Multi-year Funding Status Report 2021

Researcher: Reb Bryant (PhD student)

Advisor: Jim Bever

Title: Testing the effect of native arbuscular mycorrhizal fungi on prairie restoration

success at Nachusa Grasslands

In this project, we have planned to set up a field experiment testing our hypothesis that native AMF will promote the survival and growth of plants of conservation interest at Nachusa Grasslands. We will accomplish this by inoculating seedlings with either native AMF, non-native AMF, or sterile soil, planting them in an established and new restoration at Nachusa Grasslands, and monitoring them over two growing seasons.

In April 2021, we began this project by collecting soil from four remnant sites at Nachusa Grasslands (Doug's Knob in the Dot and Doug Wade Prairie Unit, Isabelle's Knob and another knob in the Main Unit, and Schafer Knob in the Schafer Prairie Unit) as well as a disturbed site. These remnant sites were chosen for the prevalence of certain rare plants that have been difficult to establish in restorations across the preserve. At each remnant site, Reb Bryant and Nathaniel Weickert first identified the target species and then collected four 0.5 L subsamples of soil at 2-10 cm depth. The subsamples were at least 2 m apart from each other and stored separately. At the disturbed site, four 0.75 L subsamples were also collected in a similar manner. This soil was taken on ice back to Drs. Jim Bever and Peggy Schultz's lab in Lawrence, KS at the University of Kansas where each subsample was homogenized and stored for further processing.

The collected soil was first used to make whole soil "trap" cultures that will be used to inoculate seedlings for the restoration experiment to be planted in late May 2022. Reb Bryant worked with Dr. Liz Koziol to produce the cultures. In gallon pots, sterilized soil-sand mix was added to ~70% of the total pot volume, and a total of 300 mL of soil collected from Nachusa Grasslands was added. Finally, the cultures were topped off with the sterilized soil-sand mix and planted with little bluestem (*Schizachyrium scoparium*), pale purple coneflower (*Echinacea pallida*), and prairie blazing star (*Liatris pycnostachya*) and grown in a greenhouse for six months. A total of 10 cultures were prepared: five from both the disturbed and remnant sites. For the remnants, four cultures were prepared using soil from one the four remnant sites (with equal amounts from each subsample), and a fifth culture was prepared using soil from all four sites. For the disturbed site, four cultures were prepared using one subsample each, and a fifth was prepared with an equal combination of the subsamples. These cultures were harvested and stored in a cold room in preparation for inoculation of plugs for the field experiment later this year.

In addition to whole soil cultures, we also set up single-morphotype cultures of AMF. We used 50 mL of soil from each remnant site (with equal amounts of soil from

each subsample) and disturbed site subsample to extract AMF spores. From these extractions, we isolated different morphotypes of AMF based on color, size, and other visible features. These morphotypes were added to individual pots of a sterile soil-sand mix and planted with Sorghum. These cultures will continue to grow in our greenhouse until we harvest them, extract the AMF, and confirm or reject the success of the culture and analyze the identity of the AMF spores. If the culture has only one species of AMF, then a larger culture will be started that may be used in future projects at Nachusa Grasslands.

In the next year, we plan to set up and monitor our field experiment at Nachusa Grasslands over the growing season in 2022 as well as continue with our efforts to culture AMF for future projects. In our field experiment, we expect that the planted seedlings inoculated with whole soil cultures (including AMF) from the remnant sites will both survive and grow more vigorously than the seedlings inoculated with cultures from the disturbed site or with sterile soil. We may also see increased plant biodiversity overall in plots with seedlings inoculated with AMF cultured from the prairie remnants over the next two growing seasons. We look forward to continuing to work with Dr. Elizabeth Bach, the restoration crew, and other staff over the course of this project and plan to meet the goals we set in our original proposal.