ECOLOGICAL SUCCESSION IN A CHANGING WORLD

Mycorrhizal feedbacks generate positive frequency dependence accelerating grassland succession

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Abstract
1. Plant mutualists including arbuscular mycorrhizal (AM) fungi have been postulated as being important drivers of plant community diversity and succession. Late successional plants have been shown to be more responsive to AM fungi and more sensitive to AM fungal species identity, which could generate positive feedback and potentially accelerate succession.

2. We test the effect of AM fungi on plant diversity and on frequency dependence predicted by positive plant–AM fungi feedback across a successional gradient. We created prairie mesocosms comprised of a majority of early successional, equal abundance, and a majority of late successional plant species. We inoculated these mesocosms and a field restoration experiment with 14 different communities of AM fungi from late successional prairies that varied in levels of species richness.

3. Overall, we found that AM fungi increased plant diversity and this was driven by the response of late successional plant species to mycorrhizae. Our results indicate that AM fungal composition is more important than AM fungal diversity per se. We found that the effect of inoculation with a single species or groups of AM fungi depended on whether those fungi benefited late successional plant species. Early successional plants consistently exhibited negative frequency-dependent growth regardless of fungal composition, while late successional plants demonstrated positive frequency-dependent growth in our mesocosms—but only in the presence of beneficial AM fungal species. These results are consistent with positive plant–mycorrhizal feedbacks accelerating plant community successional trajectories once late successional plants establish. Mesocosm results were mirrored with field inoculation assays where we found that beneficial AM fungi facilitated late successional plant establishment.

4. Synthesis. Our results provide support for beneficial arbuscular mycorrhizal fungi being a primary mechanism for positive plant–soil feedback driving plant community succession, as late successional seedlings grew faster and larger when their neighbours were also late successional plant species when they were associated with beneficial arbuscular mycorrhizal fungi. We found that this positive feedback thereby accelerated succession in mesocosms and in the field.

KEYWORDS
arbuscular mycorrhizal fungi, determinants of plant community diversity and structure, inoculation, microbial diversity, mycorrhizal responsiveness, plant succession, plant–fungal specificity, plant–soil (below-ground) interactions

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1 | INTRODUCTION

There is a growing recognition of the potential for below-ground soil biota to structure plant communities. This view derives support from two major research directions that predict conflicting dynamics of microbial contribution to plant species diversity. Plant–soil feedback research has identified a critical role of negative feedbacks, largely due to pathogens, in structuring plant communities and maintaining plant diversity (Bauer, Mack, & Bever, 2015; Mangan, Herre, & Bever, 2010). In contrast, widely cited evidence from mesocosm studies suggests that inoculation with symbiotic arbuscular mycorrhizal (AM) fungi generally results in increased plant diversity and productivity (e.g., Bauer, Kleczewski, Bever, Clay, & Reynolds, 2012; Mariotte et al., 2013; van der Heijden et al., 1998, Vogelsang, Reynolds, & Bever, 2006). However, positive plant–soil fungal feedbacks would be expected to lead to loss of diversity over time as it increases the abundance of common plant species (Bever, Platt, & Morton, 2012).

One possible resolution to the tension between these two sources of evidence may come from observing that mesocosm studies are usually run in as single-year studies, often involving the initiation of a plant community (e.g., Ammann & Nyberg, 2005; Mariotte et al., 2013; van der Heijden et al., 1998, Vogelsang et al., 2006). However, the expectation of loss of diversity due to positive feedback is predicted to occur with plant community succession which could take many growing seasons (Bever et al., 2010; Bever, Westover, & Antonovics, 1997). Therefore, it is possible that temporary AM fungal-mediated improvements in plant species diversity do not predict the long-term effects of AM fungi on plant community dynamics.

To understand the long-term effect of mycorrhizal fungi on plant succession and species coexistence, the effects of mycorrhizae on plant community dynamics must be studied over longer time periods. One approach has been to study these dynamics across plant species that occur early and late in succession within reciprocal feedback tests. Reciprocal feedback tests across co-occurring early and later successional grassland plant species have suggested a shift from strongly negative to less negative or even positive plant–soil feedbacks across succession (Bauer et al., 2015; Kardol, Martijn Bezemer, & Putten, 2006; Zhang et al., 2010; Zhang, Putten, & Veen, 2016). Pathogens certainly contribute to the strong negative feedbacks in early successional species (Mills & Bever, 1998; Van der Putten, 2003) and these effects can contribute to species turnover during succession when late successional species have greater resistance or tolerance to pathogens.

The AM fungi are also likely to influence plant–soil feedbacks during succession. Many late successional plants are much more responsive to mycorrhizal fungi than early successional plant species and exhibit strong positive growth benefits to AM fungi generally (Bauer, Koziol, & Bever, 2018; Koziol & Bever, 2015; Middleton & Bever, 2012) as well as increased sensitivity to mycorrhizal species identity (Koziol & Bever, 2016a). Therefore, the relative success of late successional plant species will likely depend upon the presence and species composition of mycorrhizal fungi in their environment. Together these results suggest that changes in mycorrhizal fungal composition could ultimately drive grassland community succession. There is abundant evidence that the composition of AM fungi change during succession (Hamel, Dalpé, Lapierre, Simard, & Smith, 1994; House & Bever, 2018; Johnson, Zak, Tilman, & Pfleger, 1991; Oehl et al., 2010; Schnoor, Lekberg, Rosendaal, & Olsson, 2010) and evidence that field inoculation of late successional fungi can accelerate plant succession (Koziol & Bever, 2016b; Middleton et al., 2015). However, to date, we do not have a direct test of whether AM fungal composition alters the relative success of early versus late successional plant species.

In this study, we tested the role of AM fungi in driving succession by assessing plant community diversity, productivity, and frequency-dependent plant growth across prairie grassland mesocosms. We created a successional gradient by varying initial plant community composition to reflect majority of early successional, equal abundance, and a majority of late successional plant species. This gradient allowed us to test across mesocosms in which the dominating plant species have demonstrated weak (majority of early successional plants) to strong (majority of late successional plants) benefit from mycorrhizal fungi. Because previous work has identified prairie AM fungi that consistently range from nonbeneficial to highly beneficial for late successional prairie plants (Koziol & Bever, 2016a), we were able to inoculate these mesocosms with AM fungal communities that differed in both fungal richness as well as how beneficial the AM fungal community would be to late successional plants. We also paired these data with a field inoculation study using the same AM fungal isolates. Because late successional plants are generally strongly responsive to specific mycorrhizae, we predicted that AM fungal composition would drive late successional plant productivity and that these plant species would increase their relative abundance in the presence of beneficial fungal species. We predicted that increased late successional plant growth would be strongest when late successional plants were more common due to mycorrhizal feedbacks generating positive frequency dependence on late successional plant growth.

2 | MATERIALS AND METHODS

2.1 | Plant material

Plants were classified as early or late successional based on field observations of grassland succession and coefficient of conservatism scores, a metric of how likely a plant is to be found in more or less disturbed areas (Betz, Lootens, & Becker, 1996; Schramm, 1992; Swink & Wilhelm, 1994), as supported by correlations with plant life histories (Bauer et al., 2018; Koziol & Bever, 2015).
2.2 | Fungal material

The AM fungi were isolated from remnant prairies in northern Indiana (within 50 miles of 41°34′40.49″N, 87°25′7.24″W). Pure cultures were grown with *Sorghum bicolor* in 1:1 mixture of sand and Indiana soil (see, Koziol & Bever, 2016b, Vogelsang et al., 2006 for more details on culturing methods). The AM fungi tested included: *Claroideoglomus claroideum, Funneliformis mosseae, Cetraspora pellucida, Claroideoglomus lancellosum, Acaulospora spinoa*, and *Entrophospora infrequens* based on spore morphology. Prior to planting the experiment, a mean infection percentage (MIP) was conducted to assess primary fungal infection. Fungal species were grown with a sorghum host for 4 weeks and fungal infection of roots was scored. After analysing the root infection scores using Proc GLM in SAS (SAS, 2015) using a contrast comparing difference in infection among the different fungal species, we found no significant differences in mean infection potentials considering hyphal infection ($F_{1,7} = 2.0, p = 0.1$) or arbuscules ($F_{1,7} = 2.2, p = 0.1$). Based on previous work with these same fungal species, each AM fungal species was categorized as “beneficial,” “nonbeneficial,” or “intermediate” at promoting late successional plant growth (Koziol & Bever, 2016a) (Appendix S1: Table S1).

2.3 | Experimental mesocosm design

Soil was collected from the Kankakee Sands Nature Preserve in Morocco, Indiana and was mixed 1:1 with Indiana river sand. The background soil was loamy sand with a pH of 8.2, with 0.1% organic matter, 70 p.p.m. Nitrate, 19 p.p.m. P, 33 p.p.m. K, and 102 Mg (Mehlich-3). The soil mixture was steam sterilized at 165–170°F twice for 4 hr with a 1-day rest period. Seeds were obtained from Spence Nursery in Muncie, Indiana and were cold moist stratified twice for 4 hr with a 1-day rest period. We designed mesocosms with 10,075 cm$^3$ of sterile soil, and 75 cm$^3$ of fungal inoculum placed at the centre depth. Noninoculated pots received 10,075 cm$^3$ of the sterilized soil mixture.

Mesocosms were established in the Indiana University greenhouses in May 2013. Plant size measurements, including height and the number of leaves, were taken immediately after planting and again in July 2013. The above-ground biomass of all plants was harvested during September–October 2013. At that time, two soil cores (2.54 cm diameter × 10 cm depth) were collected to assess AM fungal spore composition. Pots were individually wrapped in frost cloth and placed on outdoor benches to overwinter from October 2013–March 2014. Pots were brought back into the greenhouse during March 2014 and grown until October 2014, when the second-year above-ground biomass was harvested to assess plant productivity.

2.4 | Statistical analysis for mesocosm study

We analysed plant productivity (log transformed (1+ total above-ground biomass (g))) using a mixed model with plant species, plant community treatment, and soil treatment as predictors with soil treatment by plant species and soil treatment by plant species by successional community used as random effects, thereby testing for general patterns across all plant species. Plant height immediately after planting was used as a predictor to remove effects of variation in initial plant size. After the first growing season, we calculated total community productivity, diversity, and per capita plant growth (explained below) across successional mesocosms. We only report total community productivity for year two because of inconsistent overwintering survival (see Appendix S1 for additional information).

We used the vegan package in R to calculate the inverse Simpson’s index as a metric of plant community diversity of each mesocosm using the above-ground biomass (g) of each plant species in each mesocosm (Oksanen et al., 2007). We use this index to match previous work on field samples (Koziol & Bever, 2016b). We first tested for direct effects of AM fungal community composition and richness on plant community diversity using a generalized linear model with block, fungal community treatment, and plant community treatment (early biased, mid successional, or late biased) and their interaction as predictors. We designed a priori contrasts comparing the effects of different AM fungal compositions on plant community diversity, including: (a) the linear regression of fungal diversity; (b) whether the diverse fungal mixture was different from the average of particular fungal functional groups (i.e., nonbeneficial, intermediate, or beneficial); (c) whether the average of particular functional groups differed from the control; (d) whether functional groups were different from each other; and (e) whether mixtures were different from the average of the individual fungal species. We then tested the extent to which fungal effects interacted with the initial proportion of late successional plant species in the plant community treatment by adding the fungal inoculation*proportion late interaction term. This interaction tests for a consistent directional difference between early and late successional biased communities in their response fungal
composition. In this analysis, we include the a priori contrasts as described above and the interactions of these contrasts with the initial proportion of late successional plant species. These analyses were performed in proc GLM in SAS 9.4 (SAS, 2015).

To detect positive or negative frequency-dependent growth for each plant species across succession and when grown with different fungal communities, we found the average per capita biomass of early and late successional plants among each pot when grown in the three different successional communities. We also analysed the per capita flowering structure biomass (flower heads or spikes). The interactive effects of fungal composition and initial proportion of late successional plants on per capita biomass and flowering were assessed using the same model specifications as described above for plant diversity. We assessed changes in mycorrhizal fungal composition using spore quantification (Appendix S1).

2.5 | Experimental design for field experiment

We describe the results from a recent experiment assessing the effects of inoculation with five different native AM fungal species in an Indiana grassland restoration that was seeded with 54 prairie plant species (Koziol & Bever, 2016b). The experiment compared different AM fungal inoculation treatments comprised of either four individual AM fungal species derived from prairies in Indiana, including *Acaulospora spinosa*, *Entrophospora infrequens*, *Claroideoglomus lanellus*, and *Claroideoglomus claroideum*, or all four AM fungal species together. No effort was made to remove the existing fungi at the restoration site. The AM fungi were introduced by planting 16 inoculated prairie seedlings into each plot (eight early and eight late successional; see Koziol and Bever (2016b) for more details on this experimental design). Briefly, inoculated seedlings were planted in 150-cm³ pots that were filled with sterilized background soil and 15 cm³ of live or sterilized inocula placed at the centre depth just prior to a seedling being planted. Plants receiving sterilized inocula are henceforth referred to as noninoculated. After 3 weeks, seedlings were transplanted into the field. Each of the 16 different plant species were planted into similarly inoculated plots using replication. During the first 2 years we were able to monitor the growth of the inoculated seedlings. We collected total plot above-ground biomass and sorted it by planted, seeded, and nonseeded species in years two and three of the experiment.

2.6 | Statistical analyses for field experiment

We analysed third-year total native, early, and late successional plant above-ground biomass, plant community diversity and per capita growth rates in the plots using general linear models (GLM) in SAS. We used the second growing season biomass of planted species to calculate per capita growth rates of the planted prairie seedlings that received live or sterilized inocula. We used fungal inoculation composition and block as predictors. Inverse Simpson’s index was calculated as an estimate plant community diversity using the vegan package in R as described for the mesocosm study. We deconstructed plant growth in response to inoculation treatments using a priori contrasts comparing inoculated versus noninoculated plant growth, plant growth differences among the individual fungal species, whether the diverse mixture affected plants differently than the average effect of the individual species and each of these contrasts by successional stage within the model. Using second-year above-ground biomass data published in Koziol and Bever (2016b) and new above-ground biomass data we collected during year three of this restoration, we calculated the proportion of native plant abundance consisting of late successional species based on coefficient of conservatism score as previously described. We tested the correlation of the proportional late successional species across years using SAS.
Proc Corr (SAS, 2015) and tested whether the proportion of late successional biomass increased differently over time as a function of inoculation treatment using two-tailed t-tests of the slopes of the proportional late successional biomass.

3 | RESULTS

3.1 | Mesocosm experiment

3.1.1 | Plant community diversity responds to fungal community richness and composition

Plant community diversity was strongly affected by AM fungal composition (Appendix S1: Table S3, Figure 1a) and initial plant community structure (early, mid, or late successional mesocosms) (Appendix S1: Table S3, Figure 2). In general, diversity was lowest with the noninoculated control and nonbeneficial AM fungi regardless of the number of late successional species present in a mesocosm (Figures 1a and 2, black lines and symbols). Overall, greater AM fungal richness resulted in greater plant community diversity in growing season one (Appendix S1: Table S3, Figure 1a, linear regression contrast $F_{1,105} = 98.8, p < 0.0001$). However, the effects of AM fungal richness were strongly dependent on AM fungal composition. When the most beneficial fungi were present, plant diversity was high and not statistically different from the diverse fungal mixture regardless of AM fungal richness (Appendix S1: Table S3, $F_{1,105} = 0.0, ns.$). Inoculation with nonbeneficial AM fungi increased diversity relative to the control (Appendix S1: Table S3, $F_{1,105} = 13.5, p = 0.0005$), but not nearly as much as beneficial or intermediate fungi (Appendix S1: Table S3, $F_{1,105} = 82.7, p < 0.0001$). Thus, the overall effect of improved diversity with increasing AM fungal richness was driven by the presence of beneficial AM fungal species (fungal composition) and not fungal richness or diversity per se. In fact, the highest level of plant diversity was obtained from inoculation with a single species of beneficial AM fungi (E. infrequens, Figure 1a).

The effect of AM fungal composition on plant diversity depended on the initial proportion of late successional plant species present in the mesocosm (fungal composition × plant community composition as found in the linear model $F_{1,119} = 2.2, p = 0.015$, Figure 2). As the proportion of initial late successional seedling abundance increased, the positive effects of beneficial AM fungi on plant community diversity became even more strongly pronounced ($F_{1,119} = 10.46, p = 0.0016$). Plant community diversity increased with late successional seedling abundance when inoculated with beneficial or intermediate AM fungi, but not when inoculated with nonbeneficial AM fungi (Figure 2, $F_{1,119} = 16.9, p < 0.0001$). The positive effect of AM fungal diversity on plant diversity did not depend upon initial proportion of late successional plant species ($F_{1,119} = 1.0, p = 0.3$).

3.1.2 | Mesocosm plant productivity depended on plant successional status, initial plant community composition, and AM fungal community composition

The total mesocosm productivity in the first year depended on the plant community treatment (Appendix S1: Table S4, $F_{2,944} = 19.2, p < 0.0001$), AM fungal composition (Figure 3, $F_{13,944} = 2.8, p = 0.01$), and their interaction (Appendix S1: Table S4, $F_{26,944} = 1.7, p = 0.02$). Overall, total mesocosm productivity was lowest in the early successional biased plant treatment and the greatest productivity was found in the late successional plant-biased treatment during year one (Appendix S1: Table S4, $F_{26,944} = 19.2, p < 0.0001$). Treating individual plant species as random effects, we find that early successional plant species consistently grew larger in the first year than late successional plant species (Appendix S1: Table S4, Figure 3, $F_{1,1520} = 121.3, p < 0.0001$), confirming our initial categorization of plant species. However, the relative growth rates of early and late successional plant species depended upon the interaction of AM fungal composition and initial plant composition (Appendix S1: Table S4, $F_{28,944} = 10.8, p < 0.0001$).
3.1.3 Frequency dependency across successional and AM fungal composition

On average, late successional plants exhibited positive frequency-dependent growth rates, as seedlings grew significantly larger in pots dominated by late successional plants (Appendix S1: Table S5, Figure 4a). Positive late successional frequency dependence, as measured by per capita plant mass (Appendix S1: Table S5, Figure 4a, $F_{1,118} = 4.1, p < 0.0001$) and per capita inflorescence biomass (Appendix S1: Table S5, $F_{1,118} = 2.6, p = 0.003$), depended on AM fungal community composition. In general, late successional plants grew poorest and did not exhibit positive frequency-dependent growth in the control or when inoculated with nonbeneficial fungi (black lines in Figure 4a). However, late successional plants had strong positive frequency-dependent growth when grown with AM fungal communities containing “beneficial” and “intermediately beneficial” AM fungi (Appendix S1: Table S5, blue and red lines in Figure 4a, $F_{1,118} = 14.1, p = 0.0003$). The frequency-dependent growth observed in mixtures of two nonbeneficial fungi, mixtures of two intermediately beneficial fungi, mixtures of two beneficial fungi, and mixtures of one beneficial and one intermediately beneficial fungi were not different from null expectations from the effects of the individual fungi. However, we found stronger positive frequency dependence for late successional plants than expected with communities of AM fungi that included mixtures of beneficial and nonbeneficial AM fungi (Appendix S1: Table S5, $F_{1,118} = 21.1, p < 0.0001$)
and mixtures of intermediately beneficial and nonbeneficial AM fungi (Appendix S1: Table S5, \( F_{1,118} = 14.2, p = 0.04 \)).

In contrast to late successional plants, early successional plants experienced consistent negative frequency-dependent growth, as the average early successional plant size decreased with increasing proportion of early successional plants (Appendix S1: Table S5, \( F_{1,118} = 164.7, p < 0.0001 \)). Early successional species demonstrated similar negative frequency-dependent growth whether plants were inoculated or noninoculated with AM fungi (\( F_{1,118} = 0.0, p = 1.0 \)) and whether they were inoculated with "beneficial" or "intermediate" AM fungal species or their mixtures or not (Appendix S1: Table S5, \( F_{1,118} = 1.3, p = 0.3 \)). These results suggest that the negative frequency-dependent growth of early successional plant species was largely unaffected by AM fungal species composition. However, the level of negative frequency dependence in per capita growth rates of early successional plant species did increase with mixtures of beneficial and nonbeneficial AM fungi compared to expectations (Appendix S1: Table S5, \( F_{1,118} = 13.8, p = 0.0003 \)), indicating that AM fungal composition can impact early successional plant species performance.

3.1.4 | Shifts in plant community succession across years resulting from frequency dependence

The proportion of late successional plant abundance the second growing season was strongly predicted by the proportion of late successional plant abundance found at the end of the first growing season (Figure 5a, \( F_{1,112} = 16.6, p = 0.002 \)). The slope of this relationship was significantly greater than 1 (\( T = 4.95, p = 0.0003 \)), as is consistent with positive feedback. Pots that attained a larger proportion of late successional plant biomass the first growing season, due to beneficial AM fungal composition, promoted larger increases in the proportion of late successional plant species than pots that had low proportion of late successional plant species in year one (Figure 5a).

3.2 | Field experiment

3.2.1 | Plant community diversity is affected by fungal composition

We found that second-year plant diversity in the field was affected by AM fungal inoculation (Appendix S1: Table S6, \( F_{5,10} = 5.11, p = 0.003 \)). Mirroring our mesocosms results, the effect of inoculation with AM fungi on plant diversity in the field depended on AM fungal species identity (Figure 1b, \( F_{3,10} = 5.0, p = 0.01 \)). Our classification of "beneficial" and "non-beneficial" fungi were generally consistent across the field and mesocosm experiments, which took place on different soils and with different plant community treatments, with the most notable exception being the fungus A. spinosa. Consistent with mesocosm results, inoculation with some single AM fungi resulted in diversity as low as the noninoculated controls while inoculation with other AM fungal species generated diversity that was greater than the four species fungal mixture. Increased native abundance, richness and late successional richness due to AM fungal composition continued during year three of the restoration (Appendix S1: Table S6). As with our mesocosm study, we found that inoculation with individual fungal species resulted in large variation on per capita growth of late successional plants (Appendix S1: Table S6, \( F_{3,10} = 3.0, p = 0.05 \)), but not for early successional species (\( F_{3,10} = 0.7, p = 0.6 \)).

**FIGURE 5** The proportional abundance of late successional species was strongly predicted by the proportion of late successional species from the previous year and by fungal composition in mesocosms (a) and in the field (b). Dotted lines represent the best fit line (a, b). For mesocosms, we weighted this line by the number of replicated mesocosms per fungal composition averaged across plant community treatments. We found that the slope of this line is significantly greater than one, as is consistent with positive feedbacks facilitating plant community succession (a). This pattern was consistent field results (b). Each symbol represents the average proportion of late successional species in a given fungal composition averaged across succession (a) or among each plot (b).
3.2.2 | Shifts in plant community succession resulting from positive frequency dependence

The proportion of late successional plant abundance during year two was strongly correlated with the proportion of late successional plant abundance during year three in the field (Figure 5b, $r = 0.77$, $df = 32$, $p \leq 0.0001$), similar to what was found in mesocosms. As we found in our mesocosms, the slope of this relationship is greater than 1, as is consistent with positive feedback. That is, plots that attained relatively high compositions of late successional plant species maintained larger increases in the proportion of late successional plant species the following year relative to plots that had a low proportion of late successional plant species in year one (Figure 5b). We found that the overall pattern was only slightly more positive than the 1:1 line, but that this modest effect was dependent on fungal composition (Appendix S1: Table S7, Figure 5b).

4 | DISCUSSION

4.1 | AM fungi as a driver of plant diversity

Although previous observations have shown that plant species diversity can increase with increasing AM fungal richness (e.g., van der Heijden et al., 1998; Vogelsang et al., 2006), the presence of AM fungi does not consistently increase plant diversity (e.g., Hartnett & Wilson, 1999; O’Connor, Smith, & Smith, 2002), and attempts have been made to describe the context dependence in which inoculation with AM fungi increase plant diversity (Klironomos et al., 2011; Urcelay & Diaz, 2003; Vogelsang et al., 2006). Our work indicates that AM fungal composition is more important to plant diversity than AM fungal diversity per se. We found that inoculation with two AM fungal species could result in high or low plant species diversity—depending on whether those fungi were “beneficial” (Figure 1a). Plant diversity with a single, highly beneficial AM fungal species matched what was found with our most species-rich AM fungal mixtures in mesocosms and the field, supporting a critical role of the “sampling” effect mechanism mediated the effect of AM fungal richness on plant diversity (Vogelsang et al., 2006).

While our results affirm an important role of AM fungi in promoting plant diversity within a single growing season, we find evidence that this effect is due to an increase in both species evenness and the effective plant species pool (via increased plant species richness in the field study). We found that the presence of beneficial AM fungi can increase the representation of highly mycorrhizal plant species that would otherwise be rare, thereby increasing plant diversity. However, this benefit to plant diversity may not be sustained over the long-term as these same fungi can accelerate the dominance of these plants through positive feedbacks, which can then lead to a loss of plant community diversity over time (Bever et al., 1997). Others have found that when mycorrhizal plants are promoted by their fungal community, this can lead to dominance and the exclusion of other plants (McCain, Wilson, & Blair, 2011).

Our results suggest that the successional status of the plant community provides a reliable framework for predicting the effect of AM fungi on plant diversity. In recently disturbed, early successional environments, changing the AM fungal composition by adding generally beneficial fungal species will likely have strong effects on plant diversity by increasing the representation of late successional plant species, as shown in the present study and previous work (Bauer et al., 2012; Klironomos, McCune, Hart, & Neville, 2000; Koziol & Bever, 2016b; van der Heijden et al., 1998; Vogelsang et al., 2006). However, other studies have found that in undisturbed environments with a dominance of late successional plant species, removal of AM fungi may allow minority early successional species to increase, thereby increasing plant diversity (Hartnett & Wilson, 1999; O’Connor et al., 2002). Together, these studies suggest that while short-term inoculation studies demonstrate the potential positive impact of AM fungi on plant diversity in early successional environments, these gains may not be sustained for the long term. To assess the long-term stability of fungal influences in succession, future work should incorporate plant-soil feedbacks within a holistic framework (Bever, 1999; Bever et al., 2010; Umbanhowar & McCann, 2005).

Our classification of “beneficial” and “non-beneficial” mycorrhizal fungi were generally consistent across the field and mesocosm experiments, which took place on different soils and with different plant community treatments, with the most notable exception being the fungus A. spinosa. This suggests that while fungal composition can be a consistent predictor of plant community responses, there is some context dependency of when and where particular fungi are beneficial promoters of diversity. This difference could be due to environmental variables that include alternate forms of nutrients, as previous work has shown that AM fungi may be more or less beneficial when delivering different types of phosphorus to a host plant (Vogelsang et al., 2006) or if a plant is a dominant or subordinate species in the community (Urcelay & Diaz, 2003).

4.2 | AM fungi as drivers of succession

It has been reported that early successional plant species are less dependent on AM fungi (Janos, 1980; Koziol & Bever, 2015) and show less specificity towards specific fungal taxa than late successional species (Koziol & Bever, 2016a, 2016b). These two results are supported in the present study. We also present evidence that the dynamics of AM fungi can influence terrestrial plant succession via positive plant-AM fungal feedbacks in our mesocosm experiment and field study. Positive feedback through changes in the AM fungal community is evident in the test of the net impact of AM fungal composition on plant communities. Here, we found that early successional plants exhibited consistent negative frequency dependence, regardless of AM fungal presence or composition. This result is consistent with negative density dependence due to resource depletion driving the suppression of fast growing early successional species by other early successional species. This result is consistent with the facilitation model of succession (Connell & Slatyer, 1977), in which
early successional species differentially limit themselves. In contrast, for late successional plant species, we found evidence of positive frequency dependence. We found improved late successional plant growth in the field and in mesocosms when beneficial AM fungi were present. Moreover, in the mesocosms, late successional seedlings grew fastest when their neighbours were also late successional plant species—but only in the presence of positive growth promoting AM fungi. While negative feedback through AM fungal communities has been observed among early successional species (Bever, 2002), positive feedbacks and positive frequency dependence are a common expectation for mutualisms (Umbanhowar & McCann, 2005; Vandermeer & Boucher, 1978).

Positive feedback could be due to changes in density of AM fungi or changes in AM fungal composition (Bever et al., 2012). We found positive frequency dependence when individual beneficial AM fungi were present, supporting an important role of changes in AM fungal density affecting plant succession. However, we found strongest positive frequency-dependent growth of late successional plants when the mesocosms included mixtures of beneficial and nonbeneficial AM fungal species (Figure 3a), which is consistent with changes in AM fungal composition contributing to positive feedback (Bever, 1999). This result indicates that late successional seedlings benefit not just from increasing density of AM fungi but also from having neighbouring late successional plant species that increase the proportion of beneficial fungi relative to nonbeneficial fungi, as might be expected from observations of preferential allocation to the most effective mutualist (Bever, Richardson, Lawrence, Holmes, & Watson, 2009, Kiers et al., 2011, Zheng, Ji, Zhang, Zhang, & Bever, 2015, Ji & Bever, 2016). The net dynamic of positive feedback supports the inhibition model of succession (Connell & Slatyer, 1977), in which early successional species inhibit late successional species through limiting benefits from beneficial mycorrhizal fungi.

Our data on fungal composition facilitating plant succession align with what is known from field data on mycorrhizal succession indicating that AM fungal composition and abundance is likely to change during plant community succession. Even though our assessments of the effect of early and late successional plant communities on fungal composition were inconclusive in this experiment, previous work has shown that late successional plants support greater AM fungi colonization than early successional plants (Koziol & Bever, 2015, 2016a), that late successional soils may have higher fungal biomass and diversity (Abbott & Robson, 1991; Baer, Kitchen, Blair, & Rice, 2002; Kardol et al., 2006; Klopf, Baer, Bach, & Six, 2017) and that fungi in late successional soil may be more beneficial than the fungi in early successional soils (Johnson et al., 1991).

Although fungal abundance may recover after soil disturbance, AM fungal composition may not recover. Disturbed soils may have only half of the AM fungal species as intact sites (Säle et al., 2015) and many AM fungi do not occur in areas with soil disturbance (Hamel et al., 1994; House & Bever, 2018; Oehl et al., 2010; Schnoor et al., 2010), including species in the genera Scutellospora, Entrophospora, Gigaspora, and Acaulospora (Jansa et al., 2002; Johnson, 1993; Oehl et al., 2004). Here, we show that inoculation with late successional soil symbionts can facilitate grassland succession by promoting late successional plant species survival and growth, as others have shown (Middleton & Bever, 2012; Middleton et al., 2015). Our results suggest that this effect may be driven by the presence of specific late successional AM fungal species that are highly beneficial to late successional plants. Thus, the fungal-mediated shift towards late successional dominance we observed likely reflects a long-standing adaptation of these plants to beneficial microbes found in intact, later successional soils. In contrast, the lower overall responsiveness of early successional plants to AM fungi likely reflects an adaptation to disturbed soil environments that harbour fewer or less beneficial AM fungi. Further work assessing whether early successional plants are more responsive to AM fungi from early successional environments as well as studies assessing changes in fungal composition during grassland community succession would further illuminate the mechanisms by which the soil community drives succession.

5 | CONCLUSIONS

Our results suggest that AM fungal communities can influence plant community stability, diversity, and succession. Our results identify that the successional status of a plant community provides a predictable framework for understanding the effect of AM fungi on plant community diversity. However, we also provide evidence that these short-term diversity impacts may not represent the long-term influence of AM fungi. We find that the long-term influence of AM fungi on plant communities is governed by a positive plant-AM fungi feedback dynamic across plant successional stages. Our results support potential alternative stable states (Lewontin, 1969) of early successional plants interacting with AM fungal communities composed of nonbeneficial AM fungi or late successional plant communities interacting with AM fungal communities that include strong representation of beneficial fungal species. Our work identifies AM fungal dynamics as a potential switch point determining the trajectory of a plant community during succession and determining the success of restoration and restoration outcomes. However, AM fungi are not the only soil organisms that could drive successional processes and future work should aim to investigate the relative role of AM fungi and other soil organisms (i.e., pathogens, herbivores, etc.) in determining plant community successional processes.

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AUTHORS’ CONTRIBUTIONS

L.K. and J.D.B. planned and analysed these experiments and wrote this manuscript; L.K. initiated the research experiments and collected data.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.99ch097 (Koziol & Bever, 2018).

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REFERENCES

Abbott, L., & Robson, A. (1991). Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. *Agriculture Ecosystems & Environment*, 35, 121-150. https://doi.org/10.1016/0167-8809(91)90048-3

Ammann, R. L., & Nyberg, D. W. (2005). Vegetation height and quality of original and reconstructed tallgrass prairies. *The American Midland Naturalist*, 154, 55-66. https://doi.org/10.1674/0003-0310(2005)154[0055:VHAQQO]2.0.CO;2

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. https://doi.org/10.1890/1051-0761(2002)012[1688:CIESAF]2.0.CO;2

Bauer, J. T., Kleczewski, N. M., Bever, J. D., Clay, K., & Reynolds, H. L. (2012). Nitrogen-fixing bacteria, arbuscular mycorrhizal fungi, and the productivity and structure of prairie grassland communities. *Oecologia*, 170, 1089-1098. https://doi.org/10.1007/s00442-012-2363-3

Bauer, J. T., Koziol, L., & Bever, J. D. (2018). Ecology of Floristic Quality Assessment: Testing for correlations between coefficients of conservatism, species traits, and mycorrhizal responsiveness. *AoB PLANTS*, 10, px073. https://doi.org/10.1093/aobpla/px073

Bauer, J. T., Mack, K. M., & Bever, J. D. (2015). Plant-soil feedbacks as drivers of succession: Evidence from remnant and restored tallgrass prairies. *Ecosphere*, 6, 158. https://doi.org/10.1890/ES14-00480.1

Betz, R. F., Lootens, R. J., & Becker, M. K. (1996). Two decades of prairie restoration at Fermilab, Batavia, Illinois. Proceedings of the Fifteenth North American Prairie Conference (pp. 20–30). Bend, OR, Natural Areas Association.

Bever, J. (1999). Dynamics within mutualism and the maintenance of diversity: Inference from a model of interguild frequency dependence. *Ecology Letters*, 2, 52–61. https://doi.org/10.1046/j.1461-0248.1999.21050.x

Bever, J. D. (2002). Host-specificity of AM fungal population growth rates can generate feedback on plant growth. *Plant and Soil*, 244, 281–290.

Bever, J. D., Richardson, S. C., Lawrence, B. M., Holmes, J., & Watson, M. (2009). *Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism*. *Ecology Letters*, 12, 13–21.

Bever, J. D., Dickie, I. A., Facelli, E., Facelli, J. M., Klironomos, J., Moora, M., ... Zobel, M. (2010). Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology & Evolution*, 25, 468–478. https://doi.org/10.1016/j.tree.2010.05.004

Bever, J. D., Platt, T. G., & Morton, E. R. (2012). Microbial population and community dynamics on plant roots and their feedbacks on plant communities. *Annual Review of Microbiology*, 66, 265. https://doi.org/10.1146/annurev-micro-092611-150107

Bever, J. D., Westover, K. M., & Antonovics, J. (1997). Incorporating the soil community into plant population dynamics: The utility of the feedback approach. *Journal of Ecology*, 85, 561–573. https://doi.org/10.2307/2960528

Connell, J. H., & Slatyer, R. O. (1977). Mechanisms of succession in natural communities and their role in community stability and organization. *The American Naturalist*, 111, 1119-1144. https://doi.org/10.1086/283241

Hamel, C., Dalpé, Y., Lapierre, C., Simard, R. R., & Smith, D. L. (1994). Composition of the vesicular-arbuscular mycorrhizal fungi population in an old meadow as affected by pH, phosphorus and soil disturbance. *Agriculture Ecosystems & Environment*, 49, 223-231. https://doi.org/10.1016/0167-8809(94)90051-5

Hartnett, D. C., & Wilson, G. W. (1999). Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology*, 80, 1187–1195. https://doi.org/10.1890/0012-9658(1999)080[1187:MIPCS][2.0.CO;2]

House, G. L., & Bever, J. D. (2018). Disturbance reduces the differentiation of mycorrhizal fungal communities in grasslands along a precipitation gradient. *Ecological Applications*, 28, 736–748. https://doi.org/10.1002/eap.1681

Jans, D. P. (1980). Mycorrhizae influence tropical succession. *Biotropica*, 12, 56–64. https://doi.org/10.2307/2388157

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. https://doi.org/10.1007/s00572-002-0163-z

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

Johnson, N. C. (1993). Can fertilization of soil select less mutualistic mycorrhizae? *Bulletin of the Ecological Society of America*, 3, 749–757. https://doi.org/10.2307/1942106

Johnson, N. C., Zak, D. R., Tilman, D., & Pfleger, F. (1991). Dynamics of vesicular-arbuscular mycorrhizae during old field succession. *Oecologia*, 86, 349–358. https://doi.org/10.1007/BF00317600

Kardol, P., Martijn Bezemer, T., & van der Putten, W. H. (2006). Temporal variation in plant–soil feedback controls succession. *Ecology Letters*, 9, 1080–1088. https://doi.org/10.1111/j.1461-0248.2006.00953.x

Kiers, E. T., Duhamel, M., Beehsey, Y., Mensah, J. A., Franken, O., Verbruggen, E., ... Bücking, H. (2011). Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science*, 333, 880–882. https://doi.org/10.1126/science.1208473

Klironomos, J. N., McCune, J., Hart, M., & Neville, J. (2000). The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. *Ecology Letters*, 3, 137-141. https://doi.org/10.1046/j.1461-0248.2000.00131.x

Klironomos, J., Zobel, M., Tibbett, M., Stock, W. D., Rilling, M. C., Parrent, J. L., ... Facelli, E. (2011). Forces that structure plant communities: Quantifying the importance of the mycorrhizal symbiosis. *New Phytologist*, 189, 366–370. https://doi.org/10.1111/j.1469-8137.2010.03550.x

Klopf, R. P., Baer, S. G., Bach, E. M., & Six, J. (2017). Restoration and management for plant diversity enhances the rate of belowground ecosystem recovery. *Ecological Applications*, 27, 355–362. https://doi.org/10.1002/eap.1503

Koziol, L., & Bever, J. D. (2015). Mycorrhizal response trades off with plant growth rate and increases with plant successional status. *Ecology*, 96, 1768–1774. https://doi.org/10.1890/14-2208.1

Koziol, L., & Bever, J. D. (2016a). AMF, phylogeny and succession: Specificity of response to mycorrhizal fungi increases for later successional plants. *Ecosphere*, 7, e1555.
Koziol, L., & Bever, J. D. (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., & Bever, J. D. (2018). Data from: Mycorrhizal feedbacks generate positive frequency dependence accelerating grassland succession. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.9ch097

Lewontin, R. C. (1969). The meaning of stability. *Brookhaven Symposia in Biology*, 22, 13–24.

Mangan, S. A., Herre, E. A., & Bever, J. D. (2010). Specificity between Neotropical tree seedlings and their fungal mutualists leads to plant-soil feedback. *Ecology*, 91, 2594–2603. https://doi.org/10.1890/09-0396.1

Mariotte, P., Meugnier, C., Johnson, D., Thébault, A., Spiegelberger, T., & Buttler, A. (2013). Arbuscular mycorrhizal fungi reduce the differences in competitiveness between dominant and subordinate plant species. *Mycorrhiza*, 23, 267–277. https://doi.org/10.1007/s00572-012-0465-8

McCain, K. N., Wilson, G. W., & Blair, J. (2011). Mycorrhizal suppression alters plant productivity and forb establishment in a grass-dominated prairie restoration. *Plant Ecology*, 212, 1675–1685. https://doi.org/10.1007/s11258-011-9940-0

Middleton, E. L., & Bever, J. D. (2012). Inoculation with a native soil community advances succession in a grassland restoration. *Restoration Ecology*, 20, 218–226. https://doi.org/10.1111/j.1526-100X.2010.00752.x

Middleton, E. L., Richardson, S., Koziol, L., Palmer, C. E., Yermakov, Z., Henning, J. A., ... Bever, J. D. (2015). Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. *Ecosphere*, 6, 276. https://doi.org/10.1890/ES15-00152.1

Mills, K. E., & Bever, J. D. (1998). Maintenance of diversity within plant communities: Soil pathogens as agents of negative feedback. *Ecology*, 79, 1595–1601. https://doi.org/10.1890/0012-9658(1998)079[1595:MODWPC]2.0.CO;2

O’Connor, P. J., Smith, S. E., & Smith, F. A. (2002). Arbuscular mycorrhizal fungi influence plant diversity and community structure in a semiarid herbage. *New Phytologist*, 154, 209–218. https://doi.org/10.1046/j.1469-8137.2002.00364.x

Oehl, F., Laczko, E., Bogenrieder, A., Stahr, K., Bösch, R., van der Heijden, M., & Sieverding, E. (2010). Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biology and Biochemistry*, 42, 724–738. https://doi.org/10.1016/j.soilbio.2010.01.006

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. https://doi.org/10.1007/s00442-003-1458-2

Oksanen, J., Kindt, R., Legendre, P., O’Hara, B., Stevens, M. H. H., Oksanen, M. J., & Suggests, M. (2007). The vegan package. Community ecology package 10.

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

SAS. (2015). SAS 9.4 user’s guide: Survey data analysis. Cary, NC: SAS Institute Inc.

Schnoor, T. K., Lekberg, Y., Rosendahl, S., & Olsson, P. A. (2010). Mechanical soil disturbance as a determinant of arbuscular mycorrhizal fungal communities in semi-natural grassland. *Mycorrhiza*, 21, 211–220. https://doi.org/10.1007/s00572-010-0325-3

Schramm, P. (1992). *Prairie restoration: a twenty-five year perspective on establishment and management*. Proceedings of the Twelfth North American Prairie Conference (pp. 169–177). University of Northern Iowa Press, Cedar Falls.

Swink, F., & Wilhelm, G. (1994). *Plants of the Chicago region*. Indianapolis, IN: Indiana Academy of Science.

Umbanhower, J., & McCann, K. (2005). Simple rules for the coexistence and competitive dominance of plants mediated by mycorrhizal fungi. *Ecology Letters*, 8, 247–252. https://doi.org/10.1111/j.1461-0248.2004.00714.x

Urcelay, C., & Diaz, S. (2003). The mycorrhizal dependence of subordinates determines the effect of arbuscular mycorrhizal fungi on plant diversity. *Ecology Letters*, 6, 388–391. https://doi.org/10.1046/j.1461-0248.2003.00444.x

van der Heijden, M. G. A., Klironomos, J. N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., ... Sanders, I. R. (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396, 69–72. https://doi.org/10.1038/23932

Van der Putten, W. H. (2003). Plant defense belowground and spatial-temporal processes in natural vegetation. *Ecology*, 84, 2269–2280. https://doi.org/10.1890/02-0284

Vandermeer, J. H., & Boucher, D. H. (1978). Varieties of mutualistic interaction in population models. *Journal of Theoretical Biology*, 74, 549–558. https://doi.org/10.1016/0022-5193(78)90241-2

Vogelsang, K. M., Reynolds, H. L., & Bever, J. D. (2006). Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. *New Phytologist*, 172, 554–562. https://doi.org/10.1111/j.1469-8137.2006.01854.x

Zhang, N., Van der Putten, W. H., & Veen, G. (2016). Effects of root decomposition on plant-soil feedback of early-and mid-successional plant species. *New Phytologist*, 212, 220–231. https://doi.org/10.1111/nph.14007

Zhang, Q., Yang, R., Tang, J., Yang, H., Hu, S., & Chen, X. (2010). Positive feedback between mycorrhizal fungi and plants influences plant invasion success and resistance to invasion. *PloS ONE*, 5, e12380. https://doi.org/10.1371/journal.pone.0012380

Zheng, C., Ji, B., Zhang, J., Zhang, F., & Bever, J. D. (2015). Shading decreases plant carbon preferential allocation towards the most beneficial mycorrhizal mutualist. *New Phytologist*, 205, 361–368.

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