2014.06c Project Abstract
For the Period Ending June 30, 2017

PROJECT TITLE: Prairie Sustainability Through Seed Storage, Beneficial Microbes, and Adaptation
PROJECT MANAGER: Ruth G. Shaw
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2014, Chp. 226, Sec. 2, Subd. 6c

APPROPRIATION AMOUNT: $600,000
AMOUNT SPENT: $600,000
AMOUNT REMAINING: $0

Overall Project Outcomes and Results

The once vast MN prairie harboring tremendous genetic diversity has been drastically diminished. Society's increasing recognition of the multifarious benefits MN prairie provides has generated demand for scientifically based prairie conservation and restoration. Accordingly, this project undertook to:

- preserve seeds of 40 plant species from sites throughout MN's prairie region,
- identify microbes that promote prairie plant health,
- discover the scale of local adaptation for prairie plant species, and
- predict the rate of future adaptation of prairie plant populations.

Outcomes:
We obtained genetically representative collections from over 330 populations of 64 plant species native to MN prairie. To ensure lasting viability of these seeds, many are stored at the USDA National Center for Genetic Resources Preservation in Fort Collins, CO.

We characterized microbial communities on prairie plants, isolating and identifying over 2500 strains from prairie clover. Graduate student DeMers presented these findings at a national scientific meeting.We conducted an experiment to determine whether microbes benefit host plants that originate from the same site more than they benefit host plants from different sites; analysis is ongoing.

We established 3 field experiments to clarify the extent to which plants survive and reproduce more when they are planted near their site of origin. This study focuses on 6 prairie species, each sampled from 12 sites. Monitoring of survival and growth of plants is proceeding, as is analysis of this dataset.

To assess the genetic variation available to support adaptation, we established foundation plantings of little bluestem, in preparation for estimating the adaptive capacity of two populations.

Project Results Use and Dissemination

In accomplishing these goals, we have advanced:

1. Relationships with professional native-seed collectors and with several student groups at rural MN university campuses.
2. Scientific training of 10 undergraduates at 3 Minnesota university campuses, 7 technicians, 3 graduate students, and 2 post-doctoral associates and engagement of over 60 community volunteers and over 400 others.
3. Discussions with users and producers of native seed, aiming to increase source-identified seed available for prairie restorations in Minnesota.

This Project has expanded the diversity and volume of local, source-identified seeds and microbes from Minnesota prairies and has collected and analyzed data that will support restoration of MN prairie. All aspects of this project are being continued through new funding from the ENRTF.

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

Date of Report: September 8, 2017

Date of Next Status Update Report: N/A (this is the final report)

Date of Work Plan Approval: June 4, 2014

Project Completion Date: June 30, 2017

Does this submission include an amendment request? YES

PROJECT TITLE: Prairie Sustainability Through Seed Storage, Beneficial Microbes, and Adaptation

Project Manager: Ruth G. Shaw

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Location: Polk, Ramsey, Redwood, Stevens, Wabasha

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$600,000**
	Amount Spent:	**$600,000**
	Balance:	**$0**

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 6c

Appropriation Language:
$600,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to collect and preserve germplasm of plants throughout Minnesota's prairie region, study the microbial effects that promote plant health, analyze local adaptation, and evaluate the adaptive capacity of prairie plant populations. This appropriation is available until June 30, 2017, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Prairie Sustainability Through Seed Storage, Beneficial Microbes, and Adaptation

II. PROJECT STATEMENT:

With the goals of preserving prairie plant diversity in Minnesota and promoting restoration of thriving prairies, we propose to accomplish the following outcomes:

- collection and preservation of germplasm of 40 plant species from 12 sites throughout the prairie region of MN,
- determination and collection of microbes that promote the health of prairie plants,
- discovery of the scale of local adaptation for diverse prairie plant species, and
- evaluation of the adaptive capacity of prairie plant populations.

This work will provide fundamental information necessary to the state's efforts to establish both scientifically sound and economically feasible practices for prairie restoration. Because the generation time of prairie plant species is typically long (e.g. greater than 20 yr estimated for narrow-leaved purple coneflower, *Echinacea angustifolia*, Hurlburt 1999), we envision continuation of this program well beyond the three-year period of funding from LCCMR to begin in 2014. We here provide plans for conducting this project in its first three years.

Background: Since European settlement, the once vast expanses of MN prairie, approximately 18 million acres, have been diminished to small remnants totaling about 235,000 acres. Similarly, the once tremendous genetic diversity within each of the many species that typify prairie has been drastically reduced. Consequently, remnant populations are subject to severe inbreeding, which reduces the robustness of plants and can cause further population decline. Increasingly, society recognizes that prairies play critically important roles, such as:

1.) stable, resilient plant communities

2.) habitat for diverse wildlife, including pollinators

3.) maintenance of water quality

4.) roadside stabilization

5.) sustainable harvest of biomass for fuel production

6.) sources of novel plant products for local industries, e.g. cosmetics.

In addition to needs for these crucial ecological services, the goal of preserving the natural beauty of prairies for future generations is spurring efforts for extensive prairie restoration. However, such large-scale prairie restorations face daunting challenges, as they require large quantities of seeds adapted to the environment in which they will grow. Even small remnants of prairie retain valuable genetic resources. Protection of these remaining genetic resources can ensure this genetic variation will be available as germplasm for massive

restorations now, as the basis for future adaptation to climate change, and for human uses yet to be discovered. Minnesota's prairie plants have been adapting to their local climates and soils since the glaciers receded 14,000 years ago. Beneficial microbes adapted along with the plants. However, climate is now changing at a rate that calls into question the capacity of plants to adapt and raises concerns about losses of native plant species and invasion of noxious weeds.

Premises and Hypotheses:

1. **Efforts to collect and preserve genetic diversity of Minnesota's prairie plants are essential both to avert their loss altogether and to support prairie restoration throughout the prairie region of the state.**

2. **Symbiotic microorganisms of plants are most beneficial when originating from the same locality as the plant population. Microbes are more often pathogenic when originating from a different location than the plant population.**

3. **Populations of prairie species are adapted to local abiotic conditions, as well as to the other organisms in their local communities. The geographic scale of local adaptation is not well understood, however, and must be experimentally determined to inform the choice of germplasm for prairie restorations.**

4. **The genetic variation residing in populations of prairie species is the basis for ongoing adaptation. The amount of genetic variation, which determines rates of adaptation under natural selection, must be determined experimentally.**

III. PROJECT STATUS UPDATES:

Project Status as of January 30, 2015:

We have staffed the project, succeeding in recruiting highly capable individuals with relevant expertise to play key roles in the efforts to collect and conserve seeds and carry out the experiments. Team members visited possible locations for seed collection, identified the most promising sites, and obtained necessary permits. Our staff conducted outreach to enlist volunteers to assist in seed collection, and led groups on collection trips to Polk, Clay, Norman, Douglas, Otter Tail, Pope, Chippewa, Swift, Goodhue, Ramsey, Redwood, Stevens, Wabasha, Olmsted, Dodge, Dakota, Washington, Lyon, Brown, Pipestone, Yellow Medicine, Jackson, and Murray counties. Volunteers and staff secured statewide collections from 22 remnant prairies for eight plant species six of which will be used in the experimental studies. In preparation for the experiments, 10 staff members stored and processed seed from 8696 plants. A subset of seed from these individual plants was cleaned, scarified, and stratified, as appropriate, for germination. The seeds are currently developing into plants in a growth chamber, after which they will be transplanted and moved to a greenhouse, and then to field plots.

Amendment Request (01/30/2015):
We request approval for transfer of funds for Activity 1. As shown in the attached budget, we request that $8500 previously allocated to personnel be transferred to augment the funds for travel and for supplies for seed collecting. This is necessary so that funds will be available for Activity 1 during the coming season. The originally

allotted funds were insufficient for the seed collecting supplies even for our first season of collecting, and in this season we expended nearly all the allocated travel funds. We also request an allocation for supplies and services (greenhouse use and shipping) for Activity 3. We did not initially anticipate using greenhouse space to augment seed availability for the field experiments of Activity 3.
Amendment approved by LCCMR 2-2-2015

Project Status as of September 1, 2015:

As described in the previous update, cleaning of the seeds that were wild-harvested in autumn 2014 has continued. The majority have now been sent to the National Center for Genetic Resources Preservation (NCGRP) for viability analysis and storage. Seeds that were sown for our experiments resulted in over 52,000 seedlings that represented at least 12 populations for each of 6 native species. The seedlings were tended for 4 months in a greenhouse after which 100 per population per species (7200 total) were randomly chosen as the basis of a seed-increase generation. These 7200 individuals were transplanted into the field in June-July 2015; the remaining plants were installed into buffer zones around the field site. We will continue to harvest seed produced by the 7200 individuals for use in Activities 2, 3 and 4. Studies of microbial interactions with three species have been initiated (Activity 2). Sites have been identified for the reciprocal transplant experiment (Activity 3), and seeds will be sown into those sites during October 2015.

Project Status as of January 11, 2016:

We have now completed cleaning and processing of the seeds that were wild-harvested in autumn 2014. Of the 6 species that were transplanted into the field at Rosemount in June-July 2015, 2 species produced abundant seed in time for planting in Fall 2015. We harvested from 69 - 94 individuals from each of 12 populations of those 2 species, obtaining 2 and 8 lbs of seed, respectively. These seeds were then sown into 3 evaluation sites in western Minnesota; the resulting plants will be used in Activities 2 and 3. The remaining 4 species have established well at the transplant site; they are expected to produce sufficient seeds in 2016 for use in Activities 2 and 3.

Amendment Request (1/11/16):
We request approval for a transfer of funds within Activity 1 to cover underestimated costs. As shown in the attached budget, we request that $500 previously allocated for personnel be transferred to augment the fund for services. This is necessary to cover costs of shipping seeds to the USDA National Center for Genetic Resources Preservation (NCGRP). Due to the volume of seeds that were collected and allotted for preservation, the funds allocated for shipping through our previous amendment request were insufficient. We further request reallocation of an additional $15,000 from the Activity 1 personnel line to cover unanticipated costs of the remaining activities, as follows: for Activity 3, $4,000 to augment funds for field supplies that are required for the installation of 3 experimental sites, $1,000 for lab and greenhouse supplies related to those installations, $1,000 to augment funds for services, specifically plot fees, required for the ongoing use of field plots used to increase seed for that experiment, and $8,000 to augment funds for travel costs associated with maintenance and seed harvest at those same plots; for Activity 4, $1000 to funds for lab supplies. We note that we did not initially anticipate that the budget should reflect, through cost-splitting, overlap between Activities 3 and 4. Finally, we request allocation of $200 from the field supplies line-item in Activity 4 to augment the funds available for services, specifically greenhouse use, for Activity 2.
Amendment approved by LCCMR 1-25-2016

Amendment request (05/19/2016):
We request approval for a transfer of funds within Activity 1 to allow us to utilize services that will increase project efficiency. As shown in the attached budget, we request that $4,400 previously allocated to personnel, $1,000 previously allocated to equipment, tools, and supplies, and $600 previously allocated to travel be transferred to augment the fund for services. This will allow us to utilize services offered by professional native-seed collectors in distant parts of Minnesota. The local expertise provided by these partners will result in more efficient and cost-effective seed collection.
Amendment Approved by LCCMR 6-1-2016

Project Status as of September 1, 2016:

Seed collection efforts (Activity 1) are ongoing, and we have collected populations of 27 species during this reporting period, with additional collections planned through the autumn. Using both laboratory culturing and molecular approaches, we have been assessing the diversity and composition of the aboveground endophyte and belowground rhizobia microbial communities from the source locations of 12 *Dalea purpurea* populations, as well as the 3 evaluation sites. We also have experimental individuals planted at Lake Bella WMA (SW MN) and in the greenhouse for the 3 evaluation sites to determine whether augmenting the microbial community is necessary for the establishment of the legume species to be planted this fall for Activity 3. The 2 grass species sown into the 3 evaluation sites in 2015 have established well; we have collected survival and morphological data on over 8,000 individuals (Activity 3). All species sown as seeds at the transplant site in 2015 are flourishing. We anticipate that ample seed of the 2 legume and 2 forb species will be available for sowing at the evaluation sites, as well as second cohorts of the 2 grass species (Activity 3). Planning for the crosses required for Activity 4 is well advanced.

Amendment Request (9/1/16):
We request approval for a transfer of funds from the Activity 1 personnel line to cover unanticipated costs as follows: for Activity 2, $3,020 to augment funds for lab supplies required for microbial culturing and $3,450 for services, specifically greenhouse fees needed for rearing experimental plant populations; for Activity 3, $4,120 for services, specifically plot fees required to maintain our long-term seed-increase plots and $4,140 to augment funds for the travel needed to maintain and harvest seeds from those plots and to maintain and collect samples and data from the evaluation plots in western MN; and for Activity 4, $3,000 for services, specifically greenhouse fees, needed for establishing and maintaining the pedigreed lines. We further request the transfer of an additional $58,500 from the Activity 1 personnel line and $64,965 from the Activity 4 personnel line to augment the personnel lines in Activities 2 and 3 as follows: $31,170 to Activity 2, for the work entailed in rearing experimental plants and culturing and characterizing microbes; $92,295 to Activity 3 for experiment maintenance, sample and data collection, and data analysis required for this Activity. These transfers of funds will not affect our work on Activities 1 or 4 due to extensive support from volunteers and external partners (Activity 1) and lower-than-expected labor costs (Activity 4).
Amendment Approved by LCCMR 9/15/2016

Project Status as of January 10, 2017:
Seed collection efforts (Activity 1) are complete; over the course of this project, 336 populations of 64 species have been harvested and are being appropriately stored. Assessment of the diversity and composition of microbial communities (Activity 2) is steadily progressing. We have also isolated and sequenced roughly 2000 strains of endophytic bacteria. We have also isolated more than 1,000 strains of rhizobia bacteria; identification of these strains is approximately 10% completed. We successfully completed installation of Activity 3 by sowing cohorts of 4 species (2 legumes, 2 forbs) and second cohorts of the 2 grass species, into our 3 out-state evaluation sites. The data collected on the first cohorts of those 2 grass species, representing over 8,000 individuals, is being entered and analyzed. The plants required for the planned crosses (Activity 4) are currently in stratification and germination stages.

Amendment Request (1/31/2017):
We request approval for a transfer of funds to cover unanticipated costs in Activities 2 and 3. As shown in the attached budget, we request that $7,479 previously allocated collectively to Activity 1 ($3,890 for subcontracted seed-collection services, $388 for field supplies, $120 for seed-shipping services, and $3,081 for travel) and $857 in Activity 3 services be transferred to Activity 2 travel ($212), Activity 3 field supplies ($1,086), Activity 3 lab supplies ($162), and Activity 3 travel ($6,876). These transfers will not affect our work on Activity 1, which is essentially complete, or on Activity 3, which at this stage has less need for greenhouse space than previously expected.
Amendment Approved by LCCMR 2/3/2017

Project Status as of September 8, 2017:

Seed collection efforts (Activity 1) are complete; over the course of this project, 336 populations of 64 species have been harvested and are being appropriately stored. Assessment of the diversity and composition of microbial communities (Activity 2) is complete with over 2000 fungal and 1000 microbial cultures, and a greenhouse study to assess effects of microbial-plant associations on plant growth in Dalea purpurea. We successfully completed installation of Activity 3 (geographic scale of local adaptation), having sown one cohort of 4 species and 2 cohorts of 2 species into three out-state evaluation sites. Data on over 8,000 individuals were collected during the first field season (2016) and are under analysis; preliminary results were presented at a national conference. All cohorts will be assessed again in Aug-Sept 2017 in Phase II activities. For Activity 4 (adaptive capacity), in July 2017, we completed transplanting of 400 parental plants from each of 2 populations of little bluestem into a common garden. We will conduct planned crosses in Aug-Sept 2017. Leveraging LCCMR support to gain additional funding from the University of Minnesota Institute on the Environment ($3000, IonE), we are conducting focus groups aimed at engaging public and private entities in increasing the volume and diversity of local, source-identified seed available for prairie restorations in Minnesota.

Amendment Request (9/8/2017):
We request approval for transfer of funds to cover misestimated costs in Activities 1, 2, 3 and 4. As shown in the attached Budget, we request that:

- Within Activity 1, $48 be reallocated from Personnel to Services, to cover additional costs associated with shipping seeds collected by volunteers residing in western Minnesota
- Within Activity 2, that $11,272 be reallocated from Professional/Services/Technical Contracts to Lab Supplies. The May lab developed a more cost-effective approach for the genetic analysis of beneficial bacteria (rhizobia) that did not require sequencing services but did require purchase of minor pieces of equipment (a pump) and additional lab supplies. With this new approach, we were able to analyze many more strains of rhizobia (over 800 total) rather than the planned 200 - 300 rhizobia using sequencing approaches.
- $23,810 be reallocated to Activity 3 Personnel to cover higher-than-anticipated labor costs associated with data collection and field-site maintenance, from multiple lines as follows: $10,403 from Activity 2 Personnel; $9,546 from Activity 4 Personnel; $2,194 from Activity 2 Services and $1,593 from Activity 3 Services (greenhouse fees for both Activities were lower than anticipated); and $74 from Activity 1 Personnel.
- Within Activity 4, that $1,050 be reallocated to Services as follows to cover higher-than-estimated greenhouse fees: $853 (from Lab Supplies) and $197 (from Equipment)
- $832 be reallocated to Activity 4 Travel, from multiple lines as follows: $170 from Activity 1 personnel, $506 from Activity 2 Professional Contracts and $156 from Activity 4 Equipment.

Overall Project Outcomes and Results:

1. A diverse, genetically representative collection of germplasm from over 330 populations of 64 species of plants native to Minnesota's prairies.
2. Established relationships with professional native-seed collectors in NW and SE Minnesota and with student groups in NW (UM-Crookston), W-central (UM-Morris), and SW (SMSU) Minnesota.
3. Isolated more than 3000 strains of fungal and bacterial symbionts associated with *Dalea purpurea*. Identified more than 2500 of these strains using morphology and DNA sequence. Determining variation in effects of microbes on plants using targeted greenhouse manipulations (see #4 below).
4. Performed reciprocal combinations of five *Dalea purpurea* and nitrogen-fixing rhizobia populations to explore the potential for local co-adaptation across the geographic range in Minnesota.
5. Established 3 large field experiments for the assessment of local adaptation over the lifetimes of 6 long-lived, perennial prairie species.
6. Estimated the scale of local adaptation for juveniles of 2 native grass species.
7. Established 2 foundation populations of a native grass species, to allow long-term estimates of adaptive capacity.

8. Presented project results at 4 national conferences.
9. Supported the scientific training and education of 11 undergraduates, 7 technicians, 3 graduate students, and 2 post-doctoral research associates and contributed to the scientific engagement of over 60 community volunteers.
10. Ongoing discussion with large-scale users and producers of native seed, to improve the volume and diversity of local, source-identified seed available for prairie restorations in Minnesota.

IV. PROJECT ACTIVITIES AND OUTCOMES:

Activity 1: Collection and conservation of 480 populations of prairie plants.

Description:

We will collect seeds of at least 40 species characteristic of MN prairies, sampling moist and dry habitats in at least 3 populations in each of the 4 ecologically defined subsections of MN prairie. When sampling, we will take care to obtain genetically representative samples for each population and avoid depleting seed input to the site. To the extent possible, we will make seed gathering efficient by focusing our efforts in sites with populations of more than 200 flowering individuals for each of several of our target species, as established in preliminary visits to sites while plants are flowering. If populations this large are not available, we will gather seeds as they mature from populations no smaller than 60 individuals, both to avoid depleting populations and to ensure samples are representative. In order to secure genetic diversity within populations, we will collect seeds from plants that are minimally 3 m apart. As possible within each site, we will sample seeds from plants occupying more moist low-lying areas and also those occupying drier hilltops within each site, keeping these collections distinct. We will also gather seeds on multiple dates throughout the period of seed maturation to ensure we include plants spanning a wide range of timing of seed production. Each seed sample will be divided, and half of the sample will be shipped as soon as possible to the USDA National Center for Genetic Resources Preservation in Fort Collins, Colorado. There, they will be archived in conditions chosen to maximize the longevity of seeds. A portion of the samples will be available to researchers at NCGRP for investigations of seed viability and longevity. The other half of the seed sample will be retained as the basis for the experiment described below (Activity 3). Altogether (i.e., over multiple collecting dates), we will plan to gather at least 6,000 seeds from 60 individuals (but minimally, 30 individuals) from each population in order to include 95% of alleles having frequencies at least 0.05 (Brown and Marshall 1995). For each population, a voucher sample of leaves and flowers will be collected, pressed and archived in the University of Minnesota Herbarium.

		Budget (1/30/17)	**Revised Budget (9/8/17)**
Summary Budget Information for Activity 1:	**ENRTF Budget:**	~~$45,991~~	$45,746
	Amount Spent:	$	$45,746
	Balance:	$	$0

Activity Completion Date: June 30, 2017

Outcome	***Completion Date***	Budget (1/31/17)	**Revised Budget (9/8/17)**
1. A lasting archive of a well-designed sample of prairie genetic diversity.	October 2016	**~~$15,330~~**	**$15,248**
2. Measures of the initial viability of seed samples.	December 2016	**~~$15,330~~**	**$15,249**
3. Estimates of the longevity of the stored seeds.	August 2017	**~~$15,331~~**	**$15,249**

Activity Status as of January 30, 2015:

During late summer and fall of 2014, we began collecting and conserving seeds according to the plans outlined above. Volunteer participation in seed harvesting enabled us to reach our collection goals economizing as much as possible. Our teams, comprising 7 Healthy Prairies staff and 58 volunteers, who collectively devoted nearly 1000 person-hours to this effort, made complete collections of eight species: *Anemone cylindrica* (thimbleweed, seed from 978 individuals), *Bouteloua curtipendula* (sideoats grama, seed from 1709 individuals), *Dalea candida* (white prairie clover, seed from 799 individuals), *Dalea purpurea* (purple prairie clover, seed from 1061 individuals), *Liatris aspera* (rough blazing star, seed from 884 individuals), *Liatris punctata* (dotted blazing star, seed from 666 individuals), *Monarda fistulosa* (wild bergamot, seed from 839 individuals), and *Schizachyrium scoparium* (little bluestem, seed from 1760 individuals). In total we collected seed from 8698 individual plants, which is being cleaned (as appropriate) and stored before it is sent to the USDA seed preservation facility in Colorado.

Activity Status as of September 1, 2015:
Between January and September 2015, our team of 10 staff invested over 2,500 person-hours in cleaning, packaging, and storing the seeds harvested in 2014. Seed collections from the remaining 32 species are scheduled for 2016; in anticipation of these efforts, our teams scouted 5 remnant prairies in Washington, Dakota, and Wabasha Counties for early-flowering species including *Hesperostipa spartea* (porcupinegrass),

Lithospermum canescens (hoary puccoon), *Viola palmata* var. *pedatifida* (bearded birdfoot violet), *Phlox pilosa* (prairie phlox) and *Calylophus serrulatus* (yellow sundrops).

Activity Status as of January 11, 2016:

We scouted for populations of additional species, but did not collect seeds during 2015, focusing our efforts on Activities 2 and 3.

Activity Status as of September 1, 2016:

Between April and September 2016, 5 staff and 6 community volunteers harvested genetically-representative samples of seed from 27 species from 22 sites in 19 MN counties. In total, we have collected seed from over 1,950 individual plants during this reporting period. Seeds have been processed as appropriate and are being held in cold storage at the University of Minnesota.

Activity Status as of January 10, 2017:

Seed collection and processing have concluded. Since the inception of the Healthy Prairies project, staff and volunteers have harvested genetically-representative samples of 336 populations representing 64 species collected from 51 sites in 30 MN counties; over 14,000 individual plants were sampled. The seeds have been processed appropriately and are being held in cold storage at the National Center for Genetic Resources Preservation and at the University of Minnesota.

Activity Status as of August 15, 2017:
Seed collections are in cold storage at the National Center for Genetic Resources Preservation and at the University of Minnesota. With support from an intramural grant from the University of Minnesota Institute on the Environment (IonE) that leveraged our ENTRF funding, we held 2 focus groups for large-scale public and private users of native seed. Participants identified aspects of native-seed production and use in Minnesota that are and are not working well currently, as well as opportunities for improving the diversity and availability of local, source-identified seed for use in restoring Minnesota prairies.

ACTIVITY 2: Microbial Aids to Plant Health

Description:

A. We plan to characterize microbial communities associated with prairie plant species. From the local adaptation experiment described below (Activity 3), microbial communities associated with aerial leaves and stems for three focal plant species will be sampled. Small portions of 3 plants per population, for 3 replicate blocks (324 plants total per species) will be removed from the field and taken into the lab to determine plant-associated bacteria and fungi by culturing. These microbes, including pathogens, will be isolated from the plant and identified using morphology and DNA sequencing. Resulting cultures will be available for evaluating their effects on plant growth in B. below. To better understand the microbial communities associated with plants, and

thus focus our experimental efforts, we will use NextGen sequencing of entire microbial communities within these same plants. This method generates millions of sequences, which are then analyzed to determine community species composition.

B. Are plant-associated microbes originating from "home" environments more often beneficial than when originating from "away" environments? We will test this hypothesis by evaluating the degree to which plant-associated microbes, the endophytes, protect their plant hosts against pathogens. Endophytic bacteria and fungi live inside plants without causing disease symptoms and some may protect the plant host against disease or insects. We will germinate seeds for the 3 focal species to obtain 60 plants per species per evaluation site, or 180 plants per species in total. For each of the three experimental "home" sites, 10 plants grown from seed of each experimental site (30 total) will be retained in the greenhouse as controls, and 10 plants will be planted out (outplants) into that "home" site experimental garden, plus 10 outplants to each of the other two "away" site experimental gardens. These outplants will grow at each site for 6 weeks to "collect" symbiotic microbes. We will then plan to return all experimental plants to a University of Minnesota St. Paul campus greenhouse where we will subject both controls and microbe-associated plants to the most common pathogens from each site.

		Budget (1/31/17)	**Revised Budget (9/8/17)**
Summary Budget Information for Activity 2:	**ENRTF Budget:**	~~$203,052~~	$189,949
	Amount Spent:	$	$189,949
	Balance:	$	$0

Activity Completion Date: June 30, 2017

Outcome	**Completion Date**	Budget (1/31/17)	**Revised Budget (9/8/17)**
1. A. Determine the composition of beneficial microbial communities and how these microbial species differ across seed source populations.	October 2016	~~$135,903~~	$126,634
2. B. Evaluate the extent to which the beneficial microbes are locally adapted and protect plants against disease.	August 2017	~~$67,149~~	$63,315

Activity Status as of January 30, 2015:

Nothing to report at this time.

Activity Status as of September 1, 2015:

We will focus on the genus *Dalea (D. purpurea* (purple prairie clover) and *D. candida* (white prairie clover)) for the microbial studies because they harbor fungal symbionts in above ground tissues and nitrogen-fixing bacterial symbiont in the belowground roots. Soil microbial and foliar endophyte communities were sampled from the two *Dalea spp.* at 18 sites representing 12 focal populations in July and September, 2015. We are currently isolating soil microbes (N-fixing rhizobia) and fungal symbionts living in leaves (endophytes). Rhizobia will be isolated from each of the 12 populations representing MN prairie diversity (Kane Keller). The aboveground leaf fungal endophytes were isolated and will be identified using morphology and DNA sequence (Mara DeMers). Rhizobia and fungal isolates will be used in greenhouse experiments to determine effects on *Dalea* growth and reproduction as well as evaluations of natural community diversity. Preliminary trials of inoculation protocols are underway.

Activity Status as of January 11, 2016:

We have remained focused on the genus *Dalea* for microbial experiments. Using the microbial and foliar endophyte communities that were sampled in September 2015, we have progressed on the isolation and identification of the symbiotic microbes from 16 sites. Nitrogen-fixing rhizobia bacteria are nearly isolated from 10 populations of *Dalea purpurea* and 6 populations of *Dalea candida*. These rhizobia will be used in greenhouse experiments to assess how variation in legume-rhizobium associations across Minnesota may differentially affect *Dalea* growth and reproduction, and how they may affect other microbes associated with these legumes. *Dalea purpurea* and *Dalea candida* leaves were sampled from 16 prairie sites to identify the fungi inside them and measure trends in fungal diversity. The original 5670 leaf samples had approximately 93 percent growth rate, for a total of about 5300 isolates. Sequencing to identify them is approximately 10 percent complete.

Activity Status as of September 1, 2016:

Assessment of the diversity and composition of the endophytic and rhizobia bacterial communities associated with Dalea purpurea is steadily progressing. Using the soil samples collected in September 2015 from the same remnant prairie populations being used in Activity 3, we have isolated more than 400 strains of rhizobia bacteria via nodule capture. Identification of these strains using phylogenetic clustering and genetic sequencing is proceeding. Endophyte community sampling from the leaves of Dalea purpurea and D. candida at 16 prairie sites to identify the fungi inside them and to measure trends in fungal diversity also continues. Isolation from 2015 is complete and sequencing is about 80% finished. Greenhouse experiments exploring establishment of the endophyte community are under way, and we have begun collecting samples from greenhouse inoculation trials. Collections in 2016 of soil and leaf samples from each of the 12 focal Dalea purpurea populations are about 20% completed. Subsamples of these collections are being stored for metagenomics analysis to be conducted from November 2016 to February 2017.

Activity Status as of January 10, 2017:

Assessment of the diversity and composition of the endophytic and rhizobia bacterial communities associated with *Dalea purpurea* is steadily progressing. Using the soil samples collected in September 2015 and July 2016 from the same remnant prairie populations being used in Activity 3, we have isolated more than 1,000 strains of rhizobia bacteria via nodule capture from trap plants in the greenhouse. Identification of these strains using phylogenetic clustering and genetic sequencing is proceeding and is roughly 10% completed. Trial inoculations of these isolated rhizobia is proceeding in the greenhouse. Test inoculations of field soil from each of the three planting sites in Activity 3 is nearly completed. Endophyte community sampling from the leaves of *Dalea purpurea* and *D. candida* at 16 prairie sites to identify the fungi inside them and to measure trends in fungal diversity also continues. Isolation from 2015 is complete. Trials to select a method of inoculation have been completed and we are currently optimizing the inoculation of particular endophyte strains per plant for upcoming fitness experiments. Collections in 2016 of leaf samples from each of the 12 focal *Dalea purpurea* populations are completed, with roughly 2000 endophyte strains isolated and sequenced. Subsamples of these soil and leaf collections are being stored for metagenomics analysis to be conducted in spring 2017.

Activity Status as of August 15, 2017:

We have assessed of the diversity and composition of the endophytic and rhizobia bacterial communities associated with Dalea purpurea across 13 populations spanning the geographic range in Minnesota. Using the soil samples collected in September 2015 and July 2016 from the same remnant prairie populations being used in Activity 3, we have isolated more than 1,000 strains of rhizobia bacteria using trap plants. We have identified and assessed diversity in 800 of these strains using phylogenetic clustering and genetic sequencing ~ 300 strains of which are Rhizobium or Mesorhizobium nitrogen-fixing mutualists of Dalea purpurea. Identified strains are in cryopreserve storage for further studies (Phase II).

We completed a large-scale greenhouse experiment to determine the geographic scale of local co-adaptation between plant and rhizobia populations. This experiment utilized 5 of our Dalea purpurea plant populations and 10 rhizobia strains from each of those 5 plant populations (total of 50 strains) to determine population level effects of rhizobial variation on plant growth. The experiment is complete and the data are currently being analyzed.

Fungi living within plants (endophytes) may strongly affect plant growth and reproduction. We completed community sampling from the leaves of Dalea purpurea and D. candida at 16 prairie sites and established cultures of over 2000 fungi. . The cultures have been characterized by morphology and DNA sequence and community diversity has been assessed.

ACTIVITY 3: The scale of local adaptation.

Description:

To rigorously evaluate the degree of local adaptation, we will focus on six MN prairie species (at least one each of grass, forb, and legume), making use of the seeds collected as described above in Activity 1. We will plant seeds from all 12 populations sampled for each species in Wildlife Management Areas in at least three of the four sampling regions represented in the seed collections (near Crookston, Morris, and Lamberton). We will monitor survival and growth of plants from each sampled population at each site in order to determine the relationship between plant survival and growth, on the one hand, and, on the other, the experimental factors: region of garden, region of origin, and soil moisture in location of origin. At each location, we will establish arrays in a randomized complete block design. The 72 species-populations will be assigned to random positions within each block. Seeds will be sown into the experiment in the fall so they will undergo natural field conditions through the winter. We will monitor seedling emergence in plots early in the following year, late May/early June. Seedlings will be individually marked, and their locations will be precisely mapped. The survival and size (leaf number and length of longest leaf) of each seedling will also be recorded. For each species, the fitness of the populations in the three locations will be evaluated and compared. We will evaluate the degree of local adaptation by comparing the overall fitness of the populations sampled nearest to each experimental site with those from more remote locations. We will also test for the role of source habitat (moist vs. dry) in the expression of fitness in each location.

		Budget (1/31/17)	**Revised Budget (9/8/17)**
Summary Budget Information for Activity 3:	**ENRTF Budget:**	~~$267,622~~	$289,839
	Amount Spent:	$	$289,839
	Balance:	$	$0

Activity Completion Date: June 30, 2017

Outcome	**Completion Date**	Budget (1/31/17)	**Revised Budget (9/8/17)**
1. Experiments to evaluate effects of seed source distance on establishment and long-term success of prairie plants in restorations.	August 2017	~~$200,266~~	$217,380
2. Evaluation, for each species, of the relationship between early performance of plants and distance to source as well as habitat characteristics.	August 2017	~~$67,356~~	$72,459

Activity Status as of January 30, 2015:

From each of the sampled populations, seeds of six species, *Anemone cylindrica* (thimbleweed), *Bouteloua curtipendula* (sideoats grama), *Dalea candida* (white prairie clover), *Dalea purpurea* (purple prairie clover), *Monarda fistulosa* (wild bergamot), and *Schizachyrium scoparium* (little bluestem), have been cleaned and scarified as necessary, and stratified in preparation for growing them out. We pooled an aliquot of cleaned seed from individual plants of the same species from the same site. Therefore, a portion of seed from each individual plant remains distinct, which will allow for a future assessment of the scale of local adaptation within sites, and as well as between sites. Currently the pooled seeds are germinating in a growth chamber. When they develop into seedlings they will be transplanted into the greenhouse and in the spring moved into field plots to produce the next generation of seeds for this experiment.

Activity Status as of September 1, 2015:

Over 52,000 plants were reared in the greenhouse; of these, 7,200 (100 plants x 12 populations per species x 6 species) were transplanted into a field site in Rosemount, Minnesota in June-July 2015. The remaining plants were installed into buffer zones surrounding the field site. These plants are being allowed to naturally cross-pollinate with conspecifics from the same population. Two of the species species, *Monarda fistulosa* (wild bergamot) and *Anemone cylindrica* (thimbleweed) have established 85 - 100 healthy plants per population but have not flowered. Four of the species, *Dalea purpurea* (purple prairie clover), *Dalea candida* (white prairie clover), *Schizachyrium scoparium* (little bluestem), and *Bouteloua curtipendula* (sideoats grama), are producing seeds, which we are collecting preparatory to planting them into the reciprocal transplant experiment in October 2015. In consultation with DNR personnel who manage the Wildlife Management Areas, we have identified sites to host this experiment in the northwest, west central and southwest region, as planned.

Activity Status as of January 11, 2016:

The 12 populations of *Bouteloua curtipendula* (sideoats grama) grown at the Rosemount field site produced 8.2 lbs of seed; the 12 *Schizachyrium scoparium* (little bluestem) populations produced 1.9 lbs of seed. During October 2015, experimental plots were established at the Twin Valley, Dolven, and Lake Bella Wildlife Management Areas (WMAs); the little bluestem and sideoats seeds were sown into these plots in November-December 2015. For each species, 50-100 seeds were sown into 20-45 replicate subplots per population per WMA.

Activity Status as of September 1, 2016:

All 12 populations of both sideoats grama and little bluestem have established well at each of the 3 experimental sites where they were sown in 2015. At the 3 sites, we tagged and collected early-season data on establishment, leaf-number, and plant height on over 8,000 individuals. Data entry and analysis are ongoing. All 6 species sown at the transplant site in 2015 are flourishing; we anticipate ample seed of the 2 legume and 2 forb species will be available for sowing at the evaluation sites, as well as second cohorts of sideoats grama and

little bluestem. Seed collection at our seed-increase plots is complete for 4 of the 6 species and 50% complete for the remaining 2 species.

Activity Status as of January 10, 2017:

We collected a second, late-season round of establishment, leaf-number, and height data on 8,000 individuals of the 2 grass species established at our 3 outstate evaluation sites. Data entry is complete for the early-season observations and is underway for the late-season. Analysis is ongoing. We completed seed collection from our seed-increase plots and processed and packaged 30 packages of seed per population per species (~ 6,500 packages total). These seeds, which constituted initial cohorts of the 2 legume and 2 forb species and 2nd cohorts of the 2 grass species, were sowed into each of the 3 evaluation sites.

Activity Status as of August 15, 2017:
Entry and analysis of all first-season (2016) data is complete. Preliminary results were presented at a national conference (National Native Seed Conference, Washington, D.C., February 13-16, 2017). Estimation of the geographic scale of local adaptation, using aster modeling to estimate population-level fitness, is in progress. We are monitoring establishment of the cohort sown into our 3 out-state evaluation sites last year (12 populations each of 6 species). All cohorts will be assessed again in Aug-Sept 2017 in Phase II activities.

ACTIVITY 4: Adaptive capacity of prairie populations.

Description:

We will assess the genetic variation available to support ongoing adaptation by conducting quantitative genetic studies of plant characteristics involved in adaptation; for example, leaf thickness is often important for adaptation to drought. Given the labor-intensive nature of this kind of study, we will focus on one species that is also represented in the local adaptation experiment (Activity 3). As candidates for this component of the project, we are considering *Aster sericeus, Coreopsis palmata, Lobelia spicata*, and *Thalictrum dasycarpum*. The final choice of focal species will be based on availability and size of populations. For the focal species, we will obtain a pedigreed sample of seeds from each of three populations by carrying out formal genetic crosses between plants. Within each population, at least 60 individual plants will be chosen at random to serve as parents and crossed to produce approx. 150 seeds per mating. To prevent access of insect pollinators and herbivores to the flowers, we will cover them with netting while the flowers are receptive and again later to prevent seeds from being lost to natural dispersal. The resulting progeny will be grown at three sites, one near each source population. Seeds will be sown during the fall and monitored for germination, survival, and reproduction, as in Activity 3.

		Budget	Revised Budget (9/8/17)
Summary Budget Information for Activity 4:	**ENRTF Budget:**	~~$83,335~~	$74,465
	Amount Spent:	$	$74,465
	Balance:	$	$0

Activity Completion Date: June 30, 2017

Outcome	Completion Date	Budget
1. Prediction of rates of adaptation based on genetic variation and natural selection.	August 2017	$74,465

Activity Status as of January 30, 2015:

Nothing to report at this time.

Activity Status as of September 1, 2015:

We have identified *Schizachyrium scoparium* (little bluestem) as a probable candidate for this activity. Plants have been reared from seed that was wild-harvested in 2014 and are being grown to reproductive maturity in the greenhouse. We will then test *S. scoparium* for self-incompatibility. This condition is assumed by the scientific literature but has not been definitively established. Pending the outcome of the self-incompatibility assessment, we will conduct planned crosses on this test population to refine our methods.

Activity Status as of January 11, 2016: To evaluate self-incompatibility of *Schizachyrium scoparium* (little bluestem), we are in the process of vernalizing the plants to induce flowering.

Activity Status as of September 1, 2016:

During this reporting period, we finalized our protocols for pollen harvest and transport and for performance of planned crosses. Paternal plants growing at the site of origin (Olmsted County) were identified, GPS-located, and tagged. However, our experimental little bluestem populations – as well as those growing in at least 8 remnant prairies in southern and central MN - flowered very early this season. We are thus unable to perform the crosses with little bluestem in the field as planned and are exploring alternatives.

Activity Status as of January 10, 2017:

We are in the process of stratifying and germination seed from 2 populations of *Schizachyrium scoparium* (little bluestem). The resulting individuals will be reared in the greenhouse and used for planned crosses.

Activity Status as of August 15, 2017:
800 little bluestem individuals (400 from each of 2 populations) were transplanted into a field site in St. Paul in July 2017. These individuals will form the foundation of pedigreed lineages. We anticipate conducting planned crosses in late August-mid September.

Final Report Summary:
This Project (Prairie Sustainability Through Seed Storage, Beneficial Microbes, and Adaptation) has achieved one of its primary goals and experiments are complete to accomplish the other three as above. Additionally, we will capitalize on the project's research infrastructure (long-term field experiments, seed-increase plots, pedigreed lines) and relationships with external partners (state and federal agencies, colleges and universities, community groups, non-profit organizations) as we continue Phase 2, thanks to renewed ENRTF support.

V. DISSEMINATION:

DESCRIPTION:

The findings of this project will be promulgated via oral presentations and research papers in the scientific literature. The experimental populations established at Wildlife Management Areas will continue to be available for further research beyond the term of the funding. They will also serve as prairie restorations on these sites. The germplasm will be made available to producers of seed for restorations.

Status as of January 30, 2015:

Principal investigator Ruth Shaw discussed the project's goals and rationale at a training for Nature Conservancy staff in January.

Status as of September 1, 2015:

Principal investigators Ruth Shaw and Georgiana May discussed the project's goals and rationale at a symposium at University of Minnesota-Morris (Feb). Dr. Shaw also discussed the project, its aims, and its motivations at the 2015 annual symposium of the Native Plant Society of Minnesota (March).

Status as of January 11, 2016:

Nothing to report at this time.

Status as of September 1, 2016:

In July, we participated in Market Science, which is a collaboration between scientists at the University of MN and other scientists throughout the Twin Cities. Market Science uses a common space at the Lake Street Farmers' Market to provide market-goers with interactive explorations of topics in natural history and basic life

and physical sciences. We had two hands-on activities: creating “seed balls”, balls of mud and native seed used for restoration and native landscaping; and looking through microscopes at fungal endophytes cultured from native prairie plant leaves. We had 130 people stop by our booth and were able to disseminate informational flyers about the Healthy Prairies Project, including volunteer opportunities. We discussed our research goals and aims, while educating people on the distribution and importance of the prairie ecosystem in Minnesota.

Status as of January 10, 2017:
Principal investigator Georgiana May discussed the project and its aims at The Nature Conservancy’s first annual Science Slam in December 2016. This event was attended by TNC staff, conservation professionals, and representatives of many research projects conducted on TNC properties. The Healthy Prairies team leveraged outcomes of Activity 1 in a successful application for an intramural UMN grant. The grant supports initial steps in conveying source-identified foundation seed to growers.

Status as of August 15, 2017:
Post-doc Shelby Flint presented preliminary results from Activity 3 at a seminar given to students and faculty at the State University of New York, Plattsburgh and at a national conference (National Native Seed Conference, Washington, D.C.) in February. Post-doc Kane Keller presented results from Activity 2 at a seminar given to students and faculty at California State University, Bakersfield in March. Graduate student Mara Demers presented results for Activity 2 at national meetings in 2016 and 2017 and graduate student Nicholas Goldsmith presented work from Activity 1 (at the Ecological Society of America Conference in August. Principal investigator Ruth Shaw discussed the project’s aims with the 12 participants of 2 focus groups, who included personnel from state and federal agencies and non-profit organizations.

Final Report Summary:
We have communicated our project’s motivation and context, aims, and results to at least 400 community members, resource managers, students, and researchers throughout the duration of this project. As a result of these conversations, the citizens of Minnesota have a greater appreciation of the importance of prairies and prairie conservation.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	Revised $ Amount (1/11/16)	Revised $ Amount (5/26/16)	Revised $ Amount (9/1/16)	Revised $ Amount (1/30/17)	Revised $ Amount (9/8/17)	Explanation
Personnel:	$540,000	$535,600	$517,870	$517,870	$521,439	Faculty summer salaries, 1 postdoctoral fellow, 2 graduate students, 4 undergraduate assistants, 2 civil service employees.
Professional/Technical/Service	$17,000	$17,000	$17,000	$17,000	$5,222	Sequencing to evaluate the microbial communities (metagenomics

Contracts:						~$13,000 at the UM sequencing facility) associated with focal plant species. Individual fungal sequencing to better identify isolates (~ $4000).
Equipment/Tools/ Supplies:	$18,300	$17,300	$20,320	$21,179	$31,245	Lab (petrie dishes, reagents), field supplies (stakes, markers, envelopes for seed),
Services (5/26/16):	$2,700	$8,700	$19,270	$14,403	$11,714	Fees for greenhouse use and shipping of seeds; Subcontracting with seed collectors
Travel Expenses in MN:	$22,000	$21,400	$25,540	$29,548	$30,380	Mileage (UM-TC rental: $40/day, $0.17/mile) for travel to sites (from UM-TC St. Paul campus to Polk, Redwood, Stevens, and Wabasha Counties, altogether 1003 miles between regions) for collecting seeds (4 times in 2014 and in 2015) and to locations of experimental plots (from UM-TC St. Paul campus to Crookston, Morris, and Lamberton); (4 additional trips in 2015, 8 in 2016, and two in 2017), as well as lodging.
TOTAL ENRTF BUDGET:	$600,000	$600,000	$600,000	$600,000	$600,000	

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: 10.17

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

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
University of Minnesota-TC	$156,000	$28,594	In-kind support: salaries and office space for Shaw, May, and Wyse. Off-campus indirect cost rate, 26%.
NCGRP	$29,520		In-kind support: preparation of seed samples ($5760 = 480samples*$12/sample), cryogenic storage ($21600 = 480*3yr*$15/accession/yr), assays of a subset of seed samples ($2160 =120*$18/sample)
State			
	$0	$0	
TOTAL OTHER FUNDS:	**$185,520**	**$**	

VII. PROJECT STRATEGY:

A. Project Partners:

Project Partners not receiving funds:

UMN-TC faculty Drs. D. Wyse, S. Galatowitsch, D. Moeller, P. Tiffin; UM-D faculty Dr. J. Etterson; MN-DNR; The Nature Conservancy. USDA NCGRP (Drs. C. Walters, C. Richards).

Project Partners receiving funds: UMN-TC faculty Drs. R. Shaw, G. May; post-doctoral fellows, graduate students, undergraduate students, and civil service staff, TBD.

B. Project Impact and Long-term Strategy:

This work will provide fundamental information necessary to efforts to establish both scientifically sound and economically feasible practices for prairie restoration. Restorations that will thrive as stable, resilient plant communities that provide habitat for diverse pollinators and wildlife require large quantities of seeds adapted to the environment in which they will grow. Even small remnants of prairie retain valuable genetic resources.

Protection of these remaining genetic resources can ensure that this genetic variation will be available as germplasm for massive restorations now, as the basis for future adaptation to climate change, and for human uses yet to be discovered. We will archive the seed collections to maintain their viability for the long-term so that they will be available as the basis for restorations, as well as for further research. We intend to make them available to producers of seed for restorations as results of the experiments emerge. Because prairie species are long-lived, we intend to continue this project well beyond the initial three-year funding period in order to accomplish fully informative accounting of plant fitness to address the focal questions, and we are making this clear to the managers of the WMAs that will host the experiment of Activity 3. We will invite colleagues to conduct research that takes advantage of this long-term experiment, and we will seek further funding to support the continuation of the research.

C. Spending History:

Funding Source	**M.L. 2008 or FY09**	**M.L. 2009 or FY10**	**M.L. 2010 or FY11**	**M.L. 2011 or FY12-13**	**M.L. 2013 or FY14**
NSF grant to Shaw: development of protocol for establishing quantitative genetic experiments on prairie plants from seed	$5,000	$5,000	$5,000	$5,000	
NSF grant to Etterson (co-PI Shaw):development of protocol for collections and storage of seeds; for assessment of sites and securing permits				$30,000	$5,000
NSF grant to May: Development of methods for studying endophytic fungi and bacteria, and statistical analyses of endophyte communities.					$10,000

VIII. ACQUISITION/RESTORATION LIST: N/A

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

X. ACQUISITION/RESTORATION REQUIREMENTS WORKSHEET: N/A

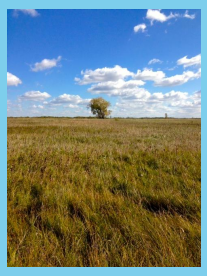
XI. RESEARCH ADDENDUM: N/A

XII. REPORTING REQUIREMENTS:

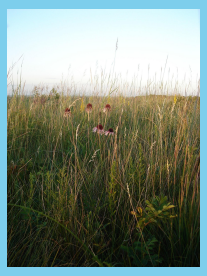
Periodic work plan status update reports will be submitted no later than January 30, 2015, September 1, 2015, January 11, 2016, September 1, 2016 and January 10, 2017. A final report and associated products will be submitted between June 30 and August 15, 2017.

Environment and Natural Resources Trust Fund
M.L. 2014 Project Budget
Project Title: Priarie Sustainability through Seed Storage, Beneficial Microbes, and Adapt
Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 6c
Project Manager: Dr. Ruth Shaw
Organization: Regents of the University of Minnesota
M.L. 2014 ENRTF Appropriation: $600,000
Project Length and Completion Date: 3 years, June 30, 2017
Date of Report:September 8, 2017

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET BUDGET ITEM	Activity 1 Budget (1/31/17) *Collection & conservation of 480 populations of prairie plants*	Revised Activity 1 Budget (9/8/17)	Amount Spent	Activity 1 Balance	Activity 2 Budget (1/31/17) *Microbial aids to plant health*	Revised Activity 2 Budget (9/8/17)	Amount Spent	Activity 2 Balance	Revised Activity 3 Budget (1/31/17) *The scale of local adaptation*	Revised Activity 3 Budget (9/8/17)	Amount Spent	Activity 3 Balance	Activity 4 Budget (9/1/16) *Adaptive capacity of prairie populations*	Revised Activity 4 Budget (9/8/17)	Amount Spent	Activity 4 Balance	TOTAL BUDGET	TOTAL BALANCE
Personnel (Wages and Benefits)	~~$36,370~~	$36,078	$36,078	$0	~~$172,170~~	$161,767	$161,767	$0	~~$233,295~~	$257,105	$257,105	$0	~~$76,035~~	$66,489	$66,489	$0	$521,439	$0
Dr. Ruth Shaw, Professor: $15,600 (75% salary, 25% benefits); one half month summer salary for 2 years																		
Dr. Georgiana May: $14,270 (75% salary, 25% benefits): one half month summer salary for 2 years																		
Dr. Donald Wyse: $0																		
2 Postdoctoral Associate: $249,000 (83% salary, 17% benefits); 100% FTE for 2.5 years																		
1 Graduate Research Assistant: $77,000 (58% salary, 42% benefits); 50% FTE for year 1 and 41% FTE for year 2																		
4 Undergraduate Research Assistants or temp/casual employees: $50,000 (93% salary, 7% benefits); Two 21% FTE for year 1 & four 21% FTE for years 2 & 3																		
2 Civil Service/Union people: $112,000 (73% salary, 27% benefits); 50% FTE for 2 years																		
Professional/Technical/Service Contracts - UM sequencing facility) metagenomics ~$13,000 to assess bacteria associated with focal plant species. Individual sequencing to identify fungal isolates (~ $4000)					~~$17,000~~	$5,222	$5,222	$0									$5,222	$0
Services (5/26/2016): Seed collection by subcontractors	$2,110		$2,110	$0													$2,110	$0
Equipment/Tools/Supplies- Field Supplies: envelopes for gathering seeds, stakes for marking field experiments	$2,612		$2,612	$0					$7,086		$7,086	$0	~~$1,300~~	$947	$947	$0	$10,645	$0
Lab Supplies: petri dishes, media reagents				$0	~~$8,020~~	$19,292	$19,292	$0	$1,162		$1,162	$0	~~$1,000~~	$147	$147	$0	$20,601	$0
Services(01/30/2015): fees for greenhouse use, plot fees, shipping of seeds	~~$580~~	$628	$628	$0	~~$3,650~~	$1,456	$1,456	$0	~~$5,063~~	$3,470	$3,470	$0	~~$3,000~~	$4,050	$4,050	$0	$9,604	$0
Travel expenses in Minnesota - Mileage (UM-TC rental: $40/day, $.17/mile) for travel to sites (from UM-TC St. Paul campus to Polk, Redwood, Stevens, and Wabasha Counties, altogether 1003 miles between regions) for collecting seeds (four times in 2014 and in 2015) and to locations of experimental plots (from UM-TC St. Paul campus to Crookston, Morris, and Lamberton; (4 additional trips in 2015, 8 in 2016, and two in 2017), as well as lodging.	$4,319		$4,319	$0	$2,212		$2,212	$0	$21,016		$21,016	$0	~~$2,000~~	$2,832	$2,832	$0	$30,380	$0
COLUMN TOTAL	~~$45,091~~	$45,746	$45,746	$0	~~$203,052~~	$189,949	$189,949	$0	~~$267,622~~	$289,839	$289,839	$0	~~$83,335~~	$74,465	$74,465	$0	$600,000	$0



Join the Healthy Prairies Project!



Less than 1 % of MN prairies remain. Those that persist are fragmented and have lost genetic diversity.

Prairies improve water quality, prevent droughts and floods, and provide habitat for wildlife.

Collaborate with the Healthy Prairies Project to harvest seed from prairie remnants in the summer/fall of 2016!!

- Learn to identify MN prairie plants
- Contribute to MN prairie conservation research
- Spend time outdoors exploring MN prairies

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND
UNIVERSITY OF MINNESOTA
Funded by the Environment and Natural Resources Trust Fund

Contact us for more information:
HealthyPrairies@gmail.com
612-626-3836