RESTORATION ECOLOGY
The Journal of the Society for Ecological Restoration

RESEARCH ARTICLE

Rhizobia inoculation increases survival, flower production, herbivory, and pollinator visitation in an annual prairie legume

Renee Dollard[1], Paul A. Price[1], Jonathan T. Bauer[3], Brian Connolly[4], Emily Grman[1,2]

Native legumes are functionally important members of grasslands, but their reintroduction into degraded systems is limited by strong establishment filters. One of these establishment filters might be rhizobia limitation, where legume seedlings are unable to find suitable rhizobia symbionts in grasslands targeted for restoration. To test links between rhizobial inoculation and legume demographic parameters in a grassland restoration context, we evaluated how inoculation with rhizobia altered survival and seed production of a native annual legume (*Chamaecrista fasciculata*) inoculated with rhizobia and transplanted into a restored prairie. Small mammal herbivory was an important filter affecting survival of *C. fasciculata* transplants, with inoculated plants 81% more likely to be grazed than uninoculated plants. Despite this heavy grazing, plants inoculated with rhizobia survived transplantation 71% more often and, as a result, produced 82% more flowers, experienced 73% more visits by pollinators, and on average produced 220% more seeds. Our results indicate that although herbivory may also shape legume population establishment, at least in some years in some places, rhizobia could alter *C. fasciculata* interactions with both herbivores and pollinators and improve population establishment.

Key words: *Chamaecrista fasciculata*, herbivory, mutualism, partridge pea, prairie restoration, soil microbial community

Implications for Practice

- Inoculation with rhizobia is not a silver bullet to increase all measures of legume performance in restored sites. Instead, like all species interactions, the effects of this mutualism are complex and multifaceted.
- Restoration practitioners considering pre-planting inoculation treatments for legumes should carefully consider conditions in their particular restoration site. Rhizobia inoculation may be most advantageous in restorations with fewer mammalian herbivores and robust pollinator networks.
- Protecting transplanted seedlings from herbivores may be a more effective use of practitioners' time and resources than inoculation with soil microbes.

Introduction

Many dispersal and establishment filters can prevent plants from establishing during restoration of degraded ecosystems (Grman et al. 2015). Understanding and alleviating these filters can improve establishment of the species upon which ecosystem functioning depends, such as legumes. Because of their symbiotic relationships with nitrogen-fixing bacteria, legumes alter nitrogen availability, with plants surrounding legumes exhibiting higher nitrogen content (Mulder et al. 2002; Temperton et al. 2007). Despite this pivotal role in stabilizing and catalyzing nitrogen flow, legumes are often underrepresented in restored prairies (Kindscher & Tieszen 1998; Grman et al. 2015). If the primary barrier for legumes in restored prairies is dispersal, then increasing seeding rates or transplanting individual seedlings should allow legume abundance to increase over time. However, the presence of legumes and other forbs often decreases with time (Baer et al. 2002; Camill et al. 2004), indicating that the primary barriers related to legume abundance in restored tallgrass prairies could be related to establishment and survival. Viable populations of legumes may fail to establish even when live seedlings are transplanted (Muir et al. 2011) or restorations are seeded with a surplus of seeds (Grman et al. 2015; Jaurena et al. 2016). Thus, it is imperative that restoration practitioners focus on establishment filters to produce successful long-term restorations.

One barrier to legume establishment may be depleted soil microbial communities, specifically the absence of effective rhizobia mutualists. Microbial communities found within young restorations and agricultural fields can differ significantly from those found in remnant prairies (Barber et al. 2017). Microbial communities from remnant prairies can promote more legume

---

Author contributions: RD, PAP, JTB, EG conceived and designed the study; RD collected the data and wrote the manuscript; RD, BC, EG analyzed the data; all authors contributed to manuscript revisions.

[1]Department of Biology, Eastern Michigan University, Ypsilanti, MI 48197, U.S.A.
[2]Address correspondence to E. Grman, email egrman@emich.edu
[3]Department of Biology, Institute for the Environment and Sustainability, Miami University, Oxford, OH 45056, U.S.A.
[4]Biology Department, Gonzaga University, Spokane, WA 99258, U.S.A.

doi: 10.1111/rec.70104
Supporting information at:
http://onlinelibrary.wiley.com/doi/10.1111/rec.70104/suppinfo

growth than microbial communities from restored prairies (Grman et al. 2020), suggesting that essential components of prairie microbial communities could be missing from restored sites. Legume spread can be limited by a lack of rhizobia mutualists (Simonsen et al. 2017; Lopez et al. 2021); for example, invasive legumes form less effective mutualisms with rhizobia from outside their ranges than with rhizobia from within their ranges (Rodríguez-Echeverría et al. 2012; terHorst et al. 2018), suggesting that effective rhizobial mutualists could be dispersal limited. Graham (2005) recommended the application of rhizobia inoculum to enhance prairie legume establishment and prairie productivity, and Beyhaut et al. (2014) showed that some forms of rhizobia inoculation can increase legume species richness in restored prairie, demonstrating that, independent of human influence, rhizobia do not disperse to all sites.

However, adding rhizobial inoculations to restorations to alleviate establishment filters may not be a simple fix: plant interactions with soil mutualists can shape interactions with other species, such as pollinators or herbivores, that affect plant performance, reproduction, and survival. We are unaware of studies following the effects of initial inoculation in restored communities to examine these species interactions and effects on population establishment. Rhizobia alter seedling germination, seedling vigor, seed yield, and seed protein mass (Kaschuk et al. 2010; Mendes et al. 2013; Ranjbar Sistani et al. 2017), potentially affecting granivory. Inoculation with rhizobia could also affect herbivory, either increasing palatability to herbivores by increasing protein content or decreasing palatability by increasing defensive compounds (Kempel et al. 2009; Godschalx et al. 2015). Rhizobia also influence production of plant growth hormones, vitamins, siderophores, and solubilization of insoluble phosphates (van der Heijden et al. 2008; Kaschuk et al. 2010). By altering plant functional traits, rhizobia may alter plant interactions with other species, including herbivores, defensive ants, and pollinators (Kempel et al. 2009; Keller et al. 2018; Magnoli et al. 2023). Thus, the ability or inability of legumes to find suitable rhizobia partners may alter the importance of filters caused by interactions with other species. Previous studies have found strong evidence for the context dependency of the effects of rhizobia on other species interactions, but it is unclear whether the net effect of rhizobia inoculation would still be positive in a restoration context.

To assess the direct and indirect effects of rhizobia inoculation on prairie legume establishment, we transplanted inoculated seedlings of a native annual legume, *Chamaecrista fasciculata*, into a restored prairie and monitored survival, flower production, pollinator visitation, seed production, and seed germinability. We hypothesized that rhizobia limitation forms a strong establishment filter and that it would interact with other establishment filters. We predicted that (1) direct effects of rhizobia inoculation on *C. fasciculata* seedlings would be strong and positive and (2) rhizobia inoculation would indirectly affect survival and seed production through interactions with herbivores and pollinators. By testing these hypotheses on an annual legume that has only 1 year in which to reproduce, we gain a clearer perspective on the demographic factors limiting population establishment.

Methods

We conducted a field study in Legacy Land Conservancy's Lloyd and Mabel Johnson Preserve (Ann Arbor, MI, U.S.A.). The 51-ac preserve contains several restored prairies, oak-hickory forest, and allotments for community gardens. Prior to restoration through seed sowing in 2017, fields were farmed in a soy–corn crop rotation. The preserve is managed for invasive species with spot-spraying of glyphosate, biennial burning, and occasional mowing. Our site was burned in early 2021 prior to the beginning of the experiment. Soils are Blount loams, slightly acidic and somewhat poorly drained. The site was dominated by plants common to restored prairies, including native bunchgrasses (Big bluestem [*Andropogon gerardii*], Little bluestem [*Schizachyrium scoparium*], and Indiangrass [*Sorghastrum nutans*]) and native forbs (New England aster [*Symphyotrichum novae-angliae*], Wild bergamot [*Monarda fistuolosa*], and Canada goldenrod [*Solidago canadensis*]).

To isolate rhizobia, we collected soil from 10 restored and remnant prairies across southwest Michigan and northwest Indiana and grew *Chamaecrista fasciculata* seedlings on prairie soil for 8–9 weeks in a growth chamber (Grman et al. in preparation). We harvested plants and isolated rhizobia from up to five nodules per plant; for this experiment, we selected six rhizobia isolates from large, healthy nodules on *C. fasciculata* plants. The isolates were most similar to reference strains of *Bradyrhizobium viridifuturi* (two isolates from different sites), *B. japonicum* (two isolates from different sites), *Rhizobium alamii*, and *R. pisi*. After isolation, we stored rhizobia isolates in 20% glycerol in a −80°C freezer. The six selected isolates grew on tryptone, yeast extract, minor salts, and experimental carbon media agar plates (Mohamed et al. 2021) in an incubator at 30°C for 3–7 days. To create slurries for inoculation, we suspended isolates in sterile water and diluted to an optical density of 0.1 at 600 nm, then combined equal parts of each isolate to create a homogenous solution.

We scarified *C. fasciculata* seeds (Prairie Moon Nursery, Winona, MN, U.S.A.) by covering them with 98% sulfuric acid for 20 minutes and rinsing with sterile, deionized water, then surface sterilized them by covering them with 10% bleach for 3 minutes and rinsing again. We germinated seeds in sterile petri dishes in the Eastern Michigan University greenhouse, then after 3–5 days transplanted seeds into 60 mL cone-tainers (Steuwe & Sons, Tangent, OR, U.S.A.) filled with an autoclaved mixture of 90% sand and 10% sieved oldfield topsoil. We watered plants with 5 mL of a weak N-free nutrient solution (SNM) 6 days a week for the first 2 weeks and 4 days a week thereafter (Price et al. 2015). After 2 weeks of seedling growth, we applied 1 mL of the inoculant mixture or 1 mL of sterile water to each pot, with treatments randomly assigned.

Three weeks after inoculation, we transplanted seedlings into existing vegetation in 1 m^2 plots in our field site in a randomized block design. Each plot contained nine evenly spaced plants inoculated with the same treatment; plots were arranged in 15 blocks containing one replicate of each treatment (two plots per block, 30 plots in total, 9 plants per plot = 270 total plants) and each block was separated by 1 m. All plots in a block were transplanted on the same date, but the blocks were planted over a 3-week span. At transplanting, we watered to aid in plant establishment; after transplanting the first three blocks and observing high mortality, we supplemented blocks planted later with additional water throughout the first week following planting to reduce mortality from drought stress. We did not replace plants that died.

After transplanting, we monitored plants for survival, pollinator activity, and seed production. To monitor survival, we surveyed plots once a week until the first frost (24 October 2021) and recorded whether plants were dead or alive and whether they were grazed. “Grazed” plants had been clipped near the ground, with remaining stems and foliage cut into small fragments surrounding the plant’s stump, a pattern consistent with grazing by voles (order Rodentia, family Cricetidae). We did not consider insect herbivory. Plants that quickly produced new foliage were considered to be “alive.” There were 13 grazed plants, distributed across both treatments, that appeared to produce new foliage several weeks after the first grazing event or were grazed multiple times; we dropped these plants from the analysis because we were unable to clearly assign a date of death.

To assess pollinator activity, we performed eight rounds of pollinator observations between 9:30 and 14:00 hours from 27 July 2021, to 17 August 2021, randomizing plot sequence for each round. Pollinator observations consisted of a 2-minute acclimation period followed by a 5-minute period during which we observed all plants in a plot and recorded the number of flowers and number of pollinator visits separately for each plant in the plot. To assess the number of seeds produced by plants, we collected seed pods at the time of ripening. From late August through the first frost, we monitored plants daily for seed pod ripening. Once seed pods appeared ripe, we clipped the pod from the stem and placed it into a coin envelope. We stored seeds at room temperature for 30 days to aid the pods in further drying out, then separated seeds from the pods, counted them, and weighed them to 0.001 g. To assess seed viability, we performed seed germination trials. We selected, at random, 20 seeds from each plot in the seven blocks where at least one plant in each treatment produced seeds (280 seeds total). We weighed and sterilized the selected seeds by dipping the seeds in a 10% bleach solution, followed by a 70% ethanol solution and sterile water, then placed seeds in petri dishes with anchor paper and 3 mL of sterile water. We did not scarify seeds because of logistical constraints. For 2 weeks, we added sterile water as needed to moisten the paper, then used a crush-cut test to assess the viability of any seeds that did not germinate (Connolly et al. 2017).

We analyzed data using R 4.0.3 (R Core Team 2020). To assess whether rhizobia influenced plant time to death, we performed a Kaplan–Meier model using the survdiff function from the survival package (Therneau 2020). We used the lmer function in the package lme4 (Bates et al. 2015) to assess the effects of treatment on transplant shock (proportion of plants in a plot that died within 14 days of transplanting), grazing (proportion of plants grazed), flower number (number of flowers produced by a plant in a plot, averaged across all plants in a plot and all sampling dates), pollinator visitation (number of visits to a plant in a plot, averaged across plants and sampling dates), seed number (number of seeds produced by a plant in a plot, averaged across plants in a plot), the total seed mass of all seeds on a plant (averaged across plants in a plot), the mass of individual seeds produced by a plant (averaged across plants in a plot), and the proportion of seeds that were viable (either germinated or passed the crush-cut test; averaged across plants within a plot in the seven replicate blocks where we had enough seeds to analyze). We plotted the residual and fitted values of each model to assess heteroscedasticity and used a square root transformation on seed number, mean seed mass, and total mass of seeds to meet the assumptions of a linear mixed model. We included block identity as a random effect in all models and tested significance using Wald *F* tests with Kenward-Roger degrees of freedom (*df*). To assess whether inoculation, grazing, or flower number predicted whether a plant produced seeds, we used a binomial mixed model with block, plot, and plant identity as nested random effects and Wald chi-square tests to assess significance.

Results

Rhizobia treatment increased survival of *Chamaecrista fasciculata* ($p = 0.006$; Fig. 1A) for nearly the entire growing season. Differences in time to death are likely related to differences in two key causes of mortality: transplant shock and grazing. Inoculated plants were 71% less likely to die from transplant shock ($F_{[1,14]} = 18.4$, $p < 0.001$; Fig. 1B) but 81% more likely to be grazed ($F_{[1,14]} = 56.7$, $p < 0.001$; Fig. 1C) than uninoculated plants (41% more likely when considering only plants that survived transplant shock; Fig. S1).

Because dead plants did not produce any flowers, rhizobia inoculation also increased the likelihood of plants producing flowers by 51% ($F_{[1,14]} = 7.1$, $p = 0.02$; Fig. 2A) and the number of flowers produced by 82% ($F_{[1,14]} = 8.8$, $p = 0.01$; Fig. 2B). Inoculated plants also received 73% more pollinator visits ($F_{[1,14]} = 6.7$, $p = 0.02$; Fig. 2C), but this effect seemed to be mediated through flower number: plants with more flowers had more visitation ($F_{[1,21.9]} = 327.2$, $p < 0.0001$; Fig. 2D) but inoculation did not increase pollinator visitation when controlling for flower number ($F_{[1,15.3]} = 1.0$, $p = 0.3$). However, these positive influences of rhizobia inoculation on pollination were mediated through effects on plant survival: when we repeated the analyses omitting the plants that died from transplant shock, there were no effects of inoculation on the probability of flowering ($F_{[1,13.7]} = 1.0$, $p = 0.9$; Fig. S2a), flower number ($F_{[1,13.6]} = 1.3$, $p = 0.3$; Fig. S2b), or visitation ($F_{[1,13.6]} = 0.7$, $p = 0.4$; Fig. S2c), even when controlling for the effect of flower number on visitation ($F_{[1,13.0]} = 0.9$, $p = 0.4$; Fig. S2d).

Most (78%) plants failed to produce seeds. However, inoculated plants, being more likely to survive and thus produce flowers, produced nearly three times the number and total mass of seeds and produced individual seeds that were almost twice as heavy as uninoculated plants, though none of these effects were statistically significant (Fig. 3; $F_{[1,14]} = 3.2$, $p < 0.1$; $F_{[1,14]} = 3.1$, $p < 0.1$; $F_{[1,14]} = 0.9$, $p = 0.3$). The effects of inoculation on these measures of plant performance, above and beyond effects on transplant shock, were in the same direction, though somewhat weaker and nonsignificant (Fig. S3; seeds

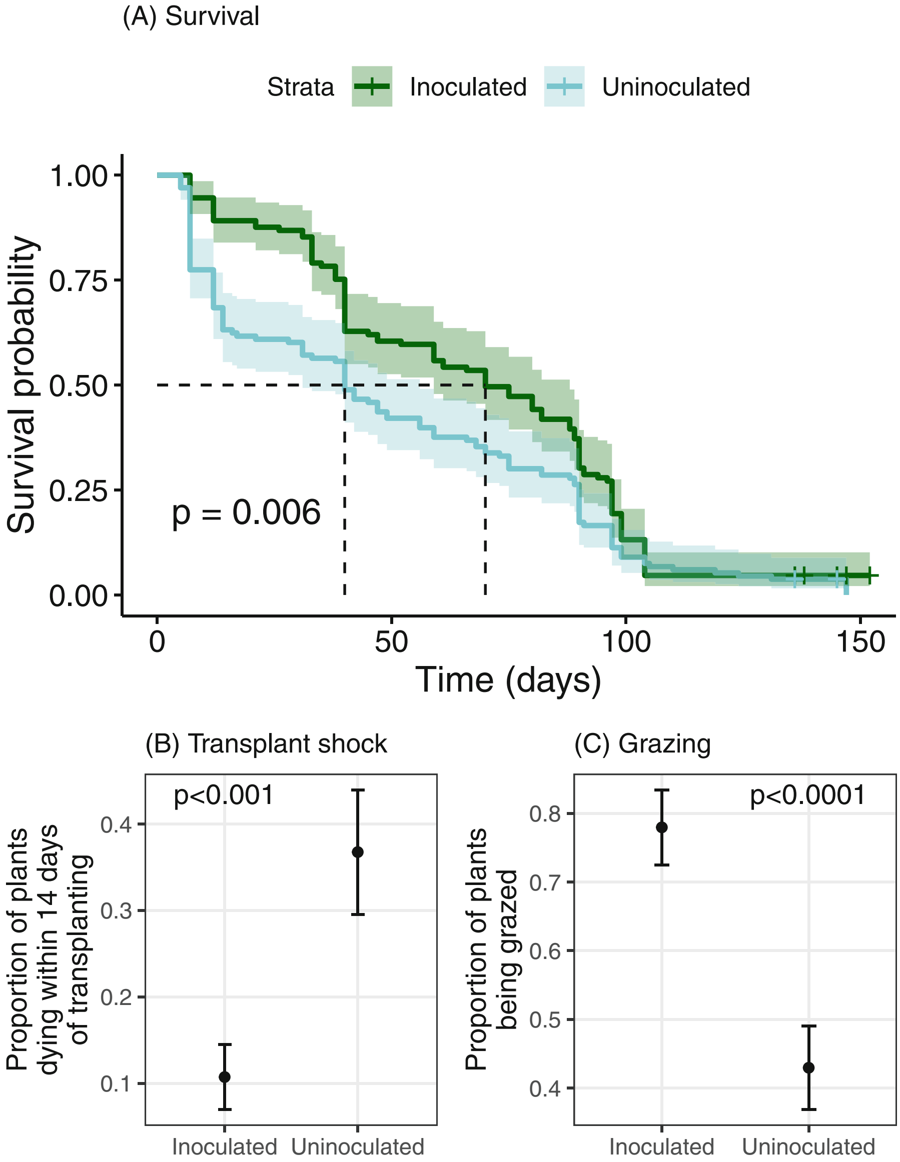

Figure 1. Effect of rhizobia inoculation on (A) survival curves, (B) proportion of plants that died within 14 days of transplanting (mean ± SE of 15 replicate plots), and (C) proportion of plants that were grazed (mean ± SE).

produced $F_{[1,13.7]} = 1.0$, $p = 0.3$; total seed mass $F_{[1,13.7]} = 1.1$, $p = 0.3$; mean seed mass $F_{[1,13.7]} < 0.0001$, $p = 1.0$). Significant predictors of whether a plant produced seeds included only flower number ($\chi^2_1 = 14.8$, $p < 0.001$) and not whether it was grazed ($\chi^2_1 = 3.1$, $p = 0.08$) or inoculated with rhizobia ($\chi^2_1 = 0.4$, $p = 0.6$). Seed germination for all seeds was very poor (3%), even though most (82%) were viable. Inoculation increased seed viability by about 10%, but this was not statistically significant ($F_{[1,6]} = 0.6, p = 0.47$; Fig 3D).

Discussion

Inoculating *Chamaecrista fasciculata* seedlings with nitrogen-fixing rhizobia before transplanting them into a restored prairie increased seedling survival and changed their interactions with other species, including herbivores and pollinators. Inoculated plants were more robust to transplant shock, were more likely to be grazed, and produced a greater number of flowers (because dead plants could not produce flowers), resulting in greater pollinator visitation and a trend toward more and heavier seeds. Therefore, inoculation can help *C. fasciculata* seedlings overcome establishment filters, but in complicated ways that affect other establishment filters such as herbivory.

Death of transplanted seedlings is of particular interest to restoration practitioners because it represents a loss of time, effort, and resources. While not unexpected, death from transplant shock can be severe (Miao et al. 1997; Davidson et al. 2019). The reasons that plants die from transplant shock are not well understood,

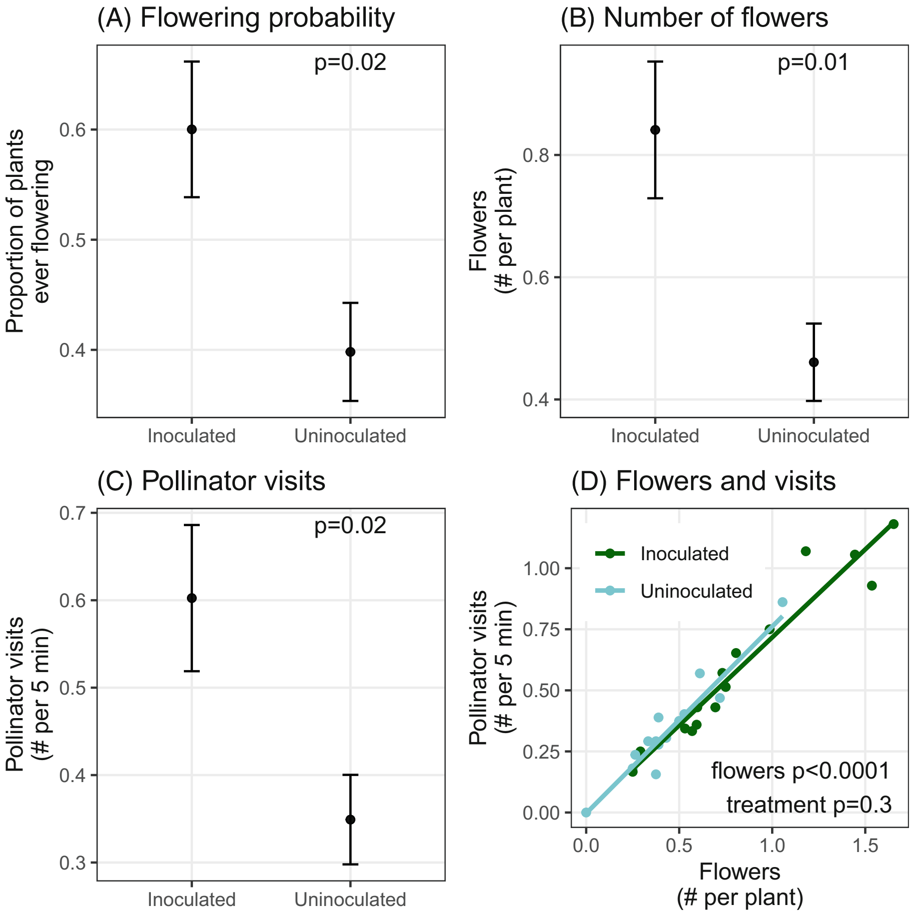

Figure 2. Effect of rhizobia inoculation on (A) the proportion of plants that ever produced flowers, (B) the number of flowers produced per plant (mean ± SE), (C) the number of pollinator visits per plant per 5 minutes observation period (mean ± SE), and (D) the relationship between flower number and pollinator visitation.

making it difficult for restoration practitioners to mediate the effects of transplant shock (Godefroid et al. 2011; Guzmán-Luna & Martínez-Garza 2016). Doyle et al. (2021) found that the use of whole soil inoculation as a pre-treatment increased the growth and health of *Hibbertia spanantha*, an Australian sub-shrub, in comparison to alternative pre-treatments. Combined with our findings, this indicates that the inoculation of seedlings with beneficial microorganisms can reduce the effect of transplant shock on translocated plants (Haase & Rose 1993; Blair et al. 2019). The exact mechanism by which these microorganisms mediate transplant shock is not known; it is possible that inoculating plants with rhizobia prior to transplanting makes plants more tolerant of low nutrient environments. Rhizobia can also aid in drought stress (Hussain et al. 2018), a widely known stressor for transplanted plants. Inoculation with rhizobia may also increase plant aboveground biomass, making them better competitors in areas with already established vegetation. Regardless of the mechanism by which rhizobia decreased transplant shock, inoculation increased the number of plants that survived the first stage of translocation and thus represents a valuable pre-planting treatment for restoration practitioners with cascading effects on flowering, pollination, and seed production.

Transplanted *C. fasciculata* also experienced intense grazing: 63% of plants were consumed by small mammals, likely voles (e.g. *Microtus pennsylvanicus*). The intensity of small mammal grazing supports the idea that these animals play an important role in the establishment and maintenance of plant diversity in restored ecosystems, particularly if intense grazing occurs early on in restoration (Howe et al. 2002). Herbivores may influence plant populations directly by removing plant individuals or indirectly by reducing the plants' ability to flower and produce seeds. Sullivan et al. (2016) found that *M. pennsylvanicus* reduced population growth and establishment of *C. fasciculata* in a tallgrass prairie and altered plant community structure. In our study, plants inoculated with rhizobia were more likely to be grazed, suggesting that inoculated plants were a preferred food source, possibly because of a higher nitrogen content (Ritchie & Tilman 1995; Knops et al. 2000; Nisi et al. 2015) or simply because they were larger and more apparent. Since herbivores may graze plants at any point in time, their activity has the potential to influence the duration and intensity of flowering as well as the timing of peak flowering (Tadey 2020), with long-term consequences for plant demography. Importantly, populations of small mammal grazers can be cyclic

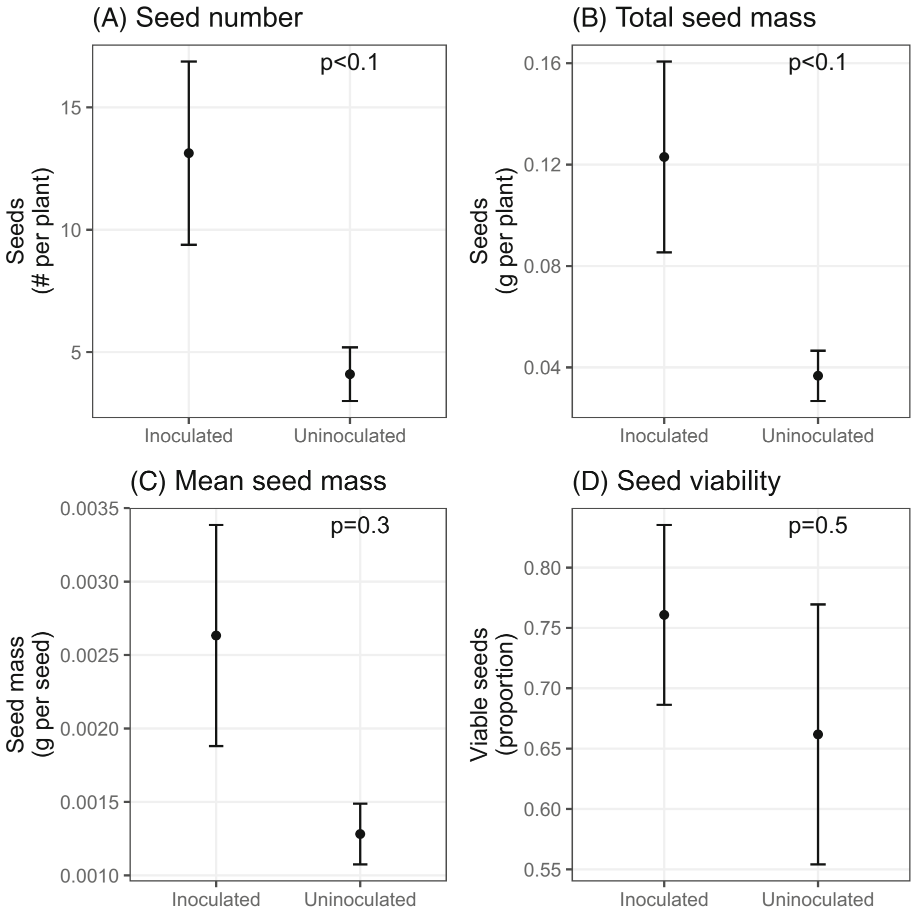

Figure 3. Effect of rhizobia inoculation on (A) the number of seeds produced per plant (mean ± SE), (B) the total mass of seeds produced per plant (mean ± SE), (C) the mean mass of all seeds produced by a plant (mean ± SE), and (D) seed viability (proportion viable).

(Howe et al. 2002), suggesting that additions of transplants or sowing seeds during nadirs in small mammal grazer populations will increase the likelihood of plant establishment in restoration. One recent study found that in their first growing season, no transplanted legume seedlings evaded herbivores whether inoculated or not (Galloway et al. 2024), suggesting that inoculations may be effective only when seedlings are protected from herbivory. Disentangling the interactive effect of rhizobia mutualisms with herbivore demography and preference, and in turn the effect of herbivory on legume pollination and seed production, will require further studies that manipulate both rhizobia mutualisms and grazers across multiple years.

Seed production in an annual plant represents the net effect of all abiotic and biotic influences on plant reproductive success. While *C. fasciculata* pollinator visitation was directly influenced only by flower number and not directly by inoculation, rhizobia increased plant survival and the number of flowers and therefore increased the probability of plants siring a greater number of offspring and producing a greater number of seeds. Although the effects we detected on seed production were only marginally significant and we were unable to test for effects on seed siring, we argue that rhizobia are likely to have a positive influence on *C. fasciculata* population growth through effects on survival and therefore reproduction. This is noteworthy because other studies have found that even if inoculated plants produce more flowers, they may not produce more seeds or sire more seeds on other maternal plants if there are insufficient pollinators in the fragmented landscapes in which restoration occurs (Steffan-Dewenter & Tscharntke 1999; Slagle & Hendrix 2009).

We observed two instances in which rhizobia altered *C. fasciculata* interactions with other species: pollinators and mammalian consumers. There are many other kinds of species interactions that rhizobia may have altered, including ants that defend against insect herbivores or arbuscular mycorrhizal fungi (Kempel et al. 2009; Godschalx et al. 2015; Fehling 2022). These other species interactions could also represent establishment filters. Thus, alleviating one establishment filter (rhizobia limitation) could alter the strength of other establishment filters (e.g. pollination, mammalian, or invertebrate herbivory, and/or arbuscular mycorrhizal fungal-mediated nutrient availability) that are themselves inherently variable in space and time. Lacking a mechanistic model and predictive understanding of when and where different establishment filters are likely to be most limiting, we can only suggest that practitioners acknowledge that rhizobia limitation may be one of many factors affecting legume establishment in restorations. However, our work here shows that even without modifying other establishment filters, rhizobia inoculation could potentially lead to higher legume population growth rates.

Acknowledgments

We thank E. Sprague, R. Bender, D. Ruffing, and A. Shukla for their help in the lab and field. We also gratefully acknowledge financial support from the EMU Biology Department's Daniel and Meta Hellwig Graduate Research Award and the National Science Foundation (DEB 1927671). Parts of this text have been previously published as partial fulfillment of academic accreditation requirements for Dollard's Master of Science from Eastern Michigan University. Data will be archived at the Environmental Data Initiative (EDI) upon manuscript acceptance.

LITERATURE CITED

Baer SG, Kitchen DJ, Blair JM, Rice CW (2002) Changes in ecosystem structure and function along a chronosequence of restored grasslands. Ecological Applications 12:1688–1701. https://doi.org/10.1890/1051-0761(2002)012[1688:CIESAF]2.0.CO;2

Barber NA, Chantos-Davidson KM, Peralta RA, Sherwood JP, Swingley WD (2017) Soil microbial community composition in tallgrass prairie restorations converge with remnants across a 27-year chronosequence. Environmental Microbiology 19:3118–3131. https://doi.org/10.1111/1462-2920.13785

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48. https://doi.org/10.18637/jss.v067.i01

Beyhaut E, Larson DL, Allan DL, Graham PH (2014) Legumes in prairie restoration: evidence for wide cross-nodulation and improved inoculant delivery. Plant and Soil 377:245–258. https://doi.org/10.1007/s11104-013-1999-z

Blair SA, Koeser AK, Knox GW, Roman LA, Thetford M, Hilbert DR (2019) Health and establishment of highway plantings in Florida (United States). Urban Forestry & Urban Greening 43:126384. https://doi.org/10.1016/j.ufug.2019.126384

Camill P, McKone MJ, Sturges ST, Severud WJ, Ellis E, Limmer J, et al. (2004) Community- and ecosystem-level changes in a species-rich tallgrass prairie restoration. Ecological Applications 14:1680–1694. https://doi.org/10.1890/03-5273

Connolly BM, Agnew LK, Orrock JL (2017) Interactive effects of contact fungicide and cold stratification on the germination rate for five dominant temperate tree species. Forest Science 63:303–309. https://doi.org/10.5849/FS-2016-110R3

Davidson BE, Germino MJ, Richardson B, Barnard DM (2019) Landscape and organismal factors affecting sagebrush-seedling transplant survival after megafire restoration. Restoration Ecology 27:1008–1020. https://doi.org/10.1111/rec.12940

Doyle CAT, Pellow BJ, Rapmund RA, Ooi MKJ (2021) Preparing threatened plants for translocation: does home soil addition and nutrient loading improve growth and flowering? Plant Ecology 222:829–842. https://doi.org/10.1007/s11258-021-01146-0

Fehling LS (2022) Reward complementarity and context dependency in multi-species mutualist interactions in partridge pea (*Chamaecrista fasciculata*). MS thesis. Miami University, Oxford, Ohio.

Galloway E, Price PA, Grman E, Bauer JT (2024) Rare legumes are missing mutualists, but herbivory and environmental filtering are more important determinants of reintroduction success. Restoration Ecology 32:e14278. https://doi.org/10.1111/rec.14278

Godefroid S, Piazza C, Rossi G, Buord S, Stevens A-D, Aguraiuja R, et al. (2011) How successful are plant species reintroductions? Biological Conservation 144:672–682. https://doi.org/10.1016/j.biocon.2010.10.003

Godschalx AL, Schädler M, Risel JA, Balkan MA, Ballhorn DJ (2015) Ants are less attracted to the extrafloral nectar of plants with symbiotic, nitrogen-fixing rhizobia. Ecology 96:348–354. https://doi.org/10.1890/14-1178.1

Graham PH (2005) Practices and issues in the inoculation of prairie legumes used in revegetation and restoration. Ecological Restoration 23:187–195. https://doi.org/10.3368/er.23.3.187

Grman E, Allen J, Galloway E, McBride J, Bauer JT, Price PA (2020) Inoculation with remnant prairie soils increased the growth of three native prairie legumes but not necessarily their associations with beneficial soil microbes. Restoration Ecology 28:S393–S399. https://doi.org/10.1111/rec.13126

Grman E, Bassett T, Zirbel CR, Brudvig LA (2015) Dispersal and establishment filters influence the assembly of restored prairie plant communities. Restoration Ecology 23:892–899. https://doi.org/10.1111/rec.12271

Guzmán-Luna A, Martínez-Garza C (2016) Performance of 15 tropical tree species recruited or transplanted on restoration settings. Botanical Sciences 94:757–773. https://doi.org/10.17129/botsci.659

Haase DL, Rose R (1993) Soil moisture stress induces transplant shock in stored and unstored 2 + 0 Douglas-fir seedlings of varying root volumes. Forest Science 39:275–294. https://doi.org/10.1093/forestscience/39.2.275

Howe HF, Brown JS, Zorn-Arnold B (2002) A rodent plague on prairie diversity. Ecology Letters 5:30–36. https://doi.org/10.1046/j.1461-0248.2002.00276.x

Hussain MB, Mahmood S, Ahmed N, Nawaz H (2018) Rhizobial inoculation for improving growth physiology, nutrition and yield of maize under drought stress conditions. Pakistan Journal of Botany 50:1681–1689

Jaurena M, Lezama F, Salvo L, Cardozo G, Ayala W, Terra J, Nabinger C (2016) The dilemma of improving native grasslands by overseeding legumes: production intensification or diversity conservation. Rangeland Ecology & Management 69:35–42. https://doi.org/10.1016/j.rama.2015.10.006

Kaschuk G, Leffelaar PA, Giller KE, Alberton O, Hungria M, Kuyper TW (2010) Responses of legumes to rhizobia and arbuscular mycorrhizal fungi: a meta-analysis of potential photosynthate limitation of symbioses. Soil Biology and Biochemistry 42:125–127. https://doi.org/10.1016/j.soilbio.2009.10.017

Keller KR, Carabajal S, Navarro F, Lau JA (2018) Effects of multiple mutualists on plants and their associated arthropod communities. Oecologia 186:185–194. https://doi.org/10.1007/s00442-017-3984-3

Kempel A, Brandl R, Schädler M (2009) Symbiotic soil microorganisms as players in aboveground plant-herbivore interactions – the role of rhizobia. Oikos 118:634–640. https://doi.org/10.1111/j.1600-0706.2009.17418.x

Kindscher K, Tieszen LL (1998) Floristic and soil organic matter changes after five and thirty-five years of native tallgrass prairie restoration. Restoration Ecology 6:181–196. https://doi.org/10.1111/j.1526-100X.1998.06210.x

Knops JMH, Ritchie ME, Tilman D (2000) Selective herbivory on a nitrogen fixing legume (*Lathyrus venosus*) influences productivity and ecosystem nitrogen pools in an oak savanna. Ecoscience 7:166–174. https://doi.org/10.1080/11956860.2000.11682585

Lopez ZC, Friesen ML, Von Wettberg E, New L, Porter S (2021) Microbial mutualist distribution limits spread of the invasive legume *Medicago polymorpha*. Biological Invasions 23:843–856. https://doi.org/10.1007/s10530-020-02404-4

Magnoli SM, Keller KR, Lau JA (2023) Mutualisms in a warming world: how increased temperatures affect the outcomes of multi-mutualist interactions. Ecology 104:e3955. https://doi.org/10.1002/ecy.3955

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiology Reviews 37:634–663. https://doi.org/10.1111/1574-6976.12028

Miao SL, Borer RE, Sklar FH (1997) Sawgrass seedling responses to transplanting and nutrient additions. Restoration Ecology 5:162–168. https://doi.org/10.1046/j.1526-100X.1997.09719.x

Mohamed OG, Dorandish S, Lindow R, Steltz M, Shoukat I, Shoukat M, et al. (2021) Identification of a new antimicrobial, desertomycin H, utilizing a modified crowded plate technique. Marine Drugs 19:424. https://doi.org/10.3390/md19080424

Muir JP, Pitman WD, Foster JL (2011) Sustainable, low-input, warm-season, grass–legume grassland mixtures: mission (nearly) impossible? Grass and Forage Science 66:301–315. https://doi.org/10.1111/j.1365-2494.2011.00806.x

Mulder C, Jumpponen A, Högberg P, Huss-Danell K (2002) How plant diversity and legumes affect nitrogen dynamics in experimental grassland communities. Oecologia 133:412–421. https://doi.org/10.1007/s00442-002-1043-0

Nisi AC, Hernández DL, English LP, Rogers ES (2015) Patterns of selective herbivory on five prairie legume species. The American Midland Naturalist 173:110–121. https://doi.org/10.1674/0003-0031-173.1.110

Price PA, Tanner HR, Dillon BA, Shabab M, Walker GC, Griffitts JS (2015) Rhizobial peptidase HrrP cleaves host-encoded signaling peptides and mediates symbiotic compatibility. Proceedings of the National Academy of Sciences of the United States of America 112:15244–15249. https://doi.org/10.1073/pnas.1417797112

R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ranjbar Sistani N, Kaul H-P, Desalegn G, Wienkoop S (2017) Rhizobium impacts on seed productivity, quality, and protection of *Pisum sativum* upon disease stress caused by *Didymella pinodes*: phenotypic, proteomic, and metabolomic traits. Frontiers in Plant Science 8:1961. https://doi.org/10.3389/fpls.2017.01961

Ritchie ME, Tilman D (1995) Responses of legumes to herbivores and nutrients during succession on a nitrogen-poor soil. Ecology 76:2648–2655. https://doi.org/10.2307/2265835

Rodríguez-Echeverría S, Fajardo S, Ruiz-Díez B, Fernández-Pascual M (2012) Differential effectiveness of novel and old legume–rhizobia mutualisms: implications for invasion by exotic legumes. Oecologia 170:253–261. https://doi.org/10.1007/s00442-012-2299-7

Simonsen AK, Dinnage R, Barrett LG, Prober SM, Thrall PH (2017) Symbiosis limits establishment of legumes outside their native range at a global scale. Nature Communications 8:14790. https://doi.org/10.1038/ncomms14790

Slagle MW, Hendrix SD (2009) Reproduction of *Amorpha canescens* (Fabaceae) and diversity of its bee community in a fragmented landscape. Oecologia 161:813–823. https://doi.org/10.1007/s00442-009-1429-3

Steffan-Dewenter I, Tscharntke T (1999) Effects of habitat isolation on pollinator communities and seed set. Oecologia 121:432–440. https://doi.org/10.1007/s004420050949

Sullivan LL, Danielson BJ, Harpole WS (2016) Mammalian herbivores alter the population growth and spatial establishment of an early-establishing grassland species. PLoS One 11:e0147715. https://doi.org/10.1371/journal.pone.0147715

Tadey M (2020) Reshaping phenology: grazing has stronger effects than climate on flowering and fruiting phenology in desert plants. Perspectives in Plant Ecology, Evolution and Systematics 42:125501. https://doi.org/10.1016/j.ppees.2019.125501

Temperton VM, Mwangi PN, Scherer-Lorenzen M, Schmid B, Buchmann N (2007) Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment. Oecologia 151:190–205. https://doi.org/10.1007/s00442-006-0576-z

terHorst CP, Wirth C, Lau JA (2018) Genetic variation in mutualistic and antagonistic interactions in an invasive legume. Oecologia 188:159–171. https://doi.org/10.1007/s00442-018-4211-6

Therneau T (2020) A package for survival analysis in R. R package version 3.4-0. https://CRAN.R-project.org/package=survival

van der Heijden MGA, Bardgett RD, Straalen NMV (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11:296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

Supporting Information

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

Figure S1. Proportion of transplant survivors that were grazed.
Figure S2. Effect of rhizobia inoculation on (a) the proportion of plants that ever produced flowers, (b) the number of flowers produced per plant (mean ± SE), and (c) the number of pollinator visits per plant per 5 minutes observation period (mean ± SE), after removing all the plants that died during the first 14 days after transplanting.
Figure S3. Effect of rhizobia inoculation on (a) the number of seeds produced per plant (mean ± SE), (b) the total mass of seeds produced per plant (mean ± SE), and (c) the mean mass of all seeds produced by a plant (mean ± SE), after removing all the plants that died during the first 14 days after transplanting.

Coordinating Editor: Louise Egerton-Warburton

Received: 6 January, 2025; First decision: 13 February, 2025; Revised: 28 May, 2025; Accepted: 28 May, 2025