M.L. 2016, Chp. 186, Sec. 2, Subd. 03a Project Abstract For the Period Ending June 30, 2020

PROJECT TITLE: Data-Driven Pollinator Conservation Strategies
PROJECT MANAGER: Daniel Cariveau
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2016, Chp. 186, Sec. 2, Subd. 03a

APPROPRIATION AMOUNT: \$520,000 AMOUNT SPENT: \$519,996 AMOUNT REMAINING: \$4

## Sound bite of Project Outcomes and Results

Our results demonstrate that emphasizing local flowering communities is critical for conserving native bee communities. We also demonstrate that restorations in areas of high agriculture can be effective for conserving native bee communities. We collected a high quality, open-access dataset of bees and plants in prairie restorations and remnant prairies.

#### **Overall Project Outcome and Results**

Tallgrass prairie in Minnesota has declined by over 98%. A critical tool for prairie conservation is ecological restoration. One goal of prairie restoration is to conserve, protect and restore native bees and the pollination services they provide. In Minnesota, there are over 450 species of bees with the prairies having the highest diversity and abundance. However, there is a lack of information available to enable land managers to effectively create and maintain restorations for bees. This project addresses three objectives. First, we test how surrounding landscape influences native bee communities in prairie restorations. This information can help land managers determine where to place restorations and which restorations to prioritize for management. Second, we examined how plant communities influence bee communities. Third, we studied pollination in restorations and evaluated which bee species were the most effective pollinators. We sampled bees at 26 sites in the summers of 2017 - 2019. We collected over 18,000 bee specimens and recorded over 11,000 bee by plant interactions. We found that surrounding landscape had less of an impact on bee communities than local flower communities. This suggests that increasing plant diversity is a critical tool for enhancing bee communities while exact placement is less important. We also found that restorations and remnants have unique bee and flower communities. This suggests that we might not be meeting restoration goals as bee communities in quite different than remnant prairies. Finally, we found large variation in the pollination effectiveness among bee groups. This research benefits Minnesotans highlights the importance of maintaining diverse plant communities in restorations. As surrounding landscape did not influence bee communities or pollination, restorations in areas of high agriculture can be effective. We have created a highly resolved open-access dataset for future studies. We have published five peer-reviewed manuscripts to date and are attached as supplementary material.

#### **Project Results Use and Dissemination**

We have disseminated our results to the scientific community as well as the general public. We gave 6 presentations at national conferences. Two of these were invited presentations. Dan Cariveau also presented these results at three departmental seminars at other universities. We have published a total of 5 peer-

reviewed manuscripts. We expect at least three more. Ian Lane will be submitting the second chapter of his dissertation in early 2021 and third chapter before May 2021. At which point he will defend his PhD thesis. Dr. Gabriella Pardee is finishing up a manuscript on pollen efficiency of prairie plants. In addition to publishing in journals, we have also made the data and code available and open access. All published work and presentations have and will continue to acknowledge this ENRTF support. We have also presented the results of this research at outreach events. Ian Lane has also been a guest on the Minnesota Department of Natural Resources Prairie Pod podcast. This podcast is focused on the science of prairie conservation. We led a highly successful field day on pollinators in prairies. This was co-led by Ian Lane and Dan Cariveau. In addition, we partnered with the United States Fish and Wildlife Service, Minnesota Department of Natural Resources and the Prairie Reconstruction Initiative. This brought in 50 natural resource professionals and was focused on prairie sites in southwestern Minnesota. Finally, this funding has supported two graduate students. Alan Ritchie defended his master's thesis in December 2019. He is now the Pollinator Coordinator for the Minnesota Department of Natural Resources. Ian Lane is finishing his PhD thesis. He has scheduled a defense date for May 2021.

Our publication list as of December 2020 is below. The papers are also attached as supplementary material in this report. Bolded names represent co-authors whose salary or graduate funding has come from this grant. All manuscripts with data and analyses have open-access data and code. These data can be found on the University of Minnesota's Digital Repository of the University of Minnesota

(<u>https://conservancy.umn.edu/handle/11299/166578</u>). There have been 99 downloads of these data as of December 22, 2020.

- **Cariveau, D.P.,** Bruninga-Socolar, B. & **Pardee, G.L**. (2020). A review of the challenges and opportunities for restoring animal-mediated pollination of native plants. *Emerg. Top. Life Sci.*, 4, 99–109.
- Lane, I.G., Herron-Sweet, C.R., Portman, Z.M. & Cariveau, D.P. (2020). Floral resource diversity drives bee community diversity in prairie restorations along an agricultural landscape gradient. J. Appl. Ecol., 57, 2010-2018.
- **Portman, Z.M., Lane, I.G., Pardee, G.L.** & **Cariveau**, D.P. (2020). Reinstatement of *Andrena vernalis* Mitchell (Hymenoptera: Andrenidae) from synonymy with *A. ziziae* Robertson. *The Great Lakes Entomologist*, 53, 25–40.
- Ritchie, A.D., Lane, I.G. & Cariveau, D.P. (2020). Pollination of a bee-dependent forb in restored prairie: No evidence of pollen limitation in landscapes dominated by row crop agriculture. *Restor. Ecol.*, 28, 919–926.
- **Portman,** Z.M., Burrows, S.J., Griswold, T., Arduser, M., Irber, A.J., Tonietto, R.K.,...**Cariveau, D.P**. (2019). First Records of the Adventive Pseudoanthidium nanum (Mocsáry) (Hymenoptera: Megachilidae) in Illinois and Minnesota, with Notes on its Identification and Taxonomy. *Great Lakes Entomol.*, 52, 6



Date of Report: December 22, 2020

**Final Report** 

Date of Work Plan Approval: June 7, 2016

Project Completion Date: June 30, 2020

Does this submission include an amendment request? Yes

## **PROJECT TITLE: Data-Driven Pollinator Conservation Strategies**

Project Manager: Daniel P Cariveau

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Location: Western Minnesota (Tallgrass Prairie Region)

Total ENRTF Project Budget: 520,000	ENRTF Appropriation:	\$520,000
	Amount Spent:	\$599,996
	Balance:	\$4

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 03a

## Appropriation Language:

\$520,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to improve understanding of the relationships and interactions between native bee pollinators and rare and declining plant species and to determine optimal placement and species plantings for pollinator habitat in order to develop guidelines for planning, designing, and planting pollinator habitat. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

Carryforward; Extension (a) The availability of the appropriations for the following projects is extended to June 30, 2020: (5) Laws 2016, chapter 186, section 2, subdivision 3, paragraph (a), Data-Driven Pollinator Conservation Strategies;

## I. PROJECT TITLE: Data driven pollinator conservation strategies

## **II. PROJECT STATEMENT:**

1. Why? With billions of dollars to be spent on Minnesota prairie restoration in coming years, there is an urgent need for more information on how to best implement costly restoration actions to achieve a variety of goals. One goal is to enhance native bee communities. In fact, the decline of some native bees species has motivated government agencies, non-profit organizations, and private landowners to create thousands of acres of pollinator habitat throughout Minnesota. However, it is expensive to install and little information exists on how maximize investments to best support native bees. The primary objective of this project is to provide foundational data and information to guide land managers in Minnesota so they can effectively plan and design habitat for native bees. A second objective is to determine the pollination effectiveness of native bees within habitat restorations. A goal of restorations is to create functioning, self-sustaining ecosystems and this relies on seed production of native plants. Many plants need bees for seed production however bee species vary in pollination effectiveness. Understanding this variation is important, as it will enable land managers to optimize habitat for bees that are the most effective pollinators and maximize overall success of habitat restorations.

2. Goals and outcomes. Currently, there are no rigorous guidelines to help land managers best implement pollinator habitat. Given this lack of information, the project we propose has four main goals. The first goal is to determine the best placement of pollinator habitat to maximize value for native bees. Correct location of pollinator habitat is critical. If a habitat is too far from a source population, native bees will not be able to colonize the new habitat. However, if bees are able to colonize new habitats far from remnant prairies, this provides land managers more opportunities to create habitat for bees. The outcomes of this goal will be guidelines on how distance from remnant prairie sites affects success of pollinator habitat. The second goal is to determine which plant species are most preferred by native bees. Most information on plants for bees are based on best guesses by land managers. However, a more quantitative approach is needed. For example, different plants may benefit different bee species. One outcome of this goal is to determine whether certain plants benefit rare or declining bee species. In addition, spring is a critical time for a number of bee species. Some early spring species are considered rare and others such as bumble bees need spring plants when they are founding new nests. Seeds of early spring plants are expensive and difficult to establish and pollinator habitat is thus usually lacking in these plants. Therefore, a third outcome is to create a targeted list of plant species that are most effective in the early spring and this will help land managers focus on certain early plants that critical to bees foraging in this season. The *third goal* is to determine which bee species provide the greatest benefit to plants within plantings. The outcome of this goal will be a list of native bees species that quantifies pollination effectiveness for two to three native prairie plant species. The *fourth goal* is to disseminate findings to land managers. The outcomes of this goal will be talks, a workshop and printed outreach documents.

3. How? In collaboration with TNC, DNR and BWSR, we will select sites near the Minnesota Prairie Conservation Plan. To determine how placement affects success of pollinator habitat, we will collect bees within newly created forb-rich habitats at varying distances from remnant prairies. We will collect bees at remnant prairie sites and at newly created forb-rich habitat using bee bowls. To determine flower use, we will also collect bees from flowers using hand nets and record the flower species each bee was visiting. A botanist will conduct flower surveys and identify plants. To determine pollination effectiveness of bee species, we will measure pollen deposition of bee species to a sample of several prairie plants, focusing on 2-3 plant species.

4. Project significance – This project will collect vital data that will be used to effectively implement restoration goals that benefit native bees. It will lead to plant lists that are targeted to important groups of bees such as those that forage in the spring as well as rare and declining species. We will work closely with land managers and landowners throughout this project to disseminate our results and we will encourage feedback for improving this project. Finally, the results of this work will be shared with other prairie ecologists to improve management and conservation of the tallgrass prairies of Minnesota.

## **III. OVERALL PROJECT STATUS UPDATES:**

## Project Status as of January 1, 2017:

In the summer of 2016, Ian Lane (PhD student) and Alan Ritchie (MSc student) began their graduate work focused on the three activities in this proposal. Ian Lane is focused on Activities 1 and 2 and Alan is focused on Activity 3. One main goal of this summer was to select sites (Activity 1). Ian Lane visited approximately 70 sites, assessed vegetation at sites and obtained permits for collection. In addition to visiting sites, he has conducted land cover analyses of these sites. He is using these analyses to finalize site selection. A second goal was to collect bees at a subset of sites (Activity 2). We collected 235 specimens of approximately 40 bee species on 31 different plant species. We also created a pollen library for future pollen identification research. Using observational data from site visits and site-level data of seed mixes, Alan has narrowed plant choices for Activity 3 to three species (*Chamaecrista fasciculata, Monarda fistulosa,* and/or *Dalea purpurea*) and is currently growing these species in the greenhouse for further evaluation. Finally, we have presented an overview of our study and findings at two outreach events.

## Project Status as of July 1, 2017:

Since the last report, we have focused on starting the first field season. Sites have been selected for all three activities. In total, we have 16 restoration sites that are managed as MN Board and Water Conservation Easements. We have also selected 6 remnant prairies for sampling. We hired 6 field technicians and began research in western Minnesota in May 2017. We sampled bee communities using hand nets, bowl traps and blue vane traps. In the first round of sampling, we have collected nearly 4,000 native bee specimens. The most common plants being visited were, in descending order: *Zizia aurea, Erigeron philadelphicus, Achillea millefolium, Gallardia aristata,* and *Barbarea vulgaris*. We conducted a total of 1,558 vegetation quadrats and recorded 59 different plant species in bloom. Finally, we conducted trial pollination experiments and will begin full experiments in July 2017.

## Project Status as of January 1, 2018:

We have completed the first field season. We sampled a total of 21 sites. Sixteen of these sites are RIM restoration sites managed by MN BWSR. Five of the remaining sites were remnant prairies. Sampling involved hand netting, bowl trapping and vane traps. We collected a total of 11,600 native bee specimens. Approximately 40% of the specimens have been identified to species and all identifications will be completed in the next few months. We have identified nearly 5,000 specimens for a total of approximately 20 species. So far, we have worked through the easiest to identify and least-species rich groups, and we expect over 100 species. In addition, we sampled plant communities at each site during five different sampling rounds. These data have all be entered and are awaiting data checking. We have recorded at least 181 blooming plant species across our sites. Alan Ritchie (MSc student) completed work on pollen limitation in restoration sites. He used the native *Chamaecrista fasciculata* as a model plant. He found that seed production in this plant was not limited by pollen. Next year, we will complete new studies on more plant species. These project results were disseminated in a number of instances. Dan Cariveau promoted this research at the Arboretum's Pollinator Symposium as well as at invited talks at the University of Illinois Urbana-Champaign, University of North Dakota, and the Conservation Sciences Seminar at the University of Minnesota. Ian Lane has given a talk at the Little Lunch on the Prairie Webinar Series.

## Amendment Request May 30, 2018

We request a reallocation of funds to increase funds for travel and supplies. In total, this will be \$30,000 more for travel and \$500 for supplies. We have sampled sites more frequently than we had predicted when writing the grant. The long distances among sites was also greater than initially predicted. In addition, Activity 3 required more supplies then predicted as we needed to construct robust deer fencing. To cover the increase in

travel and supplies, we request to use the funds for the postdoctoral research associate across the three activities. We would also like to move funds allocated to contract work for bee identification to salary and fringe. We have hired a taxonomist and we are therefore able to do this work in the lab. None of these reallocations will affect our ability to complete the objectives. We hired a highly competent field crew leader that was instrumental in getting this project started. A detailed description for reallocation requests for each activity follows below.

For Activity 1, we request that \$8,000 for contract work for bee identification be reallocated to salary. We also request that \$10,000 be reallocated from salary to travel. To cover this, we will reduce the amount of salary for the postdoctoral research associate. We request the same reallocation for Activity 2: \$8,000 for contract work for bee identification be reallocated to salary and \$10,000 be reallocated from salary to travel. We will reduce the amount of salary for the postdoctoral research associate. For Activity 3, we request that \$10,000 be reallocated from salary to travel. We will reduce the amount of salary to travel and \$500 be reallocated from salary to supplies.

We note that none of the objectives have changed in this grant.

## Project Status as of July 1, 2018:

We are starting our second field season. We have hired a field crew and have completed one round of sampling. We conducted an earlier round of sampling to assess spring bee communities. This summer, we are focusing on comparing restoration and remnant prairies. In addition, we are focusing on conducting more robust sampling techniques to better capture heterogeneity of flowering plant communities in remnant prairies. We are also conducting plant visitation studies to assess pollination of prairie plants. All bees have been identified and databased from the 2017 field season. We collected 11,666 specimens from over 159 different bee species. This comprises between 30-40% of the known bee diversity in Minnesota. We collected 7,182 bee specimens from 113 different plant species and recorded 186 blooming plants. From some of these species we will be analyzing pollen loads using metabarcoding to determine plant use by generalist and specialist bees. Finally, we are beginning new pollination studies this coming summer. We continue to disseminate the results of this research. Our group has given presentations to land managers, the general public and other scientists.

## Amendment Request November 7, 2018

We are requesting an extension as we need a full summer to meet the objectives of Activity 3: "Determine most effective native bee species for pollinating prairie plants". We have had two very productive field seasons to complete Activities 1 and 2. We have also begun Activity 3 with a focus on *Dalea purperea* - a native plant with a number of generalist and specialist pollinators. We are currently counting pollen grains and seeds. However, as getting pollinator-focused visitation rates, and will need more time. In particular, this plant species blooms in July and August. This will be our focus in summer 2019. Furthermore, by having another year, we will be better able to complete our dissemination objectives as all data will be analyzed and presented to land managers and other stakeholders. Amendment Request signed into law 5/31/19

## Project Status as of March 27, 2019:

We completed our second field season. For Activities 1 and 2 we collected nearly 11,000 native bee specimens from nearly 100 species of plants. We collected another 7,000 native bee specimens from passive trapping methods (bowl traps and blue vane traps). We have collected at least three species of note in 2018: *Bombus ternarius* - a declining bumble bee; *Eucera albata* - a prairie specialist and *Lasioglossum cattelae* – a new Minnesota state record. In the two years of this study, we will have over 18,000 specimens of bees and at least 180 species. This represents one of the largest and high-quality datasets on native bees in prairie ecosystems. We found that surrounding landscape did not affect the number of bee species in a restoration while plant richness was positively associated with bee species number In addition, we have continued to conduct a study on pollen load analysis to better quantify bee use of native plants. Finally, we conducted a field season to examine the effect of various bee species on pollination and reproductive success of the native plant *Dalea* 

*purpurea* (Fabaceae). We have finished data process of bee flower visitors, counting pollen on stigmas, and counting the number of seeds per fruit. We have continued to disseminate the results of our research in workshops and talks. We have reached over 200 people since the last report. In the summer of 2018 we are planning a land-manager workshop on the prairie restoration and native bees.

## Project Status Update as of July 1, 2019:

We have made much progress since the last update. For activities 1 and 2, all data are cleaned, checked and uploaded onto a MySQL database. For Activity 1, we collected over 18,000 bee specimens and over 193 species. For Activity 2, we recorded over 10,000 bee by flower interactions. This included over 163 bee species and 136 flower species. Using data from these two activities, we are writing two manuscripts and will have one submitted by the end of 2019. For activity 3, we have finished processing data from a field study on how fire and co-blooming plants influenced pollination of purple prairie clover (*Dalea purpurea*). We have also completed field work on single-visit pollen deposition of purple prairie clover (*Dalea purpurea*) and golden alexander (*Zizia aurea*). We are now processing these data in the lab. Alan Ritchie (MSc) student has submitted the results of his research on pollen limitation in partridge pea (*Chamaecrista fasciculata*) to the peer-reviewed journal *Restoration Ecology*. Alan Ritchie, Ian Lane, and Dr. Gabriella Pardee each presented research generated from this funding at the national Ecological Society of America meeting in Louisville, KY. The conference is attended by a few thousand ecologists. We also co-organized a field workshop in Southwestern Minnesota on habitat restoration for native bees. We hosted this field day in conjunction with the Minnesota Department of Natural Resources (Megan Benage and Gina Quiram) and the United States Fish and Wildlife Service (Paul Charland). Approximately 50 natural resource professionals attended this workshop.

## Project Status Update as of January 31, 2020:

We continue to make good progress on this project. All data for all activities are cleaned and entered. For Activity 1, Ian Lane has presented the results of his research at scientific conferences. He has also submitted the first chapter of his dissertation to a peer-reviewed journal and it is currently in review. Ian is currently analyzing these results from Activity 2. He will be submitting these findings to a peer-reviewed journal before the end of this grant. Finally, most of the progress has taken place on Activity 3. Alan Ritchie completed is master's thesis and graduated in January 2019. One of his chapters was accepted for publication in the journal *Restoration Ecology*. Dr. Gabriella Pardee has finished processing data from the 2019 summer field season. She is currently analyzing these data. She found that bee species group differ dramatically in their effectiveness as pollinators of *Zizia aurea*. She is in the process of analyzing similar data from *Dalea purperea*. These results will be written up for publication this year. For dissemination this reporting period, our main focus was on presenting to the scientific community. Ian Lane was invited to present at the Entomological Society of America meetings in 2019. Further, we had two manuscripts accepted in peer-reviewed journals and another two are currently in review.

## Amendment Request December 22, 2020

We request a reallocation of funds to increase amount for salary and supplies. In total, this would be \$5,613 more for salary and \$727 for supplies. Both of these increases are related to Activity 3. We needed more salary time to collect fine-scale resolution on pollinator effectiveness. This increase in sampling also increased the amount of time needed to count pollen grains. We also needed more supplies for Activity 3 as we did a number of stigma squashes to count pollen grains. To make up for the needed funds for salary and supplies, we were able to reduce costs in two areas. First, we needed less travel that predicted for Activity 3. We spent more time at fewer sites to get more higher quality pollinator efficiency data. This reduced the cost of travel by \$4,192. Second, we were able to partner with the United States Fish and Wildlife Service and the Minnesota Department of Natural Resources to lead a field workshop for land managers. We saved costs in terms of printing documents and creating online outreach material.

For Activity 1 and 2, we request that \$1,822 (\$911 per Activity) budgeted for workshop to reallocated to salary for Activity 3. For Activity 3, we request \$5,613 for salary and \$727 for supplies. These funds will come from the workshop funds in Activities 1 and 2 listed previously. In addition, we will reduce travel funds from Activity 3 by \$4,192 and workshop funds in Activity 3 by \$327.

## **Overall Project Outcomes and Results**

Tallgrass prairie in Minnesota has declined by over 98%. A critical tool for prairie conservation is ecological restoration. One goal of prairie restoration is to conserve, protect and restore native bees and the pollination services they provide. In Minnesota, there are over 450 species of bees with the prairies having the highest diversity and abundance. However, there is a lack of information available to enable land managers to effectively create and maintain restorations for bees. This project addresses three objectives. First, we test how surrounding landscape influences native bee communities in prairie restorations. This information can help land managers determine where to place restorations and which restorations to prioritize for management. Second, we examined how plant communities influence bee communities. Third, we studied pollination in restorations and evaluated which bee species were the most effective pollinators. We sampled bees at 26 sites in the summers of 2017 – 2019. We collected over 18,000 bee specimens and recorded over 11,000 bee by plant interactions. We found that surrounding landscape had less of an impact on bee communities than local flower communities. This suggests that increasing plant diversity is a critical tool for enhancing bee communities while exact placement is less important. We also found that restorations and remnants have unique bee and flower communities. This suggests that we might not be meeting restoration goals as bee communities in quite different than remnant prairies. Finally, we found large variation in the pollination effectiveness among bee groups. This research benefits Minnesotans highlights the importance of maintaining diverse plant communities in restorations. As surrounding landscape did not influence bee communities or pollination, restorations in areas of high agriculture can be effective. We have created a highly resolved open-access dataset for future studies. We have published five peer-reviewed manuscripts to date and are attached as supplementary material.

## **IV. PROJECT ACTIVITIES AND OUTCOMES:**

ACTIVITY 1: Determine most effective placement of pollinator plantings.

**Description:** The location of pollinator habitat plots is likely a critical factor for determining the establishment success of native bee communities. For example, if a newly created habitat patch is close to remnant prairie habitat that contains a rich and abundant native bees, it is more likely that this nearby site will be more readily colonized than a site that is distant from a remnant prairie. To examine this, we will first select 10-15 native, remnant prairie sites located in the tallgrass prairie region of western Minnesota (see Visual Component). These will serve as reference sites. At each of these remnant prairie sites, we will then select three to four forb-rich prairie restoration sites that vary in distances from the native, remnant prairie. These sites will be sites that are managed by The Nature Conservancy, United States Fish and Wildlife Service, the Department of Natural Resources, and/or the Minnesota Board of Water and Soil Resources. Cariveau has already been in contact with land managers and is in the process of choosing sites. The surrounding landscape can have a large impact on the local native bee community. Therefore, during each year of the study, we will characterize the land use surrounding each newly created habitat and native, remnant prairie. We will us GIS to quantify the amount of area in wetland, forest, soybean, corn, and Conservation Reserve Program surrounding the newly created habitat. We will use these factors as covariates in statistical models.

At the remnant prairie and restoration sites, we will place bowl traps with soapy water along a standardized transect three to four times each year. Bowls will remain in the field for 24 hours for each sampling event. Bees will be removed from bowls and placed in 70% alcohol and taken back to the lab where they will be processed, pinned and identified. After identification, pinned specimens will be housed in the University of Minnesota Insect Collection. Specimens will be added to a database that will be publicly accessible once peer-reviewed

publications have been accepted.

While the full project will take place with sampling in the summer of 2017, we will begin sampling a subset of these sites in the summer of 2016 using funds from D. Cariveau's start-up provided by the University of Minnesota. Therefore, some sites will be sampled for a total of 3 years (summer 2016, 2017, and 2018) while others will be sampled for two years (summers 2017 and 2018).

Summary Budget Information for Activity 1: ENRTF Budge		<del>\$ 194,409</del>
Req	uest	\$ 193,498
Amount Sp	ent:	\$ 193,498
Bala	nce:	\$0
Outcome		Completion Date
1. All sites selected		October 2016
2. GIS completed for each site (completed in fall of each sample year)		June 2019
3. Bee collections completed and identified. Entered into database and analyzed		January 2019
4. Travel to meeting to present results to land managers and general public		June 2019
5. Workshop to disseminate results to land managers		June 2019

## Project Status as of January 4, 2017:

A major focus for the summer of 2016 was finding restoration and remnant sites to sample native bees. Ian Lane joined the Cariveau Lab as a PhD student in June 2016. He spent the summer visiting restoration and remnant sites in western Minnesota. In total, he visited approximately 70 sites in July through September to determine each site's suitability for sampling bees. He obtained permits to collect specimens on a subset of these sites. These included lands managed by The Nature Conservancy and Minnesota DNR as well as a Scientific and Natural Area.

In addition to site visits, Ian has been selecting sites based on landscape characteristics. As our questions in this activity are focused on habitat placement, careful consideration of site selection is critical. In particular, two issues are important to control for during site selection. First, there must be enough variation across sites in the factors of interest (e.g. distance of sites from remnants and amount agricultural land surrounding sites) while also minimizing variation in other factors that would increase noise (e.g. restoration age and size). Second, sites must be distributed in a systematic manner across the landscape to eliminate spatial autocorrelation among sites. If either of these issues are not controlled, it can make statistical analyses highly problematic. To select sites, Ian has conducted landscape analysis on 70 sites and examined these sites for variation in numerous factors. He used the USDA's National Agricultural Statistics Service Cropland Data Layer in ArcGIS. Using these landscape factors while also considering the spatial distribution of sites, he has selected approximately 20 sites in eight counties in western Minnesota. He is currently working getting permissions for these sites in preparation for summer 2017.

## Project Status as of July 1, 2017:

In the spring of 2017, we selected and received permission to conduct research as 16 BWSR conservation easements that spanned a range of surrounding area in agricultural production. We also selected and received permission to sample 6 remnant prairies in western Minnesota. In May of 2017, we began sampling native bees. This involved hiring a field crew of 6 technicians in addition to Ian Lane who is a PhD student conducting this research. We have used a combination of hand netting, bowl traps and blue vane traps. In the first round of sampling, we collected nearly 4,000 bee specimens. This is an incredibly high number of specimens as we still have the majority of the summer to conduct research. Most specimens have been pinned, labeled and their associated data have been entered into spreadsheets. Once all of the data have been entered, we will upload these data into a secure MySQL database. This fall and winter, we will be working on identifying specimens to

species. In addition, we will spend the winter summarizing data and conducting analyses of our first year of sampling so as to plan summer 2018.

## Project Status as of January 1, 2018:

We completed sampling in September 2018. In total, we have collected 11,666 wild bee specimens. All specimens have been pinned, labeled, and entered into the database. Our final step is to identify all specimens to species and enter species information into the database. A newly hired taxonomist, Zach Portman, has begun species identification and he has identified nearly 5,000 specimens of approximately 20 species. Most of these specimens have been from easy-to-identify and species-poor groups. We expect to have around 75 species. While we have identified just over 40% of the specimens, we have already found a number of interesting species including the declining bumble bee *Bombus terricola*, the rare species *Xeromelecta interrupta* and three likely state records: *Perdita ignota, Lasioglossum michiganense,* and *Lasioglossum occidentale*. Species identification will be completed and entered into the database before field season begins in 2018. We are currently planning on our second full field season.

## Project Status as of July 1, 2018:

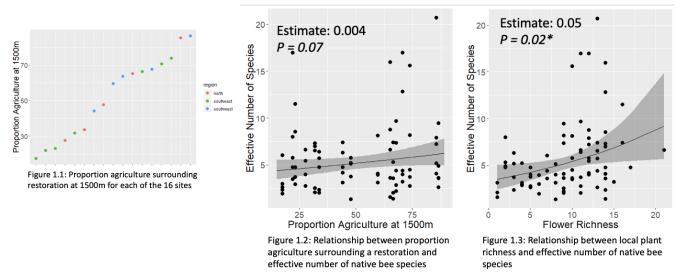
We collected and identified 11,666 specimens in the summer of 2017. All specimens have been identified to the lowest taxonomic level possible. This results in at least 159 total species. Note that this is more than double of what we predicted in January 2018. This accounts for roughly 30-40% of the total faunal bee diversity in Minnesota. The five most common species were *Augochlorella aurata* (1873 specimens), *Melissodes trinodis* (1319), *Lasioglossum admirandum* (1023), *Ceratina mikmaqi* (963), and *Lasioglossum albipenne* (789). All specimens and identifications are entered into the Cariveau Lab Native Bee Database (MySQL with multiple back-ups).

We have selected new field sites this season. We are focusing on sites near Morris, MN. This will allow us to sample a different region and will likely increase the total number of species. In addition, we focused on landscape comparisons last season. This summer our objective is to focus more on remnant and restoration comparisons. We have selected a total of 5 remnant and 5 restoration sites. We have already hired a field crew and have completed our first round of sampling. We were able to sample spring bees in a more rigorous manner this spring. Finally, we noticed that plant communities in restorations are likely less heterogenous than in remnants. To capture this variability, we have implemented an additional sampling plan for this summer. We will be conducting variable transect walks outside of our hectare plots. This will expand our sample area.

## Project Status as of March 19, 2019:

We completed our second field season. We sampled 10 sites and caught 6,533 specimens of native bees. All specimens have been labeled and sorted. We are now in the process of identification with approximately 95% of specimens identified. In total, we collected a total of 143 species. Species of note include the following. *Bombus ternarius* is a declining bumble bee. *Eucera albata* is a prairie specialist. *Lasioglossum cattelae* is a new state record for Minnesota.

The summer of 2018 is our final season for our large collecting effort. Across these two years, we collected over 18,000 specimens and approximately 190 species. We have begun data analyses and are writing the results up for publication in peer-reviewed journals. We sampled 16 restoration sites that ranged in the proportion of agriculture surrounding each site at 1500 m (Figure 1.1). In particular, as the number of One interesting, but preliminary result is that we found that landscape composition had little effect on bee abundance or the number of bee species in a restoration (Figure 1.2). However, floral community did have an effect on the number of bee species. Here plant species increased the number of bee species also increased (Figure 1.3). This finding will be the focus of our manuscript to be submitted later this year.



#### Project Status as of July 1, 2019:

Data from the 2018 field season has been cleaned, uploaded onto a MySQL database, and in a final form for analysis. We collected a total of 18,390 specimens, including multiple new species records for the state and a "near" final tally of 193 species of bees. These data will be the focus of two manuscripts examining how landscape context mediates the relationship of bee species in prairie remnants and the nearby restorations.

Continuing work another is focused on how surrounding landscape impacts the variability of the bee species found in restorations. As with before, local floral richness in the restorations is more important than landscape in structuring bee communities, suggesting that even isolated locations can serve as habitat for a diverse community of bees.

#### Project Status as of January 31, 2020:

Ian Lane has continued to analyze data for this activity. In addition to results presented above, he quantified how species composition of prairie reconstructions varied as a function of surrounding land cover and floral

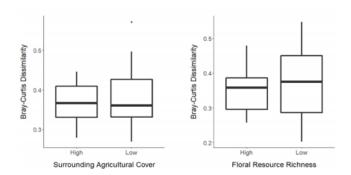


Fig 1.4 Comparison of species composition as a function of the proportion of surrounding land cover and floral resource cover. We found no effect of either factor on species composition.

resource richness (Fig 1.4). He did not find an effect of either of these factors on species composition. These results suggest that isolated prairie reconstructions may be effective habitat for native bees as there is little effect of land cover. This is lan's first chapter and he has submitted this research to a peer reviewed journal. Whether or not it gets accepted in this particular journal, this research is of high quality and we expect that this research will be published in a peer-reviewed journal in 2020.

#### Final Report Summary:

Ian Lane sampled bees on a total of 26 sites in western Minnesota. Sixteen of these sites were restorations while 10 were remnant prairies. These varied in the total amount of agriculture surrounding each site, ranging from

20% to over 90% of the total land cover being in agriculture production at 1500-meter radius. Ian predicted that sites with a higher amount of agriculture would have a lower diversity and more similar bee community compared to sites with a low amount of agriculture. At sites with a lot of agriculture, there are likely few bee species and those that are present are likely those that are adapted to agriculture. In contrast, sites without a high amount of surrounding agriculture will likely have more species. Ian also recorded the flowering plant community at each site to determine whether this local factor was also important This sampling took place over two summers. Ian found that surrounding landscape had little effect on the diversity and composition of bee communities at both restoration and remnant sites. However, floral community diversity was important in driving the diversity and composition of bee community. This is a hopeful result as it suggests land managers could effectively restore many sites, even those surrounded by a lot of agricultural. Further, these results indicate that managing restoration sites to maintain high plant diversity is critical for maintaining bee communities. A subset of these results have been published in the peer reviewed *Journal of Applied Ecology* in 2020. The remaining results are being submitted for publication in early 2020.

ACTIVITY 2: Quantify plant use by bees in remnant prairies and pollinator habitat.

**Description:** In addition to distance from remnant prairie, the local native plant community may also have an important impact on the establishment and persistence of native bees in newly created habitat. Therefore, we will sample bees that are visiting flowers in both the remnant prairie and the new habitat. We will collect bees that are visiting flowers using a hand net and then record the plant species that the bee was visiting. These collections will take place along standardized transects at each site four times each season. Collections will begin in late April and early May. Each site will be sampled four times each year. After identification, pinned specimens will be housed in the University of Minnesota Insect Collection. Specimens will be added to a database that will be publicly accessible once peer-reviewed publications have been accepted.

There is little information on the floral needs of this early spring bee community. Of particular interest is determining which plant species are used by early spring native bees. Early spring plants are expensive and difficult to establish in new prairie habitat. In addition, we will determine which plant species are most preferred by rare and declining bee species. Therefore, our results will highlight particular plants that are highly attractive to these bees and help land managers to focus on these plant species. After identification, pinned specimens will be housed in the University of Minnesota Insect Collection. Specimens will be added to a database that will be publicly accessible once peer-reviewed publications have been accepted.

We will also characterize the local flower community by placing 1m<sup>2</sup> quadrats along the collection transect. In each quadrat, we will identify each plant species and count the number of flowers blooming. One of the field technicians will be dedicated to identifying plants and collecting botanical information.

As noted in Activity 1, this project will begin with a subset of sites in the summer of 2016 using funds from D. Cariveau's start-up funds provided by the University of Minnesota. Therefore, some sites will be sampled for a total of 3 years (summer 2016, 2017, and 2018) while others will be sampled for two years (summers 2017 and 2018).

Summary Budget Information for Activity 2:	ENRTF Budget:	\$ <del>195,409</del>
	Request	\$ 194,498
	Amount Spent:	\$ 194,498
	Balance:	\$0

Outcome	Completion Date
1. Bee collections completed and identified. Entered into database and analyzed	January 2019

2. List of flowers most preferred by rare and declining bee species.	June 2019
3. List list of flowers most preferred by spring bee species.	June 2019

## Project Status as of January 4, 2017:

Ian Lane, Alan Ritchie and Dan Cariveau all participated in preliminary collections of native bees at remnant and restoration sites. In total, we collected 255 specimens from 31 plant species. We are finalizing bee identifications and expect to have approximately 40 species. There were several interesting species. We collected three rare bumble bee species: *Bombus pensylvanicus*, *B. fervidus*, and *B. borealis*. In addition, we collected other uncommon species including *Colletes robertsonii*, *Syvastra obliqua*, *Andrena quintalis*, as well as some mid to late season, large-bodied specialist *Andrena* such as *A. hirticincta* and *A. rudbeckia*.

We leveraged this ENTRF and received a small grant from the University of Minnesota's Institute on the Environment. As part of this grant, we hired an undergraduate to create a pollen library for plants in the region. We will collect pollen from bees and use this pollen library to determine which plant species these bees have been visiting. This will provide another valuable measure of plant preference.

We have created a plant and bee trait database for Minnesota using MySQL. This database has been shared with other researchers and we will continue to build this database as it furthers our ability to examine trait matching between plants and bees.

## Project Status as of July 1, 2017:

Of the nearly 4,000 specimens collected, over 2600 were from hand netting. We recorded bees visiting 38 different plant species. The most common plants being visited were, in descending order, *Zizia aurea* (1714 specimens), *Erigeron philadelphicus* (325 specimens), *Achillea millefolium* (242 specimens), *Gallardia aristata* (47 specimens), and *Barbarea vulgaris* (46 specimens). At each site, we have conducted vegetation surveys. This has already resulted in 1,558 1 m<sup>2</sup> quadrats and we have recorded 59 blooming plant species.

## Project Status as of January 1, 2018:

Of the 11,666 specimens collected, over 7,000 were from hand netting. Hand netting allows us to record which species of plant each bee species was visiting. We collected bees from a total of 118 blooming plant species. The most commonly visited plants were as follows: *Zizia aurea, Ratibida pinnata, Dalea purpurea, Erigeron philidelphicus* and *Cirsium arvense.* Furthermore, we recorded the number of blooming plants species and the total number of flowers per site. Flower sampling took place within one day of bee sampling. In total, we recorded 181 plant species blooming at our sites. The floral data has been entered into the database and is awaiting data checking and is close to analysis.

In addition, Dan Cariveau was recently awarded a federal United States Department of Agriculture Exploratory Grant as a Co-PD (\$100,000; *Developing an innovative method to unravel pollen use by native bees*. USDA-NIFA Grant# 2018-67030-27396. Dave Andow - Project Director, Dan Cariveau - Co-Project Director). We will be sampling pollen loads from native bee specimens collected from the ENRTF funding. We will be using Next Generation genetic sequencing to determine which plant species comprise pollen loads of native bees. This technique greatly improves the ability to identify pollen grains to species. This pollen sampling will further the objective of this ENRTF project. We will start this project in early spring 2018.

## Project Status as of July 1, 2018:

We collected 7,182 specimens by hand net. This technique allows us to determine which species native bees were visiting. We collected bees from 113 blooming plant species (we found some misspellings and therefore our numbers dropped slightly from the last update). We recorded a total of 186 flower species at the sites.

We are now sampling pollen loads from a select set of native bees. We will characterize pollen use through metabarcoding techniques (see grant above). We have begun the preliminary stages of the process. While this is not funded through this ENRTF project, we were able to obtain USDA funding because of the strong dataset collected from the ENRTF. Further, the findings from this USDA-funded research align with the objectives of the Activity as well.

## Project Status as of March 27, 2019:

We are still processing specimens (see above). Of the ~7000 specimens collected, nearly 3500 specimens were collected from netting and we collected these bees from approximately 100 species of flowers. All data have been databased and we are finalizing some of the plant names. We have databased and curated ~120 herbarium specimens. These will be used as reference plant collection. We are beginning to focus data analysis for this objective.

We are continuing to use bees for this project for a collaboration with D. Andow and Deborah Pires Paula (UMN Entomology). For this collaboration, we will be sampling pollen loads from 60 specimens of *Augochlorella aurata* and 60 specimens of *Melissodes trinodis*. Through this project, we will be able to determine pollen loads of a generalist bee (*A. aurata*) and a specialist bee (*M. trinodis*). We will compare pollen loads to plants that were blooming at each site. We predict that the specialist will have fewer pollen species in their pollen loads compared to the generalist. Further, we predict that pollen load diversity will be related to plant species number at a site for the generalist species but not the specialist. The specialists will likely focus on only a few species of plants and this pollen loads and are waiting on the generic data. This will also help us better understand floral use by native bees – the primary focus of this Activity.

## Project Status as of July 1, 2019

We have documented 10,035 floral interactions with bees, representing 163 species of bees interacting with 139 different species of flowers. This data set will allow us to ask detailed questions about how floral communities in prairie restorations shape bee communities, as well as how they compare to remnant prairie habitat.

In addition to our previous sampling, we also undertook an additional type of sampling in the early spring to better document floral use at this time of year. This new sampling focused only on spring flowering plants occurring between late April and mid-May. This sampling produced 936 specimens representing 72 species of bees visiting 27 different species of flowers. This more targeted data will allow us to better understand floral use at this critical part of the year.

**Project Status as of January 31, 2020** Ian Lane has continued to analyze these data. In particular, he is interested in whether there is greater variability among native bee communities in prairie remnants as compared to prairie reconstructions. He has found that species composition is in fact more variable in remnants (Fig 2.1). This suggests that each prairie remnant has a more distinct bee community while prairie reconstructions have native bee communities that are similar across locations. This is likely due to the fact that each prairie likely has a unique flowering community while prairie reconstructions have similar flower communities due to similar seed mixes being planted. Ian presented these results at the Entomological Society of America meeting in November 2019. He is close to finishing this and other analyses and we expect these results will be submitted to a peerreviewed journal by end of this project on June 30, 2020.

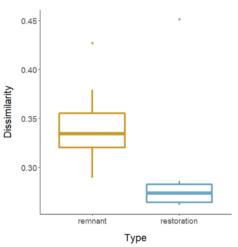


Figure 2.1. Comparison of native bee community variation across prairie remnants and prairie restorations

#### **Final Report Summary:**

Ian Lane sampled bee and plant communities at a total of 20 sites. Ten of these sites were prairie remnants and each remnant was paired with restoration plot. This pairing allowed us to directly compare plant and bee communities among restoration and remnant sites. He collected a total of 8,917 individuals represented by 164 bee species or species groups. At restorations he collected 5,458 specimens with the remaining 3,459 collected in remnant prairies. Plant sampling transects documented 155 species of blooming plants, with 122 species found in remnants and 76 species found in restorations. Ian found that bee and plant communities differed greatly between remnants and restorations. Bee communities had a higher amount of variation among remnant sites compared to restorations (Fig 2.2). In other words, each remnant site was more unique while restorations sites were more similar to each other. Interestingly, there was no difference in bee diversity among remnants and restorations (Fig 2.2). It is likely that restoration sites have more similar bee communities as the plant communities are more similar. First, plant communities are quite different in remnant versus restored prairie sites (Fig 2.3). Second, bees that are more specialized on particular plant species made up a greater proportion of bees in prairie remnants (Fig 2.3). This is likely due to the presence of unique plants in these prairie remnants. Ian also quantified the average tongue length of bees in each plot. He also predicted that there would be a higher average tongue length in prairies as they tend to have flower species with longer corollas. However, he did not find evidence for this effect (Fig 2.3). Overall, these results suggest that prairie restorations may not be mimicking prairie remnant plant communities, and this is leading to differences in bee communities. Ian is currently finishing writing these results and will submit these findings to a peer reviewed journal in early 2021.

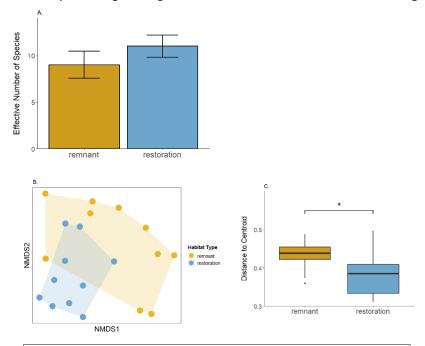
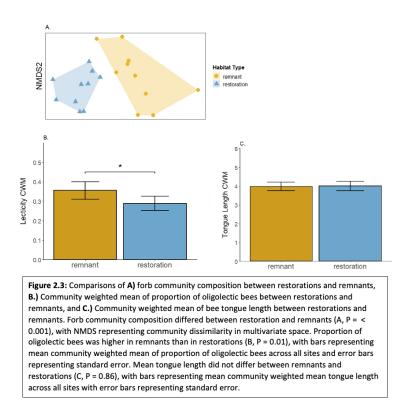


Figure 2.2: Comparison of A) bee community diversity between prairie restoration and remnants, B.) Representation of bee community composition between restorations and remnants, and C.) Bee community  $\beta$ -diversity between restorations and remnants. Bee community diversity did not differ between remnant and restoration (A, P = 0.24), with graph representing mean effective number of species and error bars representing standard error. Bee community composition differed between restoration and remnants (B, P = 0.002), with NMDS representing community dissimilarity in multivariate space (B).  $\beta$ -diversity was higher in remnants than restorations (C, P = 0.04).



**ACTIVITY 3:** Determine most effective native bee species for pollinating prairie plants

**Description:** While new habitat may be important for native bees, little is known about the importance of native bees for new habitat. We will examine this question by determining how effective different bee species are in pollinating native prairie plants. We will focus on newly created habitats as this is a likely stage at which seed production is critical. We will choose two or three species of native plants in the new prairie habitat. For each plant species, we will bag a subset of flowers while they are still in the bud stage to prevent visits from pollinators. Once they open and the stigma becomes receptive, we will present these non-visited flowers to foraging bees. We will record the bee species or bee species group that visited the flower. Once a flower has been visited, we will cover the flower and bring it back to the lab. In the lab, we will stain the stigmas and count the number of conspecific pollen grains. This will allow us to determine the number of pollen grains that each bee species or species.

In the summer of 2016, we will experiment with a number of native plants and determine the exact methodology for 2-3 plant species.

Summary Budget Information for Activity 3:	ENRTF Budget:	<del>\$ 130,</del>	<u>182</u>
	Request:	\$ 132,	004
	Amount Spent:	\$ 132,	000
	Balance:	\$	4

Outcome	Completion Date
1. Plant species selected	December 2016
2. Pollen deposition data collected and measured for 2 to 3 native plant species	June 2019

#### Project Status as of January 4, 2017:

In the late summer of 2016, Alan Ritchie, a graduate student in the Cariveau Lab, visited restoration sites throughout the tallgrass prairie region to determine plants commonly blooming in these restorations. In the fall of 2016, Alan acquired seed mix data from 50 restoration sites as well as seed mix lists from local nurseries. Combining the data from his site visits with the seed mix data, he chose a subset of plants to examine further. Alan is likely to use *Chamaecrista fasciculata* (partridge pea), *Monarda fistulosa* (bee balm) and/or *Dalea purpurea* (purple prairie clover). He is currently growing seeds in the greenhouse at the University of Minnesota, Twin Cities Campus. Alan is using these greenhouse plants to finalize protocols for pollen limitation and pollinator efficiency experiments. In the summer of 2017, Alan will conduct field experiments to assess pollen limitation and pollinator efficiency of these species.

## Project Status as of July 1, 2017:

We have begun trials of pollination experiments. Alan Ritchie purchased and grew *Penstemon grandiflorus*. Using these plants, Alan set-up a trial experiment to test methodologies and study design. The trial site had two plots – on plot had 15 plants in low-density (all plants 10 meters apart) and one plot in high density (15 plants 1 meter apart). At each plot he observed pollinator visitation, conducted hand pollination experiments, and sampled surrounding vegetation. He will then use this design and information from the experimental trail to conduct pollination experiments at 10 sites (20 plots) using *Chamaecrista fasciculata* (partridge pea). These plants will be placed in the field in mid-July 2017. Alan will also conduct this experiment in summer 2018 using both *P. grandiflorus* and *C. fasciculata*.

## Project Status as of January 1, 2018:

Master's student Alan Ritchie completed his first field season studying pollen limitation in restorations. He focused on *Chamaecrista fasciculata* (partridge pea). He placed out 30 potted plants at 10 BWSR RIM sites. At each site, he placed out two arrays. One array was a high-density with 1 meter between each plant and another low-density array with 10 meters between each plant. The low-density array was larger as there were fewer stems per meter and it proved too difficult to deter hungry deer. Therefore, we decided to use only the high-density plots. At each plot, Alan added supplemental pollen to half of the plants and left the others as open pollinated. He also observed pollinators visiting these plants. We hypothesized that sites with a greater amount of surrounding agriculture there would be fewer bees. Therefore, adding supplemental pollen would prove more beneficial at high agriculture sites due to the reduced number of bees. We found that this plant however, was not pollen-limited. This coming field season, we will expand this work to examine different plant species.

## Project Status as of July 1, 2018:

We are conducting research on pollination of native plants in remnant and restorations this summer. We have hired Gabriella Pardee as a postdoctoral research associate. She has expertise in pollination ecology. We will be comparing pollination of plants in both remnant and restoration plots. Findings of this project will be summarized in January 2019.

## Project Status as of March 27, 2019:

In summer 2018, we sampled *Dalea purperea* to examine seed set and pollination. We haphazardly chose 96 plants. Half of these plants were in a burned area of a prairie and the other half in a non-burned portion. For each plant, we observed pollinator visitation and collected pollinators over 8 field days. In total, we recorded 209 individual bees and 318 individual flies visiting *Dalea*. In addition, we collected 1168 stigmas to determine pollen deposition. We counted the number of *Dalea* pollen grains per stigma and the number of grains ranged from 0 to 107 with a mean of 7.19 *Dalea* pollen grains per stigma. Finally, we collected 308 total seed heads and found that the number of seed per plant ranged from 0 to 849 seeds with a mean of 190.53 seeds per plant. The

focus of this final season will be on Activity 3. We will be obtaining single visits and pollination data for two new plant species.

## Project Status as of July 1, 2019

In 2019, we conducted research in 4 remnants and 4 restored prairies to examine pollinator effectiveness of two focal species: *Zizia aurea* and *Dalea purpurea*. For this study, we set up a one-hectare area within each prairie and conducted pollinator observations and single visits for each focal species. For our observation period, we walked haphazardly throughout the one-hectare area for 30 mins, and collected every bee that was actively foraging on our focal species using hand nets. Each bee was collected into a separate vial. Next, we conducted single visits by cutting stems of plants that were bagged the day before and presenting the open flowers to a bee. Once the bee landed on an open flower, we recorded the duration of the visit, the identification of the bee to morphogroup, and the stigmas that the bee touched while foraging. Once the visit was complete, we used forceps to collect the stigma and stored it in a glassine envelope. Finally, we sampled the flowering community within each prairie by placing 60 1m<sup>2</sup> quadrats down throughout the one-hectare area and recording species richness and abundance of each flowering species. When we returned to the lab, we pinned and swabbed each individual bee that was collected during our observation period for pollen using fuchsin gel. We then melted the gel onto a microscope slide. We will count the number of *Zizia* and *Dalea* pollen grains and identify pollen from coflowering plant species to gain insight into foraging behavior. We also processed the stigmas collected from single visits by pressing stigmas onto microscope slides with melted fuchsin gel. We will count the number of *Zizia* and *Dalea* pollen grains collected from single visits by pressing stigmas onto microscope slides with melted fuchsin gel. We will count the number of *Zizia* and *Dalea* pollen grains to determine which bee morphogroups are providing the most pollination to our focal species.

For *Zizia aurea*, we collected over 500 bees that were visiting the flowers within the remnant and restored prairies. Further, we conducted 120 single visits from 6 morphogroups. For *Dalea purpurea*, we collected over 200 bees from our observation period within the remnant and restored prairies. Further, we conducted 160 single visits from 5 morphogroups.

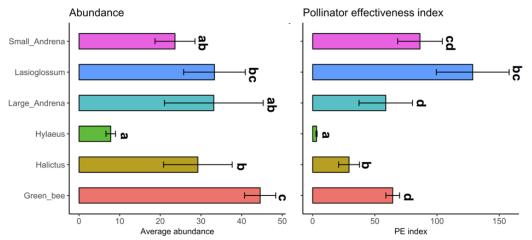
We have finished swabbing all the bees for pollen that were collected off *Zizia* and *Dalea* and are currently processing the stigmas from the single visit study. The next step is to count the pollen slides and analyze the data. We plan to do this during the Fall 2019.

Master's student Alan Ritchie and postdoctoral research associate Gabriella Pardee chose to examine pollination of *Dalea purpurea*, or purple prairie clover in 2018. The Activity 1 data indicated *Dalea* is one of the most commonly visited plant species by bees, hosts both generalist and specialist bees, and is common to both restored and remnant prairies. These qualities make it an ideal model plant for studying both bees and bee pollination in prairies. To better understand its pollination ecology, we sampled *Dalea* to examine seed set and pollination in a remnant prairie that is being managed with frequent burning. Fire is an important reproductive cue for many prairie plants, and subsequent nectar and pollen availability for bees, but there is limited data on how fire affects pollination. At this remnant prairie we haphazardly chose 96 plants. Half of these plants were in a burned area of a prairie and the other half in a non-burned portion. We predicted that fire would improve pollination by shifting the timing and duration of flowering in *Dalea*, increasing the chances that *Dalea* bloom at the same time (flowering synchrony). We have completed sampling and counting. Preliminary results show that fire shifts flowering earlier but did not increase flowering synchrony or *Dalea* pollen grain receipt relative to non-burned plants. Interestingly, we found that earlier flowering *Dalea* in the burned portion of the prairie experience lowered seed set, the opposite of what is frequently observed in other plants. These early flowering plants tended to have more pollen from other species of plant, which suggests the timing of flowering could impact which pollinators are most effective at pollinating prairie plants.

Finally, Alan Ritchie has analyzed his data on pollen limitation in Partridge Pea (*Chamaecrista fasciculata*) and has written a manuscript that has been submitted to the peer-reviewed journal Restoration Ecology.

## Project Status as of January 31, 2020

We have made a lot of progress on this third objective. Alan Ritchie defended his Master's thesis in December. The first chapter has been accepted for the peer-reviewed journal *Restoration Ecology*. Alan is in the process of making minor edits and it will be published early this year.



Dr. Gabriella Pardee completed her field research and all stigmas have been processed and counted. In total, she recorded 157 single visits to Zizia aurea from 7 different bee species groups. She calculated the mean amount of pollen deposited as well as the abundance from Ian Lane's data. This provided a *pollinator* effectiveness index. She found large differences in pollen deposition across species groups.

Fig 3.1 Abundance and pollinator effectiveness index from six groups of native bees. Pollinator effectiveness index is calculated as the abundance times the average number of pollen grains deposited for that group.

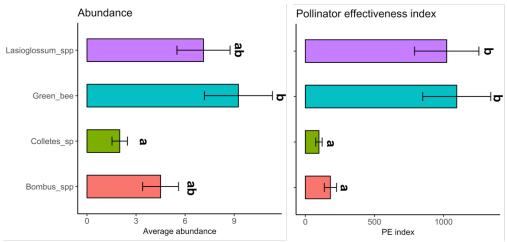
This suggests that species do differ in how effective they are at delivering pollen. In addition, Dr. Pardee is finalizing analysis on data from *Dalea purpurea*. In total, she collected 146 single visits. These data are currently being checked and are nearly ready for analysis.

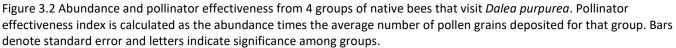
### **Final Report Summary:**

The research conducted in Activity 3 yielded a number of valuable results. First, Alan Ritchie used the funds from this project to examine pollen limitation in the annual, bee-pollinated plant *Chamaecrista fasciculata* (partridge pea). In particular, Alan predicted that there would be fewer pollinator visits at sites surrounded by a high amount of agriculture and that this would, in turn, lead to pollen limiting seed production. He conducted this research in prairie restorations as he was interested in determining whether lack of pollinators might be limiting plant reproduction within restorations. To accomplish this, he created 10 populations of potted *C. fasciculata* and hand-pollinated half of plants at each population. Each population was placed in a prairie restoration that varied in the amount of agriculture surrounding the site. He then hand-pollinated flowers on half of the plants at each site. He found that pollen limitation did not differ among sites. Further, this plant does not seem to be pollen limited. This is a hopeful result as it suggests that pollen limitation is not a factor for this plant. However, this is limited to one plant species in one year. This result was published in the peer-reviewed journal *Restoration Ecology* (see dissemination results below).

Dr. Gabriella Pardee conducted a study on the pollinator effectiveness of different pollinator groups for two species of plants: *Dalea purpurea* (purple prairie clover) and *Zizia aurea* (golden alexander). For each plant, Dr. Pardee collected bees visiting these flowers. Further, she presented unvisited flowers to foraging bees. Upon a bee visit, she would record the bee group and then place the flower stigma (pollen receiving part of the flower) inside a microcentrifuge tube. These stigmas were then counted to see how many pollen grains were deposited by each group. She used this to calculate the average pollen grains deposited per visit for each pollinator. She then multiplied this by the abundance data in Ian Lane's dataset to get a pollinator effectiveness index. For *Zizia aurea*, she collected over 500 bees that were visiting the flowers within the remnant and restored prairies. Further, she conducted 120 single visits from 6 morphogroups. For *Dalea purpurea*, we collected over 200 bees from our observation period within the remnant and restored prairies and obtained 160 single visits from 5 morphogroups. finished the analysis for *Dalea purpurea*. She was able to analyze

pollinator effectiveness for the 4 main pollinator groups that visit this species. She found large differences in pollen deposition across species groups. This suggests that species do differ in how effective they are at delivering pollen. For *Zizia aurea*, small bees in the genus *Andrena* and bees in the genus *Lasioglossum* were the most effective pollinators. She found that that two of the bee groups, *Lasioglossum* spp. and green bees, are the most effective pollinators of *Dalea purpurea*. These two groups also happen to be the more abundant bees, which suggests that prairie restoration efforts have been successful in reinstating these most effective pollinators. The bee groups differed in their effectiveness between the two plant species. Small bees in the *Andrena* genus were important pollinators for *Zizia aurea* but were totally absent for *Dalea purpurea*. Green bees were important pollinators of *Dalea purpurea* and while they were abundant *Zizia aurea* pollinators, they were not particularly effective. Interestingly, the bumble bees (genus *Bombus*) were not important pollinators for either plant. These results suggest demonstrates that pollinator effectiveness does differ among species and that different plants benefit from different pollinators. This suggests that pollinator conservation efforts should be broad enough to conserve multiple bee species to ensure adequate pollination of diverse plant communities. The results for both *Zizia aurea* and *Dalea purpurea* are currently being written up for a manuscript that will be published in a peer reviewed journal.





#### **V. DISSEMINATION:**

The funds from this proposal will result in a number of outreach deliverables. First, we will create welldocumented native plant lists based on the results of bee-plant surveys. These lists will be useful to both the general public and land managers. These plant lists will be distributed throughout Minnesota and posted online. We will also give presentations for the general public. We will also present at professional meetings that are attended by land managers such as the annual meeting of the Minnesota chapter of The Wildlife Society.

These outreach activities will be done in close collaboration with a number of partners. Along with Crystal Boyd of the DNR, we will co-organize bee identification workshops. These workshops will also leverage funds and staff from ENTRF-ML14-6a: "Enhancing Pollinator Landscapes". We will also collaborate closely on workshops and coordinate speaking events. In addition, we will work with a number of established outreach programs and personnel at the University of Minnesota. Elaine Evans (PhD student at the University of Minnesota) conducts the Minnesota Bumble Bee Survey and is conducting outreach activities for ENTRF-ML-15-3g: "Minnesota Native Bee Atlas". She will share the plant lists and results of this study during outreach events. Finally, we will work with the University of Minnesota's Bee Squad to disseminate these results.

#### **Description:**

## Project Status as of January 1, 2017:

While still early in the research, we have participated in outreach events. Ian Lane and Alan Ritchie presented their findings at a conference focusing on ENTRF-funded pollinator research. The conference was organized by Dan Cariveau on December 15, 2016 and held at the University of Minnesota. In addition, The Nature Conservancy held a symposium for scientists conducting research on their property. Ian presented an overview of his project at this meeting.

## Project Status as of July 1, 2017:

Ian Lane and Alan Ritchie presented their thesis plans to the Department of Entomology. The goal of these seminar presentations was to inform and receive feedback from faculty and students regarding their research plans.

## Project Status as of January 1, 2018:

The overall goals of the project and some of the results have been presented in front of a number of different audiences. Dan Cariveau promoted this research at the Arboretum's Pollinator Symposium as well as at invited talks at the University of Illinois Urbana-Champaign, University of North Dakota, and the Conservation Sciences Seminar at the University of Minnesota. Ian Lane has given a talk at the Little Lunch on the Prairie Webinar Series. In April, Christina Herron-Sweet will present results at the Society of Ecological Restoration regional meeting in Stevens Point, WI.

## Project Status as of July 1, 2018:

We have continued to disseminate this work through research talks. Ian Lane and Alan Ritchie presented an update on this project at an LCCCMR-themed symposium for approximately 30 attendees. Ian Lane gave a talk to approximately 50 Farm Bill biologists entitled: *Bee communities in restorations and remnants: What is the role of the agricultural matrix?* Christina Herron-Sweet presented the results of this research at the Midwest Branch Meeting for the Society for Ecological Restoration. Finally, Ian Lane will be giving a presentation and co-leading a field day. This event is sponsored by the Minnesota Department of Natural Resources.

## Project Status as of March 27, 2018:

Ian Lane (PhD Student) co-led and presented at a field day that was sponsored by the Minnesota Department of Natural Resources. This field day was targeted at land managers. In addition, Ian presented the results of this research to other scientists at the Society for Rangeland Science in February 2019. Dan Cariveau has presented the results of this work at three events. He spoke at Café Scientifique – hosted by the Bell Museum. This was attended by approximately 25 individuals. Dan also presented this research at the Pollinator Symposium at the University of Minnesota Landscape Arboretum in September. Approximately 200 attendees were present at this symposium. He presented this project at meeting hosted by the McKnight Foundation with about 40 people present. Finally, Ian Lane and Dan Cariveau are co-organizing a workshop for land managers for the summer of 2019.

## Project Status as of July 1, 2019

Graduate student Ian Lane disseminated information produced from this project to the annual Prairie Chicken Society meeting in the form of a 40 minute informational talk. We (Ian Lane and Dan Cariveau) also co-organized a field workshop in Southwestern Minnesota on habitat restoration for native bees. We hosted this field day in conjunction with the Minnesota Department of Natural Resources (Megan Benage and Gina Quiram) and the United States Fish and Wildlife Service (Paul Charland). Approximately 50 natural resource professionals attended this workshop. We produced two informational handouts for this activity.

Three scientists presented their work at the national Ecological Society of America meeting in Louisville, KY. This is the premier national ecology conference and is attended by a few thousand scientists. Dr. Gabriella Pardee presented results from activities 1 and 2 (title: *Functional diversity and plant-pollinator networks in restored prairies across an agriculturally dominated landscape*). Alan Ritchie presented the results of activity 3 (title: *Landscape context does not affect restoration of pollination function of a bee-pollinated forb in restored prairies*). Ian Lane presented results from activities 1 and 2 (title: *The role of local and landscape factors in shaping bee communities in restored prairies*). Ian has also been invited to speak at the Entomological Society of America annual meeting where he will present additional work from this project.

## Project Status as January 31, 2020

Much of our dissemination in this period has been focused on dissemination to the scientific community. Ian Lane gave an invited talk at the Entomological Society of America meeting in St. Louis, MO in November 2019. Alan Ritchie presented his thesis research in a public seminar in December 2019. Further, we have been working to publish the results of our research. We currently have two manuscripts that have been accepted to peer-reviewed journals. Another two are currently in review. We have made sure to acknowledge ENRTF funding in all cases. Bolded names represent co-authors whose salary or graduate funding has come from this grant. Once published, all data and code will be indexed with a digital object identifier (DOI) and available open-access at the Data Repository of University of Minnesota (DRUM - https://conservancy.umn.edu/handle/11299/166578).

- Lane IG, Herron-Sweet C, Portman Z, Cariveau DP. (In Review) Floral resource diversity drives bee community diversity in prairie restorations along a landscape simplification gradient. *Journal of Applied Ecology.*
- **Cariveau DP**, Bruninga-Socolar B, **Pardee GL**. (In Review) A review of the challenges and opportunities for restoring animal-mediated pollination of native plants. *Emerging Topics in Life Sciences* (Invited Review)
- **Ritchie AD**, **Lane IG**, **Cariveau DP**. (Accepted) Pollination of a bee dependent forb in restored prairie: No evidence of pollen limitation in landscapes dominated by row crop agriculture. *Restoration Ecology*.
- **Portman ZP**, **Lane IG**, **Pardee GL**, **Cariveau DP**. (Accepted) Reinstatement of *Andrena vernalis* Mitchell (Hymenoptera: Andrenidae) from synonymy with *A. ziziae* Robertson. *Great Lakes Entomologist*

## **Final Report Summary:**

Overall, we have disseminated our results to the scientific community as well as the general public. We gave 6 presentations at national conferences. Two of these presentations were invited presentations. Dan Cariveau also presented these results at three departmental seminars at other universities. We have published a total of 6 peer-reviewed manuscripts. We expect at least three more. Ian Lane will be submitting the second chapter of his dissertation in early 2021 and third chapter before May 2021. At which point he will defend his PhD thesis. Dr. Gabriella Pardee is finishing up a manuscript on pollen efficiency. In addition to publishing in journals, we have also made the data and code available and open access. All published work and presentations have and will continue to acknowledge this ENRTF support. We have also presented the results of this research at outreach events. Ian Lane has also been a guest on the Minnesota Department of Natural Resources Prairie Pod podcast. This podcast is focused on the science of prairie conservation. We led a highly successful field day on pollinators in prairies. This was co-led by Ian Lane and Dan Cariveau. In addition, we partnered with the United States Fish and Wildlife Service, Minnesota Department of Natural Resources and the Prairie Reconstruction Initiative. This brought in 50 natural resource professionals and was focused on prairie sites in southwestern Minnesota. Finally, this funding has supported two graduate students. Alan Ritchie defended his master's thesis in December 2019. He is now the Pollinator Coordinator for the Minnesota Department of Natural Resources. Ian Lane is finishing his PhD thesis. He has scheduled a defense date for May 2021.

Publication list as of December 2020 (Bolded names represent co-authors whose salary or graduate funding has come from this grant.)

- **Cariveau, D.P.,** Bruninga-Socolar, B. & **Pardee, G.L**. (2020). A review of the challenges and opportunities for restoring animal-mediated pollination of native plants. *Emerg. Top. Life Sci.*, 4, 99–109.
- Lane, I.G., Herron-Sweet, C.R., Portman, Z.M. & Cariveau, D.P. (2020). Floral resource diversity drives bee community diversity in prairie restorations along an agricultural landscape gradient. J. Appl. Ecol., 57, 2010-2018.
- **Portman, Z.M., Lane, I.G., Pardee, G.L.** & **Cariveau**, D.P. (2020). Reinstatement of *Andrena vernalis* Mitchell (Hymenoptera: Andrenidae) from synonymy with *A. ziziae* Robertson. *The Great Lakes Entomologist*, 53, 25–40.
- **Ritchie, A.D., Lane, I.G. & Cariveau, D.P**. (2020). Pollination of a bee-dependent forb in restored prairie: No evidence of pollen limitation in landscapes dominated by row crop agriculture. *Restor. Ecol.*, 28, 919–926.
- Portman, Z.M., Burrows, S.J., Griswold, T., Arduser, M., Irber, A.J., Tonietto, R.K.,...Cariveau, D.P. (2019). First Records of the Adventive Pseudoanthidium nanum (Mocsáry) (Hymenoptera: Megachilidae) in Illinois and Minnesota, with Notes on its Identification and Taxonomy. *Great Lakes Entomol.*, 52, 6

Budget Category	\$ Amount	<b>Overview Explanation</b>
Personnel:	\$ 38,140	<ul> <li>Dan Cariveau (PI); 66.2% Salary, 33.8% fringe 7.7% FTE for 3 years; Supervision of all project activities, direct supervision of graduate students and postdoctoral research associate, analyze data, write peer-reviewed papers and reports, disseminate information</li> </ul>
	\$157,599	<ul> <li>Postdoctoral Research Associate; \$157,599, 78.6% Salary; 21.4% fringe 100% FTE for 3 years, Supervision of field technicians, lead field data collection, analyze data, write peer- reviewed papers, disseminate information</li> </ul>
	\$112,430	• 3 Field Technicians; \$37,477, 73.7% salary, 26.3% fringe, 15 weeks for 3 years, collect field data, pin and identify insects, enter and manage data
	\$112,813	<ul> <li>University of Minnesota PhD Student,; 48% Salary, 52% fringe (including tuituion), 70% FTE for 3 years, conduct fieldwork, analyze data, write peer- reviewed papers, disseminate information</li> </ul>

## VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

		(32weeks/\$11hour/10 hours per week), pin insects, enter data
	\$10,200	<ul> <li>1 student worker;</li> <li>(30 weeks/\$11hour/10 hours per week), pin insects, enter data</li> </ul>
Professional/Technical/Service Contracts:	\$16,000	<ul> <li>Bee Identification by one or multiple specialists in bee taxonomy</li> </ul>
Equipment/Tools/Supplies:	\$ 1,834	<ul> <li>Equipment/Tools/Supplies: Insect collection supplies (nets, collecting jars, gps devices to find sites)</li> </ul>
	\$ 4,605	<ul> <li>Equipment/Tools/Supplies: Insect curation equipment (drawers, cabinets, insect pins, label paper, barcode software)</li> </ul>
	\$ 2,000	<ul> <li>Equipment/Tools/Supplies: Plant collection and curation for type specimens (herbarium, plant presses, herbarium supplies)</li> </ul>
	\$ 2,000	<ul> <li>Equipment/Tools/Supplies: Pollination quantification tools (pollen dye, microscope slides, flourscent UV cube)</li> </ul>
Printing:	\$ 3000	<ul> <li>Printing of outreach documents and scientific publication costs</li> </ul>
Travel Expenses in MN:	\$ 48,500	<ul> <li>Field crew travel: collect bees, identify and collect plants, collect single visit pollen deposition data, travel to present results of research</li> </ul>
TOTAL ENRTF BUDGET:	\$520,000	

## Explanation of Use of Classified Staff: N/A

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

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: <u>Postdoctoral Research</u> = 2.19 (1 @ 81% FTE for 3 years); PhD Student = 2.3 (1 @ 70% FTE for academic year; 100% FTE for summer for 3 years); Field Technicians = 2.8 (3 @ 48 weeks or 92.3% FTE for 3 years); Student worker 1 = 0.46 (1 @ 32 weeks for 10 hours/week or 15.4% FTE for 3 years); Student worker 2 = 0.42 (1 @ 30 weeks for 10 hours/week or 14.4% FTE for 3 years); Cariveau = 0.22 (1 @ 7.7% FTE for 3 years) TOTAL = 8.39 FTE

# 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			
NA	\$NA	\$ NA	
State			
Start-up funds provided from the University of Minnesota Department of Entomology to begin project. In addition, Cariveau's start-up funds will cover 30% of PhD Student Salary during academic year for 3 years. All funding is secured.	\$ 53,513	\$ 53,513 (\$2061 equipment, \$527 travel, \$24,953 salary/tuition)	Salary and fringe for field techs (\$20,461), crew leader (\$8,744), travel (\$4500), equipment (\$2061). To enable an earlier start of this project (April/May 2016), some of these will be spent prior to funding date of July 30, 2016. In addition, Cariveau's start-up funds will cover 30% of PhD Student Salary during academic year for 3 years (\$15,247)
TOTAL OTHER FUNDS:	\$ 53,513	\$ 53,512	

## VII. PROJECT STRATEGY:

**A. Project Partners:** Daniel Cariveau (U MN) will lead the project. Dr. Cariveau is a newly hired Assistant Professor, funded for first three years by ENRTF through proposal "Enhancing Pollinator Landscapes" submitted by Marla Spivak in 2014. He will directly supervise a PhD student and postdoctoral research associate. The PhD student and postdoctoral research associate will directly supervise the field technicians and student workers. Marissa Ahlering (TNC), Dan Shaw (MN BWRS), and Greg Hoch (MN DNR) will assist in selecting sites, acquiring permits, and provide technical guidance. M. Spivak (U MN) will provide scientific and administrative advice.

**B. Project Impact and Long-term Strategy:** This project will provide valuable information to help maximize success of prairie habitat restoration projects. This project will focus on the tallgrass prairie region of Minnesota, as this is the primary area of new pollinator habitat. It will inform efforts such as the Minnesota Prairie Conservation Plan, a \$3.5 billion effort with a major focus on restoration. The results from the project will provide valuable information to local, state, federal and non-profit agencies that are creating pollinator habitat. Finally, through collaborative and data-sharing efforts at the University of Minnesota and state agencies such as the Minnesota DNR, this project will be part of a larger effort to understand and better implement restoration in the tallgrass prairie region of Minnesota.

## C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	\$ Amount
	August 31, 2015 –	\$ 329,144
ENRTF award to M. Spivak in 2014, "Enhancing Pollinator	September 1 <sup>st</sup> 2018	
Landscapes"		

## IX. VISUAL COMPONENT or MAP(S): See attached

**X. RESEARCH ADDENDUM:** See research addendum to be submitted December 11<sup>th</sup>, 2015.

## XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than January 1, 2017, July 1, 2017, January 1, 2018, July 1, 2018 and January 1, 2019. A final report and associated products will be submitted between June 30 and August 15, 2019.

#### Environment and Natural Resources Trust Fund M.L. 2016 Project Budget

Project Title: Data-Driven Pollinator Conservation Strategies
Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 03a
Project Manager: Daniel P. Cariveau
Organization: University of Minnesota
M.L. 2016 ENRTF Appropriation: \$ 520,000
Project Length and Completion Date: 4 Years, June 30, 2020



ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Activity 1 Budget 06/30/2020	Amount Spent	Balance	Revised Activity 2 Budget 06/30/2020	Amount Spent	Activity 2 Balance	Revised Activity 3 Budget 06/30/2020	Amount Spent	Activity 3 Balance	Revised Total Budget 06/30/2020	TOTAL BALANCE
BUDGET ITEM	Determine most plantings	effective placeme	nt of pollinator	Quantify plant us and pollinator ha	se by bees in remn abitat	ant prairies	Determine most pollinating prairi	effective native be e plants	ee species for		
Personnel (Wages and Benefits)	\$163,773	\$163,773	\$0	\$163,773	\$163,773	\$	0 \$105,628	\$105,628	\$0	\$433,174	
Dan Cariveau (PI); \$38,140, 66.2% Salary, 33.8% fringe 7.7% FTE for 3 years											
Postdoctoral Research Associate; \$ <del>157,599</del> , 78.6% Salary; 21.4% fringe 100% FTE											
Field Technician 1; \$37,477, 73.7% salary, 26.3% fringe, 16 weeks for 3 years											
Field Technician 2; \$37,477, 73.7% salary, 26.3% fringe, 16 weeks for 3 years											
Field Technician 3; \$37,477, 73.7% salary, 26.3% fringe, 16 weeks for 3 years											
University of Minnesota PhD Student, \$112,813; 48% Salary, 52% fringe (including tuituion), 70% FTE for 3 years											
Student worker 1; \$10,880; (32weeks/\$11hour/10 hours per week)											
Student worker 2: \$10,200; (30weeks/\$11hour/10 hrs per week)											
Professional/Technical/Service Contracts											
Equipment/Tools/Supplies	\$3,219	3219	\$0	\$4,219	4219	\$	0 \$4,227	4227	\$0	\$11,665	
Equipment/Tools/Supplies: Insect collection supplies (nets, collecting jars, gps devices to find sites) - \$1834											
Equipment/Tools/Supplies: Insect curation equipment (drawers, cabinets, insect pins, label paper, barcode software) \$4605											
<b>Equipment/Tools/Supplies:</b> Plant collection and curation for type specimens (herbarium, plant presses, herbarium supplies) \$2000											
Equipment/Tools/Supplies: Pollination quantification tools (pollen dye, microscope slides, flourscent UV cube) \$2000											
Capital Expenditures Over \$5,000											
Printing											

Travel to and from field sites, renting fleet vehicles, hotel/camping, travel to share information with agencies and land managers, \$48,500 total	\$26,167	\$26,167	\$0	\$26,167	\$26,167	\$0	\$21,976	\$21,976	\$0	\$74,310	\$0
Other											
Workshop for land managers - spread across three activities as the workshop will include information from all entire project \$1,500; Plant list and outreach printing for workshops and broader use - spread across activities 1 and 2	\$339	\$339	\$0	\$339	\$339	\$0	\$173	\$169	\$4	\$851	\$4
COLUMN TOTAL	\$193,498	\$193,498	\$0	\$194,498	\$194,498	\$0	\$132,004	\$132,000	\$4	\$520,000	\$4