2007 Project Abstract
For the Period Ending June 30, 2009

PROJECT TITLE:	**Neutralization of Reed Canary Grass Root Exudates**
PROJECT MANAGER:	**Bradley J. Cook**
AFFILIATION:	**Minnesota State University-Mankato**
MAILING ADDRESS:	**Department of Biological Sciences** **242 Trafton Science Center S**
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FUNDING SOURCE:	**Environment and Natural Resources Trust Fund**
LEGAL CITATION:	**ML 2007, [Chap._30_], Sec.[_2_], Subd._4j_.**

APPROPRIATION AMOUNT: $ 115000

Overall Project Outcome and Results

Reed canary grass (*Phalaris arundinaceae*; hereafter Pa) is an aggressive plant invading wetlands in the Midwest. Invasion by Pa leads to a reduction of native plant diversity and loss of wetland functionality. Our ability to control invasion by Pa and reestablish native plant communities has been unsuccessful because of our limited understanding of the mechanisms that allow Pa to become invasive. The study of plant-soil feedbacks as a mechanism for dominance is a two-step process: plants alter their soil microbial community; and the altered soil microbial community has a positive feedback on plant growth or a negative feedback on neighboring plants. Results from three experiments comparing soil microbial communities and plant growth revealed that *Phalaris arundinacea* (Pa) used plant-soil feedbacks to outcompete tussock sedge (*Carex stricta*; hereafter Cs).

In a soil training experiment, Pa and Cs cultured their soil microbial communities in a manner that differed in both magnitude and composition. Soil training had a neutral feedback on Pa growth and a negative feedback on Cs.

In our first reciprocal transplant experiment, growth of Pa and Cs was greater in their corresponding native soils than in the soil of the other species. Thus, both plants receive positive feedback from their native soil microbial communities. Soil microbial communities were similar when cultivated by Pa regardless of soil type, and Cs soil microbial community catabolic activity depended on soil type.

In our second reciprocal transplant experiment, the effects of competition were dependent on soil microbial communities. Pa growth was best in competition with Cs in Cs-native soils and Pa-sterile soils. Competition did not affect the growth of Cs; however, Cs growth was least in native soils from Pa and Cs. In sterile soils, soil microbial communities depended on the type of competition. In native Pa soils, heterospecific competition had a greater effect on soil microbial communities than did conspecific competition.

Denaturing gradient gel electrophoresis (DGGE) analysis indicated that Pa SMCs were stable and of low diversity, but Cs SMCs were dynamic and of comparatively high diversity.

Bioassays and gas chromatography-mass spectrometry (GC-MS) analyses revealed the presence of methyl esters of fatty acids known to have antimicrobial activity.

Our results suggest that Pa does not use alleopathy, but is induced to produce an antimicrobial compound that has a strong, directional effect on soil microbial communities, which promotes its growth and inhibits the growth of neighboring plants.

Project Results Use and Dissemination

Portions of Results 1, 2, and 3 have been written as a manuscript (*A plant-soil feedback as a mechanism for the invasive success of Phalaris arundinacea*) and is being revised for publication. A second manuscript including Results 1-5 is in preparation by the investigators.

Portions of this work were presented:

1) as an invited talk at the University of Bern, Switzerland (8/08)
2) at the 93rd Annual Ecological Society of America Meeting; Milwaukee, WI. (8/08)
3) at the 13rd "Annual Conference of the Wisconsin Wetland Association; Oconomowoc, WI. (2/08)
4) (two papers) at the North American Lake Managers Society (NALMS) International Conference; Hartford, CT. (10/09)
5) (four papers) at the 2008 and 2009 Minnesota State University Undergraduate Research Conference (4/08 and 4/09)

In addition, portions of this work were used for a M.S. thesis project, as class exercises in undergraduate courses, and as several undergraduate independent research projects at Minnesota State University.

Trust Fund 2007 Work Program Final Report

Date of Report: December 18, 2009
Trust Fund 2007 Work Program Final Report
Date of Work program Approval: June 5, 2007
Project Completion Date: June 30, 2009

I. PROJECT TITLE: Neutralization of Reed Canary Grass Root Exudates

Project Manager: Bradley J. Cook
Affiliation: **Minnesota State University-Mankato**
Mailing Address: **Department of Biological Sciences**
242 Trafton Science Center S
City / State / Zip : **Mankato, MN 56001**
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Location: *Green House A on Minnesota State University-Mankato campus, Trafton Science Center South, Blue Earth County, Mankato, MN 56001*

Total Trust Fund Project Budget:	**Trust Fund Appropriation:**	**$ 115000**
	Minus Amount Spent:	**$ 79,874**
	Equal Balance:	**$ 35,127**

Legal Citation: ML 2007, [Chap._30_], Sec.[_2_], Subd._4j_.

Appropriation Language:
Neutralization of Reed Canary Grass Root Exudates
$115,000 is from the trust fund to Minnesota State University, Mankato, to assess plant-soil feedback contribution to the invasiveness of reed canary grass through identification and neutralization of inhibitory root exudates.

II. and III. FINAL PROJECT SUMMARY
Reed canary grass (*Phalaris arundinaceae*; hereafter Pa) is an aggressive plant invading wetlands in the Midwest. Invasion by Pa leads to a reduction of native plant diversity and loss of wetland functionality. Our ability to control invasion by Pa and reestablish native plant communities has been unsuccessful because of our limited understanding of the mechanisms that allow Pa to become invasive. The study of plant-soil feedbacks as a mechanism for dominance is a two-step process: plants alter their soil microbial community; and the altered soil microbial community has a positive feedback on plant growth or a negative feedback on neighboring plants. Results from three experiments comparing soil microbial communities and plant growth revealed that *Phalaris arundinacea* (Pa) used plant-soil feedbacks to outcompete tussock sedge (*Carex stricta*; hereafter Cs).

In a soil training experiment, Pa and Cs cultured their soil microbial communities in a manner that differed in both magnitude and composition. Soil training had a neutral feedback on Pa growth and a negative feedback on Cs.

In our first reciprocal transplant experiment, growth of Pa and Cs was greater in their corresponding native soils than in the soil of the other species. Thus, both plants receive positive feedback from their native soil microbial communities. Soil microbial communities were similar when cultivated by Pa regardless of soil type, and Cs soil microbial community catabolic activity depended on soil type.

In our second reciprocal transplant experiment, the effects of competition were dependent on soil microbial communities. Pa growth was best in competition with Cs in Cs-native soils and Pa-sterile soils. Competition did not affect the growth of Cs; however, Cs growth was least in native soils from Pa and Cs. In sterile soils, soil microbial communities depended on the type of competition. In native Pa soils, heterospecific competition had a greater effect on soil microbial communities than did conspecific competition.

Denaturing gradient gel electrophoresis (DGGE) analysis indicated that Pa SMCs were stable and of low diversity, but Cs SMCs were dynamic and of comparatively high diversity.

Bioassays and gas chromatography-mass spectrometry (GC-MS) analyses revealed the presence of methyl esters of fatty acids known to have antimicrobial activity.

Our results suggest that Pa does not use alleopathy, but is induced to produce an antimicrobial compound that has a strong, directional effect on soil microbial communities, which promotes its growth and inhibits the growth of neighboring plants.

OUTLINE OF PROJECT RESULTS*:*

Result 1: Soil preparation and training

Description: Soils will be collected from the rhizospheres of Pa and Tussock sedge (*Carex stricta*; hereafter Cs) communities locally. Each soil type will be sieved with a 2 cm sieve to remove coarse organic matter. Half of both Pa and Cs soils will be triple autoclaved for 20 minutes at 121ºC over the course of 3 successive days to kill all biotic organisms. Newly purchased and washed 40/70 grit silica sand will be autoclaved at 110ºC for 12 hours so that it can be used as a neutral substrate for microbial inoculation. The sterile and non-sterile Pa and Cs soils will then be mixed with the sterile sand in a 9:1 (sand: soil) volumetric proportion to produce four soil treatments, Pa soil; autoclaved Pa soil; Cs soil*;* and autoclaved Cs soil.

Summary Budget Information for Result 1: Trust Fund Budget: $ ~~21000~~
Revised Budget: $ 11000

Amount Spent:	**$**	**9825**
Balance:	**$**	**1175**

Deliverable	**Completion Date**	**Budget**	**Status**
1. Soil collected and sterilized	7/10/07	$2000	completed
	12/23/07	$2000	completed
2. Soil partitioned for treatment/storage	7/10/07	$3000	completed
	12/23/07	$3000	completed
3. Soil trained	10/31/08	$1000	completed

Final Report Summary: All the deliverables for Result 1 were completed below budget despite problems encountered with storm damage to our greenhouse. Soils were collected and sterilized three times rather than the once as proposed. We first collected and sterilized soils prior to receiving funding for this project in hope of facilitating the work if funded. This first set of soils were subsequently partitioned, stored, treated, and trained as proposed. A second set of soils were collected for additional plant-soil feedback experiments but these soils were likely contaminated as a result of a wind storm that damaged our greenhouse. Due to the likely contamination we removed this second set of soils from further study. A third set of soils were collected again for additional plant-soil feedback experiments. The third set of soils was used in three experiments. This set of soils was used for three experiments: 1) A second soil-plant feedback experiment; 2) An experiment to determine if there was an allelopathic effect of Pa on Cs; and 3) An experiment to determine if there was an effect of activated carbon on the soil microbe community.

The substantial balance of $1175 is due to cost savings from our collection and set up of an initial experiment prior to receiving funding from the LCCMR.

Result 2: *CLSU testing*

Description: – Difference in the composition of microbial communities can be revealed by comparing the sources of carbon that can be used by each community. BIOLOG plates contain 95 different carbon substrates. Microbial catabolism of each substrate is detected by a redox indicator, and the use of substrates can be quantitatively evaluated through the use of a microplate photometer. Each inoculated plate will be read twice daily for up to 7 days. Normalization procedures described by others will be used to control for differences in microbial numbers among samples (Garland and Mills, 1991).

Summary Budget Information for Result 2:	**Trust Fund Budget:**	**$ 23000**
	Amount Spent:	**$ 17564**
	Balance:	**$ 5436**

Deliverable	**Completion Date**	**Budget**	**Status**

1. CLSU data collected	2/28/08	$9000	completed
	2/28/09	$9500	completed
2. CLSU data analyzed	3/30/08	$2500	completed
	3/30/09	$2000	completed

Final Report Summary: Originally we proposed to conduct two experiments to investigate the ability of Pa to shape microbial communities. We added a third experiment to directly compare the effects of Pa and Cs on native vs. non-native soil communities.

In our reciprocal transplant experiment, we observed that in native soils, Pa cultivated similar microbial communities, regardless of whether the soil originated from Pa or Cs monoculture stands. In contrast, the microbial communities cultivated by Cs were dependent on the original plant community. Pa appeared to actively shape its soil microbial communities into one with common catabolic capability.

For our second experiment, we used sterile soils that were passively inoculated with greenhouse microbes, i.e., non-native communities, in order to investigate the role of soil training by both Pa and Cs. Two sets of soils were left fallow, while a third and fourth set were planted with Pa or Cs for training. Following a four-month training period, the plants were removed. Cs was planted into one fallow soil and the Cs-trained soil, and Pa was planted into the second fallow soil and the Pa-trained soil. At this time, samples for CLSU testing were collected from all four soils. Following a four-month growth period, a final set of samples was collected for CLSU analysis, and plants were harvested. We observed that in the soils that were not pre-trained by either Pa or Cs, soil microbial communities changed little between the start and finish of the experiment. However, CLSU patterns were markedly different between the beginning and the end of the experiment in soils that had been previously trained by either Pa or Cs. Both plants shaped not only native soil communities (as seen with the first reciprocal transplant experiment, above), but also non-native communities. In addition, one plant growth cycle of prior training of the soil appears to have a profound effect on soil microbial community composition.
In our third experiment, we investigated the effect of conspecific (same species) and heterospecific (different species) competition on soil microbial communities by performing reciprocal transplants into native and non-native soils. In non-native soils, we observed that, regardless of soil type, CLSU patterns for microbial communities were different when grown with heterospecific or conspecific neighbors. In native Cs soils, the type of competition appeared to have little effect on soil microbial community composition. However, in native Pa soils, heterospecific competition had a greater effect on soil microbial community competition than did conspecific competition. These results, when combined with those of the second experiment, suggest that the effect of Pa on soil microbial communities is induced in the presence of a heterospecific neighbor.

The substantial balance of $5436 reflects unspent graduate student stipend and tuition waiver due to the departure of a graduate student prior to the completion of

their studies and this project. The work was completed by Secott and undergraduate student volunteers.

Result 3: Plant harvest and data analysis

Description: Plant height and biomass will be measured at the end of each training phase.

Summary Budget Information for Result 3:	**Trust Fund Budget:**	**$ 4000**
	Amount Spent:	**$ 2502**
	Balance:	**$ 1498**

1. Plant height and biomass data	11/30/07	$1000	completed
	3/30/08	$1000	completed
	6/30/08	$1000	completed
	11/1/08	$1000	completed

Final Report Summary: Here we summarize the results from 4 experiments: 1) A soil-training experiment; 2) A reciprocal transplant experiment; 3) An experiment that tests for allelopathy between Pa and Cs; and 4) A second reciprocal transplant experiment.

The results from our soil-training experiment revealed that Pa biomass did not differ between sterile soils trained by Pa and untrained soils. However, Cs biomass was negatively affected in Cs trained soil. Therefore, soil training by Pa had a neutral effect on Pa growth but soil trained by Cs had a negative effect on Cs growth.

Our first reciprocal transplant experiment compared growth of Pa and Cs in their own native soil and that of the other species. Our results showed that both Pa and Cs produced 91% and 88% more biomass, respectfully, when grown in soil that had been field-cultured by conspecifics (individuals of its own species) than heterospecifics (individuals of the other species) These results are important because they indicated that both Pa and Cs had positive plant-soil feedback interactions when grown in their own soil with native microbial communities.

The results from our test for allelopathy reveled that Pa did not have an allelopathic effect on Cs, but Pa was a better competitor under our experimental conditions. Additionally, activated carbon did not affect the growth of either Pa or Cs; that is Pa and Cs biomass did not differ between soils with and without activated carbon. These results are important because they provide good evidence that Pa does not use allelopathy as a mechanism to outcompete Cs.

Our second reciprocal transplant experiment compared growth of Pa and Cs in their own native and sterile soils and in those of the other species.

Competition with either a conspecific or heterospecific neighbor did not affect Cs growth. However, growth of Cs was less in native soils of both Pa and Cs than in sterilized Pa and Cs soils.

For Pa, the effect of soil type was influenced by the species of neighboring plant. There was no difference in Pa growth in native Pa soil in the presence of either conspecific or heterospecific competitors. Pa growth was best in sterile Cs soils, regardless of the level of competition. Pa growth was higher in sterile Pa soil and native Cs soil when in competition with Cs. The latter result indicates that enhanced Pa growth may be induced in the presence of heterospecific competition in native Cs soils. This observation is significant, as it is the most likely scenario at the beginning of the invasion of a stand of native plants.

The substantial balance of $1498 is due to cost savings from our collection and set up of the first soil-training experiment and reciprocal transplant experiment prior to receiving funding from the LCCMR and due to cost savings from our decision to use student volunteers rather than graduate students to work on this part of the project.

Result 4: *T-RFLP testing*

Description: DNA will be extracted from soil, and ribosomal RNA genes (rDNA) will be amplified using fluorescently-labeled primers in the Polymerase Chain Reaction (PCR). The PCR products will be digested with restriction enzyme *Msp*I, and fragments will be separated and visualized using a LiCor 4300 DNA Analyzer. Because each taxon has a unique rDNA sequence, each may generate different banding patterns following digestion. Soils containing similar communities will have similar banding patterns; where the community compositions are significantly different, the banding patterns will also differ.

Summary Budget Information for Result 4:	**Trust Fund Budget:**	**$ 26085**
	Revised Budget	**$ 36085**
	Amount Spent:	**$ 23653**
	Balance:	**$ 12432**

Deliverable	**Completion Date**	**Budget**	**Status**
1. Protocol establishment	1/31/08	$7585	completed
2. Analysis of DGGE data	6/30/08	$9750	completed
	3/30/09	$8750	completed

Final Report Summary: We substituted denaturing gradient gel electrophoresis (DGGE) analysis for terminal restriction fragment length polymorphism (T-RFLP) analysis because the former is more commonly employed, and therefore more easily placed in the broader context of microbial community analysis. There was no apparent correlation between CLSU analysis and DGGE analysis for the soil training experiment. However, two general conclusions could be reached. First, the number of taxa present in Cs soils (as indicated by the number of discrete DGGE bands) was greater than that observed for Pa. This indicates that the microbial communities in Cs soils are more diverse than those in Pa soils. Second, the taxa present in Cs

soils within and among treatments were variable, whereas the taxa in Pa soils were relatively consistent. These results may indicate that Pa limits soil microbial community diversity more so than does Cs.

The remaining balance of $12432 resulted from using undergraduate student volunteers to conduct the procedures instead of hiring a graduate research assistant.

Result 5: *HPLC analysis*

Description: The original soil extracts and extracts prepared from soils collected periodically from the tanks and from the Pa rhizosphere will be analyzed by Reverse Phase HPLC-UV/VIS. A mixture of solvents (methanol, acetonitrile) and columns (C-18 pre-column, ODS, C-18) coupled with several different wavelengths (280 nm, universal for phenolics, 260 nm catechins, etc) will be used to separate and screen components. Those fractions that show biological activity will be analyzed by HPLC-MS. The MS fingerprint will be used to search existing libraries of compounds to identify the compounds.

Pa rhizomes will also be collected. After collection the rhizomes will be rinsed with deionized water and divided into the root section and stem/leaf section. Both sections will be weighed for biomass determination. Aliquots of roots (approximately 150 grams) will be crushed using a ball grinder. The crushed roots will be extracted with water and methanol. These extracts will be analyzed for compounds using HPLC-UV/Vis. Components will be separated and collected by HPLC. Those fractions showing biological activity will be further analyzed by HPLC-MS. The MS will give a fingerprint of the compound.

Summary Budget Information for Result 5:	**Trust Fund Budget:**	**$ 25000**
	Amount Spent:	**$ 15691**
	Balance:	**$ 9309**

Deliverable	**Completion Date**	**Budget**	**Status**
1. Protocol establishment	1/31/08	$7500	completed
2. Sample preparation/analysis	4/15/08	$12000	completed
	1/31/09	$5500	completed

Final Report Summary: Because the HPLC-MS was not functioning properly, it was decided to begin these analyses using bioassays to identify inhibitory extracts. GS-MS analyses were used to identify potentially inhibitory compounds present in the extracts Extracts of Pa roots were used to test the effect of extracted compounds on the germination of seeds known to be sensitive to bioactive substances commonly used in bioassays. Methanol extracts of Pa roots inhibited the germination of lettuce and radish seeds, as well as those of Reed Manna Grass, a wetland plant. GC-MS analyses of the inhibitory methanol extracts revealed the presence of methyl esters of linoleic acid and linolenic acid. The formation of the methyl esters was due to the methanol solvent. Both of these C18 unsaturated fatty acids are known to inhibit

both gram-positive and gram-negative bacteria, and are used as antimicrobial food additives.

The balance of $9309 resulted from using undergraduate student volunteers and undergraduate student class projects instead of hiring a graduate research assistant.

Result 6: Final Report Preparation

Description: Final report preparation, printing, and dissemination.

Summary Budget Information for Result 6:	**Trust Fund Budget:**	**$ 15915**
	Amount Spent:	**$ 10638**
	Balance:	**$ 5277**

Deliverable	**Completion Date**	**Budget**	**Status**
1. Draft report	4/30/09	$4000	completed
2. Final report	6/30/09	$11915	completed

Result Status as of *(December 18, 2009)***:** The final report is completed. Portions of Results 1, 2, and 3 have been written as a manuscript (*A plant-soil feedback as a mechanism for the invasive success of Phalaris arundinacea*) and is being revised for publication. A second manuscript including Results 1-5 is in preparation by the investigators.

Portions of this work were presented:

1) as an invited talk at the University of Bern, Switzerland (8/08)
2) at the 93rd Annual Ecological Society of America Meeting; Milwaukee, WI. (8/08)
3) at the 13rd "Annual Conference of the Wisconsin Wetland Association; Oconomowoc, WI. (2/08)
4) (two papers) at the North American Lake Managers Society (NALMS) International Conference; Hartford, CT. (10/09)
5) (four papers) at the 2008 and 2009 Minnesota State University Undergraduate Research Conference (4/08 and 4/09)

In addition, portions of this work were used for a M.S. thesis project, as class exercises in undergraduate courses, and as several undergraduate independent research projects at Minnesota State University.

Final Report Summary:

V. TOTAL TRUST FUND PROJECT BUDGET:

Staff or Contract Services:

Bradley Cook: $12787 (36 days over 2 years) = 10% of full-time employment over two years. Cook is the principle investigator of the project and is responsible for all project tasks, data collection/analysis, results, deliverables, and reports. Cook is primarily responsible for soil preparation/training, all greenhouse experiments,

data analysis, report writing and dissemination. Cook will directly co-supervise the two graduate students with Secott.

Timothy Secott: $24314 (54 days over 2 years) = 15% of full-time employment over two years. Secott is the co-principle investigator of the project and is primarily responsible for CLSU and T-RFLP testing and analysis and will assist with report writing and dissemination. Secott will directly co-supervise the two graduate students with Cook.

Beth Proctor: $12181 (14 days over 2 years) = 4.1% of full-time employment over two years. Proctor is primarily responsible for HPLC/MS analysis.

Graduate Student #1: $13412 (4 of 6 semesters stipend and tuition) = 66% of full-time employment for one year. This student will be under the direct supervision of Cook.

Graduate Student #2: $1600 (4 of 6 semesters stipend and tuition) = 66% of full-time employment for one year. This student will be under the direct supervision of Secott.

Equipment: $9730. Purchase of denaturing gradient gel electrophoresis system, replacement of equipment damaged during move.
Supplies: $15334. Supplies include HPLC columns, solvents, sand, DNA extraction reagents, PCR primers, BIOLOG plates, electrophoresis reagents (buffers, agarose, polyacrylamide, etc.) and laboratory consumables (pipet tips, gloves, etc.).

Travel: $515. This money will be used to collect plant and soils for this project. The remainder will be spent to send the graduate students to a local/regional meeting to present their research.

Development: $ N/A
Restoration: $ N/A
Acquisition, including easements: $ N/A

TOTAL TRUST FUND PROJECT BUDGET: $ 115000

Explanation of Capital Expenditures Greater Than $3,500 (October 6, 2008 Update): In May we purchased an Ingeny PhorU Denaturing Gradient Gel Electrophoresis (DGGE) System for $8,000. We are requesting retroactive approval of this purchase. We offer fiscal and technical explanations for retroactive approval. From a fiscal perspective, we simply did not read the LCCMR guidelines carefully and did not realize that we should have acquired prior approval. Additionally, prior to its purchase, my review of our expenditures indicated that we were under budget and ahead of schedule on several project results. For example, out of hopeful anticipation of receiving LCCMR funding, much of the work for Result 1 was started during the peer review process and completed before funding arrived. This preliminary work was funded by the Department of Biological Sciences at MSU. Similarly, we originally budgeted $15,134 as salary for Cook and, to date, have spent $2,900 on his salary. Much of Cook's time spent on this project to date has been covered by MSU or another grant.

From a technical perspective the DGGE system was recommended by one of the peer review panel members and will provide us with clearer results than will T-RFLP. In addition, the use of the DGGE system will allow us to identify (through DNA sequencing) those organisms that respond to reed canarygrass root exudates, rather than simple functional groups -- more and better information.

We will continue to use the DGGE system for similar analyses for its useful lifetime. If not, we commit to pay back the Environment and Natural Resources Trust Fund an amount equal to either the cash value received or the residual value approved by the LCCMR director if it is sold.

VI. OTHER FUNDS & PARTNERS:

A. Project Partners: N/A

B. Other Funds Proposed to be Spent during the Project Period: Minnesota State University-Mankato (MSUM) will contribute ~$15,000 as direct matching funds as teaching assistantships and tuition waivers for the two graduate students. MSUM will provide in-kind matching funds including office space and computer facilities for key personnel and graduate students for the duration of the project. Support services including greenhouse/laboratory, library access and services, statistical expertise, accounting services, copying costs, publication costs, some travel, and office/laboratory/greenhouse maintenance and power will be also be provided by MSUM for the duration of the project.

C. Past Spending: N/A

D. Time: N/A

VII. DISSEMINATION: At appropriate opportunities the investigators of this project will continue to present the results at local, state, regional, and international venues. Portions of Results 1, 2, and 3 have been written as a manuscript (A plant-soil feedback as a mechanism for the invasive *success of Phalaris arundinacea*) and is being revised for publication. A second manuscript including Results 1-5 is in preparation by the investigators.

Portions of this work were presented:

1) as an invited talk at the University of Bern, Switzerland (8/08)
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In addition, portions of this work were used for a M.S. thesis project, as class exercises in undergraduate courses, and as several undergraduate independent research projects at Minnesota State University.

VIII. REPORTING REQUIREMENTS:
Periodic work program progress reports will be submitted not later than January 8, 2008; June 30, 2008; and January 8, 2009. A final work program report and associated products will be submitted between June 30 and August 1, 2009 as requested by the LCCMR

IX. RESEARCH PROJECTS: See attachment B: revised research addendum

Attachment A: Budget Detail for 2007 Projects - Summary and a Budget page for each partner (if applicable)

Project Title: Neutralization of Reed Canary Grass Root Exudates - *4(i)*

Project Manager Name: *Bradley J. Cook*

Trust Fund Appropriation: $ *115000*

1) See list of non-eligible expenses, do not include any of these items in your budget sheet

2) Remove any budget item lines not applicable

2007 Trust Fund Budget	**Result 1 Budget:**	**Result 1 Budget: Revised 10/08**	**Amount Spent** *(as of 6/30/09)*	**Balance** *(as of 6/30/09)*	**Result 2 Budget:**	**Amount Spent** *(as of 6/30/09)*	**Balance** *(as of 6/30/09)*	**Result 3 Budget:**	**Amount Spent** *(as of 6/30/09)*	**Balance** *(as of 6/30/09)*	**Result 4 Budget:**	**Result 4 Budget: Revised 10/08**	**Amount Spent** *(as of 6/30/09)*	**Balance** *(as of 6/30/09)*	**Result 5 Budget:**	**Amount Spent** *(as of 6/30/09)*	**Balance** *(as of 6/30/09)*	**Result 6 Budget:**	**Amount Spent** *(as of 6/30/09)*	**Balance** *(as of 6/30/09)*	**TOTAL BUDGET**	**TOTAL BALANCE**
	Soil preparation and training			*21000*	*CLSU testing*		*23000*	*Plant harvest and data analysis*		*4000*	*T-RFLP testing*			*36,085*	*HPLC analysis*		*25000*	*Final report preparation*		*15915*		
BUDGET ITEM				0			0			0				0			0			0	0	0
PERSONNEL: wages and benefits	~~18,500~~	8,500	8,351	149	19,500	13,533	5,967	4,000	2,502	1,498	~~22,816~~	22,816	9,012	13,804	20,000	10,747	9,253	15,000	10,149	4,851	89,816	35,522
Printing				0			0			0				0			0	400	323	77	400	77
Other Supplies *(list specific categories)*				0			0			0				0			0			0	0	0
Sand	~~500~~	500	314	187			0			0				0			0				500	187
Pots	~~1,000~~	1,000	161	839			0			0				0			0				1,000	839
BIOLOG plates				0	3,000	3,000	0			0				0			0				3,000	0
PCR primers				0			0			0	~~400~~	400	1,246	-846			0				400	-846
T-RFLP primers				0			0			0	~~769~~	769	1,369	-600			0				769	-600
Molecular biology reagents and kits				0			0			0	~~1,600~~	1,600	1,796	-196			0				1,600	-196
HPLC Solvents				0			0			0				0	2,000	1,944	56				2,000	56
HPLC Columns				0			0			0				0	3,000	3,000	0				3,000	0
Disposibles (gloves, tips, etc.)	~~1,000~~	1,000	1,000	0	500	1,031	-531			0	~~500~~	500	500	0			0				2,000	-531
Travel expenses in Minnesota				0			0			0				0			0	515	166	349	515	349
Travel outside Minnesota *(where?)*				0			0			0				0			0			0	0	0
Other *(Describe the activity and cost) be specific*				0			0			0		10,000	9,730	270			0			0	10,000	270
COLUMN TOTAL	**~~$21,000~~**	**$11,000**	**$9,825**	**$1,175**	**$23,000**	**$17,564**	**$5,436**	**$4,000**	**$2,502**	**$1,498**	**~~$26,085~~**	$36,085	**$23,653**	**$12,432**	**$25,000**	**$15,691**	**$9,309**	**$15,915**	**$10,638**	**$5,277**	**$115,000**	**$35,127**
	$10,000 was transferred to other for Result 4; DGGE system.											Other = DGGE system										