The Plant Microbiome and Native Plant Restoration: The Example of Native Mycorrhizal Fungi

LIZ KOZIOL, PEGGY A. SCHULTZ, GEOFFREY L. HOUSE, JONATHAN T. BAUER, ELIZABETH L. MIDDLETON, AND JAMES D. BEVER

Ecological restoration efforts can increase the diversity and function of degraded areas. However, current restoration practices cannot typically reestablish the full diversity and species composition of remnant plant communities. We present evidence that restoration quality can be improved by reintroducing key organisms from the native plant microbiome. In particular, root symbionts called arbuscular mycorrhizal fungi are crucial in shaping grassland communities, but are sensitive to anthropogenic disturbance, which may pose a problem for grassland restoration. In the present article, we highlight the conceptual motivation and empirical evidence evaluating native mycorrhizal fungi, as opposed to commercial fungi. Reintroduction of the native microbiome and native mycorrhizal fungi improves plant diversity, accelerates succession, and increases the establishment of plants that are often missing from restored communities. The example of mycorrhizal fungi serves to illustrate the value of a more holistic view of plant communities and restoration that embraces the intricacies and dynamics of native microbial communities.

Keywords: arbuscular mycorrhizal fungi, plant microbiome, plant–microbe interactions, grassland restoration, inoculation

Grassland restorations are commonly able to increase native plant cover, but outcomes vary widely (figure 1; Brudvig et al. 2017). Generally, restorations have lower plant species diversity compared with that of nearby remnant grasslands (Kindscher and Tieszen 1998, Martin et al. 2005, Middleton et al. 2010), and plant species richness, especially forb species, can decline over time (Baer et al. 2002). In addition, some of the plant species seeded into restorations are not well represented in the resulting plant community (Grman et al. 2015). Although management strategies and site histories can explain some variation in restored plant community composition (Grman et al. 2013), much of the variation in restoration outcomes remains unexplained. This remaining variation in outcomes is likely because of restoration protocols that focus primarily on the plant community rather than on the establishment of other important ecological components of grassland ecosystems. In the present article, we will argue that the focus on the reintroduction of plants without the reestablishment of native plant microbiomes may be limiting restoration success.

Accumulating evidence identifies the plant microbiome, including both soil microbes and microbes living fully within plants, as an important driver of plant community composition. Experiments and field studies have shown that microbes play important roles in plant local adaptation (Schultz et al. 2001, Johnson et al. 2010), coexistence (Bever et al. 2015), relative abundance (Klironomos 2002, Mangan et al. 2010), succession (Kardol et al. 2007, Bauer et al. 2015, Koziol and Bever 2016b), and invasions (Callaway et al. 2004, Pringle et al. 2009, Vogelsang and Bever 2009). Given this growing realization that microbiomes can structure plant communities, it is logical that the successful restoration of native plant communities may require reestablishment of native microbiomes. Although many microbiome components might be important to plant and ecosystem function, plant mutualists such as mycorrhizal fungi are obvious first candidates to aid restoration.

It has long been known that reintroduction of mycorrhizal fungi can be crucial to the establishment of plant species in artificial and severely degraded landscapes such as in the reclamation of mine spoils (e.g., Jasper et al. 1989). However, when attempting to use mycorrhizal fungi to aid the restoration of native plant diversity, the response to inoculation can be highly variable across different studies, detailed in a recent review expressing skepticism about the value of inoculation in enhancing restoration quality (Hart et al. 2017b). In the present article, we focus on inoculation with native arbuscular mycorrhizal (AM) fungi in particular, and we suggest that some of the variation in the effectiveness of inoculation reflects the lack of attention to the source of the

BioScience 68: 996–1006. © The Author(s) 2018. Published by Oxford University Press on behalf of the American Institute of Biological Sciences.
All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com.
doi:10.1093/biosci/biy125
Advance Access publication 14 November 2018

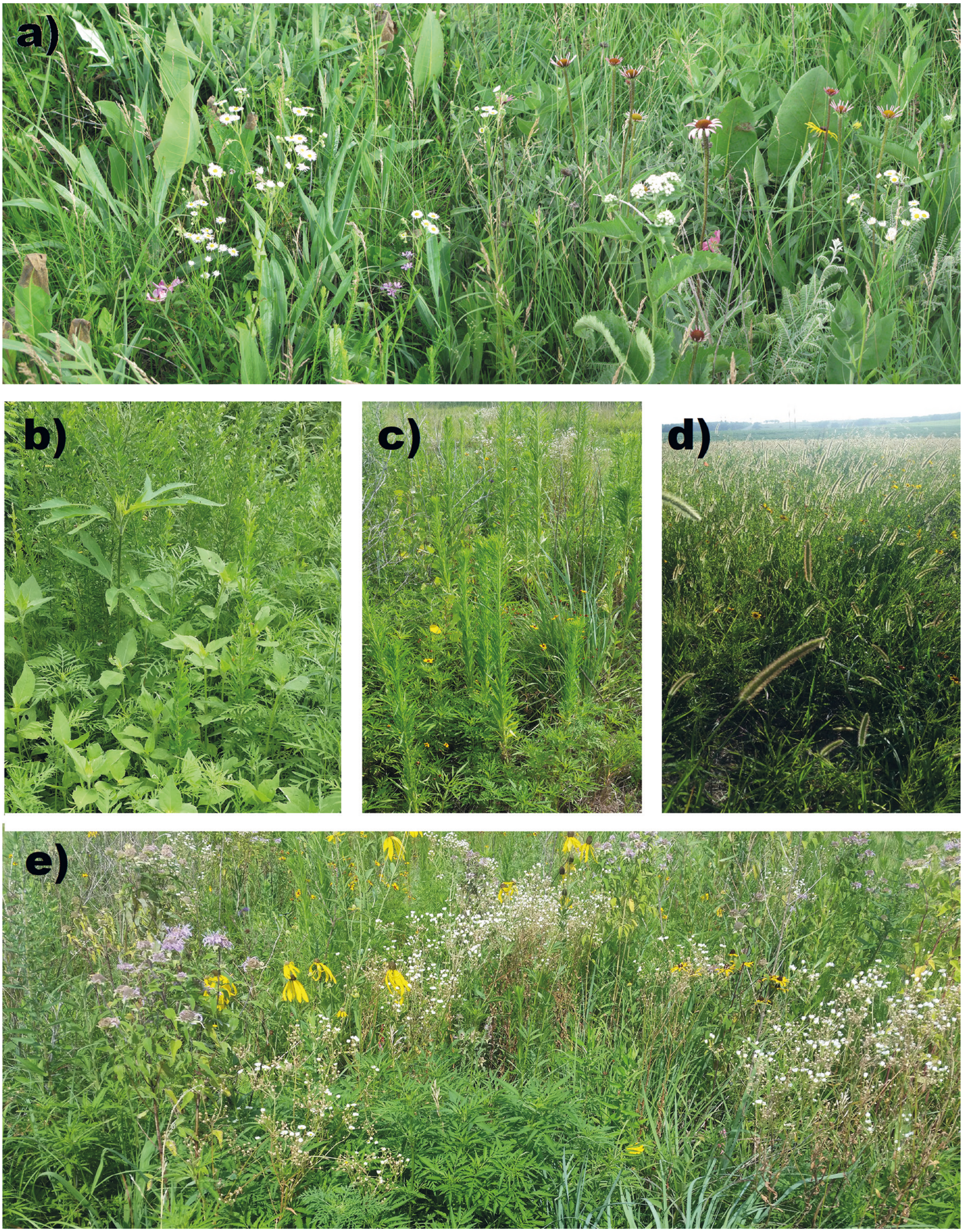

Figure 1. Undisturbed, remnant prairies have greater plant and functional diversity, with 24 native plant species being visible in this photo (a), compared with nearby prairie restorations (b–e). Restorations are often dominated by weedy native plants such as Conyza canadensis *(b);* Ambrosia *spp. (c); nonnative forbs, such as* Lespedeza cuneata *(c); nonnative grasses, such as* Setaria pumila *(d); or low-diversity native grasses and forbs (e). Many early successional and nonnative plants (b–e) have a low responsiveness to AM fungi, such as* Monarda fistulosa, Elymus canadensis, Rudbeckia hirta, Setaria *spp. and* Solidago canadensis, *whereas plants that are common in undisturbed prairies are generally more responsive to mycorrhizae, including species in the genera* Echinacea, Amorpha, Sporobolous, Silphium, *and* Dalea *(see Koziol and Bever 2015 and Bauer et al. 2018 for a more complete list of species mycorrhizal responsiveness).*

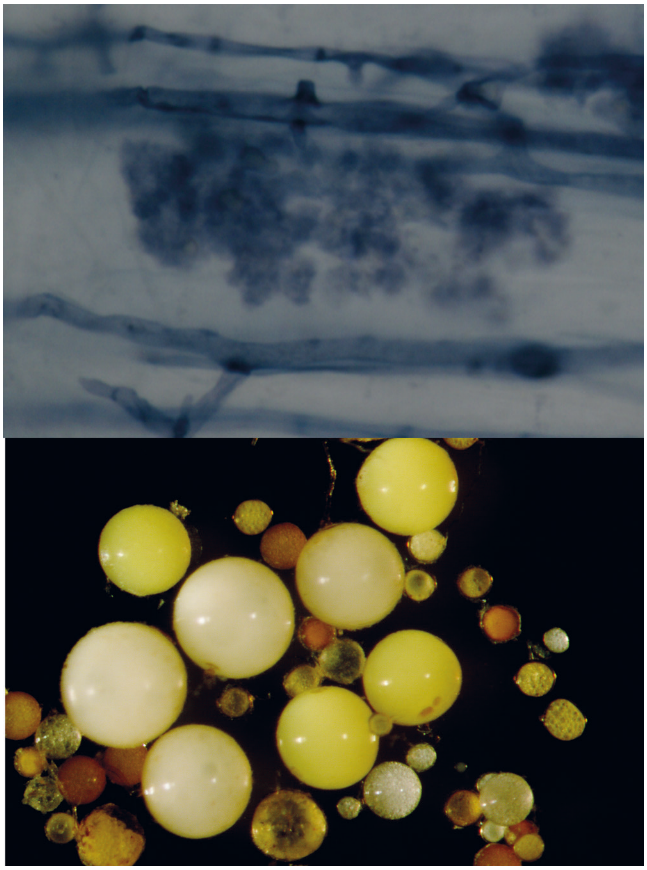

Figure 2. Long, slender fungal hyphae and highly branched arbuscules (rectangular structure) of AM fungi are stained dark blue inside and in between the plant root cells (a). Single-celled spores of nine different AM fungal species differ in size, color, and ornamentation (b).

AM fungal inocula and the ecological target of the restoration. On the basis of the last 15 years of experiments, we suggest that the value of reintroducing native AM fungi to restorations will likely depend on the context in which they are being used, including the land-use history of the site, the plant species selected, and the type of AM fungi chosen for inoculation.

In this review, we summarize what is known about inoculation treatments with AM fungi in order to determine when these inoculations are likely to enhance the establishment, growth, and survival of desirable plant species in restorations. We outline different inoculation techniques and inocula sources and discuss their costs and benefits. Most of the discussed work has taken place in the prairie grasslands of the United States, but we expect that the underlying community dynamics and the value of inoculation with native AM fungi will be similar for other ecosystems (e.g., Wubs et al. 2016). Moreover, we expect that lessons learned from AM fungal inoculation will be relevant for efforts to evaluate the effect of reintroducing of other components of the native plant microbiome in native plant restoration.

Overview of mycorrhizal mutualisms

Terrestrial plants have essential and long-standing relationships with root symbionts, including AM fungi, which are found in fossilized roots of some of the first land plants that grew nearly 300 million years ago (Remy et al. 1994). This symbiosis between plants and AM fungi represents one of the oldest and most widespread mutually beneficial interactions on earth, and most extant plant species continue to benefit from this mutualism. Plants rely on AM fungi to collect and deliver phosphorus and other nutrients that limit plant growth in exchange for carbohydrates from the plant. AM fungi infect host plant roots and then send threadlike hyphae beyond the root zone, mining nutrients from the soil and transporting them back to their host plant. Nutrient exchange occurs within plant roots in specialized structures called *arbuscules* (figure 2a). These fungi can also provide plants with nonnutritional benefits by alleviating environmental stressors such as drought (Koziol et al. 2012) and diminishing host susceptibility to herbivory (Middleton et al. 2015, Delavaux et al. 2017). Beyond benefitting the growth of a plant host, AM fungi confer other ecosystem services, such as reducing soil erosion by promoting soil aggregation through production of a sticky glycoprotein called *glomalin* (Wright and Upadhyaya 1996). Because of the diverse benefits that plant communities can either directly or indirectly receive through associating with AM fungi, the reintroduction of native AM fungi has the potential to promote native plant growth in restorations and to improve soil health and ecosystem quality.

Native, late successional AM fungi are likely to be functionally different from disturbance adapted early successional fungi

AM fungal species are ecologically and functionally distinct. Individual species vary in their effect on plant host phosphorous, nitrogen, potassium, and sodium uptake (Aggangan et al. 2010, Ji and Bever 2016); the carbon elicited from a host plant (Bever et al. 2009); and their ability to provide nonnutritional benefits such as alleviation of drought stress (Marulanda et al. 2003) and pathogen resistance (Sikes et al. 2009). Importantly, because fungi vary in the specificity of these effects, AM fungal composition can alter the outcome of plant–plant interactions and ecosystem functions. For instance, variation in plant response to specific AM fungal taxa may drive plant community diversity and productivity (van der Heijden et al. 1998, Vogelsang et al. 2006, Wagg et al. 2011). In addition, specific AM fungal species are consistently beneficial to plant species that are highly dependent on mycorrhizal fungi (Koziol and Bever 2016a), suggesting that some AM fungal species may be more important to include in an inoculum than others (Koziol and Bever 2018).

AM fungal species also differ in their sensitivity to anthropogenic disturbance. The net effect of major disturbances such as conventional agriculture or reclaimed mine landscapes, which combine multiple individual stressors, is that soils have significantly degraded AM fungal communities

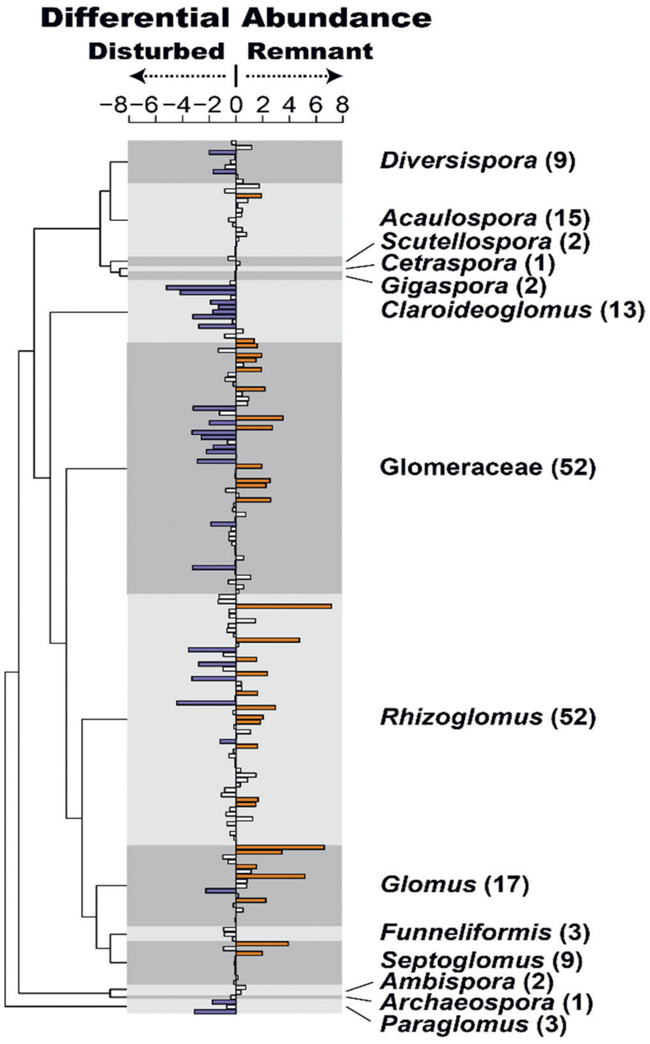

Figure 3. Differential abundance (log$_2$ scale) of AM fungal operational taxonomic units (OTUs) in remnant prairies (remnant) and nearby postagricultural fields (disturbed) sampled in Illinois and Missouri. Each bar represents an OTU: The orange bars are OTUs significantly more abundant in remnant sites, the purple bars are OTUs significantly more abundant in disturbed sites, and the unfilled bars are OTUs found equally in both site histories. OTUs were derived from ribosomal RNA gene sequences and were assigned to genera (or family Glomeraceae); the alternating gray bands group OTUs by taxon, with the number of OTUs listed in parentheses (see House and Bever 2018 for details). The branching structure to the left denotes evolutionary relationships among taxa.

that may require restoration. Mechanical disturbance such as tillage is highly destructive to soil organisms; it can decrease AM fungal density and shift fungal composition (Oehl et al. 2003). Microbial biomass may require decades to recover after tillage cessation (Baer et al. 2002). Specifically, it has been shown that frequently tilled soils have an increased abundance of non-*Glomus* species (Oehl et al. 2004) and a reduction or a loss of AM fungal species from the genera *Scutellospora*, *Entrophospora*, and *Acaulospora* (Jansa et al. 2002). Others have found that repeated fertilization can select for less beneficial AM fungi and an increase in *Rhizoglomus intraradices* (Johnson 1993). In assessing AM fungal communities in remnant prairies and nearby post-agricultural fields in Illinois and Missouri, House and Bever (2018) found that 56 of the 181 AM fungal taxa observed were sensitive to anthropogenic disturbance. Although 25 of these sensitive AM fungal taxa increased with disturbance, the remaining 31 were heavily reduced (figure 3 doi:10.5061/dryad.bs79gk5).

The changes in fungal composition due to anthropogenic change can have consequences for plant community succession and ecosystem processes. If we apply the plant ecology concept of tolerance to disturbance decreasing as succession progresses (Grime 1977) to AM fungi, we can consider those that are disturbance tolerant to represent early successional species (weedy) and those that decrease with disturbance to represent late successional species. The loss of species or a change to more disturbance adapted, early successional species may result in less beneficial or less mutualistic fungal communities, which could have negative impacts on restoration success. For instance, fungi in early successional environments failed to promote the growth and establishment of late successional plants (Johnson 1993, Middleton and Bever 2012, Koziol and Bever 2016b, Bauer et al. 2017) or soil aggregate stability (Duchicela et al. 2012). Because the changes that occur in AM fungal composition during succession or after soil disturbance can persist for long periods of time (House and Bever 2018), restoring AM fungi from native, late successional environments may improve restoration success and ecosystem function in disturbed or early successional soils. For simplicity, we refer to native, late successional fungi as *native fungi* for the remainder of this review.

Commercial fungi are likely to be functionally similar to early successional fungi

Although tremendous attention is paid to the origin and native status of the seeds used in grassland restorations, current restoration practice fails to consider the origin of microbial inoculants including commercial mycorrhizal products. Many commercial mycorrhizae are of unreported origin. In the present article, we argue that the ecology of many commercial inocula is functionally more similar to weedy, early successional native fungi and that these inocula may hinder the succession of restorations. Fungi that proliferate with disturbance may be more easily cultured and therefore are more likely to be included within commercial mixes (see box 1). Indeed, commercial mycorrhizal inocula manufacturers or sellers sometimes promote their mycorrhizae as the most “aggressive” or “high-yielding” fungal strains and some report spore counts of up to 387 fungal propagules per gram—which can be 8–10 times greater than

Box 1. Types of AM fungal Inocula.

Whole soil amendments

Whole soil is as simple as it sounds: It inoculates with intact rhizosphere soil containing the complete array of the soil community including AM fungi, beneficial bacteria such as nitrogen fixing *Rhizobia*, pathogens, soil-dwelling insects, nematodes, plant roots, and even seeds. Using whole soil from a reference late successional remnant grassland has been shown to be more beneficial to plant growth and richness in restorations than whole soils collected from disturbed old fields (Bever et al. 2003, Ji et al. 2010, Middleton and Bever 2012). This pattern has also been observed using reference ecosystem whole soils within heathland restorations (Wubs et al. 2016). Although it is beneficial, whole soil inoculations are highly destructive of remnant sites, and inoculation rates of 150–10,000 gallons of inoculum per acre have been reported in the literature (Bever et al. 2003, Middleton and Bever 2012, Wubs et al. 2016), which is beyond the scope of many restoration projects in terms of cost, effort, and inoculum availability. Because the destruction of remnant habitat to improve a restoration cannot be justified, this option may only be viable in cases in which a remnant habitat is condemned.

Trap cultures

A rarely tested alternative to whole soil inoculations in restoration is cultured whole soils called *trap cultures*. The trap culture method works to amplify the volume of whole soil microbes so that a few liters of whole soil inoculum can be used to inoculate hundreds of plants. However, this method requires some training in microbial culturing, the ability to sterilize growing media, and greenhouse environments. Although few have studied the effect trap culturing practices have on microbial communities, some have reported that AM fungal diversity can be lost over time (Trejo-Aguilar et al. 2013) and that the selection of plant hosts can change microbial community composition (Bever et al. 1996) and can lead to the buildup of plant pathogens (Bauer et al. 2015). Therefore, more study is needed to determine the best practices for producing trap cultures in addition to the benefits that they might provide to restoration.

Pure arbuscular mycorrhizal fungal culture amendments

Arbuscular mycorrhizal (AM) cultures contain only living propagules of single or multiple mycorrhizal fungal species (figure 2b). AM fungal cultures can associate with and benefit the growth of a wide range of plant species that are desirable in restoration (Koziol and Bever 2015, Middleton et al. 2015, Bauer et al. 2018). Whether AM fungal cultures benefit restoration largely depends on inocula source and composition, with native AM fungi being especially important for late successional grassland plant species (figure 4; Koziol and Bever 2016b). Often diverse mixtures have been found to provide similar benefits to the best individual fungus (e.g., figure 4). As the best individual fungus can depend on environment, mixtures provide more consistent benefits across environments (Vogelsang et al. 2006). Meta-analyses of inoculation studies show that native, locally adapted AM fungal inocula are likely to benefit plant growth more than commonly available commercial inocula even if the commercial inocula is diverse (Maltz and Treseder 2015, Middleton et al. 2015, Rúa et al. 2016). Commercial inocula may be ineffective because they are (most likely) collected from nonnative or nonreference ecosystems or because they tend to comprise "weedier" fungal species, such as *Rhizoglomus intraradices*, *Funneliformis mosseae*, and *Claroideoglomus claroideum*, that may proliferate with anthropogenic disturbance (figure 4). Commercial fungi tend to behave more like early successional fungi in restorations, likely because commercial fungi are similar to the early successional fungi already present in disturbed soils. We argue that native, late successional mycorrhizal fungi (commercial or otherwise) be used in restorations because there is accumulated evidence indicating that nonnative commercial fungi are not beneficial in restorations (Middleton et al. 2015), and there are potential negative consequences of introducing nonnative soil organisms to restoration sites and these introductions may not be reversible (Hart et al. 2017a).

what is typically observed in nature, which ranges from less than 10 to around 50 spores per gram of soil (Visser et al. 1984, Jasper et al. 1991, Johnson 1993, Oehl et al. 2003, Oehl et al. 2004, Middleton et al. 2015, Säle et al. 2015). In addition, many commercial mycorrhizae comprise either a single or a limited number of AM fungal species (box 1). Many species that are common in commercial mycorrhizal inocula are species that have been shown to proliferate and be found at high abundance in disturbed, early successional soils, such as *Rhizoglomus intraradices*, *Funneliformus mosseae*, and *Rhizoglomus aggregatus* (Johnson 1993, Jansa et al. 2002, Oehl et al. 2004). Many fungal species that are sensitive to tillage and fertilization disturbance, such as those in the genera *Scutellospora*, *Entrophospora*, *Gigaspora*, and *Acaulospora* (Johnson 1993, Jansa et al. 2002, Oehl et al. 2004), are typically absent in commercial inocula. As fungi that proliferate with disturbance may already be common at sites that are targeted for restoration, it is possible that adding commercial inoculum that comprise early successional fungi may not alter the functions already present at that site.

Consistent with what has been found with weedy, early successional fungi, some commercial fungi have been shown to inhibit late successional plant growth (Middleton et al. 2015, Emam 2016) and are ineffective at reducing erosion (Vogelsang and Bever 2010), improving species richness (Perkins and Bennett 2017), or increasing native plant cover (White et al. 2008, Ohsowski et al. 2017). However, native soil microbes, including native mycorrhizal fungi, can confer strongly positive effects on restoration quality in comparable restoration experiments (Maltz and Treseder 2015, Middleton et al. 2015). We argue that native mycorrhizal fungi (commercial or otherwise) be used as inocula in restorations because there is accumulated evidence indicating that nonnative commercial fungi are not beneficial

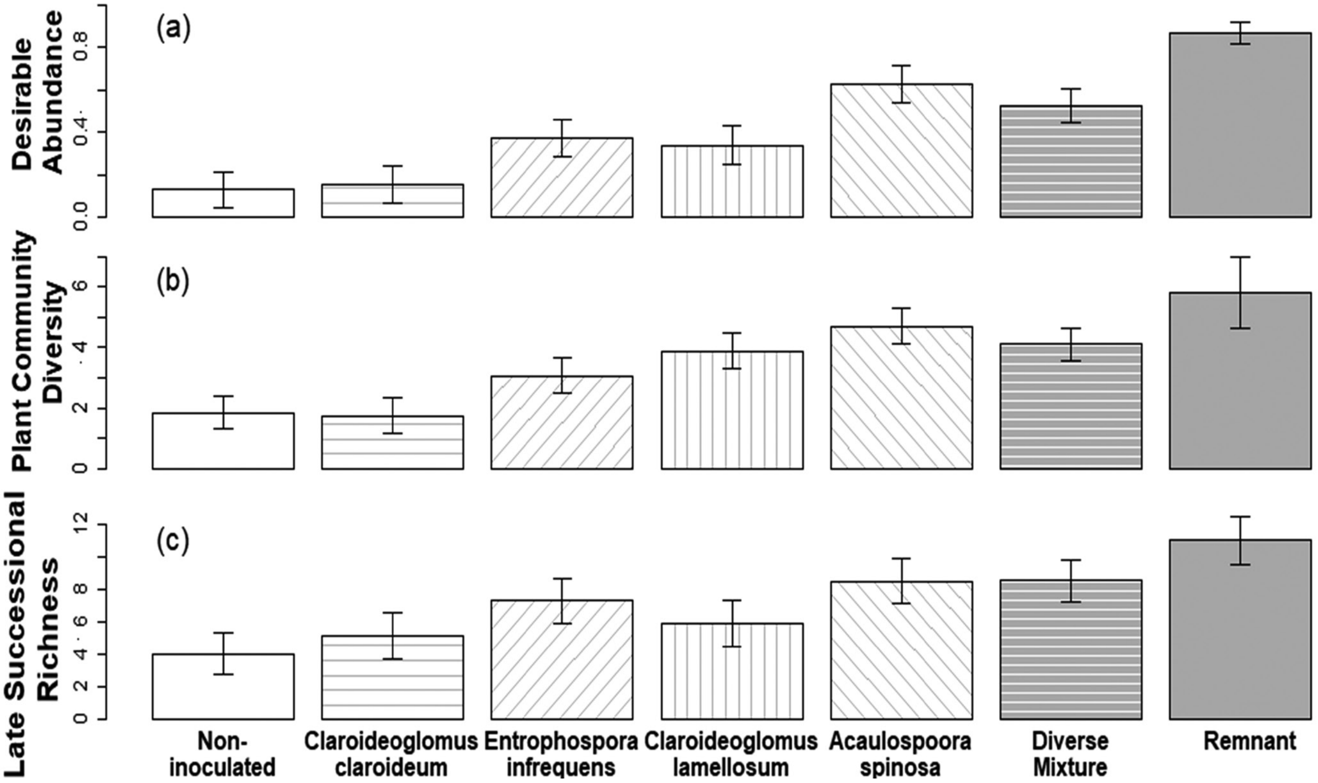

Figure 4. Restoration plots were seeded with 54 species and inoculated with either one of four different AM fungi isolated from a remnant prairie or a diverse mixture of four AM fungi, or they were not inoculated. Inoculation increased the abundance of desirable plants (a), community diversity (inverse Simpson's index; b) and increased late successional richness (c). Desirable plants are plants that were seeded in the restoration or were native in remnant habitat. Fungal inoculations moved the restored plant communities toward the plant composition found in remnant, undisturbed prairie in nearby southern Illinois (remnant). The data were adapted from Koziol and Bever (2016b) and from previously unpublished data of plant community composition in remnant prairies near Champaign–Urbana, Illinois.

in restorations and because there are potential negative consequences of introducing nonnative soil organisms to restoration sites and these introductions may not be reversible (Hart et al. 2017a). Because of variation in plant growth responses to individual fungal species, we highlight the need for more research to be conducted on the effect that the composition of native, late successional AM fungi has on restoration outcomes.

The potential benefits to grassland restoration

To illustrate the potential benefits of inoculation with native, late successional AM fungi in native grassland restoration, we describe the results from a recent experiment assessing the effects of five different native AM fungal inocula on a restoration that was seeded with 54 prairie plant species (Koziol and Bever 2016b). The study began with solarization of the area to be restored for 1 month in the early spring using black plastic that killed much of the turf. The experiment compared different native AM fungal inoculation treatments using one of four individual AM fungal species derived from native, late successional prairies in Indiana, or using all four AM fungal species together: *Entrophospora infrequens*, *Claroideoglomus lamellosum*, *Acaulospora spinosa*, *Claroideoglomus claroideum*. The AM fungi were introduced by planting inoculated seedlings of prairie plant species (nurse plants). Nurse plants inoculated with AM fungi were about 40% more likely to survive and grew three times larger than noninoculated seedlings in the first year. These benefits spread to neighboring plants because plots inoculated with AM fungi had improved establishment of desirable plants from seed, increased plant species diversity, and more species of desirable, late successional prairie plant species during the second year of the restoration (figures 4a–4c). As in laboratory studies (e.g., Vogelsang et al. 2006), this study demonstrates that inoculating restorations with a diverse mixture of native mycorrhizal fungi is as effective as inoculating with the best individual fungus with regards to improving of plant species richness and diversity to be more similar to remnant prairie communities compared with noninoculated controls, which represent conventional restoration practices (figure 4; see the supplemental material online). Because the best individual fungus

Box 2. When and How to Inoculate with AM Fungi.

When and how inoculation should take place to ensure its best chance for success requires careful consideration. As obligate symbionts, AM fungi need to infect roots of effective hosts to ensure their growth and long-term survival. Therefore, mycorrhizal inocula are best introduced into a restoration on the roots of host plants (Middleton and Bever 2012, Koziol and Bever 2016b) or during spring seed application when plant roots will be quickly available for colonization. Some have found broadcasting (Wubs et al. 2016) and tilling (Bever et al. 2003) to be effective ways to introduce microbiome inocula. However, in the absence of association with a growing host plant, AM fungal hyphae and spores are vulnerable to parasitism by bacteria and other fungi, as well as consumption by worms and insects. In addition, surface broadcasting and inconsistent distribution of inocula with ineffective tillage may leave the AM fungal inocula on the soil surface, where spores may be prone to desiccation in addition to starvation from a lack of host plant roots.

As many early successional and nonnative weedy plants are poor hosts for mycorrhizal fungi (Vogelsang and Bever 2009, Lankau et al. 2014), the initial stages of a restoration represent a particularly sensitive period for inoculations. However, the early establishment of native mycorrhizal fungi and late successional plants can generate a positive feedback that can accelerate restoration (Koziol and Bever 2016b). Therefore, inoculation early in a restoration has the potential to have the greatest positive impact.

can depend on environment, this effect may enable diverse fungal mixtures to provide more consistent benefits across varied environments.

Increased establishment of native plant species has also occurred in other independent inoculation experiments, both in North American grasslands of the tallgrass prairie and western coastal and desert plains (Richter and Stutz 2002, Bever et al. 2003, Vogelsang and Bever 2010, Middleton and Bever 2012, Middleton et al. 2015) and in grasslands elsewhere in the world (Zhang et al. 2012, Wubs et al. 2016). The reintroduction of native AM fungi in restorations can improve the establishment of native plant species, which, in turn, can improve habitat quality for wildlife and pollinators (Debinski and Babbit 1997, Tonietto and Larkin 2018). These benefits can be amplified over time, because successful establishment of long-lived late successional plant species can suppress invasive plants (Middleton et al. 2010) and thereby reduce future management costs. However, the value of reintroduction of native AM fungi to restorations will likely depend on the context in which they are being used, including the land-use history of the site, the plant species planted, and the type of AM fungi chosen for inoculation (box 1).

When and where might native AM fungal inoculation be the most beneficial?

Restorations are necessary in degraded sites that have been altered physically, chemically, or biologically. The land-use history of a restoration site will determine where mycorrhizal inoculations will improve restoration success. Chemical application, such as glyphosate or fungicides, can reduce the viability of AM fungi and other beneficial soil microbes including symbiotic *Rhizobia* (Druille et al. 2015). Although we lack a detailed understanding of the natural history of most soil microorganisms, including AM fungi, it is clear that soil microbial communities are generally negatively affected by anthropogenic disturbance (Fierer et al. 2013), and the same is true for AM fungi (Oehl et al. 2003, Moora et al. 2014, House and Bever 2018). We illustrate this problem with recent analyses of AM fungal composition of prairies and postdisturbance old fields of Illinois and Missouri (figure 3). We found that several AM fungal taxa are particularly abundant in undisturbed prairies but appear sensitive to disturbance, whereas other AM fungal taxa accumulate with anthropogenic disturbances including overgrazing, mechanical soil disturbance, and the accumulation of nonnative plant species (House and Bever 2018). AM fungal communities are slow to recover following the abandonment of agriculture, perhaps because native grasslands are often not available nearby as sources of natural colonization.

We have limited information on how the majority of AM fungi disperse naturally. A few species of AM fungi are dispersed by rodents (Mangan and Adler 2002), although some evidence suggests these fungi may disperse very slowly via underground movement—perhaps only a meter or two a year (Middleton and Bever 2012, Middleton et al. 2015). This suggests that although fungal biomass and density may recover after disturbance, some AM fungal species are effectively lost (Oehl et al. 2003) because of their low colonization ability. Therefore, areas with land-use history that includes soil disturbance, such as mechanical and chemical disturbance, may benefit from the addition of native fungi prior to restoration.

Another consequence of anthropogenic disturbance is the establishment of invasive plants. Plant invasions can have strong negative effects on AM fungal density and community composition (Hawkes et al. 2006, Pringle et al. 2009, Grove et al. 2017), the growth and establishment of native plants (Pringle et al. 2009, Vogelsang and Bever 2009), and soil aggregate stability (Duchicela et al. 2012). More work is needed to understand how often legacy effects occur and their duration (Lankau et al. 2014), but inoculation with AM fungi will likely improve restoration outcomes where species with known negative effects on AM fungal communities invade. For this reason, dominance of nonnative plant species on the landscape may be a good indicator of the value of inoculation (Vogelsang and Bever 2009, Duchicela et al. 2012). Inoculation

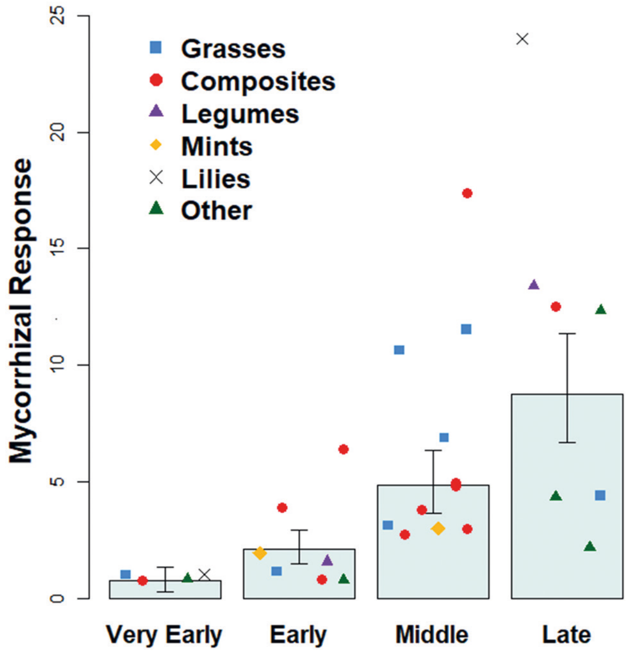

Figure 5. Mycorrhizal responsiveness (plant mass with native AM fungal inoculation or plant mass without AM fungal inoculation) is greater for later successional plants. Later successional plants (e.g., Amorpha canescens*) tend to be conservation priorties (Bauer et al. 2018) and can be difficult to establish in restoration. These results indicate that late successional plants may be inhibited in disturbed soils that have weakened mycorrhizal fungal communities, whereas early successional plants will not. This effect was consistent across plant family (the symbols of different colors and shapes). The bars represent the average mycorrhizal responsiveness within each successional stage, and the points represent each individual plant species within that successional stage. The data were adapted from Koziol and Bever (2015).*

with native AM fungi has improved establishment of native grassland species in communities dominated by nonnative species, including abandoned agricultural fields, mown lawns, road cuts, and pastures (Bever et al. 2003, Middleton et al. 2015, Koziol and Bever 2016b). See box 2 for more information on when and how to inoculate with AM fungi.

Which plant species benefit most from native AM fungal inoculation?

Many plant species have been shown to benefit from mycorrhizal fungi, including hundreds of species of grasses, composites, and legumes. Plant family or genus has been suggested as being a good predictor of whether a given species will respond to AM fungi (Hoeksema et al. 2010, Reinhart et al. 2012). For instance, species in the genus *Carex* are nonmycorrhizal and, therefore, are not expected to benefit from reestablishment of native mycorrhizal fungi (Bauer et al. 2018). Recent work in prairies has was indicated that plant successional stage may be a strong predictor of plant response to native AM fungi within plant families (figure 5). Fast-growing early successional plants are less dependent on AM fungi, and slower-growing late successional plants are highly dependent on AM fungi (Koziol and Bever 2015, Bauer et al. 2018) In addition, the growth of late successional plant species is more sensitive to changes in AM fungal species than in early successional plants (Koziol and Bever 2016a). Therefore, when planting a restoration, late successional plant species are likely to be especially vulnerable to alterations in the AM fungal community.

Late successional plant species, including *Amorpha canescens*, *Eryngium yuccifolium*, and *Sporobolis heterolepis*, are considered conservative species because of their sensitivity to anthropogenic disturbance and are highly valued in restorations as indicators of high-quality natural habitat (Bauer et al. 2018). Because late successional plants both are sensitive to mycorrhizal composition and can be missing from restorations (figure 1), we argue that inoculation with native AM fungi may be important for increasing grassland restoration quality through the promotion of these conservative plant species. Consistent with these expectations, reintroduction of native AM fungal communities and whole prairie soils have been shown to improve establishment of late successional, highly conservative plant species (Middleton et al. 2015, Koziol and Bever 2016b); substantially accelerate succession (Middleton and Bever 2012, Wubs et al. 2016); and improve restoration quality in both US and European grasslands (Middleton and Bever 2012, Wubs et al. 2016, Koziol and Bever 2016b).

Future directions for improvement of grassland restorations

We have found that locally adapted mixtures of native, late successional AM fungal strains are likely to be optimal for restoration of the most conservative plant species (Middleton et al. 2015, Koziol and Bever 2016). These benefits could cascade to pollinators and herbivorous insects of these plant species, many of which are of conservation concern (e.g., monarch butterflies and native bees), because pollinators demonstrate increased abundance within diverse native plant community habitats (Debinski and Babbit 1997, Tonietto and Larkin 2018). However, whether microbe-mediated improvements in restoration quality or diversity have cascading effects on native pollinators remains to be tested.

Although there are important benefits of reintroducing native AM fungi (summarized in table 1), major barriers remain in the widespread implementation of this approach. Currently, locally adapted, native inocula are only commercially available within the tallgrass prairie ecological region. In addition, the logistics and sensitivities of the introduction of native inocula need to be addressed further. The challenge in overcoming these barriers is accentuated by the limited expertise that most restoration practitioners have with AM fungal biology. Innovative partnerships and improved communication are needed between scientists, horticulturists with expertise in AM fungal biology and cultivation,

Table 1. Benefits and barriers of inoculation with native, late successional arbuscular mycorrhizal (AM) fungi in restoration.

Benefits	Barriers
• Improved native plant establishment	• Availability of native AM fungal inocula from reference ecosystems is limited
• Improved establishment of high-quality plant species, particularly those with late successional life-history strategies	• Introducing nonnative AM fungi or other microbes may have unintended effects
• Accelerated succession	• Native AM fungal inocula can be expensive for large projects
• Greater plant community diversity and establishment of desirable plant species	• Restorations are stochastic; return on AM fungal inoculation investment is not guaranteed
• Greater resistance to invasion by nonnative invasive plant species	• Nurse plant method of inoculation is proven, but labor intensive
• Reduced vulnerability to soil erosion	• Expertise in development and evaluation of native inocula is limited

restoration practitioners and advocates of holistic restoration of native grassland communities. Communication between these groups would facilitate the development of needed products and the technology, and the best practices in their use. All of these efforts rely on demand for native, locally adapted AM fungal inocula being generated by ecological consultants and restoration practitioners.

More generally, although the reintroduction of native, late successional AM fungi can improve grassland restoration, we do not know the extent to which successful restoration of native AM fungi will be sufficient to restore the original diversity and ecosystem services of undisturbed grasslands. The evidence that inoculation with native AM fungi accelerates restoration (e.g., Koziol and Bever 2016b) is promising, but it remains to be seen whether it is sufficient for the long-term stabilization of grassland diversity. Specifically, although reintroduction of native AM fungi can increase the establishment of late successional plant species, the resulting increase in plant diversity may only be temporary if these species are ultimately competitively excluded by native early or midsuccessional plant species. In addition, although late successional plants and native AM fungi are associated with positive ecosystem attributes such as increased soil aggregate stability (Duchicela et al. 2012), the extent to which fast-forwarding succession by using native AM fungal inoculation will confer ecosystem services similar to those of late successional grasslands remains unknown. Stable coexistence of species within high diversity grassland restorations may require the reintroduction of many components of the plant microbiome, such as plant pathogens, beneficial bacteria, or other endophytes. The dynamics of pathogens, for instance, may be crucial to long-term coexistence of species diversity (Kardol et al. 2007, Bauer et al. 2015, Bever 2015). How sensitive these organisms are to disturbance remains relatively unknown, and whether the restoration of the complete native microbiome would improve stability of grassland restorations is also an open question.

Conclusions

The plant microbiome is a new frontier in ecological restoration that has been shown to play a central role in the establishment of diverse plant communities in restorations. We suggest that the new approach of incorporating native and late successional microbiome “probiotics” to restoration environments should be added to other standard grassland restoration management practices that include restoring native plants and herbivores as well as increasing fire frequency to improve restoration quality. We have outlined how reintroduction of one particularly important component of the plant native microbiome, symbiotic mycorrhizal fungi, can improve restoration outcomes. Although the use of locally adapted and locally collected native plant seed has long been the suggested protocol for restoring native plant communities (Pearse et al. 1948), this concept is only recently being applied to plant microbes. Furthermore, research and product development are necessary for this standard to be realized on a large scale.

Supplemental material

Supplemental data are available at *BIOSCI* online.

Acknowledgments

We acknowledge support from National Science Foundation grants no. DEB 0919434, no. DEB 1556664, no. DEB 1738041 and no. OIA 1656006; SERDP grant no. RC-2330; USDA grants no. 2016-67011-25166 and no. 2016-67012-24680; and the Land Institute Perennial Agricultural Project. LK is the owner of MycoBloom LLC. We would like to thank the past and present members of the Bever-Schultz laboratory group for helping us develop these ideas.

Data accessibility

The new data included in this article are the species composition in remnant prairies presented in figure 4 and those presented in figure 3. These data will be deposited in Dryad on publication (Koziol et al. 2018).

References cited

Aggangan NS, Moon HK, Han SH. 2010. Growth response of *Acacia mangium* Willd. seedlings to arbuscular mycorrhizal fungi and four isolates of the ectomycorrhizal fungus *Pisolithus tinctorius* (Pers.) Coker and Couch. New Forests 39: 215–230.

Baer S, Kitchen D, Blair J, Rice C. 2002. Changes in ecosystem structure and function along a chronosequence of restored grasslands. Ecological Applications 12: 1688–1701.

Bauer JT, Blumenthal N, Miller AJ, Ferguson JK, Reynolds HL, Brudvig L. 2017. Effects of between-site variation in soil microbial communities and plant–soil feedbacks on the productivity and composition of plant communities. Journal of Applied Ecology 54: 1028–1039.

Bauer JT, Koziol L, Bever JD. 2018. Ecology of floristic quality assessment: Testing for correlations between coefficients of conservatism, species traits, and mycorrhizal responsiveness. AoB Plants 10 (art. plx073).

Bauer JT, Mack KM, Bever JD. 2015. Plant–soil feedbacks as drivers of succession: Evidence from remnant and restored tallgrass prairies. Ecosphere 6: 158.

Bever J, Schultz P, Miller R, Gades L, Jastrow J. 2003. Prairie mycorrhizal fungi inoculant may increase native plant diversity on restored sites (Illinois). Ecological Restoration 21: 311–312.

Bever JD. 2015. Preferential allocation, physio-evolutionary feedbacks, and the stability and environmental patterns of mutualism between plants and their root symbionts. New Phytol 205: 1503–1514.

Bever JD, Mangan SA, Alexander HM. 2015. Maintenance of plant species diversity by pathogens. Annual Review of Ecology, Evolution, and Systematics 46: 305–325.

Bever JD, Morton JB, Antonovics J, Schultz PA. 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. Journal of Ecology 84: 71–82.

Bever JD, Richardson SC, Lawrence BM, Holmes J, Watson M. 2009. Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. Ecology Letters 12: 13–21.

Brudvig LA, Barak RS, Bauer JT, Caughlin TT, Laughlin DC, Larios L, Matthews JW, Stuble KL, Turley NE, Zirbel CR. 2017. Interpreting variation to advance predictive restoration science. Journal of Applied Ecology 54: 1018–1027.

Callaway RM, Thelen GC, Barth S, Ramsey PW, Gannon JE. 2004. Soil fungi alter interactions between the invader *Centaurea maculosa* and North American natives. Ecology 85: 1062–1071.

Debinski DM, Babbit AM. 1997. Butterfly species in native prairie and restored prairie. Prairie Naturalist 29: 219–228.

Delavaux CS, Smith-Ramesh LM, Kuebbing SE. 2017. Beyond nutrients: A meta-analysis of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. Ecology 98: 2111–2119.

Druille M, Cabello MN, García Parisi PA, Golluscio RA, Omacini M. 2015. Glyphosate vulnerability explains changes in root-symbionts propagules viability in pampean grasslands. Agriculture, ecosystems and environment 202: 48–55.

Duchicela J, Vogelsang KM, Schultz PA, Kaonongbua W, Middleton EL, Bever JD. 2012. Non-native plants and soil microbes: Potential contributors to the consistent reduction in soil aggregate stability caused by the disturbance of North American grasslands. New Phytologist 196: 212–222.

Emam T. 2016. Local soil, but not commercial AMF inoculum, increases native and non-native grass growth at a mine restoration site. Restoration Ecology 24: 35–44.

Fierer N, Ladau J, Clemente JC, Leff JW, Owens SM, Pollard KS, Knight R, Gilbert JA, McCulley RL. 2013. Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. Science 342: 621–624.

Grime J. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. American Naturalist 111: 1169–1194.

Grman E, Bassett T, Brudvig LA. 2013. Confronting contingency in restoration: Management and site history determine outcomes of assembling prairies, but site characteristics and landscape context have little effect. Journal of Applied Ecology 50: 1234–1243.

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.

Grove S, Haubensak KA, Gehring C, Parker IM. 2017. Mycorrhizae, invasions, and the temporal dynamics of mutualism disruption. Journal of Ecology 105: 1496–1508.

Hart MM, Antunes PM, Abbott LK. 2017a. Unknown risks to soil biodiversity from commercial fungal inoculants. Nature Ecology and Evolution 1: 0115.

Hart MM, Antunes PM, Chaudhary VB, Abbott LK. 2017b. Fungal inoculants in the field: Is the reward greater than the risk? Functional Ecology 32: 126–135.

Hawkes CV, Belnap J, D'Antonio C, Firestone MK. 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant and Soil 281: 369–380.

Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, Koide RT, Pringle A, Zabinski C, Bever JD, Moore JC. 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecology Letters 13: 394–407.

House GL, Bever JD. 2018. Disturbance reduces the differentiation of mycorrhizal fungal communities in grasslands along a precipitation gradient. Ecological Applications 28: 763–748.

Jansa J, Mozafar A, Anken T, Ruh R, Sanders I, Frossard E. 2002. Diversity and structure of AMF communities as affected by tillage in a temperate soil. Mycorrhiza 12: 225–234.

Jasper D, Abbott L, Robson A. 1991. The effect of soil disturbance on vesicular: Arbuscular mycorrhizal fungi in soils from different vegetation types. New Phytologist 118: 471–476.

Jasper DA, Abbott LK, Robson AD. 1989. Acacias respond to additions of phosphorus and to inoculation with VA mycorrhizal fungi in soils stockpiled during mineral sand mining. Plant and Soil 115: 99–108.

Ji B, Bentivenga SP, Casper BB. 2010. Evidence for ecological matching of whole AM fungal communities to the local plant–soil environment. Ecology 91: 3037–3046.

Ji B, Bever JD. 2016. Plant preferential allocation and fungal reward decline with soil phosphorus: Implications for mycorrhizal mutualism. Ecosphere 7 (art. e01256).

Johnson NC. 1993. Can fertilization of soil select less mutualistic mycorrhizae? Bulletin of the Ecological Society of America 3: 749–757.

Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proceedings of the National Academy of Sciences 107: 2093.

Kardol P, Cornips NJ, van Kempen MM, Bakx-Schotman JT, van der Putten WH. 2007. Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. Ecological Monographs 77: 147–162.

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.

Klironomos JN. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417: 67–70.

Koziol L, Bever JD. 2015. Mycorrhizal response trades off with plant growth rate and increases with plant successional status. Ecology 96: 1768–1774.

Koziol L, Bever JD. 2016a. AMF, phylogeny and succession: Specificity of response to mycorrhizal fungi increases for later successional plants. Ecosphere 7 (art. e1555).

Koziol L, Bever JD. 2016b. The missing link in grassland restoration: Arbuscular mycorrhizal fungi inoculation increases plant diversity and accelerates succession. Journal of Applied Ecology 54: 1301–1309.

Koziol L, Rieseberg LH, Kane N, Bever JD. 2012. Reduced drought tolerance during domestication and the evolution of weediness results from tolerance-growth trade-offs. Evolution 66: 3803–3814.

Koziol L, Bever JD. 2018. Mycorrhizal feedbacks generate positive frequency dependence accelerating grassland succession. Journal of Ecology. doi:10.1111/1365-2745.13063

Koziol L, Schultz PA, House GL, Bauer JT, Middleton EL, Bever JD. 2018. Data from: The plant microbiome and native plant restoration: The example of native mycorrhizal fungi. Dryad Digital Repository. doi:10.5061/dryad.bs79gk5

Lankau RA, Bauer JT, Anderson MR, Anderson RC. 2014. Long-term legacies and partial recovery of mycorrhizal communities after invasive plant removal. Biological invasions 16: 1979–1990.

Maltz MR, Treseder KK. 2015. Sources of inocula influence mycorrhizal colonization of plants in restoration projects: A meta-analysis. Restoration Ecology 23: 625–634.

Mangan SA, Adler GH. 2002. Seasonal dispersal of arbuscular mycorrhizal fungi by spiny rats in a neotropical forest. Oecologia 131: 587–597.

Mangan SA, Schnitzer SA, Herre EA, Mack KML, Valencia MC, Sanchez EI, Bever JD. 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. Nature 466: 752–U710.

Martin LM, Moloney KA, Wilsey BJ. 2005. An assessment of grassland restoration success using species diversity components. Journal of Applied Ecology 42: 327–336.

Marulanda A, Azcón R, Ruiz Lozano JM. 2003. Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought stress. Physiologia Plantarum 119: 526–533.

Middleton EL, Bever JD. 2012. Inoculation with a native soil community advances succession in a grassland restoration. Restoration Ecology 20: 218–226.

Middleton EL, Bever JD, Schultz PA. 2010. The effect of restoration methods on the quality of the restoration and resistance to invasion by exotics. Restoration Ecology 18: 181–187.

Middleton EL, Richardson S, Koziol L, Palmer CE, Yermakov Z, Henning JA, Schultz PA, Bever JD. 2015. Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. Ecosphere 6: 276.

Moora M, Davison J, Öpik M, Metsis M, Saks Ü, Jairus T, Vasar M, Zobel M. 2014. Anthropogenic land use shapes the composition and phylogenetic structure of soil arbuscular mycorrhizal fungal communities. Federation of European Microbiological Societies Microbiology Ecology 90: 609–621.

Oehl F, Sieverding E, Ineichen K, Mäder P, Boller T, Wiemken A. 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. Applied and Environmental Microbiology 69: 2816–2824.

Oehl F, Sieverding E, Mäder P, Dubois D, Ineichen K, Boller T, Wiemken A. 2004. Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. Oecologia 138: 574–583.

Ohsowski BM, Dunfield K, Klironomos JN, Hart MM. 2017. Plant response to biochar, compost, and mycorrhizal fungal amendments in post-mine sandpits. Restoration Ecology.

Pearse CK, Plummer AP, Savage D. 1948. Restoring the range by reseeding. Yearbook of Agriculture 19: 1–7.

Perkins LB, Bennett JR. 2017. A field test of commercial soil microbial treatments on native grassland restoration. Restoration Ecology.

Pringle A, Bever JD, Gardes M, Parrent JL, Rillig MC, Klironomos JN. 2009. Mycorrhizal symbioses and plant invasions. Annual Review of Ecology, Evolution, and Systematics 40: 699–715.

Reinhart KO, Wilson GW, Rinella MJ. 2012. Predicting plant responses to mycorrhizae: Integrating evolutionary history and plant traits. Ecology Letters 15: 689–695.

Remy W, Taylor TN, Hass H, Kerp H. 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proceedings of the National Academy of Sciences 91: 11841–11843.

Richter BS, Stutz JC. 2002. Mycorrhizal inoculation of big sacaton: Implications for grassland restoration of abandoned agricultural fields. Restoration Ecology 10: 607–616.

Rúa MA, Antoninka A, Antunes PM, Chaudhary VB, Gehring C, Lamit LJ, Piculell BJ, Bever JD, Zabinski C, Meadow JF, Lajeunesse MJ, Milligan BG, Karst J, Hoeksema JD. 2016. Home-field advantage? Evidence of local adaptation among plants, soil, and arbuscular mycorrhizal fungi through meta-analysis. BioMed Central Evolutionary Biology 16: 122.

Säle V, Aguilera P, Laczko E, Mäder P, Berner A, Zihlmann U, van der Heijden MG, Oehl F. 2015. Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry 84: 38–52.

Schultz PA, Miller RM, Jastrow JD, Rivetta CV, Bever JD. 2001. Evidence of a mycorrhizal mechanism for the adaptation of *Andropogon gerardii* (Poaceae) to high- and low-nutrient prairies. American Journal of Botany 88: 1650–1656.

Sikes BA, Cottenie K, Klironomos JN. 2009. Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. Journal of Ecology 97: 1274–1280.

Tonietto RK, Larkin DJ. 2018. Habitat restoration benefits wild bees: A meta-analysis. Journal of Applied Ecology 55: 582–590.

Trejo-Aguilar D, Lara-Capistrán L, Maldonado-Mendoza IE, Zulueta-Rodríguez R, Sangabriel-Conde W, Mancera-López ME, Negrete-Yankelevich S, Barois I. 2013. Loss of arbuscular mycorrhizal fungal diversity in trap cultures during long-term subculturing. IMA fungus 4: 161–167.

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability, and productivity. Nature 396: 69–72.

Visser S, Griffiths C, Parkinson D. 1984. Topsoil storage effects on primary production and rates of vesicular-arbuscular mycorrhizal development in *Agropyron trachycaulum*. Plant and Soil 82: 51–60.

Vogelsang KM, Bever JD. 2009. Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. Ecology 90: 399–407.

Vogelsang KM, Bever JD. 2010. The Use of Native Plants and Mycorrhizal Fungi for Slope Stabilization and Topsoil Management. California Department of Transportation, Roadside Research and Innovation, Landscape Architecture Program.

Vogelsang KM, Reynolds HL, Bever JD. 2006. Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. New Phytologist 172: 554–562.

Wagg C, Jansa J, Stadler M, Schmid B, van der Heijden MGA. 2011. Mycorrhizal fungal identity and diversity relaxes plant–plant competition. Ecology 92: 1303–1313.

White JA, Tallaksen J, Charvat I. 2008. The effects of arbuscular mycorrhizal fungal inoculation at a roadside prairie restoration site. Mycologia 100: 6–11.

Wright SF, Upadhyaya A. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Science 161: 575–586.

Wubs E, van der Putten W, Bosch M, Bezemer TB. 2016. Soil inoculation steers restoration of terrestrial ecosystems. Nature Plants 2: 16107.

Zhang T, Sun Y, Shi Z, Feng G. 2012. Arbuscular mycorrhizal fungi can accelerate the restoration of degraded spring grassland in Central Asia. Rangeland Ecology and Management 65: 426–432.

Liz Koziol (lizkoziol@ku.edu), Peggy A. Schultz, and James D. Bever are affiliated with the Kansas Biological Survey, at the University of Kansas, in Lawrence. Geoffrey L. House is affiliated with the Los Alamos National Laboratory, in Los Alamos, New Mexico. Jonathan T. Bauer is affiliated with Miami University, in Oxford, Ohio. Elizabeth L. Middleton is affiliated with the Missouri Department of Conservation, in Clinton.