**PROJECT TITLE:** Supporting Pollinators and Prairies with Beneficial Soil Fungi

**I. PROJECT STATEMENT**

**Our goal** is to develop practical methods by which land managers can harness beneficial soil fungi to lower costs and improve reliability of native prairie restorations in agricultural areas. If successful, this will improve conservation of endangered pollinators and other wildlife, control non-native grasses that ruin restorations, and provide cost-effective conservation of soil and water.

**Why this is important.** Restored tallgrass prairie can provide excellent habitat for imperiled Monarch butterflies and rapidly declining native bees. Restored prairie also supports economically valuable wildlife that controls crop pests, enabling reductions in pesticides that harm pollinators. To attain these benefits, however, the restored prairie must be diverse enough to provide season-long bloom and vigorous enough to resist invasive species. Restorations that lack early season blooms cannot support bumblebees, for example, that begin establishing nests in early spring; likewise, without vigorous growth of early-season grasses and forbs, non-native grasses easily invade, ruining pollinator habitat. To address this problem, better establishment methods for spring-flowering native prairie species are critically needed. Establishment of these species is typically poor, which is a major problem because seeds of these species are scarce and expensive. Evidence suggests that better establishment could be achieved on these lands by active management of beneficial soil microorganisms, just as crops benefit from inoculation with beneficial microbes. In particular, beneficial soil fungi colonize the roots of native prairie plants and improve access to water and nutrients, resources which allow the plant to thrive, compete with invasive grasses, and to produce many flowers for pollinators and other valued wildlife. However, beneficial soil fungi can be quite specific to particular plant species. It is critical that the correct soil fungi for native prairie species are present when a restoration begins; otherwise, mismatches can lead to reduced benefits to, or harmful effects on, native prairie plant species.

**How we will achieve the goal.** Greatly lowering project costs by taking advantage of several long-term multi-site prairie restoration experiments, we will survey beneficial soil fungi that colonize native plant species in reconstructed and remnant prairies in western Minnesota and in prairies established over the past 40 years at Three Rivers Parks. We will identify fungi associated with successful restorations and healthy prairie remnants (in a recently-funded companion study) and examine the timeline of colonization in the restored prairies of different ages (this study). We will then test the effects of these fungi on native prairie plant species including hard-to-establish cool-season forbs and native cool-season grasses in competition experiments with invasive grasses. We will measure fungal effects on the key plant traits of growth, leaf area and flower number with and without competition.

**II. PROJECT ACTIVITIES AND OUTCOMES**

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| **Activity 1:** *Identify beneficial soil fungi associated with prairie plants in restorations established 5 – 45 years ago.*  To efficiently use beneficial fungal inoculum to improve prairie restorations, we need to identify not only which fungal species are beneficial, but the length of time it takes them to colonize their host plants; those that colonize rapidly on their own can be excluded from managed inoculations. Ten individuals of 20 species (emphasis on early blooming species) will be collected at a total of 10 restorations planted in the early 1970’s, 2010 and 2014 and from 4 nearby remnant prairies. DNA will be extracted from the roots of each plant and sequenced by the University of Minnesota Genomics Center to enable identification and determine abundance of beneficial soil fungi in each root sample. We will assess the effect of location, reconstruction age, and plant species on the identity and abundance of beneficial soil fungi present.  **ENRTF BUDGET: $128,532** | | |  |
| **Outcome** | **Completion Date** |
| *1. Root collection, extraction, and DNA sequencing* | **Feb. 2021** |
| *2. Identify beneficial soil fungi that are associated with prairie species at each location* | **June 2021** |

**Activity 2:** *Test the value of beneficial soil fungi for enhancing resistance of native forbs and grasses to invasion by non‑native grasses (smooth brome and Kentucky bluegrass).*

We will test effects of fungi, identified in Activity 1 and in a separately funded (USGS) companion study, on ability of spring-blooming native forbs and grasses to resist invasion by smooth brome and Kentucky bluegrass. In July 2021, soils from remnant prairies will be harvested and beneficial fungi amplified in a glasshouse; fungal inocula (chopped roots and soils) will be mixed with sand and planted with a high-performing prairie species to further amplify soil fungi. After 4 months this inoculum will be used in a competition experiment. Seedlings of 20 highly-valued native prairie species will be grown in pots containing basic potting mix with and without inoculum (of species-appropriate fungi) for 12 weeks, after which seeds of smooth brome and Kentucky bluegrass will be added. Size, leaf area, and flowering of all species will be monitored throughout the growing season, then above-ground production will be collected, dried and weighed and root samples will be collected to confirm fungal identity. If fungi improve competitive ability of native species, we expect the planted invasive grasses to be smaller and native plants larger and to produce more flowers in inoculated pots than in pots without fungi. This study will assess the effect of beneficial fungi on the ability of native prairie species to compete against non-native invasive grasses.

**ENRTF BUDGET: $202,547**

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| **Outcome** | **Completion Date** |
| *1. Preparation of inocula* | **Feb. 2022** |
| *2. Grow native prairie plant seedlings with and without inocula* | **June 2022** |
| *3. Competition experiment* | **Sept 2022** |
| *4. Identify beneficial soil fungi that provide most benefit to key prairie species* | **January 2023** |
| *5. Final report* | **June 2023** |

**III. PROJECT PARTNERS:**

**A. Partners receiving ENRTF funding**

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| **Name** | **Title** | **Affiliation** | **Role** |
| Nicholas Jordan | Professor | University of Minnesota | Project management, idenfication of fungi, field testing of beneficial fungi |
| Diane Larson | Research Wildlife Biologist | U.S. Geological Survey, St. Paul, Minnesota | Oversee field collections, monitoring, data analysis |
| Laura Aldrich-Wolfe | Plant Ecologist | Consultant, Moorhead, Minnesota | Interpretation of sequence data, data analysis |
| Stefanie Vink | Microbial Ecologist/ Bioinformatician | Consultant, Groningen, The Netherlands | Methods and interpretation of sequence data, data analysis |

**IV. LONG-TERM- IMPLEMENTATION AND FUNDING:**

We will share our results with practitioners via multiple channels, including webinars (e.g., the Prairie Reconstruction Initiative series) and podcasts (e.g., MN DNR’s Prairie Podcast). This study complements University of Minnesota’s Healthy Prairies Initiative, currently supported by an ENRTF grant, by addressing soil fungal communities and focusing on the key challenge of cost-effective reconstruction of highly diverse prairies that resist degradation by exotic grasses in the long-term.

**V. TIME LINE REQUIREMENTS:**

Roots will be collected and processed in late summer 2020 and sequenced that fall/winter. Sequence data will be analyzed during spring-summer 2021. Beneficial soil fungal increases will occur Fall 2021; seedlings will be grown in these soils Spring 2022, planted in a competition experiment in June 2022, and monitored summer-fall 2022. A final report will be completed and results disseminated to land managers by July 2023; follow-up implementation research, if warranted, will then begin.