Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2017 LCCMR Work Plan

Date of Submission: September 14, 2016

Date of Next Status Update Report: January 1, 2018

Date of Work Plan Approval: 06/07/2017

Project Completion Date: June 30, 2020

Does this submission include an amendment request? _No_

PROJECT TITLE: Assessment of Microbes for Improving Wild Rice Restoration

Project Manager: Chan Lan Chun

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Location: Northeastern

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$334,000**
	Amount Spent:	**$0**
	Balance:	**$334,000**

Legal Citation: M.L. 2017, Chp. 96, Sec. 2, Subd. 03f

Appropriation Language:
$334,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota, Natural Resources Research Institute, to evaluate the microbial communities and nutrients associated with wild rice and competing vegetation, with the goal of enhancing restoration success to increase the abundance of wild rice. This appropriation is available until June 30, 2020, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Assessment of Microbes for Improving Wild Rice Restoration

II. PROJECT STATEMENT:

Wild rice (*Zizania palustris*) is an ecologically and culturally important plant in Minnesota and its state grain. Wild rice was historically abundant in northern Minnesota but its abundance and distribution have been reduced due to environmental contaminants, habitat destruction, physical disturbance, and establishment of competitive or invasive plant species. In recent years, there have been collaborative efforts to restore wild rice wetlands by reducing competitive or invasive plant cover, seeding, and other means; this results in improved wildlife habitat and increased opportunities for wild rice harvest. Even with many ongoing restoration efforts, however, post-restoration monitoring is not common. When such monitoring does occur, the evaluation is primarily based on wild rice results, which may not be sufficient to understand other factors influencing restoration success. We hypothesize that changes in sediment microbial communities and nutrients in wild rice beds are key determinants in the re-establishment of self-sustaining wild rice populations. Microbes are known as primary mediators of plants' growth, adaptation, and competitive success. Particularly, plant-microbe interactions in the rhizosphere are directly associated with nutrient uptake and disease/stress tolerance, resulting in changes in plant community composition and ecology. Such associations, which are not well-characterized, can be important to restoration success and provide a promising opportunity to develop targeted control of competitive or invasive plants such as pickerel weed, arrowheads, and narrow-leaf cattails. The overall goal of this project is to improve restoration activities that increase the abundance and distribution self-sustaining wild rice beds by assessing re-establishment of wild rice stands in comparison with competitive or invasive plants species, and evaluating changes in sediment microbial communities and nutrients in wild rice beds. This project will examine total microbial (both fungal and bacterial) communities and nutrients in the rhizosphere of both wild rice and competitive or invasive plants in well-established wild rice wetlands as well as pre-restoration and post-restoration sites. The results of this work will identify microbial and nutrient associations in self-sustaining wild rice wetlands and apply the information to develop a management strategy to promote restoration success in the St. Louis River estuary and wild rice lakes in Minnesota.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of January 1, 2018:

Project Status as of July 1, 2018:

Project Status as of January 1, 2019:

Project Status as of July 1, 2019:

Project Status as of January 1, 2020:

Overall Project Outcomes and Results:

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Collect vegetation data and sediment samples

Description: In this project, sampling site will be selected based on the presence of self-sustaining wild rice, past restoration sites with and without success, and current restoration sites. Examples of potential sampling sites include areas with self-sustaining wild rice (such as northwest portions of Rask Bay in St. Louis River estuary; Kettle Lake in Carlton County, and St. Louis River at Norway Point and/or Skibo landing in St. Louis County) and restoration sites (such as North Bay, south portions of Rask Bay, and Radio Tower Bay in St. Louis River estuary; and Big Rice Lake in St. Louis County). At each sampling site, we will collect vegetation data to assess establishment of wild rice stands in comparison with competitive or invasive plants species and conduct plant, soil, and water sampling to examine total microbial (both fungal and bacterial) communities and nutrients in the rhizosphere of both wild rice and competitive or invasive plants. Airboat will be used to access sampling sites since it is an essential and effective way to access wetland with emergent plants without damaging wild rice plants. Key information will include the density and cover of wild rice and presence and cover of coexisting or competing emergent, floating-leaf, and submerged plants. We will choose pairs of sample sites where we can sample natural or managed wild rice stands and stands of competing or invasive plants in the same or similar wetlands. Vegetation in each stand (wild rice or competitors) will be sampled with sets of small plots (0.25 m^2); sampling the first year will be designed to determine adequate sample size using a species-area curve. Within each plot, density of wild rice will be determined by counting stems of wild rice and all floating-leaf and emergent species within the plot. The overall cover of all submerged aquatic plants (e.g. sparse, patchy, or dense) will be recorded in field form. All plants within the plots will be identified to species if possible, or to the lowest taxonomic level possible for plants that are not flowering or fruiting and therefore cannot be identified to species. Plant nomenclature will follow the current MNTaxa lists available online at the Minnesota Department of Natural Resources website.

Concurrently, sediment samples associated with wild rice and other coexisting or competing plants will be collected from the sites. Typically, plant root and sediment samples will be collected by using a soil core sampler, which is hammered into the sediment. The cores from field sites will be placed in sterile Whirlpak bags or PTFE bottles and kept in a cooler until transported to the laboratory. Roots are collected from the soil core to harvest three soil fractions: bulk soil (the soil remaining after picking out the roots from the core), rhizosphere (the soil adhering to the roots), and rhizoplane (the washed roots). The rhizoplane fraction will be collected from homogenization of the roots washed with sterile phosphate saline solution twice. Each fraction is weighed and the subsamples will be stored as it is at -20 °C for DNA extraction. To determine the influence of microbial communities in water column on rhizosphere microbial community of wild rice and coexisting plants, we will collect overlying water and sediment porewater near the sampling plots. We will monitor physicochemical parameters including water temperature, pH, dissolved oxygen, conductivity, and redox potential at sampling site and collect water samples for chemical and microbiological analyses. The water samples are collected in two collection bottles, one with acid preservatives and the other without acid preservatives. Acid bottles will be tested for total N (nitrate + nitrite), total phosphorus, cations and non-acid bottles for nitrite, soluble phosphorus, ammonia, total suspended solid, other anions, and microbial community analyses. For microbial community analyses, the water samples will be filtered through 0.22 µm, polyethane sulfonate filters to trap bacterial microorganisms.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 123,181
Amount Spent: $ 0
Balance: $ 123,181

Outcome	Completion Date

1. Monitoring and compilation of vegetation data from sampling sites	*November 2019*
2. Collection of sediment associated with wild rice and other coexisting plants	*November 2019*
3. Field water quality measurement and sampling	*November 2019*
4. Evaluate restoration success based on wild rice and vegetation data	*March 2020*

Activity 1 Status as of January 1, 2018:

Activity 1 Status as of July 1, 2018:

Activity 1 Status as of January 1, 2019:

Activity 1 Status as of July 1, 2019:

Activity 1 Status as of January 1, 2020:

Final Report Summary:

ACTIVITY 2: Identification of microbial communities and nutrient associated with wild rice
Description: We will characterize total microbial (both fungal and bacterial) communities and the level of nutrients (both macro- and micronutrients) in three fractions: bulk soil, rhizosphere soil, and the rhizoplane fraction of each sample. Total microbial communities of subset samples will be determined using culture-independent methods (amplicon-based sequencing) and microscopic analyses (fluorescence microscopy and scanning electron microscopy). Genomic DNA of microbial cells from three fractions of bulk soil, rhizospheric soil, and rhizosphane of each sample and water samples will be extracted using the Powersoil™ DNA isolation kit and will be used for Next Generation, Illumina sequencing, of total microbial community populations. DNA sequence analysis of triplicate samples of each fraction will be done using two independent primer sets targeting archaeal, bacterial and fungal populations. For archaeal and bacterial community analysis, 16S rRNA will be amplified by polymerase chain reaction (PCR) using barcoded primers. The internal transcribed spacer region will be used for amplification of eukaryotes, broadly, with a focus on microbial eukaryotic lineages for fungi. The amplicons from individual samples will be pooled together, and the multiplexed amplicons will be paired-end sequenced on an Illumina/Solexa Sequencer at the University of Minnesota Genomics Center. We will also perform total metagenomic analyses on a few selected wild rice samples to obtain functional information about microbial genes present in samples. The DNA sequence data obtained in our studies will be sorted, trimmed, verified and aligned to a taxonomic database for assessment of phylogenetic diversity using the mothur and QIIME software programs. The taxonomic signature of microorganisms in each sample will be compared within and across samples at all time points and statistically analyzed. Comparisons of bacterial constituents of environmental samples, between sites and years, will be determined by examining the numbers and types of operational taxonomic units. Species diversity, and richness, evenness, and rarefaction analysis will also be determined. Furthermore, to determine the influence of microbial communities of water column on rhizosphere microbiome of wild rice and other coexisting plants, all water and bulk soil samples collected are treated as a source and compared against a sink community of rhizosphere and rhizoplane of plant root. The relative contribution will be determined using the SourceTracker.

In addition to metagenomic analyses, high resolution microscopies will be used to examine root anatomy, spatial distribution of microbes on root, and elemental partitioning such as iron plaques. Scanning electron microscopy (SEM) will be used for high resolution imaging and microanalytical platform of rhizosphere of wild rice. Initially, the root samples will be observed using cryo-SEM system after flash-freezing them using liquid nitrogen. Furthermore, the spatial distribution and relative abundance of the bacteria-root environment will be investigated using a combination of species specific fluorescent stains, non-specific fluorescent stains, and fluorescence in situ hybridization probes for differentiating the bacteria associated with the plant root from root itself. Taken together, this activity will contribute to the overall understanding of microbial biodiversity and ecology of wild rice and competing plants. The comparison of rhizosphere microbiome and nutrients between wild rice and competitive or invasive aquatic plants will identify the contribution of plant-microbe interactions to restoration success as well as improve management strategies accordingly.

Summary Budget Information for Activity 2:

ENRTF Budget: $ 195,293
Amount Spent: $ 0
Balance: $ 195,293

Outcome	Completion Date
1. Sample processing and DNA extraction	December 2019
2. Identify rhizosphere microbial communities associated with emergent aquatic plants using DNA sequencing	May 2020
3. Determine the level of nutrients and genes relating microbial nutrient cycling	December 2019
4. Correlation analysis of microbial communities/nutrient with restoration success	March 2020

Activity 2 Status as of January 1, 2018:

Activity 2 Status as of July 1, 2018:

Activity 2 Status as of January 1, 2019:

Activity 2 Status as of July 1, 2019:

Activity 2 Status as of January 1, 2020:

Final Report Summary:

ACTIVITY 3: Project data dissemination and public outreach
Description: Findings will be disseminated and archived via reports to LCCMR, peer-reviewed publications, and presentations at conferences. A fact sheet that summarizes our findings will also be distributed to wild rice restoration managers at state, tribal, and federal agencies. Several manuscripts will be written and submitted for publication in peer-reviewed journals. Results will be presented at state and national wildlife and ecology conferences. All publications resulting from this project will be made available through Open Access journal websites.

In addition, we will develop educational materials and opportunities for community discussion about wild rice restoration efforts in collaboration with wild rice managers and research partners at Fond du Lac Natural Resources, 1854 Treaty Authority, St. Louis River Alliance, and the Great Lakes Indian Fish and Wildlife Commission. These activities will provide opportunities to engage school and community groups in small-scale projects, build community support for wild rice restoration efforts. Moreover, these partner and researchers will take the results of our study into consideration as they make management decisions, and will work with us to ensure that our data products and research papers reach a broad audience within their agencies.

Summary Budget Information for Activity 3:

ENRTF Budget: $ 15,526
Amount Spent: $ 0
Balance: $ 15,526

Outcome	Completion Date
1. Development of educational materials and community discussions	April 2020
2. Dissemination of project data and results via seminars and workshops	June 2020

Activity 3 Status as of January 1, 2018:

Activity 3 Status as of July 1, 2018:

Activity 3 Status as of January 1, 2019:

Activity 3 Status as of July 1, 2019:

Activity 3 Status as of January 1, 2020:

Final Report Summary:

V. DISSEMINATION:
Description: Findings will be disseminated and archived via reports to LCCMR, peer-reviewed publications, and presentations at conferences. A fact sheet that summarizes our findings will also be distributed to wild rice restoration managers at state, tribal, and federal agencies. Results will be presented at state and national wildlife and ecology conferences. Our activity 3 also include public outreach plans.

Dissemination Status as of January 1, 2018:

Dissemination Status as of July 1, 2018:

Dissemination Status as of January 1, 2019:

Dissemination Status as of July 1, 2019:

Dissemination Status as of January 1, 2020:

Final Report Summary:
VI. PROJECT BUDGET SUMMARY:
A. Preliminary ENRTF Budget Overview:
***This section represents an overview of the preliminary budget at the start of the project. It will be reconciled with actual expenditures at the time of the final report.**

Budget Category	$ Amount	Overview Explanation
Personnel:	$ 238,844	Chan Lan Chun, Principal Investigator: $36,043 (66.3% salary, 33.7% benefits); 7.4% FTE each year for 3 years; Randall E. Hicks, co-PI: $23,476 Co-Investigator (66.3% salary, 33.7% benefits); 3.7% FTE each year for 3 years; George Host, co-PI: $18,743 (66.3% salary, 33.7% benefits); 4% FTE each year for 3 years; Carol Reschke, co-PI: $45,669 (72.6% salary, 27.4% benefits); 20% FTE each year for 3 years; Adelle Schumann, Research Technician: $23,185(72.6% salary, 27.4% benefits); 15% FTE each year for 3 years; Graduate Research Assistant $91,728 (82.4% salary, 17.6% benefits); 50% FTE each year for 3 years.
Professional/Technical/Service Contracts:	$ 24,796	$24,796 is budgeted to use airboat service for wild rice, invasive plants, water and sediment sampling from Natural Resources Department of the Fond du Lac Band of Ojibwe. Airboat is essential for access to wild rice stands without damaging wild rice plants, as well as the most efficient access into cattail stands for sampling invasive emergents. $40/hour including fuel × 200 hours boating time = $8,000 for use of airboat; $30/hr x 2 boat operators x 200 hrs boating time=$12,000 for boat operators' salary; 10 samplings per yr x 150 mi per sampling 3 yrs x $0.54 per mi =$2,430 for FdL truck & airboat trailer mileage to sample sites; $1,602 field survey lodging (2 rooms at $89/night x 9 nights) and $764 per diem for two boat operators ($51 per day x 2 people x 7.5 days) for overnight travel.
Equipment/Tools/Supplies:	$ 28,580	$4,600 is budgeted for plant and sediment sampling supplies for activity 1: Chest waders/boots $360; field guide to aquatic plants $100; soil knife $60; plant press supplies $110; plastic collecting bags $100; gaps

		batteries $50, sediment core samplers $250, water collection bottles $500, and porewater samplers $ 350, and 2 YSI field water quality probes $1410x2 $12,000 is budgeted for DNA/RNA extraction kit and molecular biological agents for activity 2: Nucleic acid extraction $600/kit*12kits for ~1000 samples= $7,200; PCR reagents $2/reaction*2000 samples=$4000; and various molecular markers $800 $5,700 for activity 1 and $5,780 for activity 2 are budgeted for chemical and expendable lab supplies: Disposable plasticware and lab supplies (e.g., Petri dishes, etc.) and miscellaneous chemicals (e.g., stains, solvents, antibiotics) Brochure and factsheet production for public dissemination of technical research results for activity 3: $500
Travel Expenses in MN:	$ 6,770	In-state sampling: $4,482: 10 samplings/yr x 150mi/sampling x 3yrs x$0.54/mi =$2430 + vehicle rental use $10/day x 45days=$450 + field survey lodging $89/night x 3nights x 2rooms x 3 summers=$1,602 for activity 1 In-state conference attendance: $2,288: Registration 2 people: $750; lodging $89/night x 3nights x 2rooms=$1,284; per diem/meals for 3 days $38.25+$51+$38.25=$127.50 x 2people = $255) for activity 3
Other: DNA sequencing and chemical analyses	$ 35,010	$22,010 is budgeted for Illumina sequencing and supercomputer usage fee: UMN Genomic Center (UMGC): Illumina Sequencing and library preparation ~ $4670/lane + $10 library prep/sample: ~800 samples per project = $8,000 (sample prep) + 3 lanes ($14,010) $13,000 is budgeted for chemical and nutrient analyses: UMD NRRI Central Analytical Lab: $65/sample x 200 samples
TOTAL ENRTF BUDGET:	**$334,000**	

Explanation of Use of Classified Staff: not applicable

Explanation of Capital Expenditures Greater Than $5,000: not applicable

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 3.0 FTEs

Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: not applicable

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
State	$159,766	$	Indirect fee (53% UMN Modified Total Direct Costs 7/1/17-6/30/18; 54% UMN Modified Total Direct Costs 7/1/18-6/30/20) are provided in-kind.
TOTAL OTHER FUNDS:	**$159,766**	**$**	

VII. PROJECT STRATEGY:

A. Project Partners:

Partners receiving ENRTF funding

- Chan Lan Chun, Assistant Professor, UMD, $36,044: Oversee the project and take lead on plant-microbe interaction and metagenomic analysis
- Randall Hicks, Professor, UMD, $23,476: Provide expertise on microbial ecology in St. Louis River Estuary (SLRE) wetland
- George Host, Initiative Director - Forest and Land, UMD, $18,743 and Carol Reschke, Senior Scientist, UMD, $45,669: provide expertise on distribution of wild rice and other emergent plants in SLRE and inland lakes and streams, plant community sampling and analysis.
- Adelle Schumann, Research Scientist, UMD, $23,185: Conduct microscopic study of plant-microbe interactions using SEM and fluorescence microscopy
- Graduate student, TBD, $91,728: Collect plants and sediment and take lead on the analysis of nutrient and metagenomic analyses.

Partners NOT receiving ENRTF funding

John Lindgren (MNDNR), Darren Vogt (1854 Treaty Authority), and Tom Howes (Fond du Lac Natural Resources), St. Louis River Alliance who are involved in restoration of wild rice will provide consultation and assistance for site selection, sampling and outreach program.

B. Project Impact and Long-term Strategy:

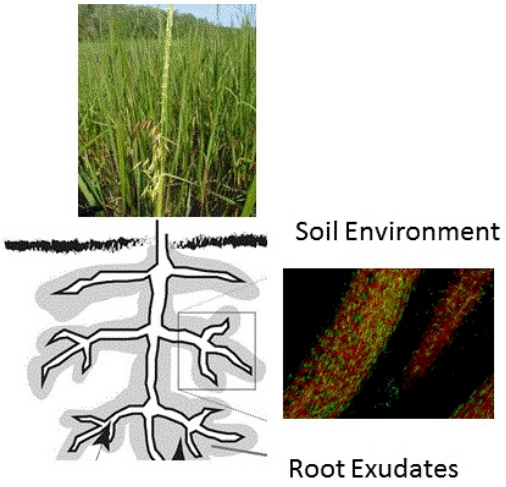
This project will provide key information on the microbial communities and sediment nutrients associated with wild rice and competing vegetation, with the goal of promoting restoration success to increase the abundance of self-sustaining wild rice in the SLRE and inland wild rice habitats in Minnesota. Restoration of wild rice wetlands will improve long-term protection of native species and aquatic biodiversity, and support management of this culturally and ecologically important natural resource in Minnesota.

VIII. REPORTING REQUIREMENTS:

- **The project is for 3 years, will begin on July 1, 2017, and end on June 30, 2020.**
- **Periodic project status update reports will be submitted January 1 and July 1 of each year.**
- **A final report and associated products will be submitted between June 30 and August 15, 2020**.

IX. VISUAL COMPONENT or MAP(S):

Soil nutrients and microbial communities will be key determinants in the re-establishment of self-sustaining wild rice populations.



- Wild rice growth, adaptation and competitive success
- Above-ground plant community composition and ecology

Microbial association with roots in soil environment

- Nutrient availability and cycling
- Enhanced mutualist (novel symbiosis)
- Disease defense

Environment and Natural Resources Trust Fund
M.L. 2017 Project Budget

Project Title: Assessment of Microbes for Improving Wild Rice Restoration
Legal Citation: M.L. 2017, Chp. 96, Sec. 2, Subd. 03f
Project Manager: *Chan Lan Chun*
Organization: *University of Minnesota Duluth*
M.L. 2017 ENRTF Appropriation: **$** *334,000*
Project Length and Completion Date: *3 years, June 30, 2020*
Date of Report:

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	Activity 3 Budget	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	***Collect vegetation data and sediment***			***Identification of microbiota and nutrient***			***Data dissemination and public outreach***				
Personnel (Wages and Benefits)	**$83,603**	**$0**	**$83,603**	**$142,503**	**$0**	**$142,503**	**$12,738**	**$0**	**$12,738**	**$238,844**	**$238,844**
Chan Lan Chun, Principal Investigator: $36,043(66.3% salary, 33.7% benefits); 7.4% FTE each year for 3 years.											
Randall E. Hicks,co-PI: $23,476 Co-Investigator (66.3% salary, 33.7% benefits); 3.7% FTE each year for 3 years											
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Graduate Research Assistant: $91,728 (82.4% salary, 17.6% benefits); 50% FTE each year for 3 years											
Professional/Technical/Service Contracts	**$24,796**	**$0**	**$24,796**	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$24,796**	**$24,796**
$24,796 is budgeted to use airboat service for wild rice, invasive plants, water and sediment sampling from Natural Resources Department of the Fond du Lac Band of Ojibwe. Airboat is essential for access to wild rice stands without damaging wild rice plants, as well as the most efficient access into cattail stands for sampling invasive emergents. $40/hour including fuel × 200 hours boating time = $8,000 for use of airboat; $30/hr x 2 boat operators x 200 hrs boating time=$12,000 for boat operators' salary; 10 samplings per yr x 150 mi per sampling* 3 yrs x $0.54 per mi =$2,430 for FdL truck & airboat trailer mileage to sample sites; $1,602 field survey lodging (2 rooms at $89/night*9 nights) and $764 per diem for two boat operators ($51 per day x 2 people x 7.5 days) for overnight travel.											
Equipment/Tools/Supplies	**$10,300**	**$0**	**$10,300**	**$17,780**	**$0**	**$17,780**	**$500**	**$0**	**$500**	**$28,580**	**$28,580**

$4,600 is budgeted for plant and sediment sampling supplies for activity 1: Chest waders/boots $360; field guide to aquatic plants $100; soil knife $60; plant press supplies $110; plastic collecting bags $100; gaps batteries $50, sediment core samplers $250, water collection bottles $500, and porewater samplers $ 350, and 2 YSI field water quality probes $1410x2											
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Brochure and factsheet production for public dissemination of technical research results for activity 3: $500											
Travel expenses in Minnesota	**$4,482**	**$0**	**$4,482**	**$0**	**$0**	**$0**	**$2,288**	**$0**	**$2,288**	**$6,770**	**$6,770**
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Other	**$0**	**$0**	**$0**	**$35,010**	**$0**	**$35,010**	**$0**	**$0**	**$0**	**$35,010**	**$35,010**
$22,010 is budgeted for Illumina sequencing and supercomputer usage fee: UMN Genomic Center (UMGC): Illumina Sequencing and library preparation.~ $4670/lane + $10 library prep/sample: ~800 samples per project = $8,000 (sample prep) + 3 lanes ($14,010)											
$13,000 is budgeted for chemical and nutrient analyses: UMD NRRI Central Analytical Lab: $65/sample x 200 samples											
COLUMN TOTAL	**$123,181**	**$0**	**$123,181**	**$195,293**	**$0**	**$195,293**	**$15,526**	**$0**	**$15,526**	**$334,000**	**$334,000**