RESEARCH ARTICLE

UN Decade on Ecosystem Restoration

Manipulating plant microbiomes in the field: Native mycorrhizae advance plant succession and improve native plant restoration

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Abstract

1. The plant microbiome is critical to plant health and is degraded with anthropogenic disturbance. However, the value of re-establishing the native microbiome is rarely considered in ecological restoration. Arbuscular mycorrhizal (AM) fungi are particularly important microbiome components, as they associate with most plants, and later successional grassland plants are strongly responsive to native AM fungi.

2. With five separate sites across the United States, we inoculated mid- and late successional plant seedlings with one of three types of native microbiome amendments: (a) whole rhizosphere soil collected from local old-growth, undisturbed grassland communities in Illinois, Kansas or Oklahoma, (b) laboratory cultured AM fungi from these same old-growth grassland sites or (c) no microbiome amendment. We also seeded each restoration with a diverse native seed mixture. Plant establishment and growth was followed for three growing seasons.

3. The reintroduction of soil microbiome from native ecosystems improved restoration establishment.

4. Including only native arbuscular mycorrhizal fungal communities produced similar improvements in plant establishment as what was found with whole soil microbiome amendment. These findings were robust across plant functional groups.

5. Inoculated plants (amended with either AM fungi or whole soil) also grew more leaves and were generally taller during the three growing seasons.

6. *Synthesis and applications.* Our research shows that mycorrhizal fungi can accelerate plant succession and that the reintroduction of both whole soil and laboratory cultivated native mycorrhizal fungi can be used as tools to improve native plant restoration following anthropogenic disturbance.

KEYWORDS
arbuscular mycorrhizal fungi, microbial inoculation, nurse plants, plant microbiome, plant succession, restoration, rhizosphere, soil amendments

1 | INTRODUCTION

The plant microbiome, such as leaf endophytes, mycorrhizal fungi, beneficial soil bacteria, invertebrates and other biota, can influence plant local adaptation (Johnson et al., 2010), coexistence (Bever et al., 2015), relative abundance (Klironomos, 2002; Mangan et al., 2010), succession (Bauer et al., 2015) and invasion of plant communities (Callaway et al., 2004; Pringle et al., 2009). Recent evidence highlights how plant microbiome biota are sensitive to anthropogenic change and soil disturbance. For instance, agricultural processes including tillage (Jansa et al., 2002), planting crop monocultures (Oehl et al., 2003), the use of fertilizers (Johnson, 1993; Säle et al., 2015) and glyphosate applications (Druille et al., 2015) have been linked to less abundant, less resilient and/or less beneficial soil microbes. As most restoration efforts take place in areas with disturbed soils, changes in soil biota prior to restoration may affect restoration outcomes (Lekberg & Koide, 2005), where some native plant species fail to establish with conventional (no microbiome amendment) restoration practices (Grman et al., 2015; Kindscher & Tieszen, 1998). Given the growing realization that soil microbiomes contribute to plant community structure, the successful restoration of native plant communities may require the re-establishment of the native soil microbiome (Wubs et al., 2016). Applying microbiome amendments via whole soil transplant or trap cultures has led to promising improvements in restoration quality (Emam, 2016; Middleton & Bever, 2012; Vahter et al., 2020; Wubs et al., 2016), but these studies are few and often restore small areas of <1 ha. This is likely because transporting enough soil for large restorations is highly destructive to native ecosystems that are often scarce and protected. To address these challenges, researchers have begun to identify and isolate the key microbiome biota to include in restoration efforts.

Within grassland ecosystems, arbuscular mycorrhizal (AM) fungi are considered a keystone microbial guild, as plants and mycorrhizal fungi have an essential and long-standing relationship (nearly 400 million years; Remy et al., 1994), and mycorrhizae continue to benefit many grassland species (Bauer et al., 2018; Wilson & Hartnett, 1998). Although AM fungi are commonly present in most soils, AM fungi in early successional environments can have reduced abundance, lower infectivity, reduced mutualistic capabilities and altered composition relative to fungi in undisturbed soils (Abbott & Robson, 1991; Pärtel et al., 2017; Säle et al., 2015; Tipton et al., 2018), and some native AM fungal species are lost after disturbance (House & Bever, 2018; Säle et al., 2015). When AM fungi are amended into restored soils, native and locally collected mycorrhizal fungi improve restoration outcomes more than commercial fungi (Emam, 2016; Maltz & Treseder, 2015; Middleton et al., 2015) or fungi from previously disturbed soils, such as old fields or restored sites (Grman et al., 2020; Middleton & Bever, 2012; Smith et al., 2018). The composition of native fungal amendments can also determine plant response to inoculation (Koziol & Bever, 2017, 2019). Native mycorrhizal communities might be especially important for native late successional plants and plants of conservation concern, as these plants have been shown to have greater sensitivity to and dependency on AM fungal species of their microbiomes relative to early successional and non-native plant species (Cheeke et al., 2019). Therefore, reintroducing native AM fungi and/or whole soil communities from intact native communities could support the restoration and development of late successional plant communities.

Here, we test for the generality of soil microbiome influence on plant succession and improvements in restoration outcomes in the tallgrass prairie. Five novel prairie restorations were initiated in Illinois (one site), Kansas (two sites) and Oklahoma (two sites) across sites with different soil characteristics, management histories and invading plant communities to test whether inoculation effects may depend on sites with varying characteristics. Mid- to late successional prairie seedlings were inoculated with one of three types of native microbiome amendments: (a) whole rhizosphere soil collected from local old-growth, undisturbed grassland communities in Illinois, Kansas or Oklahoma, (b) laboratory cultured mycorrhizal fungi from these same old-growth grassland sites or (c) no microbiome amendment. We present data on inoculated plant growth and establishment from the first 3 years of restoration to test the hypothesis that that late successional plant survival and growth will be improved after inoculation with native microbiome amendments. We also conducted vegetation surveys on the restored plant community to test the hypothesis that microbial amendment would improve restoration quality.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Four experimental restorations were initiated during April–May 2014 (IL, KS A, KS B and OK A) and one during April 2015 (OK B) in Illinois, Kansas and Oklahoma, USA (Supplement 1, Figure S1). Because of the different existing vegetation (plant invaders Old World Bluestem [*Bothriochloa* spp.], Brome [*Bromus inermis*] and Fescue [*Festuca arundinacea*]) and land use present at each site, several methods were used to prepare sites for restoration, including herbicide application, disking, solarization with black plastic tarps or no vegetation removal (Figure S1 and Supplement 2). Nine replicate blocks were planted at the Illinois site (27 plots) and seven replicate blocks at all other sites (21 plots per site), where each inoculation treatment was randomized within each block. All nurse plants transplanted in each 4 m^2 plot had the same inoculation treatment, where 16 inoculated nurse plants were planted equidistantly along the centre line of each plot and individually labelled with a metal tag (see seedling and inoculation preparation below). Because nurse plant survival was low in the first growing season, four nurse plants were replanted in 2015 in Kansas restorations as effort allowed. All nurse plants transplanted in 2015 were germinated and inoculated in 2015 as previously described but using inocula collected or cultured in spring 2015. In total, 1,944 inoculated nurse plants were monitored for these experiments. In addition, each site was seeded with

a diverse native seed mixture containing 58 (Kansas and Oklahoma) or 64 (Illinois) prairie species (Supplement 3) by hand broadcasting seed.

2.2 | Background sampling, inoculum creation and inoculum assessment

Prior to planting the restorations, the AM fungal communities of disturbed sites and remnant prairie sites were assessed by collecting soils from three or more different locations in disturbed (prior to restoration) and old-growth remnant sites (Supplement 1 Methods) during 2014. AM fungal DNA was used to compare each disturbed site to its corresponding remnant site. Molecular techniques utilized for background AM fungi sampling were also used to confirm the AM fungal communities were present in inocula and AM fungi present in roots sampled from inoculated nurse plants at the end of first growing season (September). For methods on AM fungal DNA analyses, see Supplement 1. PLFA analyses were conducted on soils in between the nurse plants the first 2 years of the experiment. See Supplement 2 for soil analyses.

AM fungal culturing was started in 2012, when we sampled AM fungi from old-growth prairie sites near each of the restorations (Supplement 1, Table S1). For each state (Illinois, Kansas and Oklahoma), 0.5 L of soil was sampled from five locations within 4 m of each other at one or more old-growth prairie locations by collecting the top 12.7 cm of topsoil, chopping roots into 1 cm fragments and homogenizing. Species of AM fungi were sorted microscopically, and single species cultures were established (Supplement 1, Culture Propagation). Whole soil inocula were collected using the same sampling methods but were collected directly prior to plot establishment during the spring of 2014 or 2015 depending on restoration start date (Supplement 1, Table S2). Whole soil or cultured fungal communities were homogenized and pooled within each state to create old-growth whole soil or AM fungal mixtures from Illinois, Kansas and Oklahoma.

2.3 | Seedling preparation and inoculation

Nurse plant species seed (Supplement 1, Table S3) was cold moist stratified for 30–60 days before germination in sterilized sand and were planted into inocula treatments within 20 days following germination. Seedlings were inoculated with whole soil inocula, AM fungal inocula or sterilized inocula from background soil. Henceforth, inoculated plants are referred to as 'nurse plants'. Nurse plants were inoculated with microbial treatments at 15% by volume (23 cm^3) at the centre pot depth of 150 cm^3 containers™ with the remaining volume containing sterilized soil:sand mixtures. Soils used in the nurse plant pots were collected from each restoration site and sterilized via autoclaving for 2 hr (Supplement 2). Mid-late successional plants were selected to use as nurse plants that are common targets for diverse native tallgrass restorations. Two forbs, one grass and one legume

were included at each restoration site (Table S3) and included *Allium cernuum*, *Amorpha canescens*, *Schizachyrium scoparium*, *Echinacea pallida*, *Echinacea angustifolia*, *Allium stellatum*, *Andropogon gerardii*, *Lespedeza capitata*, *Dalea purpurea* and *Ratibida pinnata*. Initial plant size (height and/or leaf/tiller number) was measured at the time of planting and then annually at the end of each growing season. IL grass height the second growing season and grass tiller number the third growing season were not collected (see Supplement 1).

2.4 | Plant community sampling

Restored plant community richness, abundance, diversity and the proportion of plot recolonization by the dominant invading plant prior to restoration were analysed at the end of each growing season using the point intercept method (see Supplement 1).

2.5 | Statistical analyses

Mixed models were used to analyse nurse plant growth with initial plant height at transplant, inoculation treatment, plant functional group (grass, legume or forb [non-legume forbs henceforth called 'forbs']), block × site interactions, site × inoculation treatment interactions, site × functional group interactions, functional group × inoculation treatment interactions and site × functional group × inoculation treatment interactions as fixed predictors. To control for non-independence of replicate plants within plots, we identify plot × inoculation treatment × block × site and the interaction with plant functional group and plant species as random effects. In repeated measures analyses of survival across years (Supplement 1, Table S7), we add a third random effect to control for non-independence of multiple measures of the same individual over time. This random effect is specified as plot × block × treatment × site × functional group × plant number. All analyses were performed using proc mixed and proc glimmix in SAS 9.4.

To compare the effects of different soil microbes on nurse plant growth and survival, orthogonal a priori contrasts were designed comparing plant growth with living inocula (whole soil and AM fungal cultures) versus non-inoculated, growth difference when inoculated with AM fungi versus whole soil inocula, and these contrasts by site and by plant functional group. As survival decreased, paired with unsymmetrical data collection, the resulting unbalanced data structure would not support analyses of high-order interactions, and therefore required reduced models for one growth measurement during the second and third growing seasons. The higher-order interactions including interactions of inoculation with site were not significant prior to removal (Supplement 4), with one exception (site × functional group, $p = 0.05$) for leaf number third growing season. LS means and standard errors were back transformed from model estimates for data visualization. If plants were missing from a sampling but counted as present at a later sampling, survival was adjusted to include the survival of that nurse plant in previous

samplings. Survival results were consistent with and without this adjustment. See Supplement 1 for statistical methods on plant community composition, AM fungal composition and PLFA data.

3 | RESULTS

3.1 | Microbiome communities of the sites and inocula

Prior to restoration, the background AM fungal communities of the restoration sites (before planting) were significantly different from the old-growth, remnant grasslands from which the whole soil inocula was collected regardless of the geographic location of the different sites (PERMANOVA $p < 0.0001$, Figure 1). Many of the AM fungal species present in the initial AM fungal inocula were confirmed to be present in the whole soil inocula as well as detected in the roots of the inoculated nurse plants after being planted in the restoration (Supplement 1, Table S2 and Figures S2 and S9). The AM fungal OTUs identified from nurse plant roots at the end in the first growing season had 98%, 93%, 96% and 64% of the AM fungal OTUs found in the AM fungal inocula well as 89%, 90%, 98% and 62% of fungal OTUs found in the whole soil inocula for the IL, KS A, KS B and OK B sites, respectively (Supplement 1, Table S2). Despite the consistent presence of the inocula at each site, PLFA analyses indicated total AM fungal biomass did not differ among plots, regardless of inoculum treatment ($F_{2,167} = 0.136$, $p = 0.9$). Site was a strong predictor of PLFA abundance (Supplement 1 Results).
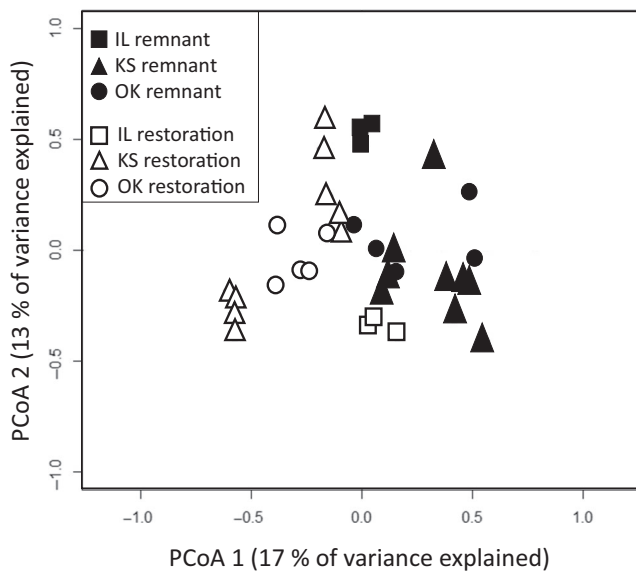
FIGURE 1 Differences in AM fungal communities between prairie remnants and restoration sites (before planting) as visualized by principal coordinates analysis (PCoA) and coloured by both site status (remnant [black] or restoration [white]) and site location (Illinois [IL, squares], Kansas [KS B, diamonds] or Oklahoma [OK B, circles])

TABLE 1 The effect of inoculation, restoration site and plant functional group on nurse plant survival at the end of each growing season using proc glimmix.

Effect	First growing season				Second growing season				Third growing season			
	Num df	Den df	F value	p	Num df	Den df	F value	P	Num df	Den df	F value	p
Initial size	1	1,553	5.87	0.0155	1	1,553	9.78	0.0018	1	1,117	6.93	0.0086
Site	4	98	38.9	<0.0001	4	98	23.38	<0.0001	3	79	21.36	<0.0001
Inoculation treatment	2	98	15.43	<0.0001	2	98	8.68	0.0003	2	79	4.63	0.0125
Inoculated versus non-inoculated	1	98	29.19	<0.0001	1	98	14.31	0.0003	1	79	6.6	0.0121
Whole soil versus AM fungi	1	98	2.65	0.107	1	98	2.83	0.0954	1	79	1.75	0.1891
Site × inoculation treatment	8	98	3.96	0.0004	8	98	2.15	0.038	6	79	0.7	0.6466
Plant functional group	2	197	37.34	<0.0001	2	197	70.41	<0.0001	2	157	88.92	<0.0001
Site × plant functional group	8	197	7.11	<0.0001	8	197	3.63	0.0006	6	157	1.92	0.0805
Plant functional group × inoculation treatment	4	197	1.45	0.2182	4	197	0.31	0.8711	4	157	1.88	0.117
Inoculated versus non-inoculated × plant functional group	2	197	2.71	0.069	2	197	0.2	0.8161	2	157	1.1	0.3369
Whole soil versus AM fungi × plant functional group	2	197	0.37	0.6913	2	197	0.47	0.6269	2	157	1.95	0.1459

Bold text represents model predictors and non-bold text represents designed contrasts

3.2 | The effects of soil microbiome inoculation on nurse plant survival

The inclusion of living soil microbiome (either whole soils or AM fungal inocula) isolated from remnant prairie communities improved nurse plant survival relative to controls during the first, second and third growing seasons (Table 1, Figure 2a, $F_{1,98} = 29.19$, $p < 0.0001$, $F_{1,98} = 14.31$, $p = 0.0003$, $F_{1,79} = 6.6$, $p = 0.01$, respectively). Although plant survival declined over time across all treatments (Figure 2a), average nurse plant survival was increasingly improved with inoculation over time relative to the controls (first, second and third growing season survival was 26%, 26% and 52% improved with AM fungi and 36%, 44% and 107% improved with whole soil inoculation, respectively; Figure S3a). Plant survival was not statistically different between living inoculation treatments (inoculation with whole microbiome soil compared to AM fungal inocula) any year (Table 1; Figure 2a).

Restoration location was a significant predictor of survival (Table 1). Significant site × inoculation interactions were observed the first and second growing seasons (Table 1, Figure 2b,c, $F_{8,98} = 3.96$, $p = 0.0004$ and $F_{8,98} = 2.15$, $p = 0.04$). These interactions were likely driven by IL being unresponsive to inoculation and the other sites being more responsive to inoculation the first 2 years (Figure 2b,c). The site where vegetation was not removed prior to restoration, OK A, had the lowest survival of all the sites each year. The site by inoculation treatment interaction declined over time and was non-significant during the third growing season where all sites benefited from inoculation relative to the controls (Table 1, $F_{6,79} = 0.7$, $p = 0.6$, Figure S3b,c).

Plant functional group was consistently a significant predictor of plant survival (Table 1). All functional groups benefited from inoculation and therefore no significant inoculation treatment × functional group interactions were found on plant survival any year (Table 1). However, in the first growing season, a marginally significant trend for plant functional groups to interact with inoculation differently was observed, where forb survival was most strongly improved with inoculation (~50%) relative to legumes (20%–30%) and grasses (10%) (Table 1, $F_{2,197} = 2.71$, $p = 0.069$, Figure S4).

3.3 | The effects of microbiome inoculation on plant productivity

Surviving plants produced more leaves and tillers following microbiome inoculation with either whole soil or AM fungi in the first year (Figure 3a), with inoculated AM fungi and whole soil plants having 30% more leaves relative to controls (Supplement 1, Table S5, $F_{1,95} = 38.46$, $p < 0.0001$). This pattern of improved growth with inoculation persisted each year (Figure 3a–c) but was marginally significant in year 3 as plant mortality increased (Supplement 1, Table S5, $F_{1,3} = 7.60$, $p = 0.07$, Figure 3c). Plants inoculated with whole soil or AM fungi had consistently similar effects on leaf production each year (Table S5, whole soil versus AM fungi contrasts always non-significant, Figure 3a–c).

Patterns observed for plant height closely matched those observed for plant leaf or tiller production (Figure 3a–f). Plant height was improved with inoculation, with significantly taller plants he first and second growing seasons (Supplement 1, Table S5, $F_{1,95} = 25.74$, $p < 0.0001$, Figure 3d and $F_{1,83} = 13.69$, $p = 0.0004$, Figure 3e). However, plant height the third growing season was no longer affected by inoculation (Supplement 1, Table S5, $F_{1,3} = 0.01$, $p = 0.9$, Figure 3f). There were not significant differences between AM fungi and whole soil in any year for plant height (Supplement 1, Table S5, Figure 3d–f).
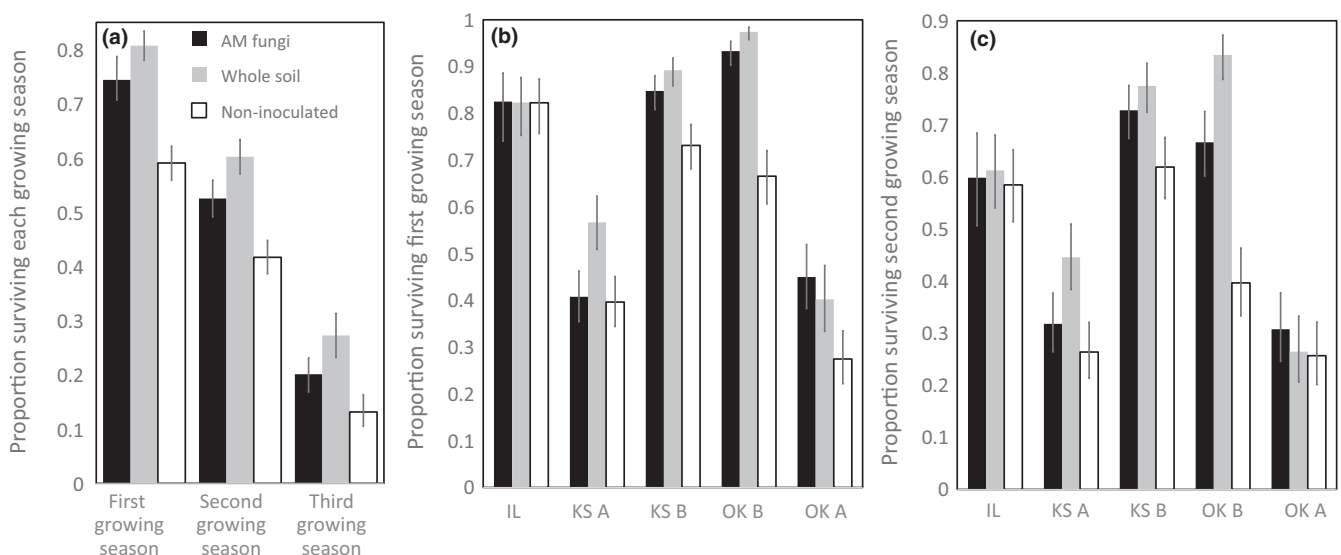
FIGURE 2 Effects of inoculation on plant survival each growing season for all sites (a), and interactions with site the first (b) and second growing seasons (c). Bars represent the average proportion of nurse plants surviving and error bars are standard error derived from back transformed logits in the proc glimmix models. Shading on bars represents AM fungi (black), whole soil (grey) and non-inoculated (white)
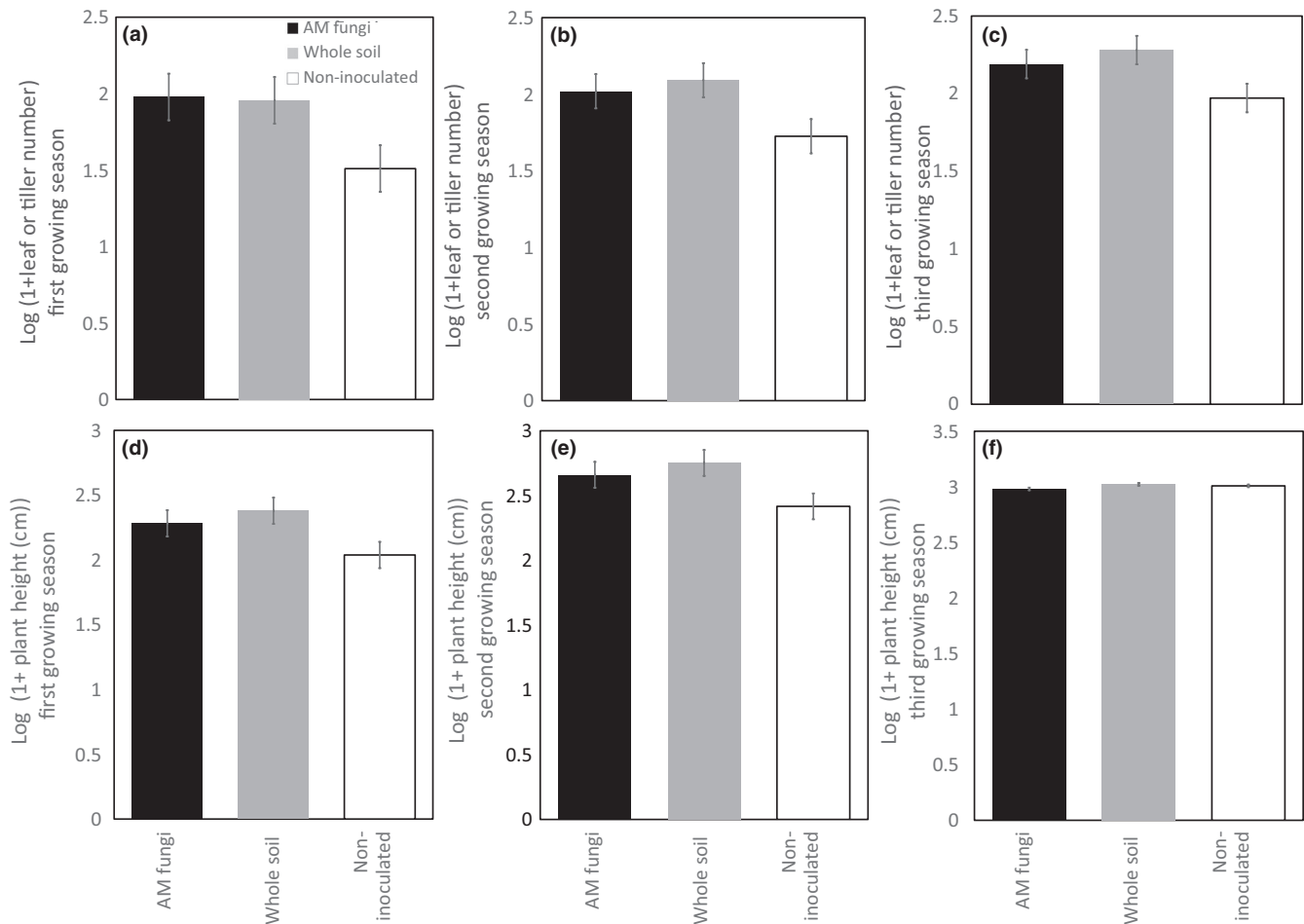
FIGURE 3 The effect of inoculation each growing season on plant leaf or tiller number (a–c) and plant height (d–f). Bars represent the LS means of plant growth and error bars are standard error from the proc mixed models. Shading on bars represents the different inoculation treatments of AM fungi (black), whole soil (grey) and non-inoculated (white)

Plant functional group was a significant predictor of plant height all growing seasons and leaf number the first and second growing seasons (Supplement 1, Table S5). Plants from different functional groups always responded to inoculation in a similar way for all metrics analysed (Supplement 1, Table S5, plant functional group × inoculation treatment interactions all non-significant), generally benefitting from native microbes (Figure S5a–c), but plant height the third year being unaffected by inoculation (Figure S5b).

Site was an important predictor of plant height and leaf and tiller number all three growing seasons (Supplement 1, Table S5, all $p < 0.01$), with the exception being height during the third growing season (Supplement 1, Table S5, $F_{3,3} = 3.14$, $p = 0.2$). Plant leaf or tiller number and plant height improvements with inoculation were generally consistent across sites (Supplement 1, Table S5, site × inoculation all non-significant, Figure S6a,c,d). The exception was plant height in the first growing season, where plant height at some sites was more strongly affected by inoculation than at other sites (Supplement 1, Table S5, $F_{8,95} = 3.05$, $p = 0.004$, Figure S6b), and plants at IL grew largest regardless of inoculation.

3.4 | Plant community response

Inoculation had significant effects on the density of the restored plant community as well as the proportion of recolonization by the invading plant species that had dominated prior to restoration (Table S6). AM fungi inoculated plots had significantly less recolonization by the non-native dominant plant species relative to plots inoculated with whole soil (AM fungi vs. whole soil contrast, $F_{1,655} = 6.43$, $p = 0.012$, AM fungi 30% less than control, Figure S7). Plant community diversity differed among the inoculation treatments over time (Table S6, Figure S8, $F_{4,665} = 3.27$, $p = 0.012$), where AM fungi increasingly improved diversity, diversity in the whole soil and control plots declined the third growing season.

4 | DISCUSSION

Anthropogenic disturbance can alter the plant microbiome by changing composition and reducing the abundance and effectiveness of symbionts (Abbott & Robson, 1991; Baer et al., 2002; Druille et al., 2015; Oehl et al., 2003). These changes can affect restoration

success, as many plants with a late successional life-history strategy are strongly responsive to native soil microbes (Bauer et al., 2018; Grman et al., 2020; Koziol & Bever, 2015). In the current study, it was confirmed that disturbed soils (restoration sites before planting) harbour different AM fungal communities than adjacent remnant native grassland soils, as is consistent with House and Bever (2018). We also found support for the hypothesis that later successional prairie plants are more likely to establish and grow larger in a restoration following inclusion of native microbiome amendments. Given that late successional plants often fail to establish with conventional (no microbiome amendment) restoration practices (Grman et al., 2015; Kindscher & Tieszen, 1998; Koziol & Bever, 2017), this study indicates that native microbiome amendments may be useful in restorations that target late successional plant establishment and richness. While other studies have tested microbiome amendment in restoration at individual sites (Bever et al., 2003; Emam, 2016; Wubs et al., 2016), we are the first to test for the general effect of native microbiome amendments in restoration using common microbiome application methods across distinct restoration sites with varying soil characteristics, invading plant communities and site management histories. Although site was important for plant survival and growth, all five restoration sites were found to benefit from native microbiome restoration by having increased growth and/or survival during the study period. Additionally, site × inoculation interactions for growth and survival were generally weak and were not significant in the third growing season. Therefore, this study suggests that late successional plant reliance on components of their soil microbiome is consistent across the North American tallgrass prairie. Given that evidence is accumulating that plants from many grassland habitats across the globe benefit from inoculation, including in other North American grasslands such as coastal dunes (Crawford et al., 2020) and semi-arid grasslands (Richter & Stutz, 2002) as well as grasslands on other continents (Neuenkamp et al., 2019; Smith et al., 2018; Vahter et al., 2020; Wubs et al., 2016; Zhang et al., 2012), additional research is needed to assess whether native microbial amendments can be used as a tool to aid in the establishment of difficult to establish late successional plants in these other ecoregions as well as within other understudied grasslands globally.

This study is the first to directly compare locally derived, native ecosystem AM fungi and whole soil as restoration amendments. We found that inoculation with either locally derived AM fungi or whole soil similarly and consistently improved nurse plant establishment and/or growth, and that inoculation with AM fungi resulted in less invasive recolonization and greater plant community diversity at the end of the study. In contrast, previous studies have compared locally collected whole soil inocula and commercial mycorrhizal inocula (of non-local origin; Emam, 2016; Rowe et al., 2007) and found commercial inocula to be less effective. AM fungi can be adapted to particular soil conditions (Johnson et al., 2010; Rúa et al., 2016). Therefore, the use of non-local inoculum may lead to fungal and soil mismatches between soil characteristics and fungal capabilities. Supporting this hypothesis, the few studies that have compared locally adapted versus commercial AM fungi have found locally adapted AM fungi improve

restoration success (Maltz & Treseder, 2015; Middleton et al., 2015). These mycorrhizal amendments were all locally adapted, potentially giving them an advantage in the restored soils, and each was effective at promoting plant growth and survival, compared to the non-inoculated control, despite differences in inocula composition and OTU richness. Together, these results suggest that native, locally adapted mycorrhizal mixtures may generally improve the establishment of plant species with high conservation value in grassland restorations, supporting past work (Neuenkamp et al., 2019). However, future work should assess the spatial scale over which being 'local' confers advantages, determines the degree of similarity among native mycorrhizal inocula from different 'remnant' ecosystems and assesses unintended effects of using non-local sources of mycorrhizal amendments in restoration (Hart et al., 2017).

The whole soil inocula contained an inclusive sampling of the diverse biota in old-growth prairie soils, likely including pathogens, *Rhizobia*, mycorrhizal fungi, soil bacteria, nematodes as well as other invertebrates. However, across these five restoration sites, nurse plants generally responded positively to whole soil inoculation regardless of nurse plant functional group, suggesting that the benefit from the addition of native soil mutualists is greater to late successional plants than the harm from native pathogens. While negative feedbacks, which have been shown to be commonly driven by host-specific pathogens (Bauer et al., 2015; Bever et al., 2015; Crawford et al., 2019; Kardol et al., 2007; Klironomos, 2002), may increase with introduced native pathogens in future years, these results indicate that plant benefits from whole soil inoculations are largely driven by the presence of beneficial mycorrhizal species, as has been shown previously (Bauer et al., 2015; Kardol et al., 2006). Moreover, these benefits may outweigh the negative effects of plant pathogens found in whole soils over a 3-year period.

Although the benefits of inoculation treatments persisted through three growing seasons, these benefits were realized with subtle impacts on the composition of the soil communities. While we confirmed that inoculated AM fungi were detected in the roots of inoculated plants after planting, inoculation produced little alteration in overall AM fungal biomass, based on AM fungal (PLFA) biomarker, indicating that the shifts in the relative abundances of AM fungal taxa were not associated with altered overall AM fungal biomass. Others have found that the overall density of AM fungi did not change following inoculation (Fernández et al., 2012; Vahter et al., 2020). Although microbial amendment did not influence AM fungal biomass, the introduction of native AM fungi resulted in large increases in productivity of late successional plants, indicating the disturbed functioning of AM fungal taxa that dominate following anthropogenic soil disturbance.

The benefits of pairing responsive late successional plants with beneficial native AM fungi may generate a positive feedback and spread to neighbouring plants (Bauer et al., 2015; Kardol et al., 2007; Koziol & Bever, 2019; Zhang et al., 2016), although there is limited evidence of this in the field (Koziol & Bever, 2017; Neuenkamp et al., 2019; Wubs et al., 2019). Previous work in grasslands has found that the effects of inoculation can spread up to 2 meters a year to

reduce non-native plant species, and increase nearby plant community diversity, seed recruitment and the growth of neighbouring late successional plants (Koziol & Bever, 2017; Middleton & Bever, 2012; Middleton et al., 2015). Although inoculation effects were observed in all 3 years for plant survival, inoculation did not alter plant height during the third growing season. This could be due to plant height being a poor predictor of fitness differences as plants mature. It is also possible that inoculum differences were minimized by spread of AM fungi across isles to other plots over time. Dissection of the conditions under which benefits of inoculation spread to neighbouring plants and spatial extent of this spread will require further work.

4.1 | Implications to applied practices

To date, several restoration ecologists have found positive effects of whole rhizosphere soils amendments in novel restorations and recommended their use in future restoration practices (Bever et al., 2003; Middleton & Bever, 2012; Rowe et al., 2007; Vahter et al., 2020; Wubs et al., 2016). These studies have tested field inoculation rates ranging from 500 to 45,000 L (150–12,000 gallons) of whole rhizosphere inocula per hectare. Harvesting whole soil inocula from endangered remnant grasslands soils could decimate the native soil communities of the tallgrass prairie system very quickly. For instance, it is estimated that <1,000 ha of high-quality remnant prairies remain in Illinois (one of the study sites), with 83% of these prairies being 4 ha or less (IDNR, 2021). Thus, harvesting whole soil from these increasingly threatened systems for restoration should be approached cautiously. However, the whole soil inoculation approach would support the restoration of late successional plants in systems that have sufficient whole soil for donation to restoration.

For North American grasslands, these results suggest that adding locally derived AM fungal amendments may provide the same restoration benefits as whole soil inoculations. We find evidence that AM fungi are keystone components of the soil microbiome for native prairie plants and that the inclusion of this soil guild alone can improve restored plant establishment and productivity for later successional plant as well as improving diversity and limiting reinvasion in the restored plant community. These data contribute to the growing body of work indicating that grassland plant species with later-successional strategies are dependent on and responsive to below-ground microbiome biota, especially AM fungi, relative to early successional plant species (Abbott & Robson, 1991; Bauer et al., 2015; Kardol et al., 2007; Koziol & Bever, 2017; Middleton et al., 2015; Zhang et al., 2012, 2016). Based on these results, we suggest that native mycorrhizal fungi can be used as a tool for the restoration of difficult to establish later successional plants in degraded grasslands and to improve restoration quality. Developing cost-effective methods to cultivate native mycorrhizae either via fungal cultures or whole soil culturing methods is needed for microbiome amendments to be a tangible tool for future revegetation efforts.

Future directions in applied practices should focus on the long-term effects of native inocula in grassland restoration, including the long-term effects on plant and soil communities and ways in which inoculation can be optimized as a tool for future efforts to restore grasslands. Long-term benefits may be promising, as recent evidence suggests that the effects of inoculation can spread from site of inoculation to increase nearby native diversity, establishment and future seed recruitment (Koziol & Bever, 2017; Middleton et al., 2015). Although overall survival diminished during this study period, the relative benefits of inoculation on survival increased over time as well as increased restored plant community diversity with AM fungal inoculation. Recent meta-analysis suggests that the benefits of inoculation may improve overtime (Neuenkamp et al., 2019). To be used as an effective restoration tool, more work is needed to assess whether applying native mycorrhizal inoculum can be effective using other application techniques likely to be utilized for large-scale restoration, such as via seed drill, the use of trap cultures and other broadcast methods (Koziol et al., 2020; Vahter et al., 2020; Wubs et al., 2016).

CONFLICTS OF INTEREST
L.K. owns MycoBloom LLC. MycoBloom did not participate in this research.

AUTHORS' CONTRIBUTIONS
All authors initiated and designed the field experiment; L.K., G.L.H., J.T.B., A.G.T., and E.B.D. implemented the experiments and/or collected the data; L.K., A.G.T., G.L.H., E.B.D. and J.D.B. analysed the data and L.K. wrote the first draft. All authors contributed to the final manuscript.

DATA AVAILABILITY STATEMENT
Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.9kd51c5hz (Koziol et al., 2021).

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