

## **2019 Project Abstract**

For the Period Ending June 30, 2023

**PROJECT TITLE:** Improving Nitrogen Removal in Greater Minnesota Wastewater Treatment Ponds

**PROJECT MANAGER:** Paige J. Novak

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**FUNDING SOURCE:** Environment and Natural Resources Trust Fund

**LEGAL CITATION:** M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04e as extended by M.L. 2022, Chp. 94, Sec. 2, Subd. 19 (c.1) [to June 30, 2023]

**APPROPRIATION AMOUNT: \$ 325,000**

**AMOUNT SPENT: \$ 293,029**

**AMOUNT REMAINING: \$ 31,971**

### **Sound bite of Project Outcomes and Results**

Inadequately treated wastewater in rural communities contributes to environmental/human health issues. We studied how to improve rural wastewater treatment pond performance. Our results suggested that manually increasing oxygen supply when temperatures are greater than 10°C should improve ammonia biodegradation; if temporary, total nitrogen removal should be possible, improving rural water quality.

### **Overall Project Outcome and Results**

Inadequately treated wastewater discharges can contain high concentrations of nitrogen, including ammonia and nitrate, which contribute to negative human and environmental effects. Little research has been conducted on nitrogen removal/degradation in rural wastewater treatment ponds, which could be helpful to improve rural water quality. Although both ammonia and nitrate are problematic, ammonia is more toxic than nitrate and must biodegrade first for total nitrogen to be biodegraded. In this study, we studied how low oxygen levels and low temperature, both of which can occur in ice-covered wastewater treatment ponds in Minnesota during winter and spring, impacted ammonia biodegradation. Well-controlled laboratory experiments were conducted and samples from real wastewater treatment ponds were analyzed.

Our research showed that both low temperature (10-6°C) and oxygen (0.5-1 mg/L) resulted in little-to-no ammonia biodegradation. As mentioned, unless the ammonia is biodegraded, total nitrogen biodegradation cannot occur. Low temperature critically limited ammonia biodegradation, while the negative effect of low oxygen concentration was less important, increasing the time before ammonia started to biodegrade, but not changing how fast it degraded. Unfortunately, temperature cannot be controlled in these large treatment ponds during the cold winter months. Oxygen, however, can be manually added. We observed that if the oxygen concentration was 3 mg/L or higher, ammonia could be biodegraded at a temperature of 10°C. Field results were consistent with laboratory results. Results showed that low ammonia concentrations could be achieved with low temperature and low oxygen concentrations in most of the ponds. Below ≈10°C, ammonia concentrations increased with decreasing oxygen. Above 20°C, low ammonia was observed regardless of the oxygen concentration. These results suggest that interventions, such as sparging with air, can improve ammonia biodegradation in winter/early spring. Nevertheless, at temperatures below 10°C, oxygen addition may not improve ammonia biodegradation, resulting in ammonia volatilization instead, contributing to poor air quality.

### **Project Results Use and Dissemination**

Results from this research have been presented at local, regional, and national conferences via posters and oral presentations. Notable dissemination activities include presentations at the Association of Environmental

Engineering and Science Professors (poster and podium), the Air & Waste Management Association, Upper Midwest Section, Conference on the Environment, the Central States Water Environment Association Annual Meeting, and an invited technical workshop given at the Minnesota Wastewater Operators Association Conference. Three papers are expected to be submitted on this research and are currently in progress. They will be submitted to LCCMR when accepted for publication.



# Environment and Natural Resources Trust Fund (ENRTF) M.L. 2019 ENRTF Work Plan Final Report (Main Document)

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**Date of Submission:** August 10, 2023  
**Final Report**  
**Date of Work Plan Approval:** June 5, 2019  
**Project Completion Date:** June 30, 2023

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**PROJECT TITLE:** Improving Nitrogen Removal in Greater Minnesota Wastewater Treatment Ponds  
**Project Manager:** Paige J. Novak  
**Organization:** University of Minnesota  
**College/Department/Division:** Department of Civil, Environmental, and Geo- Engineering  
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**Location:** Minneapolis, MN 55455 and McLeod or Sibley counties

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<b>Total Project Budget:</b>	\$325,000
<b>Amount Spent:</b>	\$293,029
<b>Balance:</b>	\$31,971

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**Legal Citation:** M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04e as extended by M.L. 2022, Chp. 94, Sec. 2, Subd. 19 (c.1) [to June 30, 2023]

**Appropriation Language:** \$325,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to assess cold weather nitrogen cycling and different aeration methods to improve the efficacy of Minnesota's underperforming wastewater treatment ponds.

M.L. 2022 - Sec. 2. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2023]

## I. PROJECT STATEMENT:

In Minnesota there are over 1000 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, including ammonia and nitrate. Ammonia can decrease the oxygen levels in the receiving water body and is also toxic to fish. Nitrate can eventually lead to eutrophication and can also

contaminate groundwater supplies, rendering well water unsafe to drink. It is therefore important to treat, and thereby remove, these nitrogen species to protect surface and groundwater quality in greater Minnesota.

An option for treating wastewater in small communities is treatment ponds. In fact, there are currently more than 300 wastewater treatment pond systems that help serve the needs of Minnesota's small communities. These systems are very simple to operate, relying on wind and surface transfer of air to help bacteria within the pond treat the nitrogen species present in the wastewater. The ponds are emptied periodically, according to guidelines from the Minnesota Pollution Control Agency, and are discharged to surface water such as creeks or streams.

Unfortunately, 23% of Minnesota's ponds under-perform with respect to total nitrogen removal. Very little research has been conducted on wastewater treatment ponds to understand how they operate with respect to nitrogen removal and to understand 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 bacterial nitrogen removal. If nitrogen cycling in pond systems was well understood, these systems could be managed more precisely. If 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.

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 at both the laboratory scale at the University of Minnesota and full scale with the assistance of project partner Minnesota Rural Water Association (MRWA). Subsequent laboratory research will focus on how simple interventions such as mixing and oxygen addition improve or change nitrogen cycling. Recommendations based on the laboratory work will be provided to MRWA, to assist in developing and field-testing improved nitrogen removal practices in the future. 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 some of Minnesota's 1000 small communities that need wastewater management.

## **II. OVERALL PROJECT STATUS UPDATES:**

### **Amendment Request (09/13/2019):**

This formal amendment request is to seek approval for the purchase of a computer as part of the materials needed to construct wastewater reactors for use in our experiments. For this project it is critical to control the dissolved oxygen concentration in the reactors. For this we have planned to purchase a control system that will turn on and off an air flow for precise control; this is coupled with a data logger to collect the data generated and track the dissolved oxygen concentration over time and also with a pH control system. As part of the control system, we need a simple (inexpensive) computer (<\$300). The computer will only be used to run the LabView software and iSense software used for pH and dissolved oxygen control and to log data from the sensors monitoring pH and dissolved oxygen over time. We have changed the wording in Attachment A to reflect this purchase. No funds need to be re-allocated or re-budgeted and this amendment will not alter the project outcomes or time-line.

### **Amendment Approved by LCCMR 9/13/2019**

### **First Update March 1, 2020**

Project activities over the last seven months have included method development and construction of reactors with precise oxygen and temperature feedback control. Initial trouble-shooting experiments have been performed to fine-tune the control system. Sediment and pond water have been collected with the help of project partner Minnesota Rural Water Association to seed the reactors. Experiments at different dissolved

oxygen concentrations and temperatures will begin shortly. In addition, during the first project period the recruitment and hiring of a graduate research assistant has also taken place.

### **Second Update September 1, 2020**

Project activities since the previous update have included replicate experiments at room temperature with high dissolved oxygen concentrations as a set of “controls”. These experiments have yielded reproducible data and meet our performance expectations with respect to nitrification. A walk-in refrigerator has been prepared for experiments requiring low temperature conditions, while dissolved oxygen control system refinement is ongoing. Experiments with varying dissolved oxygen and temperature conditions will begin shortly.

Additionally, our partnership with the Minnesota Rural Water Association has been leveraged to provide data from a series of wastewater treatment ponds in Minnesota throughout the year, including six different pond systems sampled at different times of the year. Samples and sonde measurements for each pond in each pond system are being collected seasonally at the influent, effluent, transfer points between ponds, and pond centers. Samples include both water column and sludge samples. The samples are analyzed by our partners and by us and we anticipate this data set to provide excellent information regarding how nitrogen cycles in these different ponds throughout the year.

Laboratories at the University of Minnesota were shut down as a result of the COVID-19 pandemic from March 16, 2020 to May 11, 2020. During the laboratory shut-down the student working on the project performed a review of the literature and worked with project partners on the design of the field sampling. The student began lab work again in July of 2020.

### **Third Update March 1, 2021**

Project activities since the previous update have included responding to unexpected obstacles and continued experimental operation. The dissolved oxygen (DO) control system required further optimization and reconfiguration to provide reliable low-DO operation. In addition, the walk-in refrigerator that was going to be used for all low-temperature experiments was found to be capable of only achieving temperatures of approximately 10°C in the reactors. As a result, an additional chiller system had to be purchased for use in the experiments. Experiments have been performed at room temperature at several DO set-points, including low (0.5 mg/L) set-points. Results showed that, as expected, nitrification proceeds slowly under low DO conditions and does not begin until the carbonaceous oxygen demand has been removed by heterotrophic bacteria. Nevertheless, even at the very low DO concentration of 0.5 mg/L, nitrification does occur. This suggests that our hypothesis that the provision of low quantities of DO during winter under the ice-cover of wastewater treatment ponds will stimulate nitrification and improve nitrogen removal in these systems. Experiment are planned for a range of DO concentrations at several low temperature set-points as well and should begin mid-spring and be performed over the course of the summer months.

The field study has been progressing according to plan, with the winter sampling period finished on February 18, 2021. The collaboration with industry partners described in the Second Update has been expanded to encompass collaborative meetings between the graduate student working on this project and the Minnesota Technical Assistance Program analytical team. Since the last update, data for the summer and fall sampling periods have been processed and organized. The development of statistical analytical and graphical frameworks using the open-source software program R is in progress for examining seasonal trends in pond performance, with an emphasis on low oxygen and low temperature conditions. To our knowledge this is the first comprehensive data set on pond performance at low temperatures and low oxygen levels and should serve as a great resource for understanding how these ponds function under these challenging temperature and DO conditions so that their performance can be improved.

The recruitment and hiring of an undergraduate laboratory assistant took place over this period.

#### **Fourth Update September 1, 2021**

Work has continued on the experiments performed with varying DO and temperature. The chiller system purchased in winter 2021 received repairs for agitation malfunction upon initial testing, then it was calibrated for use in the low-temperature experiments. Challenges arose with organic matter leaching from the tubing used in the experiments and these have now been addressed. National supply-chain issues affected our ability to quickly make purchases and alter experimental operation and have slowed down our experiments. We anticipate that this will be resolved in the coming project period. We are in the process of replicating several of the experiments performed at low DO and will then proceed with the experiments performed at low temperature.

Since the previous update, work has continued with respect to analyzing the field study data, with data collection now complete. All of the data have been checked for accuracy and statistical and graphical analysis of the data is in progress. A manuscript describing these results should be written and submitted for publication in early 2022.

#### **Amendment Request (09/30/2021):**

This formal amendment request is to seek approval for a rebudget of \$22 from “Travel expenses in Minnesota” to “Equipment/Tools/Supplies” and \$10,211 from “Personnel” to “Equipment/Tools/Supplies”. This movement of funds will not impact the project outcomes or timeline, decreasing the summer funding of Novak and LaPara slightly. The funds are needed for additional equipment because the pH controllers and dissolved oxygen control system was more expensive than initially anticipated (a total of (\$18,727), and even with moving funds away from laboratory services and decreasing expenditures of laboratory consumables, additional funds were needed. We would also like to seek approval to pay an undergraduate student researcher on the project. The graduate student working on the project was able to secure a very limited time teaching assistantship that allowed her to deepen her knowledge of environmental microbiology by reviewing the concepts critical to this LCCMR project with students in the course “Environmental Microbiology.” As a result, a small amount of funding is available to pay an undergraduate research assistant to help the graduate student analyze some of her samples, allowing more of her time to be spent on data and statistical analysis. Again, this will not impact the project outcomes or timeline of the project.

#### **Amendment approved by LCCMR 10/21/21**

#### **Fifth Update March 1, 2022**

Experiments performed with varying DO and temperature setpoints have continued. Pandemic-related supply chain issues have been recently resolved, and the final room temperature experiments are underway. Statistical methods have been selected for hypothesis testing for both the benchtop experiment and the field study. Coding for data analysis is in progress.

#### **Update as of June 30, 2022**

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

#### **Sixth Update September 1, 2022**

Results thus far suggest that even at low temperatures, a DO of 0.5 mg/L is enough to facilitate nitrification of ammonium to nitrate, albeit slowly. This serves as a guide for enhancing nitrification in the field over winter months during which DO in the ponds can drop as a result of ice cover. During this period the chiller used for the low-temperature experiments needed to be repaired. One low DO experiment at room temperature is currently being repeated. Low-temperature experiments will re-start in the coming weeks now that the chiller is repaired.

Pond data analysis is ongoing. Results analyzed so far indicate that stratification was most prevalent during winter and spring and that nearly all ponds in each system were simultaneously stratified or mixed. In both

summer and fall, five out of six pond systems were mixed. One system during spring experienced differential stratification/mixing among ponds, and another system did not “perform” like the others with respect to stratification during both winter and spring. The latter system was also the only ice-covered system during winter. We suspect that these discrepancies are related to date of sample collection and system-specific parameters, such as location and operating retention time. Further investigation is underway to determine what mechanisms may cause these observations and how these factors are related to nitrification activity and functional potential.

**Amendment Request (09/21/2022):**

This formal amendment request is to seek approval for a rebudget of \$2,500 from “Personnel” to “Equipment/Tools/Supplies”. This movement of funds will not impact the project outcomes or timeline, decreasing the summer funding of Novak and LaPara slightly. The funds are needed for additional materials to maintain the pH and dissolved oxygen control systems given the longer operating period of the project and to repair the chiller used in the project.

**Amendment approved by LCCMR 10/11/22**

**Seventh Update March 1, 2023**

Low temperature, low DO experiments have resumed with no additional delays. Experiments are proceeding as scheduled and are scheduled to be completed by May 31, 2023. Data quality has been validated for experimental control systems, and data processing and treatment methods have been selected as appropriate. Chemical analyses, including un-analyzed samples from previous experiments, have been proceeding simultaneously with ongoing experimentation. Stalled DNA extractions have resumed in preparation for beginning qPCR analyses in the coming weeks.

Analyses of pond field study data are proceeding. During this period, the focus was on finishing the nitrification experiments and repairing instruments critical to timely analysis of experimental samples. A plan for examining pond data using nitrogen mass balances has been developed.

Additionally, the recruitment and hiring of an undergraduate laboratory assistant took place over this period.

**Amendment Request (03/01/2023):**

This formal amendment request is to seek approval for a rebudget of \$7,000 from “Personnel” to “Equipment/Tools/Supplies”. This movement of funds will not impact the project outcomes or timeline, decreasing the funding that had been reallocated from the Subcontract slightly. The funds are needed for additional sampling materials, given the longer operating period of the project.

**Amendment Approved by LCCMR 3/15/23**

**Overall Project Outcomes and Results**

Inadequately treated wastewater discharges can contain high concentrations of nitrogen, including ammonia and nitrate, which contribute to negative human and environmental effects. Little research has been conducted on nitrogen removal/degradation in rural wastewater treatment ponds, which could be helpful to improve rural water quality. Although both ammonia and nitrate are problematic, ammonia is more toxic than nitrate and must biodegrade first for total nitrogen to be biodegraded. In this study, we studied how low oxygen levels and low temperature, both of which can occur in ice-covered wastewater treatment ponds in Minnesota during winter and spring, impacted ammonia biodegradation. Well-controlled laboratory experiments were conducted and samples from real wastewater treatment ponds were analyzed.

Our research showed that both low temperature (10-6°C) and oxygen (0.5-1 mg/L) resulted in little-to-no ammonia biodegradation. As mentioned, unless the ammonia is biodegraded, total nitrogen biodegradation cannot occur. Low temperature critically limited ammonia biodegradation, while the negative effect of low oxygen concentration was less important, increasing the time before ammonia started to biodegrade, but not changing how fast it degraded. Unfortunately, temperature cannot be controlled in these large treatment ponds during the cold winter months. Oxygen, however, can be manually added. We observed that if the oxygen concentration was 3 mg/L or higher, ammonia could be biodegraded at a temperature of 10°C. Field results were consistent with laboratory results. Results showed that low ammonia concentrations could be achieved with low temperature and low oxygen concentrations in most of the ponds. Below ≈10°C, ammonia concentrations increased with decreasing oxygen. Above 20°C, low ammonia was observed regardless of the oxygen concentration. These results suggest that interventions, such as sparging with air, can improve ammonia biodegradation in winter/early spring. Nevertheless, at temperatures <10°C, oxygen addition may not improve ammonia biodegradation, resulting in ammonia volatilization instead, contributing to poor air quality.

### III. PROJECT ACTIVITIES AND OUTCOMES:

**ACTIVITY 1 Title:** Laboratory assessment of how nitrogen cycles during winter/spring months

**Description:**

Model pond reactors (5-liter) will be set up in the laboratory, containing sediment and/or water obtained from two wastewater treatment ponds: a well performing and poorly performing pond. Reactors will be operated to model pond conditions when the temperature is low and ice cover decreases the amount of oxygen that can reach the deeper regions of the pond. It is during these months that the nitrogenous compounds that can cause surface water and groundwater damage are thought to build up. Nitrogen removal and the types and quantities of bacteria that perform various nitrogen cycling steps will be measured to understand how cold temperatures and low oxygen impact these processes.

**ACTIVITY 1 ENRTF BUDGET:**

**ENRTF Budget:                    \$184,109**

<b>Outcome</b>	<b>Completion Date</b>
<i>1. Experiments performed at a variety of oxygen levels at low (2°C) temperature</i>	<i>7/31/20</i>
<i>2. Experiments repeated at 7°C</i>	<i>1/31/21</i>
<i>3. Experiments repeated at 12°C</i>	<i>6/31/21</i>

**First Update March 1, 2020**

Methods have been developed to quantify organic carbon, ammonia, nitrate, nitrite, and specific bacteria in our system: total bacteria, anammox bacteria, denitrifying bacteria, aerobic ammonia oxidizing bacteria, and aerobic nitrite oxidizing bacteria. Methods have been shown to be reproducible and can be applied with confidence to our system.

Six bench-scale wastewater pond reactors have been constructed with precise oxygen and pH feedback control. The reactors can also be maintained at a given temperature. This enables three duplicate set-points (oxygen or temperature) to be tested simultaneously. Oxygen control is sophisticated and precise and allows for control within 20% of the target concentration and is capable of maintaining dissolved oxygen target concentrations between 0.5 and 2.0 mg/L. Reactors were seeded with pond water collected with the assistance of MRWA and initial trouble-shooting experiments have been performed to fine-tune the control system and determine the general time-frame of carbon and ammonia degradation.

**Second Update September 1, 2020**



Despite delays resulting from laboratories closing at the beginning of the COVID-19 pandemic, methods have been developed for bench-scale wastewater pond reactor and control system maintenance as well as sample collection.

Eighteen replicate experiments (as described in the First Update) at room temperature with high dissolved oxygen concentrations have been performed as experimental controls. Results indicate that the data are reproducible and confirm that the oxidation of ammonia to nitrite and nitrate is occurring readily by biological nitrification, as expected. Further optimization of the dissolved oxygen control system to maintain low-dissolved-oxygen setpoints is in progress.

A lab bench with storage was constructed in a walk-in refrigerator capable of maintaining temperatures as low as 3.3°C. The six bench-scale wastewater pond reactors and the associated control equipment will be moved into the walk-in-refrigerator for experiments requiring low temperature conditions. Experiments with varying dissolved oxygen and temperature conditions will begin shortly.

### **Third Update March 1, 2021**

Optimization of the dissolved oxygen (DO) control system has indicated that each experiment must be run at a constant DO set-point for all six reactors, rather than with three sets of duplicates as initially planned. Data indicate that experimental results are not statistically significantly different ( $p > 0.05$ ) among different experiments that are seeded with the same pond water and sediment and operated under the same conditions. Thus, the statistical integrity of the experiments is not compromised by adjusting the replication structure.

Following DO control system optimization, experiments with varying DO concentrations have been performed at room temperature. Initial results suggest that, as expected, nitrification proceeds slowly under low DO conditions, and does not begin until the carbonaceous oxygen demand has been removed by heterotrophic bacteria. Nevertheless, as mentioned above, even at the very low DO concentration of 0.5 mg/L, nitrification does occur. Experiments with additional DO concentration setpoints performed at room temperature will continue and experiments performed at low temperatures will begin shortly.

The walk-in-refrigerator described in the Second Update is in fact only able to maintain a temperature of approximately 10°C within the reactors while stirred and biologically active; this is in contrast to the 3.3°C anticipated. While reactor temperatures of 10°C will be useful as a midpoint in identifying the relationship between nitrification rates and temperature, we must achieve lower reactor temperatures to adequately represent full-scale pond operation during winter months. To accomplish this, a refrigerated shaking water bath was ordered. The bath can accommodate three reactors, and will be set up in the walk-in-refrigerator such that three reactors are operating at 4°C while the other three reactors operate at 10°C. As mentioned above, all six reactors will have the same DO set-point. This experimental set-up will allow multiple experiments to be performed at different DO set-points at both 10°C and 4°C.

### **Fourth Update September 1, 2021**

The chiller system purchased in winter 2021 malfunctioned upon delivery, so it was sent away for repairs. Following repairs, the chiller system was calibrated for use in the low-temperature experiments. Challenges arose with organic matter leaching from the tubing used in the experiments. As pH adjustments were made to the reactors to maintain a constant pH, organic matter leached from the tubing and resulted in the presence of erratic and sometimes high concentrations of organic matter in the reactors. This interfered with the analysis of chemical oxygen demand and may have also interfered with nitrification. This issue took some time to troubleshoot but has now been resolved. Nevertheless, national supply-chain issues have affected our ability to quickly obtain new tubing, which has slowed down the replication of our experiments. We anticipate that this will be resolved in the coming project period and the low-temperature experiments will proceed as planned.

### **Fifth Update March 1, 2022**

Statistical testing has confirmed that the DO concentration for each setpoint is controlled with an appropriate level of precision at the desired concentrations. Appropriate statistical methods for data analysis have been selected based on characteristics of the data and the literature. Multiple linear regression will be used for variable selection to identify the factors that correspond most closely with nitrification. Writing the analytical and graphical code in R has begun, although final hypothesis testing cannot be performed until all experiments have been completed. The supply chain issues that were slowing down the experimental progress appear to be resolved and experiments are in progress. Low-temperature experiments will begin in the coming weeks.

#### **Update as of June 30, 2022**

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

#### **Sixth Update September 1, 2022**

The chiller used for the low-temperature experiments broke and needed to be repaired. One low DO experiment is currently being repeated. Low-temperature experiments will begin again in the coming weeks now that the chiller is repaired. The focus of the project for the past few months has been on analyzing the pond data but is now shifting back to the laboratory experiments.

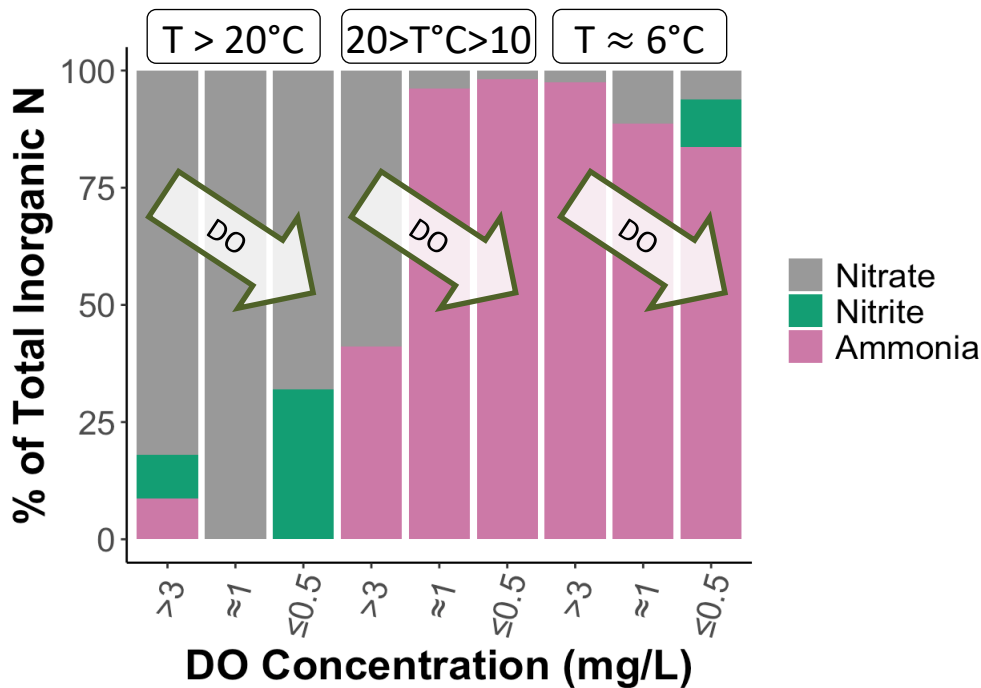
#### **Seventh Update March 1, 2023**

Nitrification experiments at low temperatures and low DO concentrations have resumed. The experiments' equipment and instruments have required occasional troubleshooting, but these issues have been resolved. For example, a method for recalibrating DO probes onsite was developed. With only two month-long experiments remaining, experimentation will be completed by the end of May, 2023. Data quality has been verified using R statistical software with DO, pH, and temperature measurements within 10%, 1%, and 30% of their respective setpoints, on average. A backlog of nitrite and nitrate samples were analyzed following extensive repair and recalibration of the ion chromatograph. These data are now being used for mass balance-based examination of the fate of inorganic nitrogen in the reactors. Outstanding chemical analyses for previous experiments have been completed, and DNA extractions are in progress for qPCR to begin in the coming weeks. Additionally, data treatment mechanisms for missing, unresolvable, and below-detection-limit data have been selected in accordance with literature precedent. The analytical and graphical code is currently being written in R.

#### **Final Report Summary**

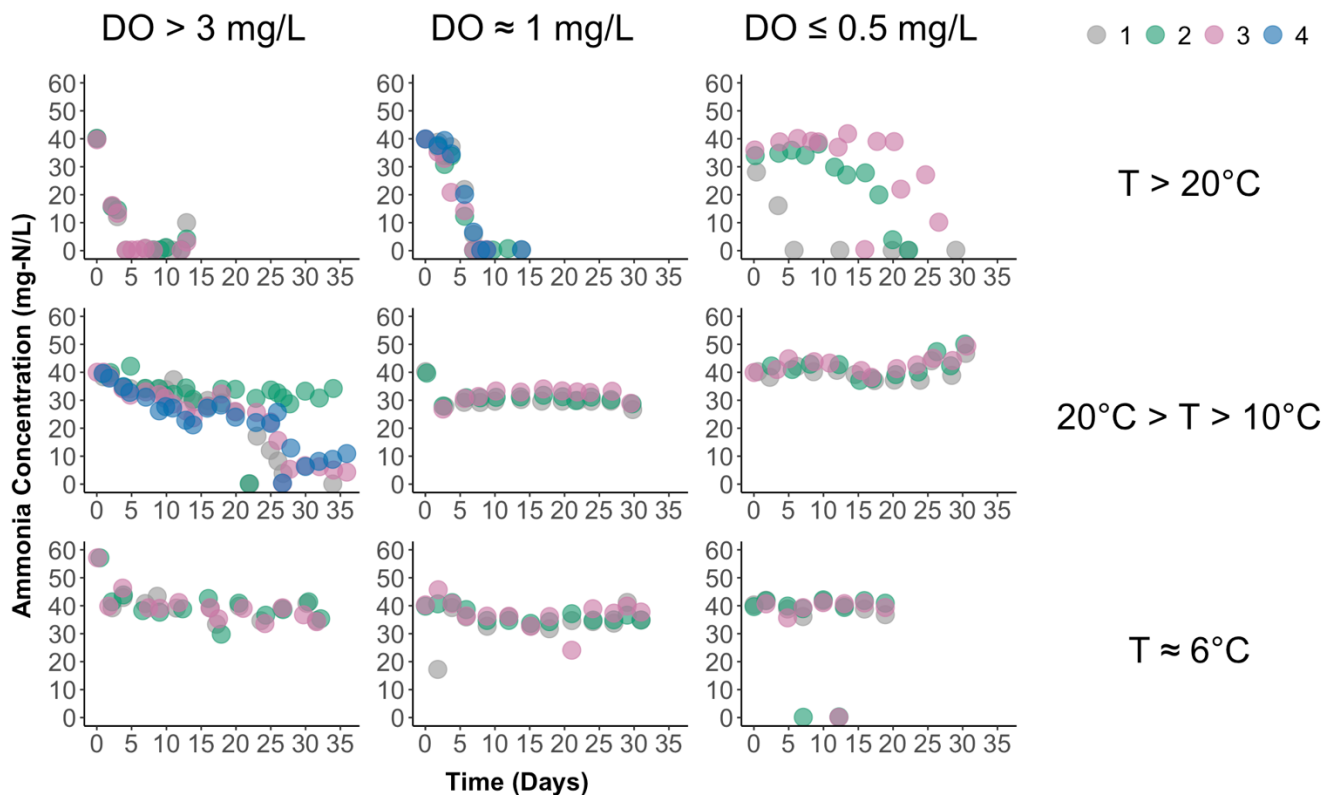
All remaining nitrification experiments and subsequent analyses were successfully completed during the final project period. Experimental outcomes confirmed that complete nitrification is possible even at extremely low DO concentrations (0.5 mg/L) and moderately low temperatures (10°C).

All experiments that demonstrated nitrification were operated at temperatures  $\geq 10^\circ\text{C}$ . Of these experiments, those at lower DO concentrations nitrified to a lesser extent. Less ammonia was converted to either nitrite (the first step of nitrification) or nitrate (the second step of nitrification) after one month, as shown in Figure 1. There was a dramatic shift in the extent of nitrification between experiments operated at temperatures  $>20^\circ\text{C}$  and experiments operated between 10 and  $20^\circ\text{C}$ . Experiments operated at temperatures  $<10^\circ\text{C}$  and those operated between 10 and  $20^\circ\text{C}$  with  $\leq 1$  mg/L DO did not remove ammonia. Between 10 and  $20^\circ\text{C}$ , however, ammonia was degraded at DO  $>3$  mg/L. Thus, it is likely that at this temperature range, "winter/spring" limitations could be partly ameliorated with oxygen addition.



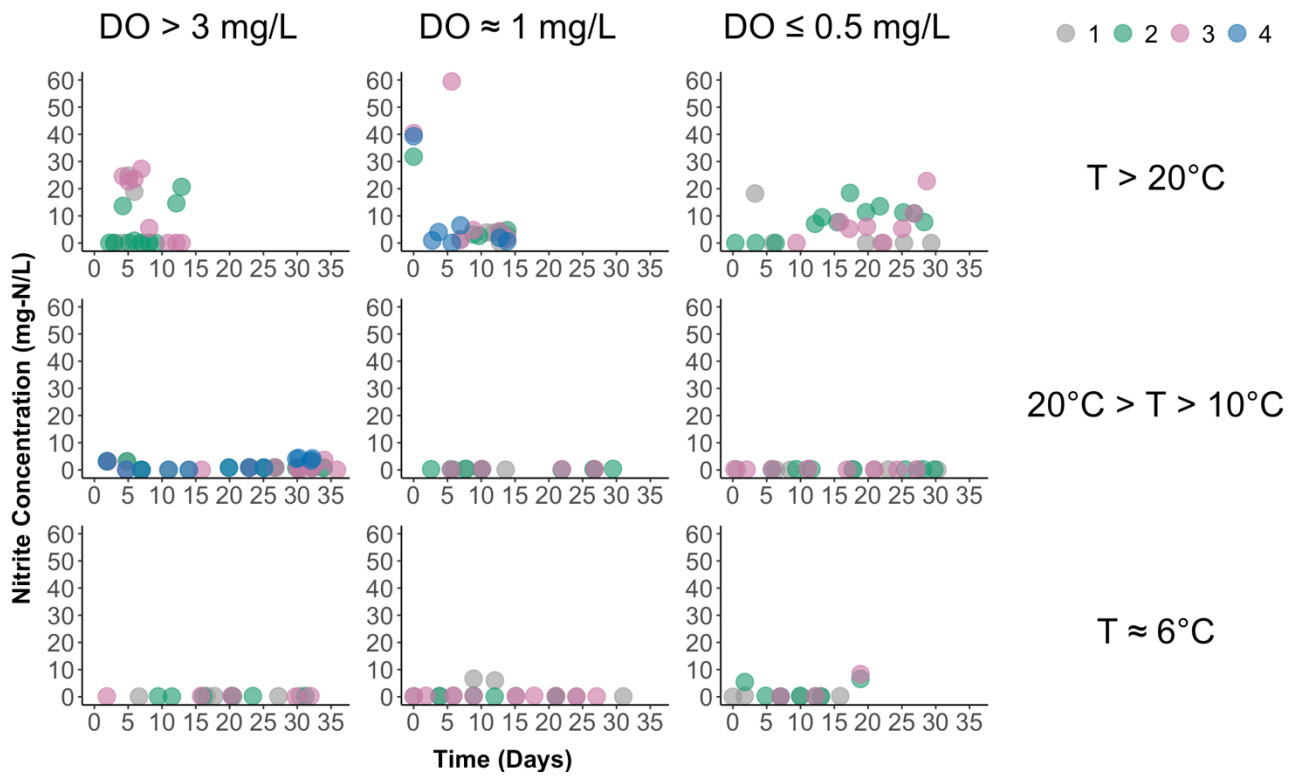
**Figure 1.** Inorganic nitrogen speciation at the conclusion of each experiment as a fraction of total inorganic nitrogen. Experiments are arranged in groups of three by temperature (top) with the approximate DO concentration for each experiment on the x-axis. Translucent white arrows qualitatively indicate the range of DO concentrations for the 3 experiments operated at each temperature setpoint.

From these experiments, we also gained insight into the different effects DO and temperature have on nitrification. Low DO concentration significantly increased the “lag time” before ammonia removal began, as well as its variability, as shown by the three experiments operated with temperatures >20°C (Figure 2). Nevertheless, once ammonia degradation began in these experiments, no difference in the rate of ammonia removal per quantity of ammonia oxidizing bacteria (AOB) was observed. Although low temperatures significantly decreased the rate of ammonia removal, as shown by the three experiments operated at DO >3 mg/L (Figure 2), this effect is less significant with higher AOB abundance. Therefore, improving AOB retention could reduce the time required for complete nitrification at low DO concentrations.

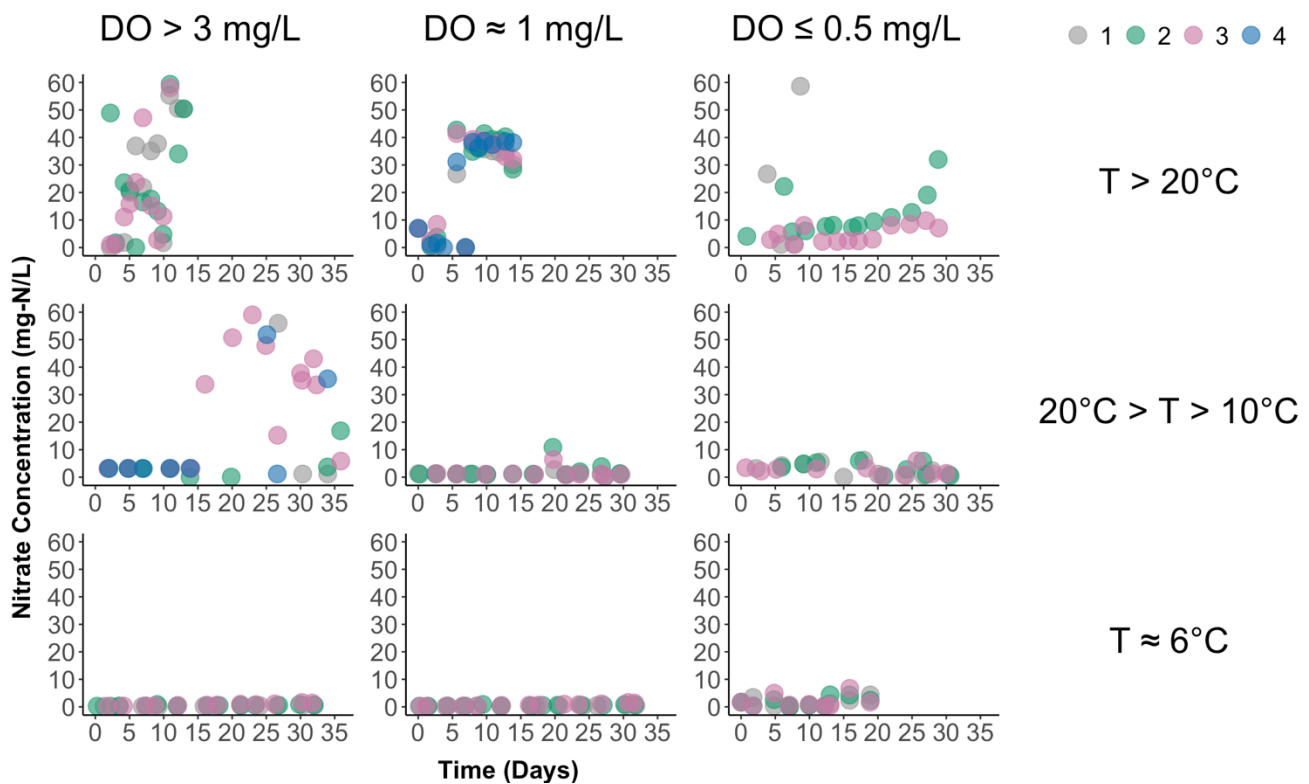


**Figure 2.** Ammonia concentration for each reactor (1 – 4) throughout each experiment is shown as a matrix of experimental conditions.

For experiments that demonstrated ammonia removal, nitrification was identified based on the accumulation of nitrite and/or nitrate (Figures 3, 4). Complete nitrification occurred in the experiment with  $T > 20^\circ\text{C}$  and  $\text{DO} > 3$  mg/L, followed by ammonia production from decaying organic matter. Partial nitrification was observed in the experiment at  $T > 20^\circ\text{C}$  and  $\text{DO} \leq 0.5$  mg/L,  $T > 20^\circ\text{C}$  with  $\text{DO} \approx 1$  mg/L, and  $20^\circ\text{C} > T > 10^\circ\text{C}$  with  $\text{DO} \leq 0.5$  mg/L.

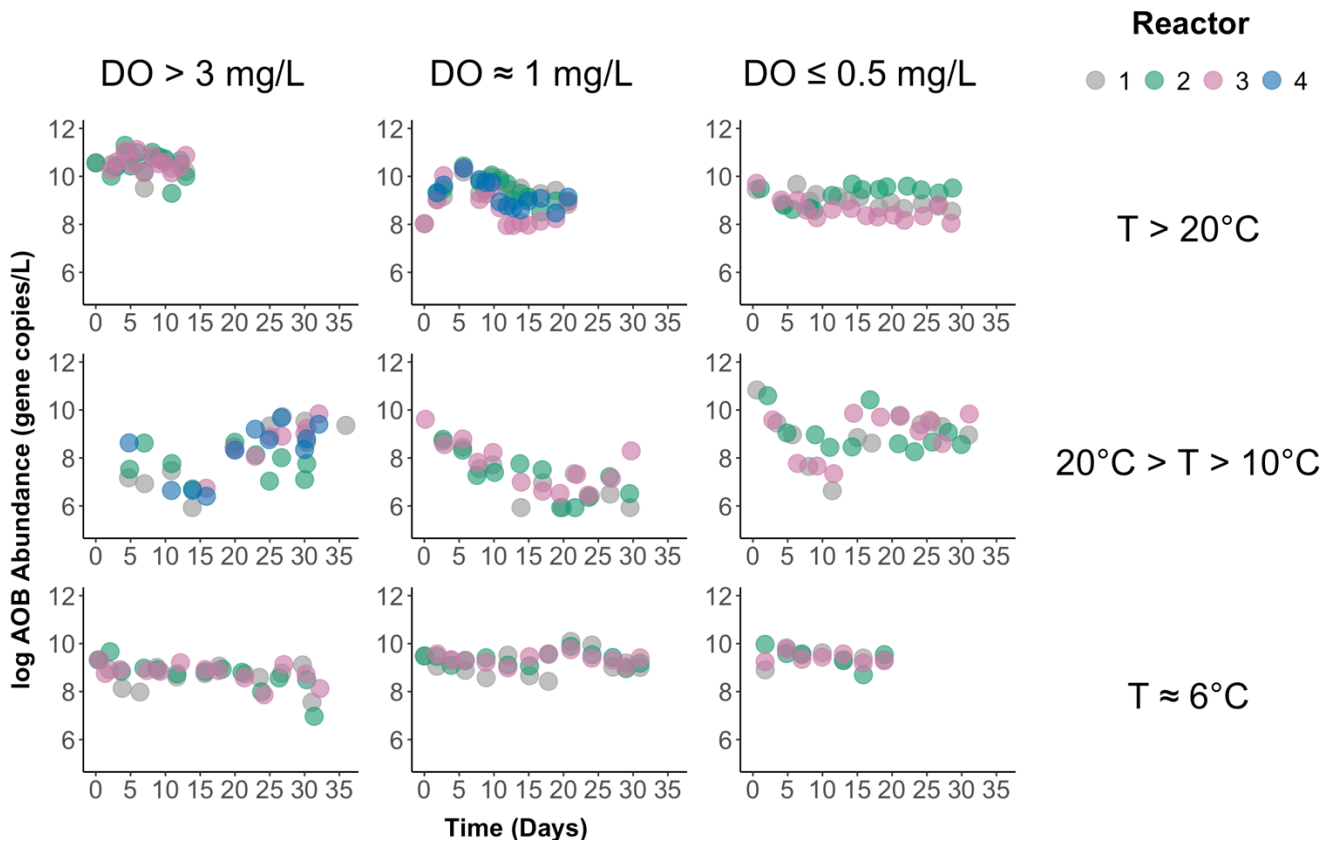


**Figure 3.** Nitrite concentration for each reactor (1 – 4) throughout each experiment is shown as a matrix of experimental conditions.



**Figure 4.** Nitrate concentration for each reactor (1 – 4) throughout each experiment is shown as a matrix of experimental conditions.

Interestingly, the abundance of AOB was relatively stable regardless of temperature or DO concentration (Figure 5). This suggests that there is biological potential for nitrification in ponds even under winter conditions. The constant presence of AOB suggests that nitrification is likely to proceed with sufficient hydraulic residence time and oxygen. Although pond systems are typically operated with extended detention times during the winter months to accommodate diminished performance, systems that consistently under-perform may need to shift to even longer hydraulic residence times and/or add oxygen. Such a change in operational strategy would likely necessitate expanding storage capacity, which may be cost-prohibitive and still may not be adequate in cases of both low DO and temperature <10°C.



**Figure 5.** Absolute abundance of *amoA* genes for *N. oligotropha*-like bacteria is shown for each reactor (1 – 4) throughout each experiment as a matrix of experimental conditions.

**ACTIVITY 2 Title:** Full-scale pond assessment of nitrogen cycling during winter/spring

**Description:**

We will work with our project partner, the Minnesota Rural Water Association, to obtain samples from the same two ponds over time. Water-column samples will be taken for analysis of nitrogen species; temperature and oxygen concentrations will be measured by Minnesota Rural Water Association. Samples will be taken over time after wastewater loading during the winter and spring months when temperatures are expected to range from 2°C to 12°C and the ponds range from free-surface to ice-covered. Several samples will also be taken during the summer and fall months to serve as “warm water controls”. Samples of the water column and pond sediment will be taken to measure the nitrogen-cycling bacteria present in the samples.

**ACTIVITY 2 ENRTF BUDGET:****ENRTF Budget:****\$100,000**

<b>Outcome</b>	<b>Completion Date</b>
<i>1. Sampling the oxidation ponds during winter and spring</i>	<i>6/30/21</i>
<i>2. Analysis of the bacteria present in pond samples</i>	<i>6/30/21</i>

**First Update March 1, 2020**

The graduate student working on the project has been coordinating with our project partners to plan for sampling in the upcoming summer and next cold-water season. She has also been in contact with the Minnesota Pollution Control Agency to coordinate purchase and use of sondes that will enable sampling of multiple water chemistry parameters within the ponds during the winter and spring of 2020-2021.

**Second Update September 1, 2020**

The graduate student working on the project has leveraged our partnership with the Minnesota Rural Water Association to develop additional partnerships with the Minnesota Pollution Control Agency, Saint Cloud Wastewater Treatment Plant, and Minnesota Technical Assistance Program to coordinate a field study of six wastewater treatment pond systems in Minnesota: Sandstone, Balaton, Middle River, Winthrop, Rushmore, and Jeffers. MRWA project partners collect samples and sonde measurements for each pond seasonally at the influent, effluent, transfer points between ponds, and pond centers including both water column and sediment samples. Water column samples are taken at seven depths throughout each pond's cross-section. Fall sampling will be complete on November 17. Chemical parameter analyses are conducted by the Saint Cloud Wastewater Treatment Plant and Minnesota Valley Testing Laboratory with procedures analogous or identical to the methods used in our laboratory experiments. This will facilitate comparison of field study data with the laboratory experiments of Activity 1. During sampling, the graduate student picks up and processes samples for microbial analyses from the Saint Cloud facility on a weekly basis. Analyses of microbial, sonde, and chemical parameter data for the first two sampling periods will be completed during the next project period.

**Third Update March 1, 2021**

The field study has been progressing according to plan, with the winter sampling period finished on February 18, 2021. During the sampling period, the graduate student picked up and processed samples for microbial analyses from the Saint Cloud Wastewater Treatment facility on a weekly basis. Since the last update, data for the summer and fall sampling periods have been processed and organized. Data visualization has begun, along with the development of the frameworks to be used to analyze the large quantity of data generated. Statistical analyses and graphical techniques are being implemented through the open-source software package R using RStudio. The graduate student working on the project attends bi-weekly project meetings with the collaboration team and the Minnesota Technical Assistance Program analytical team to discuss project updates and analytical strategies. Examination of trends in the pond field study data is underway, and analyses of seasonal variations in pond performance, with an emphasis on low oxygen and low temperature conditions, are expected later this year.

**Fourth Update September 1, 2021**

All the samples for the field study (summer 2020, fall 2020, winter 2021, and spring 2021) have been collected and analyzed. All the data has been checked for accuracy and reviewed with respect to units. Statistical and graphical analyses of the data are in progress. A manuscript describing these results should be written and submitted for publication in early 2022.

**Fifth Update March 1, 2022**

Efforts have been focused on exploring the possible analytical strategies for field data analysis. The data exhibit high multicollinearity and nonparametric characteristics, as expected for such a complex engineered environment. Cluster analysis will be used to identify patterns with respect to nitrogen cycling across treatment stages, pond systems, and seasons. The analytical and graphical code is currently being written in R.

### **Update as of June 30, 2022**

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

### **Sixth Update September 1, 2022**

Data analysis has been ongoing. Results indicate that location and seasonal trends in environmental factors (e.g., temperature) play a significant role in mixing status of full-scale pond systems. For example, three of four ice-free pond systems at a similar latitude followed nearly identical seasonal stratification/mixing patterns, except for one system (J) in the fall and one pond within one system (R) in the spring. System J's stratification at the time of fall sampling might be explained by the fact that it was sampled two weeks later than the other three ponds due to a snowstorm making the sampling trip too dangerous. This time lapse likely included a drop in temperature that led to stratification for the winter; this will be confirmed as part of our ongoing analyses. In fact, five of the six systems studied were stratified in winter, which is consistent with this observation. The primary pond of system R remained stratified at the time of spring sampling. This may be because R was the first system sampled in spring, so the conditions may have actually been more winter-like, and as a result, the primary pond was stratified. These results suggest that the timing of sampling is as important in the analysis as the "season" of sampling.

Despite uncertain "seasonality" at the time of sampling, it is likely that these systems follow a predictable pattern of mixing and stratification throughout the year and that all ponds within each system experience similar conditions. Only one system experienced differential stratification/mixing among its ponds during one season. Five of six pond systems were well-mixed in both summer and fall, four of five systems were mixed in spring (one omitted due to lack of data), while five of six systems were stratified during winter. Only 1 pond system performed differently from the others in more than one season. This pond was located at a unique geographical position in the forested region of northeast Minnesota, supporting the idea that system location influences pond conditions and therefore performance. Further investigation is underway to determine what trends in nitrogen speciation are associated with these environmental factors.

### **Seventh Update March 1, 2023**

The primary focus of the past few months has been on setting and maintaining an aggressive schedule for nitrification experiments (Activity 1) but is shifting toward a more even division between Activities 1 and 2. With this in mind, an analytical approach has been devised based on nitrogen mass balances over differential liquid volumes in each pond to analyze the data gathered by our project partners. Relevant assumptions are being tested, such as those regarding phase-partitioning and complexation.

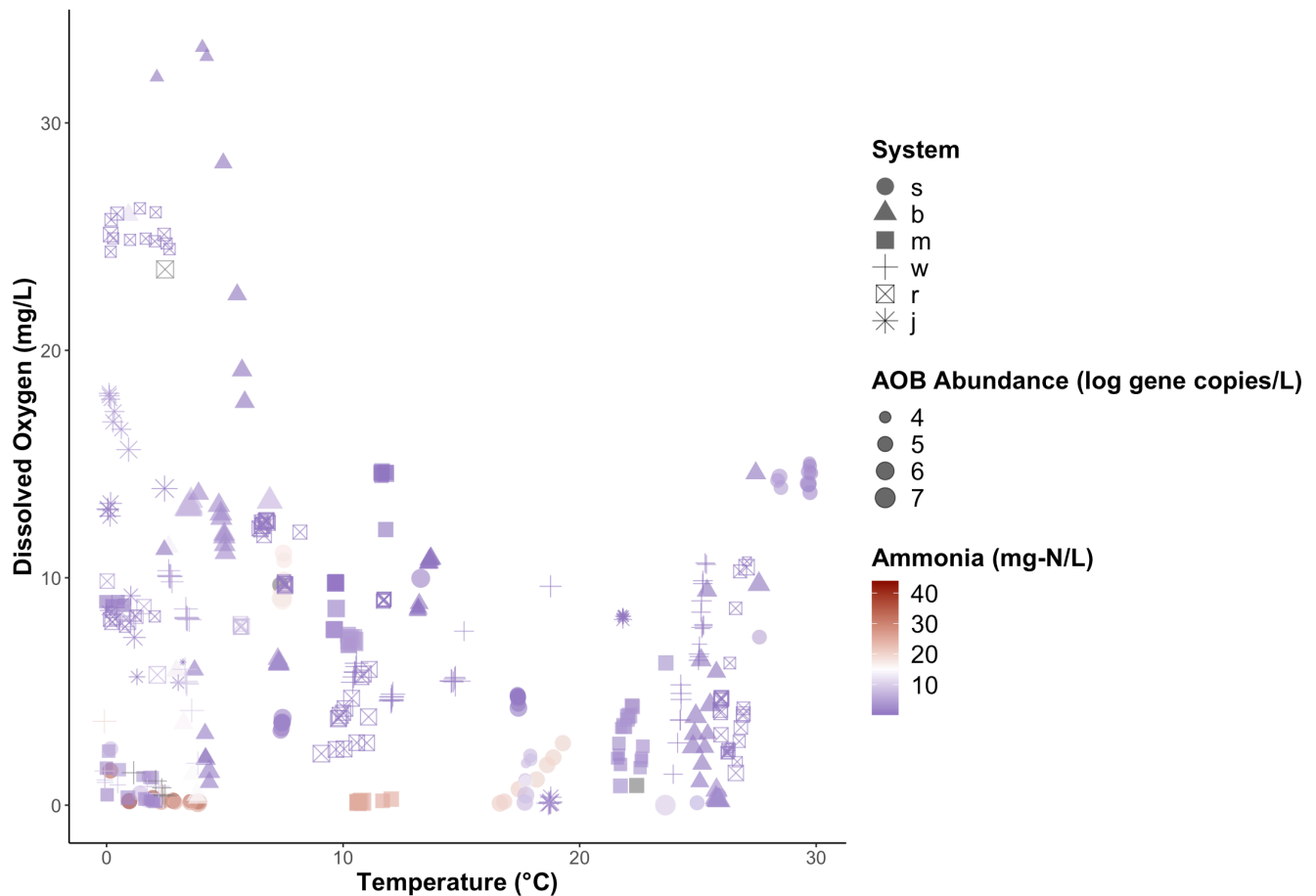
### **Final Report Summary**

Our results confirm the complexity of full-scale facultative pond systems, with mixing status and treatment stage emerging as factors associated with inconsistent performance at low temperatures and DO concentrations. Ponds within a single system tended to behave similarly with respect to mixing and stratification, and there was an associated seasonal pattern among systems.

Dissolved oxygen concentration generally followed the expected negative association with temperature (Figure 6), with generally higher DO concentrations with low temperature, when oxygen is more soluble in water and microbial activity is lower. DO can be supersaturated at extremely low winter temperatures and saturated even during fall and spring. During summer, oxygen saturation is possible for some systems, likely a result of algal activity. DO availability also follows the expected pattern with respect to extent of treatment: Primary ponds have lower DO than secondary ponds. This is expected as there is more organic matter to "feed" the bacteria in the primary ponds, resulting in the bacteria "breathing" more oxygen and decreasing DO concentrations accordingly.



Low ammonia concentrations were achieved under low temperature and low DO conditions in most systems. Nevertheless, Activity 2 results are generally consistent with Activity 1 results in that below  $\approx 10^{\circ}\text{C}$ , ammonia concentration had a negative association with DO. Above  $20^{\circ}\text{C}$ , low ammonia concentrations were found regardless of DO concentration, affirming that low temperature more significantly affects ammonia removal than low DO concentration.



**Figure 6.** AOB relative abundance (size) and ammonia concentration (color) are shown in relation to DO concentration and temperature for each system.

At temperatures below  $20^{\circ}\text{C}$ , systems with a high relative abundance of AOB had lower ammonia concentrations, while above  $20^{\circ}\text{C}$ , ammonia concentrations were low, regardless of AOB relative abundance. It was possible to have low ammonia concentrations at low temperatures despite low AOB abundance (small purple symbols at the low temperature range), when DO was plentiful. Interestingly, this relationship was not consistent for all systems: At extremely low temperature and DO, only System S had high ammonia concentrations. System S was likewise the only system with elevated ammonia concentration at approximately  $7^{\circ}\text{C}$  with moderate DO, although both Systems S and M had elevated ammonia concentrations between  $10^{\circ}\text{C}$  and  $20^{\circ}\text{C}$  with low DO concentrations.

The relative abundance of AOB seems to matter a lot at very low DO concentrations. DO availability and AOB relative abundance seem to become increasingly important at lower temperatures. In fact, winter conditions showed a notably low AOB relative abundance regardless of DO availability. Spring-like moderately high DO concentrations with temperatures between  $5^{\circ}\text{C}$  and  $15^{\circ}\text{C}$  had the highest AOB relative abundance, suggesting AOB growth was favored under those conditions. This would make sense, as the water warms up enough for AOB growth, but it is still cold enough that DO concentrations would be high. This is also in line with our COD data,

which showed low COD concentrations in spring (because the ponds have been sitting all winter long). Under these conditions (low COD), slow-growing AOB should be able to out-compete other (organic carbon-utilizing) bacteria for the available DO. In winter, spring, and summer, S and M had high ammonia concentrations and low relative abundance of AOB. Because of the predominance of stratification during winter, it is likely the potential for nitrification is mass-transfer-limited. System S was mixed in winter and stratified in spring, which was opposite to the pattern observed in the other systems, and this seems important to ammonia removal.

**ACTIVITY 3 Title:** Evaluation of simple methods (mixing and aeration) at the laboratory scale to stimulate total nitrogen removal during periods of low oxygen concentration and low temperature.

**Description:**

Laboratory experiments 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. The effect of such simple stimulation methods on the transformation patterns of nitrogen species under conditions least favorable to nitrogen removal in the winter and spring will be determined.

**ACTIVITY 3 ENRTF BUDGET:**

**ENRTF Budget: \$40,891**

<b>Outcome</b>	<b>Completion Date</b>
1. <i>Evaluation of effect of ORC addition and mixing on nitrogen removal in the laboratory</i>	3/30/22
2. <i>Discussion of results with project partner to determine strategies for field assessment in the future</i>	4/30/22

**First Update March 1, 2020**

No work has begun on Activity 3.

**Second Update September 1, 2020**

No work has begun on Activity 3.

**Third Update March 1, 2021**

No work has begun on Activity 3.

**Fourth Update September 1, 2021**

No work has begun on Activity 3.

**Fifth Update March 1, 2022**

No work has begun on activity 3. Experimental design will begin during the next project period.

**Update as of June 30, 2022**

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

**Sixth Update September 1, 2022**

Results from the experimental work indicate that even at dissolved oxygen concentrations as low as 0.5 mg/L nitrification can proceed, albeit slowly. As a result, the quantity of ORC needed for a given pond should be easily calculated. Nevertheless, ORC does release phosphorus with O<sub>2</sub> and this is likely to negatively impact the effluent quality from wastewater treatment ponds. As a result, other methods (sparging) are likely to be more practical. As the pond data from winter continues to be analyzed (Activity 2) we expect to have a better understanding of the oxygen addition needs and timing of oxygen addition for better ammonium removal over the cold winter months.

### **Seventh Update March 1, 2023**

Nitrification experiment results suggest that temperature may have a more significant effect on nitrification rate than hypothesized relative to the effect of DO availability. It is suspected that the abundance of biomass and, specifically, ammonia- and nitrite- oxidizing microorganisms may modulate these effects. Ongoing microbial analyses of both experimental and field study data should allow us to decipher likely nitrogen transport and transformation mechanisms under these varied conditions and the possible effects nitrification-inducing interventions, including ORC, may have on these systems.

### **Final Report Summary**

Our research suggests that the combination of low temperature and low oxygen is responsible for poor nitrification during winter. Oxygen amendment does appear to be able to partially address this problem, if the temperature is  $>10^{\circ}\text{C}$ . While there is no 'silver bullet' in stimulating winter nitrification, oxygen amendment via sparging, in combination with a mixing regime, may render pond conditions more favorable to nitrification during winter. Future research should investigate the relative effects of water column paddle mixing, sediment agitation via mixing, and implementing baffles or other flow controls that affect mass transfer.

Activity 1 results confirm that temperature has a more significant effect on rate and extent of nitrification than hypothesized relative to the effect of DO availability. A substantial community of ammonia oxidizing microorganisms is present even under low temperature and low oxygen conditions and they can be active as low as  $10^{\circ}\text{C}$  if adequate oxygen is present. Activity 2 results indicate that stratification does not limit ammonia removal under winter DO and temperature conditions. Interestingly, Minnesota tends to see a mid-winter thaw. As climate change accelerates such weather patterns, it may be that a well-timed oxygen amendment in the winter, corresponding to an increase in temperature, may be able to increase nitrification over the winter months.

Project progress was hindered by the interruption of the Covid-19 pandemic and the resulting supply chain disturbances, as well as ongoing equipment malfunctions that required shifts in experimental methods. As a result, no laboratory-scale interventions were tested to determine their effects on nitrogen transformation. Nevertheless, the high quality and consistent results achieved in Activity 1 and 2 are extremely helpful in showing where and how interventions should be used.

## **IV. DISSEMINATION:**

### **Description:**

The target audience for results from this research will be environmental engineers and scientists in academia, professionals in the area of wastewater treatment, city managers and other local government officials, and the Minnesota Pollution Control Agency. Results will be disseminated through scholarly publications in peer-reviewed journals such as *Environmental Science and Technology* and *Environmental Science: Water Research and Technology*. Results from the research project will also be presented at regional conferences such as the *Conference on the Environment*. MRWA will disseminate the research findings in the quarterly Today magazine and monthly TA Times newsletter as needed. MRWA will participate in joint presentations with the University of Minnesota as needed to present the research findings. MRWA will also make research material available on the MRWA website as needed or deemed appropriate.

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](#).

### **First Update March 1, 2020**

The graduate student working on the project was named an ARCS scholar and therefore presented her research plan at the ARCS scholar award event in October, 2019. No other dissemination of this work has taken place.

#### **Second Update September 1, 2020**

The graduate student working on the project was named an ARCS scholar for a second year and therefore presented her research plan at the ARCS scholar award event in October, 2020. No other dissemination of this work has taken place.

#### **Third Update March 1, 2021**

The graduate student working on the project has continued networking and sharing research updates as an ARCS scholar. She is also preparing abstracts and presentation materials for upcoming national conferences, including the biannual conference of the Association of Environmental Engineering and Science Professors and the Gordon Research Conference on Applied and Environmental Microbiology. It is currently unclear whether these conferences will proceed as a result of the COVID-19 global pandemic.

#### **Fourth Update September 1, 2021**

The graduate student working on the project has continued networking and sharing research updates as an ARCS scholar and within the research group and academic unit. As a result of the COVID-19 global pandemic, conferences did not proceed as expected. The graduate student working on the project anticipates publication of field study findings in early 2022 and anticipates presenting at one to three conferences during 2022, regardless of modality (in person or virtual).

#### **Fifth Update March 1, 2022**

The graduate student working on the project has continued networking and sharing research updates as an ARCS scholar, within the research group and academic unit, and among local colleagues. The ongoing pandemic continues to limit opportunities for dissemination. The graduate student will begin developing a manuscript for publication describing the field study results over the next project period. The graduate student working on the project has applied to present her research at several conferences this coming summer.

#### **Update as of June 30, 2022**

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

#### **Sixth Update September 1, 2022**

The graduate student working on this project was accepted to present at two conferences in Summer 2022 and chose to present a poster at the AEESP conference, declining the other opportunity to focus on research. Additionally, the graduate student gave a technical presentation at the Minnesota Wastewater Operators Association Conference upon invitation. A manuscript for publication describing field study results is in progress.

#### **Seventh Update March 1, 2023**

The graduate student working on this project presented her results at the Air & Waste Management Association, Upper Midwest Section, Conference on the Environment in November 2022 and has been accepted to present her results at the Central States Water Environment Association 96<sup>th</sup> Annual Meeting in May 2023. She has applied for an additional podium presentation at the Association of Environmental Engineering and Science Professors in June 2023. A manuscript for publication describing the results of nitrification experiments (Activity 1) is in progress.

#### **Final Report Summary**

Results from this research have been presented at local, regional, and national conferences via posters and oral presentations. Notable dissemination activities include presentations at the Association of Environmental Engineering and Science Professors (poster and podium), the Air & Waste Management Association, Upper Midwest Section, Conference on the Environment, the Central States Water Environment Association Annual Meeting, and an invited technical workshop given at the Minnesota Wastewater Operators Association Conference. Three papers are expected to be submitted on this research and are currently in progress. They will be submitted to LCCMR when accepted for publication.

**V. ADDITIONAL BUDGET INFORMATION:**

**A. Personnel and Capital Expenditures**

**Explanation of Capital Expenditures Greater Than \$5,000:** N/A

**Explanation of Use of Classified Staff:** N/A

**Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:**

Enter Total Estimated Personnel Hours for entire duration of project: 6,480	Divide total personnel hours by 2,080 hours in 1 yr = TOTAL FTE: 1.04 FTE/yr
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**Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation:**

Enter Total Estimated Contract Personnel Hours for entire duration of project: N/A	Divide total contract hours by 2,080 hours in 1 yr = TOTAL FTE: N/A
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**VI. PROJECT PARTNERS:**

**A. Partners outside of project manager’s organization receiving ENRTF funding**

Name	Title	Affiliation	Role
Minnesota Rural Water Association			Responsible for sampling in activity 2 and assisting with dissemination activities

**B. Partners outside of project manager’s organization NOT receiving ENRTF funding**

None

**VII. LONG-TERM- IMPLEMENTATION AND FUNDING:**

The proposed work fits into a larger research agenda at UMN on the development and evaluation of treatment technologies for water and wastewater. The proposed research expands the focus to outstate and rural infrastructure support, coordinating with several other proposals. **This research will also be coordinated with the Minnesota Pollution Control Agency’s (MPCA’s) work on the optimization of rural wastewater systems. The MPCA does not have the capacity to perform longer-term and well controlled experiments and this research will complement their work well.**

**VIII. REPORTING REQUIREMENTS:**

- Project status update reports will be submitted March 1 and September 1 each year of the project
- A final report and associated products will be submitted between June 30 and August 15, 2023

**IX. SEE ADDITIONAL WORK PLAN COMPONENTS:**

**A. Budget Spreadsheet**

**B. Visual Component or Map**

**C. Parcel List Spreadsheet: N/A**

**D. Acquisition, Easements, and Restoration Requirements: N/A**

**E. Research Addendum**

**Attachment A:**

**Environment and Natural Resources Trust Fund**

**M.L. 2019 Project Budget -Final**

**Legal Citation:** M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04e as extended by M.L. 2022, Chp. 94, Sec. 2, Subd. 19 (c.1) [to June 30, 2023]

**Project Manager:** Paige J. Novak

**Project Title:** Improving Nitrogen Removal in Greater Minnesota Wastewater Treatment Ponds

**Organization:** University of Minnesota

**Project Budget:** \$325,000

**Project Length and Completion Date:** 4 years, June 30, 2023

**Today's Date:** 08/10/23



<b>ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET</b>	<b>Budget 3/15/23</b>	<b>Amount Spent</b>	<b>Balance</b>
<b>BUDGET ITEM</b>			
<b>Personnel (Wages and Benefits)</b>	\$ 170,067	\$ 169,231	\$ 836
Novak (PI, 1.3% time per year for three years, salary 75% of cost, fringe benefits 25% of cost). Project supervision, provide guidance on the reactor construction and operation. Total estimated cost is \$11,104. LaPara (PI, 1.2% time per year for three years, salary 75% of cost, fringe benefits 25% of cost). Project supervision, guidance on microbial analysis aspects of the project. Total estimated cost is \$7,918. Graduate student (50% time per year for three years, 57% salary, 32% tuition, 11% fringe benefits) and undergraduate student researchers. Conducting laboratory experiments and analyzing samples collected from oxidation ponds by the Minnesota Rural Water Association. Total estimated cost is \$142,523.			
<b>Professional/Technical/Service Contracts</b>			
Minnesota Rural Water Association will work with us to take treatment pond samples and do chemical analysis in the field. 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).	\$ 82,000	\$ 52,011	\$ 29,989
<b>Equipment/Tools/Supplies</b>			
Laboratory supplies are budgeted including, but not limited to: kits for in-field nitrogen species analysis, chemicals for synthetic wastewater generation, materials to construct wastewater reactors, including a system for pH (\$2,566) and dissolved oxygen control (\$16,161) and the software and computer needed to operate the control system, 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 (\$12,502/yr). Additional funds budgeted for equipment repair and maintenance (\$3,200),	\$ 70,933	\$ 69,929	\$ 1,004
<b>Travel expenses in Minnesota</b>			
Mileage charges to oxidation pond sites for sample collection, and pond water, wastewater, and pond sediment collection. Mileage will be reimbursed \$0.55 per mile or current U of M compensation plan.	\$ 2,000	\$ 1,858	\$ 142
<b>Other</b>		\$ -	
<b>COLUMN TOTAL</b>	\$ 325,000	\$ 293,029	\$ 31,971

<b>OTHER FUNDS CONTRIBUTED TO THE PROJECT</b>	<b>Status (secured or pending)</b>	<b>Budget</b>	<b>Spent</b>	<b>Balance</b>
<b>Non-State:</b>		\$ -	\$ -	\$ -
<b>State:</b>		\$ -	\$ -	\$ -
<b>In kind:</b> Novak and LaPara will provide unpaid time to the project (including 2% cost share). Because the project is overhead-free, laboratory space, electricity, and other overhead costs are provided in kind. The University of Minnesota overhead rate is 54%.		\$ 175,500		

<b>PAST AND CURRENT ENRTF APPROPRIATIONS</b>	<b>Amount legally obligated but not yet spent</b>	<b>Budget</b>	<b>Spent</b>	<b>Balance</b>
<b>Current appropriation:</b>		\$ -	\$ -	\$ -
<b>Past appropriations:</b>		\$ -	\$ -	\$ -

# Outstate Wastewater: Improving Nitrogen Removal in Treatment Ponds

*With this research, nitrogen removal in Minnesota's rural wastewater treatment ponds when subject to cold weather and ice cover was better understood*

Full-scale pond sampling



Controlled laboratory experiments seeded with pond sediment and water

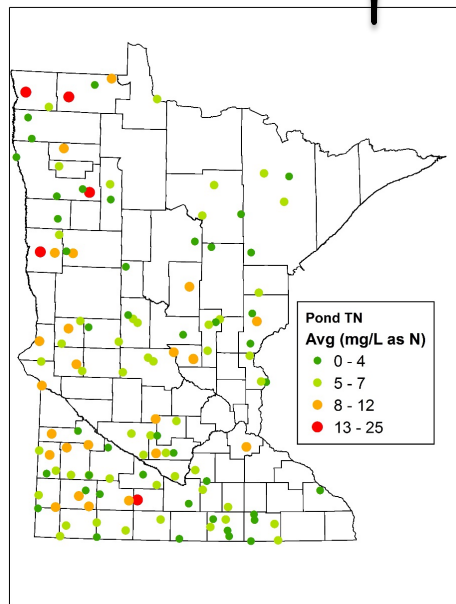
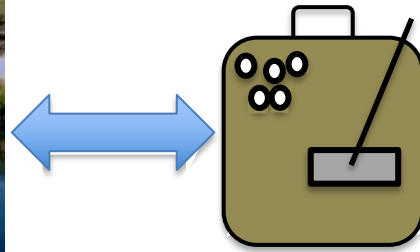


Figure from the MPCA

## Result:

Ammonia might build up in ponds when the T is less than 10°C and oxygen levels are low. If oxygen can be added and the bacteria that biodegrade ammonia can be better retained, this should improve ammonia removal



Department of  
**Civil, Environmental,  
and Geo- Engineering**  
UNIVERSITY OF MINNESOTA

Partners for water  
quality solutions