M.L. 2017, Chp. 96, Sec. 2, Subd. 08a Project Abstract
For the Period Ending June 30, 2021

PROJECT TITLE: Optimizing the Nutrition of Roadside Plants for Pollinators
PROJECT MANAGER: Emilie Snell-Rood
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2017, Chp. 96, Sec. 2, Subd. 08a

APPROPRIATION AMOUNT: $815,000
AMOUNT SPENT: $774,735
AMOUNT REMAINING: $40,265

Sound bite of Project Outcomes and Results
This research shows that, from a nutritional perspective, Minnesota roadsides are promising habitat for native bees and monarchs. To minimize the negative effects of roadside pollutants on insect pollinators, managers should prioritize low- to moderate-traffic roads for restoration, mow a buffer strip, and support efforts to ban the pesticide chlorpyrifos.

Overall Project Outcome and Results

Insect pollinators have suffered steep declines over the last two decades. Roadsides are a promising opportunity for pollinator conservation, potentially providing millions of acres of habitat, and acting as dispersal corridors. However, roadside habitat also contains pollutants such as heavy metals from car wear and past leaded gasoline use, sodium from road salt application, and pesticides from adjacent agriculture. In this research, we combined surveys of roadsides across Minnesota, with controlled lab and field experiments, to test how such roadside pollution impacts insect pollinators, and implications for restoring roadside habitat for monarch butterflies and native bees. Our results suggest that plants alongside the majority of Minnesota roadsides have sodium and metal content below which is worrisome to bees and monarchs. However, plants along very high traffic roads, especially those right next to the road, likely have negative effects on pollinator health. Our data also suggest that pesticides may be a significant concern for 5-10% of roadside plants. This research suggests roadside restoration efforts should focus on roads with low to moderate traffic volumes (<20K cars daily) and that mowing a buffer on the road edge should eliminate the most toxic plants. Recent national efforts to ban the pesticide chlorpyrifos would also be beneficial for Minnesota roadsides as this was the most commonly detected insecticide. Finally, this research suggests benefits to planting a diversity of roadside plants as species accumulate different toxins to different degrees, although on higher traffic roads, managers may want to avoid a handful of high accumulating species (e.g., yellow coneflower). Overall, from a nutritional perspective, Minnesota roadsides are promising habitat for insect pollinators, for instance, potentially producing 14M migratory monarchs annually. Future work should consider management methods that may minimize vehicle collisions, as currently pollinator mortality from collisions likely far exceeds that from plant toxicity.

Project Results Use and Dissemination
This project directly led to six publications in print and twelve in progress. Data are publically available on either DRYAD or Mendeley. This work was presented in over 25 seminars, conference presentations, and webinars presented locally, nationally and internationally. The conclusions of the work are available in online talks, such as the Cedar Creek "Lunch with a Scientist" series and the Rights-of-Way working group research series on

pollinator habitat. This research will be featured in a popular science book on road ecology and resulting management recommendations shared as a brief report to relevant agencies later this year.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2017 LCCMR Work Plan

Date of Submission: August 16, 2021

Date of Next Status Update Report: NA

Date of Work Plan Approval: 06/07/2017

Project Completion Date: June 30, 2021

Does this submission include an amendment request? no

PROJECT TITLE: Optimizing the Nutrition of Roadside Plants for Pollinators

Project Manager: Emilie Snell-Rood

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Location: Anoka, Becker, Benton, Big Stone, Blue Earth, Brown, Carver, Cass, Chippewa, Chisago, Clay, Clearwater, Cottonwood, Crow Wing, Dakota, Dodge, Douglas, Faribault, Fillmore, Freeborn, Goodhue, Grant, Hennepin, Houston, Hubbard, Isanti, Jackson, Kandiyohi, Lac qui Parle, Le Sueur, Lincoln, Lyon, McLeod, Mahnomen, Martin, Meeker, Mower, Murray, Nicollet, Nobles, Norman, Olmsted, Otter Tail, Pipestone, Pope, Ramsey, Redwood, Renville, Rice, Rock, Scott, Sherburne, Sibley, Stearns, Steele, Stevens, Swift, Todd, Traverse, Wabasha, Wadena, Waseca, Washington, Watonwan, Wilkin, Winona, Wright, Yellow Medicine

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$815,000**
	Amount Spent:	**$774735**
	Balance:	**$40,265**

Legal Citation: M.L. 2017, Chp. 96, Sec. 2, Subd. 08a as extended by M.L. 2020, First Special Session, Chp. 4, Sec. 20

Appropriation Language:
$815,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota in cooperation with the Departments of Agriculture, Natural Resources, and Transportation and the Board of Water and Soil Resources to produce site-specific recommendations for roadside plantings in Minnesota to maximize the nutritional health of native bees and monarch butterflies that rely on roadside habitat corridors. This appropriation is available until June 30, 2020, by which time the project must be completed and final products delivered.
M.L. 2020 - Sec. 2. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2021]

I. PROJECT TITLE: ***Optimizing the nutrition of roadside plants for pollinators***

II. PROJECT STATEMENT:

Pollinators have suffered steep declines over the last two decades. We rely on bees for pollination of over 70% of crops; however, some species have declined by 90% over the last two decades. Monarch butterflies, which are so loved by the American public that they would spend over $4 billion to save them, have declined more than 80% over the last 20 years. From the White House's recent "Pollinator Research Action Plan" to state-specific legislation from Minnesota to California, there are increasing calls for pollinator conservation, including for 7 million acres of new pollinator habitat. One promising direction is the use of roadside habitat as a source of pollinator larval and adult resources. Within Minnesota alone, there are over a million acres of roadside habitat.

This research will result in site-specific recommendations for roadside plantings in Minnesota to maximize the health of bees and butterflies that rely on such habitats as conservation corridors. While roadsides are promising pollinator habitat, there is a concern they may act as ecological sinks, which attract pollinators, but result in declines in pollinator health. For instance, plants along roadsides can accumulate sodium from salt runoff. Sodium is a potent attractant for many animals, and while it is an important micronutrient, they can feed on it to the point of toxicity. Nitrogen also accumulates on roadsides, potentially attracting animals with protein-poor diets to areas that also suffer from heavy metal buildup from brake and tire wear. Pesticide spillover from adjacent agricultural fields may additionally limit the quality of roadside habitats. Because different plants accumulate chemicals to different degrees, this research aims to develop recommendations for plantings for various road use intensities that optimize the value of roadside milkweed for monarchs and flower pollen and nectar for bees.

The first activity of this work will survey roadside plants and pollinators at 50 sites across Minnesota, measuring the nitrogen, sodium, heavy metal, and insecticide content of leaves, nectar, and pollen from four species of plants favored by pollinators. The second activity of this work will use data from the roadside surveys to rear monarchs and bumblebees in nutritional conditions simulating high, medium, and low use road intensities to determine levels at which nutrients and heavy metals become toxic. The third activity of this work will use controlled field manipulations to determine how plants of different families accumulate nutrients and heavy metals under conditions simulating high, medium, and low road use intensity. Taken together, these data will speak to which species of plants should be prioritized in the restoration of roadsides for pollinators.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of *[January 1, 2018]***:** As detailed below, we have made substantial progress with our roadside surveys (Activity 1), sampling soil, milkweed, goldenrod, monarchs, and bumblebees from over 50 sites across the state, ranging in traffic volumes from 500-20,000 vehicles per day. We are currently processing these samples and expect to have data on nutrient and heavy metal content of the leaves and soil by the end of December 2017, and similar data for the lower mass samples by early 2018. Analyses of these data will be run Jan-March so we can focus our priorities for the 2018 field season.

With respect to pollinator rearing (Activity 2), we have primarily made progress in terms of monarch rearing under high and low sodium conditions, showing that there is substantial variation in how susceptible monarchs are to increasing sodium, and for many lines, moderate increases in dietary sodium can have somewhat positive effects on flight performance. With respect to controlled plant manipulations (Activity 3), we piloted manipulative plots of milkweed at Cedar Creek field station in 2017, mostly learning that the heavy metal treatments representative of high traffic sites were more stressful to the plants than expected – for future manipulations we will focus on establishing the plants more completely prior to the start of the manipulations (and/or spreading out the manipulations).

Project Status as of *[July 1, 2018]***:** As detailed below, we have made substantial progress over the last 6 months on all three of our activities. We have completed preliminary analyses of 2017 field season data and set several 2018 field season goals based on these analyses. With respect to Activity 1, we have begun our 2018 roadside sampling, focusing on roadside sites with greater native species richness and choosing sites to maximize our power in determining the effects of traffic volume and adjacent road use. With respect to Activity 2, we have focused our 2018 monarch experiments on manipulating zinc and sodium, in part using an artificial diet we have been perfecting for monarchs. With respect to Activity 3, we have established our field plots and have begun experimental manipulation of nitrogen, sodium, and zinc to investigate plant family-specific reactions to these nutrients/metals in a controlled setting.

Amendment Approved by LCCMR 6/29/2018: We are requesting a budget amendment because two of our spending categories have changed. First, we had originally allocated $8000 in consulting fees to cover our collaborative discussions with the DNR. Since starting these meetings, our contacts at the DNR have determined that the effort involved in the meetings is better served as a *pro bono* consultation effort. Given that our supply costs are running higher than expected to date, we would like to re-allocate that money towards supplies across all three activities. In particular, costs of soil, tools, pots, and watering associated with plantings and rearing in Activity 2 and 3 are all running higher than we had originally estimated. For example, greenhouse fees associated with space, pots, soil, cleaning, and pest control for Activity 2 and 3 have been running $250-$500 *per month* (400+ potted milkweeds for Activity 2 and 900+ potted 14 species for Activity 3). Second, we had originally budgeted for our field technician to be employed through September 2018, one month after the end of the 2018 field season. Lauren Agnew (our current technician) will be transitioning to become a graduate student research assistant (working on the bee component of Activity 2) starting at the end of August. Thus, we have an extra month of her salary (and fringe). We would like to re-allocate this towards travel expenses, both for Activity 1 and Activity 3, both of which are on track to run higher than originally projected. We are having to take more trips to Cedar Creek this field season (and likely in 2019) to setup, weed, and monitor our plots (e.g., we had thought it would take a day to plant all of the field experimental plots for Activity 3, but it took 4 days!). Similarly, our 2018 costs for monitoring roadside sites are on track to run higher than 2017 because we needed a larger vehicle to hold more samples ($912/month vs $780), and we have planned an extra month of sampling to monitor June blooming plants (and associated costs of hotel/food during this time). With this amendment request, there are no changes to the goals or expected outcomes of this work. Please see summary of changes in the budget as well.

Project Status as of *[January 1, 2019]***:** As detailed below, we have made progress in several areas over the last 6 months. We have completed all sample collection for Activity 1 and are immersed in data analysis and manuscript preparation for samples collected in 2017 and are in the midst of submitting and analyzing samples from the 2018 field season. We have completed diet manipulations for sodium and zinc for monarchs and a suite of experiments testing sodium discriminability in monarchs. We have installed and harvested plant tissue from a large fully factorial experiment manipulating sodium, zinc and nitrogen for a dozen plant species used in roadside restoration. While many results are pending, data so far suggests that sodium and zinc levels along roadsides are potentially concerning for monarchs, but only for high traffic roads and plants close to roadsides. While egg-laying monarchs deposit eggs on high sodium plants, caterpillars are able to avoid the most toxic plants to some degree. In the next year, we will turn our attention more to bees, investigating a wider range of plants, nectar chemistry and the developmental effects on bumblebees.

Amendment Approved by LCCMR January 17, 2019: In our summer 2018 field season, our travel expenses for Activity 1 were higher than expected (by $1840). This is because we covered way more ground than originally estimated in order to resample sites at different times of the summer and obtain replicate sites within each of our categories of interest. Field technician Lauren Agnew drove 13,575 miles for Activity 1 this summer (we had estimated 9000) so fleet services charges were higher than originally budgeted. At the same time, travel expenses for Activity 2 were lower than expected as all needed butterflies were collected on campus and travel expenses for Activity 3 were also lower than expected because most travel to Cedar Creek field station was done with personal vehicles, which was cheaper than using fleet services. Thus, we are re-allocating money from the travel budget of Activity 2 and Activity 3 to the deficit in Activity 1. Note that all travel for Activity 1 has now concluded. With this amendment request, there are no changes to the goals or expected outcomes of this work. Please see summary of changes in the budget as well.

Project Status as of *[July 1, 2019]***:** As discussed below, we have made substantial progress in several areas. With respect to Activity 1, we are in the midst of writing for data from 2017 and data analysis for samples from 2018. Preliminary analyses show strong signatures of road characteristics (traffic volume, distance from road) for the sodium and zinc content of plants with some differences depending on the type of plant. We have received pesticide data back for about 50% of tests we plan to run. Preliminary analyses show pesticide residues on at least 25% of roadside milkweed, although it is dominated by herbicides and fungicides. With respect to Activity 2, we are in the midst of writing and analysis for data on metal and sodium toxicity and behavioral responses in butterflies (monarchs and cabbage whites). This field season, we are focused on measuring sodium toxicity in nectar and pollen for bumblebees, looking at interactions between metal exposure and nitrogen, and testing effects of sodium exposure on performance in the field in monarchs. Finally, with respect to Activity 3, we are in the process of analyzing data and writing a paper on species-specific plant variation in responses to nitrogen, sodium and zinc from a controlled 2018 field experiment. We are in the middle of a large greenhouse experiment to further test these ideas but also considering nectar allocation. We are very excited about the results we have to date and the directions this research is taking us this summer. We are on track for having basic roadside

restoration recommendations by 2020. Indeed, data to date has already entered into a GIS-based model and a habitat calculator for roadside restorations for monarchs.

Amendment to change project manager's FTE Approved by LCCMR 8/13/2019. This Amendment request includes two parts. First, we would like to begin the process for a legislative extension, which we are seeking primarily because we need more time to complete Activity 2. We were delayed by one year in recruiting a student to head up the component of this aim looking at sodium and metal toxicity in bumblebees. We will need the additional field season (2020) and following year to complete experiments and sample analysis. Second, we would like to shift some of the salary funds across personnel categories, although not between activity codes. In our original budget, we had allocated funds to cover 6 summer research assistantships across several graduate students. However, students on the project have been fortunate to receive both fellowship and department support and it is now clear that only 2 of those 6 RAships are necessary. At the same time, the number of papers associated with the project has grown larger than anticipated (we now project at least 16 resulting papers, half of which are actively in prep or revision. To manage some of the analysis and writing workload, the PI (ESR) is dedicating a portion of her 2019 sabbatical to leading at least three of these papers and contributing heavily to all additional papers and experiments. If approved, this amendment request would increase ESR's FTE from 12% to 22% for the 2019 fiscal year, distributed between summer salary 2019 and fall sabbatical salary 2019.**Project Status as of** ***[January 1, 2020]*****:** We have made progress summarizing our findings in manuscripts, submitting papers on the metal and salt content of milkweeds found on roadsides across Minnesota, and the impacts of zinc (the most elevated roadside metal in our survey) on monarch development. We continue work on manuscripts investigating the effects of roadsides on the nutrient, metal and salt content of a range of other native plant species used by pollinators in roadsides, and a survey of pesticide residues on roadside plants. We have also worked the last 6 months to wrap up a very productive 2019 field season where we a) studied the effects of road salt on monarch performance in the field, b) considered interactions between nitrogen, metals and salt on butterfly development, c) evaluated the effect of nectar and pollen sodium on bumblebee development, and d) tested for impacts of roadside nutrient conditions on plant leaf and nectar chemistry in a controlled greenhouse study. PI ESR summarized findings and recommendations to-date at the 2019 annual Center for Transportation Studies conference. Overall, our findings are supporting the value of roadsides as habitat for pollinators – it is likely that plants on only the highest traffic roads (and those directly adjacent to the roadside) are toxic to pollinators with respect to metal and salt content. However, we are concerned about the frequency of pesticide residues on roadside plants, although the most worrisome chemical is one slated to be banned in the by the EPA.

Amendment Approved by LCCMR February 4, 2020: As a result of our intense 2019 field season rearing butterflies and bees, we overspent our Activity 2 supply budget by $380. However, we have extra funds allocated to supplies in our Activity 1 budget, which is mostly complete apart from remaining costs to post associated with dry ice for shipping samples for analysis. We would like to reallocate $1200 in supply costs from Activity 1 to Activity 2. Activity 2 has been somewhat more expensive than originally projected, mostly because we have been rearing thousands of insects to assess effects of sodium, metals, and interactions with nitrogen. Rearing supplies include cages, tubes/stands to hold plants/milkweed, insect tags, and artificial diet ingredients.

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

Project Status as of ***[July 1, 2020]*****:** During the last six months, the progress of the project has been substantially altered by the Covid-19 pandemic, which has altered our plans for the 2020 field season and significantly delayed three sets of samples due to lab closures. More details are provided below, but these issues led to a Covid-related grant extension to June 30, 2021 as approved by the MN Legislature in mid-June. We have made progress over the last six months in terms of data analysis and publications. Manuscripts now in print describe how salts and metals in roadside milkweeds tend to be lower than levels that are toxic to monarchs. Pending analyses follow-up on this work by showing that traffic effects tend to be more important than adjacent land use (for plant metal/salt level) and levels of salts and heavy metals in roadside nectar and pollen tends to be lower than reported in studies on heavily polluted sites. These observations overall support the idea that roadside habitat is promising for monarchs and bumblebees, at least with respect to toxicity of plants. However, plants from higher traffic roads (>20,000 cars/day) or closer to the road edge are more toxic, so restoration efforts for pollinators should be prioritized to lower traffic roads (e.g., avoiding urban highways). Over the next six months, we will work to catchup on analyses and resulting writing that was delayed due to lab closures in addition to working through a revised field season plan to assess metal toxicity levels for bumblebees (see Activity 2).

Project Status as of *[January 1, 2021]***:** Over the last six months we have successfully caught up with respect to covid-complications – labs re-opened and pending samples and datasets were processed and analyzed. We performed one field experiment on the interacting effects of sodium and zinc on monarchs, a field experiment on heavy metal loads in bees, and a lab experiment on zinc toxicity and immunity in butterflies (all related to Activity 2). We continued data analysis and writing with respect to all activities: two papers were published with relevance to Activity 2, two were revised/submitted with respect to Activity 2, one was submitted with respect to Activity 1 and one completed with respect to Activity 3. Over the final six months of the grant we plan to finish as many analyses and papers as possible and potentially run one final experiment on metal toxicity in bees (which was cancelled this summer due to Covid.

Overall Project Outcomes and Results [June 30, 2021]**:** Insect pollinators have suffered steep declines over the last two decades. Roadsides are a promising opportunity for pollinator conservation, potentially providing millions of acres of habitat, and acting as dispersal corridors. However, roadside habitat also contains pollutants such as heavy metals from car wear and past leaded gasoline use, sodium from road salt application, and pesticides from adjacent agriculture. In this research, we combined surveys of roadsides across Minnesota, with controlled lab and field experiments, to test how such roadside pollution impacts insect pollinators, and implications for restoring roadside habitat for monarch butterflies and native bees. Our results suggest that plants alongside the majority of Minnesota roadsides have sodium and metal content below which is worrisome to bees and monarchs. However, plants along very high traffic roads, especially those right next to the road, likely have negative effects on pollinator health. Our data also suggest that pesticides may be a significant concern for 5-10% of roadside plants. This research suggests roadside restoration efforts should focus on roads with low to moderate traffic volumes (<20K cars daily) and that mowing a buffer on the road edge should eliminate the most toxic plants. Recent national efforts to ban the pesticide chlorpyrifos would also be beneficial for Minnesota roadsides as this was the most commonly detected insecticide. Finally, this research suggests benefits to planting a diversity of roadside plants as species accumulate different toxins to different degrees, although on higher traffic roads, managers may want to avoid a handful of high accumulating species (e.g., yellow coneflower). Overall, from a nutritional perspective, Minnesota roadsides are promising habitat for insect pollinators, for instance, potentially producing 14M migratory monarchs annually. Future work should consider management methods that may minimize vehicle collisions, as currently pollinator mortality from collisions likely far exceeds that from plant toxicity.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: *Survey of roadside plants and pollinators*
Description:

The first aim of this research is to document the nutrient content of roadside plants and pollinators along Minnesota roadways. Drawing from an existing network of roadside sites randomly distributed across non-forested areas of the state, we will survey 50 sites across varying degrees of road use intensity and adjacent agricultural use. Plant samples will be taken of four focal native species commonly used by pollinators, including common milkweed (Apocynaceae: *Asclepias syriaca*), gray-headed coneflower (Asteraceae: *Ratibida pinnata*), bee balm (Lamiaceae: *Monarda fistulosa*), and showy tick trefoil (Fabaceae: *Desmodium canadense*). Importantly, these plants are in bloom in July (when sampling will occur) and are commonly found along Minnesota roadsides. We will measure the nutrient and heavy metal composition of leaves, pollen and nectar of these samples, including nitrogen, phosphorus, sodium, nickel, zinc, cadmium, and lead. A subset of milkweed samples will be used to measure pesticide content.

At each site we will additionally sample pollinators using these roadsides to test whether a nutrient and heavy metal signature can be seen in insect tissue. We will focus our pollinator sampling efforts on larval monarchs (*Danaus plexippus*), a common native bumblebee that flies in July (*Bombus impatiens*), and an easily identifiable native bee with a smaller foraging range (*Agapostemon virescens*). At each site, we will take data on additional factors known to affect nutrient and heavy metal deposition, including roadside slope, wind direction, and adjacent areas where traffic changes speed. We predict that the nitrogen, sodium, and heavy metal content of plant leaves, nectar and pollen, along with insect tissue itself, will increase as road use intensity increases.

Summary Budget Information for Activity 1: **ENRTF Budget: $378,030**

Amount Spent: $ 365,305
Balance: $ 12,725

Outcome	Completion Date
Collection of roadside plants, soil, and pollinators across two field seasons	Aug. 2018
Nutrient, heavy metal & pesticide content of roadside plants and soils	Dec 2018
Measurement of pollinator nutrient, heavy metal content	Dec 2018

Activity 1 Status as of *[January 1, 2018]***:** We have made substantial progress on Activity 1. Field technician Lauren Agnew led a successful first field season with assistance from Rebecca Meyer. They sampled over 50 roadside sites from across the state of Minnesota, covering approximately 6,000 miles over a 6-week period (July-August). Sites were chosen based on pilot data of randomly selected roadside plants that contained milkweed, but structured to span a range of traffic volumes, from 500 to 20,000 cars per day. At each site, soil, plants, and pollinators were sampled at three widths from the road. In sum, they collected over 200 soil samples, 200 milkweed leaf samples, 100 milkweed nectar samples, 100 monarch caterpillars, 80 bumblebees, and 20 goldenrod leaf, nectar and pollen samples. Since September, Lauren has been working to organize and process the samples in the lab. 100 soil samples and 200 milkweed leaf samples have been submitted to the University of Minnesota Research Analytical lab for ICP analysis of sodium, phosphorus, nickel, lead, cadmium, and zinc. We are in the process of preparing these samples for measurement of carbon and nitrogen on an element analyzer. We expect data on element content of these samples to be available by 12/31/17. The pollinator samples have been weighed, and test samples submitted to determine which of two machines to use for these much lower mass samples. A test nectar sample has also been submitted to a lab collaborating with co-PI Clay Carter. We hope to have the methods worked out for these small samples by January 2018.

Looking ahead, we have hired a postdoc (see further details under Activity 3) to start processing this dataset in January. All roadside site data has already been compiled, and we expect the postdoc (Kristin Sikkink) to run statistical models to determine the major contributing factors that determine roadside plant nutrition using predictor variables such as traffic volume, distance to road, soil characteristics, adjacent agriculture, adjacent speed changes, roadside slope, road width, and geographic location. These analyses will inform the next steps for all three activities. Based on these results, we will meet as a team in March (also with the DNR) to set priorities for the 2018 field season for all activities. Given our success at sampling milkweed, but our limited success with sampling other native roadside plant (very low – there are very few other natives that are as common), for Activity 1, we may take a more targeted approach, driving until we locate focal species such as coneflower and beebalm, which we rarely encountered in our 2017 sampling. Depending on our statistical power, we may also supplement our existing dataset with additional data for milkweed, especially in June, for which we have no samples yet.

Activity 1 Status as of *[July 1, 2018]***:** We have made substantial progress in data analysis from our 2017 field season and have already begun sampling for the 2018 field season. Analyses to date suggest that levels of sodium and zinc in roadside milkweed are tightly linked to road use – Na and Zn content in leaves increases with traffic volume and distance from the road from sites surveyed across the state of Minnesota. Levels of other heavy metals (Cd, Pb, Ni) are low and not noticeably tied to traffic along roads, but possibly to adjacent land use. Relative to data we have from our lab (and other studies) on butterfly and moth survival with increasing sodium and heavy metal exposure, we are most concerned with the levels of sodium measured in roadside leaves (see Activity 2). Measurement of nitrogen, sodium, and heavy metals is complete for our 2017 leaf and soil samples. We are still working out methods to measure our low mass samples and have switched to using a different lab with more extensive expertise using ICP-MS with very small volumes for these samples. Postdoc Tim Mitchell (see also Activity 3 below) has taken over data analysis and writing up results from the 2017 field season and has an active draft of the main paper coming out of the 2017 field work, which will focus on roadside milkweed and monarchs, while Lauren Agnew will direct another paper coming out of this activity, focused on bee and pollen nutrient/heavy metal content.

Based on analyses of 2017 data, and meetings as a grant team, we had two priorities for the 2018 field season. First, a random sampling of MN roadsides resulted in sampling of few native plants used by pollinators, apart from common milkweed. Thus, we have revised our site selection protocol for the 2018 field season to focus on roadside areas that were previously seeded with native seeds by MnDoT or are otherwise enriched for native plants, for instance because they are adjacent to high quality prairie. To locate such areas, we have met with both MnDoT and DNR and generated a list of 30+ roadside sites to target for a greater diversity of roadside plants. To round out our sampling for each plant family (3-4 species each), we may also substitute 1 or 2 non-native species (e.g., Fabaceae). Second, the power of our statistical analyses to detect the effects of adjacent road use was

greatly limited by our 2017 site selection protocol (randomly pulled sites) and the failure to focus on just a few land use categories. Thus, we have substantially focused our site selection procedure for the 2018 field season to sample plants in a fully factorial way from three categories: high/low traffic volume (<2000 cars/day and >10000 cars/day), with or without adjacent agriculture, with or without adjacent active railroad. Preliminary analyses suggested land use category may be associated with roadside plant heavy metal content for some heavy metals, but our analyses were limited by the fact that some of these variables were confounded for the 2017 field season – our sampling protocol for 2018 will allow us to tease apart these factors. We decided to include adjacent rail use because so many of the high diversity sites for native plants are also adjacent to railroads. Finally, given the high snowfall of the 2017-2018 winter, we are re-sampling a subset of our 2017 sites to determine a closer-to-upper limit for sodium content of roadside milkweed and we are starting our sampling earlier in the season (June) to account for early blooming species and temporal changes in plant sodium content.

Activity 1 Status as of *[January 1, 2019]***:** In 2018, we completed all of our sample collection for this Activity. Field technician Lauren Agnew and assistant Annika Herdtle traveled over 13,000 miles around the state of Minnesota June-August 2018 collecting samples for this activity. They aimed to sample roadsides in a fully factorial design of high-low traffic volume, adjacent agriculture, and adjacent railroad in at least four regions throughout the state. They collected leaf, nectar and pollen samples from plant species from four different families (milkweeds, several legumes, asters, and mints) in addition to bees and milkweed for pesticide analysis. A subset of sites were sampled several times during the season. Analysis of 2018 samples is pending.

In addition, we have made substantial progress in the analysis of 2017 samples. First, all milkweed leaf samples and soil samples have been analyzed and postdoc Tim Mitchell is heading up the paper using these samples to describe the nutritional content of roadside milkweed for monarchs. Results suggest that sodium and zinc levels in milkweed (which scale with traffic and distance to roadside) are the most concerning, sometimes reaching levels that are toxic to monarchs – however, prioritizing restorations on low to medium traffic roads and creating a buffer strip just adjacent to the road will likely minimize this toxicity problem. We anticipate this paper to be submitted by February 2019. Second, we have submitted our pollen, nectar, bee and monarch caterpillar samples for ICP-MS analysis. It took a while to find a lab capable of reliably measuring nutrient and metal content of our very small pollen and nectar samples and we are awaiting the results (samples were submitted in September). Now grad student Lauren Agnew will be leading this manuscript (to be written in 2019). Third, we have submitted our milkweed samples to a USDA facility for profiling of 150 pesticides to test for insecticide spillover from adjacent agricultural fields. So far, we see no pesticide residues on milkweeds adjacent to residential sites, and natural areas, however, about a third of samples contain pesticide residues (of varying chemicals) when they are collected adjacent to corn and soybean. We will be sending a larger sample of our milkweed samples to the lab for analysis in January 2019 for a more complete picture of which pesticides may be an issue (and whether they reach toxic levels for monarchs). We anticipate this dataset to lead to a smaller publication on pesticide spillover issues for roadside milkweed.

Activity Status as of [July 1, 2019]. While field work for this activity is complete, we have allocated time towards sample processing, sample submission, waiting for sample analysis (labwork has been taking 4-8 months), and results analysis and writing. We have made several advances in this activity over the last 6 months. First, we have completed two of four sets of analyses for pesticide residues on roadside milkweed. Results from the USDA lab shows that, depending on the site and year, 25-50% of roadside milkweeds have pesticide residue, although the most common residues are herbicides and fungicides (not insecticides). We are currently working to analyze these data and determine which levels of insecticide residues are of concern. Second, we have just received back data on leaf chemical composition (metals, sodium, phosphorus, nitrogen) for almost 200 plant samples collected in our 2018 field season teasing apart effects of adjacent land use (agriculture and railroad) and traffic volume for a range of plants important to insect pollinators (not just milkweed, the focus of our 2017 sampling). We will be analyzing these data over the next few months. Third, we have submitted nectar and pollen samples from our 2017 field season (still waiting for results) and are processing nectar samples from our 2018 field season for ICP-MS analysis of sodium and metal content. Fourth, we have received back data on the metal and sodium content of roadside monarch larvae and bees (the former data piece is going into a current paper in prep and the latter will be analyzed this summer to go into a paper focused on roadside bumblebees).

Activity Status as of [January 1, 2020]. We have three manuscripts related to this activity in prep or in review. First, our manuscript on the nutrient, metal and salt content of roadside milkweed is currently in review at *Ecological Applications*. Here, we show that leaf sodium and zinc content is predicted by traffic volume and distance from the road, but the majority of sites have levels below that which are harmful for monarchs. Second, we have made substantial progress on a manuscript investigating nutrient and metal content of 6 species of roadside plants used by pollinators, also considering the effects of adjacent agriculture and railroad more

explicitly. Here, metal and sodium content of most species is predicted by road characteristics more so than adjacent land use, and except for the highest traffic roads (and just adjacent to the road), metal and salt levels tend to be at non-toxic levels (we hope to submit this paper by May 2020). Finally, we have started work on a paper summarizing pesticide residues of roadside milkweed. We are awaiting data back on an additional 20 leaf samples, and also plan to run analyses of nectar for one insecticide of particular concern (chlorpyrifos). Of the toxins we have surveyed for as part of activity 1, we are currently most concerned about the pesticide piece. We see insecticide residues on 30-40% of roadside milkweed leaves and a quarter of these may contain levels that are lethal to developing monarchs (we hope to submit this paper by May 2020). One final note: we hope to have data in Feb/March on the metal and sodium content of roadside nectar and pollen. After submitting samples to a lab earlier this year, the lab unexpectedly closed. We had to find two alternate labs to run the samples, but we are back on track.

Activity Status as of [July 1, 2020]. Our milkweed findings have been published in Mitchell et al 2020 Science of the Total Environment: *Traffic influences nutritional quality of roadside plants for monarch caterpillars*. This manuscript show that the sodium (and zinc) content of roadside milkweed increases with traffic volume and distance from road; monarch caterpillars feeding on high sodium plants also have higher sodium body content. However, most milkweed metal and salt content is significantly below that which is highly toxic to monarchs, so the majority of Minnesota roadways are suitable monarch habitat with respect to salt and heavy metal toxicity. The spread of Covid-19 significantly impacted progress on this aim because three labs with pending sample analysis shut down. All three labs have since re-opened and are in the process of finishing samples or transmitting data back to our lab. *Three sets of analyses (and corresponding papers) are in progress with respect to this activity*: a) analysis of metal and salt content of roadside nectar, pollen, and bumblebees (led by PhD student Lauren Agnew), b) analysis of roadside forbs metal and salt content to tease apart relative effects of traffic, or adjacent agricultural or railroad (now led by PhD student Alex Shephard), and c) analysis of pesticide residues in roadside milkweed and nectar (led by recent graduate Megan Kobiela). The most relevant findings across these analyses so far include a) heavy metal content of roadside pollen and nectar is lower than expected based on previous research based on highly polluted sites, b) traffic has greater effects than adjacent railroad or agriculture in determining plant nutritional content, and c) while milkweed leaves have some pesticide residues, they are lower in frequency than recent studies in the Western US and the residues do not appear to be present in nectar.

Activity Status as of [January 1, 2021]. After the spring Covid-lockdowns of several labs processing our samples, we have since received data back on nectar pesticides, bee and pollen heavy metals, and remaining leaf sodium/metal content. We made significant progress over the last six months on several papers and data analyses relevant to this aim. First, we submitted a paper (currently in review at Ecological Applications), led by Alex Shephard, detailing the heavy metal content of several roadside forb species commonly used by pollinators – the findings are that traffic volume, more than adjacent landuse – impact the element content of plants used by monarchs and bees. Levels of metals and salts are generally lower than what is toxic to these species. Second, we have analyzed preliminary results of pesticide residues in nectar, finding that the commonly insecticide chlorpyrifos, does seem to be getting into the nectar of roadside plants (we are currently awaiting GC-MS data on the oxidized form of this pesticide, and this manuscript is in preparation). Third, we received our final set of bee and pollen metal ICP-MS data and have begun analyses for a manuscript on the threats of roadside pollen and nectar for native bumblebees. Preliminary analyses so far suggest that copper may be the only heavy metal of potential concern, as it does appear to be elevated in nectar from high traffic roadsides. Over the final six months of this grant, we plan to finish our analyses, and hope to submit the remaining two papers relevant to this activity (one on pesticides and one on bees/pollen/nectar).

Final Report Summary *[June 30, 2021]:* Research within this activity has generated six publications (three in revision or in prep). Several themes have emerged across these studies. First, the salt and metal content of roadside plants increases with traffic volume and decreases with distance from the road and traffic variables tend to be more important for plant traits than adjacent landscape use. Second, field observations suggest pollutant content of roadside plants varies with species (highest in some species in Asteraceae) and soil type (lower in sandy soils). Third, roadside salt (sodium) and copper pollutants appear to move into plant nectar, suggesting that both plant leaves (e.g., feeding monarch caterpillars) and nectar (e.g., feeding adult butterflies or bees) are potential inputs for insect pollinators using roadsides. Data on variation in roadside plant nutrients, metal and salt content across the state of Minnesota are combined with data on toxicity (Activity 2) and experimental work on these same plants (Activity 3) to generate overall conclusions and management recommendations (see abstract).

ACTIVITY 2: *Pollinator health in different nutrient conditions*
Description:

The second aim of this work seeks to determine levels at which nutrients and heavy metals become harmful to pollinators. Data from Activity 1 will be used to parameterize diets representative of low, medium, and high road use intensity for both monarchs and bumblebees.

We will use *Bombus impatiens* as a model bumblebee system. Nitrogen, sodium, nickel, and zinc will be manipulated in pollen balls of 60 queens, mimicking concentrations measured in pollen in activity 1. We will measure development time and body size of 10 offspring per queen. *Danaus plexippus* (monarchs) will be reared on milkweed grown in conditions meant to simulate low, medium, and high road use intensity. Leaves will be harvested from field plots for rearing of monarchs under controlled lab conditions where only nutrition is manipulated. We will measure survival, development time, and adult body size for at least 30 individuals per treatment.

Data on size and development time will address whether roadside nutrient levels are harmful to pollinators. We predict that growth parameters will be highest at the medium-level road use intensity, and lowest at the high level. Additionally, we will compare levels of pesticides measured in Activity 1 to levels found in other studies to be toxic in bumblebees and monarchs.

Summary Budget Information for Activity 2:

ENRTF Budget: $195,200
Amount Spent: $ 187,875
Balance: $ 7,324

Outcome	Completion Date
Determine optimal nutrient conditions for developing bumblebees	Dec 2020
Determine optimal nutrient conditions for developing monarchs	Dec 2019

Activity 2 Status as of ***[January 1, 2018]*****:** To date, our efforts on Activity 2 have focused on monarch performance with respect to dietary sodium. This summer and fall (2017), grad student Megan Kobiela conducted a large rearing experiment, rearing monarchs on control of sodium-treated milkweed. She reared over 300 individuals from 10 family lines, measuring a wide range of performance traits, such as survival, body size, development time, cold tolerance, flight muscle and flight abilities. So far, she has found that increasing sodium somewhat reduces survival, but in a family-specific way. Some families are completely tolerant of increasing sodium content. In addition, preliminary results so far are also consistent with previous observations that at least moderate increases in dietary sodium can have positive influences on flight muscle – males on the higher sodium diet were able to fly faster and for longer than those on the control diet, consistent with the role of sodium as an important micronutrient. In addition, student assistant Max Marckell spent several weeks this summer compiling existing data on the toxicity levels of heavy metals and common pesticides for both bees, butterflies and moths. We expect this literature to complement the data we are generating from our rearing experiments in determining hazardous levels of roadside chemicals.

Looking ahead, we are currently trying to recruit a graduate student to focus on the rearing of bumblebees. It is likely that another student in the Snell-Rood lab will take over the monarch manipulations starting summer 2018, focusing on manipulating sodium, nitrogen, and heavy metal content of the diet. Given some of the challenges with field manipulations of milkweed (see Activity 3), we also plan to explore the possibility of using both a plant-based and an artificial diet for the monarch rearing so we can more precisely control the nutrient content of their diet. Regardless, we expect the data analyses from Activity 1 to be complete in March – this information will inform the upcoming diet manipulations. Finally, given the delays in recruiting all of our grad student support for this aim, we will likely extent the completion date to 2019 for this aim and plan to commit two more field seasons. Please note that we classify university greenhouse charges related to Activity 2 and 3 in our “supply” budget as these costs include costs of pots and soil used to raise plants for monarch rearing and field manipulations.

Activity 2 Status as of ***[July 1, 2018]*****:** Over the last 6 months, we have worked to align our roadside milkweed data (Activity 1), with what we know one optimal values for butterflies and moths, to determine our next experimental steps. Based on research to-date by Megan Kobiela (see above), levels of sodium in roadside milkweed seem to be the most alarming – we start to see survival impacts at 250 ppm Na, but have been measuring sodium concentrations up to 2000 ppm. Values from the literature on other species of butterflies and moths suggest that the levels of heavy metals we have measured so far (for Zn, Ni, Pb, Cd) may not be concerning. Zinc appears to be the one heavy metal most tightly linked to traffic use and we will be running

experiments this summer to determine whether the levels we have measured (up to 70 ppm) are concerning for monarch performance (for other butterflies/moths, we don't see effects until 200 ppm). Grad student Alex Shephard has been refining an artificial diet for monarchs in the lab, which will allow us to precisely manipulate dietary Zn content in rearing experiments planned for July-Sept 2018. Alex also plans to test a greater range of sodium and heavy metals using this artificial diet (possibly Cd or Ni if links with adjacent road use pan out).

With respect to measuring performance of bumblebees, our main update is with respect to planning. Lauren Agnew (current field technician, and bee expert on the grant) will be joining the lab as a PhD student fall 2018. She will begin nutritional manipulations of bumblebees starting in 2019 and going into 2020. Given this delayed timing, we will revisit in 2019 whether we will need to extend the timeline for Activity 2. In the meantime, we have started planning for these manipulations, for instance by discussing queen vs. worker manipulations and obtaining rearing boxes.

Activity 2 Status as of *[January 1, 2019]***:** We have made substantial progress in our quantification of toxicity levels for monarchs. Our roadside analyses to date suggest zinc is the roadside element of most concern (based on levels in milkweed and how it scales with traffic and distance to the road). Based on this, we ran several experiments in 2018, focusing on using an artificial diet to measure zinc toxicity in monarchs – it begins to show effects on survival between 50 and 100 ppm (our max values are 70 ppm on very busy roads, but generally less than 50 ppm). We repeated the experiments using a species of less conservation concern and found these levels to be non-toxic – this, coupled with data from the literature on pest moth and butterfly species suggests monarchs are more sensitive to heavy metal exposure than disturbance-adapted pest species. Grad student Alex Shephard led these experiments and already has a draft paper written up of the results – we expect to submit this paper spring 2018. On the sodium front, Megan Kobiela made progress in completing her monarch measurements and analyses in looking at sodium toxicity and will be writing up this paper in the spring.

With respect to bumblebee performance, we have started conversations in regards to the design of the rearing experiments we will start in 2019. Given that grad student Lauren Agnew just started in the lab in September, we are hoping to defer her additional year of funding by one year so we can have two full field seasons of rearing work with bumblebees. I would like to talk with LCCMR staff about this process as I understand we will have to go through the main LCCMR process in order to extend Aim 2 [spoke with staff in January and June 2019].

Activity 2 Status as of *[July 1, 2019]***:** Over the last 6 months, we have made significant progress in terms of analysis, writing and publication of Activity 2 results from previous years. First, we have a paper in press investigating sodium preferences in adult and larval monarchs – these data suggest that egg-laying monarchs are not being actively attracted to sodium, however, they also fail to discriminate against milkweeds containing toxic levels of sodium (e.g., as along high traffic roads). There is a tendency for caterpillars to avoid the most toxic sodium plants. We are working with citizen scientists to follow-up on these results by determining how far individual caterpillars move and thus whether they may regularly disperse away from the most toxic plants on the immediate road edge. Second, we have made progress on two papers mentioned above on zinc and sodium toxicity in monarchs. We hope to submit these papers soon.

Most of our current experimental efforts underway fall within Activity 2 (for the summer 2019 field season). First, we are currently running an experiment investigating sodium toxicity for bumblebees (*Bombus impatiens*) using locally collected queens. As there is little to no existing data on this, we are looking at a large gradient of sodium exposure in pollen and effects on developing larvae. We also plan to run trials this summer on adult worker bees, exposing wild-collected individuals to gradients of sodium in nectar. Second, we are running a number of butterfly experiments this summer looking at interactions between nitrogen (which is elevated along high traffic roads) and stress from either sodium or heavy metals (zinc in particular) along roadsides. We are using a number of butterfly species in these tests, always monarchs, but also other species such as cabbage whites, sulfurs, and painted ladies to test the broader generality across species that feed on other plants. We predict that elevated nitrogen will decrease stress associated with road salt and metals. Finally, we are attempting to run an experiment looking at the impacts of sodium on monarch performance in the field. To date, our results suggest that sodium from road salt runoff is the most concerning stressor for pollinators in roadside habitat. Results from lab work to date suggest moderate increases in sodium could be beneficial but not large increases. In addition, moderate increases in sodium have positive influences on some traits (muscle mass, cold tolerance), but negative influences on other traits (body size). We plan to test how these lab results play out in the field through an outdoor rearing experiment where monarchs are exposed to control milkweed, or milkweed sprayed with salt to mimic a low traffic volume road or a high traffic volume road typical of the I-35 "monarch corridor" in rural Minnesota (e.g., 20,000 cars/day). We will raise 1000-3000 individuals, measure individuals at emergence, tag and release individuals timed with the fall migration (centered around August 20th). An existing network of citizen scientists

(through the Monarch Watch program) will help to monitor and track individuals, potentially allowing unprecedented tests of survival in the field and flight performance all the way to the wintering grounds in Mexico. We predict that individuals reared on the low sodium treatment will have relatively higher performance in the field, but those from the high sodium treatment will have relatively lower performance (survival and flight speed).

Activity 2 Status as of *[January 1, 2020]***:** We have made substantial progress on several manuscripts and experiments related to this activity. With respect to effects of heavy metals, we have submitted a manuscript studying the effects of dietary zinc on monarch development, completed an experiment testing for interactions between zinc and nitrogen (which are both elevated along roadsides), and also worked with an independently funded postdoc in the lab to investigate the effects of metals on immunity. Here, we see that monarchs are more sensitive to heavy metals in their diet than pest butterfly and moth species, but they can tolerate levels of zinc that are seen along high traffic roads in Minnesota. With respect to the effects of road salt, we have completed two papers on the effects of sodium on butterfly performance in the lab (submitted as part of a graduate student's dissertation), and have started work on two manuscripts summarizing a large 2019 experiment looking at the effects of sodium on performance in the wild. Here we see substantial genetic variation in responses to dietary salt in local butterfly populations. Additionally, many of the positive effects of salt seem to cancel out some of the negative effects in the field. These results, coupled with the data from Activity 1, are leading to the general conclusion that with respect to butterflies, roadside plants on low to moderate traffic Minnesota roads are not toxic (<20,000 cars/day). Finally, 2019 was our first field season working with rearing bumblebees (*Bombus impatiens*). We reared offspring from queens on 6 sodium/salt treatments in addition to wild-collected workers. So far, our results suggest that we don't start to see negative effects of salt on bee performance until we reach levels much higher than we have detected so far in roadside pollen/nectar. However, we are still working on this piece. We are additionally in the process of analyzing 2019 data looking at interactions between dietary nitrogen and salt) on four species of butterflies.

Activity 2 Status as of *[July 1, 2020]*: We have made progress with respect to several manuscripts coming out of this activity. First, Shephard et al. 2020 Insect Conservation and Diversity; *Assessing zinc tolerance in two butterfly species* showed that while monarchs are more sensitive to zinc exposure than pest butterfly species, they are likely not negatively suffering from elevated levels of zinc in milkweed along most Minnesota roadsides (which tend to be <75 ppm Zn and negative impacts in monarchs are seen >200 ppm). Second, Shepard et al. 2020 (in revision, Evolutionary Applications) adds to this work by showing the local butterfly populations harbor substantial genetic variation in response to levels of zinc seen in roadside plants.

The closure of the lab due to Covid-19 has resulted in some revisions to planned experiments for summer 2020 with respect to this activity. We were planning to manipulate dietary salt and heavy metals for bumblebees to determine toxicity levels. Social distancing requirements and closures of the bee lab in May prevented the running of these experiments. We instead are taking a revised three-pronged approach: a) collecting bees from parks across the Twin Cities that vary in soil lead levels and using body metal content and measures of body condition to infer variation across species in toxicity and levels at which plant metal levels are concerning, b) fall/winter plans for rearing drone *Bombus impatiens* to infer toxicity levels (from commercial sources), and c) a meta-analysis of recent metal toxicity data from across insects to infer levels that are toxic for different pollinators.

Activity 2 Status as of *[January 1, 2021]*: Despite Covid-related shutdowns, we have made substantial progress in two areas on this activity. First, we have two additional papers on metal tolerance, led by Alex Shephard, that will be submitted within the next month or two. Both papers focus on zinc (the metal of highest concern in roadside milkweeds in MN) and find no evidence of zinc toxicity in monarchs for the levels we see in MN. Both papers consider additional interactions between zinc and other roadside pollutants (salt and nitrogen), and while there is some evidence for combined effects of multiple stressors (e.g., Zn and N stress), the levels that are concerning in the lab are rarely seen on MN roadsides. This is good news for heavy metal stress on roadsides and monarch survival. Second, we have successfully shifted our approach for inferring metal toxicity for bees (in light of Covid). Grad student Lauren Agnew collected about 300 bees this summer along an urban metal gradient and will infer levels of toxicity based on correlations between bee metal content and condition (body size). This approach will be complemented by pending meta-analyses of metal toxicity in several bee species, and a May/June 2021 lab experiment on toxicity of one metal (likely copper, see above) in *Bombus impatiens*. Finally, we published a paper (Snell-Rood et al 2020 Evolution) that includes tests of nitrogen-sodium interactions in four species of butterflies.

Final Report Summary *[August 16, 2021]***:** Research within this activity has generated 14 publications (eight in print or in press). Several themes have emerged across these studies. First, in general, the levels of salts and metals seen in the field (Activity 1) tend to be below lethal levels, especially for metals we were initially concerned

about (Pb, Cd). However, levels of salts and metals along very high traffic roads may have substantial effects on survival and performance of individuals that do happen to survive. Second, levels of pesticides measured in roadside plants are generally more concerning for insect toxicity than the metals and salts we measured, in particular for the pesticide chlorpyrifos, which also seems to be getting into nectar. Third, we find little to no evidence of roads acting as a "trap" in terms of attraction of insects to toxic places they cannot "escape" from. For example, butterflies are not attracted to high salt plants in the context of egg laying, and several species of butterflies we measured harbor substantial genetic variation in ability to deal with high salt and metal levels. These data suggest populations are robust to the pollution on most Minnesota roadways, which is good news for pollinator habitat on roadways.

ACTIVITY 3: *Plant nutrient status and pollinator preferences*
Description:

Given that different plants concentrate nutrients to different degrees, the final aim of this research is to determine which plant species would maximize pollinator health along roadsides. We will work with the DNR, MnDoT, and the Minnesota Board of Soil and Water Resources to choose 12 focal plant species with established success in roadside restorations in Minnesota. We will focus on four plant families favored by pollinators: Asteraceae, Fabaceae, Apocynaceae, and Laminaceae, choosing three different species within each family to make generalizations about how a given plant family reacts to different simulated roadside conditions.

We will grow 12 different plant species at three sites of varying soil conditions at the Cedar Creek Ecosystem Science Reserve. At each site, we will simulate the different nutrient and heavy metal conditions at low, medium, and high road use intensities, adding nitrogen, sodium, nickel and zinc to the soil at three different times. Following the experiment, heavy metal addition will be mitigated with the application of phosphorus and organic matter.

Three replicate plants per site per species will be sampled for leaves, pollen and nectar, for nutrient and heavy metal analyses. We will additionally work with the Cedar Creek internship program to survey pollinators in these plots, measuring the abundance of pollinating bees for least 10 time points during flowering. As expected for Activity 1, we predict that the nutrient and heavy metal content of plants will increase as simulated road use intensity increases**.** As pollinators tend to be attracted to high sodium and high nitrogen resources, but often fail to discriminate against heavy metal presence, we predict that pollinators will favor the high road use intensity plots.

Finally, with data from all three activities in hand, we will meet with the DNR, MnDoT, and the Minnesota Board of Soil and Water Resources to discuss optimal roadside plantings for different road use intensities.

Summary Budget Information for Activity 3:

ENRTF Budget: $ 241,770
Amount Spent: $ 221,555
Balance: $ 20,215

Outcome	Completion Date
Measure nutrients in different plant species in controlled conditions	Dec 2019
Determine pollinator preference for different plant nutrient contents	Sept 2019
Recommendations for roadside plantings to maximize pollinator health	June 2020

Activity 3 Status as of ***[Jan 1, 2018]*****:** During summer 2018, student research assistant Max Marckell ran a pilot manipulation plot at Cedar Creek. With assistance from grad student Megan Kobiela, we set up plot types meant to simulate sodium, zinc, and nickel inputs characteristic of low, medium and high road use intensities – initial values were based on the literature, future values will be based on analyses from Activity 1. We planted in milkweeds into these test plots and watered them three times with treatment salts in July and August. We harvested leaf tissue at two stages for analysis (pending) to try to determine which watering inputs result in which leaf levels. This information will be critical to the milkweed-based rearing of monarchs for Activity 2. We also learned some important lessons for the plant manipulations for Activity 3, namely that plants will need to be established in the greenhouse prior to planting into amended plots so they are well established prior to the start of the treatments, which were more stressful to these plants than anticipated. Based on this, we are also exploring other options for Activity 3 manipulations, such as in situ manipulations in roadside soils (pending discussions with MnDoT) and/or manipulations of already established plants (which worked well this summer with respect to milkweed and NaCl watering for Activity 2).

Although it took much longer than expected, we have also recruited a postdoc to direct the main components of this activity, with field seasons in 2018 and 2019. We initially interviewed three candidates in August, but unfortunately, over the following three months, these candidates either took other offers or ended up not being able to come due to personal reasons. In late December we hired Tim Mitchell, who will be starting in January with plans for experimental design for the summer. We initially hoped to have a postdoc start in this position in Oct/Nov 2017, but, due to the delays in hiring a postdoc for this project, we also decided to hire a local postdoc for just three months (Jan-March) to start pushing things forward with the data analysis necessary to start this aim (see notes in Activity 1), reducing this appointment by a corresponding three months.

Activity 3 Status as of ***[July 1, 2018]*****:** We have make extensive progress on this activity. Postdoc Kristin Sikkink directed the preliminary analysis of Activity 1 data in January-March to determine the context for the field manipulations for this Activity. Postdoc Tim Mitchell, who started in January, has been directing this activity throughout the spring semester. Our first field manipulation is well underway after first establishing experimental plants in the greenhouse in soil mixes that approximated nutrient conditions of low use roads. Plants were transferred into field plots at Cedar Creek, with 3 species per plant family for 3 plant families and 1 replicate species for one of our families (mints had very low germination success). After much discussion following our preliminary analyses of Activity 1, we are manipulating nitrogen, sodium, and zinc in a fully factorial manner where the “high level” corresponds to the 90th percentile for measured nutrient/metal levels in soil along Minnesota roadsides. For a subset of plants (1 sp. per family), we are including two additional levels, including one level higher than that measured in the 2017 field season. We will harvest plant tissue approximately 1 month after treatment and continue various plant measures (e.g., plant height) and pollinator visitation throughout the field season (once blooming has started). To increase sample size for measures of pollinator visitation, Tim will also be setting up field plots of sodium treated milkweed to measure field oviposition on sodium-enriched milkweed as preliminary analysis suggest butterflies are attracted to plants with very high sodium content.

Activity 3 Status as of ***[January 1, 2019]*****:** We have made progress on two fronts with respect to this Activity. First, Tim Mitchell led a very successful field season at Cedar Creek where we manipulated sodium, nitrogen and zinc in field plots of 12 species in a fully factorial manner. The plots were setup over a 4-day period in May and treated three separate times throughout the growing season. Leaf samples were taken from over 650 individual plants and have been dried, weighed, ground and submitted for elemental analyses (results pending). The plants have been left in the ground to overwinter and we plan to run additional manipulations and harvests or nectar and pollen in 2019 (very few plants bloomed during the first field season they were in the ground). Given the low rate of blooming on these plots in 2018, we may setup additional manipulative plots at Cedar Creek in 2019 using plants that are already established to make sure we get enough nectar samples.

Second, Tim Mitchell and PI Emilie Snell-Rood led a study looking at monarch preference for sodium, which gets at whether monarchs can avoid (or are possibly attracted to) plants with levels of sodium that are potentially toxic. We looked at preferences in both caterpillars and egg-laying females with respect to levels of increasing sodium that were toxic (found along high traffic roads) and non-toxic (found along low traffic roads) using lab, greenhouse and field assays. Results showed that female butterflies do not make egg-laying decisions with respect to milkweed sodium content, thus potentially exposing offspring to toxic levels of sodium. However, late instar monarch caterpillars are able to avoid the highest levels of sodium when making decisions about which leaves to feed on. We may run additional experiments this summer looking at how far caterpillars can move, which will get at whether individual caterpillars can leave a toxic plant close to the road and choose a less toxic milkweed further from the road. Tim has written up these results and plans to submit this manuscript February 2019.

Activity 3 Status as of ***[July 1, 2019]*****:** Over the last 6 months, we have made progress with processing samples and data analysis associated with this activity. We have received chemical data on leaf samples harvested from our 2018 field experiment at Cedar Creek and have run a preliminary analysis that reveals differences across plant species in responses to sodium and metal variation in the soil, for instance, as expected from previous studies, plants in the family Asteraceae (sunflowers, black-eyed susans) accumulate more heavy metals. Second, we are in the midst of a large greenhouse experiment to push forward the goals of this activity. In our 2018 field season, we had great success in collecting leaf tissue for analysis, but only one of the 12 species we planted bloomed in any numbers in our field plots, limiting tests of predictions about nectar and pollen. To address this, we are running a greenhouse experiment using a subset of prairie species and agricultural representatives from the same plant families (that we know will bloom in a short period of time) to make inferences about how different plant families allocate sodium and metals into nectar versus leaf tissue. We are currently growing 14 species in 4 different treatments and will be harvesting nectar and leaf tissue throughout the summer. Finally, we plan to resample our Cedar Creek field plots, although only about 1/3-1/2 of the plants survived the winter, so this will be

a scaled back experiment, perhaps just focusing on the three milkweed species that had the highest over-winter survival.

Activity 3 Status as of *[January 1, 2020]***:** We have made progress on two components of activity 3. First, we have made been working on a paper using a 2018 field experiment to investigate contrasting effects of salt (sodium), metals (zinc), and nitrogen on plant nutrient content. Here, we see significant variation across species in how the accumulate metals and sodium in their leaves. We see some hints that related species perform similarly across the treatments (e.g., legumes). Second, we completed a complementary greenhouse experiment testing similar questions. Here, we were also able to harvest nectar, allowing us to test whether we see similar patterns in nectar and leaves. While overall conclusions are pending, our results so far support the idea of planting a diversity of plants in roadsides because species vary in their nutritional responses.

Activity 3 Status as of *[July 1, 2020]***:** This activity suffered from two significant data delays due to covid-19 – closures of labs with both plant leaf and nectar samples. Both labs are now back open and data in hand (or almost so). We have two papers in progress for this activity: a) data from a 2018 field study experimentally studying the effects of salt and heavy metal inputs on plant salt/metal levels (paper draft complete, hope to submit soon), b) data from a 2019 greenhouse experiment focusing on effects of salt on leaf sodium and nectar sodium levels across several species of plants (data pending/delayed from covid-19 lab closures). We hope to submit paper (a) this coming grant period in addition to starting/completing analyses for paper (b). Both are led by postdoc Tim Mitchell who will soon be transitioning to work on a MnDoT/LRRB funded project also on roadside habitat for pollinators.

Activity 3 Status as of *[January 1, 2021]***:** We now have a complete draft of the paper summarizing our field work experimental findings – major findings are that species of native roadside plants differ in their uptake of sodium and zinc inputs (and those that take up more sodium also take up more zinc, offering some suggestion for which species should be avoided along high traffic roads). We are about to submit an additional (and likely final) round of samples for our greenhouse study (flower parts to complement nectar and leaf data we received back over the summer). The postdoc leading this aim (Tim Mitchell) transitioned to another position (a MnDoT roadside project) in July, but continues to push forward these two papers in his spare time.

Final Report Summary *[August 16, 2021]:* Research within this activity has generated two publications (both in prep). Several themes have emerged across these studies. First, plant species used in roadside pollinator plantings vary widely in uptake of salts and metals. Some species that accumulate high levels of both salts and metals (e.g. yellow coneflower) should perhaps be avoided in seed mixes along high traffic sites. Second, while roadsides generally contain elevated levels of nitrogen, salts and metals, there appear to be very few interactions between these nutrients and toxins in terms of plant uptake, drastically simplifying predictions about how plant chemistry should vary along roadsides. Finally, these controlled experiments, in both field and greenhouse settings, clarify how salts and metals accumulate not only on plants (from dust), but also from direct uptake into plant tissue (leaves and nectar).

V. DISSEMINATION:

Description:

Scientific publications. All results will published as open access papers in peer-reviewed scientific journals such as Ecology, Proceedings of the National Academy of Sciences, or PLoS One. We expect at least 2 publications to arise from each activity. *Update:* the cost of open-access is significantly higher for most publications than the publication fees allowed. However, we are making publications open-access where we can, and providing manuscripts on websites such as research gate whenever possible.

Publically available data. All data will be publically available, either in databases such as DRYAD (http://www.datadryad.org/) and/or through the Department of Natural Resources Observation Database.

Collaborative meetings with relevant agencies and the development of management best practices. Throughout the project planning, and as data become available, we will talk with the DNR, MnDot, and Board of Water and Soil Resources through email, conference calls, and in person meetings. Such discussions have already fruitfully led to the sampling strategy for Activity 1. Future meetings will further refine the proposed methods in addition to discussing the broader management implications of the findings. We will consider what the survey data (Activity 1), pollinator rearing (Activity 2), and plant manipulations (Activity 3) mean for pollinators along roadsides – are some plants more likely than others to maximize the health of pollinators along roads? Are some road use

intensities more or less likely to compromise the health of pollinators feeding there? How might the present results interact with other management considerations such as traffic death or mowing? We aim to develop site-specific recommendations that can be used to guide roadside management for pollinators.

Status as of *[January 1, 2018]***:** We are in periodic communication with the DNR, having met with them once over the summer (2017) and having regular email correspondence with contacts there. We have not yet started writing manuscripts as we are waiting for results and analyses (see above). Some of the planned experiments and preliminary findings have been presented at meetings, including the Animal Behavior Society (June 2017) and the Society for Integrative and Comparative Biology (January 2018).

Status as of *[July 1, 2018]***:** We presented analyses of preliminary data at two local conferences in March that included several of our contacts at MnDoT, DNR and BWSR (the LCCMR pollinator update meeting at UMN and the annual update meeting for a separate project funded through the Department of Transportation's on roadside habitat for monarchs). We met with MnDoT and DNR in May to discuss our plans for the 2018 field season and get input in site selection and sampling protocol.

On the publication front, we have made progress on two manuscripts resulting from this work a) summer 2017 field season data (Activity 1) on roadside milkweed and monarchs (directed by postdoc Tim Mitchell), b) tolerance of monarchs to dietary sodium (Activity 2, directed by grad student Megan Kobiela). A related paper on tolerance of butterflies to dietary nickel (Ni) was also submitted this past semester (first authored by Megan Kobiela).

Status as of *[January 1, 2019]***:** Lauren Agnew presented preliminary data analyses at a pollinator summit at the University of Minnesota Arboretum and at the Entomological Society annual conference in Vancouver. Tim Mitchell presented data analyses at the Center for Transportation Studies conference at the University of Minnesota in November. Megan Kobiela presented her monarch sodium results at the International Society for Behavioral Ecology conference in Minneapolis in August. Tim, Megan, and Alex Shephard will be presenting data on metal and sodium toxicity at the upcoming January Society for Integrative and Comparative Biology conference. Three manuscripts have been prepared and will be submitted spring semester 2019 (roadside milkweed, sodium preferences, and zinc toxicity); several others are in preparation.

Status as of *[July 1, 2019]***:** Over the last 6 months, we presented some of this work at the Integrative and Comparative Biology meeting and at an informal luncheon at the Minnesota Zoo. Two papers have been published (or are in press) and we are actively working on 6 other papers. Mapping out our current experiments, we are projecting 16 manuscripts to come out of this work (at least). Two of these we plan as synthetic reviews. Given that we originally allocated $6000 to publishing papers as open access, we plan to prioritize these funds towards the papers that will be of most interest to roadside managers (e.g., the review papers and the roadside surveys). However, we also plan to make all of the work available as individual papers and a summary report to MnDoT and other interested agencies.

In addition, PI ESR is also involved in a separate project on roadsides as habitat for monarchs nationwide. The data from this ENTRF project has been important in parameterizing underlying assumptions in development of a GIS-based model and also a habitat calculator that give roadside managers tools for prioritizing roadsides for restoration. As part of this project, two webinars were hosted in May to department of transportation officials from around the country. Those webinars can be found here:
https://www.youtube.com/watch?reload=9&v=BRpIcAM4pgQ&feature=youtu.be
https://www.youtube.com/watch?v=FtLqST0MYec&feature=youtu.be
PI ESR fielded questions during and after the webinar on the nutrition of roadside plants for pollinators.

Status as of *[January 1, 2020]***:** Over the last six months, we have submitted two manuscripts to peer-reviewed journals (see above), an in-press paper has been published, and two additional papers were submitted as part of a PhD dissertation (to be submitted to journals in Jan/Feb). We are actively working on an additional six papers (see summaries above under each activity). In addition, PI ESR summarized results to-date at the annual Center for Transportation Studies conference. She summarized current management recommendations for roadside managers interested in habitat for pollinators: prioritize low- or moderate traffic roads (<20,000 cars/day), mow plants within 10' of the road (these are the most toxic plants), support current efforts to pan the pesticide chlorpyrifos, plant a diversity of plants as they respond to roads in different ways. These results also informed a proposal application to MnDoT/LRRB on evaluating cost-effective roadside revegetation techniques to support pollinator communities (together with co-PI Dan Cariveau).

Status as of *[July 1, 2020]*: As mentioned above, two papers have been published over the last 6-month period, links: https://www.sciencedirect.com/science/article/pii/S0048969720315588 and https://onlinelibrary.wiley.com/doi/full/10.1111/icad.12404. PI Snell-Rood also summarized some of the relevant results during a Transportation Research Board webinar attended by approximately 350 roadside managers from across the country, https://webinar.mytrb.org/Webinars/Details/1379 (the final 10 minutes of the webinar).

Status as of *[January 1, 2021]*: Relevant publications from the last six months include Kobiela and Snell-Rood 2020: https://setac.onlinelibrary.wiley.com/doi/abs/10.1002/etc.4845 and Snell-Rood et al 2020 (Experiment 2 and Figure 4 in particular): https://onlinelibrary.wiley.com/doi/abs/10.1111/evo.14072. Results were presented at the Ecosystem Health seminar in December (seminar series within Vet-Med school at UMN) and virtual department seminars at the University of Arkansas, University of Cincinnati, and University College, Cork, Ireland).

Final Report Summary [August 16, 2021]: This project has directly led to six publications in print and twelve in revision or in prep that acknowledge ENRTF support. This project intersected with five additional research projects from 2017 onwards that were supported by additional sources (e.g., undergraduate or graduate research funds or other research support). This project spurred the development of two currently funded projects, including a MnDoT/LRRB funded project on roadside revegetation methods for pollinators and an NSF-funded project on urban salt and metal contamination. Each of these outputs is listed in the "list of products" document. This work also contributed to the content of over 25 seminars, conference presentations, and webinars (see "list of products") presented both within the state, nationally and internationally. The overarching conclusions of the work are available in several talks online, such as the Cedar Creek LTER "Lunch with a Scientist" series and the Rights-of-Way working group research series on pollinator habitat (see links in list of products document). Finally, this research will be featured in a forthcoming book on road ecology (due 2022, https://www.bengoldfarb.com/roadecology). Author Ben Goldfarb visited the lab for three days July 2021 to visit field sties, see experiments in progress and learn about the general findings of the work. We plan to summarize these general findings and management recommendations in a brief report that we will distribute to relevant agencies (MnDoT, DNR, others), by the end of 2021. Additional papers that we are currently working on should all be submitted for publication by 2022 and will be sent to LCCMR staff once published. Data for each publication are publically available, linked via a data accessibility statement for each manuscript, generally on either DRYAD (https://datadryad.org/search?q=snell-rood) or Mendeley (e.g., https://data.mendeley.com/datasets/4g4jw6kb56/1).

VI. PROJECT BUDGET SUMMARY:

A. Preliminary ENRTF Budget Overview:

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

Budget Category	$ Amount	Overview Explanation
Personnel:	$83340 $21,000 $7,500 $23,000 $167,000 $213,600 $38,000	• Emilie Snell-Rood, project manager: 12% FTE for year 2, 22% FTE in year 3, ~~4~~% FTE Year 1 – oversee and coordinate entire project • Clay Carter, Asst Prof: 4% FTE for 3 years – oversee all nectar and pollen analyses • Karen Oberhauser, Prof: 4% FTE for year 1 – oversee and advise on all monarch rearing and milkweed analysis • Elizabeth Borer, Prof: 4% FTE for 3 years – oversee and coordinate plant nutrient manipulations at Cedar Creek • Postdoctoral Associate: 100% FTE for years 2 and 375% FTE year 1 – Run all nutrient field manipulations, coordinate data analysis and writing • Two graduate research assistants: avg 42% FTE for 3 years – Run all bee and monarch rearing experiments, assist with plant collection and manipulations

	$67,000	• Two undergraduate research assistants, 92% FTE & 23% FTE for 2 years; Field assistants, help with rearing and sample processing • Lab technician: 100% FTE for 1 year, 25% FTE for year 2 -- run all roadside surveys and sample processing
Professional/Technical/Service Contracts:	$8000	• The Department of Natural Resources will provide valuable consulting and advice across 9 total meetings (3 per year, 2 hrs/meeting, 4 people/meeting)
Equipment/Tools/Supplies:	$6000 $6000 $3000	• Supplies for collecting and processing plants and pollinators (Activity 1): vials, dry ice, cooler, paper bags, forceps, safety vests, weather gauge, meter sticks, capillary tubes, microcentrifuge tubes, gloves, paintbrushes, clipboards and writing utensils • Supplies for rearing monarchs and bees (Activity 2): queen bees from Koppert, CO2 tanks, plastic rearing containers, paper towels, ethanol, bleach, pollen, honey, sugar, sharpies, bee labels, super glue, plastic bags, chemical reagents (NaCl, NiCl2, ZnCl2) • Supplies for field manipulations of plants and pollinator observations (Activity 3): field guides, NaCl, NiCl2, ZnCl2, nitrogen fertilizer, watering cans, plant seeds, soil amendments, clipboards
Printing:	$6000	• Open access publications (2 papers per activity)
Travel Expenses in MN:	$17,000	• Travel to 50 roadside sites for sampling plants and pollinators in Activity 1 (approximately 3000 miles over a 4-month period over two field seasons, includes lodging and meal allowance for technician and field assistant) • Travel to Cedar Creek field site for plot manipulations in Activity 3 and plant collection for rearing in Activity 2
Other:	$98,500	• Plant, soil and insect samples for measurement of nitrogen, sodium and heavy metals content ($20/sample). A subset of plant samples will be assayed for insecticides ($300/sample). Activity 1 includes approx 3 species across 50 sites (nectar, pollen, leaves, N = 3/sp/site). Activity 3 includes 12 species across at least 3 subplots at 3 sites (N = 3/sp/treatment)
TOTAL ENRTF BUDGET:	**$815,000**	

Explanation of Use of Classified Staff: N/A

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

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: Grad students = 3 (2 people half time for 3 years), Snell-Rood = 0.375 (1.5 mos. for 3 years), Oberhauser = 0.042 (0.5 mos. for 1 year), Borer = 0.126 (0.5 mos. for 3 years), Carter = 0.126 (0.5 mos. for 3 years), Postdoc = 2.75 (1 person full time for 2.75 years), technician = 1.25 (1 full time for 1 year and second field season), Undergrads = 1.75 (1 person @ half time during the academic year and 2 people at full time during the summer - for 2 years): total = **9.5**

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

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
In kind services	$372,000	$372,439	
Indirect costs associated with this proposal			
	$	$	
State			
	$	$	
TOTAL OTHER FUNDS:	**$372,000**	**$**372,439	

VII. PROJECT STRATEGY:

A. Project Partners:

Partners receiving ENRTF funding

- Emilie Snell-Rood, Associate Professor, University of Minnesota, Project leader, $50,000
- Karen Oberhauser, Professor, University of Minnesota, Oversee monarch work, $7.500
- Elizabeth Borer, Professor, University of Minnesota, Oversee nutrient manipulations, $23,000
- Clay Carter, Assistant Professor, University of Minnesota, Oversee nectar measurements, $21,000
- Graduate student assistants, University of Minnesota, Run rearing experiments, $297,000
- Postodoctoral researcher, University of Minnesota, Run field manipulations, $167,000
- Lab Technician, University of Minnesota, Roadside surveys, $67,000
- Undergraduate assistants, University of Minnesota, Run site surveys, $38,500

Partners NOT receiving ENRTF funding

- Dan Cariveau, Assistant Professor, University of Minnesota, advise on bee surveys
- Marla Spivak, Professor, University of Minnesota, Oversee bee rearing
- Dan Shaw, Minnesota Board of Water and Soil Resources, advise on plant selection
- Ken Graeve, Minnesota Dept. of Transportation, advise on plant selection

B. Project Impact and Long-term Strategy:

The final outcome of this project will be recommendations for roadside plantings to maximize pollinator health. For example, preliminary data show that milkweed can accumulate very high levels of sodium, suggesting plantings along less intensely used rural roads may be ideal. Datasets will be made publically available on the digital repository “DRYAD” and the DNR observation database; publications will be open access. Throughout the project planning, and as data become available, we will talk with the DNR, MnDoT, and Board of Water and Soil Resources through email, conference calls, and in person meetings. At the conclusion of the data collection, we will discuss the broader management implications of the findings. We will consider what the survey data (Activity 1), pollinator rearing (Activity 2), and plant manipulations (Activity 3) mean for pollinators along roadsides – are some plants more likely than others to maximize the health of pollinators along roads? Are some road use intensities more or less likely to compromise the health of pollinators feeding there? How might the present results interact with other management considerations such as traffic death or mowing? We will develop site-specific recommendations that can be used to guide roadside management for pollinators. We will additionally communicate these findings to all relevant stakeholders. In doing so, this research will contribute to the development and use of roadsides as habitat for monarchs and native bees.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	$ Amount
Funds supplied from the Office of the Vice President for Research (University of Minnesota) that funded this project during its first two years (spent by 2013). Resulted in publication: Snell-Rood et al. 2014 PNAS	2011-2013	$28,000
		$
		$

VIII. REPORTING REQUIREMENTS:

- **The project is for 3 years, will begin on 07/01/17, and end on 06/30/21.**
- **Periodic project status update reports will be submitted** *[January 1]* **and** *[July 1]* **of each year.**
- **A final report and associated products will be submitted between June 30 and August 15, 2021**.

IX. VISUAL COMPONENT or MAP(S):

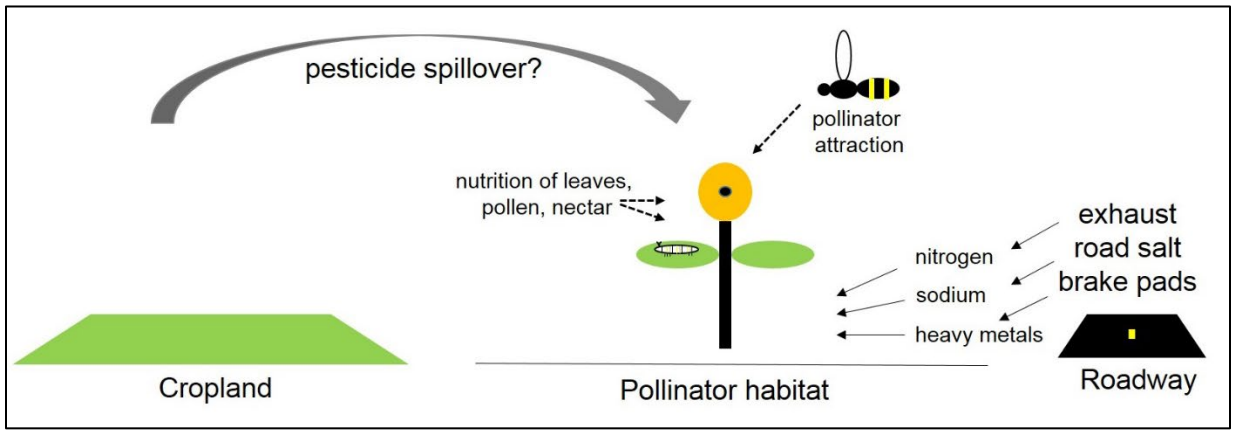

Figure 1. ***Research overview***. Some elements of road runoff such as nitrogen and sodium may attract pollinators to roadsides where toxins such as pesticides and heavy metals may additionally be present. This research addresses the nutrition of roadside plants through roadside surveys, developmental manipulations of monarchs and bumblebees, and plant trials to optimize which species to plant along roadways of different use profiles.

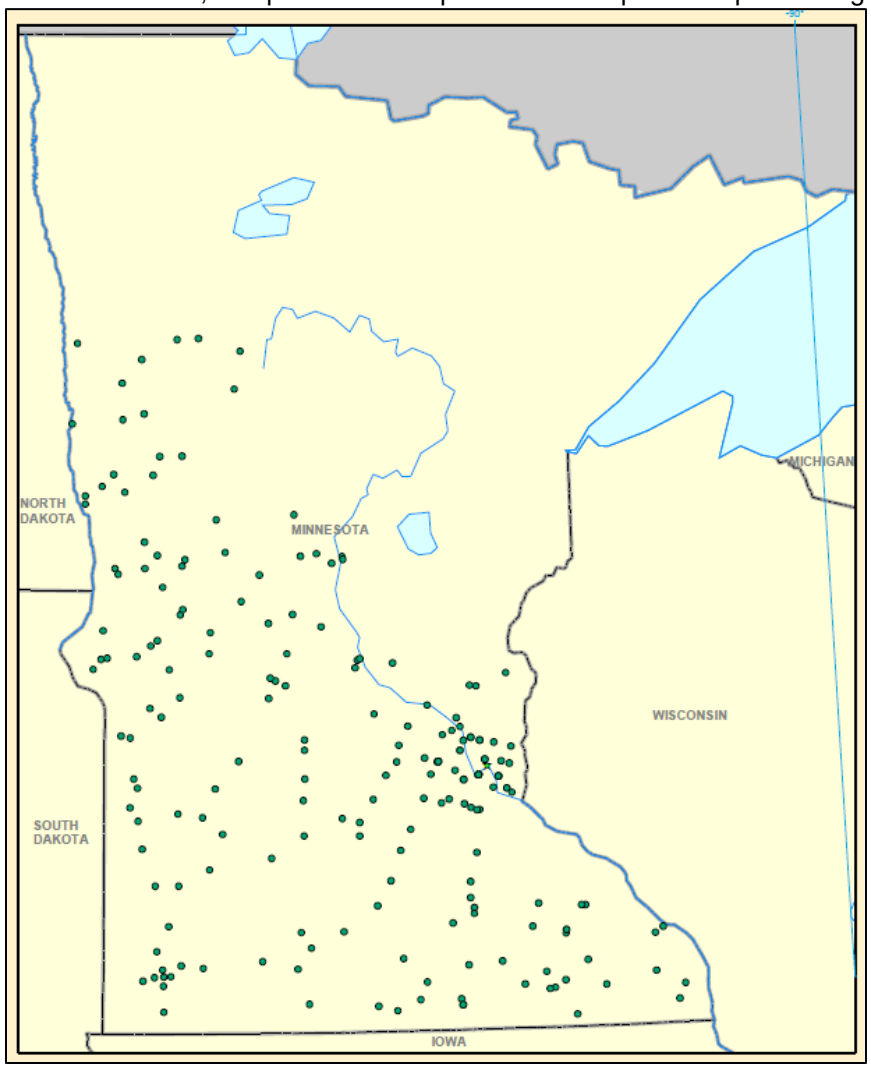

Figure 2. ***Roadside sites***. Activity 1 of this research will focus on 50 roadside sites drawn from an existing network of sites (shown here). These sites represent a random sample of sites within prairie and open habitats within 200 miles of the Twin Cities. We will build on existing data for these sites, which will include two field seasons of surveys of milkweed and monarchs. Over 50% of these sites contain milkweed. The sites include a mixture of road use intensities and adjacent land use (>50% agriculture).

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

Project Title: Optimizing the Nutrition of Roadside Plants for Pollinators
Legal Citation: M.L. 2017, Chp. 96, Sec. 2, Subd. 08a
Project Manager: Emilie Snell-Rood
Organization: University of Minnesota
M.L. 2017 ENRTF Appropriation: $815,000
Project Length and Completion Date: 4 years, June 30, 2021
Date of Report: August 16, 2021

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 budget	Amount Spent	Activity 1 Balance	Activity 2 budget	Amount Spent	Activity 2 Balance	Activity 3 Budget	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM											
Personnel (Wages and Benefits)	$282,890	$273,107	$9,783	$182,500	$176,567	$5,933	$201,000	$200,436	$564	$666,390	$16,280
Emilie Snell-Rood, project manager: $83340 (75% salary, 25% benefits), 4% FTE in Year 1, 12% FTE for year 2, 22% year 3											
Clay Carter, Asst Prof: $21,000 (75% salary, 25% benefits), 4% FTE for 3 years											
Karen Oberhauser, Prof: $7,500 (75% salary, 25% benefits), 4% FTE for 1 year											
Elizabeth Borer, Prof: $23,000 (75% salary, 25% benefits), 4% FTE for 3 years											
Postdoctoral Associate: $167,000 (82% salary, 18% benefits), 75% FTE in Year 1, 100% FTE in years 2 & 3											
Two graduate research assistants: $213660 (52% salary, 48% benefits during academic year; 85% salary, 15% benefits during 1/3 summers), avg 42% FTE for 3 years											
Two undergraduate research assistants, $38,000, one 50% time during academic year and 2 full-time in the summer (100% salary, 0% benefits) 92% FTE & 23% FTE for 2 years											
Lab technician: $67,000 (79% salary, 21% benefits), 100% FTE year 1, 25% FTE for year 2											
Professional/Technical/Service Contracts	$0	$0	$0				$0	$0	$0	$0	$0
The Department of Natural Resources will provide valuable advice across 2-3 meetings per year											
Equipment/Tools/Supplies	$6,800	$6,722	$78	$10,200	$10,100	$100	$6,000	$5,948	$52	$23,000	$229
Supplies for rearing monarchs and bees (Activity 2): queen bees from Koppert, CO2 tanks, plastic rearing containers, paper towels, ethanol, bleach, pollen, honey, sugar, sharpies, bee labels, super glue, plastic bags, chemical reagents for manipulations (NaCl, NiCl2, ZnCl2), diet ingredients for monarch artificial diet (vitamin mix, cholesterol, etc), lab and wild type lines of monarchs, greenhouse fees for pots, soil, cleaning, watering, pest control and space for milkweed & adult butterflies ($200-500/month)											

Supplies for collecting and processing plants and pollinators (Acitivity 1 & 3): vials, dry ice, cooler, field microscopes, paper bags, forceps, safety vests, weather gauge, meter sticks, capillary tubes, microcentrifuge tubes, freezer boxes&bags, gloves, paintbrushes, clipboards and writing utensils, drierite, fan&shelf for soil drying, chemicals & pH meter for soil pH, dyes and reagents for pollen analyses											
Supplies for field manipulations of plants and pollinator observations (Activity 3): field guides, NaCl, NiCl2, ZnCl2, nitrogen fertilizer, watering/spray cans for treatments, plant seeds, soil amendments/soil, clipboards, fees for irragation of plots at Cedar Creek (and treatment of plots for weed control), soil corers/shovels for planting, "cone-tainers" for plant growth, flagging/rebar for plot marking, greenhouse fees associated with rearing field plants											
Capital Expenditures Over $5,000											
None											
Fee Title Acquisition											
NA											
Easement Acquisition											
NA											
Professional Services for Acquisition											
NA											
Printing	$2,000	$1,020	$980	$2,000	$1,020	$980	$2,000	$1,020	$980	$6,000	$2,939
Open-access publications											
Travel expenses in Minnesota	$17,840	$17,838	$2	$500	$188	$312	$2,770	$591	$2,179	$21,110	$2,493
Travel to 80+ roadside sites for sampling plants and pollinators in Activity 1 (6000 miles in 2017 and estimated 9000 miles in 2018, includes lodging and meal allowance for technician and field assistant); travel to field site for plot manipulations in Activity 3 (approx weekly visits to Cedar Creek) and plant/butterfly collection for monarch rearing Activity 2											
Other	$68,500	$66,617	$1,883	$0	$0	$0	$30,000	$13,560	$16,440	$98,500	$18,324
Plant, soil and insect samples for measurement of nitrogen, sodium and heavy metals content ($20/sample). A subset of plant samples will be assayed for insecticides ($300/sample). Activity 1 includes approx 3 species across 50 sites (nectar, pollen, leaves, N = 3/sp/site). Activity 3 includes 12 species across at least 3 subplots at 3 sites (N = 3/sp/treatment)											
COLUMN TOTAL	**$378,030**	**$365,305**	**$12,725**	**$195,200**	**$187,876**	**$7,324**	**$241,770**	**$221,555**	**$20,215**	**$815,000**	**$40,265**