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

Date of Report: January 15, 2014

Date of Next Status Update Report: January 12, 2015

Date of Work Plan Approval:

Project Completion Date: August 31, 2017

Does this submission include an amendment request? No

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:	**$0**
	Balance:	**$600,000**

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: Healthy prairies: 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.

Restorations that will thrive 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. 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 12, 2015:

Project Status as of September 1, 2015:

Project Status as of January 11, 2016:

Project Status as of September 1, 2016:

Project Status as of January 10, 2017:

Project Status as of August 15, 2017:

Overall Project Outcomes and Results:

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, and taking care both to obtain genetically representative samples for each population and to 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 that 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 that we include plants spanning a wide range of timing of seed production. Each seed sample will be divided, and half 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. Half 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.

Summary Budget Information for Activity 1:

ENRTF Budget: $125,000

Amount Spent: $ 0

Balance: $125,000

Activity Completion Date: October 15, 2016

Outcome	Completion Date	Budget
1. A lasting archive of a well-designed sample of prairie genetic diversity.	October 2016	$125,000
2. Measures of the initial viability of seed samples.	December 2016	$0
3. Estimates of the longevity of the stored seeds.	August 2017	$0

Activity Status as of January 12, 2015:

Activity Status as of September 1, 2015:

Activity Status as of January 11, 2016:

Activity Status as of September 1, 2016:

Activity Status as of January 10, 2017:

Activity Status as of August 15, 2017:

ACTIVITY 2: Microbial Aids to Plant Health

A. 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 to 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 UM St. Paul greenhouse where we will subject both controls and microbe-associated plants to the most common pathogens from each site.

Summary Budget Information for Activity 2: **ENRTF Budget: $150,000**

Amount Spent: $ 0

Balance: $150,000

Activity Completion Date: June 30, 2017

Outcome	Completion Date	Budget
1. A. Determine the composition of beneficial microbial communities and how these microbial species differ across seed source populations.	October 2016	$90,000
2. B. Evaluate the extent to which the beneficial microbes are locally adapted and protect plants against disease.	August 2017	$60,000

Activity Status as of January 12, 2015:

Activity Status as of September 1, 2015:

Activity Status as of January 11, 2016:

Activity Status as of September 1, 2016:

Activity Status as of January 10, 2017:

Activity Status as of August 15, 2017:

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. 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 that they will undergo natural field conditions through the winter. We will monitor seedling emergence in the 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.

Summary Budget Information for Activity 3:

ENRTF Budget: $225,000

Amount Spent: $ 0

Balance: $225,000

Activity Completion Date: June 30, 2017

Outcome	Completion Date	Budget
1. Experiments to evaluate effects of seed source distance on establishment and long-term success of prairie plants in restorations.	August 2017	$175,000
2. Evaluation, for each species, of the relationship between early performance of plants and distance to source as well as habitat characteristics.	August 2017	$50,000

Activity Status as of January 12, 2015:

Activity Status as of September 1, 2015:

Activity Status as of January 11, 2016:

Activity Status as of September 1, 2016:

Activity Status as of January 10, 2017:

Activity Status as of August 15, 2017:

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 it 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, 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 are 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.

Summary Budget Information for Activity 4: **ENRTF Budget: $100,000**

Amount Spent: $ 0

Balance: $100,000

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	$100,000

Activity Status as of January 12, 2015:

Activity Status as of September 1, 2015:

Activity Status as of January 11, 2016:

Activity Status as of September 1, 2016:

Activity Status as of January 10, 2017:

Activity Status as of August 15, 2017:

Final Report Summary:

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 12, 2015:

Status as of September 1, 2015:

Status as of January 11, 2016:

Status as of September 1, 2016:

Status as of January 10, 2017:

Status as of August 15, 2017:

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Explanation
Personnel:	$564,000	Faculty summer salaries, 1 postdoctoral fellow, 2 graduate students, 4 undergraduate assistants, 2 civil service employees
Professional/Technical/Service Contracts:	$17,000	Sequencing to evaluate the microbial communities (metagenomics ~$13,000 at the UM sequencing facility) associated with focal plant species. Individual fungal sequencing to better identify isolates (~ $4000).
Equipment/Tools/Supplies:	$9,000	Lab (petrie dishes, reagents), greenhouse (pots, labels), and field supplies (stakes, markers)
Travel Expenses in MN:	$10,000	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 (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 iin 2015, 8 in 2016, and two in 2017), as well as lodging.
TOTAL ENRTF BUDGET:	**$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	$	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 = 480 samples*$12/sample), cryogenic storage ($21600 = 480*3 yr*$15/accession/yr), assays of a subset of seed samples ($2160 = 120*$18/sample)
State			
	$0	$0	
TOTAL OTHER FUNDS:	**$**	**$**	

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 fellow, graduate students, undergraduate students, and civil service staff, TBD.

B. Project Impact and Long-term Strategy: *T*

This work will provide fundamental information necessary to efforts to establish bothscientifically sound and economically feasible practices for prairie restoration. Restorationsthat will thrive as stable, resilient plant communities that provide habitat for diversepollinators and wildlife require large quantities of seeds adapted to the environment inwhich they will grow. Even small remnants of prairie retain valuable genetic resources.Protection of these remaining genetic resources can ensure that this genetic variation willbe available

as germplasm for massive restorations now, as the basis for future adaptationto climate change, and for human uses yet to be discovered. We will archive the seedcollections to maintain their viability for the long-term so that they will be available as thebasis for restorations, as well as for further research. We intend to make them available toproducers of seed for restorations as results of the experiments emerge. Because prairiespecies are long-lived, we intend to continue this project well beyond the initial three-yearfunding period in order to accomplish fully informative accounting of plant fitness toaddress the focal questions, and we are making this clear to the managers of the WMAsthat will host the experiment of Activity 3. We will invite colleagues to conduct researchthat takes advantage of this long-term experiment, and we will seek further funding tosupport 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_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

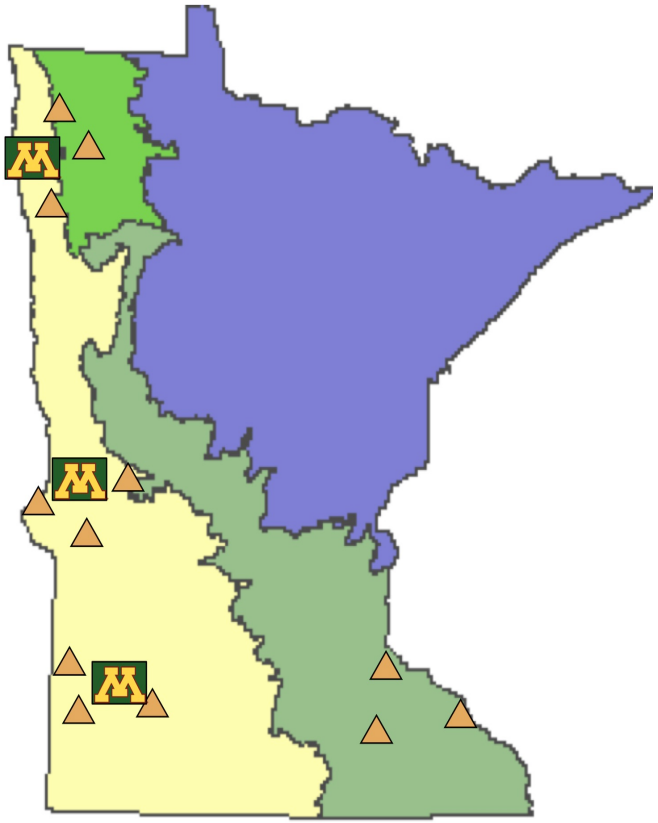
XI. RESEARCH ADDENDUM: N/A

XII. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than January 12, 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 Adaptation
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, August 31, 2017*
Date of Report: January 15, 2014

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET BUDGET ITEM	Activity 1 Budget *Collection and conservation of 480 populations of prairie plants*	Amount Spent	Activity 1 Balance	Activity 2 Budget *Microbial aids to plant health*	Amount Spent	Activity 2 Balance	Activity 3 Budget *The scale of local adaptation*	Amount Spent	Activity 3 Balance	Activity 4 Budget *Adaptive capacity of prairie populations*	Amount Spent	Activity 4 Balance	TOTAL BUDGET	TOTAL BALANCE
Personnel (Wages and Benefits)	$141,000	$0	$141,000	$141,000	$0	$141,000	$141,000	$0	$141,000	$141,000	$0	$141,000	$564,000	$564,000
Dr. Ruth Shaw, Professor: $23,000 (75% salary, 25% benefits); one half month summer salary for 3 years														
Dr. Georgiana May: $21,000 (75% salary, 25% benefits): one half month summer salary for 3 years														
Dr. Donald Wyse: $0														
1 Postdoctoral Associate: $149,000 (83% salary, 17% benefits); 100% FTE for 3 years														
2 Graduate Research Assistants: $153,000 (58% salary, 42% benefits); 50% FTE for year 1 and 41% FTE for year 2														
4 Undergraduate Research Assistants: $49,000 (93% salary, 7% benefits); Two 21% FTE for year 1 & four 21% FTE for years 2 & 3														
2 Civil Service people: $169,000 (73% salary, 27% benefits); 50% FTE for 3 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		$17,000								
Equipment/Tools/Supplies- Field Supplies: envelopes for gathering seeds, stakes for marking field experiments	$500	$0	$500		$0	$0	$2,000	$0	$2,000	$1,500	$0	$1,500	$4,000	$4,000
Lab Supplies: petri dishes, media reagents				$5,000		$5,000							$5,000	$5,000
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.	$4,000	$0	$4,000	$2,000	$0	$2,000	$2,000	$0	$2,000	$2,000	$0	$2,000	$10,000	$10,000
COLUMN TOTAL	$145,500	$0	$145,500	$165,000	$0	$165,000	$145,000	$0	$145,000	$144,500	$0	$144,500	$600,000	$600,000



Evaluation sites
– UM Outreach stations

Collection sites

Plant-associated microbes
nutrient uptake
N-fixation
protection against disease

Soil microbes
decomposition
mineralization