2014 Project Abstract
For the Period Ending June 30, 2017

PROJECT TITLE: Methods to Protect Beneficial Bacteria from Contaminants to Preserve Water Quality
PROJECT MANAGER: Paige Novak
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2014, Chp. 226, Sec. 2, Subd. 03b

APPROPRIATION AMOUNT: $ 279,000
AMOUNT SPENT: $ 277,935
AMOUNT REMAINING: $ 1,065

Overall Project Outcome and Results

We studied the effect of common pollutants, perfluorinated alkyl substances or perfluorinated substances (PFCs), on bacteria. PFCs are found throughout the environment from concentrations of 0.00001 mg/L in rivers to 10 mg/L at heavily polluted sites. Because humans rely on bacteria for nutrient cycling and waste degradation, it is important to understand whether PFCs affect bacteria. We studied PFCs with known health impacts, those containing 7-8 fully fluorinated carbons, along with shorter, 4- and 6-carbon "replacement" PFCs.
We discovered that PFCs with 3-8 fully fluorinated carbons increased the permeability of bacterial membranes at high (mg/L) concentrations. We observed that these compounds deposited into model bacterial membranes and changed their fluidity at concentrations of 0.1 to 50 mg/L. This is significant because bacterial membranes form semi-permeable barriers; it is the semi-permeable nature of membranes that enable bacteria to communicate with one another and control metabolism. Increased membrane fluidity and permeability in bacteria induced by PFC exposure could have impacts on the bacterial functions that humans rely on.

Perhaps not surprisingly, we also discovered that PFCs with 3-8 fully fluorinated carbons altered the bacterial response the external chemical signal used for bacteria-to-bacteria communication. This occurred at PFC concentrations of 0.01 to 50 mg/L. Finally, exposure to high (50 mg/L) concentrations of PFCs with 7-8 fully fluorinated carbons and PFC-containing aqueous film-forming foam changed microbial metabolism, decreasing the amount of carbon degraded to methane. The presence of a co-contaminant lessened this effect, likely as a result of the co-contaminant displacing the PFCs from the bacterial surface. This research indicates microbial metabolism is not likely to be affected by PFCs unless these compounds are present at high concentrations, such as at fire-fighting training sites. Nevertheless, the effects of PFCs on bacterial membranes and bacteria-to-bacteria communication at lower concentrations could cause unanticipated impacts.

Project Results Use and Dissemination

Three peer-reviewed manuscripts are expected to be published from this work; these will be

submitted to the LCCMR when accepted for publication. Multiple presentations about the research have been given at both regional and national/international conferences.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2014 Work Plan

Date of Report: August 11, 2017

Final Report

Date of Work Plan Approval: June 4, 2014

Project Completion Date: June 30, 2017

PROJECT TITLE: Methods to Protect Beneficial Bacteria from Contaminants to Preserve Water Quality

Project Manager: Paige J. Novak

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Location: Hennepin, Statewide impact

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$279,000**
	Amount Spent:	**$277,935**
	Balance:	**$ 1,065**

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 03b

Appropriation Language:
$279,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to research how and why bacteria that provide ecological functions humans depend on for water quality are affected by exposure to certain man-made perfluorinated chemicals entering the wastewater treatment system in order to identify methods that can be implemented to protect those bacterial functions from being degraded. This appropriation is available until June 30, 2017, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Methods to Protect Beneficial Bacteria from Contaminants to Preserve Water Quality

II. PROJECT STATEMENT:

Humans depend on bacteria to cycle nutrients and carbon. In doing so, bacteria perform critical ecological functions that enable life to exist. Bacteria are also harnessed for use in engineered systems such as wastewater treatment plants and landfills. In fact, it is through the activity of bacteria in engineered systems that engineers protect surface water from excess nitrogen pollution, decompose solid waste, and treat wastewater so that its discharge is cleaner and therefore better supports aquatic life. Unfortunately, the environments where these critical bacteria live are also environments filled with a complex "soup" of chemicals. The chemicals present in personal care products, medicines, and products such as clothing and packaging are eventually found in wastewater, solid waste, and the wastewater-derived biosolids that are applied to agricultural land. These chemicals can negatively affect bacterial function, and can be particularly damaging when present in mixtures. One common class of chemicals that are present throughout the environment is perfluorinated chemicals (PFCs); based on other research, it is hypothesized herein that PFCs can cause other co-contaminants to be more toxic to bacteria.

The proposed research will study how bacterial function, namely the oxidation of ammonia and the anaerobic degradation of a mixture of carbonaceous compounds, is affected when bacteria are exposed to a mixture of PFCs in the presence and absence of other co-contaminants (model contaminants of different structures). This research will help us understand why/when critical bacterial functions such as nitrogen cycling and carbon decomposition are lost as a result of chemical exposure. It will also help us understand which bacteria are more resistant to such harmful affects and why, with the goal of developing engineered methods to protect critical bacterial functions.

III. PROJECT STATUS UPDATES:

Amendment Request (12/11/2014):

The addendum is to formally request a re-budgeting of funds for this project.

As part of the project, we would like to establish one additional personnel category: temporary worker, within the subcategory of undergraduate-level researcher. The temporary worker will essentially fulfill the role of the already-budgeted undergraduate researcher (assisting the graduate student researcher with routine activities (running reactors, culturing organisms) to enable the graduate student researcher to spend more time on higher-level functions such as data analysis and mechanistic studies involving artificial membranes. By expanding the category of undergraduate researcher to included "temporary worker," we will be able to pay a post-undergraduate student, with a higher skill set, to perform this work or pay a non-University of Minnesota undergraduate student to work on the project in the summer. This simply enables more flexibility in hiring the most qualified and skilled worker to assist the graduate research assistant. No funds are actually rebudgeted, the Personnel subcategory of "undergraduate researcher" is simply expanded to include "temporary worker" as well.

This amendment request will not affect project objectives or timelines.

Amendment Approved: 12/12/14

Project Status as of January 31, 2015:

Project activities over the first six months have focused on method development and the establishment of required source cultures. Experiments with positive and negative controls have been performed with model cell membranes (liposomes) to assess the permeability of the liposomes to fluorescent dyes outside of the

membrane. Additional experiments are currently being performed with similar positive and negative controls to assess the leakage of fluorescent dyes out of the liposomes to determine which method is most robust. Liposomes created with and without PFCs have also been successfully generated in the laboratory and characterized.

Source reactors of nitrifying and methanogenic cultures have been established both in the presence and absence (control) of PFOS. These reactors are being monitored and have been used to perform preliminary experiments to determine the appropriate concentrations of PFCs and co-contaminants that will be subject to further study.

Project Status as of July 31, 2015:
Recent project activities have been focused on activity one. The addition of PFOS to nitrifying cultures has resulted in no statistically significant difference in oxygen consumption (as compared to the control). Preliminary experiments have been run with methanogenic cultures. This method needs more refinement to reduce the standard deviation of replicates. Investigation into selection of antibiotic resistance genes in cultures exposed to contaminants is underway.

Preliminary experiments using the liposomes have demonstrated that low concentrations of PFOS do not increase permeability of liposomes to the fluorescent dye ANTs. Additional experiments will be performed using higher concentrations of PFOS as well as exposure to additional membrane stressors to investigate the effect of multiple stressors on membrane integrity.

We have obtained a strain of *Aliivibrio fischeri* that can be used to determine the effect of PFCs on quorum sensing. Because the signaling molecules need to diffuse through the membrane it is hypothesized that PFCs may alter natural signaling processes in bacteria. A method to test this is being developed.

Amendment Request (11/6/2015):
The addendum is to formally request a re-budgeting of funds for this project.

As part of the project, we use an ion chromatograph (IC) to monitor the concentration and transformation of nitrogen species and other charged species in solution (sulfate, etc.). The IC that we had in the department for research use has become inoperable and unfixable. We would like to purchase a new instrument ($35,000). I would like to re-budget $1,000 of this grant to put towards the purchase of this shared-use instrument to cover the samples analyzed in this project. For reference, we can analyze samples by IC for $20/sample in another laboratory. At this rate, and given the fact that standards need to be analyzed when samples are analyzed, we would quickly exceed the $1,000 requested in this re-budget (50 samples), more than recouping the funds spent on a portion of the new instrument, which will then be available for unrestricted use for the work described herein. Funds would be moved within the category “Equipment/Tools/Supplies” to a new subcategory labeled “equipment” from the current subcategory describing supply/service/repair expenditures.

This amendment request will not affect project objectives or timelines.

Approved by LCCMR 11-12-2015

Amendment Request (12/11/2015):
The addendum is to formally request a re-budgeting of funds for this project.

In this project we would like to use a novel technique that will allow us to very precisely determine the fluidity of a model microbial membrane in the presence of different perfluorinated compounds at different concentrations. This technique would enable us to understand our macro-scale results (microbial function/elemental cycling) based on what occurs at a molecular level with microbial membranes. The

technique involves placing a constructed model membrane on an incredibly sensitive oscillator, a quartz crystal microbalance (QCM), and then heating it to determine how the integrity of the membrane changes with different treatments. This instrumentation exists for us to use at the University of Minnesota in the Characterization Facility. Currently, however, only one person in the world (Nathalie Tufenkji at McGill University in Montreal, Canada, http://people.mcgill.ca/nathalie.tufenkji/) is using the QCM in this manner. We would like to send the student working on this LCCMR project to train for a week or less in Dr. Tufenkji's laboratory to learn this technique so that she can then bring this knowledge back to the University of Minnesota for use here. We would therefore like to request a re-budget of funds for international travel to complete this training. Funds ($1,000) would be moved from the subcategory "Equipment repair and maintenance" under "Equipment/Tools/Supplies" to a new category "Travel" and subcategory labeled "International travel for methodological training".

This amendment request will not affect project objectives or timelines but will enable us to very clearly determine what occurs in microbial cells at a molecular level, vastly improving our ability to translate and extrapolate our current laboratory results.

Amendment Approved: 12/14/15

Project Status as of January 31, 2016:
Current activities have focused on activity one, specifically work with the methanogenic culture. It has been concluded that the presence of PFCs and co-contaminants in batch-type reactors does not affect the presence of antibiotic resistance genes. Additionally, it appears that only high levels of PFCs (>1mg/L) have a detectable, toxic effect on cultures. When 2,4-dichlorophenol (a compound that is known to disrupt the separation of H^+ across cell membranes) is added in conjunction with high levels of PFCs an antagonistic interaction is observed, *i.e.*, PFCs become less toxic. More work is being done to confirm these results and pinpoint the concentration where the effect of PFCs becomes relevant and also to determine why PFCs reduce the impact of 2,4-dichlorophenol. Our current hypothesis is that PFCs make cells less fluid and resistant to the diffusion of compounds across the cell membrane, counteracting compounds that make cells leaky, such as 2,4-dichlorophenol.

Preliminary work has been completed to determine the role that PFCs have in quorum sensing. The mutant strains of *Allivibrio fischeri* that we have received have been exposed to PFOS and PFOA overnight. It appears that while the PFCs have little effect on growth (*i.e.*, they are not toxic, as observed with methanogenic cultures), they significantly reduce luminescence, the response to the signaling molecule that must diffuse into the cells before luminescence is induced. Several tests still need to be run to confirm these results and make sure this not a byproduct of toxicity; nevertheless, these results are consistent with the hypothesis that PFCs make cells less fluid and resistant to the diffusion of compounds across the cell membrane.

We have been developing a new collaboration with Nathalie Tufenkji to prove or disprove our hypothesis. Dr. Tufenkji has developed a method utilizing a quartz crystal microbalance that allows the fluidity of a constructed cell membrane to be directly measured. This method will be used to investigate changes in membrane fluidity in response to the addition of PFCs to the membrane.

Project Status as of July 31, 2016:
Dilutions of anaerobic digester communities have shown increased susceptibility to co-contaminant toxicity in the presence of some PFCs. A toxic synergistic relationship was observed when communities were exposed to 1 mg/L of 2,4-dichlorophenol (DCP) and 50 mg/L PFOS. Synergism was not observed when communities were exposed to PFOA, PFBS, and PFBA in combination with DCP. Synergism was not observed when communities were exposed to other co-contaminants (2,4-dichloroaniline, 2,4-dinitrophenol, and pentachlorophenol). DCP is neutral at the operating pH, which may explain its synergistic toxicity with PFOS. Additional experiments are planned to determine if this is the cause of the observed DCP synergistic toxicity. Using the quartz-crystal

microbalance at McGill University, we have demonstrated that all PFCs deposit onto a model cell membrane, with PFOS depositing in the greatest quantity. With respect to the other PFCs tested, deposition quantity followed the following trend: PFOA > PFBS > PFBA. Additionally, PFOS, PFOA, and PFBS all altered the phase transition temperature of the model cell membrane at 50 mg/L, meaning that these compounds had the ability to alter cell membrane fluidity. Membrane fluidity is critical for the diffusion of various compounds into the cell, such as cell signaling chemicals, oxygen, and some sources of food. Once again, PFOS displayed the greatest effect on the membrane with respect to fluidity. These results help explain why PFOS increases toxicity to a co-contaminant: it likely increases cell permeability, exacerbating the toxic effect of DCP.

Preliminary experiments have also been completed to assess the changes in surface charge in liposomes after exposure to PFCs. These results will clarify whether PFCs can also interfere with cell function by interacting with membrane proteins in addition to the membrane lipids. Results thus far indicate that exposure to 50 mg/L PFOA creates a greater negative charge to liposomes. Experiments with liposomes containing proteins will be performed over the next reporting period, as well as experiments with bacteria.

Amendment Request (7/19/2016):
The addendum is to formally request a re-budgeting of funds for this project.

The travel requested in the Amendment Request on 12/11/15 (approved 12/14/15) has been completed with very exciting and successful results. Indeed, as a result of this travel, the diffusion cell that we expected to construct ($4,000 budgeted in the project) will not be needed and excellent results showing the effect of PFCs on cell membranes have been obtained. We would therefore like to rebudget the $4,000, as it is no longer needed for diffusion cell construction. We would like to move $3,569 into “Laboratory Supplies” to cover costs associated with the new experiments performed with the quartz crystal microbalance (QCM) and we would like to move $431 to “International Travel for Methodological Training” to cover a slight cost overrun from airfare increases that occurred when the student funded on this project went to McGill University for training.

This amendment request will not affect project objectives or timelines, but will cover costs associated with us being able to very clearly determine what occurs in microbial cells at a molecular level, vastly improving our ability to translate and extrapolate our current laboratory results.

Amendment Approved: 7/21/16

Project Status as of January 31, 2017:
Significant progress has been made in activities one and two. Anaerobic digester cultures have been assessed for synergistic effects when exposed to PFCs and co-contaminants. PFOS altered the toxicity of 2,4-dichlorphenol (DCP) in a variable manner—at times acting synergistically and at other times antagonistically. Experiments are being performed to determine if the variability is due to dose variation or community composition. Likewise, the effect of PFCs in mixtures has been tested. It has been determined that changes in toxicity as a result of aqueous film forming foam (AFFF) can be described by PFOS and PFHxS. This shows that while PFOS is the component that causes the greatest toxicity, PFHxS also plays a small role. The degradation of DCP was also inhibited by the presence of PFNA, PFOA, PFOS, and PFHxS. When AFFF was added, significant inhibition occurred that could not be explained by the presence of only PFOS and PFHxS, with very little DCP degradation occurring over a month-long period.

Quorum sensing experiments have also been performed and showed that the organisms were more sensitive to the presence of signaling chemicals when PFCS were present. The effect was dependent on both the head group and the chain length of the PFCs, with greater PFCs concentrations and longer fluorinated chain lengths increasing the sensitivity of the organisms.

The effect of the PFCs head group and chain length on lipid bilayers (model cell membranes) was determined. PFCs that contain a sulfonate head group resulted in greater changes to the model cell membranes than carboxylic acid groups. Experiments are being performed to determine the have effect of PFCs on bacteria surface charge. Preliminary results show that PFCs increase the negative charge of bacteria in a manner dependent on concentration, functional group, and fluorinated chain length. These results are being verified.

All of these results point to the same conclusion: PFCs alter microbial function at a range of concentrations that are relevant and realistic in different environments. For example, bacteria are not able to degrade toxic chemicals when in the presence of a range of PFCs at concentrations that one might find in a highly contaminated site (landfill or fire training area), their respiration is affected at these high concentrations as well. Perhaps most interesting is the ability of a large range of PFCs, both those compounds that have been phased out and those currently being used, to interfere with microorganism-to-microorganism communication or signaling. This type of communication is important in many microbial functions, including virulence and biofilm development. When in the presence of PFCs at much lower concentrations (relevant in sediment, landfills, and contaminated sites), bacteria respond to much lower quantities of "signal," in essence, they turn on certain functions (like biofilm development) sooner than they otherwise would when PFCs are present.

Overall Project Outcomes and Results:
We studied the effect of common pollutants, perfluorinated alkyl substances or perfluorinated substances (PFCs), on bacteria. PFCs are found throughout the environment from concentrations of 0.00001 mg/L in rivers to 10 mg/L at heavily polluted sites. Because humans rely on bacteria for nutrient cycling and waste degradation, it is important to understand whether PFCs affect bacteria. We studied PFCs with known health impacts, those containing 7-8 fully fluorinated carbons, along with shorter, 4- and 6-carbon "replacement" PFCs.

We discovered that PFCs with 3-8 fully fluorinated carbons increased the permeability of bacterial membranes at high (mg/L) concentrations. We observed that these compounds deposited into model bacterial membranes and changed their fluidity at concentrations of 0.1 to 50 mg/L. This is significant because bacterial membranes form semi-permeable barriers; it is the semi-permeable nature of membranes that enable bacteria to communicate with one another and control metabolism. Increased membrane fluidity and permeability in bacteria induced by PFC exposure could have impacts on the bacterial functions that humans rely on. Perhaps not surprisingly, we also discovered that PFCs with 3-8 fully fluorinated carbons altered the bacterial response the external chemical signal used for bacteria-to-bacteria communication. This occurred at PFC concentrations of 0.01 to 50 mg/L. Finally, exposure to high (50 mg/L) concentrations of PFCs with 7-8 fully fluorinated carbons and PFC-containing aqueous film-forming foam changed microbial metabolism, decreasing the amount of carbon degraded to methane. The presence of a co-contaminant lessened this effect, likely as a result of the co-contaminant displacing the PFCs from the bacterial surface. This research indicates microbial metabolism is not likely to be affected by PFCs unless these compounds are present at high concentrations, such as at fire-fighting training sites. Nevertheless, the effects of PFCs on bacterial membranes and bacteria-to-bacteria communication at lower concentrations could cause unanticipated impacts.

Retroactive Amendment Request (08/11/2017):
The addendum is to formally request a re-budgeting of funds for this project.

We would like to move funds ($495 total) from the laboratory supplies category of Activity 2 to the personnel category of Activity 2 to cover an over-expenditure of $495. A raise associated with the final semester of the graduate research assistant working on the project had not been fully accounted for and there was a very slight overrun in expenditure in this category. We have now completed all work associated with this project. The graduate student working on the project studies did not require all of the funds originally budgeted for supplies, leaving funds that could be re-allocated to personnel.

The movement of funds between these categories did not affect project objectives or timelines.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Understand how and why perfluorinated chemicals alter bacterial function (nitrogen cycling and carbon decomposition) alone or in mixtures with a co-contaminant

Description: Five nitrifying and five methanogenic source cultures will be established. They will be seeded with either activated sludge and fed ammonium and trace nutrients in well-buffered medium or seeded with sludge from a local full-scale anaerobic digester and fed a blend of organic acids, alcohols and glucose (0.18 g COD/L-day) in minimal media. One set of experiments will be performed with strongly flocculating nitrifiers and methanogenic communities cultured as granules so that the physical protection of the biological floc/granule itself can be assessed. Of the five source reactors, one will be fed perfluorooctane sulfonate (PFOS), one perfluorooctane sulfonamide (FOSA), and one perfluorbutane sulfonate (PFBS), each at approximately 400 µg/kg, and a fourth will be fed PFOS at 2000 µg/kg. One reactor will be maintained in the absence of PFCs. Reactors that are chronically-exposed to the PFCs will be maintained for a period of three retention times before experiments are performed.

Aliquots of the chronically PFC-exposed source cultures will be added to triplicate reactors to which either (1) nothing or (2) melamine will be added. Aliquots of the unexposed source cultures (i.e. no chronic PFOS, FOSA, or PFBS exposure) will also be added to triplicate batch reactors to which the following is added: (1) no chemical contaminants, (2) single compounds (PFOS, FOSA, PFBS, or melamine), or (3) mixtures of each of the PFCs and melamine. PFCs will be added at concentrations of approximately 400 µg/kg. Melamine will be added at a concentration that is approximately 10% of the dose that results in a significant decrease in respiration (determined separately). Nitrifying reactors will be fed with oxygen and ammonia and the biomass-normalized rate of oxygen use and ammonia consumption will be determined for each batch reactor. Replicate experiments will be performed for dispersed and biofilm-forming nitrifying cultures. Methanogenic reactors will be fed the mixture of organic acids, alcohols, and glucose fed to the source reactors and the biomass-normalized rate of methane production will be determined for each batch reactor. As with the nitrifiers, replicate experiments will be performed for dispersed and granulated methanogenic cultures. Samples will also be taken to determine whether the melamine and PFCs are outside of, loosely bound to, or inside the cells.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 190,000
Amount Spent: $ 190,000
Balance: $ 0

Activity Completion Date: January 31, 2017

Outcome	Completion Date	Budget
1. In cultures of nitrogen-cycling bacteria, determine which perfluorinated chemicals are most harmful, alone and in mixtures, and at what concentrations	06/30/2015	$46,500
2. In cultures of carbon-cycling bacteria, determine which perfluorinated chemicals are most harmful, alone and in mixtures, and at what concentrations	06/30/2016	$50,500
3. Determine whether certain common bacterial traits (different types and thicknesses of protective "coats") make bacteria more resistant to this type of toxicity	01/31/2017	$93,000

Activity Status as of January 31, 2015:
Source reactors have been constructed and nitrifying and methanogenic cultures have been established. Each community has been enriched in the presence and absence (control) of PFOS. These reactors are being monitored, and thus far, are behaving similarly with respect to microbial respiration and growth.

Smaller reactors have also been developed from the source reactors and have been used for method development. Methods to measure respiration (oxygen consumption, ammonia oxidation, and methane production) have been developed. Additionally, preliminary experiments have been performed to target the co-contaminant concentrations that will be used to assess the effect of PFC concentration and co-contaminant exposure on respiration.

Activity Status as of July 31, 2015:
Respiration experiments have been run with nitrifying and methanogenic cultures. High concentrations of PFOS and triclosan have been tested in nitrifying cultures to determine if respiration is altered. While high concentrations of triclosan (5 mg/L) significantly slowed respiration, no statistically significant effect was seen in the presence of PFOS up to 10 mg/L. Experimentation with methanogenic cultures has shown variable results and further work is being performed to refine the experimental methods to produce less variability.

DNA has also been extracted from nitrifying and methanogenic cultures. The DNA will be examined for the number of antibiotic resistance genes present. We hypothesize that when exposed to multiple contaminants, bacteria will develop more resistance genes as a defense mechanism.

Activity Status as of January 31, 2016:
Experiments have been completed with methanogenic cultures. Methane production and antibiotic resistance genes were analyzed. We found no significant change in the number of *inti1* (gene associated with the ability of bacteria to integrate genes) and *mexB* (gene associated with multiple antibiotic resistance) genes present between control cultures and cultures to which triclosan and PFOS were added. At high concentrations (mg/L range) of PFC and 2,4 dichlorophenol (DCP, a compound that is known to "uncouple" cells, or make them leaky to H^+) antagonistic interactions were observed. 50 mg/L of PFOA and PFOS decreased the rate of methane production, while 50 mg/L of PFBS and PFBA appeared to have a minimal effect. When DCP was present in conjunction with PFOS or PFOA, toxic effects decreased. We are repeating these experiments with more data collection to enable the more precise calculation of rate coefficients. We are also performing additional experiments over a wider range of PFC concentrations to more precisely determine when these interactions occur.

We have also been studying the effects of PFCs on the quorum sensing pathway in bacteria. We have completed experiments organisms that are able to luminesce upon the diffusion of an added signal molecule into the cell. PFOS and PFOA (at 0.1 mg/L) decrease the ability of *Aliivibrio fischeri* to luminesce upon the addition of the signaling molecule. Follow up experiments are being performed to verify the concentrations at which this phenomenon is observed.

Activity Status as of July 31, 2016:
Experiments are being run with dilute methanogenic cultures. It has been demonstrated that community function is more susceptible to the effects of PFCs and co-contaminants when dilute, potentially due to increased probability of PFC interaction with microorganisms. With this knowledge, the effects of PFCs and co-contaminants can be minimized by maintaining bacteria at higher densities.

Experiments with PFCs and co-contaminants have been performed. Thus far the interactions between the co-contaminants 2,4-dichlorophenol (DCP), 2,4-dinitrophenol, 2,4-dichloroaniline, and pentacholorphenol at 1 mg/L have been tested with PFOS, PFOA, PFBS, and PFBA at 50 mg/L. At 50 mg/L only PFOS and PFOA display toxicity. Additionally, only the combination of DCP and PFOS resulted in synergistic toxicity (i.e., greater than additive toxicity). More co-contaminants and two additional PFCs ((1) perfluorohexane sulfonate, which has an identical head group to PFOS and PFBS, but a six-carbon fluorinated tail and (2) perfluorononanoic acid which has an identical fluorinated tail group to PFOS but a head group identical to PFOA and PFBA) will be tested to determine the characteristics that lead to synergistic toxicity. Data also shows that when present at only 5 mg/L, PFOS and PFOA are slow the degradation of DCP.

Activity Status as of January 31, 2017:
Experiments are continuing with dilute anaerobic digester cultures. Results have shown that the combination of 50 mg/L PFOS and 1 mg/L 2,4-dichlorophenol (DCP) consistently interact in an unusual way, with the organisms being effected both synergistically and antagonistically. Community structure and density, as well as small changes in PFC dose may play a role in this variability. Experiments are in progress pinpoint the reasons for this variability. The degradation of DCP was also tested in the dilute anaerobic digester cultures. DCP degrationa was inhibited at 5 and 50 mg/L PFNA, 50 mg/L PFHxS, 50 mg/L PFOS and 50 mg/L PFOA.

The effect of PFC mixtures was examined by adding aqueous film-forming foam (AFFF) to cultures. Because PFOS and PFHxS are the main ingredients of AFFF, the effect of AFFF was compared to that of PFOS, PFOS + PFHxS, and PFHxS. It was determined that when looking at the effect of PFCs on co-contaminant toxicity, AFFF behaved most closely to the combination of PFOS and PFHxS. This is significant as small chain (less than six carbon) PFCs have been thought to have minimal biological effects. In terms of the impact on degradation, AFFF behaved significantly different from PFOS and the combination of PFOS and PFHxS. In fact, little DCP degradation occurred in vials containing AFFF over a period of a month. The other compounds present in AFFF are therefore also important to consider, depending on the biological effect investigated.

Finally, a pure culture of the mutant bacteria *Aliivibrio fischeri* has been used to determine whether PFCs have an effect on quorum sensing, an important microbial communication strategy that is responsible for virulence and biofilm growth, among other things. Results showed that PFCs increase the quorum sensing response when signaling molecules are added. This effect appears to be dependent on the concentration and type of PFC as well as the growth state of bacteria. Results showed that the higher the concentration of PFC and the longer the chain length, the greater the quorum sensing response. PFOS also became toxic as concentrations were increased.

Again, these results show that PFCs alter microbial function at a range of concentrations that are relevant and realistic in different environments. For example, bacteria are not able to degrade toxic chemicals when in the presence of a range of PFCs at concentrations that one might find in a highly contaminated site (landfill or fire training area), their respiration is affected at these high concentrations as well. Perhaps most interesting is the ability of a large range of PFCs, both those compounds that have been phased out and those currently being used, to interfere with microorganism-to-microorganism communication or signaling. This type of communication is important in many microbial functions, including virulence and biofilm development. When in the presence of PFCs at much lower concentrations (relevant in sediment, landfills, and contaminated sites), bacteria respond to much lower quantities of “signal,” in essence, they turn on certain functions (like biofilm development) sooner than they otherwise would when PFCs are present.

Final Report Summary:
Experiments were performed with PFCs of varying fluorinated chain length and functional group. Perfluorobutanoate (PFBA), perfluorooctane (PFOA), perfluorononane (PFNA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS), containing 3, 7, 8, 4, 6, and 8 fully fluorinated carbons, respectively, were investigated. These compounds were studied because there are concerns about the health effects of long-chain PFCs and as a result, industries are switching to the use of more short chain PFCs such as the 4- and 3-carbon PFBS and PFBA, assuming that these compounds, being less hydrophobic, will be benign.

Because PFCs are surfactants, we hypothesized that they would interfere with bacterial membranes and membrane function, as bacterial membranes are essentially an assembly of surfactant-like phospholipids. Quorum sensing is a process that relies on intact bacterial membranes to work effectively. Quorum sensing is a process by which bacteria communicate, with many quorum sensing pathways requiring that signaling molecules diffuse passively through the bacterial cell membrane to reach a receptor within the cell. Quorum

sensing plays a role in a variety of bacterial processes, such as biofilm formation, pathogenicity, antibiotic production, and bioluminescence.

When a pure culture of a bacteria active in quorum sensing (*Aliivibrio fischeri*) was exposed to PFCs, effects on both bacterial metabolism and quorum sensing were observed. Exposure to higher concentrations (mg/L) of the 4-carbon and 6-carbon sulfonated PFCs increased metabolism, perhaps as a result of proton leakage across the membrane. Exposure to the 8-carbon PFOS, however, significantly decreased metabolism at 50 mg/L, suggesting that at this high concentration PFOS was toxic to *A. fischeri*. For the carboxylated PFCs, there was no metabolic indication of toxicity, but increases in metabolism were observed for the 8-carbon PFNA at 0.3-50 mg/L. The quorum sensing response, luminescence in *A. fischeri*, increased in a statistically significant manner with exposure to PFCs, increasing with PFC fluorinated chain length and exposure concentration. This effect was observed for the 4-carbon PFBS as well. Our results are particularly important because few functional effects have been documented with the shorter chain, 4-carbon and 6-carbon PFCs. Additionally, our results demonstrated that luminescence increased in PFOS- and PFNA- amended cultures at concentrations as low as 0.010 mg/L, a concentration that is frequently detected in human serum and environments such as landfills.

The observed increase in the quorum sensing response could be a result of several different mechanisms, only one of which is an increase in cell membrane permeability. To determine if membrane permeability did in fact increase as a result of PFC exposure, experiments were also performed to directly measure membrane permeability in live PFC-exposed *A. fischeri*. Live *A. fischeri* exposed to 50 mg/L of PFOS or PFNA were statistically more permeable to a membrane semi-permeable dye than unexposed bacteria. Similar results were observed for the PFCs with shorter fluorinated chains, though the increase in cell permeability was only statistically significant with 87% confidence in bacteria exposed to PFBS and PFHxS and with 91% confidence in bacteria exposed to PFOA. This trend mimics that observed with luminescence, with live cell permeability to dye increasing with increasing fluorinated chain lengths. Differences in the effect of sulfonated versus carboxylated PFCs were not evident in the permeability experiments. These results support the hypothesis that an increase in the quorum sensing response in *A. fischeri* upon PFC exposure is a result of increased bacterial membrane permeability.

Additional experiments were performed with mixed anaerobic cultures to determine the effect of PFC addition on metabolism in a complex culture. PFCs were tested alone and in mixtures, including the complex PFC-containing aqueous film forming foam (AFFF) used by firefighters. AFFF, PFOS, and PFNA were toxic to mixed anaerobic cultures, as evidenced by decreased methane production. The effect of AFFF on methane production could be modeled by the dominant PFC in the AFFF formula, PFOS. Because PFCs can be found in complex mixtures with other co-contaminants, the effect of PFCs when in the presence of a possible co-contaminant, dichlorophenol (DCP) was also tested. DCP was chosen as a model co-contaminant because we wanted to test the hypothesis that certain longer-chain and hydrophobic PFCs (i.e., PFOS or AFFF) could compete with DCP for sorption sites on/in the cell membrane, where these compounds would both be likely to have a negative effect. Interestingly, the combination of DCP with either PFOS, PFOS+PFHxS, or AFFF was less toxic than PFOS, PFOS+PFHxS, or AFFF only. In addition, we saw that the presence of PFOS or PFOS+PFHxS significantly decreased the sorption of DCP to the bacterial solids when compared to DCP only. The results with AFFF are complex and likely clouded by the many different, and unknown, compounds present in AFFF. These results suggest that PFOS does compete with, and potentially displace DCP on/in the cell membrane, perhaps moderating the toxicity observed with PFC-co-contaminant mixtures. More work is needed to understand exactly how AFFF moderates co-contaminant sorption into/onto cell surfaces. Finally, AFFF and the longer-chain PFOS, PFHxS, PFOA, and PFNA also had an impact on the anaerobic community's ability to degrade DCP. The magnitude of degradation inhibition was correlated to fluorinated chain length, functional group, and concentration, with the longer the fluorinated chain and the higher the concentration of PFC present, the more dramatic the degradation inhibition. The 4- and 3-carbon PFBS and PFBA, respectively, did not significantly inhibit DCP degradation. The effect of AFFF on DCP degradation was much greater than that observed for PFOS (the PFC most prevalent in

AFFF), suggesting that the hydrocarbons in the AFFF also slowed DCP degradation beyond that observed with only the PFCs.

ACTIVITY 2: Understand the chemical properties of co-contaminants that make them more harmful to bacterial function (nitrogen cycling and carbon decomposition) in the presence of perfluorinated chemicals

Description: We will also investigate the effect of size and hydrophobicity on transport in the presence of PFCs. This will be done by repeating the experiments described for activity 1 for two additional compounds that allow us to investigate a range of similarly structured co-contaminants with different hydrophobicities and sizes: atrazine and hexazinone. Additional experiments will be performed with a diffusion chamber to measure the transport of H^+ or co-contaminants across a model artificial cell membrane. The diffusion chamber will be created such that the upstream chamber has a large volume in which the concentrations of various dissolved species are held relatively constant and the downstream chamber is very small, allowing for the rapid concentration of species as they diffuse from the upstream chamber into the downstream chamber. The two chambers will be separated by a plate containing a small (1 mm diameter) hole. An artificial cell membrane can be created in the hole with phospholipids, much like a soap bubble, resulting in a system in which chemicals can be placed in only the upstream chamber (H^+ or melamine for example) and their diffusion across the artificial membrane to the downstream chamber can be monitored. Experiments will be performed with no membrane present (only a hole), with membranes containing phospholipids only (modeling a healthy unaffected cell membrane), and with membranes containing both phospholipids and various quantities of the three PFCs present (modeling a chronically PFC-exposed cell membrane). In this manner the transport of materials across a model cell membrane can actually be measured and used to corroborate the results observed above.

Summary Budget Information for Activity 2:

ENRTF Budget: $ 89,000
Amount Spent: $ 87,935
Balance: $ 1,065

Activity Completion Date: March 31, 2017

Outcome	Completion Date	Budget
1. In cultures of nitrogen-cycling bacteria, determine how co-contaminant chemistry affects bacterial function when present in mixtures containing perfluorinated chemicals	03/31/2016	$46,500
2. In cultures of carbon-cycling bacteria, determine how co-contaminant chemistry affects bacterial function when present in mixtures containing perfluorinated chemicals	03/31/2017	$42,500

Activity Status as of January 31, 2015:
Experiments using the diffusion cell as referenced in this proposal were not reproducible, hence we have begun to develop alternative methods to mechanistically investigate diffusion across model cell membranes. As a result, we are currently collaborating with Dr. Lisa Prevette at the University of Saint Thomas to develop methods using liposomes. Liposomes are uniform lipid bilayer suspensions of approximately 100 nm diameter. We have been able to reliably and reproducibly produce liposomes, both with and without PFCs as component of the bilayer. We have monitored the uptake of fluorescent dyes into intact and “leaky” liposomes and are currently working on developing methods to monitor the leakage of fluorescent dyes out of similar intact and “leaky” liposomes. Once fully tested with negative and positive controls, these methods will then be used to determine the permeability of liposomes made with a variety of types of PFCs over a range of concentrations. These results will provide a mechanistic underpinning to the results obtained in Activities 1 and 2, allowing us to extrapolate effectively to systems other than those that we have studied.

Activity Status as of July 31, 2015:
Methods have been established to monitor liposome “leakiness” using a fluorescent dye and quencher. Liposomes serve as a model for bacterial cell membranes. Low concentrations of PFOS do not appear to

significantly increase the leakiness of liposomes. Additional experiments are planned with higher concentrations of PFOS and a range of concentrations of additional perfluorinated compounds (PFCs). Additionally, we are looking at other methods to check liposome stability so that the response of liposomes to changes in temperature and ionic strength can be monitored upon PFC exposure as well as the permeability of liposomes to hydrogen ions.

Activity Status as of January 31, 2016:
Experiments have been performed with the method described previously; nevertheless, the method was not able to provide clear data as it could not be confirmed that the PFCs were incorporated into the liposomes. Plans have been made with Dr. Nathalie Tufenkji to complete experiments to determine if PFCs increase or decrease membrane fluidity, and at what concentration. This method will utilize a quartz crystal microbalance (QCM).

Activity Status as of July 31, 2016:
Experiments with model bilayers using the Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) technique have been performed at McGill University. Results show that all PFCs deposit onto model cell membranes and that deposition is dependent on the dose of the PFC (more deposition at higher PFC concentrations). Additionally, when PFCs are present at 50 mg/L, PFOS shows the most deposition, followed by PFOA. PFBA deposits the least amount onto the model cell membrane. This technique was also used to look at changes in phase transition temperature of the model membrane upon exposure to PFCs. A membrane's phase transition temperature is dependent on the interactions between the phospholipids and is a measurement of membrane disruption and membrane fluidity; if compounds alter the interactions between phospholipids in the model membrane, they may also alter phase transition temperature. When exposed to concentrations of PFOS above 5 mg/L and PFOA at 50 mg/L, no phase transition was detected. This suggests that at these concentrations, the model cell membranes were highly disrupted. At a concentration of 50 mg/L PFBS, the phase transition temperature of the model membrane shifted to lower temperatures (indicating greater membrane fluidity in the presence of PFBS, a newer PFC); PFBA had very little effect on the phase transition temperature of the model cell membrane at concentrations up to 50 mg/L.

Preliminary experiments have also been completed to assess the changes in surface charge in liposomes after exposure to PFCs. These results will clarify whether PFCs can also interfere with cell function by interacting with membrane proteins in addition to the membrane lipids. Results thus far indicate that exposure to 50 mg/L PFOA creates a greater negative charge to liposomes. Changes in the surface charge of the membrane could impact permeability and surface interactions. Experiments with liposomes containing proteins will be performed over the next reporting period. Methods are also being refined to investigate changes in the surface charge of bacteria as well.

Activity Status as of January 31, 2017:
Experiments utilizing the quartz crystal microbalance (described above) were repeated at the University of Wisconsin – Madison to assess the impact of PFNA on model cell membranes. PFNA was tested to specifically investigate the effect of PFC functional group on model cell membranes, as PFNA has the same number of fluorinated carbons as PFOS, but the same functional group as PFOA. It appears that the functional group is an important component with respect to lipid disruption, as PFOS caused greater model membrane disruption than PFNA at lower concentrations.

Experiments have been performed to assess the effect of PFCs on bacteria surface charge. Preliminary results show that PFCs increase the negative charge of *Aliivibrio fischeri* and *Staphylococcus epidermidis*. Similar to the results seen in the quorum sensing and QCM-D experiments, the effect is dependent on concentration and PFC chain length: the higher the PFC concentration and the longer the chain length, the greater the effect. *A. fischeri* is Gram negative, while *S. epidermis* is Gram positive. Preliminary results showed that Gram positive bacteria may accumulate more PFCs than Gram negative. Experiments are being repeated for confirmation.

The partitioning behavior of PFCs to bacteria will also be determined using liquid-chromatography mass spectrometry. This could help to determine where PFCs are accumulating (surface of bacteria vs. bacteria interior). The shorter chain PFCs did not seem to cause differences in surface charge which could because (1) they are more hydrophilic and do not accumulate in bacteria or (2) they are being taken up into bacteria and are not sticking to the bacterial surface. These results will be completed in the next two months.

These results show that PFCs stick to bacteria and that they interact with model microbial membranes and disrupt those membranes, even at lower concentrations. Because intact membranes are critical for controlling microbial signaling and respiration, this is likely the reason why we observe the functional effects that we do in Activity 1.

Final Report Summary:
PFCs partitioned to liposomes (spheres of model bacterial membranes) and bacteria, but only changed the surface charge of the liposomes. This indicates that PFCs likely incorporated into the cell membranes of the bacteria, which are located inside the cell wall and did not appreciably associate with/bind to the outer bacterial surface. PFC incorporation was greater for longer fluorinated chain lengths, most likely as a result of increased hydrophobicity. The effect of PFCs on the surface charge of the liposomes was also greater for the PFCs with longer fluorinated chains, again, indicating that the longer, more hydrophobic PFCs, such as PFOS and PFOA, associated more strongly with cell membranes (real and model). Differences in the PFC functional groups (carboxylates versus sulfonates) played a less dramatic role, although the sulfonated PFCs did change the surface charge of liposomes to a greater extent and also incorporated more into bacteria than the carboxylated PFCs. Finally, incorporation of PFCs into living and dead bacteria was statistically equivalent, indicating that PFAS incorporation into cells is a passive process.

Experiments utilizing the quartz crystal microbalance and model lipid bilayers (flat chemical models of bacterial membranes) also supported the hypothesis that PFCs incorporate inside bacterial membranes. Deposition of PFCs did not occur on the surface of the model lipid bilayers, but rather, the PFCs deposited within the lipid bilayer itself. In addition, once deposited, the PFCs changed the fluidity of the lipid bilayers, again showing that the PFCs integrated into the lipid bilayer where they altered lipid-lipid interactions. The change in fluidity is indicative of a change in membrane permeability, which supports the results observed in Activity 1. Deposition and fluidity increased with PFC exposure concentration, with measurable deposition and changes in fluidity occurring at concentrations as low as 0.1 mg/L for PFOS. This suggests that this process is an equilibrium-type chemical process, which was also indicated by the similar partitioning values with live and dead bacteria. Once again, PFC deposition was greater for longer fluorinated chain lengths and for the sulfonated PFCs as compared to the carboxylated PFCs. Interestingly, deposition and changes in lipid bilayer fluidity were observed with both PFBS and PFBA, the short-chain “replacement” PFCs. Nevertheless, experiments with PFBS and PFBA were only performed with high concentrations (50 mg/L) that under typical conditions are not likely to be environmentally relevant.

This research shows that PFCs incorporate into bacterial membranes as a function of PFC chain length and functional group. This research also suggests that once incorporated, these chemicals alter membrane properties. This could lead to microbial toxicity, stress, and changes in function as demonstrated in Activity 1. The short-chain PFCs are not likely to significantly impact bacteria unless present at high concentrations, such as those found at spill sites or at fire-fighting training areas, but this may change as more of these short-chain “replacement” PFCs are used.

V. DISSEMINATION:

Description: The target audience for results from this research will be professionals in the area of wastewater treatment, landfill management, and industry. Specific targets will be environmental engineers and scientists in

academia, industry, state agencies such as the MDA and MPCA, and environmental consultants. Results will be disseminated through scholarly publications in peer-reviewed journals such as *Environmental Science and Technology*. Results from the research project will also be presented at regional conferences such as the *Minnesota Water* conference. Results will be used to target what compounds are most problematic to microbial function, when, where, and how to best culture bacteria to protect them.

Status as of January 31, 2015:
No dissemination efforts have been made, as the project is not advanced enough at this point.

Status as of July 31, 2015:
No dissemination efforts have been made, as the project is not advanced enough at this point.

Status as of January 31, 2016:
No dissemination efforts have been made, as the project is not advanced enough at this point.

Status as of July 31, 2016:
This research was presented at the Gordon Environmental Sciences: Water Conference. No papers have been published as of yet, but two manuscripts are expected to be submitted for publication by the next project update.

Status as of January 31, 2017:
This research is near the publication stage. Two papers are currently being prepared for submittal within the next few months. A third paper will be submitted by the end of the project.

Final Report Summary:
This research was presented at the Fourth International Symposium on Bioremediation and Sustainable Environmental Technologies (international in scope) and the Association of Environmental Engineering and Science Professors (AEESP) Biannual Conference (international in scope). The graduate student presenting at the AEESP conference was awarded a $650 travel award based on the quality of her research. This research was also presented at the regional Conference on the Environment. Three papers will be submitted for publication from the project and are being finalized. They should be submitted in the next 2 months and will be sent to LCCMR upon acceptance for publication.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Explanation
Personnel:	~~$ 235,500~~ $ 235,995	Over the course of the 3-year project, support for one graduate student for three years, undergraduate support for the summers, and support for the two PIs is budgeted. The PI (Novak) will require 4 weeks of salary a year and the Co-PI (Simcik) will require 10% salary per year. Fringe benefits for the PIs at UMN are set at 33.6% by the University of Minnesota. The PIs will be responsible for project oversight, guidance of the graduate student, data interpretation and analysis, and report preparation and submission. One graduate student research assistant will devote 100% of

		their research time to the project over the 3-year project. Fringe benefits for the graduate student include tuition, health insurance, and summer FICA. Undergraduate/temporary worker support is also budgeted to assist the graduate student with experimental set-up, reactor maintenance, and sample processing for analysis.
Equipment/Tools/Supplies:	~~$ 42,069~~ $ 41,574	Funds ($12,500, $13,500, and ~~$15,500~~ 15,005) are requested for materials, supplies, consumables, analytical costs and repair/upkeep associated with the LC-MS. Required materials include, but are not limited to: pipette tips, glassware, solid phase extraction cartridges for extractions, chemicals for standards and experiments, analytical consumables, analytical fees, solvents, reagents, gloves, digital data storage media, and laboratory notebooks. Funds are requested for $1,000 to be allocated to the purchase of a shared-use ion chromatograph ($35,000 total cost) for use on this project.
Travel	$ 1,431	Funds are requested for travel to McGill University in Montreal, Canada, to learn a technique to measure the integrity of model microbial membranes using a quartz crystal microbalance in Nathalie Tufenkji's laboratory.
TOTAL ENRTF BUDGET:	**$ 279,000**	The total proposed project amount is $279,000. No indirect costs for the University of Minnesota are included in the budget.

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: 2.79

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

Other Funds: N/A

VII. PROJECT STRATEGY:

A. Project Partners: The project team consists of the Principal Investigator (PI) Paige Novak (University of Minnesota) and co-PI Dr. Matt Simcik (UMN). Novak is an expert on microbial systems and Simcik is an expert on the analysis of trace chemicals, including perfluorinated chemicals. The PI and co-PI will work together on all aspects of the research.

B. Project Impact and Long-term Strategy: The proposed research fits into a larger research agenda centered at the University of Minnesota that is focused on the problem of Contaminants of Emerging Concern. When taken

together, this research and other complementary current and prior research in this area will provide a more complete picture of how to safeguard our environment through engineering different types of treatment systems (systems that encourage the development of protective bacterial "coats" for example). In particular, an emphasis on understanding threats to water quality in Minnesota can help us to then engineer better treatment methods to promote cleaner water and therefore healthier fish and aquatic ecosystems.

C. Spending History: N/A

VIII. ACQUISITION/RESTORATION LIST: N/A

IX. VISUAL ELEMENT or MAP(S): See attached graphic.

X. ACQUISITION/RESTORATION REQUIREMENTS WORKSHEET: N/A

XI. RESEARCH ADDENDUM: See attached Research Addendum

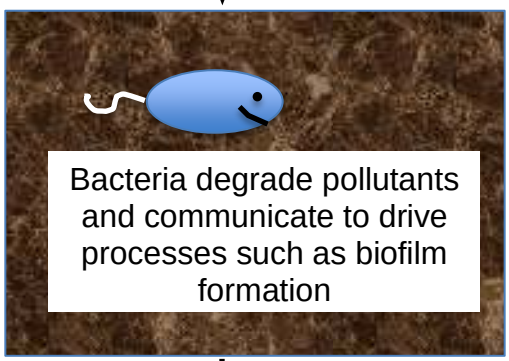
XII. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than January 31, 2015, July 31, 2015, January 31, 2016, July 31, 2016, and January 31, 2017. A final report and associated products will be submitted between June 30 and August 15, 2017.

Environment and Natural Resources Trust Fund
M.L. 2014 Project Budget

Project Title: Methods to Protect Beneficial Bacteria from Contaminants to Preserve Water Quality
Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 03b
Project Manager: Paige J. Novak
Organization: University of Minnesota
M.L. 2014 ENRTF Appropriation: $279,000
Project Length and Completion Date: 3 Years, June 30, 2017
Date of Report: August 11, 2017

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Revised Activity 2 Budget (8/11/17)	Amount Spent	Activity 2 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	***Understand how and why perfluorinated chemicals alter bacterial function (nitrogen cycling and carbon decomposition) alone or in mixtures with a co-contaminant***			***Understand the chemical properties of co-contaminants that make them more harmful to bacterial function (nitrogen cycling and carbon decomposition) in the presence of perfluorinated chemicals***					
Personnel (Wages and Benefits)	$157,000		$0	~~$78,500~~	$78,995		$0	$235,995	$0
Paige Novak, PI (~~$39,800~~ $27,384 salary, ~~$13,400~~ $9,302 fringe, 33.6% fringe rate; total for 3 years; 8% effort). Project supervision, provide guidance on the experimental set-up and microbial culturing)		$29,588				$7,098			
Matt Simcik, Co-PI (~~$26,500~~ $38,284 salary, ~~$8,900~~ $12,788 fringe, 33.6% fringe rate; total for 3 years; 10% effort). Project supervision, guidance on the analysis methods),		$30,416				$20,656			
One Graduate Research Assistant (~~$75,500~~ $79,790 salary, $55,800 fringe (includes healthcare and tuition); total for 3 years; will conduct laboratory experiments, analyze results)		$86,817				$48,772			
One Undergraduate Research Assistant or Temporary Worker (~~$15,600~~ $12,646 salary; 13 weeks (i.e., summer), full time per year for three years; will assist with analysis and laboratory experiments)		$10,178				$2,469			
Equipment/Tools/Supplies									
Equipment allocation for the purchase of a shared-use ion chromatograph ($35,000 total cost) for use on this project	$1,000	$1,000	$0					$1,000	$0
Laboratory supplies including, but not limited to: chemicals for experiments (PFCs and co-contaminants, media constituents), oxygen probes, analysis needs such as standards, gas tanks, needles, septa, consumables such as gloves and solvents.	$28,569	$28,569	$0	~~$9,000~~	$8,505	$7,440	$1,065	$37,074	$1,065
Diffusion cell construction	$0	$0	$0	$0	$0	$0	$0	$0	$0
Equipment repair and maintenance	$2,000	$2,000	$0	$1,500	$1,500	$1,500	$0	$3,500	$0
Travel									
International travel for methodological training	$1,431	$1,431	$0	$0	$0	$0	$0	$1,431	$0
COLUMN TOTAL	**$190,000**	**$190,000**	**$0**	**~~$89,000~~**	**$89,000**	**$87,935**	**$1,065**	**$279,000**	**$1,065**

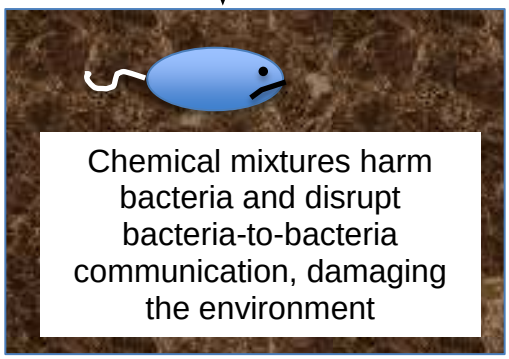
Waste



Clean water

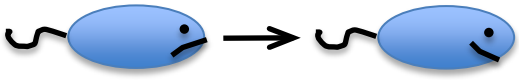
The benefit of bacteria

Waste (containing manmade chemical mixtures, perfluorinated compounds)



Dirty water

The problem



Determine why bacteria are harmed by chemical mixtures and perfluorinated compounds so that it can be prevented

Our proposed project