals may be terminated by the LCCMR as permitted under Minnesota Statutes 3.225, subdivision 6, in whole or in part, whenever the LCCMR determines that termination is in the interest of the LCCMR. The LCCMR will pay all reasonable costs associated with the contract that the consultant has incurred up to the termination date of the contract and all reasonable costs associated with termination of the contract.

EXHIBIT A

Environment and Natural Resources Trust Fund

Research Addendum for Peer Review

Project Manager Name:
Project Manager Email Address:
Project Title:

Project number:

1. Abstract

In Minnesota there are over 1,000 small communities with unmet wastewater management needs, ranging from no treatment to inadequate treatment. If inadequately treated, wastewater discharges can contain high concentrations of nitrogen species. Ammonia and nitrate can negatively impact surface and groundwater quality by decreasing oxygen levels in the receiving water body, causing eutrophication, and rendering well water unsafe to drink as a result of contamination. It is therefore important to remove these nitrogen species by efficient and effective treatment. An option for treating wastewater in small communities is treatment ponds, which are very simple to operate and relatively low-cost, relying on phenomena such as wind to provide oxygen, and thereby stimulate bacterial treatment of nitrogen species in the wastewater. Unfortunately, 23% of Minnesota's over 300 existing treatment ponds under-perform with respect to total nitrogen removal, especially during the winter and spring months. We propose to study how pond systems operate with respect to nitrogen cycling under conditions of low oxygen and/or low temperature. This work will be performed on the laboratory scale at the University of Minnesota and will be coupled with samples from full-scale treatment ponds with the assistance of project partner Minnesota Rural Water Association (MRWA). Laboratory research will focus on how simple interventions such as mixing and oxygen addition affect nitrogen cycling. Recommendations based on the laboratory work will be provided to MRWA to assist in developing and, in the future, field testing improved nitrogen removal practices. The overall goal of this research is to better understand nitrogen cycling in wastewater treatment ponds, improving their management, so that they can serve as a well-operating solution for Minnesota's small communities in need of wastewater management.

2. Background

Wastewater Treatment Ponds

Domestic wastewater is primarily comprised of sewage, which must be treated to remove various pollutants, including nutrients, pathogens, and chemical oxygen demand (Verbyla et al., 2018). Of particular concern is the release of nutrients, especially nitrogen, to the environment (Elser et al., 2007). If inadequately treated, wastewater discharges can contain high concentrations of nitrogen species, including ammonia and nitrate (Metcalf and Eddy, 2003; Bowman et al., 2002). Ammonia can decrease the oxygen levels in the receiving water body and is also toxic to fish (Metcalf and Eddy, 2003). Nitrate can eventually lead to eutrophication and can also contaminate groundwater supplies, rendering well water unsafe to drink (Metcalf and Eddy, 2003). It is therefore important to treat, and thereby remove, these nitrogen species to protect surface and groundwater quality.

An option for treating wastewater in small communities is treatment ponds. This is a treatment technique consisting of an open basin that uses natural processes to treat domestic wastewater

(Verbyla et al., 2018). These systems are very simple and inexpensive to operate and maintain. They rely on wind and surface transfer of air to provide oxygen to the microorganisms present in the pond sediment and water column. This is particularly important for the microorganisms that use oxygen to oxidize ammonia to nitrate (Verbyla et al., 2018). Ponds generally do not rely on mechanized equipment or make use of expensive material or energy inputs. These attributes make them attractive treatment solutions for communities lacking public infrastructure and funds (Bowman et al., 2002; Tharavathy et al., 2013; Verbyla et al., 2018). Nevertheless, because treatment is based to a large extent on microbial processes, the performance of a pond depends on climatological conditions such as light, temperature, rain, and wind (Tharavathy et al., 2013).

Very little research has been conducted on wastewater treatment ponds to understand how they fundamentally operate with respect to microbiological nitrogen removal. It is also unclear how low oxygen levels and low temperature—both of which can occur in wastewater treatment ponds in Minnesota during the winter and spring months—impact microbiological nitrogen removal (Bowman et al., 2002). Observations of increased nutrient release following cold seasons have been made, however, and previous research has indicated that dissolved oxygen (DO) concentrations and temperature significantly affect nitrogen removal processes (Wu et al., 2018; Smyth et al., 2018; Pennsylvania Department of Environmental Protection, n.d.). Our own research has shown that in a traditional wastewater treatment plant, ammonia oxidation occurs throughout the winter months in Minnesota if adequate oxygen is supplied (Figure 1). It is not known, however, if a similar phenomenon exists in treatment ponds and whether the denitrification of nitrate to nitrogen gas will proceed under low temperature conditions as well.

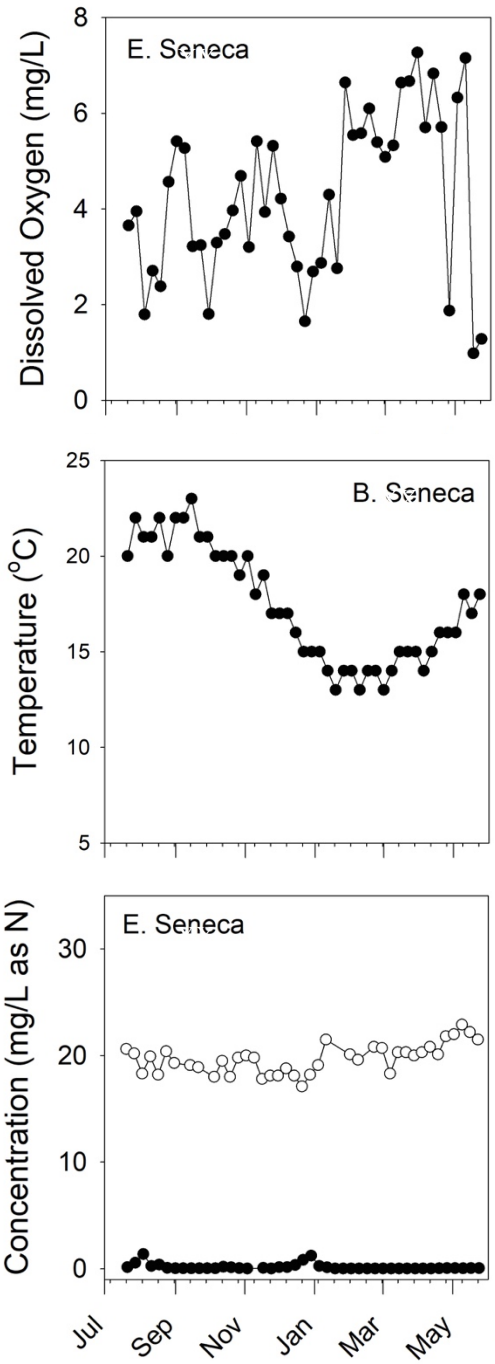

Figure 1. Various parameters measured in the Seneca Wastewater Treatment Plant in Minnesota during the 2016-2017 year. The top panel shows the DO concentration in the wastewater over time, the middle panel shows the temperature in the wastewater over time, and the bottom panel shows the ammonium (dark symbols) and nitrate (open symbols) concentrations in the wastewater over time.

Wastewater Treatment Ponds in Minnesota

In Minnesota there are over 1,000 small communities with unmet wastewater management needs, ranging from no treatment to inadequate treatment (McCarthy and Gillingham, 2008). An option for treating wastewater in small communities is treatment ponds, because they can be used in centralized or semi-centralized sewage systems (Verbyla et al., 2018). In fact, there are currently more than 300 wastewater treatment pond systems that help serve the needs of Minnesota's small communities (McCarthy and Gillingham, 2008). The ponds are emptied periodically, according to guidelines from the Minnesota Pollution Control Agency, which implements the federal Clean Water Act in Minnesota, and are discharged to surface water such as creeks or streams (MPCA, 2009). While some ponds are continuously discharged, ponds in cold climates are not discharged for months at a time because it is thought that extended retention time accounts for the slower rate of nitrogen removal during colder months of the year (Verbyla et al., 2018). Unfortunately, 23% of Minnesota's ponds under-perform with respect to total nitrogen removal, as shown in Figure 2:

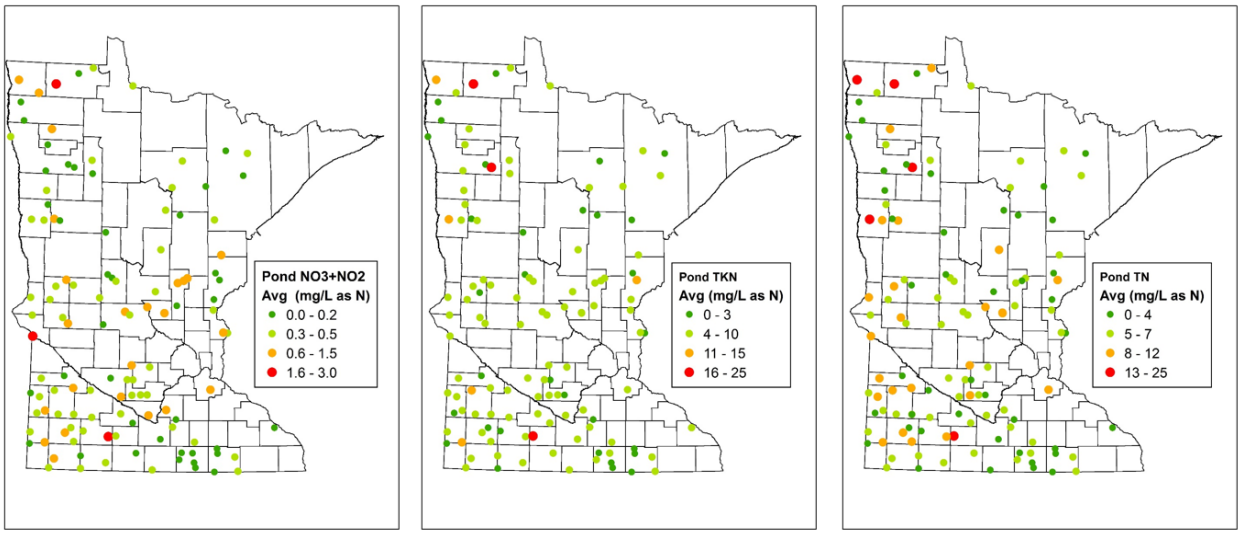

Figure 2. Oxidation ponds' effluent in Minnesota: These diagrams depict the nitrogen being released from wastewater treatment ponds in Minnesota as nitrate and nitrite; total Kjeldahl nitrogen, which accounts for ammonia and organic nitrogen; and total nitrogen; from left to right. Green circles indicate acceptable nitrogen release, while orange and red circles indicate the release of unacceptable quantities (McCarthy and Gillingham, 2008).

Because nitrogen pollution is detrimental to the environment and human health, it is important to treat, and thereby remove, these nitrogen species in the treatment ponds to protect surface and groundwater quality in greater Minnesota (McCarthy and Gillingham, 2008). If nitrogen cycling in pond systems were well understood, these systems could be managed more precisely. When needed, simple interventions such as adding oxygen and mixing the sediment of the pond could also be used to stimulate total nitrogen removal during times of poor performance, such as the winter and spring months.

3. Hypothesis

Minnesota Pollution Control Agency (MPCA) data, shown in Figure 2, indicate that nitrogen is not adequately removed from wastewater in some treatment ponds during the winter and spring months (McCarthy and Gillingham, 2008). These data indicate that the majority of nitrogen being released is in the form of ammonia and organic nitrogen. **We therefore hypothesize** that bacterial nitrification, the first step in bacterial nitrogen removal, proceeds slowly, if at all, in the winter. Furthermore, because some ponds do not underperform during winter months, **we hypothesize** that the DO concentration controls nitrification of ammonia to nitrate, and eventually, total nitrogen removal in treatment ponds, rather than temperature. **If oxygen can be amended to ponds during periods of ice cover and concomitant low oxygen concentration total nitrogen removal will be stimulated in ponds.**

4. Methodology

Laboratory assessment of nitrogen cycling in model pond reactors under conditions of low temperature and DO

The sediment and wastewater from two wastewater treatment ponds, a well-performing (WP) and poorly-performing pond (PP) that are geographically close to each other and geometrically similar, will be collected in November of the first year of the project. The project partner, MRWA, will assist with identifying ponds for sampling.

Batch reactors will be constructed (5-liter), amended with the pond sediment to a depth of 3 cm and water from one of the two ponds (PP or WP), filled so that they are operated headspace-free, and capped. Caps will contain sampling ports and ports for gas input lines and DO/temperature probes. Reactors will be gently bubbled with gas (either nitrogen gas or different blends of oxygen and nitrogen gas) and DO and temperature will be monitored continuously via a DO probe and a thermocouple connected to a data logger. The following six treatments will be set-up, with each treatment consisting of triplicate reactors: WP incubated at 4ºC, 8ºC, and 12ºC, PP incubated at 4ºC, 8ºC, and 12ºC. If needed, the operation of the 18 reactors will be staggered, but all will be set up with the same sediment and water, which will be held under refrigeration if needed.

The pond water will be spiked with 40 mg/L ammonium (as N) prior to adding it to the reactors. The initial DO concentration will be maintained at 2 mg/L. pH, total organic carbon (TOC), dissolved organic carbon (DOC), ammonium degradation and nitrate and nitrite formation and degradation will be monitored over time in the water column. Samples (2 mL) will be taken from the sediment and the water column over time for microbial analysis. Once the nitrogen species have either decayed or reached a steady concentration, the DO in the reactors will be changed (first to approximately 1 mg/L then to 0.25 mg/L), additional ammonium will be amended, and the nitrogen species present will again be monitored for degradation/formation. If 2 mg/L is inadequate to stimulate complete nitrification, an additional experimental period during which high concentrations of DO (5 mg/L) are maintained will be run to serve as a positive (no oxygen limiting) control.

Ammonium will be monitored by ammonia-specific electrode. pH will be monitored via pH probe. Nitrate and nitrite will be monitored via ion chromatograph (Metrohm). TOC and DOC will be monitored via a Shimadzu TOC analyzer. We have experience with all of these methods in our laboratory (Peterson et al., 2017). Water column samples taken for microbial analysis will be centrifuged, decanted and frozen; sediment samples will be frozen. DNA will be extracted using a Fast DNA Spin Kit (Qbiogene; Vista, Calif.) per manufacturer's instructions. The quantities of total bacteria, nitrifiers, denitrifiers, and anammox bacteria will then be quantified via quantitative

real-time PCR (qPCR) using a CFX Connect Real-Time System (Bio-Rad; Hercules, CA). qPCR will be used to quantify the number of hydrazine synthase genes (*hzsA*, specific for anammox bacteria (Harhangi et al., 2012)), the ammonia monooxygenase genes (*amoA*) of both ammonia oxidizing *Bacteria* and *Archaea* (Meinhardt et al., 2015; Rotthauwe et al., 1997***)***, the 16S rRNA genes belonging to NOB (Pellicer-Nacher et al., 2013), anammox bacteria (Bagchi et al., 2016), denitrifying bacteria (Harter et al., 2014) and total *Bacteria* (Muyzer et al., 1993). The number of gene copies in each sample will be determined by creating a standard curve of 10-fold dilutions. Standards will be purchased (gBlocks, Integrated DNA Technologies). Select samples will be diluted and quantified with each of the primers to ensure there are no PCR inhibitors present. If the samples show signs of inhibition, they will be diluted until this is no longer an issue. Detection limits will be determined by either the lowest quantifiable standard or the detection level in the no template control. A melting curve analysis will be completed at the end of each run for quality assurance.

Data will be analyzed statistically to determine whether DO and/or temperature affect nitrification or denitrification activity (determined via chemical analysis) or the number of the different nitrogen cycling populations present. Based on this data the ability to stimulate nitrogen removal via oxygen addition, even when the temperature is low, and the quantities of oxygen addition required for stimulation, will be determined.

<u>Full-scale treatment pond sampling to understand how, and how rapidly, nitrogen cycles during cold weather months</u>

The two ponds used to start the laboratory-scale reactors will also be sampled for analysis of the water chemistry and microbial community. We will work with our project partner, MRWA, to obtain samples over time (approximately 1 sample/1-2 weeks) after wastewater loading during the winter and spring months when temperatures are expected to range from 4°C to 12°C and the ponds range from free-surface to ice-covered. Water-column samples will be taken at multiple locations within each pond and at multiple depths. A YSI meter will be used to measure DO, temperature, and nitrate. Samples will be taken and immediately filtered and stored in an ice-filled cooler for transport back to the UMN laboratory for ammonia and nitrite analysis via Hach colorimetric kits. Pond sediment samples will also be taken from several locations within each pond and transported back to the laboratory. Once at UMN, DNA will be extracted from the sediment samples and they will be analyzed via qPCR as described above. Statistical trends will be analyzed over time to determine how various microbiological populations and water quality parameters vary (or co-vary).

Samples will be taken over two separate winter/spring seasons.

<u>Evaluation of simple methods (oxygen addition and mixing) at the laboratory scale to stimulate total nitrogen removal during periods of low oxygen concentration and low temperature</u>

Laboratory experiments, identical to those described above, will be performed to evaluate the effect of oxygen addition, via the solid and easily deployed “oxygen-release compound” (ORC®, Regenesis), and the effect of gentle sediment mixing via a paddle mixer on nitrogen removal. The use of ORC is particularly attractive because it can support aerobic biodegradation for up to 12 months with no operations or maintenance costs (Regenesis, n.d.). Experiments will be repeated at 4ºC, 8ºC, and 12ºC with both the WP and PP sediments/water that have been held under conditions of low DO such that nitrogen removal is poor. If ammonium oxidation to nitrate does not occur readily, ORC will be added in small amounts to determine what level of DO needs to be reached and for how long to stimulate ammonium oxidation. Likewise, if nitrate reduction to nitrogen gas does not occur readily, the effect of gentle mixing (the area impacted

and the duration of the mixing) will be studied to determine what is required to stimulate nitrate reduction. Again, all treatments will be tested in triplicate to facilitate statistical analysis of the data.

5. Results and Deliverables

In this project we will determine 1) the pattern of nitrogen cycling in two full-scale wastewater treatment ponds in rural Minnesota, 2) the effects of cold temperature and low DO on nitrogen cycling activity and on the numbers of nitrogen cycling microorganisms present in laboratory-scale model pond reactors, and 3) whether the addition of oxygen can stimulate nitrogen removal when the temperature is low. This information could be used directly by utility personnel, environmental consultants, and state agency personnel for achieving total nitrogen removal while maintaining the low-cost and low-infrastructure attributes that make wastewater treatment ponds an attractive solution for outstate wastewater treatment in rural Minnesota.

6. Timetable

This project is a three-year endeavor beginning in July 2019. The timetable for project completion is divided into three-month increments in the following table.

Task	Quarter											
	1	2	3	4	5	6	7	8	9	10	11	12
Method development and quality assurance testing for microbiological methods	x											
Sediment and Water collection		x										
Set-up of laboratory-scale reactors		x										
Experiments at 4ºC, 8ºC, and 12ºC with 2 mg/L DO			x	x								
Experiments at 4ºC, 8ºC, and 12ºC with 1 mg/L DO				x	x							
Experiments at 4ºC, 8ºC, and 12ºC with 0.25 mg/L DO					x	x						
Analysis of microbiological community in laboratory-scale experiments via qPCR						x						
Full-scale pond sampling		x	x			x	x					
Analysis of microbiological community in samples from full-scale pond via qPCR				x				x				
Experiments at 4ºC, 8ºC, and 12ºC with ORC addition and gentle paddle mixing								x	x	x	x	

Report writing		x		x		x		x		x		x
Manuscript preparation									x	x	x	x

7. Budget

The budget is as outlined on the previously submitted proposal (see Attachment A). A budget justification is provided below.

Personnel
Over the course of the 3-year project, support for one graduate student for three years, and support for the two PIs is budgeted (1 week/year for each). Fringe benefits for the PIs at UMN are set at 33.7% by the University of Minnesota. The PIs will be responsible for project oversight, guidance of the graduate student, data interpretation and analysis, and report preparation and submission. The graduate student research assistant will devote 100% of their research time to the project over the 3-year project. Fringe benefits for the graduate student include tuition, health insurance, and summer FICA.

Equipment, Tools, and Supplies
Funds ($18,000, $20,000, and $13,200 for years 1, 2, and 3) are requested for materials, supplies, consumables, microbial sequencing services, analytical costs and repair/upkeep associated with the ion chromatograph. Funds are also included for a hand-held YSI analyzer that will facilitate real-time DO, temperature, and nitrate analysis during pond sampling. Required materials include, but are not limited to: pipette tips, kits for in-field nitrogen species analysis, materials to construct wastewater reactors, chillers to maintain low reactor temperatures, analysis needs such as standards, sample vials, columns and guard columns, supplies for culture-independent bacterial enumeration and identification, consumables such as gloves and solvents, analytical fees, sequencing fees, digital data storage media, and laboratory notebooks.

Travel
Funds ($700, $700, and $600 for years 1, 2, and 3) are requested for mileage charges to travel to oxidation pond sites for sample collection, and pond water and sediment collection. Mileage will be reimbursed $0.55 per mile or current U of M compensation plan.

Professional/Technical/Service Contracts
Funds ($40,000, $30,000, and $30,000 for years 1, 2, and 3) are requested for Minnesota Rural Water Association (MRWA). MRWA will work with us to take treatment pond samples and perform chemical analyses in the field (dissolved oxygen, temperature, and some nitrogen species). A 25% engineer will be paid to perform this work. They will also help with disseminating findings through their outreach programs using staff time (25% FTE/year).

Total amount proposed
The total proposed project amount is $325,000 over the three-year duration of the grant. No indirect costs for the University of Minnesota are included in the budget.

8. Credentials

LCCMR Staff Note: 1 page CVs for each of the co-PIs and 1 paragraph each for other collaborators for a total of 3.5 pages of credentials. Credential pages removed for privacy considerations.

9. Dissemination and Use

The target audience for results from this research will be professionals in the areas of wastewater treatment and natural resource management. Specific targets will be environmental engineers and scientists in academia, industry, state agencies such as the DNR and MPCA, and environmental consultants. Results will be disseminated through scholarly publications in peer-reviewed journals such as *Environmental Science and Technology*. Results from the research project will also be presented at regional conferences such as the Minnesota Water conference and, if possible, at targeted seminars at the DNR and MPCA. Results will be used to determine which methods of stimulating nitrogen removal, such as ORC addition and/or artificial aeration, effectively reduce nitrogen release in pond effluent while maintaining the low-cost and low-infrastructure attributes that make wastewater treatment ponds an attractive solution for outstate wastewater treatment in Minnesota.

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EXHIBIT B

Peer Review Comment sheet
Environment and Natural Resources Trust Fund
Peer Review

(To be completed by individual peer reviewers)

Project Title:
Project Number:
Project Manager:
Reviewer Name:
Date:

A. Project Design

1. How well organized and complete is the project design and/or the experimental design?
2. How scientifically and technically sound is this proposal?
    a. Will the proposed structure answer the research question/s or hypotheses?
3. Suggestions for changes (please be specific):

B. Methodology

1. To what extent will the proposed methodology yield useful information and data?
    a. Are proposed methods current and supported in the literature? If not, are the proposed variations a reasonable approach considering the circumstances (such as time, funding levels, the age of the participants)?
    b. Is there sufficient replication?
2. Is the proposed approach to the data analysis adequate and appropriate?
3. Suggestions for changes (please be specific):

C. Probability of Completion as Proposed

1. How reasonable is the schedule of the project?
2. Are the technical aspects possible as proposed?
3. Is there sufficient access to needed resources?
4. Suggestions for changes (please be specific):

D. Qualifications

How well qualified are the project manager and cooperators to successfully carry out all phases of the project?

E. Broader Impacts

1. How well will results be broadly shared to promote protection and preservation of Minnesota's environment and natural resources?
2. What are the proposed benefits to Minnesota?

F. Intellectual Merit

1. How important is the proposed work to fundamental advances or understanding in its field or across fields?
2. To what extent does this project explore creative or original concepts?

G. Overall proposal quality:

H. Additional Comments

EXHIBIT C

I'm pleased to be able to share your project reviews with you.

Attached to this email are a compilation of peer review comments and a Peer Review Response template.

Please complete the following:

1. Using the attached template, provide a written response to each of the reviewer's substantive comments as appropriate and note if you made modifications to a revised addendum as a result of the comments.
2. Provide a copy of a revised addendum using 'track changes' so we can easily see where you made any changes and edits.
3. *Note that you do not need to implement all the reviewer's suggested changes. These comments are intended to improve the project, but if the suggestions are not feasible because of budget, time, facility limitations, or other factors, explaining why such changes cannot be made is a reasonable response.*

Please plan to return your response and updated research addendum to me by **Monday, December 2, 2024**. If you cannot make this deadline, please let me know right away. And, as always, feel free to reach out with any questions.

Best regards,