2016 Project Abstract
For the Period Ending June 30, 2019

PROJECT TITLE: Wastewater Treatment Process Improvements
PROJECT MANAGER: Timothy M. LaPara
AFFILIATION: University of Minnesota
MAILING ADDRESS: 500 Pillsbury Drive SE
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2016, Chp. 186, Sec. 2, Subd. 04k

APPROPRIATION AMOUNT: $ 398,000
AMOUNT SPENT: $390,777
AMOUNT REMAINING: $7,223

Sound bite of Project Outcomes and Results
This project demonstrated that specific microbial populations can be quantified in near real-time at full-scale municipal wastewater treatment facilities. Knowledge of quantities of these microbial populations should enable wastewater treatment plant operators and engineers to better optimize treatment performance, thereby improving surface water quality throughout the State of Minnesota.

Overall Project Outcome and Results
This project included the participation of 25 different wastewater treatment facilities located throughout the State of Minnesota. A total of 623 samples were collected from 38 wastewater treatment bioreactors, from 13 anaerobic digesters, and from 2 aerobic digesters. Metagenomic DNA was extracted and purified from all of these samples and used as template from which we were successfully able to quantify the numbers of total Bacteria, total Archaea, ammonia oxidizing bacteria, ammonia oxidizing archaea, polyphosphate accumulating organisms, denitrifying bacteria, and several different organisms known to be important to anaerobic digestion. DNA samples were also used to amplify and to sequence 16S rRNA gene fragments to characterize the microbial community composition in detail. In total, this project generated 19,064,646 DNA sequences that have been deposited in publicly-available databases. This project demonstrated that wastewater treatment bioreactors and anaerobic digesters generally contain similar levels of total bacteria and archaea, as expected. Substantial differences, however, were observed in the quantity of ammonia oxidizing bacteria, organisms that are critical in the removal of nitrogenous pollution. More importantly, perhaps, is that the quantity of ammonia oxidizing bacteria was connected to specific wastewater treatment process designs, suggesting that the removal of nitrogenous pollution can be controlled. Similarly, the quantity of polyphosphate accumulating organisms varied significantly between different treatment plants. Similar quantities of denitrifying bacteria were observed in all of the wastewater treatment bioreactors, independent of system design; this suggested that denitrifying bacteria are ubiquitous throughout all wastewater treatment bioreactors and their presence/quantity is neither enhanced nor suppressed by system design. This research makes a seminal advance in our understanding of the ecology of wastewater treatment bioreactors by delineating the factors that we can (process design, operating conditions, etc.) and the factors that we cannot (geographic location, weather, etc.) control and their effects on bacterial community composition.

Project Results Use and Dissemination
We have presented our preliminary results at numerous conferences and technical meetings throughout the State of Minnesota. We will continue to make these presentations over the next 2-5 years (at no cost to the

LCCMR). We have published one manuscript in the peer-reviewed literature so far, and we have drafted an additional five manuscripts that have been or will be soon submitted for publication.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2016 Work Plan Final Report

Date of Report: January 31, 2019

Final Report

Date of Work Plan Approval: June 7, 2016

Project Completion Date: June 30, 2019

PROJECT TITLE: Wastewater Treatment Process Improvements

Project Manager: Timothy M. LaPara

Organization: University of Minnesota

Mailing Address: 500 Pillsbury Drive SE

City/State/Zip Code: Minneapolis, MN 55455

Telephone Number: (612) 624-6028

Email Address: lapar001@umn.edu

Web Address:

Location: Statewide

Total ENRTF Project Budget:	**ENRTF Appropriation:**	**$398,000**
	Amount Spent:	**$390,777**
	Balance:	**$7,223**

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04k

Appropriation Language:

$398,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to characterize and quantify the nutrient-removing microorganisms used for municipal wastewater treatment, in order to improve the process used to reduce total nitrogen discharge. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Wastewater treatment process control improvements

II. PROJECT STATEMENT:

In the near future (5-10 years), new regulations are expected on Minnesota's municipal wastewater discharges for total nitrogen (ammonia, nitrite, and nitrate), which is needed to prevent the eutrophication of the Gulf of Mexico. The goal of this project is to understand the composition of the microbial communities used for municipal wastewater treatment and to provide baseline information of the quantities of nutrient-removing microorganisms used for wastewater treatment in the State of Minnesota. The benefit of this research will be a useful set of tools that can be used to better control wastewater treatment operations.

All wastewater treatment facilities in Minnesota are currently regulated with respect to the release of biodegradable organic compounds (known as "BOD" – biochemical oxygen demand). These regulations ensure that the harmful impacts of wastewater are avoided, particularly the consumption of oxygen in the receiving water body (oxygen is needed for fish and other aquatic fauna and flora to thrive). Presently, many wastewater treatment facilities are also seasonally regulated for ammonia (due to fish toxicity) and continuously regulated for phosphorus, which contributes to the eutrophication (the excessive growth of algae) in lakes.

The most cost-effective way to treat municipal wastewater is to utilize microorganisms to metabolize pollutants of concern. Wastewater treatment facilities use microorganisms to remove the nutrients (BOD, phosphorus, nitrogen) of concern from the wastewater. From an engineering and operational perspective, it is relatively straight-forward to achieve either BOD and phosphorus removal or BOD and total nitrogen removal, but it is **much more difficult problem** to achieve BOD, total nitrogen, and phosphorus removal because all three processes occur only over a very narrow range of operating conditions. Future wastewater treatment operations, therefore, will likely require better process control; the research performed in this project will delineate the tools needed to provide this better process control.

Surprisingly, wastewater treatment bioreactors are currently operated with very little knowledge of the microorganisms that provide the treatment. The scientific reason for the lack of monitoring has been the inability of microbiologists to culture organisms from environmental samples. Over the past 5-10 years, however, microbiologists have developed next-generation DNA sequencing technology to generate 50,000+ sequences per sample to address the question of "*who is there*?" In addition, quantitative polymerase chain reaction (qPCR) can be used to determine the precise quantities of specific organisms, such as those responsible for phosphorus and nitrogen removal, allowing us to address "*how many of them are there*?"

Of particular importance, qPCR techniques are now relatively affordable (less than $30,000 for all of the needed instrumentation – on par with other lab techniques) and practical, requiring no special skills beyond those possessed by typical laboratory technicians. The final activity will be to disseminate the research results to Minnesota's wastewater treatment plant managers and operators (and providing training, if requested), with the goal of using these new technologies for better wastewater treatment process control.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of January 1, 2017:

The project has thus far focused on collecting wastewater bioreactor samples from wastewater treatment facilities throughout Minnesota. Genomic DNA has been extracted and purified from the majority of these samples and the quantities of nutrient-removing organisms have been quantified. Activated sludge bioreactors have thus far contained 10^9 bacteria per milliliter, of which 10^8 are capable of denitrification (i.e., contain a *nosZ* gene) and 10^6 are bacteria capable of ammonia-oxidization (i.e., contain a bacterial *amoA* gene). In addition,

about 10^6 ammonia-oxidizing archaea (i.e., contain an archaeal *amoA* gene) were detected at one facility, but were not detectable at other facilities.

Project Status as of July 1, 2017:

The project manager received an email from LCCMR staff stating that a project status update was not required for this date.

Project Status as of January 1, 2018:

Samples are now being collected, weekly, from 25 different wastewater treatment facilities, encompassing 29 aeration basins, 14 anaerobic digesters, and 2 aerobic digesters. To date, more than 500 samples have been collected, of which DNA has been extracted and purified from more than 95% of the samples. Quantification of specific bacterial populations and characterization of bacterial community composition should commence soon. Detailed investigation of bacterial community dynamics of a single wastewater treatment facility (from north-central Minnesota) is nearing completion. An analogous study has been initiated at this wastewater treatment plant to connect the presence of specific bacterial populations (based on their DNA) with their activity (based on their RNA).

Project Status as of July 1, 2018:

Sample collection has been completed from 24 wastewater treatment plants (37 activated sludge basins and 13 anaerobic digesters; 1 wastewater treatment plant withdrew from participating in the study). Metagenomic DNA has been extracted and purified from these samples. We selected 467 samples from the activated sludge basins and 156 samples from anaerobic digesters to analyze monthly changes in microbial community composition from each sampling location. Those 623 samples will be submitted for Illumina sequencing at University of Minnesota Genomics Center shortly. Among the 467 activated sludge samples, total bacteria, ammonia monooxygenase genes (*amoA*) from bacteria, and *amoA* from *Archaea* were quantified from 385 samples. Polyphosphate-accumulating organisms (16S rRNA/PAO), polyphosphate kinase genes (*ppk*), nitrous oxide reductase genes (*nosZ*), hydrazine oxidoreductase genes (*hzo*) were quantified from 234 samples. Among the 156 anaerobic digester samples, total bacteria, total Archaea, sulfate reducing bacteria, propionic acid bacteria, *Methanomicrobiales*, *Methanobacteriales*, *Methanococcales*, *Methanosarcinales*, *Methanosarcinaceae*, and *Methanosaetaceae* were quantified from 34 samples. Once samples have been fully analyzed for our various gene targets, we will analyze the data to draw conclusions about the presence of these microorganisms as a function of wastewater treatment design parameters.

Project Status as of January 1, 2019:

All of the 623 samples have been analyzed by qPCR and by Illumina sequencing at the University of Minnesota Genomics Center. We are currently analyzing the large quantity of data that has been generated recently.

Amendment Request (2/12/2019)
Amendment Approved by LCCMR 3/8/2019

We are requesting an amendment to our budget. In our original budget, we overestimated the amount of funds required to analyze our samples but underestimate the amount of funds and effort that would be required to analyze our data. We have $14, 814 of unspent funds remaining in the category of Equipment/Tools/Supplies; we would like to shift these funds to the Personnel (Wages and Benefits) category. This would allow us to hire a 50% graduate research assistant for the Spring 2019 semester as well as the first portion of Summer 2019 (i.e., until June 30, 2019). This student would be dedicated to analyzing our incredibly large datasets and authoring manuscripts, reports, and other deliverables.

Overall Project Outcomes and Results:

This project included the participation of 25 different wastewater treatment facilities located throughout the State of Minnesota. A total of 623 samples were collected from 38 wastewater treatment bioreactors, from 13 anaerobic digesters, and from 2 aerobic digesters. Metagenomic DNA was extracted and purified from all of these samples and used as template from which we were successfully able to quantify the numbers of total Bacteria, total Archaea, ammonia oxidizing bacteria, ammonia oxidizing archaea, polyphosphate accumulating organisms, denitrifying bacteria, and several different organisms known to be important to anaerobic digestion. DNA samples were also used to amplify and to sequence 16S rRNA gene fragments to characterize the microbial community composition in detail. In total, this project generated 19,064,646 DNA sequences that have been deposited in publicly-available databases. This project demonstrated that wastewater treatment bioreactors and anaerobic digesters generally contain similar levels of total bacteria and archaea, as expected. Substantial differences, however, were observed in the quantity of ammonia oxidizing bacteria, organisms that are critical in the removal of nitrogenous pollution. More importantly, perhaps, is that the quantity of ammonia oxidizing bacteria was connected to specific wastewater treatment process designs, suggesting that the removal of nitrogenous pollution can be controlled. Similarly, the quantity of polyphosphate accumulating organisms varied significantly between different treatment plants. Similar quantities of denitrifying bacteria were observed in all of the wastewater treatment bioreactors, independent of system design; this suggested that denitrifying bacteria are ubiquitous throughout all wastewater treatment bioreactors and their presence/quantity is neither enhanced nor suppressed by system design. This research makes a seminal advance in our understanding of the ecology of wastewater treatment bioreactors by delineating the factors that we can (process design, operating conditions, etc) and the factors that we cannot (geographic location, weather, etc) control and their effects on bacterial community composition.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Characterize Minnesota's wastewater treatment microbiome.

Description:

The goal of this activity is to provide a general characterization of microorganisms in Minnesota's biological wastewater treatment facilities. This information will be critically useful to corroborate the organism-specific quantifications performed in Activity 2. This work is needed because the organism-specific quantifications are based on well-described model organisms, but these organisms might not be the pertinent nutrient-removing organisms in Minnesota's wastewater treatment facilities. This Activity will also allow us to compare the bacterial community composition of wastewater bioreactors throughout the State, determining if there are correlations between bacterial community composition and treatment facility performance, size, design, and other factors.

Samples will be collected from the bioreactors treating wastewater and wastewater sludges at selected wastewater treatment facilities on a weekly basis. We will specifically collect samples from the wastewater treatment facilities both in St. Cloud and in Brainerd, because these two facilities were recently upgraded to perform simultaneous removal of BOD, nitrogen, and phosphorus. We will also collect samples from wastewater treatment facilities in Little Falls, Duluth, St. Peter, and Mankato; these facilities do not currently perform simultaneous BOD, nitrogen, and phosphorus removal. We also intend to get samples from as many as 50 wastewater treatment facilities throughout the State. Our intention is to collect samples weekly from each of their bioreactors (many facilities have a single bioreactor, others have multiple bioreactors). We anticipate collecting ~5,000 samples (50 facilities $\times$ 1 sample per week per bioreactor $\times$ 2 bioreactors per facility $\times$ 100 weeks = 5000) for this Activity.

Assessment of the bacterial community composition will be achieved by using next-generation DNA sequencing will be performed using an Illumina MiSeq analyzer at the University of Minnesota Genomics Center. We will then use software that is available at the Minnesota Supercomputing Institute to statistically analyze the data and correlate our data to process performance (which is routinely collected from each facility). The goal is to obtain 50,000-100,000 DNA sequences per sample, which should allow us to characterize the bacterial community composition of these bioreactors in considerable detail.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 186,594
Amount Spent: $ 186,594
Balance: $ 0

Outcome	Completion Date
1. *Sample collection and Genomic DNA extractions (5,000 samples)*	December 31, 2017
2. *Next-Generation DNA sequencing (20 Illumina MiSeq runs)*	December 31, 2018
3. *Data Analysis at the Minnesota Supercomputing Institute*	April 30, 2019

Activity Status as of January 1, 2017:

To date, relatively little progress has been made on this Activity. The accomplishments, so far, have included sample collection and DNA extraction/purification from numerous wastewater treatment facilities. These DNA samples have been stored and will be used for Illumina sequencing in subsequent project periods.

Activity Status as of July 1, 2017:

The project manager received an email from LCCMR staff stating that a project status update was not required for this date.

Activity Status as of January 1, 2018:

The bacterial community composition has been determined and analyzed for more than 200 bacterial communities from a wastewater treatment facility in North-Central Minnesota. These results demonstrate that bacterial communities reproducibly adapt to changing seasons. Another 500 samples have been collected and processed (i.e., DNA has been extracted and purified) from other 25 other wastewater treatment facilities and are awaiting analysis on the Illumina MiSeq instrument.

Activity Status as of July 1, 2018:
The samples have been collected and processed and are awaiting processing at the University of Minnesota Genomics Center for Illumina MiSeq analysis. We anticipate getting these results in the next 1-3 months, after which we will commence data analysis.

Activity Status as of January 1, 2019:

Data analysis has commenced and is on-going. To date, we have preliminary indications that bacterial communities develop in wastewater treatment bioreactors due to specific reactor conditions rather due to random factors. This is practically pertinent because it suggests that wastewater treatment engineers and operators have the potential to manipulate the bacterial communities used in wastewater treatment bioreactors for a specific purpose.

Final Report Summary:

This activity generated 19,064,646 DNA sequences. The analysis of these DNA sequences allowed us to demonstrate that wastewater treatment bioreactor communities are generally reproducible and strongly affected by system design and geographic location. These results were also used to confirm the quantitative analysis performed in Activity 2.

ACTIVITY 2: Quantify nutrient-removing microbial populations in wastewater bioreactors.

Description:

In this activity, we will quantify the presence of specific microbial populations that are known to perform nitrogen and phosphorus removal. These organisms will include all bacteria, ammonia-oxidizing bacteria, ammonia-oxidizing Archaea, nitrite-oxidizing bacteria, three different types of denitrifying bacteria, and phosphate-accumulating organisms. That is, we will quantify the presence of as many as 8 different 'types' of bacteria in each sample. These quantitative values will allow us to directly compare the 'treatment capacity' of each biological wastewater treatment process (i.e., the ability to treat wastewater is usually proportional to the quantity of organisms of each type). This research will also allow us to compare how different treatment processes are affected by seasonal variation in temperature, process design, etc.

A very similar process will be used for Activity 2 as for Activity 1. We will use the same samples as used in Activity 1. We will perform these assays using the 384-well real time PCR system at the University of Minnesota Genomics Center (approximately 40,000 quantifications). Data will then be correlated to the process performance data provided to us from the treatment facilities.

Summary Budget Information for Activity 2:

ENRTF Budget: $ 201,406
Amount Spent: $ 197,417
Balance: $ 3,989

Outcome	Completion Date
1. *Sample collection and Genomic DNA extractions*	December 31, 2017
2. *qPCR targeting specific nutrient-removing microorganisms*	June 30, 2018
3. *Data Analysis at the Minnesota Supercomputing Institute*	April 30, 2019

Activity Status as of January 1, 2017:
As with Activity 1, the primary accomplishments Activity 2 have included sample collection and DNA extraction/purification from numerous wastewater treatment facilities. These DNA samples have been stored and will be used for Illumina sequencing in subsequent project periods. Numerous samples have been used to quantify total bacteria (16S rRNA genes: 10^9/mL), ammonia-oxidizing bacteria (*amoA* genes: 10^6/mL), ammonia-oxidizing archaea (*amoA* genes: < 10^6/mL), denitrifying bacteria (*nosZ*: 10^7/mL; *nirS*: 10^7/mL;*nirK* genes: 10^5/mL), and polyphosphate accumulating organisms (16S rRNA genes specific to these organisms: 10^6/mL). In general, the quantities of these populations were stable over time.

Activity Status as of July 1, 2017:

The project manager received an email from LCCMR staff stating that a project status update was not required for this date.

Activity Status as of January 1, 2018:

Relatively little progress has been made towards this goal in the past year beyond collecting samples (> 500 samples) and extracting genomic DNA.

Activity Status as of July 1, 2018:

Now that the samples have been collected and DNA has been extracted from them, we have made substantial progress with this activity. We have developed and optimized numerous methods for qPCR analysis.

Activity Status as of January 1, 2019:

All samples have been analyzed by qPCR analysis. The data, however, has not been analyzed. We intend to hire a new graduate student to analyze these data to reach conclusions on how qPCR can be used to monitor and operate full-scale wastewater treatment facilities in Minnesota.

Final Report Summary:

This project demonstrated that wastewater treatment bioreactors and anaerobic digesters generally contain similar levels of total bacteria and archaea, as expected. This research also identified specific assays that could be used to quantify populations of ammonia oxidizing bacteria and phosphorus accumulating organisms. These assays should be particularly useful for Minnesota's wastewater treatment plants as they attempt to optimize the removal of nitrogenous and phosphorous pollutants.

ACTIVITY 3: Disseminate our results to Minnesota's wastewater treatment facilities.
Description:

The first two activities will demonstrate the value of tracking bacterial populations in Minnesota's wastewater treatment facilities. The final activity will be to disseminate these results at local conferences and then to work with Minnesota's wastewater treatment facilities to perform these assays in-house. The techniques used in this project are relatively new, but they are ready to be used at wastewater treatment laboratories throughout the State for process monitoring and control. The cost of these assays is similar to those used for other laboratory assays used by wastewater treatment operators and managers. The skills required are similar to those needed by laboratory technicians.

Ideally, we will convince some facilities to purchase the equipment to perform these assays; in this case, we would provide training at no cost to the participating utility.

Summary Budget Information for Activity 3:		
	ENRTF Budget:	**$ 10,000**
	Amount Spent:	**$ 6,766**
	Balance:	**$ 3,234**

Outcome	**Completion Date**
1. *Presentations at local wastewater treatment conferences*	December 31, 2018
2. *Train laboratory technicians at wastewater treatment facilities to use qPCR*	June 30, 2019

Activity Status as of January 1, 2017:

There has been no progress with this activity at this time.

Activity Status as of July 1, 2017:

The project manager received an email from LCCMR staff stating that a project status update was not required for this date.

Activity Status as of January 1, 2018:

Public presentations have been given to the North-Central Branch of the Minnesota Water Operators Association and to Barr Engineering.

Activity Status as of July 1, 2018:

We have visited several wastewater treatment facilities to informally discuss progress on our project.

Activity Status as of January 1, 2019:

We have a planned presentation at the Central States Water Environment Association/Minnesota Wastewater Operators Association Innovative Conference in St. Cloud on February 5, 2019.

Final Report Summary:

We have presented our preliminary results at numerous conferences and technical meetings throughout the State of Minnesota. We will continue to make these presentations over the next 2-5 years (at no cost to the LCCMR). We are currently in the process of drafting numerous manuscripts to publish our findings in the peer-reviewed technical literature.

V. DISSEMINATION:

Description:

Findings will be disseminated and archived via reports to LCCMR, peer-reviewed publications, and presentations at conferences. We will also, when appropriate, disseminate results via press releases to the media. The audience is not only the scientific community, but also the public, policymakers, and practitioners. The work will also be of interest to the wastewater treatment community and we will seek avenues to share the results with this community (such as the Minnesota Wastewater Operators Conference). We would also like to work one-on-one with individual wastewater utilities to start performing these assays; our hope is that we can get actual wastewater treatment plants performing the analyses that we explore herein.

Status as of January 1, 2017:

There has been no progress with this activity at this time.

Status as of July 1, 2017:

The project manager received an email from LCCMR staff stating that a project status update was not required for this date.

Status as of January 1, 2018:

Public presentations have been given to the North-Central Branch of the Minnesota Water Operators Association and to Barr Engineering. A manuscript has been initiated that will be submitted for publication to the peer-reviewed, archival literature.

Status as of July 1, 2018:

We have visited several wastewater treatment facilities to informally discuss progress on our project.

Status as of January 1, 2019:

We have a planned presentation at the Central States Water Environment Association/Minnesota Wastewater Operators Association Innovative Conference in St. Cloud on February 5, 2019.

Final Report Summary:

We have presented our preliminary results at numerous conferences and technical meetings throughout the State of Minnesota. We will continue to make these presentations over the next 2-5 years (at no cost to the LCCMR). We have published one manuscript in the peer-reviewed literature so far, and we have drafted an additional five manuscripts that have been or will be soon submitted for publication.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview: Please see attached budget spreadsheet

Explanation of Use of Classified Staff: N/A

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

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 1.75

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: N/A

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
National Science Foundation fellowship to Julie Johnston (informational only; not intended to represent committed cost share)	$121,225	$121,225	NSF fellowship for graduate student working on this project
State			
	$197,064	$193,488	In-kind contribution; indirect costs not charged to this project
TOTAL OTHER FUNDS:	**$318,289**	**$314,713**	

VII. PROJECT STRATEGY:

A. Project Partners: N/A

B. Project Impact and Long-term Strategy:

The goal of the project is to get Minnesota's wastewater treatment plants to monitor their biomass for specific bacterial populations by qPCR. This will be achieved by demonstrating and verifying the technology and then presenting the results (including the costs for the analyses) to Minnesota's wastewater treatment facilities. If needed (or requested), we will even visit treatment facilities to train their personnel to perform the assays.

C. Funding History: N/A

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: N/A

IX. VISUAL COMPONENT or MAP(S): See attached visual

X. RESEARCH ADDENDUM: See attached

XI. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than January 1, 2017, July 1, 2017, January 1, 2018, July 1, 2018, and January 1, 2019. A final report and associated products will be submitted between June 30 and August 15, 2019.

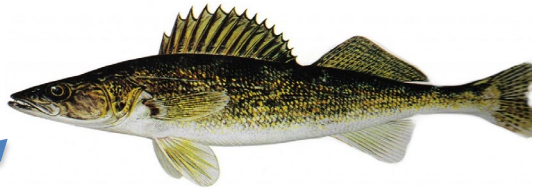
Environment and Natural Resources Trust Fund
M.L. 2016 Project Budget

Project Title: Wastewater Treatment Process Improvements
Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04k
Project Manager: Timothy M. LaPara
Organization: University of Minnesota
M.L. 2016 ENRTF Appropriation: $398,000
Project Length and Completion Date: 3 Years, June 30, 2019
Date of Report: Expenditures Through 6/30/2019

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Activity 1 Budget 3/8/2019	Amount Spent	Activity 1 Balance	Revised Activity 2 Budget 3/8/2019	Amount Spent	Activity 2 Balance	Activity 3 Budget	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	***Characterize Minnesota's wastewater***			***Quantify nutrient-removing microbes***			***Disseminate results***				
Personnel (Wages and Benefits)											
Overall	$151,888	$151,888	$0	$166,701	$162,712	$3,989	$7,750	$5,000	$2,750	$326,339	$6,739
Project Management, Timothy LaPara ($77,844: $53,149 in year 1, $12,165 in year 2, $12,530 in year 3;35% of time in year 1, 7.7% of time in years 2 and 3; 75% to salary, 25% to fringe benefits)											
Project Management, Sebastian Behrens ($44,292; 7.7% of time each year; 75% to salary, 25% to fringe benefits)											
Graduate Student at U of M ($47,451; 50% of time; 50% to salary, 50% to benefits)											
Graduate Student at U of M ($22,350.57; 50% of time; 56% to salary, 44% to benefits)											
Post-Doc at U of M ($115,704; 100% of time; 82% to salary, 18% to benefits)											
Equipment/Tools/Supplies											
General lab supplies ($5,000), reagents for qPCR ($17,500), use of UMGC's facilities for qPCR and Illumina sequencing ($36,976), DNA extraction kits ($12,500), PCR purification kits ($10,000)	$33,581	$33,581	$0	$33,580	$33,580	$0	$0	$0	$0	$67,161	$0
Travel expenses in Minnesota											
Travel to wastewater treatment facilities in Minnesota to collect bioreactor samples; travel to MN wastewater conferences to present research; travel to wastewater treatment facilities to train lab personnel	$1,125	$1,125	$0	$1,125	$1,125	$0	$2,250	$1,766	$484	$4,500	$484
COLUMN TOTAL	**$186,594**	**$186,594**	**$0**	**$201,406**	**$197,417**	**$3,989**	**$10,000**	**$6,766**	**$3,234**	**$398,000**	**$7,223**

Wastewater Treatment Process Improvements

Wastewater Treatment



Biodegradable organic carbon removal protects fish and other aquatic organisms

Nitrogen removal prevents eutrophication of coastal water

Phosphorus removal prevents eutrophication of lakes

- This project identified and validated assays for characterizing and quantifying the bacteria that remove carbon, nitrogen and phosphorus, leading to essential improvements in wastewater treatment process control
- Better wastewater treatment will lead to better surface water quality throughout Minnesota