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Mycorrhizal colonization and its relationship with plant performance differs between exotic and native grassland plant species

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Abstract Many grasslands have been transformed by exotic species with potentially novel ecological interactions. We hypothesized that exotic and native plant species differ, on average, in their percentage mycorrhizal colonization, and that mycorrhizal colonization is positively related to plant performance in the field. We compared colonization by arbuscular mycorrhizae (AM) fungi in perennial native and exotic species that were paired phylogenetically and by functional groups and grown under a common environment in field plots in Central Texas, USA. Roots were collected from plants in monoculture plots, stained, and percent colonization was assessed with a microscope. Aboveground biomass and dominance in mixture were used as measures of plant performance.

Exotic species had significantly higher colonization of AM than native species, and this result was consistent across functional groups. Percent colonization was positively correlated with biomass and dominance in mixture across native species, but not across exotic species. Our results indicate that mycorrhizal dependence is a more important predictor of competitive balance among native than exotic plant species in the subhumid grasslands of the USA.

Keywords Arbuscular mycorrhizae · Invasive species · Invasion success · Grassland · Novel ecosystem · Aboveground biomass

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Introduction

Many grasslands have been transformed by exotic species with potentially novel ecological interactions (Hobbs et al. 2006; Seastedt et al. 2008; Wilsey et al. 2009; Vogelsang and Bever 2009). Exotic-dominated grasslands in the tallgrass prairie region of North America have lower plant diversity, higher niche overlap and altered functional group composition compared to native grasslands (Wilsey et al. 2009, 2011; Daneshgar et al. 2013; Martin et al. 2014). Analyses of average differences between native and exotic plant species find consistent differences in leaf and whole plant traits (Liao et al. 2008; Rout and

Callaway 2009; Daneshgar et al. 2013). Exotic grasses in the southern Plains can have higher aboveground productivity and N uptake, but lower deep root biomass and root–shoot ratio than native grass species (Wilsey and Polley 2006). These trait differences could lead to differences in nutrient cycling and plant–microbe interactions between sites dominated by native versus exotic species. Nutrient mineralization, litter decomposition, and resource uptake are all affected by plant–microbe interactions. Plant–microbe associations have been found to differ between some native and exotic plant species pairs (Kourtev et al. 2002; Yannarell et al. 2011; Arthur et al. 2012; Lekberg et al. 2013).

Exotic species abundances and their impacts can be associated with altered pathogen to mutualist ratios (Klironomos 2002; van der Putten et al. 2013; Anacker et al. 2014; Larios and Suding 2015). Both pathogen and mutualist groups include bacteria and fungi. Among the latter, arbuscular mycorrhizal (AM) fungi form potentially mutualistic relationships with most plants, and therefore play an important role in grassland ecosystem functioning and carbon cycling (Wilson and Hartnett 1998; Miller et al. 2012). AM fungi of the *Glomeromycotina* clade (Mucoromycota, Spatafora et al. 2016), deliver nutrients for plants, and provide protection from pathogens and environmental stressors in exchange for photosynthetic carbon substrates (Smith and Read 2008). However, the strength and direction of the relationship can vary from strongly mutually beneficial, to neutral, to parasitic in some cases due to plant–fungal species–specific interactions (Klironomos 2003). This leads to variable predictions on how native and exotic species might differ in their mycorrhizal colonization rates. On average, native plant species were found to have a more positive growth enhancement to mycorrhizal colonization than exotic species (Pringle et al. 2009), although this may be explained by exotic species being more likely to be in the *Asteraceae* and *Poaceae* families (Anacker et al. 2014; Reinhart et al. 2017).

If AM colonization is important to plant growth and competitive ability (Marler et al. 1999; Bray et al. 2003), then it may lead to greater dominance in mixtures, especially under low water availability (Hartnett and Wilson 1999). Previous studies have shown mixed results on whether AM fungal abundance is correlated with performance measures during plant invasions (Inderjit and van der Putten 2010;

Anacker et al. 2014). Studies have been few and have focused largely on comparisons of single native–exotic species pairs (e.g., Lekberg et al. 2013; Duell et al. 2016). Relationships between mycorrhizal colonization and plant biomass have seldom been tested in the field under natural soil conditions (Shah et al. 2009) as we do here. A recent meta-analysis by Bunn et al. (2015) noted 'that all reviewed studies that assessed plant growth responses to AM fungi were done in greenhouses' (but see Marler et al. 1999; Callaway et al. 2003). Therefore, further progress in this area requires field studies comparing multiple exotic and native species.

Exotic species that achieve high biomass, spread widely, and achieve high dominance in their new environment (i.e., that are invasive) could have differences in mycorrhizal colonization compared to non-invasive exotic species (Bunn et al. 2015; Richardson et al. 2000). Lower water availability under irrigation should enhance differences (Hartnett and Wilson 1999). Alternatively, exotic species biomass could be unrelated or even negatively related to mycorrhizal colonization if there is a cost involved. Exotic species are sometimes non-mycorrhizal or facultatively mycorrhizal (Pendleton and Smith 1983), or may not be affected by mycorrhizae in the invaded environment due to the generalist nature of common mycorrhizal species (Richardson et al. 2000). We tested these alternate predictions using a long-term field experiment in Central Texas, the Maintenance of Exotic versus Native Diversity (MEND) experiment. Our objective was to compare mycorrhizal colonization under field conditions in a common garden experiment in order to exclude factors that sometimes confound comparisons involving invaded and non-invaded field sites. We compared multiple exotic and native plant species under similar environmental conditions, using species that were paired by phylogenetic and functional group (Table 1, online materials). In so doing, we minimized edaphic variation known to influence the microbial community, e.g., soil pH, temperature and moisture. Dominance in mixture and average aboveground biomass in monoculture, which were found to be strong predictors of species diversity decline in this system (Wilsey et al. 2009, 2011), were used as measures of invader performance. We hypothesized that (1) exotic and native plant species differ, on average, in their percent mycorrhizal colonization, and (2) that colonization by

Table 1 Origin of exotic species and native species pair for species used in mycorrhizal analyses

	Shared taxon	Origin	Planting	Aboveground biomass g/m^2
C$_4$ grasses				Mean 2008–2015
Bothriochloa ischaemum	And.	Asia	2008*	276.9
Schizachyrium scoparium	And.	Native	2008*	193.7
Cynodon dactylon	Cyn.	Africa	2008*	231.4
Buchloe dactyloides	Cyn.	Native	2008*	179.1
Eragrostis curvula	Era.	Africa	2008*	331.1
Sporobolus asper	Era	Native	2008*	396.6
Panicum coloratum	Pa.	Africa	2008*	573.1
Panicum virgatum	Pa.	Native	2008*	200.2
Paspalum dilatatum[a]	Po.	S. America	2008	182.1
Eriochloa sericea	Po.	Native	2008	313.9
Sorghum halapense	And.	Mediter.	2008*	481.3
Sorghastrum nutans	And.	Native	2008*	299.1
C$_3$ grasses				
Dactylus glomerata	And.	Europe	2014*	26.2
Nasella luecotricha	And.	Native	2008*	285.2
Festuca arundinacea	Po.	Europe	2014	39
Elymus canadensis[a]	Po.	Native	2008	211.3
Forbs				
Leucanthemum vulgare	Ast.	Eurasia	2008*	278.6
Ratibida columnifera	Ast.	Native	2008*	373.4
Taraxacum officinale	Ast.	Europe	2014*	2
Marshallia caespitosa	Ast.	Native	2014*	28.5
Cichorium intybus	Ast.	Eurasia	2014*	170.9
Vernonia baldwinii	Ast.	Native	2008*	289.9
Nepata cataria	Lam.	Eurasia	2014*	14.8
Salvia azurea	Lam.	Native	2008*	223.5
Ruellia brittoniana	Aca.	Mexico	2008	500.9
Ruellia humilis[a]	Aca.	Native	2008	3.7
Marrubium vulgare	Lam.	Eurasia	2008	124.2
Monarda fistulosa[a]	Lam.	Native	2008	19.8
Leguminous forbs				
Lotus corniculatus	Fab.	Eurasia	2014	106
Dalea purpurea[a]	Fab.	Native	2008	52
Trifolium repens	Fab.	Europe	2014	6.4
Dalea candidum[a]	Fab.	Native	2008	64.3
Medicago sativa	Pap.	Asia	2008*	147.1
Desmanthus illinoensis	Pap.	Native	2014*	64.8
Coronilla varia	Fab.	Mediter.	2008	196.4
Astragalus canadensis[a]	Fab.	Native	2008	17.2

Mean aboveground biomass is presented for monoculture plots across years. Each mean represents the average aboveground biomass across block and irrigation treatments. Bolded species strongly dominated at least one plot (Wilsey et al. 2011, 2014), or were 'invasive' in this environment

*Denoted species used in paired comparison (Table 2)

[a]Denoted species not used in regressions (Fig. 3)

AM fungi would correlate positively with species dominance in mixture and aboveground biomass for both native and exotic species.

Materials and methods

Study site

Plots were sampled from a long-term field experiment, Maintenance of Exotic versus Native Diversity (MEND), at the USDA-ARS Grassland, Soil, and Water Research Laboratory near Temple, TX. The site is located in the southern portion of the tallgrass prairie region, and receives 864 mm of precipitation per year in a bimodal rainfall pattern, with very hot temperatures in summer. The soils are Vertisol usterts with relatively high fertility, high carbonate concentrations (Wilsey et al. unpublished data), and joint P and N limitation (Fay et al. 2015). Mycorrhizal colonization rates were found to be higher at this southern Plains site (> 50% on average) than in Kansas and Minnesota (< 40% on average) in a previous study of the grass little bluestem (*Schizachyrium scoparium*) (Frater et al. 2018), perhaps due to summer droughts. Fields in the region are a patchwork of rangeland dominated by exotic grasses with scattered remnants of native prairie (Wilsey et al. 2011).

Experimental design

The MEND experiment was described in full by Wilsey et al. (2011, 2014) and is designed to test whether there are ecological differences between native and exotic species under ambient and altered rainfall. Briefly, equal-sized seedlings of perennial species were transplanted into 1 m × 1 m field plots (72 individuals/m^2) in 9-species mixture or monoculture in two blocks, one planted in October 2007 and one in March 2008. Irrigation was applied by hand during the typically dry period of mid-July to mid-August each year at a rate of 128 mm per month to half the plots, and half remained as controls. Irrigation was applied as eight events per year of 16 mm each beginning in 2008. Volunteer plants were removed by hand, and no fertilizer was added.

Aboveground biomass by species was estimated in all plots with point intercept methods. During June and October of each year, pins were dropped in a 5 × 5 grid in the 80 cm × 80 cm center of each plot. Regression equations, which were linear and had an average r^2 of 0.89 across species (see Wilsey et al. 2011; Xu et al. 2017 for equations and results), were used to convert hits to biomass (Wilsey et al. 2011; Xu et al. 2017).

Monocultures

To test our hypothesis that exotic and native plant species differ, on average, in their percent mycorrhizal colonization, we sampled monoculture plots. Plant species were planted in monoculture in each of the two blocks, and were irrigated or non-irrigated as explained above. Species biomass was sampled in all plots through eight growing seasons ending in 2015. All exotic species were included on invasive species lists and are considered invasive in the area, but they are called 'exotic' or 'non-native' here because some are economically valuable as forage species. Bolded species in Table 1 strongly dominated at least one plot in mixtures, or were the most invasive in this environment.

Roots from monocultures were sampled for mycorrhizae. Some species had gone locally extinct during the study due to a major drought in 2011 (Xu et al. 2017), so we sampled the remaining monocultures of the original, and some additional native and exotic plots that were replanted in October 2014 (Table 1). This resulted in a comparison of 11 species pairs, with species paired by phylogenetic and functional groups.

Sample collections for mycorrhizal colonization assessment

Root samples were collected during April 20–24, 2015. Root samples were cut from under individual plants in monoculture plots (2 plants per plot, 100–200 g per plant) and transported in coolers to Iowa State University in Ames, IA for mycorrhizal colonization assessment (Vierheilig et al. 1998). The roots were first washed to remove all soil. A subsample of approximately 1 g of roots from each plant was randomly selected for further analysis (Hodge 2003). Newer, and more elastic roots were chosen over older, stiffer roots including thin roots that branched off thicker main roots. The subsample was cut into lengths between 1.5 and 2.5 cm, rinsed, and then placed into a plastic cassette. The cassettes were placed into a

beaker of tap water to keep roots from drying. This process was repeated for all subsamples, and cassettes were collected 20 at a time. A 5% KOH solution was heated to 90 °C. Cassettes were placed into the solution, ensuring they were not floating at the top. The beaker was covered with foil and allowed to sit for 3 h; the heat was then turned off and the solution left to sit for 12–16 h at room temperature. The KOH was then decanted and the cassettes rinsed with deionized water 6–7 times. A 5% ink stain solution in white vinegar was stirred and heated to a boil (Vierheilig et al. 1998). The cassettes were placed into the ink for 3 min and rinsed thoroughly with deionized water. The cassettes were then placed into deionized water acidified with a few drops of vinegar for 20 min.

Roots were placed randomly into a grid-lined petri dish. To determine percent colonization, we examined roots at 100× to identify mycorrhizae, and once they were verified, we counted them at 40× under a dissecting microscope (Stemi 2000-C, Carl Zeiss, Oberkochen, Germany) to ease in counting (Giovannetti and Mosse 1980). Roots were covered in water to ensure that they remained moist. Every time a root intersected a grid-line it was examined for mycorrhizae, 28–57 intersections per sample (mean of 40). The horizontal and vertical lines were followed in the same pattern to ensure consistency. The roots were

counted as mycorrhizal, non-mycorrhizal, or unknown (roots that were obscured or damaged). The percentage of mycorrhizal colonization was obtained by dividing the number of positively identified mycorrhizal roots by the total number of roots examined, and multiplying this number by 100 (Giovannetti and Mosse 1980).

Relationships between mycorrhizal colonization and plant performance

To test our second hypothesis, colonization by AM fungi would correlate positively with species performance, we calculated plant performance in two ways—with average aboveground biomass of each species across years in monocultures and with estimates of species dominance in mixture. Dominance in mixture was estimated by monitoring mixtures over time. Mixtures were established using random draws with the condition that the relative abundances of functional groups of species (C_4 grasses, C_3 grasses, legumes, non-leguminous C_3 forbs) remain constant across mixture patches (Wilsey et al. 2009, 2011, 2014). Four draws were included in each of the two blocks, and each draw was replicated within each treatment (origin, irrigation) for a total of 32 mixture plots per block (4 draws × 2 origin treatments × 2 irrigation treatments × 2 replicates). Dominance in mixture was estimated as: D_i = aboveground biomass of species$_i$/aboveground biomass of all species in mixture.

Statistical analysis

All analyses were done with SAS 9.4 (SAS Institute Inc., Cary, NC). To test our first hypothesis, we conducted a mixed model ANOVA of average colonization between native and exotic species across functional group and irrigation treatments while pairing species by phylogenetic and functional groups. Functional groupings (C_3 grasses, C_4 grasses, forbs, leguminous forbs) were considered as a blocking term due to the paucity of C_3 grasses in our system (i.e., only one pair prevented generalization). We tested for origin (native, exotic), irrigation (0, 128 mm) and their interactions as fixed effects. Species pair and pair × origin × irrigation were random terms. An initial model tested the three-way interaction among origin, irrigation and functional group, and found that
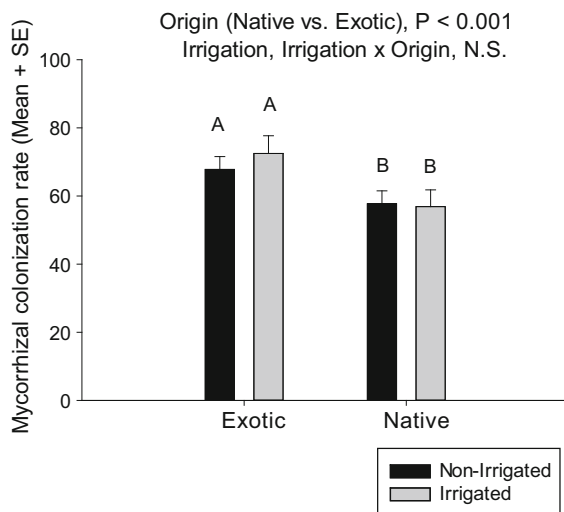
Fig. 1 Average mycorrhizal colonization rates (means + SE) between 11 native and 11 exotic species that were not irrigated or irrigated during the summer at a rate of 128 mm/year. Native and exotic species were paired by phylogenetic and functional groupings

Fig. 2 Mean aboveground biomass in monoculture plots against dominance in mixture plots across species for native (top) and exotic species (bottom) in summer irrigated plots (Y) or control plots (N)
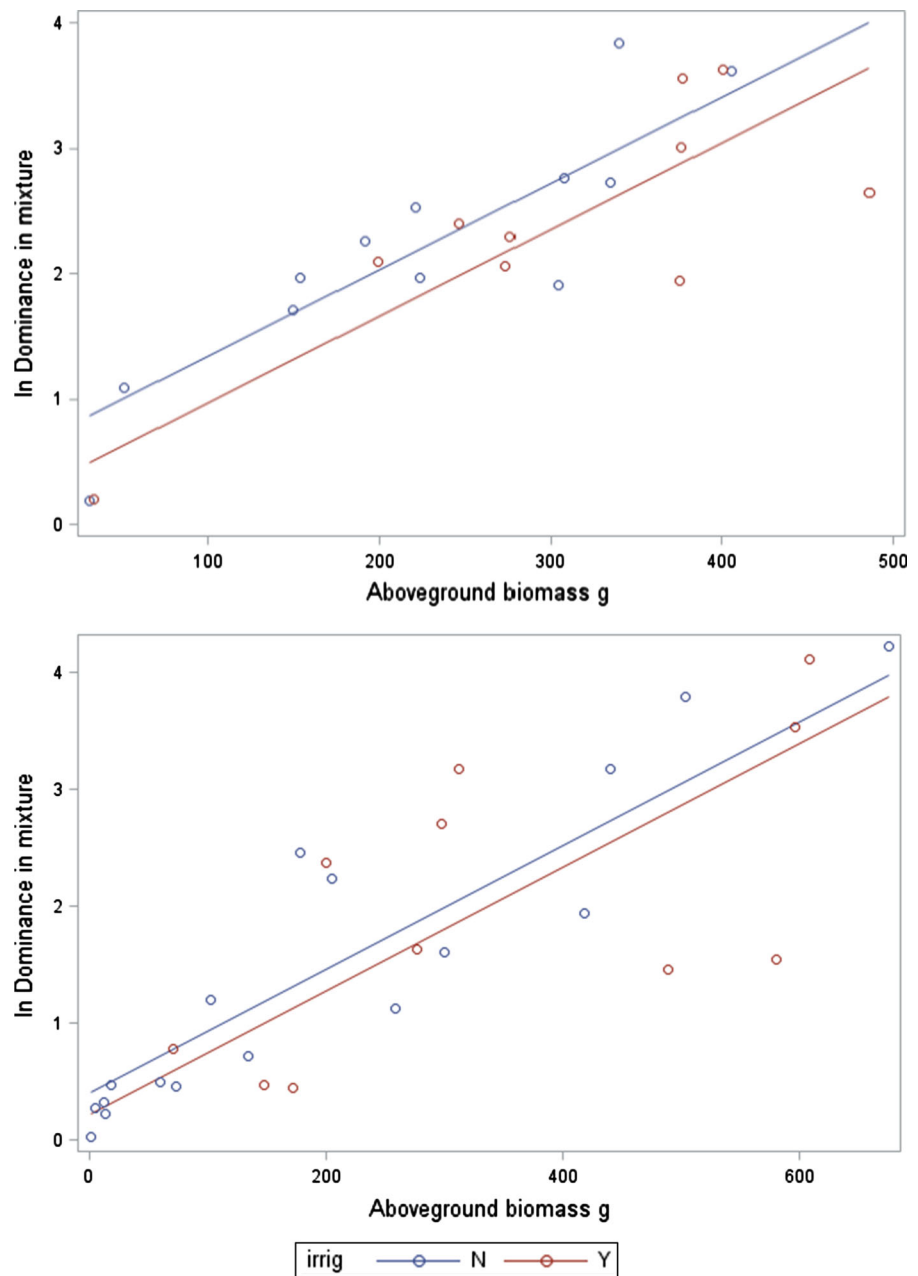
they it was not significant (all P values > 0.15). Therefore, the interaction term was pooled into the error term in the final model. The proportion of colonization was logit transformed ($ln[Y/1 - Y]$) before all analyses to improve normality (Shapiro–Wilks, $P > 0.213$) and reduce heteroscedasticity. Kenwood–Roger corrections were used to calculate degrees of freedom due to having unbalanced data.

To test our second hypothesis, we regressed species' monoculture biomass and dominance in mixture against colonization in an ANCOVA using sequential (Type 1) sums of squares. The ANCOVA had colonization as a covariate, and irrigation and origin as fixed categorical effects. Biomass was $ln(Y = 1)$ transformed, and dominance was logit transformed before analyses. The common slopes assumption was tested with the interaction between

colonization and origin. The interaction was significant in both cases ($P < 0.05$), so separate slopes were fit for native and exotic species. Relationships between biomass and dominance, and colonization were then tested individually for native and exotic species to test whether the slopes differed from 0.

Results

All species showed high mycorrhizal colonization (Fig. 1), which was consistent with past studies in the southern Plains. Mycorrhizal colonization was significantly higher overall in the 11 exotic species than in the 11 native species in the paired test (Table 2, Fig. 1). This native–exotic colonization difference was consistent across the blocking functional group term (i.e., there was no origin × functional group interaction, Table 2). Colonization was similar between summer irrigated and non-irrigated plots (means: 0.66, colonization without irrigation; 0.67, colonization with irrigation; Table 2).

There was large variation in aboveground biomass and dominance in mixture among both native and exotic species (Table 1, Fig. 2). Biomass in monoculture by species was positively related to species dominance in mixture (Fig. 2). Average native species biomass varied from near 0–600 g/m^2, and exotic species biomass varied from near 0 to > 700 g/m^2 (Fig. 2).

Table 2 Analysis of variance (ANOVA) results for percent mycorrhizal colonization between native and exotic species (origin), between summer irrigated (128 mm yearly applied from July 15th to August 15th) and non-irrigated treatments

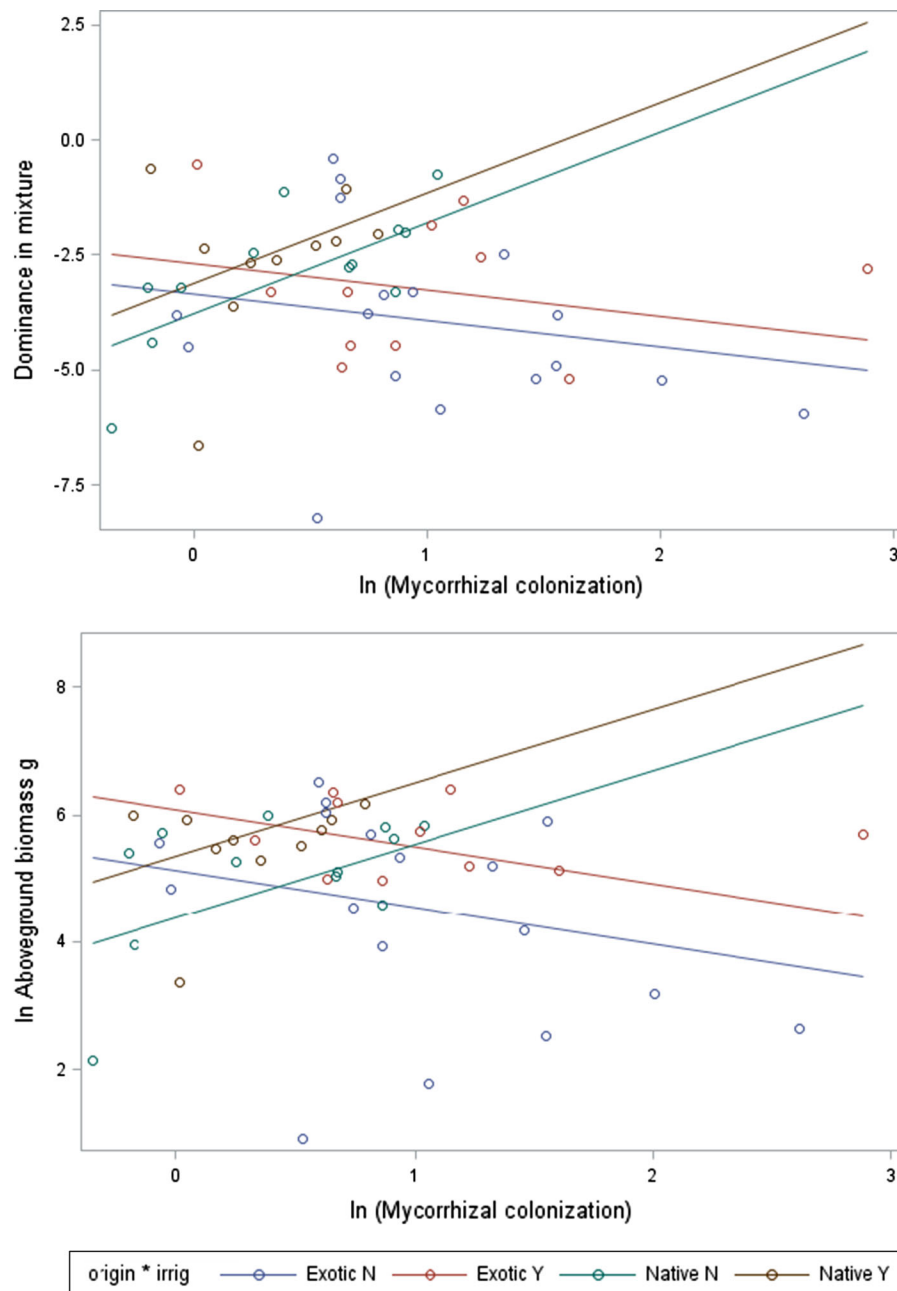| Source	df[a]	F	P value
Block (October vs. March)	1, 71.7	< 0.1	0.92
FG (block)	3, 17.1	3.2	0.05
Origin	1, 24.7	16.3	**< 0.01**
FG × origin	3, 24.8	1.4	0.27
Irrigation	1, 32.4	0.1	0.74
Origin × irrigation	1, 33	0.9	0.35

Functional groups (C$_3$ grasses, C$_4$ grasses, forbs, leguminous forbs) were included as a blocking term

Bolded P values denote significant differences

[a]df degrees of freedom

The relationship between plant performance measures and AM colonization differed significantly between native and exotic species. Dominance in mixture and AM colonization varied significantly between native and exotic species (colonization × origin interaction, $P < 0.05$, Table 3). Aboveground biomass and AM colonization also varied significantly between native and exotic species (colonization × origin interaction, $P < 0.05$) (Table 3). The positive slope of regressions between dominance and AM colonization percentage for native species was reduced by − 2.58 in exotic species (t test comparing slopes, t = − 2.62, $P = 0.0121$). The positive slope of regressions between aboveground biomass and AM colonization percentage for native

Table 3 Analysis of covariance (ANCOVA) results regressing plant success (average biomass in monocultures and dominance in mixture across years) against mycorrhizal colonization rates, irrigation (128 mm of water added in summer of each year), and origin (native or exotic species) (Type 1 SS)

Source	df[a]	Biomass F	P value	Dominance F	P value
Colonization	1	1.5	0.23	1.1	0.30
Irrigation	1	**7.7**	**< 0.01**	1.6	0.21
Origin	1	0.2	0.70	2.7	0.11
Colonization × origin	1	**6.6**	**0.01**	**6.9**	**0.01**
Colonization × irrigation	1	1.9	0.18	0.4	0.53
Origin × irrigation	1	0.2	0.63	< 0.03	0.87
Error	43				
r^2		0.30		0.22	

Data were averaged across blocks and reps before analyses

Bolded P values denote significant differences

[a]df degrees of freedom

Fig. 3 Plots of relationship between AM colonization (*logit* transformed) and two plant performance measures by species, dominance in mixtures (top) and aboveground biomass in monocultures (bottom) across exotic species and native species that were irrigated in summer (Y) or not (N)

species was reduced by − 1.78 in exotic species (t test comparing slopes, t = − 2.59, *P* = 0.0129). That is, the positive relationship between performance measures and mycorrhizal colonization found across native species was much weaker (and trending negative) across exotic species. Mycorrhizal colonization was significantly positively related to dominance and aboveground biomass across native species (dominance: $F_{1,19} = 7.6$, *P* = 0.01, biomass: $F_{1,19} = 5.8$, *P* = 0.03), but was not significantly

negative related across exotic species (dominance: $F_{1,25} = 1.3$, $P = 0.26$, biomass: $F_{1,25} = 2.6$, $P = 0.12$).

Discussion

We found greater colonization by AM fungi, on average, in exotic plant species than in comparable native species in a field experiment in Central Texas. This difference was consistent across functional groups. The relationships between colonization and measures of plant performance across species also differed significantly between native and exotic species. Across native species, mycorrhizal colonization was positively correlated with a species dominance in mixture, supporting previous work that used the fungicide benomyl by Hartnett and Wilson (1999). However, across exotic species, mycorrhizal colonization was not positively correlated with biomass or dominance in mixture. This indicates that, although exotics tended to have higher average percent mycorrhizal colonization than natives, colonization did not predict exotic plant performance in the field. Irrigation during summer had no significant effects on mycorrhizal colonization. Overall, our estimates of mycorrhizal colonization were higher than many estimates in the literature, but were consistent with other studies of Texas grasslands (Frater et al. 2018).

Exotic species with the highest levels of dominance did not differ from less dominant exotic species in their mycorrhizal colonization. The dominant exotic species in experimental mixtures were the C_4 grasses *Panicum coloratum*, *Sorghum halepense*, *Cynodon dactylon*, or *Eragrostis curvula* (Wilsey et al. 2014, Table 1). These are common dominant exotics in the Southern Plains and in other parts of the southern temperate regions (Tognetti et al. 2010; Martin et al. 2014). *Ruellia brittoniana* had high biomass in our monocultures, and is invasive in Florida and other southeastern US states. Low mycorrhizal colonization in exotic dominants implies either that mycorrhizae are not critical to exotic plant success in the southern Plains, or successful exotics efficiently grow with a smaller colonization level. We must emphasize that our results are correlative, and future studies with fungicides (e.g., Hartnett and Wilson 1999; Yang et al. 2014), especially those that target mycorrhizae and not all fungi, are needed to delve more deeply into the native versus exotic differences.

Our prediction that AM fungal colonization rates should be positively related to performance across species was supported by the positive relationship we observed across native species in this subhumid system. Fungal hyphae extend beyond plant roots because hyphae proliferation does not require as much carbon and nitrogen as the production of new roots. Moreover, as fungal hyphae are thinner, they penetrate soil better. This spatial extension of the soil volume inhabited by hyphae allows for more nutrient uptake (e.g., NH_4^+) and transfer to plants. Also, it has been discovered that *Glomus hoi* and *Rhizophagus intraradices* can uptake nitrogen from organic material (Hodge et al. 2001; Leigh et al. 2009). Combined with efficient nutrient absorption, AM fungi can competitively suppress plant pathogens in soil (Wehner et al. 2011), which could increase aboveground biomass in native grassland species.

Our finding that the correlations between plant performance and mycorrhizal colonization were weakened across exotic species was opposite of our original prediction. The weak correlation between colonization and aboveground biomass of exotic plants suggests that there may be a less mutualistic or even parasitic relationship with AM fungi in some species (Klironomos 2003). It might also indicate that exotic species encounter fewer AM fungal species with which they can associate in the invaded range (Shah et al. 2009). Native plant species, despite their lower colonization, could have developed long-term associations by sharing the same evolutionary history with AM fungi, and therefore, their biomass increased as percent colonization increased. The detailed dynamics that explain why exotic species have different relationships with their mycorrhizal partners compared to native species deserves further testing. A complementary step towards understanding the influence of AM fungi on exotics would be molecular identification of the AM fungi (Busby et al. 2013; Checinska Sielaff et al. 2018; Gornish et al. 2016). This approach would help to determine if the fungal composition of highly-colonized roots of exotic plants differs from that of native species, and if exotics are dominated by a single species or diverse group of fungi.

To conclude, we found in our long-term field experiment that percent colonization was positively correlated with aboveground biomass and dominance in mixture for native but not exotic species. However,

future work is needed to determine why the plant–mycorrhizal mutualism weakens when exotic plant species replace natives in grassland ecosystems.

A lack of a relationship does not suggest that exotic species are not impacting mycorrhizae. Stinson et al. (2006) and Vogelsang and Bever (2009) found that exotic species have important impacts on native species by degrading AMF communities, creating a long-term legacy effect. Finally, it is important to remember that our analyses were made across species, and there may be significant correlations across genotypes within species, either negative or positive. Looking at within species variation in mycorrhizal colonization and plant biomass or dominance was beyond the scope of the current study.

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