2017 Project Abstract
For the Period Ending June 30, 2021

PROJECT TITLE: Preserving Minnesota Prairie Plant Diversity – Phase II
PROJECT MANAGERS: Ruth G. Shaw, Georgiana May
AFFILIATION: Department of Ecology, Evolution and Behavior, University of Minnesota
MAILING ADDRESS: 1479 Gortner Ave., 140 Gortner Laboratory
CITY/STATE/ZIP: St. Paul, MN 55108
PHONE: (612) 624-7206 (Shaw)
E-MAIL: shawx016@umn.edu
WEBSITE: https://ruthgshaw.wordpress.com/research/
FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2017, Chp. 96, Sec. 2, Subd. 03c as extended by M.L. 2020, First Special Session, Chp. 4, Sec. 2

APPROPRIATION AMOUNT: $900,000
AMOUNT SPENT: $900,000
AMOUNT REMAINING: $0

Sound bite of Project Outcomes and Results

We gathered seeds of prairie plants and shared them with producers who are expanding seed availability for restorations. We collected, identified and studied many microbes that prairie plants harbor, documenting their effects on their hosts. Our experiments have clarified the geographic scale of plant adaptation and genetics underlying ongoing adaptation.

Overall Project Outcome and Results

Minnesota prairies harbor extraordinary diversity of plants and microbes, while also nurturing wildlife, retaining water and topsoil, and beautifying landscapes. Yet habitat loss threatens the persistence of the once vast prairies and their stunning biotic diversity. Limited understanding of this diversity and insufficient seed availability hinder sustainable management of this iconic Minnesota biome. We conducted Healthy Prairies (HP) Phase II to expand availability of seeds for prairie restorations and study approaches to increase success of restorations. Building on our prior accomplishments under ENRTF funding, we have:

1. Preserved diverse seed from 57 rarer prairie species, gathering them from widely separated locations.
2. Obtained, archived, and studied 2,600 naturally occurring microbial partners from two species.
3. Gathered data to assess the geographic scale important to plant survival and reproduction in MN.

Our extensive collections of source-identified seeds and microbes across a wide range of MN's prairie region help to conserve the diversity of MN prairies. We have provided seeds to seed producers, who have, in turn, used them in establishing fields and are seeking certification of the seeds that they obtain from them.

Our studies of effects of microbial associates on prairie plants have indicated that the bacteria providing nitrogen to prairie clover (*Dalea purpurea*, *D. candida*) disperse widely across MN prairies. Consequently, we can recommend to growers an inoculum that need not be site-specific. In contrast, the communities of fungi associated with roots of *S. scoparium* are spatially restricted, indicating that a regionally-based inoculum may be preferable.

We continued our large-scale experiment to elucidate the geographic scale of adaptation of six prairie species. We gathered extensive data from this experiment and began analyses of the data. We implemented experiments to investigate genetic structure of two populations of little bluestem (*Schizachyrium scoparium*), including genetic variance for fitness and the fitness consequences of inbreeding and of crossing between populations.

Project Results Use and Dissemination

HP team members have participated in varied opportunities to disseminate findings from this project. These include informal events to communicate with members of the public who are not all well-versed in science and may not be aware of prairies (Market Science), as well as workshops involving other scientists and land managers (Nature Conservancy 'Science Slams', Local Adaptation Workshop, held at UM-TC, March 2019, discussions of seed sourcing guidelines led by staff of MN DNR).

A paper providing an overview of the Local Adaptation Workshop has been published in *New Phytologist* (2020) 225:2246–2248*.* A manuscript reporting findings about geographic scale of local adaptation has been submitted to *Restoration Ecology* and has received positive reviews. A second manuscript reporting on a study that used focus groups to identify impediments to use of source-identified seeds for prairie restorations has been submitted to *Restoration Ecology* and has received positive reviews. Both manuscripts are under revision and will be resubmitted soon.

Environment and Natural Resources Trust Fund (ENRTF)

M.L. 2017 LCCMR Work Plan Final Report

Date of Submission: March 17, 2021
Final Report
Date of Work Plan Approval: 06/07/2017
Project Completion Date: June 30, 2021
Does this submission include an amendment request? Yes

PROJECT TITLE: Preserving Minnesota Prairie Plant Diversity – Phase II

Project Managers: Ruth G. Shaw, Georgiana May

Organization: Department of Ecology, Evolution and Behavior, University of Minnesota

Mailing Address: 100 Ecology Bldg.

1987 Upper Buford Circle

University of Minnesota

City/State/Zip Code: St. Paul, MN 55108

Telephone Number: (612) 624-7206 (Shaw)

Email Address: shawx016@umn.edu

Web Address: https://ruthgshaw.wordpress.com/research/

Location: WCentral, SE,SW,NW

Total ENRTF Project Budget:

ENRTF Appropriation:	**$900,000**
Amount Spent:	**$900,000**
Balance:	**$0**

Legal Citation: M.L. 2017, Chp. 96, Sec. 2, Subd. 03c as extended by M.L. 2020, First Special Session, Chp. 4, Sec. 2

Appropriation Language:
$900,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to continue collecting and preserving 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, 2020, by which time the project must be completed and final products delivered.

M.L. 2020 - Sec. 2. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2021]

I. PROJECT TITLE: Healthy Prairies II: Preserving Prairie Plant Diversity

II. PROJECT STATEMENT:
Minnesota prairies harbor an extraordinary diversity of plant and microbial life, while also nurturing wildlife, retaining water and topsoil, and beautifying rural landscapes. Yet habitat loss and environmental variability threaten the persistence of the once immense prairie landscape and its stunning biotic diversity. Moreover, limited understanding of this diversity and insufficient seed availability hinder cost-effective and sustainable management of this iconic Minnesota biome.
Healthy Prairies (HP) Phase II will build on the accomplishments under current funding (2014-2017). Our team and volunteers spent over 1000 hours scouting 27 prairie remnants and cataloging locations over MN prairie regions for 40 of the more common and widespread native prairie species. We collected seed from thousands of individuals, retaining extensive genetic variation while tracking locality. For experimental work, we have cultured over 5000 plant-associated microbes. We established seed-increase plots for 6 plant species (from 12 sites) and used these in experimental plantings at three locations spanning the latitudinal range of MN prairies. To realize this investment in the preservation of MN prairie plant diversity, while providing essential resources and information for prairie restoration, we will:

- Preserve diverse seed from 20 of the rarer prairie species.
- Obtain and maintain cultures of an additional 5000 naturally occurring microbial partners for grasses.
- Determine the geographic scale important to plant survival and reproduction in a varying environment.

Four major MN geographic regions across the native prairie will be served. Providing locally-sourced seed, the project will help restore and conserve the diversity of MN prairies and their associated wildlife, pollinator and microbial diversity.

III. OVERALL PROJECT STATUS UPDATES:
Amendment Request (8/14/17):
We request approval of the attached budget and the Summary Budgets for each Activity in this workplan. These items adjust our proposed budget to the amount allocated ($900,000).
We also request approval for an additional item under the "Other" line, Activity 1, on the attached budget, namely "Fees for independent contractors collecting seeds". Utilizing services offered by professional native-seed collectors in distant parts of Minnesota will result in more efficient, cost-effective, and diverse seed collection.
Finally, we request approval of revised deadlines for Project Status updates. The previous version of this workplan included due dates of Dec 1, 2018, Dec 1, 2019, and Dec. 1, 2020. These appear to be errors; we have changed these dates to Dec. 1, 2017, Dec. 1, 2018, and Dec. 1, 2019.
Amendment Approved by LCCMR 8/14/2017

Project Status as of *Dec. 1, 2017***:** Report deferred per communication from LCCMR staff on July 24, 2017.

Project Status as of *June 1, 2018***:**
We have staffed the project with highly capable individuals who have the necessary expertise to collect and conserve seeds, carry out the experiments, and maintain the research infrastructure established during Healthy Prairies I (July 2014-June 2017). We have scouted seed collection sites, obtained necessary permits, and are collaborating with partners and citizen groups to obtain collections of seeds that represent much of the geographic extent of MN prairie, as well as the genetic variability of target species. We are currently installing field experiments to test conclusions reached during Healthy Prairies 1; namely, that use of seed sourced at the regional scale (e.g., southwestern MN) could improve prairie restoration outcomes and that there is sufficient variation in the rhizobial populations within sites to support healthy growth of prairie clovers (*Dalea* species). In July 2017, we installed 800 individuals from 2 populations of little bluestem into a field site in St. Paul; these individuals form the basis of our experiment on the adaptive capacity of these populations. In fall 2017, we collected data and samples related to local adaptation from over 7,000 individuals of 6 species at our 3 field experiments; data analysis is proceeding. We are maintaining the field experiments installed during Healthy

Prairies I that assess the geographic scale of local adaptation in perennial plant species and evaluate the extent of adaptive potential to environmental change. Additional data and samples will be collected from these experiments in July – October 2018.

Amendment request (June 1, 2018)
We request approval for transfer of funds as follows: $10,000 from Activity 1 Personnel, $4,000 from Activity 2 Personnel, and $3,000 from Activity 3 Equipment/Tools/Supplies to cover previous and anticipated future Activity 3 Travel costs. We will be working with citizen groups and external partners to meet our Activity 1 goals despite this decrease in the Personnel line. Similarly, we have recruited undergraduate employees to help us meet Activity 2 goals more cost-efficiently than anticipated. Activity 3 Equipment costs are lower than expected, as we have been able to use materials purchased under Healthy Prairies Phase I to a greater degree than we anticipated.
Amendment Approved by LCCMR 6/7/2018

Project Status as of *January 18, 2019***:**
With the help of volunteers and UM-Morris undergraduates and in cooperation with The Nature Conservancy, we made substantial collections of 34 species of prairie plants from 23 sites in 9 counties in western and south-central Minnesota. We are in early stages of conveying these seeds to native seed producers, for use in establishing seed-increase populations to support prairie restoration. We completed a field experiment with prairie clovers (*Dalea* spp.) finding that when seed is sourced closer to the planting site, the rate of association with beneficial nitrogen-fixing rhizobia is increased. We are currently designing a greenhouse experiment to test whether beneficial bacteria from local plant populations results in healthier plants. During summer 2018, 2000 new microbes were collected from little bluestem (*Schizachyrium scoparium*) with the plan to test their effect on plant drought resistance. We also collected pedigreed seed from 400 little bluestem plants at our field site in St. Paul; these seeds will form the basis of greenhouse and field experiments in 2019. In fall 2018, we collected data from over 7,000 individuals of 6 species at our 3 field sites in western Minnesota. These data are being jointly analyzed with data collected in 2015-17.

Amendment request (January 18, 2019)
We request approval for transfer of funds as follows: $2,000 from Activity 1 Travel, $4,000 from Activity 1 Other, and $3,000 from Activity 3 Other to cover anticipated future Activity 3 Travel costs. We will be transitioning our Activity 1 efforts from collection of seed to distribution, requiring less travel money and maximizing efficiency of the time of employees working on both Activities 1 and 3. As Activity 3 requires little new infrastructure at this stage, resources in the "Other" line may now be used for travel expenses.
Amendment Approved by LCCMR 2/1/2019

Project Status as of *June 1, 2019*
We continue to make strong progress toward the project goals, which are on-track for completion June 2020. Our goal of enhancing availability of source-identified seed for prairie restoration advanced through drafting of material transfer agreements (MTAs) and related documents, which we circulated to select native seed producers, revised in response to their comments, and re-issued to additional external partners for further input. The 2019 seed collection plan has been finalized; staff and collaborators have been recruited to harvest seeds at 14 sites in 7 western MN counties.
Results from an Activity 2 field experiment with prairie clovers (*Dalea* sp.) and beneficial nitrogen-fixing bacteria show that plant and bacterial growth responses are predicted both by spatial distance to the source and differences specific to source sites**.** A greenhouse experiment to evaluate the impacts of differing combinations of plant and bacteria from the same and different source sites is in progress. Diverse fungi have been isolated from little bluestem (*Schizachyrium scoparium*) in conjunction with quantitative genetic studies of the host grass to evaluate its capacity for ongoing evolutionary adaptation. A greenhouse study to evaluate the contribution of

these symbiotic fungi to drought tolerance of host plants is planned. For Activity 3, entry of data collected during the 2018 censuses at the 3 field sites in western Minnesota has been completed. Joint analysis of data from 2015-2018 is completed for thimbleweed (*Anemone cylindrica)* and is underway for the other 5 species. Preliminary results suggest local adaptation of southwest-origin populations of *D. candida;* size declines with northward distance from their origin. At this early stage of our experiments, we do not detect local adaptation in the other 5 species. *Outreach and dissemination*: Our findings were presented at a workshop on local adaptation attended by staff from BWSR, DNR, The Nature Conservancy, USFW, and USGS, as well as researchers from UM-TC, UM-Duluth, UM-Morris, NDSU, SDSU, the Chicago Botanic Garden, the U. of Missouri-Columbia, and Michigan State U.

Project Status as of *Dec. 1, 2019*

- Activity 1: We successfully transferred 77 collections of seed from 41 native prairie species to four native-seed producers and as we continue these outreach efforts, source-identified seed for use in Minnesota prairie restorations will be increasingly available. In 2019, seed collections from 40 species (seven entirely new) across 15 sites in seven western Minnesota counties were made.
- Activity 2: Results of field studies show that prairie clovers (*Dalea* species) are adapted to a broad range of local conditions and that the critical seedling-establishment phase depends on the availability of beneficial nitrogen-fixing bacteria. Our results suggest that the addition of beneficial bacteria from regional sources to restoration sites will increase establishment and pose few risks.
- Activity 3: Data were collected from 7,000+ individuals of 6 species at our 3 outstate field sites. Results to date suggest that like Dalea spp above, these slow-growing perennial species, are able to survive a broad range of environmental conditions. Further, our genetic analysis of variation in little bluestem (*Schizachyrium scoparium*), suggest significant genetic variation in the capacity of populations to adapt to new environments. *Together* our results to date suggest that prairie plants have the capacity to adapt to new environments in which they find themselves (e.g. restorations) and emphasize the critical importance of both maintaining diversity and expanding seed resources.

Amendment request (December 10, 2019)

We request approval to transfer $2,432 from Activity 1 Other to cover unanticipated expenses as follows: $15 (Activity 1, Equipment/Tools/Supplies), $738 (Activity 1, Travel) and $1,679 (Activity 3, Travel). Because the Project Completion Date falls well before the height of the seed-collection season, we expect to minimally engage the services of our collection partners during the remainder of this Work Plan; the Activity 1 Other funds can thus be reallocated without compromising project goals.

Project extended to June 30, 2021 by LCCMR 6/18/20 as a result of M.L. 2020, First Special Session, Chp. 4, Sec. 2, legislative extension criteria being met.

Project Status as of July 1, 2020:

- **Activity 1:** Since December, 2019, the Healthy Prairies Project has distributed seed to two seed producers. Due to COVID restrictions and uncertainty in funding, seed collections planned for Spring and early Summer 2020 have been rescheduled for the same period in 2021.
- **Activity 2:** We obtained over 7000 cultures for fungi potentially beneficial to little bluestem (LBS) (*Schizachyrium scoparium*) from prairie sites across a water availability gradient from Western to Eastern MN. We used DNA sequencing to identify these and preliminary analyses show that these fungal communities associated with LBS may be responsive to drought. For this project, we were able to complete Outcome 2 but not Outcome 3 before COVID delays. The status for the project with prairie clovers (Dalea *sp.*) is the same as reported Dec. 1, 2019 and a publication is in preparation by Dr. Pozzi. However, Outcome 3 in which we planned to evaluate the role of beneficial microbes in *Dalea,* and LBS

early seedling establishment and survival could not be carried out due to COVID restrictions, and have been rescheduled to greenhouse studies (LBS) Winter 2021, and local field studies (Dalea) Spring 2021.

- **Activity 3:** Analysis of data has continued. Plans have been developed for field research in August. This planning has included design of experiments to evaluate effects of inbreeding and of crossing between populations of little bluestem plants (*Schizachyrium scoparium*).

Project Status as of February 1, 2021:

- **Activity 1:** Due to COVID restrictions and uncertainty in funding, seed collections intended to be completed in late summer and fall of 2020 could not be made.
- **Activity 2:** Graduate student Cedric Ndinga-Muniania identified over 1200 fungal cultures obtained from roots of little bluestem. In addition, he has shown that these beneficial fungal communities change across a gradient of water availability from western to eastern MN using Next-Generation sequencing methods. We obtained additional funding from the U. Minnesota to allow Mr. Muniania to screen these fungi for beneficial effects on plant growth in drought conditions during Winter 2021.
- **Activity 3:** Through July and August, graduate student Wes Braker carried out experimental crosses between little bluestem plants (*Schizachyrium scoparium*) growing in field plants on the St. Paul campus of UMN-TC. Mr. Braker gathered the resulting seeds and has proceeded to prepare them for germination to evaluate their viability. A small team (Shaw, May, Braker, and technician Em Daily) traveled in mid-August to the Lake Bella field site to gather data on the plants in the experimental garden there, taking extreme precautions against Covid-19 and completing the census in four days. Data have been prepared for analysis.

Amendment request (March 17, 2021)
We request approval to transfer $1,056 from Activity 2 Other and $467 from Activity 2 Travel to Activity 2 Personnel ($132) and to Activity 2 Equipment/Tools/Supplies ($1,391). These small shifts are needed to cover expenses for lab supplies and personnel involved in this activity. The pandemic led to reductions in travel and greenhouse use from the original plan.

Amendment Approved by LCCMR on 4/1/2021

Overall Project Outcomes and Results: through February 1, 2021
Minnesota prairies harbor extraordinary diversity of plants and microbes, while also nurturing wildlife, retaining water and topsoil, and beautifying landscapes. Yet habitat loss threatens the persistence of the once vast prairies and their stunning biotic diversity. Limited understanding of this diversity and insufficient seed availability hinder sustainable management of this iconic Minnesota biome. We conducted Healthy Prairies (HP) Phase II to expand availability of seeds for prairie restorations and study approaches to increase success of restorations. Building on our prior accomplishments under ENRTF funding, we have:

1. Preserved diverse seed from 57 rarer prairie species, gathering them from widely separated locations.
2. Obtained, archived, and studied 2,600 naturally occurring microbial partners from two species.
3. Gathered data to assess the geographic scale important to plant survival and reproduction in MN.

Our extensive collections of source-identified seeds and microbes across a wide range of MN's prairie region help to conserve the diversity of MN prairies. We have provided seeds to seed producers, who have, in turn, used them in establishing fields and are seeking certification of the seeds that they obtain from them.

Our studies of effects of microbial associates on prairie plants have indicated that the bacteria providing nitrogen to prairie clover (*Dalea purpurea*, *D. candida*) disperse widely across MN prairies. Consequently, we can recommend to growers an inoculum that need not be site-specific. In contrast, the communities of fungi associated with roots of *S. scoparium* are spatially restricted, indicating that a regionally-based inoculum may be preferable.

We continued our large-scale experiment to elucidate the geographic scale of adaptation of six prairie species. We gathered extensive data from this experiment and began analyses of the data. We implemented experiments to investigate genetic structure of two populations of little bluestem (*Schizachyrium scoparium*), including genetic variance for fitness and the fitness consequences of inbreeding and of crossing between populations.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Preserving prairie plant diversity for conservation and restoration

Description: We will increase availability of source-identified seed for use in MN prairie restorations by working with partners to increase seed collection, distribution, and to develop transfer agreements. Twenty of the less common but important prairie species, in addition to the 40 species obtained in 2014-17, are targeted, and these will entail greater time and scouting to collect. Efforts will be evaluated via the amount and diversity of seed collected and by the level and quality of partner involvement.

Summary Budget Information for Activity 1:	**Revised ENRTF Budget:**	**$102,821**
	Amount Spent:	**$118,374**
	Balance:	**-$15,553**

Outcome	**Completion Date**
1. *Increase availability of diverse, source-identified seed for prairie restorations by expanding our network of collectors and collection locations. Collect seed for 20 additional, relatively common species.*	October, 2019
2. *Implement material transfer agreements with producers.*	December, 2019
3. *Collect source-identified seed for 20 rarer prairie plant species. Deposit voucher specimens at UM herbaria, deposit seed at USDA facility for long-term storage, transfer seed to producers.*	June, 2020

Activity 1 Status as of *Dec. 1, 2017***:** Report deferred per communication from LCCMR staff on July 24, 2017.

Activity 1 Status as of *June 1, 2018***:**
By leveraging our support from LCCMR, we were able to obtain funding from the University of Minnesota's Institute on the Environment to conduct a series of focus groups with native seed producers and consumers. One outcome of these conversations was a list of less-common but important prairie species of which producers are interested in developing commercially viable, source-identified populations for use in restorations. We developed our list of target species for 2018 collections by combining the producers' suggestions with input from other collaborators. In addition to Dr. Kuchenreuther at UMM, who collected populations in west central MN in 2017, we have partnered with a seed collector based in northwestern Minnesota; by subcontracting to him seed collections in that region, we can make most efficient use of project resources. In 2017, he collected seeds of 19 species from populations in NW MN. In addition, we have established a partnership with the Minnesota Master Naturalists in anticipation of multiple seed collection events scheduled for June – October 2018. We have completed collection of one early-flowering species, pasque flower.

Activity 1 Status as of *January 18, 2019***:**
We have produced a preliminary report summarizing and analyzing the findings of the focus groups; this report has been submitted to focus group participants for their review and further revision. We met our 2018 seed collection goals: through the combined efforts of Margaret Kuchenreuther, 2 UM-Morris undergraduates, 9 community volunteers, and Healthy Prairies staff, we collected 34 species from 21 sites in south-central and west-central MN. In addition, we again partnered with a seed collector based in northwestern MN, allowing us

to make efficient use of project resources. In 2018, he collected 8 species from an additional 2 sites. We are in the process of drafting material transfer agreements that will facilitate conveyance of these seeds to native-seed producers, who will use them to establish seed-increase fields from which additional generations of seeds can be harvested and used for prairie restoration in MN.

Activity 1 Status as of *June 1, 2019***:**
Recent Ph.D. recipient Nicholas Goldsmith has prepared a report on the results of the focus groups for submission to *Restoration Ecology* in June 2019. With the collaboration of Dr. Goldsmith, we drafted material transfer agreements (MTAs) that were informed by the focus group report and reviewed by external partners. We have distributed the draft MTAs and associated documents to other partners for additional feedback. We finalized the 2019 seed collection plan; in this last full collection season under current funding, we aim to supplement and broaden geographic representation of collections from previous years as well as to increase the number of species available for conveyance to seed producers. Three UM-Morris undergraduates supervised by Dr. Kuchenreuther, and a collaborator in northwest Minnesota have been recruited to support our goal of collecting 38 species from 14 sites in 7 western MN counties.

Activity 1 Status as of *Dec. 1, 2019***:**
The manuscript on the results of the focus groups was submitted to *Restoration Ecology* and is currently in revision. Material transfer agreements and associated documents were finalized in September 2019. Using these documents, we executed agreements with 4 native seed producers, resulting in the transfer of 77 seed lots representing 41 species collected from 9 counties in western Minnesota. Producers will use these seed lots to establish commercial-scale, source-identified populations that will supply geographically and genetically diverse seed to Minnesota prairie restorations. We are in the process of contacting additional producers with the aim of disbursing additional seed lots. The 2019 seed collection team (3 undergraduates from UM-Morris, 2 undergraduates from UM-Twin Cities, Dr. Kuchenreuther, and a collaborator from northwest Minnesota) collected seeds from 40 species (7 of them new to the Healthy Prairies inventory) from 15 sites in 7 western Minnesota counties.

Activity 1 Status as of July 1, 2020:
Since December, 2019, the Healthy Prairies Project has distributed seed to two seed producers. Each of these producers responded to email contact about available seeds in the Healthy Prairies Project collection. The requests from each were fulfilled in full and mailed to the respective producers with no competing requests.
As part of the permit that allows the Healthy Prairies Project to collect seeds on property that The Nature Conservancy owns and managers, ½ of all seed collected must be returned to The Nature Conservancy for their own use. In January 2020, the Healthy Prairies Project returned 57 species from 9 locations, with a total of 147 collections. These collections included seed that was collected in the summer of 2019 directly from Nature Conservancy sites as well as seed from plants that were originally collected as seed at Nature Conservancy sites and have been grown at the University of Minnesota as part of the Healthy Prairies Project.
Due to COVID restrictions and uncertainty in funding, seed collections planned for Spring and early Summer 2020 have been rescheduled for the same period in 2021.

Activity 1 Status as of February 1, 2021:
Due to COVID restrictions and uncertainty in funding, seed collections planned for late Summer and Fall 2020 could not be made.

Final Report Summary:
By leveraging our support from LCCMR, we obtained funding from the University of Minnesota's Institute on the Environment to conduct a series of focus groups with native seed producers and consumers. One outcome of these conversations was a list of less-common but important prairie species of which producers are interested in

developing commercially viable, source-identified populations for use in restorations. Based on this and other considerations, we made extensive seed collections in a wide range of MN's prairie region. We have offered these collections to seed producers and distributed seed to those who requested them. These producers have established fields from these seeds and are seeking certification of the seeds that they obtain from them. In this way, this project will have substantively increased availability of source-identified seeds for use in prairie restorations in Minnesota.

Activity 2: Finding your friends in unlikely places – beneficial microbes for prairie plants
Description: We will assess the diversity and effect of naturally occurring plant-associated microbes for two types of plants essential to healthy prairies – legumes and grasses. Results will inform land managers about the use of microbes to improve prairie plant establishment in restorations, a practice common in agriculture but not widely applied to natural systems.

Summary Budget Information for Activity 2:	**ENRTF Budget:**	**$387,680**
	Amount Spent:	**$352,561**
	Balance:	**$35,119**

Outcome	**Completion Date**
1. *Use previously collected microbes to determine beneficial microbes' potential for enhancing prairie clover (*Dalea spp.*) survival and reproduction in experimental plantings and greenhouse studies.*	November, 2019
2. *Determine the diversity of microbial communities associated with little bluestem grass (*Schizachyrium scoparium*) and collect 5000 new microbes. Store living cultures at UM and USDA.*	December, 2019
3. *Determine effects of plant-associated microbes on little bluestem establishment and reproduction in experimental plantings and in greenhouse studies.*	June, 2020

Activity 2 Status as of *Dec. 1, 2017:*
Report deferred per communication from LCCMR staff on July 24, 2017.

Activity 2 Status as of *June 1, 2018***:**
In Activity 2, we ask the role of microbial symbionts in the ability of prairie plants to live in the varied environments of MN prairies. Specifically, we are conducting experiments with *Dalea purpurea*, the legume purple prairie clover, and its nitrogen-fixing bacterial partner, rhizobium. Results of a greenhouse experiment and genotyping of rhizobium isolates from 15 prairie sites in MN in Phase I demonstrated that each site harbors extensive variation in rhizobia and that plants have growth patterns characteristic of sites and regions (Kane Keller, postdoc in Phase I). The results suggest that locally sourced seed (on a regional scale) could improve prairie restoration efforts and that there is sufficient variation in the rhizobial populations within sites to support healthy growth of *Dalea* plants. We are currently testing these conclusions with plantings of *Dalea* from different source sites into 2 of the 3 experimental sites established under Phase I of the Healthy Prairies project (Adrien Pozzi, postdoc Phase II). To accomplish goal 2 (above), Cedric Ndinga-Muniania (UM graduate student) will conduct extensive collections and culturing from little bluestem in summer 2018.

Activity 2 Status as of *January 18, 2019***:**
For Outcome 1, seed representing different populations of *Dalea purpurea* and *Dalea candida* were planted in 2 experimental sites established under Phase I of the Healthy Prairies project and were harvested in September 2018 (Adrien Pozzi, postdoc Phase II). Preliminary analyses of data for plant growth and the rate at which these plants associate with nitrogen-fixing bacterial partners suggest that locally sourced seed could favor beneficial

associations between plant and soil microbes. In addition, 600 new bacterial partners were cultured and will be used in a greenhouse experiment in Spring 2019 to test whether more beneficial associations and better plant growth result from matching source microbes and plants. For Outcome 2, Cedric Ndinga-Muniania (UM graduate student) collected and cultured over 2,000 new fungal endophytes from little bluestem collected in 5 remnant prairie sites across southern MN.

Activity 2 Status as of ***June 1, 2019*****:**
For Outcome 1, field experiments conducted by postdoc Adrien Pozzi were completed Fall 2018. Results of analyses to date suggest that growth and survival of prairie clovers (*Dalea* spp.) and of beneficial nitrogen-fixing bacteria depend not only on distance to the source site, but also on source identity**.** A manuscript is being drafted. Greenhouse studies are underway to evaluate the impact of differing plant and bacterial source populations on plant growth (to be completed Fall 2019). For Outcome 2, fungal endophytes have been isolated from little bluestem (*Schizachyrium scoparium*) (UM graduate student Cedric Ndinga-Muniania). Results will inform differences in fungal communities in *S. scoparium* across a drought gradient (completion Dec 2019). For Outcome 3, Ndinga-Muniania will evaluate the fungi isolated under Outcome 2 for their tendency to confer drought tolerance to *S. scoparium* (greenhouse studies completed by June 2020).

Activity 2 Status as of ***Dec. 1, 2019*****:**
Results from the research of Adrien Pozzi (postdoc) and the May Lab show that *Dalea purpurea* and *D. candida* populations are adapted to a broad range of local conditions. In addition, we find that the seedling establishment phase is critical and dependent on the availability of beneficial nitrogen-fixing bacteria in the soils. Along with previous information on the geographic scale of diversity in these bacteria, we conclude that there are few risks of adding beneficial bacteria to restorations sites from regional sources. We hypothesize that adding beneficial bacteria to soils at the time of seed germination will increase recruitment success in Dalea plantings. We plan to test this question in Spring 2020 using our collections of beneficial bacteria made under LCCMR funding.

Activity 2 Status as of July 1, 2020:
We obtained over 7000 cultures for fungi potentially beneficial to little bluestem (LBS) (*Schizachyrium scoparium*) from prairie sites across a water availability gradient from Western to Eastern MN (Ph.D. student Cedric Ndinga-Muniania). We used DNA sequencing to identify these and preliminary analyses show that these fungal communities associated with LBS may be responsive to drought. For this project, we were able to complete Outcome 2 but not Outcome 3 before COVID delays. The status for the project with prairie clovers (Dalea *sp.*) is the same as reported Dec. 1, 2019 and a publication is in preparation by Dr. Pozzi. However, Outcome 3 in which we planned to evaluate the role of beneficial microbes in *Dalea,* and LBS early seedling establishment and survival could not be carried out due to COVID restrictions, and have been rescheduled to greenhouse studies (LBS) Winter 2021, and local field studies (Dalea) Spring 2021.

Activity 2 Status as of February 1, 2021:
Graduate student Cedric Ndinga-Muniania identified over 1200 fungal cultures obtained from roots of little bluestem. In addition, he has shown that these beneficial fungal communities change across a gradient of water availability from western to eastern MN using Next-Generation sequencing methods. Given that our ENRTF funding is exhausted, we obtained funding from the U. Minnesota to allow Mr. Muniania to screen these fungi for beneficial effects on plant growth in drought conditions during Winter 2021.

Final Report Summary:
We accomplished the goals outlined above. Because we find that the bacteria providing nitrogen to prairie clover (Dalea purpurea, D. candida, legume) are widely dispersed across the varied environments of MN prairies, we can recommend an inoculum to growers that need not be site-specific. In contrast, the communities of fungi

associated with roots of *S. scoparium* are more spatially structured, and, pending the results of the above screens, it may be best to develop a regionally-based inoculum.

ACTIVITY 3: Adaptive genetic diversity of prairie plants

Description: Continue field experiments established under Phase I to characterize the spatial scale of local adaptation for 6 prairie perennials. Evaluate genetic variation for survival and reproduction of little bluestem grass. Results will inform methods of prairie conservation and healthy prairie restoration that maintain diversity of prairie plant species.

Summary Budget Information for Activity 3:	**Revised ENRTF Budget:**	**$409,499**
	Amount Spent:	**$429,065**
	Balance:	**-$19,556**

Outcome	Completion Date
***1**. Monitor survival, growth, and reproduction in established experiments with 6 species and over 6000 plants to evaluate effect of seed source on establishment and success of prairie plants in restorations.*	November 30, 2019
***2.** Plant pedigreed little bluestem seed into field experiments to assess its capacity to adapt to varied environmental conditions, and the role of microbes (identified in Activity 2) in that process.*	November 30, 2019

Activity 3 Status as of *Dec. 1, 2017***:** Report deferred per communication from LCCMR staff on July 24, 2017.

Activity 3 Status as of *June 1, 2018***:**

To assess the adaptive capacity of little bluestem, which is a key component of upland prairies, we installed 800 little bluestem individuals into a field site in St. Paul in July 2017. These individuals will form the basis of formal genetic crosses to produce pedigreed seeds from which we can evaluate the extent of genetic variation that is present and could support the species' ongoing adaptation to environmental change. Also in fall 2017, as part of our effort to estimate the geographic scale of local adaptation in important prairie species, we collected data & samples from over 7,000 individuals of 6 species that were installed at 3 field sites during Healthy Prairies Phase I. 2017 was the 2nd year of data for 2 grass species and the 1st year of data for 2 forb and 2 legume species. Sample analyses (seed germination trials) were conducted in Dec 2017 – February 2018. Data analysis is under way. We are currently maintaining our field sites, including the common garden plots (Rosemount Research & Outreach Center) that provide seed for Activity 2 and Activity 3 experiments.

Activity 3 Status as of *January 18, 2019***:**

In August 2018, we performed formal genetic crosses on 400 little bluestem plants at our field site in St. Paul. Seeds were harvested in October 2018; these will form the basis of greenhouse and field experiments to evaluate the extent of standing genetic variation. Also in fall 2018, we collected data from over 7,000 individuals of 6 species at our 3 field sites in western Minnesota. These data have been combined with data collected in 2015-2017 and analysis is under way. During May – August 2018, we continued maintenance of our common garden plots at the Rosemount Research and Outreach Center; these provide seed for Activity 2 and Activity 3 experiments. This work included installation of substantial fencing at and below ground level to deter gopher herbivory.

Activity 3 Status as of *June 1, 2019***:**

To advance our assessment of local adaptation, we have completed entry of the 2018 census data collected on over 7,000 plants at our 3 field sites in western Minnesota. Analysis of all data collected to date for *Anemone cylindrica* is complete; joint analysis of data collected 2016-2018 is underway for the other 5 species. Preliminary results suggest local adaptation of southwest-origin populations of *D. candida;* size declines with planting distance further northward from their origin. At this early stage in the lives of these long-lived species, we do not detect local adaptation in the other 5 species. We advanced our study of capacity for ongoing adaptation of little bluestem by establishing plantings of the pedigreed seed obtained from crosses during summer 2018. In early May, we initiated routine maintenance of our nursery plots at the Rosemount ROC. We have hired 2 highly qualified UM-TC undergraduates to assist with field and greenhouse work. A third UM-TC undergraduate will earn academic credit working with our team this summer.

Activity 3 Status as of *Dec. 1, 2019*:
We collected census data on 7,000 plants from our 6 focal species at our 3 field sites in western Minnesota. Entry of the 2019 data is complete. Joint analysis of the 2016-2019 data is under way. To date, we have not detected evidence of local adaptation in these slow-growing perennial species, which take several years to reach reproductive maturity. We maintained and collected data on seedlings that were established from seed produced by the 2018 crosses among little bluestem plants (*Schizachyrium scoparium*). In July 2019, we transplanted these 1,100 individuals into a field site in St. Paul. We also sowed additional seed produced by the 2018 crosses into an adjacent field site; this resulted in over 780 individuals on which we collected survival and morphological data. These data have been entered and analyzed. Results from the juvenile stage of this perennial grass indicate significant genetic, as opposed to environmental, influence on survival and differences between the two populations in adaptive capacity.

Activity 3 Status as of July 1, 2020:
Analysis of data, as described above, has continued. Plans have been developed for field research in August. This planning has included design of experiments to evaluate effects of inbreeding and of crossing between populations of little bluestem plants (*Schizachyrium scoparium*).

Activity 3 Status as of February 1, 2021:
Through July and August, graduate student Wes Braker carried out experimental crosses between little bluestem plants (*Schizachyrium scoparium*) growing in field plants on the St. Paul campus of UMN-TC. Mr. Braker gathered the resulting seeds and has proceeded to prepare them for germination to evaluate their viability. A small team (Shaw, May, Braker, and technician Em Daily) traveled in mid-August to the Lake Bella field site to gather data on the plants in the experimental garden there, taking extreme precautions against Covid-19 and completing the census in four days. Data have been prepared for analysis.

Final Report Summary:
As planned, we have continued our large-scale experiment to elucidate the geographic scale of adaptation of six prairie species. We have gathered extensive data from this experiment, and Dr. Shelby Flint has made progress on analyses of the data. Mr. Braker plans to complete analyses of part of this dataset in his dissertation work. We have also implemented experiments to investigate several aspects of the genetic structure of two populations of little bluestem (*Schizachyrium scoparium*), including genetic variance for fitness and the fitness consequences of inbreeding and of crossing between populations. Further work on these experiments will contribute to Mr. Braker’s doctoral dissertation.

V. DISSEMINATION:
Description: Information and materials gained in Healthy Prairies II will be disseminated as follows. Seed collected from 20 prairie species will be deposited at UM and NCGRP (Activity 1). Information on microbial collections and their effects on prairie plant survival and reproduction information on the establishment will be communicated as written reports. Microbial collections will be maintained at UM and USDA (Activity 2). Information on the survival, and reproduction of 6 prairie plants in 3 outstate locations will be communicated to the MN-DNR, The Nature Conservancy, private land managers, and seed companies as written reports (Activity

3). The research findings will be disseminated through peer-reviewed papers published in major journals of evolution and ecology. A publicly accessible website giving collection locations and approximate population densities for prairie species will be maintained. In addition, public outreach will be conducted for all 3 Activities via Market Science, a program of UM presenting results at farmers markets throughout the Twin Cities.

Status as of *Dec. 1, 2017:* Report deferred per communication from LCCMR staff on July 24, 2017.

Status as of *June 1, 2018***:**
Accession of 20 species have been deposited at UM.

Status as of *January 18, 2019***:**
Dr. Ruth Shaw, Dr. Adrien Pozzi, John Benning and Anna Peschel (UMN graduate students) designed and took part in a Market Science session, a 3-hour UMN science outreach initiative where 20-25 people (children and adults) were made aware of prairie fragmentation and the need for restoration to reconnect habitat patches, as well as how fungi facilitate acquisition of plant nutrients (9/27/18, Tiny Diner farmers market, Minneapolis, MN). Accessions of 34 species have been deposited at UM. A preliminary technical report on the focus groups (discussion summaries and analysis) has been sent to participants for their review. Dr. Adrien Pozzi presented an overview of Healthy Prairies Project objectives and preliminary findings to staff and academic attendees (1/17/19, TNC office, Minneapolis, MN).

Status as of *June 1, 2019***:**
Preliminary results from the local adaptation experiment (Activity 3) were presented at a workshop on local adaptation (March 21, 2019, on the St. Paul campus of UM-TC). Participants included staff from BWSR, DNR, The Nature Conservancy, and USGS, as well as researchers from UM-TC, UM-Duluth, UM-Morris, NDSU, SDSU, the Chicago Botanic Garden, the U. of Missouri, and Michigan State. Manuscript reporting on the results of focus groups identifying and relieving impediments to production and use of source-identified seeds will be submitted to *Restoration Ecology* June 2019.
Market Science session planned at Tiny Diner 7/18/19.
A manuscript on Activity 2 – Outcome 1 expected submission Fall 2019.

Status as of *Dec. 1, 2019***:**
77 seed lots, representing 41 species, have been delivered to four commercial producers of native seeds. Results to date from all Activities were presented at a symposium (November 19, 2019, St. Paul campus of UM-TC). Attendees included collaborators from UM-Morris, past and current undergraduate, graduate, and professional project staff, and potential collaborators from other UM-TC research groups. A manuscript that reports on the focus group discussions aimed at identifying and relieving impediments to production and use of source-identified seeds was submitted to *Restoration Ecology* and is currently in revision. A manuscript on Activity 2, Outcome 1, is in progress. A Market Science session held on July 18, 2019 involved 88 members of the public participating in 3 activities that showcased MN's tallgrass prairies as well as their extreme fragmentation and its consequence of severely restricting seed dispersal. Visitors were also shown microbial partners of prairie plants with emphasis on their role in nutrient acquisition.

Status as of July 1, 2020:
A manuscript titled "Factors limiting the availability of native seed for reconstructing Minnesota's prairies: Stakeholder perspectives" was resubmitted, following revision, to Restoration Ecology.
Two additional manuscripts are nearing completion.

Status as of February 1, 2021:

A manuscript titled “Factors limiting the availability of native seed for reconstructing Minnesota’s prairies: Stakeholder perspectives” was further revised and resubmitted. It is under consideration for publication in the journal, *Restoration Ecology*. Graduate student Naomi Rushing submitted a manuscript, "Latitude of seed source impacts flowering phenology and fitness in translocated plant populations", to be considered for publication in *Restoration Ecology*.

Final Report Summary:
HP team members have participated in varied opportunities to disseminate findings from this project. These include informal events to communicate with members of the public who are not all well-versed in science and may not be aware of prairies (Market Science), as well as workshops involving other scientists and land managers (Nature Conservancy ‘Science Slams’, Local Adaptation Workshop, held at UM-TC, March 2019, discussions of seed sourcing guidelines led by staff of MN DNR).

A paper providing an overview of the Local Adaptation Workshop has been published in *New Phytologist* (2020) 225:2246–2248. A manuscript reporting findings about geographic scale of local adaptation has been submitted to *Restoration Ecology* and has received positive reviews. A second manuscript reporting on a study that used focus groups to identify impediments to use of source-identified seeds for prairie restorations has been submitted to *Restoration Ecology* and has received positive reviews. Both manuscripts are under revision and will be resubmitted soon.

VI. PROJECT BUDGET SUMMARY:
A. Preliminary ENRTF Budget Overview:

***This section represents an overview of the preliminary budget at the start of the project. It will be reconciled with actual expenditures at the time of the final report.**

Budget Category	$ Amount	Overview Explanation
Personnel:	$ **818,500**	Labor intensive field work, lab analyses
Professional/Technical/Service Contracts:	$ **31,000**	Sequencing (microbes, Activity 2), greenhouse, seed collection by local harvesters
Equipment/Tools/Supplies:	$ **36,000**	Field and lab supplies, postage
Capital Expenditures over $5,000:	$	
Fee Title Acquisition:	$	
Easement Acquisition:	$	
Professional Services for Acquisition:	$	
Printing:	$	
Travel Expenses in MN:	$ **14,500**	Travel to experimental and seed collection sites
Other:	$	
TOTAL ENRTF BUDGET:	**$ 900,000**	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than $5,000: N/A

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 14.1

Total 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			
	$	$	
State			
Indirect costs – In kind services 53%/54% of total direct costs	$477,000	$460,492	Office, lab, and meeting space, accounting and secretarial services, phone & office equipment, security, and library access, for all project personnel.
TOTAL OTHER FUNDS:	**$**	**$**	

VII. PROJECT STRATEGY:

A. Project Partners:

Partners receiving ENRTF funding

- UMN-TC faculty and Project Managers - Drs. R. Shaw ($52,000, summer salary), G. May ($49,000, summer salary); Collaborator Dr. Margaret Kuchenreuther, UM Morris ($37,000, outstate seed collections); 2 post-doctoral fellows ($157,500 each; plant adaptation, beneficial microbes); 2 graduate students ($91,500 each, plant adaptation, beneficial microbes); 4 undergraduate students ($32,000, field assistance, lab and greenhouse studies of plant – microbe interactions); Coordinator of Personnel ($103,000, recruit, train, and work with volunteers, field assistance); Technical assistant ($81,000, conduct lab and field research, maintain cultures, ordering, equipment management). *Amounts shown reflect 3 years funding.*

Partners NOT receiving ENRTF funding

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

B. Project Impact and Long-term Strategy: The impact of the proposed work will be to preserve MN prairie plant diversity, and to provide a knowledge base for restoration and maintenance of prairie plant diversity, for future generations' use. The project will enhance land management efforts that maintain prairie lands for wildlife, provide sources of new plant and microbial products, and provide databases on distributions and abundances of many iconic prairie plant species.

The strategy for accomplishing these goals is to:

- Collect seed from 20 plant species, additional to those collected in the previous project and to include those considered more rare. This will be accomplished by the Healthy Prairies team, and our outstate collaborators.
- Determine survivorship, growth, and reproduction of 6 prairie plant species at experimental plots established under previous funding at three outstate locations. These locations represent a north-south gradient across the western prairie area of MN (see Visual). We will continue seed increase plots at the Rosemount Research and Outreach Center (UM)
- Investigate the role of beneficial microbes in plant survival and reproduction. Make collections of microbial isolates, identify these, and use in experimental greenhouse studies. Beneficial microbes will be deposited at UM and USDA culture collections for public use.
- Determine the scale of genetic variation for plant survival and reproduction across the varied MN landscape as represented by the experimental plots. The results will be communicated in publications, to the public such as native plant groups, and to prairie seed companies.

Together, the results and information generated in Healthy Prairies II will have the intended impacts as we work with land managers, seed companies, and collections resources to increase the production and success of seed sources for prairie plantings across Minnesota.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	$ Amount
Healthy Prairies I: Seed storage, beneficial microbes, and adaptation	7/1/14 - 6/30/2017	$ 600,000
		$
		$

VIII. REPORTING REQUIREMENTS:

- **The project is for 4 years, will begin on 07/01/2017, and end on 06/30/2021.**
- **Periodic project status update reports will be submitted** *Dec. 1* **and** *June 1* **of each year.**
- **A final report and associated products will be submitted between June 30 and August 15, 2021**.

IX. VISUAL COMPONENT or MAP(S):

X. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS:

A. Parcel List:

B. Acquisition/Restoration Information:

<u>**Fee Title Acquisition**</u>

4. **Describe the selection process for identifying and including proposed parcels on the parcel list, including explanation of the criteria and decision-making process used to rank and prioritize parcels.**

5. **List all adopted state, regional, or local natural resource plans in which the lands included in the parcel list are identified. Include a link to the plan if one is available.**

6. **For any parcels acquired in fee title, a restoration and management must be prepared. Summarize the components and expected outcomes of restoration and management plans for parcels acquired by your organization, how these plans are kept on file by your organization, and overall strategies for long-term plan implementation, including how long-term maintenance and management needs of the parcel will be financed into the future.**

7. **For each parcel to be conveyed to a State of Minnesota entity (e.g., DNR) after purchase, provide a statement confirming that county board approval will be obtained.**

8. **If applicable (see M.S. 116P.17), provide a statement confirming that written approval from the DNR Commissioner will be obtained 10 business days prior to any final acquisition transaction.**

<u>Conservation Easement Acquisition</u>

1. **Describe the selection process for identifying and including proposed parcels on the parcel list, including explanation of the criteria and decision-making process used to rank and prioritize parcels.**

2. **List all adopted state, regional, or local natural resource plans in which the lands included in the parcel list are identified. Include a link to the plan if one is available.**

3. **For any conservation easement acquired, a restoration and management must be prepared. Summarize the components and expected outcomes of restoration and management plans for parcels acquired by your organization, how these plans are kept on file by your organization, and overall strategies for long-term plan implementation, including how long-term maintenance and management needs of the parcel will be financed into the future.**

4. **For each parcel to be conveyed to a State of Minnesota entity (e.g., DNR) after purchase, provide a statement confirming that county board approval will be obtained.**

5. **If applicable (see M.S. 116P.17), provide a statement confirming that written approval from the DNR Commissioner will be obtained 10 business days prior to any final acquisition transaction. A copy of the written approval should be provided to LCCMR.**

6. **Provide a statement addressing how conservation easements will address specific water quality protection activities, such as keeping water on the landscape, reducing nutrient and contaminant loading, protecting groundwater, and not permitting artificial hydrological modifications.**

7. **Describe the long-term monitoring and enforcement program for conservation easements acquired on parcels by your organization, including explanations of the process used for calculating conservation easement monitoring and enforcements costs, the process used for annual inspection and reporting on monitoring and enforcement activities, and the process used to ensure perpetual funding and implementation of monitoring and enforcement activities.**

<u>Restoration</u>

1. **Provide a statement confirming that all restoration activities completed with these funds will occur on land permanently protected by a conservation easement or public ownership.**

2. **Summarize the components and expected outcomes of restoration and management plans for the parcels to be restored by your organization, how these plans are kept on file by your organization, and overall strategies for long-term plan implementation.**

3. **Describe how restoration efforts will utilize and follow the Board of Soil and Water Resources "Native Vegetation Establishment and Enhancement Guidelines" in order to ensure ecological integrity and pollinator enhancement.**

4. **Describe how the long-term maintenance and management needs of the parcel being restored with these funds will be met and financed into the future.**

5. **Describe how consideration will be given to contracting with Conservation Corps of Minnesota for any restoration activities.**

6. **Provide a statement indicating that evaluations will be completed on parcels where activities were implemented both 1) initially after activity completion and 2) three years later as a follow-up. Evaluations should analyze improvements to the parcel and whether goals have been met, identify any problems with**

the implementation, and identify any findings that can be used to improve implementation of future restoration efforts at the site or elsewhere.

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

Project Title: Healthy Prairies II: Preserving MN prairie plant diversity
Legal Citation: *M.L. 2017, Chp. 96, Sec. 2, Subd. 03c*
Project Manager: Dr. Ruth Shaw
Organization: Regents of the University of Minnesota
M.L. 2017 ENRTF Appropriation: $ *900,000*
Project Length and Completion Date: 4 Years, June 30, 2021
Date of Report: December 31, 2020

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget (12/10/19)	Amount Spent	Activity 1 Balance	Activity 2 Budget (2/1/19)	Revised Budget 12/31/20	Amount Spent	Activity 2 Balance	Activity 3 Budget (12/10/19)	Amount Spent	Activity 3 Balance	Total Budget (12/10/19)	Revised Budget 12/31/20	TOTAL BALANCE
BUDGET ITEM				*Fill in your activity title here.*									
Personnel (Wages and Benefits)	$98,500	$113,640	-$15,140	~~$341,180~~	$341,312	$306,229	$35,083	$364,820	$384,763	-$19,943	$804,500	$804,632	$0
Dr. Ruth Shaw, Co-PI: $52,000 (75% salary, 25% benefits); 8% FTE. 1 month per year for three years.													
Dr. Georgiana May, Co-PI: $49,000 (75% salary, 25% benefits); 8% FTE. 1 month per year for 3 years.													
Dr. Margaret Kuchenreuther, UM Morris, collaborator: $37,000 (75% salary, 25% benefits); 8% FTE. 1 month per year, for 3 years.													
2 Postdoctoral Associates: $315,000 (82% salary, 18% benefits); 100% FTE, 3 years													
2 Graduate Students: $183,000 (51% salary, 49% benefits during the academic year & 85% salary, 15% benefits during the summer); 50% FTE, 2 years.													
4 Undergraduate Students: $32,000 (100% salary, 0% benefits); 2 @ 8% FTE (UM Twin Cities) and 2 @ 15% FTE (UM Morris), 3 years.													
Coordinator of personnel: $103,000 (79% salary, 21% benefits); 100% FTE, 2 years.													
Technical assistant: $81,000 (79% salary, 21% benefits); 100% FTE, 2 years.													
Equipment/Tools/Supplies	$15	$15	$0	~~$25,000~~	$26,391	$27,442	-$1,051	$6,000	$4,949	$1,051	$31,015	$32,406	$0
Lab Supplies: $25,000. Supplies for microbial culturing and storage (~ 6000 cultures per year), microbial detection in plant materials and identification of organisms using molecular methods and microscopy.													
Field supplies and prep work: $18,000. Envelopes and bags, blaze hats and vests, galvanized nails and landscape staples, tape measures, fencing materials, knee pads, mallets, field notebooks, etc.													
Travel expenses in Minnesota	$738	$738	$0	~~$5,500~~	$5,033	$4,090	$943	$34,679	$35,621	-$942	$40,917	$40,450	$0
Travel to field sites for seed collection (Activity 1), and microbial sampling (Activity 2). Monitoring experimental plots (Activities 2, 3), and seed increase plots in Rosemount. Total travel estimated: 25,000 miles in MN, with 150 hotel-person overnights, over 3 years.													
Other	$3,568	$3,981	-$413	~~$16,000~~	$14,944	$14,800	$144	$4,000	$3,732	$268	$23,568	$22,512	$0
Fees for independent contractors collecting seeds ($8,000)													
Postage/Shipping Fees: $2,000. Shipping seeds to Nat'l Center for Genetic Resources Preservation (NCGRP), USDA facility in Ft. Collins, CO. $100 per shipment x 20 shipments													
Sequencing (UMN-TC facility): $10,000. Detection, indentification, and distribution of naturally occurring microbes in native prairie plants using rapid, cutting edge "metagenomics" approaches													
Greenhouse space rental (UMN-TC): $13,000. Evalutating microbial effects on plant growth and reproduction (Activity 2), seedlings for outplanting, plant genetic variation analyses (Activity 3). 500 sq. ft. x $0.81/sqft per month x 30 months. Seed increase plot fees													
COLUMN TOTAL	**$102,821**	**$118,374**	**-$15,553**	**$387,680**	**$387,680**	**$352,561**	**$35,119**	**$409,499**	**$429,065**	**-$19,566**	**$900,000**	**$900,000**	**$0**

RESTORATION ECOLOGY
The Journal of the Society for Ecological Restoration

SER SOCIETY FOR ECOLOGICAL RESTORATION

RESEARCH ARTICLE

Factors limiting the availability of native seed for reconstructing Minnesota's prairies: stakeholder perspectives

Nicholas E. Goldsmith[1,2], Shelby A. Flint[1,3], Ruth G. Shaw[1]

Views about sourcing plant material for restoration, habitat reconstruction, and revegetation have developed substantially in recent years. In particular, recognition of the prevalence of local adaptation has been incorporated into guidelines that now often recommend local sourcing of germplasm. Demand for these materials frequently outstrips supply, and land management professionals repeatedly report inadequate availability of plant materials at appropriate geographic scale and affordable price. Here, we use focus group interviews to investigate the obstacles impeding production and use of source-identified native seeds in Minnesota prairie. Focus groups included both producers and users of locally sourced seeds and allowed for open-ended conversations among professionals within each group. Participants emphasized that unpredictability in demand severely restricts supply. To increase use of locally sourced seeds in restorations, participants identified key priorities: working toward more consistent standards and policies, including revising those of agencies that manage lands; promoting awareness of large ramifications from small changes to relevant laws; increasing communication and education; and increasing the number of seed producers.

Key words: climate change, demand, local adaptation, seed sourcing, source-identified seed, supply

Implications for Practice

- Uncertainty in demand for locally sourced native seeds hinders the long-term planning that producers require and aggravates their risks, compromising availability of locally sourced seeds. Processes that reduce uncertainty in demand or that reduce risks undertaken by producers would aid in increasing supplies of these materials.
- Buyers of native seeds face challenges in predicting their needs due to variation in funding requirements and timelines. Across funding agencies, project planning that recognizes the lead time required for commercial production would improve predictability of demand, producers' ability to meet demand, and, thus, availability of seeds as needed.
- Harmonization of seed-sourcing requirements across funding agencies and programs may increase predictability of demand for producers by clarifying where specific seed lot origins are most likely to be utilized.

Introduction

Habitat destruction and fragmentation are among the largest anthropogenic changes across the planet. The effects of this habitat loss range from pollen limitation that reduces reproduction (Wagenius 2006) to extinction of species (Seabloom et al. 2002). These impacts have prompted an increased focus on prairie conservation and reestablishment of prairie habitat. We use the term restoration to encompass varied revegetation and reconstruction practices that entail planting native species on the landscape.

As restoration practices have developed, recognition of the importance of local sourcing of native plant materials has also grown (Richards et al. 1998; Peppin et al. 2010; De Vitis et al. 2017). Across the globe, there is concern that using plant materials originating far from a restoration site could compromise adaptation of the restored population to the local environment, such that survival and reproduction of individuals would be inadequate to maintain a robust population, or that nearby remnant populations could be at risk of genetic admixture (McKay et al. 2005; Bucharova et al. 2019; Hamilton et al. 2020). While adaptation of populations to their local environment has been amply documented (reviewed in Kawecki & Ebert 2004; Leimu & Fisher 2008; Hereford 2009), the geographic scale of local adaptation is poorly understood (McKay et al. 2005). As a result, land managers have justified concerns about the

Author contributions: NEG, SAF, RGS conceived and designed the research; NEG conducted and SAF, RGS assisted in conducting the focus group interviews; NEG analyzed the data; NEG wrote and NEG, SAF, RGS edited the manuscript.

[1]Department of Ecology, Evolution, and Behavior, University of Minnesota—Twin Cities, 140 Gortner Laboratory, 1479 Gortner Avenue, St. Paul, MN 55108, U.S.A.
[2]Address correspondence to N. E. Goldsmith, email golds306@umn.edu
[3]Present address: Science Department, Southwest Minnesota State University, Science and Math 178, 1501 State Street, Marshall, MN 56258, U.S.A.

doi: 10.1111/rec.13554
Supporting information at:
http://onlinelibrary.wiley.com/doi/10.1111/rec.13554/suppinfo

genetic consequences of introducing novel populations to a restoration site.

Awareness of the benefits of using local plant materials is now keen, but demand for such materials outstrips supply (Broadhurst et al. 2015; Camhi et al. 2019; Elzenga et al. 2019). High cost and scarcity of suitable seeds frequently confront land managers, motivating efforts to augment availability and affordability of native plant materials at an appropriately fine spatial scale (Peppin et al. 2010; Tishew et al. 2011; Camhi et al. 2019; Elzenga et al. 2019). Efforts to augment native plant production, or increase availability of native plant materials through public funding, have been under way in various countries, including Australia (Broadhurst et al. 2015), Brazil (Schmidt et al. 2019), Germany (Mainz & Wieden 2019), and the United States (BLM 2009). The state of Minnesota, U.S.A., where prairies once occupied 7.3 million hectares, of which approximately 1% remain (MnDNR 2018*a*, Fig. 1), funded market research concerning native seeds in the 1990s (Dale 1993). More recently, the state has supported seed collection from populations throughout the state's prairie region as a basis for expanding native plant production (Minnesota Law 2014, 2017). Despite these national and regional efforts, multiple obstacles impede the use of locally sourced plant materials.

Previous research on supply and demand of source-identified seed has been most prevalent in the western United States and employed surveys of practitioners. This research identified constraints, including market uncertainty, policy inconsistencies, and technical challenges (Richards et al. 1998; Hooper 2003; Peppin et al. 2010; White et al. 2018; Camhi et al. 2019). For example, in the western United States, the yearly need for plant

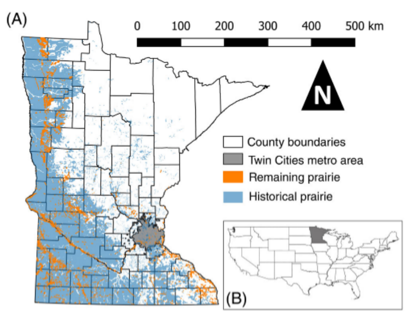

Figure 1. (A) Map of Minnesota showing extent of prairie around 1870 and present. Orange shapes represent remnant prairies as identified by the Minnesota Biological Survey (not restricted to public land). The blue regions represent the native prairie distribution before European settlement. (B) Map of the contiguous United States with the state of Minnesota shaded gray. Geographic Information System (GIS) layers are from the Minnesota Geospatial Commons (MnDNR 1895; 2013; 2018*b*; MDA 2014).

materials varies depending on extent and severity of wildfires (Richards et al. 1998; Peppin et al. 2010). In the Chicago, IL region, seed sourcing policies ranged from strictly on-site collection to a set radius from the county containing the restoration site (Saari & Glisson 2012). This inconsistency is due to uncertainties about the scale of local adaptation (McKay et al. 2005; Peppin et al. 2010; Hamilton et al. 2020), practical considerations, and the distinct goals of the varied organizations involved (Hooper 2003; Peppin et al. 2010). Additionally, technical challenges have been identified, including the need for more information on propagating, growing, and harvesting species (Dale 1993; BLM 2009; Peppin et al. 2010). Impediments to local seed sourcing are not restricted to the United States; similar obstacles are observed in other countries (Tishew et al. 2011; Broadhurst et al. 2015; Elzenga et al. 2019; Mainz & Wieden 2019; Schmidt et al. 2019).

We present research on obstacles to the production and use of locally sourced, native seeds for prairie restoration, focusing on Minnesota. In this state, there is strong public interest in conservation as demonstrated by the electorate's passage of constitutional amendments dedicating funds to natural resources (Noe et al. 2017) and by the aforementioned governmental support for native seed production. Additionally, policy, practice, and seed purchasers have changed during the 27 years since the previous study, a report to the state legislature, on this topic in Minnesota (Dale 1993), necessitating an update. We report on the results of interviews using focus groups. Participants included producers and users of native seeds sourced in the Minnesota prairie. Our methods differ from previous studies, which used surveys (e.g. Dale 1993; Hooper 2003; Smith et al. 2007; Peppin et al. 2010; Saari & Glisson 2012; De Vitis et al. 2017), databases of available seeds (e.g. White et al. 2018), or records of seed purchases (Camhi et al. 2019). We posed open-ended questions, allowing participants to steer the conversation and insights to arise through interactions among participants (Krueger & Casey 2015).

Methods

Rationale

Our goal was to characterize impediments to the production and use of locally sourced native seed, using Minnesota as an example. Rather few people lead efforts to either produce locally sourced native seed or use it to restore Minnesota prairies. Consequently, our conversations could include nearly all key decision-makers. Because this small number of actors restricts sample sizes appropriate for techniques such as surveys, we chose to use focus group interviews. Such interviews capture individual responses to open-ended questions and additional insights from interactions among participants (Krueger & Casey 2015). Our focus groups were exempt from institutional review, because individuals were asked to discuss their expertise and organizational processes, not personal information (UMN 2015).

Focus Group Participants

We invited participants based on their roles in restoring Minnesota prairies: either the production of source-identified native seeds, or the acquisition and use of such seeds (Table 1). Producers involved in a regional trade group, the Minnesota Crop Improvement Association's (MNCIA's) Native Plant Committee, were selected as participants. Additionally, we identified potential participants from the Minnesota Department of Natural Resources plant supplier list (MnDNR 2016) and through nominations. We included individuals at the major governmental, nonprofit, and for-profit organizations that acquire large quantities of native seed to restore Minnesota prairie. Smaller-scale users were nominated by individuals familiar with Minnesota prairie restorations.

Focus Group Sessions

We followed the focus group methods of Krueger and Casey (2015). The approximately 2-hour focus group interviews occurred in person and by conference call. Sessions included three to nine participants from similar institutions plus the authors (Table 1), began with the same introduction (Supplement S1), and followed the same questions (Supplement S2); for convenience, these questions are given in each Results subsection. The session involving members of the MNCIA Native Plant Committee immediately followed and frequently referenced a scheduled committee meeting; consequently, meeting notes were included in the analysis as if part of that focus group. Sessions were recorded with a Zoom H2N device (Zoom North America: Hauppauge, NY, U.S.A.).

Analysis

Audio recordings of the sessions were transcribed using Express Scribe Transcription Software (NCH Software: Greenwood Village, CO, U.S.A.) or by High Fidelity Transcription (Minneapolis, MN, U.S.A.). We analyzed the focus group sessions qualitatively. While quantitative metrics for analyzing focus group interviews exist, our sessions focused on eliciting the variety of experiences and opinions rather than assessing the frequency of certain experiences. During analysis, we merged questions 1 and 2, which addressed predicting and meeting needs, and questions 5 and 6, which addressed demand for species. We then grouped similar responses to each question, reassigning comments to different questions as appropriate, and distilled responses into the following results. Results, including anecdotes, are from participants' comments during the focus group sessions (Supplement S3).

Table 1. Number of participants in each focus group, totaling 33, exclusive of the researchers. Large-scale users represent the major government and nonprofit entities that use large volumes of seeds for restoration. Users were selected based on entities that perform large restorations and snowball sampling. Producers were identified based on the MNCIA, DNR producer list, and snowball sampling.

Session	*Participants*
Users	
Large-scale users	5
Regional users	7
Other users	4
Producers	
Minnesota Crop Improvement Association	9
Hand collectors	3
Other producers	5

Results

Predicting and Meeting Needs

How do you predict your needs for plant materials? What is the timeline of steps you need to take to be able to meet your needs?

Both users and producers of source-identified seeds repeatedly emphasized that unpredictability of needs seriously undermines the reliability of supply (Supplement S3). Users of native seeds reported that they cannot predict their needs more than a year in advance, due to variable funding and guidelines. Variation in acquisition methods—which include purchase of commercially produced material, hand collecting, and bulk harvesting from wildlands—also affect planning timelines.

Uncertainty in demand compromises the availability of commercially produced seeds. Producers and users attributed the limited availability of source-identified seeds to the challenges of planning and implementing production. These arise from varying policies, changing consumer demands, and the biology of particular species. Demand volatility most constrains production, due to the financial risks. Producers report that they start with species they can reliably produce and sell. Depending on available resources and anticipated financial return, they may expand production into other species. For some species, these efforts are constrained by insufficient knowledge of germination and propagation methods. Overall, unpredictability can obstruct entry into and expansion of the seed production business.

Demand for and the Definitions of "Local"

What geographic scale do you consider local?

Definitions of "local" vary among agencies and funding sources. Sourcing guidelines, which are periodically revised, range from restricting to seed originating within 24 km (15 miles) of a restoration site to having no restriction. For example, sourcing guidelines from Minnesota governmental agency programs range from specifying a 40-km (25-mile) radius (Department of Natural Resources, MnDNR), to allowing seeds originating from anywhere in Minnesota and bordering counties of neighboring states (Department of Transportation, details in MacDonagh & Hallyn 2010), to using predefined ecological regions (details in BWSR 2017). Individuals also expressed their opinions regarding the definition of "local." These included preference for sourcing from the same county and surrounding counties; from within 320 km (200 miles); and from an oval 480 km (300 miles) east–west and 320 km (200 miles) north–south, reflecting climatic variables.

Producers and users of native seed recommended considering local sourcing within the context of nearby conditions, intended use of the seeds, and species. The extent and quality of prairie remnants vary throughout the state; local sourcing may obviate the risk of genetically contaminating nearby remnants. Conversely, if a species no longer grows in the area, there is no risk of genetic contamination of populations. Seed collection from wildlands may compromise prairie remnants, and some land managers strictly limit harvesting. Producers viewed local sourcing as more important to long-term than potentially shorter-term restorations, such as those funded under the Conservation Reserve Program (CRP). Producers and users also stated that the definition of "local" should vary among species, given differences in pollination and seed dispersal distances.

Native seed users discussed potential effects on their practice of climate change, adaptive potential, and production location. Climate change may profoundly influence seed sourcing. Practitioners want plant materials that are adapted to both initial and future environmental conditions. However, they also noted the planning required to respond to climate change and acknowledged the risks that assisted migration may impose on extant populations. Users and producers expressed concern that populations' genetic variation declines due to genetic bottlenecks and unconscious selection during collection and propagation. They also questioned whether production site should be considered, in addition to material origin, when sourcing seeds.

Demand and Location

Are there particular parts of the state you anticipate demand changing for?

When asked about geographical change in demand, participants stressed the unpredictability. Some producers and users were unprepared for intensified concern for pollinators and demand for seeds of associated plants, including for revegetation around solar panel arrays. One user speculated that ongoing tree loss due to invasive pathogens and insects may increase demand for savanna species. Some users anticipate that climate change may shift demand indirectly via managed relocation (sensu Richardson et al. 2009). In general, participants expect demand for native seed to increase, though this may depend on marketing, state programs, and large Federal programs such as CRP.

Demand and Species

Are there particular species you anticipate demand changing for?

What seeds or plant materials are you interested in acquiring for use or production but do not have access to?

Participants noted that prairie restorations often include relatively few of the species historically present in tallgrass prairie and identified contributing factors (Supplement S3). Producers reported needing about 5 years to bring seeds of a new species to market. The corresponding delay in recovering their investment means that producers must balance risks and rewards when choosing species to produce (Supplement S4). Producers reported that, for some species, the selling price required to recover their investments is prohibitive for many purchasers. Thus, desirable species that must be sold at higher cost may be harder to sell. Moreover, idiosyncratic biology of individual prairie species can present challenges to commercial-scale production (Supplement S4), due to insufficient information on methods for effective collection, germination, growth, and harvest. Producers and users both noted low availability of species that flower early in the season, have small stature, or occupy wet prairies. Furthermore, seed yield varies interannually, and phenology, weather, and other phenomena affect harvests.

Increasing species diversity in restorations will involve decisions and actions by both producers and users of native seed. These include overseeding and efforts to support pollinators and other invertebrates, such as bulk harvesting via haying, which can collect invertebrates along with the plant materials. Demand for greater species diversity will depend on the resources available to restoration projects, especially for species that have high production costs. Currently, some users of native seeds address this by harvesting expensive species that grow on their own land and distributing them to other areas.

Current Strengths

What is currently working well in the processes for producing and using source-identified seed?

Seed sourcing is improving; more seeds, species, and populations are available, and at higher quality. Participants appreciate Minnesota's system of standards and certifications. While not all seeds meet current guidelines, users stated that seed is now regularly sourced closer to the restoration site than in the past. Demand is also strong in the broader region; producers can often sell seeds outside of Minnesota when unable to sell them within Minnesota.

Users emphasized the employment opportunities associated with grassland conservation and the increased demand for restoration work, due partly to Minnesota's state programs (e.g. Outdoor Heritage Fund). Producers were concerned, however, that increased government involvement in production could harm their business; their consensus was that private entities will grow to meet demand if not challenged by government competition. Additionally, producers voiced concern that potential government-run seed storage facilities, though intended to reduce annual variation in demand, could harm private seed brokering businesses.

Partnerships and cooperation within the restoration sector were viewed positively. Examples included the Glacial Ridge Project, a joint effort of The Nature Conservancy, government agencies, and a commercial seed producer. Users reported valuing relationships with trusted producers, volunteer seed collectors, and nonprofit organizations (e.g. Conservation Corps). Producers also reported cooperative efforts to fill orders.

Current Weaknesses

What would you change about the current source-identified seed system?

Users are often unable to obtain seeds in the quantities they need, while producers face uncertain demand. One user reported receiving bids for seed purchases that lacked some requested species (Supplement S3). Users discussed establishing guidelines to influence production choices. Producers noted the limited incentive to produce species for which demand is uncertain.

Both users and producers expressed concern over staffing (Supplement S3). Users have insufficient staff to harvest multiple times annually; this limits availability of species that are difficult to produce due to unusual phenologies or explosive seed dispersal. Users also need staff to maintain conditions that support robust, reproductive plants and discussed losing sites and genetic resources due to inadequate maintenance. Producers report difficulty retaining experienced employees, who can find higher-paying jobs elsewhere. Producing multiple genetic sources of the same species requires isolating production fields, which complicates production logistics.

Legal and bureaucratic factors also restrain expansion of supply; these include restricted seed collection on public land and varied sourcing guidelines. Participants recognized that standardizing sourcing regulations would be a complex process, especially because of the sparse data available for many species. In Minnesota, commercial producers are currently barred from obtaining foundation seed from state-owned land; whether nonprofits may collect seeds from public land for use in restoration is unclear. Producers also expressed concern that some populations or species, which could be used as a source for production, may be lost despite conservation efforts. However, some users also expressed concerns about overcollecting from wild populations. Focus group participants implicitly recognized that maintaining the genetic variation of natural populations—through avoiding genetic contamination and overharvesting—is an important part of natural resource conservation. Sourcing guidelines can create barriers. One restoration project was reportedly canceled due to inability to meet a 40-km (25-mile) sourcing restriction. Some programs may also restrict management practices that would support prairie species (e.g. restrictions to burning CRP land).

Insufficient technical information is an obstacle to use of locally sourced native seed (Supplement S3). Practitioners hold strongly differing opinions about seeding density and the sequence and timing of steps for restoring prairies. Seed testing is a further concern; results often differ among laboratories, and for many species, tests are unavailable. Producers, having noticed that certain species sometimes fail to establish, discussed the role of microorganisms in restoration and whether they should be included in production. Native seed users, being unsure of the scale of local adaptation, use rough guidelines that they suggested may be unnecessarily narrow.

Participants were concerned about the introduction of non-native species and genotypes. There are multiple vectors for unintentional introduction, including restoration equipment and contaminated seed supplies. One user discussed “seed bombing,” the well-intentioned practice of introducing plants via hurling lumps of substrate and potentially non-native seeds, that highlights the need for public education about risks of indiscriminate introduction. One producer was concerned that unscrupulous producers may include non-native species in seed mixes, to reduce costs.

Possible Solutions

What should someone focus their energy on if they want to improve the source-identified seed market?

Participants suggested the following as high-priority actions: developing more consistent standards, being aware of ramifications from changes to certain laws, revising internal agency policies, increasing communication and education, and promoting increased numbers of producers.

Greater consistency and feasibility of standards would help producers meet them and reduce risks of contamination. Greater investment in the standards is also needed—absent financial benefits from certification programs (see MNCIA 2017), producers may not commit resources to produce source-certified seeds.

Changes in two particular laws could have large ramifications (Supplement S3). One is CRP, a Federal program that pays farmers to keep land out of agricultural production. Existing and future CRP rules have broad impact on demand; e.g. producers were concerned that demand for seed will severely decrease if the cap on the amount of CRP land stays constant. The other is noxious weed law, which can potentially have large impacts because production fields may contain weeds. For example, the presence of *Cirsium arvense* (Canada thistle) in a bulk-harvested field could cause the seed lot to fail inspection and not be sold.

Native seed users recognized that agency rules, such as the restriction on private entities collecting seeds from public lands, can result in reduced seed availability. Concerns about privatizing public goods and favoritism underlie these policies, but some users find the policies counterproductive for restoration. Users speculated on contracts and easements that could alleviate these restrictions.

Improved communication was raised in two contexts: availability of research and increased dialogue between native seed producers and users. Communication between practitioners and researchers about research needs could promote development of germination, production, and tissue culture protocols. Users anticipate benefiting from research on the scale of local adaptation and the long-term effects of sourcing decisions, while researchers could benefit from conducting experiments at restorations. Producers envisioned collaboratively developing methods for producing recalcitrant species. Communication between producers and users was viewed as one way to mitigate risk. Producers discussed the value of having greater advance notice of planned projects, and users discussed sharing seeds or cooperatively harvesting their own lands.

Participants identified a need for public education about the importance of native species and locally sourced populations in neighborhood and roadside projects. Increased installation of rain gardens and pollinator gardens may increase the planting of non-native species. There is a need to stimulate landowners' interest in their prairie remnants and help them realize the potential of the seeds from them. Although the expense of planting

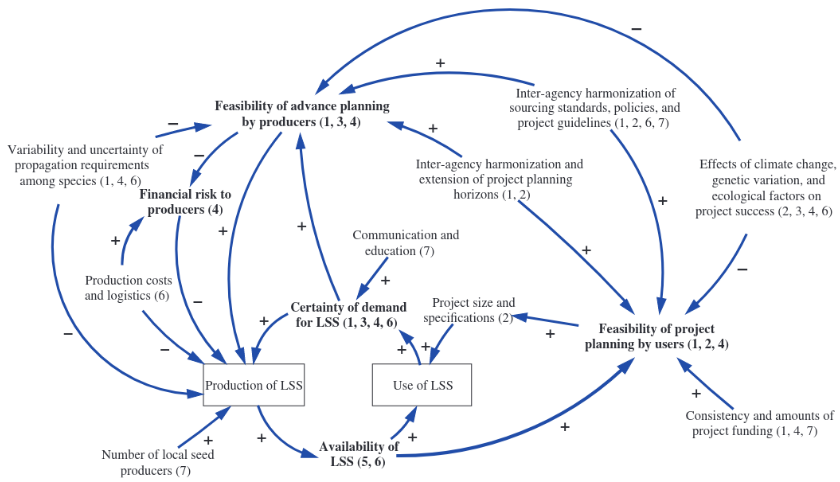

Figure 2. Conceptual model of major factors affecting production and use of locally sourced seeds (LSS) in restoration, and their interrelationships, as identified by expert focus groups (see text for details). Bolding indicates factors of particularly strong effect. Arrows and plus/minus signs indicate the direction and polarity of relationships between factors. Parenthetical numerals denote corresponding sections in Results section of the text: (1) predicting and meeting needs; (2) demand for and definitions of "local"; (3) demand and location; (4) demand and species; (5) current strengths; (6) current weaknesses; (7) insights for possible solutions.

native species on roadsides is considerable, it is small compared to the total cost of a transportation project and to the importance of maintaining native biodiversity.

Participants emphasized the need for more producers, who could increase seed availability and, thus, reduce prices. Increasing the number of producers may facilitate the production of populations sourced on finer geographic scales. Some participants opined that producers of various sizes and scales could coexist. (Fig. 2)

Discussion

Focus group participants acknowledged that increased interest in local sourcing of native seeds for prairie restorations is spurring production and use of these materials, but several issues limit seed availability. Producers emphasized that market unpredictability constrains production, while users discussed inability to obtain requisite quantities of seeds. Conversations encompassed the definition of "local," importance of key laws, role of internal policies, research needs, and importance of education, communication, and partnerships. Many of these topics have also been identified in other parts of the world (e.g. Broadhurst et al. 2015; Mainz & Wieden 2019; Schmidt et al. 2019).

Unpredictability in demand affects supply and is a barrier to launching new commercial entities. This persistent challenge was noted in the survey by Dale (1993) and is not unique to Minnesota prairie, having been identified in Australia (Broadhurst et al. 2015), Brazil (Schmidt et al. 2019), and the western United States (Richards et al. 1998; Peppin et al. 2010; Camhi et al. 2019). Mitigating this volatility may require consistent project funding and much longer planning horizons. The results of efforts elsewhere will be informative. The Seeds of Success program is increasing seed warehousing efforts (BLM 2009; Tishew et al. 2011). Federal agencies are implementing new agreements, such as indefinite-delivery/indefinite-quantity contracts, stewardship contracts, and buy-back options (Peppin et al. 2010).

Restoration goals, definitions of "local," and sourcing decisions vary considerably among agencies and organizations that fund or implement projects. Improved consistency would ameliorate unpredictability of demand. The U.S. National Seed Strategy promotes development of seed transfer zones, whether empirically, for commonly used species, or through modeling (PCA 2015). Kramer et al. (2015) suggested using provisional seed zones that incorporate the U.S. Environmental Protection Agency's level III ecoregions when seed transfer zones have not been established empirically. In Germany, regional admixture provenancing is being implemented, which uses both seed transfer zones and mixing seeds from multiple populations in these zones (Bucharova et al. 2019). For Minnesota, participants called for sourcing guidelines that are compatible, realistic, and scientifically sound, a goal that will require cooperation among diverse stakeholders.

Changes to certain laws, such as CRP and the noxious weed law, may have an outsized effect on practices. Since its establishment, CRP has varied in its size, peaking in 2007 at 14.9 million hectares (Hellerstein 2015). The program's purpose, eligibility criteria, and enrollment and reimbursement mechanisms have been altered, all of which impact large areas. In other jurisdictions, laws having an outsized impact on native seed production have been identified. For example, regulations concerning fodder in the European Union may not be consistent with restoration goals (Abbandonato et al. 2018). The variability that is important in native seeds for restoration may conflict with regulations designed for agricultural species and may need to be considered in regulations and testing (Pedrini & Dixon 2020).

Participants suggested addressing agency policies; a salient policy in Minnesota is the restriction on sourcing commercial foundation populations from public land. Relevant concerns include risk of overharvesting, privatizing public goods, and inequitable benefit from public resources. The Iowa (U.S.) Ecotype Project addressed some of these concerns by sourcing seed from sites that included public land, developing ecotypes from those seeds, and licensing ecotype foundation seed to private producers (Houseal & Smith 2000). Alternatively, some U.S. Federal agencies permit public harvest for commercial use (Robertson 2013). Overall, reconciling internal policies will depend on policymakers and stakeholders from nonprofit organizations.

The need for increased communication and education on topics concerning locally sourced seeds could be partially met by trade and producer associations, such as the MNCIA, which could communicate, educate, and help producers meet requirements (Abbandonato et al. 2018; Mainz & Wieden 2019). Additional actions, elaborated in the communication plans of the U.S. National Seed Strategy (PCA 2015), are aimed at both internal and external audiences. These include creating an electronic toolbox for briefings and presentations, utilizing social media, creating an expert speaker's bureau, and reaching out to local stakeholders through extension offices, botanic gardens, and relevant special interest organizations (PCA 2016).

Expanded research, scientific communication, and collaboration are needed. The need for more research on seed production and testing, was identified by Dale (1993) regarding Minnesota and subsequently across the globe (Broadhurst et al. 2015; Elzenga et al. 2019; Pedrini & Dixon 2020). A survey of European seed producers found that 75% of the producers who lack active collaboration with a researcher would be interested in forming a collaboration (De Vitis et al. 2017). Scientists studying the effects of climate change, seed sourcing decisions, local adaptation, seed viability tests, and germination protocols should communicate their research to a range of stakeholders through various media including focus groups, such as those used here; this is one advantage of focus groups over conventional surveys. The local knowledge that is available for some species should be valued (Schmidt et al. 2019).

There is strong interest in the production and use of locally sourced native seeds. Users of native seeds generally prioritize purchasing based on immediate funding, which they cannot accurately predict. Producers operate based on reliability of production and sales and reduction of risk. Prairie restoration will benefit from the experience of programs such as Seeds of Success, just as other systems have looked to prairie restoration in the Midwestern United States (White et al. 2018) and the United States as a whole (Tishew et al. 2011). Ultimately, citizens, subnational, and national governments, through funding and policy decisions, will have profound impacts on the future of seed production and sourcing systems.

Acknowledgments

The authors thank the Environment and Natural Resources Trust Fund of Minnesota for funding their work and the University of Minnesota's Institute on the Environment for funding this project and assisting with logistics. The authors thank K. Nelson for her guidance. The authors also thank G. Quiram, H. Bernardo, D. Larson, P. Tiffin, C. Geyer, members of the Ruth Shaw lab, P. Török, and four anonymous reviewers for feedback. The authors are tremendously grateful to the participants for devoting their time and open communication in this process.

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Supporting Information

The following information may be found in the online version of this article:

Supplement S1. Introductory statement at the beginning of each focus group session.
Supplement S2. The questioning routine.
Supplement S3. Selected quotes.
Supplement S4. Notable species from the discussion.

Coordinating Editor: Peter Török

Received: 2 July, 2020; First decision: 25 May, 2021; Revised: 25 May, 2021; Accepted: 10 August, 2021

New Phytologist

Meetings

Evolutionary approaches to seed sourcing for grassland restorations

An organized workshop in Minneapolis, MN, USA, 21 March 2019

Large-scale conversion and fragmentation of biologically diverse, productive, temperate grasslands has impaired key ecosystem services, including carbon storage (Ahlering *et al.*, 2016), pollination (Hendrickson *et al.*, 2019), and maintenance of soil structure, and hydrological services (Power, 2010; Lark *et al.*, 2015; Comer *et al.*, 2018). With increased anthropogenic stresses, including climate disruption, the need for grassland restoration has increased. Applying restoration strategies that establish and maintain long-term resiliency will be critical to regaining some of the lost ecosystem services. One of the major challenges to establishing restorations is an apparent tension that exists between evolutionary theory and restoration practice. Maintenance of evolutionary potential may require introduction of genetic variation following decades of reduced gene flow due to anthropogenic fragmentation or inbreeding (Ralls *et al.*, 2018). However, evolutionary studies have yielded abundant evidence of local adaptation, which implies that local selective pressures have contributed to differentiation in traits important to contemporary adaptation across environmentally heterogeneous landscapes (Hufford & Mazer, 2003; McKay *et al.*, 2005). Balancing the prevalence of local adaptation while maintaining evolutionary potential is necessary to sustain long-term adaptability in restored grassland communities (Aitken & Bemmels, 2016; Bucharova *et al.*, 2018). Moreover, to meet the demands of restoration, the collection, propagation and production of seed for restoration poses its own evolutionary challenges (Espeland *et al.*, 2017; Breed *et al.*, 2018). The goal of this workshop was to ask how key evolutionary processes contribute to individual-, population-, and community-level variation across the landscape and to ask how restoration practice may affect these processes and ultimately restoration success.

This workshop focused on the role of evolution in restoration, including understanding the scale and extent of adaptation to current, local conditions, estimating the impact of gene flow across scales, and quantifying the capacity for adaptation to novel selective environments. A large body of work has demonstrated that plant populations tend to be adapted to local conditions (Leimu & Fischer, 2008; Hereford, 2009); however, the eco-geographic scale of adaptation is virtually unknown for most species (McKay *et al.*, 2005). In addition, as restoration site conditions commonly diverge from pre-disturbance environments, locally sourced populations could be maladapted following restoration (Lesica & Allendorf, 1999). Whether conditions change for these or other reasons, genetic variation is a prerequisite for adaptive evolution (Lewontin, 1974). While gene flow may hamper adaptive divergence or cause outbreeding depression due to the breakup of co-adapted gene complexes (Aitken & Whitlock, 2013; Janes & Hamilton, 2017), it can also mitigate the deleterious effects of inbreeding and genetic drift to which small, fragmented populations are especially susceptible (Falk *et al.*, 2006; Hamilton & Miller, 2016). Thus, a core challenge remaining, for both restored and natural populations, especially in fragmented landscapes, is to minimize maladaptation to current conditions while maintaining adaptive potential in uncertain environments.

Oral presentations focused on the intersections of adaptation, gene flow, and the maintenance of adaptive capacity at varied levels of biological organization. A number of research programs are currently addressing the question 'How local is local?'. Marissa Ahlering (The Nature Conservancy, Minneapolis, MN, USA) noted that there is a substantial range in how we define local, and this has bearing on local, regional, and national seed management efforts. Shelby Flint (University of Minnesota, Saint Paul, MN, USA) summarized ongoing evaluation of the geographic scale of local adaptation in common grassland perennials. Flint noted that the signature of local adaptation is not consistent across species in an ongoing study. Jill Hamilton (North Dakota State University, Fargo, ND, USA) presented assessments of the eco-geographic scale of differentiation for a range of quantitative traits. Hamilton identified differences in the scale of trait differentiation across landscapes for different quantitative trait classes, including morphological, resource allocation, and stomatal traits. Hamilton suggested that different functional trait classes may be suitable for establishing seed transfer guidelines and that suitability may depend on climate–trait associations (Yoko *et al.*, In press). Lars Brudvig (Michigan State University, East Lansing, MI, USA) discussed a recently established experiment examining the consequences of intra- and inter-specific diversity on restored populations, communities, and ecosystem functions. Establishing this experiment as a large-scale restoration, Brudvig will be evaluating the impact population genetic diversity and species diversity may have on community diversity across restored ecosystems over time. Similarly, Ahlering described a new project comparing short-term success and longer-term persistence of single- and multi-source seed mixtures in large-scale restorations. These studies address fundamental questions regarding the scale of adaptation across levels of biodiversity while applying large-scale tests of composite provenancing approaches in restorations (Bucharova *et al.*, 2018).

Understanding the balance between adaptation, gene flow and demographic variation can require long-term empirical studies, particularly when considering the maintenance of connectivity across dynamic landscapes. Stuart Wagenius (Chicago Botanic Garden, Glencoe, IL, USA) discussed feedbacks between evolution and demography. Wagenius's long-term studies combining natural population observations with common garden experiments indicated substantial consequences of inbreeding depression and considerable variability in fitness across different life history stages in the long-lived perennial, *Echinacea angustifolia* (Wagenius *et al.*, 2010). Lauren Sullivan (University of Missouri, Columbia, MO, USA) presented ongoing research into the consequences of pollen and seed dispersal using a range of grassland species. Sullivan's fine-scale assessment of the impact of dispersal mode and distance on connectivity within and among populations of prairie forbs has implications for landscape-level site acquisition and management. While gene flow is important to the maintenance of diversity and connectivity across grassland ecosystems, it can be associated with risk, particularly if seed transfer increases the likelihood of introducing nonnative species into areas they have not reached. Holly Bernardo (US Geological Survey, Reston, VA, USA) discussed existing seed availability and the use of spatially explicit models to evaluate the risk of introducing nonnatives and its dependence on seed transfer distances. Bernardo's research identifies an optimized geographic distance for seed transfer that balances the trade-offs between distance, seed availability, and the risk of nonnative introductions. Additionally, range shifts can establish gene flow between previously allopatric taxa, leading to inter-specific hybridization (Hamilton & Miller, 2016). For rare species, hybridization with more widespread congeners may be undesirable (Zlonis & Gross, 2018). Briana Gross (University of Minnesota Duluth, MN, USA) summarized the population genetic consequences of gene flow between rare, isolated disjunct populations with their more common relatives asking whether hybridization is a threat to native population genetic structure. Understanding when hybridization may be viewed as a conservation threat or a conservation tool will be important to species conservation (Chan *et al.*, 2019).

Considering the maintenance of adaptive capacity, Charles Fenster and Michele Dudash (South Dakota State University, Brookings, SD, USA) advocated the use of genetic rescue, the introduction of genetic variation to counter the genetic and demographic consequences of small, fragmented populations, as a management tool for native plant populations (Carlson *et al.*, 2014; Ralls *et al.*, 2018). They offered a decision tree considering environmental conditions, breeding system, and risk of outbreeding depression as a basis for decisions on the use of genetic rescue within a restoration context (Frankham *et al.*, 2017). Taking a direct approach to estimating evolutionary potential, Ruth Shaw (University of Minnesota, St Paul, MN, USA) discussed predicted and experimentally estimated values of additive genetic variance for fitness (Fisher, 1930; Lewontin, 1974) using *Chamaecrista fasciculata* and *E. angustifolia*. Shaw suggested targets for evolutionary rescue, which differs from genetic rescue in its reliance on evolutionary change from standing genetic variation, would be populations where observed fitness is lower than predicted. Interestingly, Shaw noted that estimates of additive genetic variance for fitness based on a number of life history traits suggest a substantial capacity for adaptation. Together, this research points to the importance of maintaining genetic variance in native populations not only for current conditions, but also considering the maintenance of adaptive potential across generations.

Several participants addressed the interface between applied and theoretical considerations in the context of seed sourcing for restoration. One of the current challenges facing restoration is seed availability as demand consistently surpasses supply (Broadhurst *et al.*, 2008, 2016). Nicholas Goldsmith (University of Minnesota, St Paul, MN, USA) characterized obstacles faced by users and producers of locally sourced seed, which included uncertainty and risks associated with funding and production of seed, limited lead time on project-specific needs, and variable growing conditions that can dramatically affect seed supply and demand. Julie Etterson (University of Minnesota Duluth, MN, USA) discussed the extent and consequences of genetic bottlenecks and unconscious selection during accession, propagation, and production of farmed seed for restoration. In an experiment, Etterson noted farmed seed exhibited reduced fecundity and stress tolerance relative to wild collected seed. Etterson identified approaches to minimize selection during propagation; including increasing the number of maternal families sampled per population, harvesting at multiple times across a season, and mixing hand collections with mechanical harvesting for large-scale restorations (Espeland *et al.*, 2017). Despite growers' efforts to maintain genetic diversity, Jill Hamilton presented evidence of genomic differences between native and commercial seed sources. Although the consequences of these differences to quantitative trait variation remain to be tested, the effective population size of commercial seed sources was reduced relative to native populations. Finally, although accessibility of native seed was identified as a major limitation to implementation, new regional initiatives have the potential to improve seed availability. The newly established Native Plant Initiatives at South Dakota State University addresses some of the concerns associated with farmed sources of native seed pairing research with production (Lora Perkins, South Dakota State University, Brookings, SD, USA). Efforts that integrate research and application with education of local communities and stakeholders will be key to establishing, implementing, and maintaining these new initiatives.

Among workshop participants, there was consensus that, especially now as environmental conditions change rapidly, it is crucial to maintain and in some cases supplement existing genetic variation to enable adaptive evolutionary change. Genetic and evolutionary rescue may combat the combined impact of drift, inbreeding, and reduced gene flow due to fragmentation, ameliorating the risk of local extinctions and promoting resilience (Whitely *et al.*, 2015; Hamilton *et al.*, 2017). In addition, considering the spatial and temporal scale over which responses to changing conditions are evaluated will be important (Baythavong, 2011). Many existing experimental studies reflect seasonal weather responses, rather than long-term responses to climatic variation. Considering short- and long-term responses to selection, as well as plasticity, will be needed, both for assessing adaptive potential, designing seed mixes, and establishing seed transfer guidelines. There are clear benefits to establishing seed selection, production and transfer guidance for native grassland species, and there is much to be learned from the existing expertise implemented across different systems (Breed *et al.*, 2018; Bucharova *et al.*, 2018). With increasing need for native seed, the impact of unconscious selection on seed production will require evaluation. Finally, focusing restoration on capacity for continuing adaptation, rather than on 'local' sourcing alone, appears key to maintaining evolutionary potential. While there is debate over the definition or scale of 'local', there is consensus that maintaining and enhancing the adaptive capacity of our native grasslands is necessary. As evidence accumulates that species are maladapted to contemporary environments, identifying and implementing restoration strategies that consider the capacity for ongoing adaptation will be necessary to preserving grassland ecosystems and their evolutionary potential.

Jill Hamilton[1]*, Shelby Flint[2,3], Jessica Lindstrom[1], Kate Volk[1], Ruth Shaw[2] and Marissa Ahlering[4]

[1]Department of Biological Sciences, North Dakota State University, Fargo, ND 58102, USA;
[2]Department of Ecology, Evolution and Behavior, University of Minnesota, St Paul, MN 55108, USA;
[3]Science Department, Southwest Minnesota State University, 1501 State Street, Marshall, MN 56258, USA;
[4]The Nature Conservancy, 1101 West River Parkway, Suite 200, Minneapolis, MN 55415,USA
(*Author for correspondence: tel +1 701 231 7160; email jill.hamilton@ndsu.edu)

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Key words: climactic variation, ecosystem restoration, grasslands, Meeting report, seed sourcing.

RESTORATION ECOLOGY
The Journal of the Society for Ecological Restoration
SER SOCIETY FOR ECOLOGICAL RESTORATION

RESEARCH ARTICLE

Latitude of seed source impacts flowering phenology and fitness in translocated plant populations

Naomi S. Rushing[1,2], Shelby A. Flint[3], Ruth G. Shaw[1]

Seed sourcing strategies have received considerable attention in the restoration literature and are a key component of effective management for restoration and conservation of natural areas. Research and discussion tend to focus on optimal distances and environmental similarities between seed sources and planting sites. However, given the increasing calls for assisted gene flow and assisted migration, greater consideration of translocating populations in specific directions across climatic gradients is warranted. To the extent that local adaptation proceeds primarily in response to climatic conditions, assisted gene flow across climatic gradients is likely to promote species persistence in the face of climate change. However, if species are adapted to other abiotic and biotic factors, translocating populations across climatic gradients may have unintended and potentially maladaptive consequences. Here, we used extensive collections of seed materials from across the state of Minnesota, a field planting that established common conditions at a location that was near the southern extreme of all the source locations, and subsequent aster modeling of fitness data to examine the overall fitness consequences of translocating populations across the landscape. We found that populations from cooler, northern sources tended to have higher fitness than those from warmer, more southern locations. In addition, populations from more northern locations tended to have earlier flowering phenology relative to populations from more southern sources, perhaps conferring a fitness advantage. Taken together, our results suggest that latitude of origin may be an important factor to take into consideration during seed source selection for restoration work, and that the direction of the effects can be at odds with expectations based on climatic considerations.

Key words: assisted gene flow, assisted migration, fitness, flowering phenology, latitude, seed source

Implications for Practice

- Use of geographic distance to guide seed sourcing decisions can be problematic as it ignores other environmental and spatial variables that do not covary cleanly with geographic distance. Latitude of origin may also impact success of translocated populations.
- There is limited evidence supporting an inference that poleward assisted gene flow/migration is generally adaptive.
- Future studies investigating the relative importance to different species of the many environmental factors affected by climate change would provide valuable insight into assisted gene flow and assisted migration practices.
- When using assisted gene flow to augment populations' size and genetic variation, practitioners are advised to limit latitudinal distance between source populations and target populations to ensure sufficient overlap of flowering phenologies and maintain potential for admixture.

Introduction

Grasslands are diverse ecosystems that provide valuable ecosystem services. They are home to thousands of plant and animal species and aid in water infiltration, carbon storage, erosion control, and nutrient retention, as well as offering opportunities for hunting and other recreational activities (Tester 1995; Schulte et al. 2017; Bengtsson et al. 2019). However, grassland habitats are dwindling. Globally, over 45% of grasslands have been lost to agriculture and other uses (Hoekstra et al. 2005), while in Minnesota, U.S.A., less than 1% of original prairie habitat remains relative to pre-European settlement (Samson & Knopf 1994). The drastic loss of native prairie poses concerns about not only the resulting impairment of ecosystem services, but also the persistence of native species. Ecological restoration has become a critically important approach to mitigate habitat loss.

One of the keys to successful habitat restoration is choosing seeds that are likely to thrive in conditions at the restoration site.

Author contributions: NSR designed the study in consultation with RGS; RGS designed the Healthy Prairies Project experimental framework; SAF oversaw and implemented HPP seed collections, common garden planting, and maintenance; NSR conducted the field work for this study; NR analyzed the data with help from RGS; NSR wrote the manuscript with editing from RGS, SAF.

[1]Department of Ecology, Evolution and Behavior, University of Minnesota, St Paul, MN 55108, U.S.A.
[2]Address correspondence to N. S. Rushing, email rushi013@umn.edu
[3]Department of Biology, Southwest Minnesota State University, Marshall, MN 56258, U.S.A.

doi: 10.1111/rec.13464
Supporting information at:
http://onlinelibrary.wiley.com/doi/10.1111/rec.13464/suppinfo

A common practice is the preferential use of local seed, assuming a similar environment between the seed source and the restoration site (McKay et al. 2005; Broadhurst et al. 2008; Herman et al. 2014). According to evolutionary theory, if divergent selection outweighs gene flow, populations are expected to adapt to their local environments (Kawecki & Ebert 2004). As a result, the fitness of local populations in their home environment is expected to exceed that of foreign populations, and the fitness of populations planted at home sites is expected to exceed their fitness when planted at away sites. Local adaptation has been demonstrated to be widespread among plant species, although not ubiquitous (Leimu & Fischer 2008; Hereford 2009). However, the geographic scale of local adaptation is less clear, as is the magnitude of local adaptation (i.e. size of the fitness difference between local vs. foreign populations and fitness growing at home vs. at an away site).

The success of chosen seed sources may also be influenced by ongoing environmental change such as climate change. If current site conditions no longer match historical conditions, local seeds may no longer thrive. There is concern that local populations may not evolve quickly enough to keep pace with environmental changes, resulting in adaptational lag (Aitken et al. 2008). In response, it has been suggested that restoration practitioners engage in assisted migration (Aitken & Bemmels 2016), translocating seed from locations whose historical conditions more closely match the restoration site's current and predicted future conditions. This generally implies moving populations poleward, or in montane areas to higher elevations, with the idea that they are already adapted to the warmer temperatures predicted for these locations. Assisted gene flow is a related concept where populations are translocated along a climate gradient for the purpose of augmenting a resident population rather than initiating a new one. Assisted gene flow and assisted migration have been widely discussed (Aitken & Whitlock 2013; Breed et al. 2013; Vitt et al. 2016) and considerable work has been done with tree species (Williams & Dumroese 2013; Aitken & Bemmels 2016). However, there is a need for additional research into the logistics and effectiveness of this approach, particularly for herbaceous species (Hewitt et al. 2011; Bucharova 2017).

One potential consequence of translocating plant populations latitudinally is altered phenology, i.e. changes to timing of key life history events. Many species cue on photoperiod to initiate different life history stages, such as flowering. Moving populations latitudinally can cause their timing of flowering to differ from that of resident populations. For example, in a recent study Wadgymar and Weis (2017) translocated *Chamaecrista fasciculata* from Minnesota, Missouri, North Carolina, and Pennsylvania, to a common garden in Ontario. They found that in this extreme northerly location, plants from higher latitudes tended to begin flowering earlier than those from more southern latitudes, as Etterson (2004) also found for this species. Scalone et al. (2016) found a similar trend for *Ambrosia artemisiifolia*. As the shift in flowering timing increases, so do possible repercussions, such as an inability to complete fruit production before the onset of cold weather in the fall.

With the goal of informing choice of seed sources, the objective of this research was to address the following questions: (1) How does translocation along a latitudinal gradient affect flowering phenology? (2) How does translocation across latitudes and geographic distances impact fitness? (3) How does the degree of adaptation vary among populations sampled across an extensive geographic scale? A common garden approach was used to address these questions. Seed of three perennial prairie species, *Anemone cylindrica*, *Dalea candida*, and *Dalea purpurea*, were each collected from 12 remnant prairies in Minnesota and then grown in a field planting in southeastern Minnesota. Date of first and last flower were recorded in order to assess the impact on phenology of translocating populations along a latitudinal gradient. Survival and seed head production were used to estimate fitness. Local adaptation was detected if populations from nearby sites had higher fitness in the common garden than those from more distant sites, or if populations from sites having similar climatic conditions to the common garden had higher fitness in the common garden than populations from dissimilar sites. A better understanding of the impacts on fitness of translocating populations will contribute to improving seed sourcing practices such as assisted gene flow and assisted migration, aiding future restoration and conservation work.

Methods

Study Species

As part of a larger study assessing the geographic scale of local adaptation of six species of prairie plants in Minnesota (U.S.A.), three perennial forb species were chosen for this study: *Anemone cylindrica* A. Gray (thimbleweed), *Dalea candida* Michx. ex Willd. (white prairie clover), and *Dalea purpurea* Vent. (purple prairie clover). These three species are all native to North American grasslands and are commonly used in prairie restoration.

D. candida and *D. purpurea* are both members of the legume family, Fabaceae, which form symbiotic relationships with nitrogen fixing rhizobia. Both *Dalea* species are found throughout the central United States, ranging from Texas to Minnesota, and from Indiana to Colorado (*D. purpurea*) and Utah (*D. candida*). Within Minnesota, *D. candida* and *D. purpurea* are present throughout the southern, central, and northwestern portions of the state (USDA n.d.). Both *Dalea* species attract numerous pollinator species, which gather both nectar and pollen (Cane 2006; Applegate et al. 2007; Pearce et al. 2012). *D. purpurea* is known to be mainly xenogamous (Cane 2006); breeding system for *D. candida* is not definitively known but is generally described as cross-pollinating (Wynia 2008; Molano-Flores et al. 2011).

A. cylindrica is a member of the buttercup family, Ranunculaceae. *A. cylindrica* is native throughout Minnesota and can be found from New England to the Rocky Mountains (USDA n.d.). Unlike *D. candida* and *D. purpurea*, *A. cylindrica* is primarily autogamous (Molano-Flores & Hendrix 1998) and receives far fewer pollinator visits.

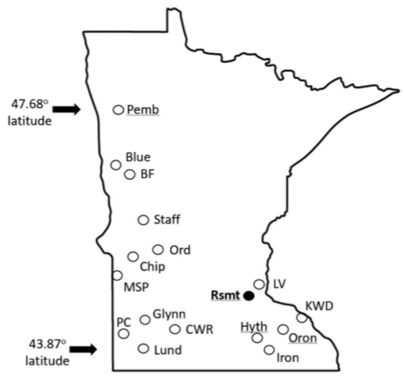

Figure 1. Map of seed sources for *Anemone cylindrica*, *Dalea candida*, and *Dalea purpurea* (open circles) and common garden site (filled circle) in Minnesota, U.S.A.

Seed Collection

A. cylindrica, *D. candida*, and *D. purpurea* seed were each collected from 12 native remnant prairies (16 prairies total) in Minnesota in 2014, under permit from the Minnesota Department of Natural Resources and The Nature Conservancy. Source sites were chosen to span much of Minnesota's native prairie, with three in southeastern Minnesota, three in southwestern Minnesota, three in west central Minnesota, and three in northwestern Minnesota (Fig. 1). At each collection site, seed was collected from at least 60 individuals per species. Seeds were collected from plants at least 3 m apart in order to minimize the chance of collecting seed from closely related individuals (Fenster 1991).

Plant Propagation

For each species and each of its 12 collection sites, 50 seeds were randomly chosen from each of 40 individuals. These seed were then pooled by site before cleaning for *A. cylindrica* and *D. candida* and after cleaning for *D. purpurea*. For *D. candida* and *D. purpurea* seed, hulls were removed and seeds were triple scarified using a sandpaper-lined drum. Seed for all three species was then stratified as follows: All seed from one species at one site was placed in a labeled, one gallon Ziploc bag containing a wet paper towel. Bags were placed in a cold room at 4°C for 30 days (*D. candida* and *D. purpurea*) or 60 days (*A. cylindrica*).

After stratification, seeds were planted in flats containing MVP Sungro Professional Growing Mix in January 2015. Each flat contained seed from one species at one site. Flats were then placed in the growth chamber in a randomized array. Growth chamber settings alternated between 12 hours at 16°C with lights off and 12 hours at 25°C with lights on. Relative humidity was maintained at 40%. In January–June 2015, seedlings were transplanted into D19 Deepot Cells containing MVP Sungro Professional Grow Mix and moved to the greenhouse. Seedling arrangement was randomized in the greenhouse. Once seedlings had at least two true leaves, they were transplanted into field plots in June–July 2015.

Field Planting Design

The field planting was located in Rosemount, MN at the Rosemount Research and Outreach Center. Within the common garden there were 12 plots, each separated by at least 100 m in order to minimize cross pollination between plots (Figs. 2 & S1). Before planting, plots were sprayed with Roundup at a rate of 3.5 L per hectare and then tilled to kill agricultural weeds. The soil in each plot was tested for phosphorus (P) and potassium (K). Plots were then fertilized with P_2O_5 and K_2O as needed based on soil tests (application rates ranged from 0 to 45 kg/ha). Plots were covered with weed barrier to minimize competition with agricultural weeds at the site. In addition, plots were weeded as needed throughout the growing season and in subsequent years to reduce weed pressure, and fenced to minimize herbivory by deer and rabbits.

Each plot was divided into subplots, each of which contained 100 individuals from one of the three study species: *A. cylindrica*, *D. candida*, or *D. purpurea*. Seed sources for each species were randomly assigned to the 12 plots such that each plot had a random combination of seed sources for the three species, while each subplot comprised individuals of a single species from a single source. Seedlings were transplanted into holes cut in the weed barrier and watered as needed after transplanting. Seedlings were arranged in alternating rows of 12 and 13 plants in hexagonal spacing. Seedlings were 0.3 m apart from each other within each row and also from their two nearest neighbors in both flanking rows. Seedling spacing was consistent across all three study species.

Climate Data

Climate data were gathered from publicly available sources for each of the source sites. Mean July maximum temperatures and mean January minimum temperatures are from the 1981–2010 Normals Map Tool on the Minnesota Department of Natural Resources website (https://www.dnr.state.mn.us/climate/summaries_and_publications/normalsportal.html). Mean annual precipitation was calculated as the mean of annual observed precipitation totals for the years 1995–2014 from the National Weather Service's database (https://water.weather.gov/precip/). The coefficient of variation (CV) for mean annual precipitation was calculated as σ/μ, the standard deviation divided by the mean. Mean annual evapotranspiration was calculated as the mean of annual evapotranspiration for the years 2000–2017. Evapotranspiration data was acquired from the Simplified Surface Energy Balance Actual Evapotranspiration data for the Conterminous United States available at the USGS Geo Data Portal webpage (https://cida.usgs.gov/gdp/). Climate data are displayed in Table 1.

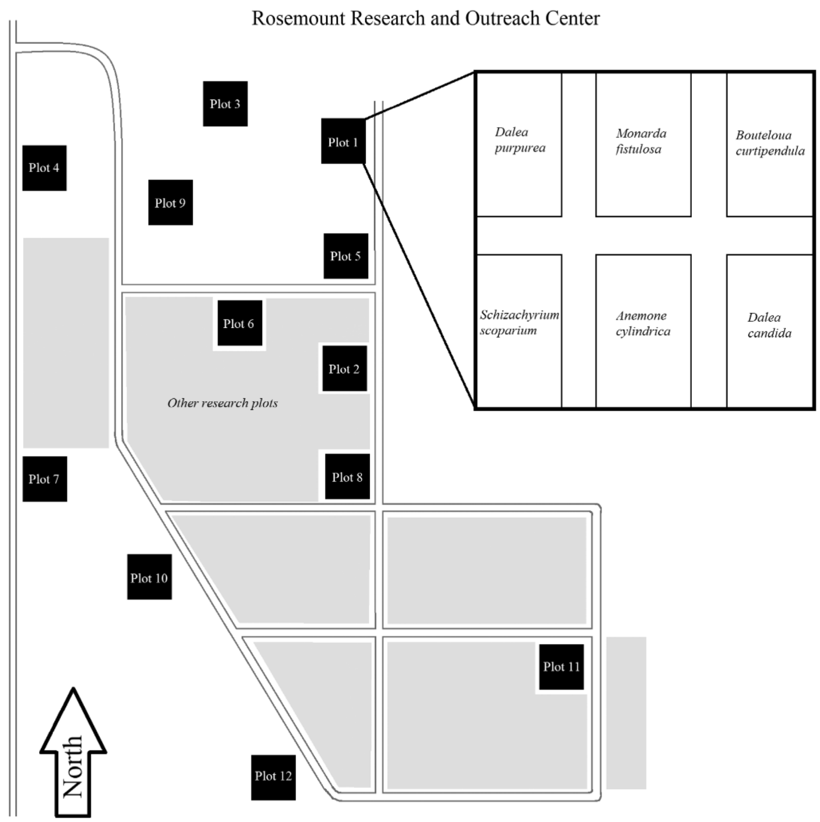

Figure 2. The field planting was located at the Rosemount Research and Outreach Center in Rosemount, MN, U.S.A. The field planting consisted of 12 plots. All plots were separated by at least 100 m to minimize cross pollination between plots. Each plot was divided into six subplots, each of which contained 100 individuals from one species. Seed sources for each species were randomly assigned to the 12 plots such that each plot had a random combination of seed sources for the six species, while each subplot was comprised of individuals of a single species from a single source. *Anemone cylindrica*, *Dalea candida*, and *Dalea purpurea* were the focal species for this project.

Phenology Data

Phenological data were collected in 2017 for *A. cylindrica*, *D. candida* and *D. purpurea*. Plots were visited one to two times a week beginning on 27 April 2017, around the time of emergence for *A. cylindrica*, and ending on 6 September 2017, at the conclusion of flowering for *D. purpurea*. The following data were recorded for all three species: date of emergence in spring and presence or absence of a living plant during each census. In addition, for each plant, the number of flower heads with open flowers was recorded at each census for *A. cylindrica* and *D. candida*. Length of flowering period was calculated as date of last flower – date of first flower +1. For *D. purpurea*, only

Table 1. Location and climate aspects of source locations. Distance given is distance between source site and common garden location in Rosemount, MN, U.S.A. (rsmt*).

Seed Source	*Latitude*	*Distance (km)*	*Mean July High (°C)*	*Mean Jan. Low (°C)*	*Mean Annual Precip. (cm)*	*CV of Mean Annual Precip.*	*Mean Annual Evapotranspiration (mm)*
Pemb	47.68	417	26.39	−20.00	54.61	0.14	423.21
Blue	46.85	356	27.22	−18.33	66.04	0.19	470.90
BF	46.69	330	26.67	−18.89	68.58	0.19	456.79
Staff	45.82	243	27.22	−17.78	40.64	0.26	503.05
Ord	45.45	192	27.22	−16.67	67.95	0.19	536.47
Chip	45.15	237	27.78	−16.67	48.90	0.16	552.84
LV	44.80	24	27.78	−14.44	75.57	0.15	503.74
MSP	44.77	269	28.06	−15.56	77.47	0.69	487.05
Rsmt*	44.70	0	27.78	−15.00	101.60	0.13	555.95
Glynn	44.26	214	27.78	−15.56	66.04	0.27	537.47
KWD	44.26	103	27.78	−14.44	81.28	0.19	488.00
CWR	44.20	171	27.78	−15.56	72.39	0.23	509.16
Oron	44.14	77	27.22	−15.00	82.55	0.14	498.11
PC	44.12	253	27.78	−15.56	77.47	0.22	503.11
Hyth	44.02	74	26.94	−14.44	87.00	0.17	496.05
Lund	43.93	227	26.67	−15.00	67.31	0.18	604.16
Iron	43.87	93	26.67	−14.44	88.90	0.15	487.11

the presence/absence of flowers on each plant was recorded due to time constraints and the very large number of flowerheads on *D. purpurea*.

Fitness Data

Fitness data were collected in 2017 and 2018 for *A. cylindrica* and *D. candida*. Fitness data were not collected for *D. purpurea* in either year due to time limitations. Number of seed heads per plant was used as an approximation of reproductive fitness. Seed head number has been shown to approximate an individual's contribution of seed to the next generation (Clark & Watkins 2010; Mahajan et al. 2020), while avoiding the laborious process of counting the very small seeds on each head individually and determining viability of each seed. Seed heads were counted at the end of the growing season for each species when the majority of plants had finished flowering. This occurred in July for *A. cylindrica* and August for *D. candida*.

Analysis of Phenology Data

Phenology data for *A. cylindrica*, *D. candida* and *D. purpurea* were analyzed using linear models in R (R Core Team 2019). Date of first flower, date of last flower, and length of flowering period were regressed on latitude of seed source in order to assess the relationship between latitude and flowering phenology. Adjusted R^2 values are reported. In addition, least squares means for date of first flower, date of last flower, and length of flowering period were estimated for each population using linear models including population as the sole predictor. Plants that did not emerge in spring, or that did not flower after emergence were excluded from these analyses.

Analysis of Fitness Data

Aster analysis (Shaw et al. 2008) was used to model mean fitness of *A. cylindrica* and *D. candida* populations in the field plantings. Individual fitness comprises multiple components—germination, survival, flowering, and fruiting—each characterized by a particular statistical distribution (e.g. Bernoulli, Poisson, normal). This situation is further complicated in perennial species because these components are expressed over the course of multiple growing seasons and include multiple rounds of seed production. Aster models use appropriate statistical distributions for each component of fitness, with later elements of fitness dependent upon earlier elements, thereby allowing analysis of overall fitness in a single model that encompasses all fitness components. Aster is available as a package in R (Shaw et al. 2008; R Core Team 2019).

A. cylindrica and *D. candida* are perennial species. Their fitness is dependent on survival and seed head production in multiple years, as depicted in the graphical model (Fig. 3). Bernoulli distributions were used to model survival from 2015 to 2017 and from 2017 to 2018. Poisson distributions were used to model number of seed heads per plant in 2017 and 2018 for analyses (1), (2), and (3) (described further below). Negative binomial distributions were used for analysis (4) (described further below) as they were a better fit for these relationships. Fitness of individual plants, comprising survival and seed head production, was modeled using subsets of the predictors: seed source, latitude of source site, geographic distance between source site and common garden site, mean July maximum temperature of source site, mean January minimum temperature of source site, mean annual precipitation of source site, CV of mean annual precipitation at source site, mean annual evapotranspiration of source site, and date of first flower. Statistical significance of factors was tested using R's ANOVA procedure to compare nested models. Separate analyses were conducted for (1) source site, (2) latitude of source site and geographic distance between source site and common garden, (3) mean July maximum

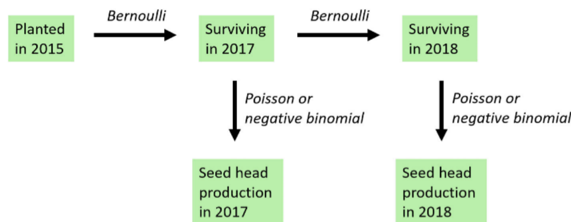

Figure 3. Graphical aster model depicting dependence of later fitness components (seed head production in 2017, survival in 2018, seed head production in 2018) on earlier fitness components (planting in 2015 and survival in 2017), for the study species *Anemone cylindrica* and *Dalea candida*. Bernoulli distributions were used to model survival from 2015 to 2017 and from 2017 to 2018. Poisson distributions were used to model seed head production for aster analyses involving latitude of source, distance between source and common garden site, and source climate conditions. Negative binomial distributions were used to model seed head production for aster analyses involving date of first flower.

temperature, mean January minimum temperature, mean annual precipitation, CV of mean annual precipitation, and mean annual evapotranspiration, and (4) date of first flower.

Assessment of Local Adaptation

In a common garden experiment, the strength of local adaptation of the study species is determined by examining fitness in the common garden along with attributes of the sources, such as geographic distance or environmental characteristics. Here, we focused on geographic distance between seed source and common garden site and five climate measures (described previously in Climate Data section). We detect the presence of local adaptation in a particular species if populations that have higher fitness in the common garden come from sources having similar climatic conditions and/or come from a shorter geographic distance, while populations that have lower fitness in the common garden come from sources having dissimilar climatic conditions and/or are from a more distant source. During the years of this study, our common garden location tended to have climatic conditions that were extreme relative to historic conditions at our source sites: high mean July maximum temperature (Fig. S2), high mean January minimum temperature (Fig. S3), extremely high mean annual precipitation (Fig. S4), low CV of mean annual precipitation (Fig. S5), and high mean annual evapotranspiration (Fig. S6). Therefore, in the comparisons of aster models, evidence of local adaptation for a given species was indicated by the following relationships with fitness: positive relationship with mean July high temperature, positive relationship with mean January low temperature, positive relationship with mean annual precipitation, negative relationship with mean CV of annual precipitation, and positive relationship with mean annual evapotranspiration.

Results

Phenology

The regression analysis showed a significant negative relationship between date of first flower and source latitude, with northern populations tending to start flowering earlier than southern populations in all three species (*A. cylindrica*, $p = 0.0033$, $R^2 = 0.025$, *D. candida*, $p = 0.0099$, $R^2 = 0.015$, *D. purpurea*, $p < 0.0001$, $R^2 = 0.10$) (Fig. 4). (Detailed *p*-values for all instances where $p < 0.0001$ are given in Table S1.) The estimated difference in average date of first flower between the northernmost and southernmost populations is 2 days for *A. cylindrica*, 4 days for *D. candida*, and 7 days for *D. purpurea*. The mean date of last flower was also earlier in plants from northern sources for *D. candida* (11-day difference, $p < 0.0001$, $R^2 = 0.20$) and *D. purpurea* (7-day difference, $p < 0.0001$, $R^2 = 0.076$) but not for *A. cylindrica* ($p = 0.37$) (Fig. S7). Mean length of flowering period was shorter for plants from northern sources for *D. candida* (8-day difference, $p < 0.0001$, $R^2 = 0.078$) but was not significantly different by source latitude for *A. cylindrica* ($p = 0.52$) or *D. purpurea* ($p = 0.62$) (Fig. 5). There was substantial variation around the linear prediction of population means for all flower phenology data (Figs. 4, 5, & Fig. S7). Three populations had sample sizes of less than five for phenology data as few plants survived and/or flowered in these populations (*D. candida* populations from Ord and KWD, *D. purpurea* population from Glynn).

Fitness and Local Adaptation

In aster models with latitude as the sole predictor, plants from northern sources had higher fitness in the common garden than those from southern sources for *A. cylindrica* ($p < 0.0001$) and marginally so for *D. candida* ($p = 0.084$). With distance as the sole predictor, plants from more distant sources had higher fitness for *A. cylindrica* ($p < 0.0001$) while there was no relationship between distance and fitness for *D. candida* ($p = 0.47$). Aster models containing both latitude and distance had a better fit than either the distance-only model ($p < 0.0001$, $p < 0.0001$) or the latitude-only model ($p < 0.0001$, $p = 0.00033$) for *A. cylindrica* and *D. candida*, respectively. These models showed that fitness depended positively on latitude of origin and negatively on distance from source site for both species. For a given latitude of

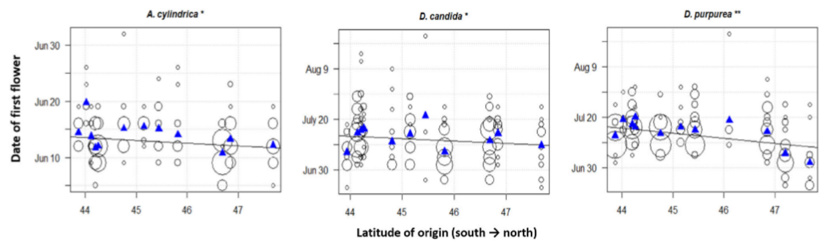

Figure 4. Date of first flower of *Anemone cylindrica* (n = 645), *Dalea candida* (n = 569), and *D. purpurea* (n = 453) overlaid with regression of first date of flowering by latitude (*$p < 0.01$, **$p < 0.0001$). Blue triangles indicate predicted population means based on linear models using source as the sole predictor.

origin, closer plants tended to have higher fitness, as can be seen with the *D. candida* populations from sources around 44° of latitude in Figure 6. Similarly, for a given distance from the source, those from higher latitudes expressed higher mean fitness, such as the *A. cylindrica* populations from sources in the 200–300 km range in Figure 6. For *A. cylindrica*, the direction of the effect for distance differs depending on whether latitude is included in the model. In the joint model, fitness has a negative relationship with distance, while in the model with distance as the sole predictor, fitness has a positive relationship with distance. The change in the effect of distance is likely due to the strong impact of latitude of origin on fitness and the fact that the more distant sources in this study also tended to be from more northerly locations.

Aster analysis of the relationship between fitness and climatic factors yielded contrasting results for *A. cylindrica* and *D. candida*. For *A. cylindrica*, seeds from sources having a higher mean July maximum temperature, lower mean January minimum temperature, higher mean annual precipitation, and higher mean annual precipitation CV tended to have higher fitness at the common garden site ($p < 0.0001$) (Table S2). (Mean annual evapotranspiration did not improve model fit for *A. cylindrica*, $p = 0.077$.) For *D. candida*, on the other hand, seeds from sources having a lower mean July maximum temperature, higher mean January minimum temperature, lower mean annual precipitation, higher mean annual precipitation CV, and higher mean annual evapotranspiration tended to have higher fitness at the common garden site ($p < 0.0001$) (Table S2). In comparison to source sites, environmental conditions at the common garden site during the study period were characterized by a high mean July maximum temperature, high mean January minimum temperature, very high mean annual precipitation, low mean annual precipitation CV, and high mean annual evapotranspiration. For *A. cylindrica*, seeds from sources having climatic conditions more similar to the common garden site tended to have higher fitness (with the exception of January minimum temperatures), evidence of local adaptation of this species to climatic factors within the geographic area covered by this study. For *D. candida*, seeds from sources with contrasting climatic conditions to the common garden site tended to have higher fitness (again, with the exception of January minimum temperatures), evidence not consistent with local adaptation to climatic factors for this species. Predicted mean population fitness values for climate and latitude/distance analyses are displayed in Table 2.

Aster analysis of fitness in relation to date of first flower indicates that fitness decreases as date of first flower gets later in

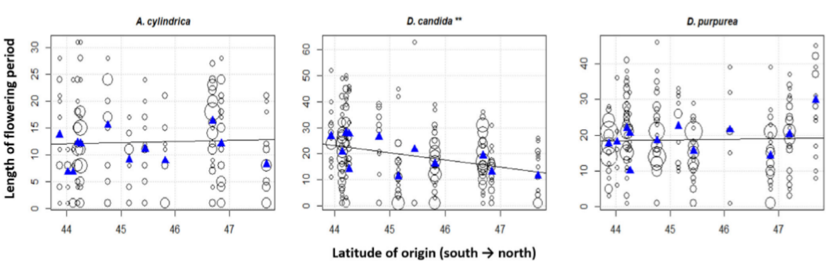

Figure 5. Length of flowering period of *Anemone cylindrica* (n = 645), *Dalea candida* (n = 569), and *D. purpurea* (n = 453) overlaid with regression of length of flowering period by latitude (**$p < 0.0001$). Blue triangles indicate predicted population means based on linear models using source as the sole predictor.

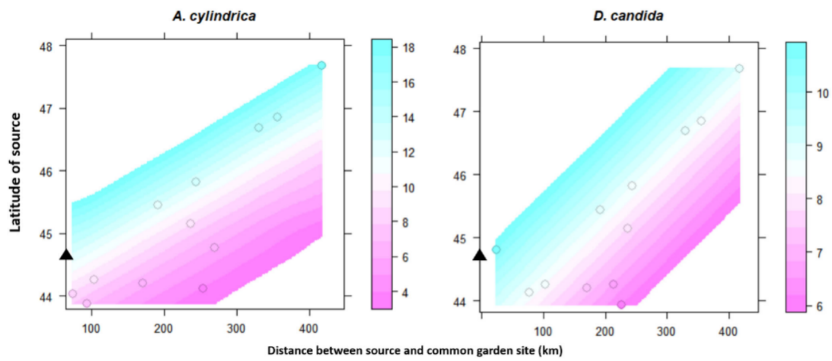

Figure 6. Predicted mean fitness of *Anemone cylindrica* ($n = 645$) and *Dalea candida* ($n = 569$) populations based on latitude of their source sites and distance between common garden and seed source. The colored bar to right of each figure is the fitness scale for each species, with higher numbers indicating higher fitness levels. Black triangles indicate latitude of common garden site.

both *A. cylindrica* and *D. candida* (Fig. 7). A quadratic model provided a better fit than the linear model for both species (*A. cylindrica*: $p = 0.00024$, *D. candida*: $p < 0.0001$).

Discussion

Seed sourcing strategies have received considerable attention in the restoration literature and are a key component of effective management for restoration and conservation of natural areas. Research and discussion tend to focus on optimal distances and environmental similarities between seed sources and planting sites (e.g. McKay et al. 2005; Broadhurst et al. 2008; Herman et al. 2014). However, given the increasing discussion of assisted gene flow and assisted migration (Whitely et al. 2015; Vitt et al. 2016; Bell et al. 2019), greater consideration of translocating populations in specific directions across climatic

Table 2. Mean population fitness is given for each source based on aster models. Sources are listed by latitude, with the most northern latitude at the top of the table. Empty cells indicate locations where seed was not collected for a given species. Columns 2 and 5 use source as the sole predictor. Columns 3 and 6 use latitude of source and distance between source and common garden site as the predictors. Column 4 uses the following environmental factors of source sites as the predictors: mean July maximum temperature, mean January minimum temperature, mean annual precipitation, and CV of mean annual precipitation. Column 7 uses mean annual evapotranspiration, in addition to the previous four environmental factors, as the predictors.

	Mean Population Fitness					
	Source	*Lat. + Dist.*	*Environment*	*Source*	*Lat. + Dist.*	*Environment*
Seed Source	A. cylindrica	A. cylindrica	A. cylindrica	D. candida	D. candida	D. candida
Pemb	2.90	17.49	12.35	2.71	8.98	12.69
Blue	18.06	14.70	22.98	4.70	8.60	5.78
BF	41.76	14.79	22.90	18.18	8.75	8.35
Staff	2.18	12.83	0.32	22.46	8.72	16.74
Ord	5.76	12.63	6.95	0.51	8.93	5.17
Chip	3.62	8.89	1.02	7.76	7.78	4.74
LV				7.44	10.62	7.05
MSP	7.96	5.91	8.33			
Glynn				5.37	6.81	7.97
KWD	28.59	8.90	10.90	0.01	8.43	8.16
CWR	5.36	6.28	9.57	5.91	7.33	7.03
Oron				19.21	8.63	7.23
PC	3.04	3.95	18.24			
Hyth	1.11	8.64	4.84			
Lund				5.52	6.19	8.84
Iron	1.78	7.12	3.95			

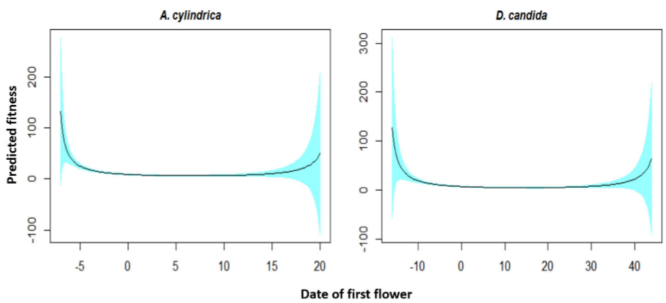

Figure 7. Predicted fitness of *Anemone cylindrica* ($n = 645$) and *Dalea candida* ($n = 569$) based on date of first flower. Initial flowering dates in figure are adjusted such that a zero date of first flower is the median date of flowering for that species. Light blue area indicates ± 95% CI.

gradients is warranted. To the extent that local adaptation proceeds primarily in response to climatic conditions, assisted gene flow across climatic gradients is likely to promote species persistence in the face of climate change. A growing body of literature has addressed this issue, particularly in tree species, using genecological models to inform translocation along climatic gradients (e.g. Hamann et al. 2011; Kilkenny 2015; Mahoney et al. 2020). However, if species are adapted to other abiotic and biotic factors, translocating populations across climatic gradients may have unintended and potentially maladaptive consequences (Bucharova 2017; Wadgymar & Weis 2017). Here, we used extensive collections of seed materials from across the state of Minnesota, a field planting that established common conditions, and subsequent aster modeling of fitness data to examine the fitness consequences of translocating populations across the landscape. We found that, in the common garden near the southern limit of the source locations, populations from cooler, northern source sites tended to have higher fitness than those from warmer, more southern locations. In addition, populations from more northern locations tended to have earlier flowering phenology relative to populations from more southern sources, perhaps conferring a fitness advantage. Evidence of local adaptation to climate was less clear, with *A. cylindrica* showing signs of local adaptation to three out of five climate factors, while *D. candida* showed signs of local adaptation to only one of five factors. Taken together, our results suggest that latitude of origin may be an important factor to take into consideration during seed source selection for restoration work.

Local adaptation is commonly found in plants, although it is not universal. In Hereford's (2009) quantitative survey, local adaptation was found in 65% of 892 estimates, while Leimu and Fischer (2008) found local adaption in 45% of 1,032 pairwise comparisons in their meta-analysis. Although it is useful to have an understanding of the general prevalence of local adaptation, in restoration ecology it is also helpful to understand the spatial extent to which specific species manifest local adaptation. In our study, evidence of local adaptation within Minnesota for *A. cylindrica* and *D. candida* was mixed. Latitude of origin had a positive effect for both species, with populations from more northern locations (which were also generally more distant) tending to outperform populations from more southern locations. Once latitude was accounted for, populations from more local sources tended to have higher fitness than those from more distant sources, again for both species. Our results provide evidence of local adaptation for both *A. cylindrica* and *D. candida* within the 75,000 km^2 area (approximately 420 km north to south and 220 km east to west) of our study. For these species there is likely to be a decrease in fitness when seed is translocated over greater distances, a finding which supports the use of more local seed during restoration work. These results align with the current seed sourcing guidelines of both the Minnesota Department of Natural Resources (Schulte & Westbrook 2013) and the Minnesota Board of Water and Soil Resources (Shaw 2019). Both organizations use a tiered approach to seed sourcing where collections are preferentially made in local areas and only from farther away when local sources are unavailable, with a maximum recommended seed transfer distance of 175 miles (282 km). Our work also demonstrates the importance of latitude of origin, which will be discussed further below.

Local adaptation to climatic factors differed between the two species. We detected evidence of local adaptation in *A. cylindrica* to July temperature highs and annual precipitation, but not to January low temperatures or annual evapotranspiration. *D. candida*, in contrast, showed evidence of local adaptation only to January low temperatures. Our results highlight the difficulty of trying to generalize about effects of environmental distance between sites. In addition, our results for *D. candida* indicate that environmental similarity as measured between source and planting site may not always aid in predicting transplant success, contrary to common assumptions. The differing responses of these two species underscore the difficulty and importance of developing species-specific restoration guidelines.

Several important environmental factors vary with changing latitudes, including photoperiod, temperature, growing season length, and biodiversity (De Frenne et al. 2013). As a result, translocating populations across latitudes may result in fitness differences based on latitude of origin. However, the direction of potential fitness differences is variable. In a study of two *Quercus* species in Minnesota, Etterson et al. (2020) found that seedlings from further south outperformed seedlings from more northern locations when planted in northern common gardens. Similarly, McGraw et al. (2015) found that the optimum temperature for populations of long-lived *Eriophorum vaginatum* in Alaska was at locations 140 km north of their home sites. In contrast, Torang et al. (2015) and Colautti and Barrett (2013) found strong evidence of local adaptation in *Arabis alpina* and *Lythrum salicaria*, respectively, as regional populations outperformed nonlocal populations in common gardens in both studies. Our findings, that northern populations tended to outperform southern populations, present yet a third scenario (although it is important to note that our study included only a limited range of sources from south of our common garden location). Our findings are similar to those of Wadgymar and Weis (2017) who found that northern populations of *Chamaecrista fasciculata* outperformed southern populations when grown in a common garden. A common assumption in the literature on assisted gene flow is that when southern populations are translocated northwards, they will outperform resident populations because they are already adapted to the warming temperatures that climate change is imposing on northern locations (Parmesan & Hanley 2015). The variability among the studies mentioned here indicates that poleward assisted gene flow may not always successfully address population declines due to climate change and may in some cases be maladaptive.

Latitude of origin can impact flowering phenology of translocated populations, either due to changes in photoperiod or changes in accumulation of growing degree days (Griffith & Watson 2006; Wadgymar et al. 2015). Flowering phenology, in turn, can impact fitness via temporal mismatch with key pollinators and other mutualists (Rafferty & Ives 2012), reproductive isolation from resident populations (Weis 2015; Wadgymar & Weis 2017), and inability to complete fruit production before the onset of cold weather in the fall (Griffith & Watson 2006). For *A. cylindrica*, *D. candida*, and *D. purpurea*, we found that populations from northern sources tended to flower earlier than populations from southern sources when grown in our field planting. This may be due to changes in the accumulation of growing degree days, where northern sources have a lower threshold of growing degree day accumulation required to initiate flowering relative to southern sources. Earlier flowering was associated with increased fitness for *A. cylindrica* and *D. candida*, the two species for which we collected fitness data. Some research has indicated that earlier flowering may be associated with increased fitness due to pollinator preferences (Elzinga et al. 2007). Alternatively, it may be that earlier flowering aligns the plant's life history with the earlier start to the growing season that is resulting from increased temperatures due to climate change. Parmesan and Yohe (2003) conducted a meta-analysis of recent phenological change, looking at 172 species including plants, birds, butterflies, and amphibians. They found a mean shift toward earlier spring timing of 2.3 days per decade, matching climate change predictions. Perhaps the northern populations in our study are better suited to the earlier spring occurring to their south, resulting in increased fitness relative to more southern populations which tend to flower later. These results suggest that when considering the impacts of climate change, it may be informative to look beyond increases in temperature per se and consider additional factors such as changes to the start and end of the growing season.

In addition to the fitness decrease and flowering delay associated with populations from more southern sources in this project, latitude of origin also determined the degree of flowering overlap between populations from different latitudes, particularly for *D. candida* and *D. purpurea*. The difference between the predicted initiation of flowering of the northernmost and southernmost populations was four and seven days, while predicted completion of flowering differed by eleven and seven days for *D. candida* and *D. purpurea*, respectively. This limits the degree of flowering overlap between the two populations, thereby limiting the potential for admixture. Although in this experiment there was still substantial overlap between populations, sourcing populations over greater distances could result in the complete separation of flowering. For example, Wadgymar et al. (2015) grew *C. fasciculata* from Minnesota, Missouri, North Carolina and Pennsylvania in a common garden in Ontario. They found that the North Carolina population was almost completely reproductively isolated from the Minnesota and Pennsylvania populations in the common garden due to its later flowering schedule. In similar work, Weis (2015) collected *Brassica rapa* from three sites along a flowering phenology cline in California and grew them in a common garden. Weis (2015) found that differences in flowering time limited admixture between the populations by up to half in the common garden. Such differences in flowering schedule could disrupt plans for assisted gene flow if immigrant populations do not hybridize with resident populations (Way & Montgomery 2015). Our research, along with that of Wadgymar et al. (2015) and Weis (2015) suggest that impacts of flowering phenology on the admixture of translocated populations should be taken into consideration in plans for assisted gene flow.

Assisted gene flow and assisted migration are two seed sourcing strategies that have been suggested to mitigate the adverse impacts of climate change that are affecting many native plant and animal populations. In this study, we used three native prairie plant species and a common garden approach to demonstrate that latitude of origin can impact the success of populations that are translocated across latitudes. In particular, we found that populations from northern sources tended to have earlier flowering schedules and higher fitness than those from southern sources. Our findings suggest that despite the current focus on northward assisted gene flow and migration, there may be situations where translocation in different directions would be beneficial. Climate change causes many environmental factors to differ from historic means, but these factors do not consistently covary across latitudes. Future studies that investigate the relative importance of these different factors to populations and species would provide valuable insight for assisted gene flow and assisted migration practices. In addition, one limitation of this study was the use of a single common garden. Further studies that incorporate multiple common gardens at different latitudes could provide a more nuanced understanding of the impacts of movement of populations across latitudes.

Acknowledgments

We thank the Minnesota Department of Natural Resources and The Nature Conservancy for permit issuance; Healthy Prairies volunteers and staff for their invaluable assistance with seed collection; and the staff of the Rosemount Research and Outreach Center for their assistance with the common garden. We thank C. Geyer for his extremely helpful assistance with aster analysis. This research was supported by the Environment and Natural Resources Trust Fund (ENRTF) of Minnesota as M.L. 2017 CH. 96 SEC. 2 SUB.03C, a University of Minnesota Ecology, Evolution and Behavior Research Award, and a University of Minnesota Ecology, Evolution and Behavior Summer Fellowship.

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Supporting Information

The following information may be found in the online version of this article:

Table S1. Detailed values for all *p*-values of less than 0.0001.
Table S2. Results of climate analysis using ANOVA to compare nested aster models.
Figure S1. One of the twelve plots in the field planting.
Figure S2. Mean July maximum temperature (°C) of seed sources, graphed by longitude and latitude of source location.
Figure S3. Mean January minimum temperature (°C) of seed sources, graphed by longitude and latitude of source location.
Figure S4. Mean annual precipitation (cm) of seed sources graphed by longitude and latitude of source location.
Figure S5. Mean annual precipitation CV of seed sources graphed by longitude and latitude of source location.
Figure S6. Mean annual evapotranspiration (mm) of seed sources graphed by longitude and latitude of source location.
Figure S7. Date of last flower of *A. cylindrica* ($n = 645$), *D. candida* ($n = 569$), and *D. purpurea* ($n = 453$) overlaid with regression of last date of flowering by latitude.

Coordinating Editor: Siegy Krauss

Received: 10 December, 2020; First decision: 8 March, 2021; Revised: 6 April, 2021; Accepted: 21 May, 2021