Sensitivity to AMF species is greater in late-successional than early-successional native or nonnative grassland plants

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Abstract. Sensitivity of plant species to individual arbuscular mycorrhizal (AM) fungal species is of primary importance to understanding the role of AM fungal diversity and composition in plant ecology. Currently, we do not have a predictive framework for understanding which plant species are sensitive to different AM fungal species. In two greenhouse studies, we tested for differences in plant sensitivity to different AM fungal species and mycorrhizal responsiveness across 17 grassland plant species of North America that varied in successional stage, native status, and plant family by growing plants with different AM fungal treatments including eight single AM fungal isolates, diverse mixtures of AM fungi, and non-inoculated controls. We found that late successional grassland plant species were highly responsive to AM fungi and exhibited stronger sensitivity in their response to individual AM fungal taxa compared to nonnative or early successional native grassland plant species. We confirmed these results using a meta-analysis that included 13 experiments, 37 plant species, and 40 fungal isolates (from nine publications and two greenhouse experiments presented herein). Mycorrhizal responsiveness and sensitivity of response (i.e., variation in plant biomass response to different AM fungal taxa) did not differ by the source of fungal inocula (i.e., local or not local) or plant family. Sensitivity of plant response to AM fungal species was consistently correlated with the average mycorrhizal response of that plant species. This study identifies that AM fungal identity is more important to the growth of late successional plant species than early successional or nonnative plant species, thereby predicting that AM fungal composition will be more important to plant community dynamics in late successional communities than in early successional or invaded plant communities.

Key words: arbuscular mycorrhizal fungi; coefficient of variation; grassland; inoculation; mycorrhizal responsiveness; plant sensitivity; plant successional stage; prairie.

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi form symbiotic relationships with most plant species and can benefit plant growth by increasing access to nutrients, providing protection from pathogens, and improving drought tolerance (Smith and Read 2008). However, plant growth response to individual fungal taxa is known to vary (Klironomos 2003, Koziol and Bever 2016), and as plants encounter different fungi in soil, variation in growth response to individual AM fungal taxa may influence plant community composition and promote or inhibit plant coexistence. AM fungal taxa also differ in the benefit that they deliver to their hosts (Vogelsang et al. 2006, Smith et al. 2015). For example, some fungal species appear to be more efficient at delivering phosphorus to roots (Ji and Bever 2016), and split root studies have shown that plants preferentially allocate carbon to the more beneficial fungal species (Bever et al. 2009, Kiers et al. 2011, Ji and Bever 2016). However, exactly how resources are allocated between partners is still an active area of research (Walder and van der Heijden 2015). Variation in plant growth response to individual AM fungal taxa (i.e., sensitivity) is ecologically important because it can lead to feedbacks, the direction and strength of which can influence plant community dynamics (Bever 1999, 2002, Castelli and Casper 2003, Mangan et al. 2010). Given that plant sensitivity to AM fungal species determines the ecological importance of AM fungal composition and diversity, a predictive framework for understanding the importance of AM fungal biodiversity will depend upon an understanding of the patterns of sensitivities to AM fungal species.

In the few studies that aimed to test for sensitivity in plant response to individual AM fungal taxa, results vary. In an analysis of mycorrhizal responsiveness and plant sensitivity to different AM fungi in 10 North American grassland plant species, Klironomos (2003) found that plant growth response was dependent on specific plant-fungal combinations (Fig. 1a), and that co-occurring plant and fungal combinations were more variable than naïve combinations. This suggests that AM fungal composition is more important to plant–plant interactions for coevolved plant and fungal combinations. Pringle and Bever (2008) also found significant variation in sensitivity of response, and this effect did not overwhelm the variation in average responsiveness of plant species (Pringle and Bever 2008). However, only nonnative and early successional native grassland plant species were included in these two studies, which have been shown to be less responsive to mycorrhizal fungi than many later successional grassland plant species (Koziol and Bever 2015, Bauer et al. 2018). In a separate study, Koziol and Bever showed that late successional grassland plant species had greater mycorrhizal responsiveness (Koziol and Bever 2015) and greater sensitivity of mycorrhizal response to individual fungal taxa than early successional native plant species (Koziol and Bever 2016), however, nonnative plants were not included in their analysis (Fig. 1b). Reynolds et al. (2006) also reported a positive correlation of mycorrhizal responsiveness and plant-fungal sensitivity. Other studies point to variation in mycorrhizal response being driven by native vs. nonnative plant status (Pringle et al. 2009), plant functional group (Hoeksema et al. 2010, Bunn et al. 2015), or evolutionary history (Hoeksema et al. 2018) but it is still not known how plant sensitivity to AM fungi may vary among these groups. An integrated analysis of the relative importance of plant successional status, plant family, plant functional group, and plant nativeness to sensitivity of response is critical to understanding how differences in AM fungal composition may influence plant–plant interactions and diversity in plant communities.

Here, we tested for differences in plant growth response to individual AM fungal taxa using 17 grassland plant species from different successional stages, plant families, functional groups, and native and nonnative statuses. Grassland plants were chosen for this study because many grassland plant species have been shown to vary in their mycorrhizal responsiveness and/or variation in response to different AM fungal taxa in previous experiments (Wilson and Hartnett 1998, Vogelsang et al. 2006, Koziol and Bever 2016). In two greenhouse experiments, we grew single plants inoculated with one of eight different AM fungal taxa that had been isolated from nearby remnant prairies to test the hypotheses that (1) late successional grassland plant species are more responsive to grassland AM fungi than nonnative plants and early successional native plants and that (2) variation in plant growth response to different AM fungal species is greater for late successional plants than in early successional native or nonnative plants. We combined our greenhouse data with 11 other studies in a meta-analysis to evaluate sensitivity of growth response to different AM fungal species in grassland plants of North America. This work improves our understanding of when we would expect AM fungal composition to influence plant-plant interactions and the diversity in plant communities by linking microbial diversity to ecosystem function.

METHODS

Experimental overview

In two greenhouse experiments, we tested for differences in plant growth response to individual fungal isolates using 17 grassland plant species from different successional stages, plant families, and native and nonnative status (see Appendix S1: Table S1 for a list of plants used in each experiment). Individual plants were grown in a factorial design with one of eight single AM fungal isolates, a mixture of AM fungal isolates, or no inocula on greenhouse benches for four months. At harvest, root samples were assessed for percentage AM fungal colonization and total plant biomass was determined for each plant/fungal treatment combination. Total biomass was used to calculate average mycorrhizal responsiveness and plant sensitivity of response to different AM fungal species for each plant species, as detailed in Mycorrhizal responsiveness and plant sensitivity of response (below). Data from our greenhouse experiments were combined with data from 11 other greenhouse studies in a meta-analysis to evaluate mycorrhizal responsiveness and sensitivity of response to different AM fungal species in grassland plants of North America.

Plant material

We chose 17 plant species from four plant families (Amaryllidaceae, Asteraceae, Commelinaceae, and Poaceae) that varied in plant successional stage (three levels: nonnative, early successional native, late successional native) and native plant status (two levels: native and nonnative) to use in our greenhouse experiments (Appendix S1: Table S1). Nonnative plant species were *Allium vineale*, *Bromus inermis*, *Cichorium intybus*, *Lactuca serriola*, *Leucanthemum vulgare*, *Setaria faberii*, *Taraxacum officinale*, and two cultivars of *Festuca arundinacea*. We included two cultivars of *Festuca arundinacea*, with and without a fungal endophyte, because the majority of *F. arundinacea* (tall fescue) contains a vertically transmitted alkaloid producing fungal endophyte (*Neotyphodium coenophialum*; Clay 1990, Belesky and Bacon 2009) that has been shown to inhibit AM fungi in roots and soil (e.g., Mack and Rudgers 2008). Early successional native plant species were *Elymus canadensis*, *Panicum capillare*, *Rudbeckia hirta*, and *Tradescantia ohiensis*. Late successional native plant species were

Allium cernuum, *Andropogon gerardii*, *Echinacea pallida*, *Liatris spicata*, and *Schizachyrium scoparium*. Successional stage for each plant species was determined using the coefficient of conservatism scores obtained from the Universal Floristic Quality Assessment Calculator for the Chicago Region (Swink and Wilhelm 1994, Freyman et al. 2016; calculator *available online*).[7] Seeds were collected locally or obtained from local sources (Spence Nursery, Muncie, Indiana, USA; *Bromus inermis* was obtained from Deer Creek Seed, Windsor, Wisconsin, USA; *F. arundinacea* was provided by K. Clay, Indiana University; local weed seeds were provided by H. Reynolds and L. Koziol, Indiana University). Seeds were cold-moist stratified and germinated in sterilized potting media (Metro-Mix 360, Sun Gro Horticulture, Agawam, Massachusetts, USA) prior to transplanting into treatments.

Fungal material

Arbuscular mycorrhizal fungi were isolated from prairies near the Kankakee Sands Nature Preserve (Morocco, Indiana, USA) and in Illinois and maintained as cultures on sorghum grass at Indiana University (Vogelsang et al. 2006, Koziol and Bever 2016). The fungal isolates used in the greenhouse experiments were *Claroideoglomus lamellosum*, *Claroideoglomus claroideum*, *Racocetra fulgida*, *Entrophospora infrequens*, *Funneliformis mosseae* (Indiana, USA), *Funneliformis mosseae* (Illinois, USA), *Cetraspora pellucida*, and *Acaulospora spinosa* (Appendix S1: Table S2). Inocula consisted of chopped up dried sorghum roots, hyphal fragments, and spores. For the mixed fungal treatments, we mixed equal proportions (by volume) of eight fungal isolates for Experiment 1 and five fungal isolates for Experiment 2 (Appendix S1: Table S2). Mycorrhizal inoculation potential assays showed no difference in inoculation potential among these fungal isolates (Koziol and Bever 2016).

Background soil

The soil for Experiment 1 was collected from an old field (Bloomington, Indiana, USA), mixed 1:2 with Indiana river sand, and sterilized by heating twice for 2 h to 121°C with a 24-h rest period in between. Soil nutrients were as follows: 8 ppm nitrate, 2 ppm ammonium, and 6 ppm available phosphorus (P, Bray-1 equivalent; A and L Great Lakes Laboratories, Fort Wayne, Indiana, USA). The soil for Experiment 2 was collected from an agricultural field in Rantoul, Illinois, USA, mixed 1:1 with Indiana river sand, and sterilized twice for 12 h with a 24-h rest period in between. Soil properties and nutrients include: pH 8.1, 0.8% organic matter, 4 ppm nitrate, 12 ppm ammonium, 8 ppm P (Bray-1 equivalent), 76 ppm potassium, and 205 ppm magnesium (A and L Great Lakes Laboratories, Fort Wayne, Indiana, USA).

[7] http://universalFQA.org

Experiment 1

Experiment 1 had a factorial design of 13 plant treatments × 10 AM fungal treatments. The plant factor consisted of one of 13 plant species/cultivars (Appendix S1: Table S1) and the AM fungal factor consisted of one of eight AM fungal isolates, an eight-isolate fungal mixture, or a non-inoculated control (Appendix S1: Table S2). Each experimental unit consisted of a 1-L pot (Stuewe and Sons, Tangent, Oregon, USA) containing sterile sand/soil mix, 200 cm³ of one AM fungal treatment added at center depth, and an individual plant. The non-mycorrhizal control pots contained an individual plant and sterile sand/soil mix. Each treatment combination consisted of seven replicates for a total of 910 pots in the experiment. Pots were positioned on greenhouse benches in a randomized complete block design and initial measurements (height and leaf number) were recorded at the time of transplanting. Plants were grown in a greenhouse from June to October 2013 (natural lighting, 24°–28°C in daytime, 18°–22°C at night; Indiana University, Bloomington, Indiana, USA). Plants were watered two times per day, for 4 min each, using an automated drip irrigation system (drip rate 2 L/h.). Forty-five days after transplanting, each pot was fertilized with 10 mL of a P-free fertilizer solution (100 mg N/L; Peters Shade Fertilizer, ICL Fertilizers, Dublin, OH, USA; 20-0-20, with micronutrients). Because some plants died over the course of the experiment, numbers of replicates for each treatment ranged from four to seven at the end of Experiment 1 ($N = 858$ surviving plants; Data S1).

Experiment 2

Experiment 2 had a factorial design of 12 plant treatments × 7 AM fungal treatments. The plant factor consisted of one of 12 plant species/cultivars (Appendix S1: Table S1) and the AM fungal factor consisted of one of five AM fungal isolates, a five-isolate fungal mixture, or an uninoculated control (Appendix S1: Table S2). Each experimental unit consisted of a 1-L pot (Stuewe and Sons) containing sterile sand/soil mix, 50 cm³ of an AM fungal treatment added at center depth, and an individual plant. The non-mycorrhizal control pots contained an individual plant and sterile sand/soil mix. There were five replicates of each treatment combination for each fungal isolate and control treatment. However, there were only three to five replicates of each plant/mixed fungal treatment due to the limited availability of the mixed inocula at the time of planting. Thus, we had a total of 405 pots in Experiment 2 (12 plant treatments × 7 AM fungal treatments × 3 to 5 replicates; Data S2). Pots were positioned in a randomized block design on greenhouse benches and maintained as above from May to August 2015. No fertilizer was applied to plants in Experiment 2. Each pot received 5 mL of a microbial filtrate prepared from the fungal inocula mixture (Koide and Li 1989). By including a microbial filtrate in
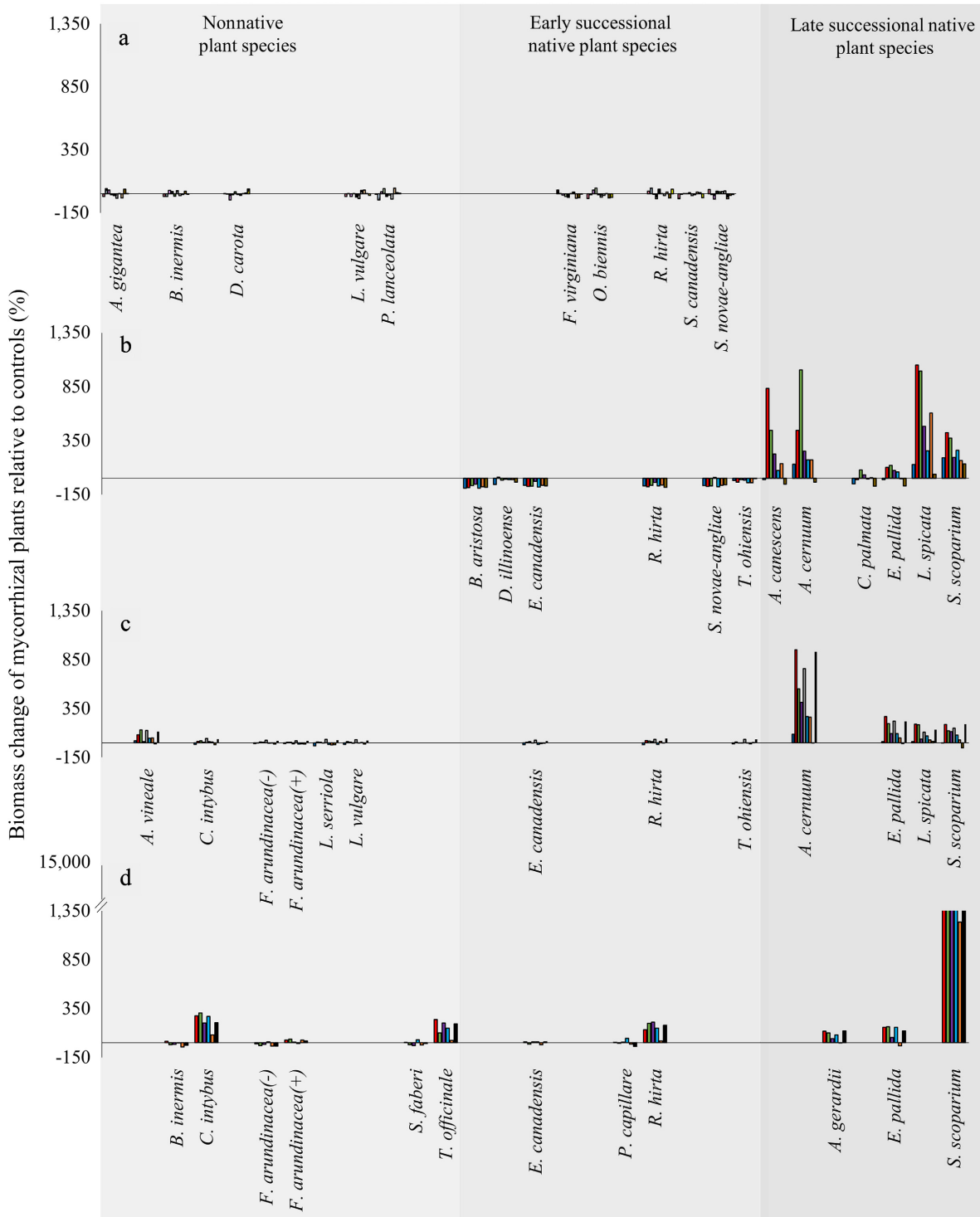
Fig. 1. Across studies, late successional native plants (dark gray panel, right) had a greater change in biomass when grown with arbuscular mycorrhizal (AM) fungi than nonnative plant species (white panel, left) or early successional native plant species (light gray panel, middle) relative to uninoculated controls. Studies shown in this figure include (a) Klironomos (2003; data from Fig. 1); (b) Koziol and Bever (2016; data from Fig. 1); and greenhouse experiments presented herein, (c) Experiment 1 and (d) Experiment 2. Different colored bars in each graph represent the percentage change in biomass of plants inoculated with individual AM fungal isolates or mixtures of AM fungal isolates relative to uninoculated controls. This figure shows that nonnative and early successional native grassland plant species have no or low response to AM fungi while the late successional native grassland plant species across studies are highly responsive to AM fungi and are sensitive to different AM fungal isolates. AM fungal species are (a) *Acaulospora*

(FIG. 1. *Continued*)

denticulata (light red), *Acaulospora morrowiae* (light blue), *Gigaspora margarita* (light purple), *Gigaspora rosea* (light green), *Rhizophagus intraradices* (dark green), *Claroideoglomus etunicatum* (dark gray), *Funnelformis geosporum* (light gray), *Funnelformis mosseae* (Canadian isolate, light orange), *Cetraspora pellucida* (brown), and *Scutellospora calospora* (yellow); (b–d) *Acaulospora spinosa* (blue), *Entrophospora infrequens* (red), *Claroideoglomus lamellosum* (green), *Claroideoglomus claroideum* (purple), *Funnelformis mosseae* (Illinois, USA isolate, gray), *F. mosseae* (Indiana, USA isolate; turquoise), *Racocetra fulgida* (orange), *Cetraspora pellucida* (brown), and AM fungal species mixtures (black). All plant-fungal combinations were co-occurring in their respective studies.

Experiment 2, we account for potential variation contributed by bacteria in our AM fungal cultures and by not including a microbial filtrate in Experiment 1, we offer a conservative estimate of the benefits of AM fungi (Hoeksema et al. 2010).

Harvest, percentage colonization of roots, and plant biomass

At harvest, soil was washed from the roots, subsamples of roots were collected for microscopic assessment of fungal structures, and the remaining roots and shoots were separated and dried at 70°C for 72 h and weighed for biomass. A Trypan Blue solution was used to visualize fungal structures in roots (Phillips and Hayman 1970) and samples were assessed for AM fungal colonization using the slide–intersect method (McGonigle et al. 1990, Data S2, Data S3). Plant biomass was used to calculate average mycorrhizal responsiveness and plant sensitivity to different fungal isolates for each plant/fungal treatment combination, as detailed in Mycorrhizal responsiveness and plant sensitivity of response (below).

Mycorrhizal responsiveness and plant sensitivity of response

Mycorrhizal responsiveness (MR) for each plant/fungal isolate combination was determined using the following equation:

$$MR = \frac{\text{average total plant biomass with fungal inoculation}}{\text{average total plant biomass without fungal inoculation}}. \tag{1}$$

The average and variance of mycorrhizal responsiveness were then calculated for each plant species, across each of the single AM fungal isolates (but not the AM fungal mixture). Because variance scales with the mean (Appendix S1: Fig. S1), plant sensitivity to different fungal isolates was measured by the coefficient of variation (CV), which was determined for each plant species using the following equation:

$$CV = \frac{\text{variance in mycorrhizal responsiveness}}{\text{average mycorrhizal responsiveness}}. \tag{2}$$

Using the coefficient of variation (which includes the variance term) provides a more conservative estimate of

plant sensitivity to different fungal isolates than using variance alone. The variation in response of one plant species to different fungal isolates (i.e., sensitivity) can be partitioned into variation in the average effect of fungi across all plant species (statistically this corresponds to the main effect of fungi) and the differential responses of plant species to different fungi (statistically this corresponds to the plant species × fungal species interaction). This latter effect corresponds to what might best be called specificity of plant response. While the broader literature in ecology often conflates sensitivity with specificity, in our study, we use sensitivity to refer to the variation in plant growth response to individual AM fungal taxa.

Data from our two greenhouse studies were analyzed separately. The MR and CV from these studies were then included in a meta-analysis with 11 other experiments from nine publications (one paper contained three experiments; Klironomos 2003) to examine variation in growth response to different AM fungal species in grassland plants of North America.

Meta-analysis selection criteria and data extracted

For the meta-analysis, we used Web of Science with the search terms [arbuscular mycorrhiza* and specific* and grassland*] and [arbuscular mycorrhiza* and specific* and prairie*] and [arbuscular mycorrhiza* and plant growth response* and prairie*] and the MycoDB database (Chaudhary et al. 2016) to identify papers that examined variation in response to specific plant–AM fungal relationships in grassland plants of North America (see PRISMA flow diagram in Appendix S1: Fig. S2). To be included in the meta-analysis, experiments had to have (1) plants grown with different isolates of AM fungi and uninoculated controls in a full factorial design in a greenhouse; (2) data on shoot or total biomass for plants grown in each treatment; and (3) contain only one plant species per pot. Field experiments, common garden, and community pot studies were excluded because we wanted to examine single plant species × single fungal species combinations only. Total biomass (roots + shoots) was used as a measure of plant growth response to AM fungi in most of the studies but in one study, total biomass was calculated as the sum of leaf dry mass (g), stem dry mass (g), and root fresh mass (g) (Shockley et al. 2004). When data for more than one experiment were presented in the same paper (e.g., Klironomos 2003), the experiments were analyzed separately (i.e., considered independent) in the

meta-analysis (Data S4). When papers contained experiments with different nutrient treatments, only the no-fertilizer treatments (e.g., Reynolds et al. 2006) or data averaged across fertilizer treatments (e.g., Reynolds et al. 2005) were used in the meta-analysis. Our Web of Science search on 30 January 2019 using the search terms above resulted in a total of 239, 90, and 120 papers, respectively (384 papers in total after duplicates were removed; Appendix S1: Fig. S2). However, after systematic screening using our selection criteria, the majority of the papers (307 papers) were eliminated based on information provided in the title and abstract (e.g., studies conducted in the field or outside of North America, studies with trees and/or with ectomycorrhizal fungi, experiments using whole soil or mixed inocula only, studies with multiple plant and/or fungal species per pot, meta-analysis or literature reviews, studies with plants grown under extreme conditions, such as in mine tailings, acidic soil, with pesticide applications, etc.). The remaining full text articles (77 papers) were assessed for eligibility by reviewing the methods and results sections of each paper. We also checked the references of eligible articles to determine whether there were additional publications that would meet our selection criteria. In the end, we identified six papers through our Web of Science searches that met the requirements of our selection criteria (Appendix S1: Fig. S2). Three additional papers were found in the MycoDB database (Chaudhary et al. 2016) that were not identified using our search terms in Web of Science (Appendix S1: Fig. S2). In total, we included 13 different experiments in the meta-analysis (Data S4), including the two greenhouse experiments presented herein, representing grassland plants from three successional stages (nonnative, early successional native, and late successional native), 11 plant families, 37 plant species, co-occurring or not co-occurring plants/fungi (e.g., fungi that would normally be co-occurring with a particular plant in the field vs. fungi obtained from an external source), and different numbers of fungal isolates used in each experiment. For each study, plant biomass data or data on percentage difference in plant growth between inoculated and uninoculated treatments were extracted from figures using webplotdigitizer (version 3.11) or taken directly from the manuscript (digitizer *available online*).[8] Data extracted from each paper are provided in the supplemental information (Data S4, Data S5).

Statistical analysis

While there has been debate on the differences in responsiveness of native and nonnative plant species (e.g., Pringle et al. 2009, Bunn et al. 2015), these comparisons have not accounted for the evidence of very strong differences in mycorrhizal responsiveness between early- and late- successional native plant species (e.g., Koziol and Bever 2015, 2016). We, therefore, test for

[8] http://arohatgi.info/WebPlotDigitizer

differences taking into account successional status of natives, thereby dividing plant species among three categories (nonnatives, early successional native, late successional native). These models allow tests for differences between these three categories. By way of comparison to the previous papers on differences between native and nonnative plant species, we also analyzed a second model that only has two levels (native vs. nonnative). Comparison of AIC values between these two models allows comparison of which of these two model structures best fits the data. Statistical tests were performed using SAS, version 9.4 (SAS Institute, Cary, North Carolina, USA).

Greenhouse experiments

Treatment effects on plant biomass.—Differences in plant growth response to fungal treatments in our greenhouse experiments (Experiment 1 and Experiment 2) were analyzed using a linear mixed model with the Proc Mixed procedure of SAS. To test for overall differences in plant biomass ($\log(x + 1)$ total biomass) among AM fungal treatments, fixed effects in the model were block, plant successional stage (three levels: nonnative, early successional native, late successional native), AM fungal treatment, and successional stage $\times$ fungal treatment and random effects were plant species $\times$ successional stage and plant species $\times$ successional stage $\times$ fungal treatment (Appendix S1: Table S3). Initial leaf number and initial height were $\log(x + 1)$-transformed prior to analysis and were included as covariates in each model to remove the influence of variation in size at the time of planting. A $\log(x + 1)$ transformation was used because some of the biomasses were <1 g, and a log transformation would have made them negative. The consistent use of the $\log(x + 1)$ transformation also reduces the variance of large numbers relative to small numbers and ensures that all numbers are positive before analysis.

For each greenhouse experiment, we also deconstructed treatment effects on plant biomass into a priori, orthogonal contrasts comparing inoculated vs. non-inoculated treatments, differences among fungal species, and single fungal species vs. multispecies fungal communities (Appendix S1: Table S3). Because we included two cultivars of tall fescue in each of our greenhouse experiments, we also tested whether total biomass of endophyte-infected fescue differed from endophyte-free fescue in AM fungal treatments overall or in treatments of individual isolates of AM fungi.

Tests of mycorrhizal responsiveness, variance in mycorrhizal response, and plant sensitivity to different AM fungal isolates.—Mycorrhizal responsiveness for individual plant and fungal combinations in each greenhouse experiment was determined from the back transformed best linear unbiased predictor (BLUP) means, as in Koziol and Bever (2015, 2016) using Eq. 1. The average mycorrhizal responsiveness and variance in mycorrhizal

responsiveness were then calculated for each plant species. Plant sensitivity to different fungal isolates was determined by the coefficient of variation, which was calculated for each plant species as described in Eq. 2. Average mycorrhizal responsiveness, variance in mycorrhizal responsiveness, and coefficient of variation were analyzed separately for each of our greenhouse experiments using linear mixed models first identifying plant successional stage (three levels: nonnative, early successional native, and late successional native) as a fixed effect and with random effects of plant family and successional stage × plant family × plant species interactions (Appendix S1: Table S4a). All pairwise differences between the three levels of plant successional stage were tested using contrasts. As described above, we compared the fit (via AICC score) of the successional status model to a second model in which the fixed effect was plant native status (two levels: nonnative and native) and random effects were plant family and native status × plant family × plant species interactions (Appendix S1: Table S4b). Average mycorrhizal responsiveness, variance in mycorrhizal responsiveness, and coefficient of variation were log(x + 1)-transformed prior to analysis to fit the assumptions of the models.

Meta-analysis

We calculated the average mycorrhizal responsiveness, variance in mycorrhizal response, and the coefficient of variation of responsiveness for each study in the meta-analysis, including our two greenhouse studies, as above. The (1) average mycorrhizal responsiveness, (2) variance in mycorrhizal response, and (3) the coefficient of variation (a measure of plant sensitivity to different AM fungal isolates) were then analyzed using unweighted meta-regressions. We first tested the fixed effects of plant successional stage (three levels: nonnative, early successional native, late successional native), number of fungal isolates, and fungal source (co-occurring or not co-occurring) and random effects of experiment, plant family, successional stage × plant family × plant species interactions and experiment × successional stage × plant family × plant species interactions (Appendix S1: Table S5). All pairwise differences between the three levels of plant successional stage were tested using contrasts. We compared the fit (via Akaike information criterion corrected for sample size, AIC$_c$) of this model to a second model, which was identical except for replacing successional stage with native status (two levels: native, nonnative) of the plant species (Appendix S1: Table S6). Response variables were log(x + 1)-transformed prior to analysis to fit the assumptions of the models.

Correlation of sensitivity of plant response to AM fungi in the greenhouse to that in mesocosms and in the field.—
Finally, we tested whether the sensitivity of plant response to individual AM fungal species in greenhouse studies was correlated with the sensitivity of plant response to AM fungi under more complex environmental conditions. To do this, we identified studies that used the same combinations of plant and AM fungal species and then tested for correlations between the coefficient of variation in greenhouse assays (Koziol and Bever 2016), mesocosms (Koziol and Bever 2019), and in the field (Koziol and Bever 2017). See Appendix S2 for additional details on these analyses.

RESULTS

Greenhouse experiments

Treatment effects on plant biomass.—Soil treatment was a significant predictor of total plant biomass at the end of both greenhouse experiments (Experiment 1, $F_{9,90}$ = 13.45, P < 0.0001; Experiment 2, $F_{6,54}$ = 7.51, P < 0.0001; Appendix S1: Table S3). Late successional native plant species generally grew larger in pots inoculated with AM fungi compared to uninoculated controls while nonnative and early successional native plant species often exhibited no growth benefit or even grew smaller in the inoculated treatments compared to controls (Appendix S1: Figs. S3, S4). In single-species inoculations in Experiment 1, *E. infrequens* and *C. lamellosum* were the most beneficial fungi for late successional plant growth (Fig. 1c; Appendix S1: Fig. S3) while *C. pellucida* and *A. spinosa* were the least beneficial fungal isolates for late successional plant growth (Fig. 1c; Appendix S1: Fig. S3). In single-species inoculations in Experiment 2, *E. infrequens* was the most beneficial to late successional plant growth while *R. fulgida* was the least beneficial for late successional plant growth (Appendix S1: Fig. S4). Biomass data for individual plants in the greenhouse experiments are in Data S1 and Data S2. The back-transformed biomass data used to calculate mycorrhizal responsiveness for each plant/fungal treatment is available in Data S6. The remainder of the results section focuses on mycorrhizal responsiveness, variance in mycorrhizal response, and sensitivity of plant response to different AM fungal isolates across plant successional stage (nonnative, early successional native, late successional native) and native plant status (nonnative, native).

Mycorrhizal responsiveness across plant successional stage and native plant status.—In Experiment 1, we found that plant successional stage was a strong predictor of plant growth response to AM fungi (i.e., mycorrhizal responsiveness; $F_{2,7}$ = 19.16, P = 0.001; Fig. 1c, Appendix S1: Table S4a, Fig. S3). Late successional native plant species had a stronger growth response to AM fungi than early successional native plant species ($F_{1,7}$ = 35.88, P < 0.001; Fig. 1c, Appendix S1: Table S4a; Fig. S3) and nonnative plant species ($F_{1,7}$ = 17.60, P < 0.01; Fig. 1c; Appendix S1: Table S4a; Fig. S3). Early successional native plant species and nonnative plants had a low growth response to AM fungi (Fig. 1c, Appendix S1: Fig. S3) and there was no difference in mycorrhizal

responsiveness between them ($F_{1,7} = 0.11$, $P = 0.75$; Appendix S1: Table S4a; Fig. S3). Plant family was not a significant predictor of mycorrhizal responsiveness ($P = 0.15$; Appendix S1: Table S4a). When analyzed by native plant status in Experiment 1, mycorrhizal responsiveness was higher in native plant species than in nonnative plant species ($F_{1,8} = 7.41$, $P = 0.03$; Appendix S1: Table S4b). Comparing the AIC values of the successional status model ($AIC_c = 3.0$) vs. the native plant status model ($AIC_c = 11.1$), we found that successional status is a much better fit for the mycorrhizal responsiveness data in Experiment 1 ($\Delta AIC_c = 8.1$).

In Experiment 2, trends in plant growth among plant successional stage were in the same direction as Experiment 1 (Fig. 1d, Appendix S1: Figs. S4, S5), but differences in mycorrhizal responsiveness were not statistically significant ($F_{2,8} = 1.96$, $P = 0.20$; Fig. 1d, Appendix S1: Fig. S4a), likely due to the substantial variance of the plant species × fungal species interaction (Appendix S1: Table S3, Table S4). When analyzed by native plant status in Experiment 2, there were no differences in mycorrhizal responsiveness between native and nonnative plant species ($F_{1,9} = 1.10$, $P = 0.32$; Appendix S1: Table S4b). Comparing the AIC values of the successional status model ($AIC_c = 35.2$) vs. the native plant status model ($AIC_c = 38.8$), we found that successional status is also a better fit for the mycorrhizal responsiveness data in Experiment 2 ($\Delta AIC_c = 3.6$).

Variance in mycorrhizal responsiveness across plant successional stage and native plant status.—In Experiment 1, plant successional stage was a strong predictor of variance in mycorrhizal responsiveness ($F_{2,7} = 6.25$, $P = 0.03$; Appendix S1: Table S4a). Late successional native plant species had higher variance in their response to AM fungi than early successional native ($F_{1,7} = 11.44$, $P = 0.01$; Appendix S1: Table S4a) and nonnative plant species ($F_{1,7} = 6.42$, $P = 0.04$; Appendix S1: Table S4a). Variance in mycorrhizal responsiveness did not differ between early successional native plant species and nonnative plant species ($F_{1,7} = 0.02$, $P = 0.90$; Appendix S1: Table S4a). When analyzed by native plant status, variance in mycorrhizal responsiveness did not differ between native and nonnative plant species in Experiment 1 ($F_{1,8} = 4.35$, $P = 0.07$; Appendix S1: Table S4b). By comparing the AIC values of the successional status model ($AIC_c = 24.2$) vs. the native plant status model ($AIC_c = 29.1$), we found that successional status is a much better fit for the variance data in Experiment 1 ($\Delta AIC_c = 4.9$).

In Experiment 2, variance in mycorrhizal responsiveness did not differ among plant successional stage ($F_{2,8} = 1.91$, $P = 0.21$; Appendix S1: Table S4a) or between native and nonnative plant species ($F_{1,9} = 1.06$, $P = 0.33$; Appendix S1: Table S4b). By comparing the AIC values of the successional status model ($AIC_c = 48.3$) vs. the native plant status model ($AIC_c = 53.3$), we found that successional status is also a better fit for the variance data in Experiment 2 ($\Delta AIC_c = 5.0$).

Plant sensitivity to different AM fungal isolates across plant successional stage and native plant status.—In Experiment 1, late successional native plant species had higher sensitivity of response to individual fungal isolates than nonnative or early successional native plant species, as determined by the coefficient of variation ($F_{2,7} = 9.35$, $P = 0.01$; Appendix S1: Table S4a). Sensitivity of response did not differ by plant family ($P = 0.19$; Appendix S1: Table S4a). When analyzed by native plant status, native plant species had a higher level of sensitivity to different fungal isolates than nonnative plant species ($F_{1,8} = 5.26$, $P = 0.05$; Appendix S1: Table S4b). By comparing the AIC values of the successional status model ($AICC = 6.6$) vs. the native plant status model ($AIC_c = 11.6$), we found that successional status is a much better fit for the plant sensitivity data in Experiment 1 ($\Delta AIC_c = 5$).

In Experiment 2, there were no differences in plant sensitivity to different AM fungal isolates among plant successional stage ($F_{2,8} = 2.06$, $P = 0.19$; Appendix S1: Table S4a) or between native and nonnative plant species ($F_{1,9} = 1.13$, $P = 0.32$; Appendix S1: Table S4b). By comparing the AIC values of the successional status model ($AIC_c = 32.8$) vs. the native plant status model ($AIC_c = 36.3$), we found that successional status is also a better fit for the plant sensitivity data in Experiment 2 ($\Delta AIC_c = 3.5$).

Endophyte infected vs. non-infected Festuca arundinacea.—Because previous research showed that endophyte-infected tall fescue had a negative impact on AM fungi in roots and soil, we also tested the mycorrhizal responsiveness and sensitivity of response of an endophyte-free cultivar and an endophyte-infected cultivar of *F. arundinacea*. In Experiment 1, there was no difference in the average growth response to AM fungi ($F_{1,696} = 0.05$, $P = 0.80$; Appendix S1: Fig. S3) or in sensitivity of response to individual fungal isolates ($F_{8,696} = 0.66$, $P = 0.70$) of *F. arundinacea* with and without a fungal endophyte. However, endophyte-infected *F. arundinacea* had on average a more positive growth response to AM fungi than *F. arundinacea* without an endophyte in Experiment 2 ($F_{1,303} = 4.28$, $P = 0.04$; Appendix S1: Fig. S4) and the sensitivity of response to individual species of AM fungi varied between *F. arundinacea* with and without the endophyte ($F_{5,303} = 2.89$, $P = 0.01$).

Meta-analysis

Average mycorrhizal responsiveness across plant successional stage and native plant status.—In the meta-analysis, we found that plant successional stage was a very strong predictor of mycorrhizal responsiveness in grassland plants of North America ($F_{2,22} = 21.88$, $P < 0.0001$; Fig. 2a, Appendix S1: Table S5, Fig. S6). Mycorrhizal responsiveness was significantly higher in late successional native plant species compared to nonnative and

early successional native grassland plant species (Fig. 2a; Appendix S1: Fig. S6). Inoculation with AM fungi often inhibited the growth of nonnative and early successional native plant species (Appendix S1: Fig. S6). There was no difference in mycorrhizal responsiveness between nonnative and early successional native plant species ($F_{1,22} = 0.20$, $P = 0.66$; Appendix S1: Table S5). When analyzed by plant native status, the meta-analysis showed no difference in mycorrhizal responsiveness between nonnative and native plant species overall ($F_{1,23} = 1.57$, $P = 0.22$; Appendix S1: Table S6). There were also no differences in mycorrhizal response among plants inoculated with local (co-occurring) vs. non-local (not co-occurring) AM fungi ($F_{1,2} = 0.06$, $P = 0.83$; Appendix S1: Table S5). By comparing the AIC values of the successional status model ($AIC_c = 107.6$) vs. the native plant status model ($AIC_c = 134.1$), we found that successional status is a much better fit for the mycorrhizal responsiveness data in the meta-analysis ($\Delta AIC_c = 26.5$).

Variance in mycorrhizal responsiveness across plant successional stage and plant native status.—The meta-analysis showed that variance in mycorrhizal responsiveness differed across plant successional stage ($F_{2,22} = 16.62$, $P < 0.0001$; Appendix S1: Table S5) and was much higher in late successional plant species than in early successional native plant species ($F_{1,22} = 31.24$, $P < 0.0001$; Appendix S1: Fig. S1, Table S5) and nonnative plant species ($F_{1,22} = 26.48$, $P < 0.0001$; Appendix S1: Fig. S1, Table S5). Variance in mycorrhizal response did not differ between nonnative and early successional native plants ($F_{1,22} = 0.16$, $P = 0.70$; Appendix S1: Table S5) or by the source of the AM fungal inocula (co-occurring vs. not co-occurring; $F_{1,2} = 0.01$, $P = 0.94$; Appendix S1: Table S5). When analyzed by plant native status, native plant species had higher variance in mycorrhizal responsiveness than nonnative plant species ($F_{1,23} = 4.31$, $P = 0.05$; Appendix S1: Table S6). By comparing the AIC values of the successional status model ($AIC_c = 215.2$) vs. the native plant status model ($AIC_c = 237.8$), we found that successional status was a much better fit for the variance data in the meta-analysis ($\Delta AIC_c = 22.6$).

Plant-fungal sensitivity across plant successional stage and plant native status.—The meta-analysis showed that the coefficient of variation, a measure of plant sensitivity to different AM fungal isolates, differed significantly with plant successional stage ($F_{2,22} = 15.90$, $P < 0.0001$; Fig. 2b; Appendix S1: Table S5). The coefficient of variation in mycorrhizal responsiveness was significantly higher in late successional native plant species compared to early successional native and nonnative plant species ($F_{1,22} = 30.71$, $P < 0.0001$ and $F_{1,22} = 23.58$, $P < 0.0001$, respectively; Appendix S1: Table S5) and did not differ between nonnative and early successional native plants ($F_{1,22} = 0.57$, $P = 0.46$; Appendix S1: Table S5). The coefficient of variation in mycorrhizal responsiveness was strongly correlated with mycorrhizal responsiveness ($R^2 = 0.66$, $P < 0.0001$; Fig. 3), but was not affected by the number of fungal isolates used in each study ($F_{1,2} = 2.28$, $P = 0.27$; Appendix S1: Table S5), by plant family ($P = 0.47$, Appendix S1: Table S5), or by the source (co-occurring vs. not co-occurring) of the AM fungal inocula ($F_{1,2} = 0.14$, $P = 0.75$; Appendix S1: Table S5). When analyzed by plant native status, there was higher sensitivity to individual fungal isolates in native relative to nonnative plant species ($F_{1,23} = 5.43$, $P = 0.03$; Appendix S1: Table S6). By comparing the AIC values of the successional status model ($AIC_c = 95.2$) vs. the native plant status model ($AIC_c = 111.8$), we found that successional status was a much better fit for the plant-fungal sensitivity data in the meta-analysis ($\Delta AIC_c = 16.6$).
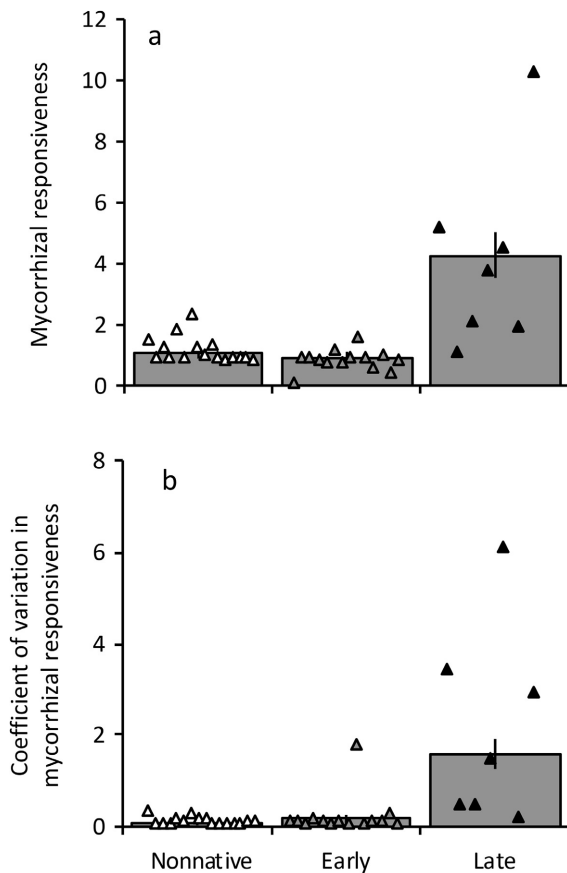

Fig. 2. Plant successional stage was a strong predictor of (a) plant growth response to arbuscular mycorrhizal fungi (i.e., mycorrhizal responsiveness) and (b) coefficient of variation in responsiveness among different fungal isolates. Bars and error bars represent plant successional stage means ± SE; symbols represent individual nonnative plant species (white symbols), early successional native prairie plant species (gray symbols), and late successional native prairie plant species (black symbols).
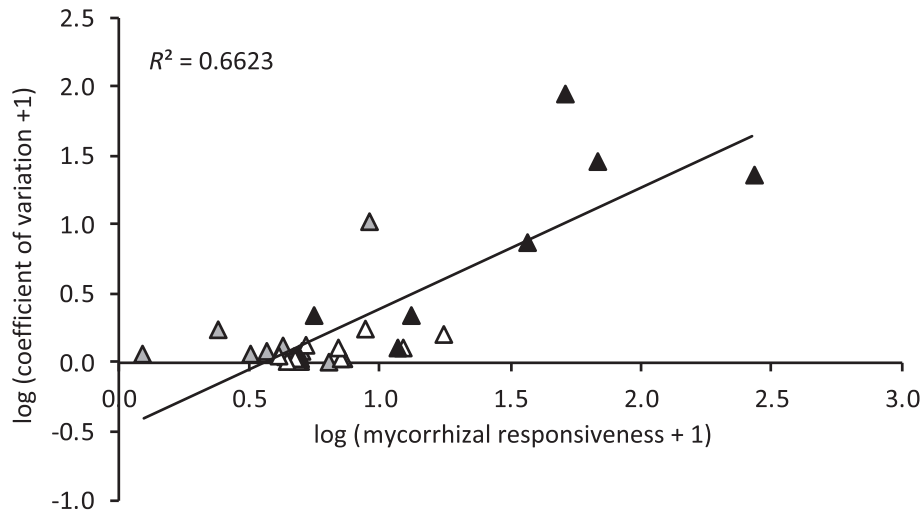
FIG. 3. Late successional native prairie plant species (black symbols) had higher mycorrhizal responsiveness and higher coefficient of variation in mycorrhizal responsiveness than early successional native plant species (gray symbols) and nonnative plant species (white symbols). Coefficient in variation of mycorrhizal responsiveness, a measure of plant sensitivity to different arbuscular mycorrhizal (AM) fungal isolates, was highly correlated with mycorrhizal responsiveness (R^2 = 0.66, P < 0.0001).

Correlations of coefficient of variation observed in the greenhouse with a mesocosm study and a prairie restoration in the field.—Finally, we examined whether plant sensitivity to different fungal isolates in greenhouse experiments is correlated with sensitivity to different fungal isolates under more complex experimental conditions (e.g., mesocosm, field). To do this, we identified studies that included the same plant/fungal species combinations in a greenhouse, mesocosm, and field experiment. We found that the coefficient of variation for a plants response to individual fungal taxa in the greenhouse was significantly correlated with the coefficient of variation calculated from the same plant treatment combinations in a mesocosm community (r = 0.74, df = 7, P = 0.02) and in a field experiment (r = 0.89, df = 3, P = 0.05; Appendix S2: Fig. S1).

DISCUSSION

With increasing levels of disturbance in terrestrial ecosystems (e.g., climate change, invasive species, and agricultural management practices), understanding how plant–AM fungal interactions shape above and below-ground communities is critically important. Through two large greenhouse studies and a meta-analysis of all relevant studies, we show that late successional native grassland plant species have more positive growth responses to AM fungi, exhibit higher variance in response to individual AM fungal taxa and are more sensitive to different AM fungal isolates than early successional native or nonnative plant species. Our results are consistent with results of Koziol and Bever (2015, 2016) in identifying the high responsiveness and sensitivity of late successional grassland plant species. As the impact of variation in AM fungal composition on plant

community dynamics depends on the strength and sensitivity of plant response to individual AM fungal species, our work identifies that AM fungal composition is particularly important to the success of late successional grassland plant species. While changes in AM fungal composition have been shown to generate significant feedbacks on plant composition (Bever 2002, Castelli and Casper 2003, Mangan et al. 2010), our work suggests that such feedbacks could be particularly important to late successional grassland plants. In support of this prediction, a recent study found that mycorrhizal composition mediated the positive frequency dependence observed in late successional grassland species (Koziol and Bever 2019).

The sensitivity of late successional plants to variation in fungal community composition could be especially important for plant community dynamics in landscapes where mycorrhizal community composition has been disrupted, such as by land management practices or invasive species (Hamel et al. 1994, Oehl et al. 2010, Schnoor et al. 2010, House and Bever 2018). Agricultural management processes such as tilling (Abbott and Robson 1991, Jasper et al. 1991) can lead to reduced AM fungal abundance and diversity as well as selecting for less mutualistic AM fungi (Johnson 1993). Our work suggests that these changes in mycorrhizal communities that occur with soil disturbance may disadvantage late successional species relative to early successional or nonnative plant species, which are less sensitive to variation in AM fungal composition. We found that many exotic plant species in the United States, including common forage crops such as *Bromus inermis* (smooth brome), exhibited little growth response or sensitivity of response to different AM fungi. This lack of responsiveness to mycorrhizal fungi in nonnative and early successional

plants may enable a competitive advantage against late successional grassland plant species, especially in disturbed or invaded areas where mycorrhizal communities have been disrupted. We found that plant sensitivity to mycorrhizal taxa in the greenhouse was strongly correlated with plant sensitivity to mycorrhizal species identified in more complex environments including a mesocosm and a field restoration experiment. These data suggest that targeted inoculations with AM fungal species that are known to improve the growth of a mycorrhizally sensitive plant species may be important in ecological restorations. Consistent with this expectation, inoculations with native AM fungal isolates in grassland restorations have been found to differentially improve the success of late successional species relative to early successional and nonnative plant species (Middleton et al. 2015, Koziol and Bever 2017).

Predictors of plant sensitivity to different AM fungal isolates

We found that the grassland plant species with the highest growth responses to AM fungi exhibited higher levels of sensitivity to the identity of AM fungal taxa, consistent with observations of Koziol and Bever (2016) and Reynolds et al. (2006). In our meta-analysis, sensitivity of plant response to AM fungal species was consistently correlated with the average mycorrhizal response of that plant species. Our results are in contrast with previous studies that found no correlation between mycorrhizal responsiveness and sensitivity to different fungal isolates (e.g., Klironomos 2003). Variation in results may be due to the choice of plant species included in each experiment. For instance, studies that included only early successional and/or nonnative plant species may not have found correlations in mycorrhizal responsiveness and sensitivity in response because these weedy plants showed little variation in response to AM fungi overall (Fig. 1). Because our study included grassland plant species from across successional stages, we captured greater variation in responsiveness and thereby generated a more powerful test of the relationship between sensitivity in response to AM fungal species and overall responsiveness to AM fungi.

In contrast to Klironomos (2003), we found that the origin of the AM fungal isolates (e.g., fungi that would normally co-occur with a particular plant in the field vs. fungi obtained from an external source) did not affect plant growth, as neither the responsiveness or the sensitivity of plant species to AM fungi depended on the match of origin of plants and fungi. Rather, in a meta-analysis that combines Klironomos' results with additional studies, we find that nonnative plant species are similar in their interactions with AM fungi as early successional native plant species, both of which are less sensitive to AM fungal identity than late successional native plant species. It should be noted, however, that no greenhouse studies have been conducted that include late

successional plants and both local (co-occurring) and non-local (not co-occurring) fungi and that, based on our data, these plants are most likely to exhibit sensitivity to mycorrhizal fungi. In this context, it is relevant to note that late successional grassland species have been found to be particularly responsive to AM fungal species isolated from late successional grassland environments (Koziol et al. 2018).

Effect of AM fungal mixtures vs. isolates on plant response

In our two large-scale greenhouse studies, we found that multispecies AM fungal inoculations elicited growth benefits similar to the most beneficial fungus in a mixture (Fig. 1 c, d; Appendix S1: Figs. S3, S4). This result is consistent with the mesocosm results of Vogelsang et al. (2006), which suggested that the sampling effect was a primary mechanism through which AM fungal diversity improved plant productivity and diversity. Together these results suggest that mixtures of AM fungal species may be superior for inoculation applications. We also found general consistency in which AM fungal species were most beneficial to late successional grassland plant species. In Experiment 1, *E. infrequens* and *C. lamellosum* were the most beneficial fungi for late successional plant growth while *C. pellucida* and *A. spinosa* were the least beneficial (Appendix S1: Fig. S3). *E. infrequens* was the most beneficial to late successional plant growth in Experiment 2 while *R. fulgida* was the least beneficial (Appendix S1: Fig. S4). These results are in congruence with those from Koziol and Bever (2016) where *E. infrequens* and *C. lamellosum* improved growth of late successional prairie plant species and suggest specific AM fungal species that should be included in AM fungal mixtures that aim to accelerate succession during prairie restoration.

Mycorrhizal responsiveness of endophyte-infected and non-endophyte infected tall fescue

The mycorrhizal growth response between endophyte-infected and non-endophyte infected tall fescue differed between our two greenhouse studies. We included both fescue cultivars in our experiments because the majority of tall fescue contains an alkaloid producing fungal endophyte (Clay 1990, Belesky and Bacon 2009) that has been shown to inhibit AM fungi in roots and soil (e.g., Chu-Chou et al. 1992, Guo et al. 1992, Müller 2003, Mack and Rudgers 2008). This potential for inhibition of AM fungi in the field may present a challenge in the ecological restoration of tall fescue-dominated landscapes for plant species that are highly dependent on AM fungi for survival and growth. In our first greenhouse experiment, we found no response of endophyte infected and endophyte-free cultivars of fescue in the mycorrhizal treatments. However, in our second greenhouse experiment, the endophyte-infected fescue had an

overall more positive response to AM fungi than the non-endophyte infected fescue and a different pattern of responsiveness to individual AM fungal species. The endophyte-infected fescue had slightly lower levels of AM fungal colonization than the non-endophyte-infected fescue, with an average of 23% and 28% root colonization, respectively. This supports the findings of Mack and Rudgers (2008), who showed that mycorrhizal colonization levels were lower in endophyte-infected tall fescue relative to the non-entophyte infected cultivar; however, in their study, there was no benefit of AM fungal inoculation on tall fescue growth or biomass. As we used the same endophyte-infected cultivar as Mack and Rudgers (2008), differences in results between the different studies may be due to the variation in AM fungal inocula used, background soil, and/or experimental conditions.

Comparison of results between our two greenhouse experiments

Although the experimental design between our two greenhouse studies was similar, Experiment 1 showed stronger differences between successional categories than Experiment 2. This difference may be due to the number and identity of plant species used (Appendix S1: Table S1), the number and identity of AM fungal isolates used (Appendix S1: Table S2), the amount of inocula added to each pot (200 cm^3 in Experiment 1 and 50 cm^3 in Experiment 2), the source of the background soil, the addition of a microbial filtrate, and the addition of fertilizer. While we did not have the power to test these factors individually in our meta-analysis, the most powerful meta-analysis of plant response to AM fungi to date did not find consistent effects of microbial filtrate or fertilizer (Hoeksema et al. 2018). With the greater power enabled by our meta-analyses, we were able to confirm that the significance patterns observed in Experiment 1 are consistent across published studies in grassland plants of North America.

Meta-analyses of mycorrhizal responsiveness

Many recent meta-analyses have suggested that plant native status, plant family and/or plant functional group are important in predicting plant species response to AM fungi (e.g., Hoeksema et al. 2010, 2018, Reinhart et al. 2012, Bunn et al. 2015). While our analysis of mycorrhizal response does not have many plant species compared to these studies (because we only included greenhouse experiments with grassland plant species and AM fungal combinations in a full factorial design), our results are relevant to the interpretation of these studies in two fundamental ways. First, by showing that the responsiveness of late-successional grassland plants depends highly on the identity of AM fungi, our work highlights that taxon sampling of the AM fungi in studies included within meta-analyses may constrain

confidence in their conclusions. Even in the most comprehensive meta-analysis of mycorrhizal response to date (Hoeksema et al. 2018), AM fungal taxa are not consistently tested across all plant families (Chaudhary et al. 2016), which could generate spurious patterns of mycorrhizal responsiveness. Second, consistently across all of our analyses, we find that models that separate out early from late successional native plant species provide superior fits to data compared to models that lump these categories of plant species. By identifying that successional status of native plant species is a very strong predictor of mycorrhizal responsiveness, our results identify that taxon sampling of plant species could constrain confidence in results from meta-analyses. Specifically, we show that whether native plant species differ in responsiveness from nonnative plant species, a focus of recent analyses (Bunn et al. 2015), will depend upon which native plant species are included. We show that nonnative plant species have similar responsiveness as early successional plant species, both of which are less responsive to AM fungi than late successional grassland plant species. Further, because we included a relatively balanced sampling of life histories within plant families, our study offers a fairer test of the relative influence of plant successional stage and plant family, and we found that the effect of plant family was not significant. Future studies should include a greater number of plant families with balanced sampling across plant successional stage to determine the strongest predictors of mycorrhizal responsiveness to different AM fungal taxa.

Conclusions

We show through greenhouse studies and meta-analyses that late successional grassland plant species not only have strong positive growth responses to AM fungi, but are also more sensitive to variation in AM fungal community composition than early successional or nonnative plants, as evidenced by their strong differences in growth response to individual AM fungal taxa. The sensitivity of late successional plants to variation in fungal community composition could have important consequences for plant species coexistence, especially in disturbed ecosystems where legacies of management practices have had a negative impact on soil communities. Our study highlights the fact that not all AM fungal species confer the same benefits to plants (Fig. 1, Appendix S1: Figs. S3, S4). Thus, targeted inoculations with AM fungal species that are known to improve the growth of a particular plant species may be important in ecological restorations and agroecosystems. While our work suggests that AM fungal composition may be more important to the dynamics of late successional plant communities compared to early successional or invasive plant communities, this inference is based on relatively few studies. More full factorial tests that include multiple plant species and multiple mycorrhizal fungal species are required to assess whether these patterns hold for other ecosystems.

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Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.2855/suppinfo

Data Availability

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.t8q43fv