


# Plant endophytes and arbuscular mycorrhizal fungi alter plant competition

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## Abstract

1. In nature, grasses simultaneously establish multiple symbiotic associations with endophytic fungi and arbuscular mycorrhizal fungi (AMF). The effect of these multiple interactions on competitive interactions between plants remains poorly understood.
2. In this study, we tested whether endophytes and AMF (*Glomus mosseae* or *Glomus etunicatum*) alter plant competition between a subordinate plant species that associates with both symbionts (*Achnatherum sibiricum*) and a dominant plant species, *Stipa grandis*, that only associates with one symbiont (AMF). And we hypothesized that endophytes can facilitate the coexistence of the subordinate plant species (*A. sibiricum*) and the dominant plant species (*S. grandis*).
3. The results demonstrated that endophyte infection significantly enhanced the competitive ability of the subordinate plant species compared to the dominant plant species. The effects of AMF on plant competition were variable and depended on the identity of the AMF species. *Glomus etunicatum* gave *A. sibiricum* plants a higher competitive ability, while *G. mosseae* gave *S. grandis* a higher competitive ability. Simultaneous infections of both endophytes and AMF in *A. sibiricum* also altered the competitive relationships with *S. grandis*.
4. In conclusion, these results suggest that endophytic fungi can facilitate the coexistence of a subordinate plant species with a dominant plant species. Moreover, endophytes could not only affect the competitive ability of the host plant directly but also indirectly by interacting with different AMF to change the growth of competing plant species.

## KEYWORDS

*Achnatherum sibiricum*, AMF species, arbuscular mycorrhizal fungi, endophyte, plant competition, *Stipa grandis*

## 1 | INTRODUCTION

Endophytic fungi are ubiquitous fungi that inhabit the aboveground tissues of healthy plants without causing obvious disease (Arnold, Maynard, Gilbert, Coley, & Kursar, 2000). The common symbiosis between cool season grasses and *Epichloë* endophytes has

important ecological implications (Clay & Schardl, 2002). In this symbiosis, the host grasses provide shelter, photosynthates and nutrients to the endophytes (Cheplick & Faeth, 2009; Schardl, Leuchtman, & Spiering, 2004). The endophytes benefit grasses in turn through increased growth (Clay, 1990; Saikkonen, Wali, Helander, & Faeth, 2004) and by providing tolerance to abiotic and

biotic stresses including drought (Gibert & Hazard, 2011; Hesse et al., 2003; Worchel, Giauque, & Kivlin, 2013), nutrient deficiency (Cheplick & Faeth, 2009; Malinowski & Belesky, 2000), herbivory (Burns & Fisher, 2006) and plant pathogens (Clay & Schardl, 2002; Pérez, Gundel, Ghersa, & Omacini, 2013; Rúa, McCulley, & Mitchell, 2013). Several studies have reported that endophyte infection can affect the competitive ability of host grasses (Brem & Leuchtman, 2002; Cripps, Edwards, & Mckenzie, 2013; Malinowski, Butler, & Belesky, 2011; Richmond, Grewal, & Cardina, 2003; Vázquez-de-Aldana, Zabalgoeazcoa, Garcia-Ciudad, & Garcia-Criado, 2013; Wu et al., 2016).

To date, most research has focused on economically important pasture grasses and turf grasses. These studies observed that the presence of endophytes had a generally positive effect on the intra- or interspecific competitive ability of their host. For instance, several studies found that endophyte infection increased the intraspecific competitive ability of infected genotypes of *Lolium arundinaceum* Darbyshire ex. Schreb. (Tall fescue) (Hill, Belesky, & Stringer, 1991, 1998; Marks, Clay, & Cheplick, 1991) and *Festuca pratensis* Huds. (Meadow fescue) (Malinowski, Leuchtman, Schmidt, & Noesberger, 1997). Other studies found that the presence of endophytes increased the ability of tall fescue and meadow fescue to compete with other plant species (Hoveland, Bouton, & Durham, 1999; Saikkonen et al., 2013). In contrast, some research has demonstrated conflicting results, showing neutral or negative effects of endophytes on the competitive ability of their hosts (Cheplick, Harrichandra, & Liu, 2014; Moraes, Witt, Phillips, Rossi, & Panozzo, 2014; Richmond et al., 2003).

Compared to agronomic grasses, relatively few studies have investigated the effects of endophyte infection on the competitive ability of native grasses, and the results are inconsistent. Faeth, Helander, and Saikkonen (2004) reported that endophyte-free (E-) *Festuca arizonica* Vasey (Arizona fescue) plants outperformed endophyte-infected (E+) plants when grown together in field and glasshouse experiments. Moreover, Brem and Leuchtman (2002) found that endophyte infection had a significant negative effect on the intraspecific competitive ability of *Brachypodium sylvaticum* (Huds.) P.B., but a significant positive effect on that of *Bromus benekenii* (Lange) Trimen. In addition, a greater competitive ability of plants harbouring the endophytes has been documented for *Poa alsodes* Gray (Craig et al., 2011), *Festuca rubra* L. (Red fescue) (Vázquez-de-Aldana et al., 2013) and *Leymus chinensis* (Trin.) Tzvel. (Wu et al., 2016).

The impact of endophytes on the competitive ability of host grasses may be related to direct impacts on characteristics produced by endophytes, such as modifications in shoot and root growth (Craig et al., 2011; Malinowski et al., 1997; Takai, Sanada, & Yamada, 2010), physiological responses to abiotic stresses (Saari & Faeth, 2012; Wu et al., 2016), resistance to herbivory (Clay, Holah, & Rudgers, 2005), or production of allelopathic substances (Aschehoug, Callaway, Newcombe, Tharayil, & Chen, 2014; Bao et al., 2015; Rudgers & Clay, 2007; Vázquez-de-Aldana, Romo, Garcia-Ciudad, Petisco, & Garcia-Criado, 2011). Endophyte-mediated effects may also be indirect.

For instance, endophytes could influence other symbionts, such as arbuscular mycorrhizal fungi and rhizobial bacteria, which influence plant growth and fitness (García Parisi, Lattanzi, Grimoldi, & Omacini, 2015; Omacini, Eggers, Bonkowski, Gange, & Jones, 2006).

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with 80% of all terrestrial plant species and are widespread in almost all terrestrial ecosystems (Brundrett, 2009; Smith & Read, 2008; van der Heijden, Martin, & Sanders, 2015). AMF receive photosynthate from the plant and deliver mineral nutrients to the plant in return. AMF form extensive hyphal networks in the soil and are well equipped to capture soil nutrients, phosphorus (P) in particular (Johnson, 2010; Smith & Read, 2008). Both endophytes and AMF can form mutualistic associations with grasses and receive shelter and carbon from their host plants (Binet et al., 2013; Mack & Rudgers, 2008). Numerous studies have reported interactions of host grasses with either one of these fungi. However, studies considering the simultaneous interaction between host grasses and both endophytes and AMF are rare. To date, these studies have primarily investigated the interaction between endophytes and AMF and largely ignored the effects of this interaction on plant performance. For example, studies of tall fescue and *Lolium perenne* L. (Perennial ryegrass) have reported that endophyte infection reduces sporulation and mycorrhizal colonization (Arrieta, Iannone, Scervino, Vignale, & Novas, 2015; Chu-Chou et al., 1992; Liu et al., 2011; Mack & Rudgers, 2008; Müller, 2003; Novas, Cabral, & Godeas, 2005; Novas, Iannone, Godeas, & Cabral, 2009; Vignale, Iannone, Pinget, Battista, & Novas, 2016).

Moreover, Larimer, Bever, and Clay (2012) found that the effects of endophyte infection on AMF also depended on AMF identity. Endophyte infection reduced the colonization of *Glomus claroideum* N.C. Schenck & G.S. Sm. (= *Claroideoglomus claroideum*), which was detrimental to the host plant, but increased the colonization of *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe (= *Funneliformis mosseae*), which had no effect on host plant growth. In a recent study, we detected that endophyte infection alleviated the detrimental effects of *G. mosseae* colonization on the host grass *Achnatherum sibiricum* (L.) Keng, but there was no significant interaction between endophytes and another AMF, *Glomus etunicatum* W.N. Becker & Gerd. (= *Claroideoglomus etunicatum*), on host growth (Zhou et al., 2016). The simultaneous infections of both endophytes and AMF on a common host plant may affect not only the plant itself but may also alter competitive relationships with other plant species. A study by Omacini et al. (2006) showed that interactions between endophytes and AMF altered competitive interactions between E+ and E- neighbouring *Lolium multiflorum* Lam. (Italian ryegrass) plants. However, whether interactions of AMF and endophytes also influence interspecific competition remains unclear.

Here, we investigate the effects of simultaneous infections by *Epichloë* endophytes and AMF on competitive interactions between two plant species (*Stipa grandis* and *Achnatherum sibiricum*), which are both characteristic of Inner Mongolia steppe vegetation. One of these plants species, *S. grandis*, associates only with AMF and naturally dominates the vegetation, while the other plant species,

*A. sibiricum*, is a subordinate species that associates both with AMF and *Epichloë* endophytes. Specifically, we aimed to test (1) whether the presence of both symbionts facilitates coexistence of the subordinate species with the dominant plant species and (2) whether the effects of the endophyte on competitive interactions between both plant species depended on the presence of particular AMF species.

## 2 | MATERIALS AND METHODS

### 2.1 | Plant and fungal material

*Achnatherum sibiricum* (L.) Keng is a caespitose perennial grass native to the Inner Mongolia steppe of China. This grass is normally a subordinate species in grassland but can sometimes become dominant (Ma, Fu, & Chen, 1985). *Achnatherum sibiricum* is usually colonized by *Epichloë* endophytes with high infection rates (86%–100%) in natural habitats (Wei et al., 2006). In addition, *A. sibiricum* can be colonized by several AMF taxa (Su & Guo, 2007); in the previous investigations, we have also observed mycorrhizal colonization (between 55% and 59%) of *A. sibiricum* collected from two populations in the Inner Mongolia steppe of China. For this study, mature seeds of *A. sibiricum* were collected from natural populations in the National Hulunber Grassland Ecosystem Observation and Research Station (119.67°E, 49.10°N) in north-east China, where the annual mean temperature is roughly –2°C and the mean annual precipitation is approximately 367 mm. Within each population, 300 plant individuals were chosen at random. The seeds were collected in August 2013 and stored at 4°C. The aniline blue staining method (Latch, Christensen, & Samuels, 1984) was used to analyse the endophyte infection frequency of the seeds. One hundred seeds from different plant individuals were tested. All of them were colonized by endophytes, resulting in an infection frequency of 100%. Previous studies by our laboratory reported that at least 10 fungal taxa were discovered in *A. sibiricum*; *Epichloë gansuensis* C.J. Li & Nan and *Epichloë sibirica* X. Zhang & Y.B. Gao were identified as the two most dominant endophytic species of *A. sibiricum* (Li et al., 2015; Zhang et al., 2009). For this study, we did not check the identity of endophytes in the *A. sibiricum* seeds, so endophyte infection responses are based on the overall endophyte community within the plants. To eliminate the endophyte, we heated a subset of randomly chosen seeds for 30 days at 60°C in a convection drying oven as per Ren et al. (2011) and Li et al. (2013). Earlier studies showed that this is an effective disinfection method for *A. sibiricum* (Li et al., 2013; Ren et al., 2011). Moreover, our earlier work showed that the heat treatment had no significant effect on germination rate, germination potential or germination index (Li, Han, Ren, & Gao, 2010).

The other plant species used for this experiment is *Stipa grandis*. *Stipa grandis* P. Smirn. is a perennial bunch grass and is one of the dominant species in the Inner Mongolia steppe. This species is widely distributed in northern China and has important nutritional forage value for sheep and cattle (Ma et al., 1985). It can form symbiotic relationships with AMF (Su & Guo, 2007) but is usually not colonized by endophytes in its leaves (Su, Guo, & Hyde, 2010). In our

previous investigation, the mean endophyte infection rate of *S. grandis* was only 1.11% at eighteen sites in the Inner Mongolia steppe of China (Wei et al., 2006). The seeds used in the present study were collected from natural populations in the National Hulunber Grassland Ecosystem Observation and Research Station in August 2012 and stored at 4°C.

Regular untreated (E+) and heat treated (E–) *A. sibiricum* seeds and *S. grandis* seeds were selected, surface-sterilized with 0.5% sodium hypochlorite solution for 10 min and washed with sterilized water. Seeds were planted in plastic pots (280 mm in diameter, 220 mm in depth), filled with 5 kg of 2 mm sieved autoclaved sand. Each pot received either eight seedlings of one species (the plant monocultures) or four seedlings of *A. sibiricum* and four seedlings of *S. grandis* (the plant mixtures). We examined endophyte status of each *A. sibiricum* plant following staining the leaf peel with aniline blue (Latch et al., 1984) at the beginning of the experiment to ensure endophyte treatment effectiveness.

We used two AMF species: *Glomus mosseae* (GM, currently *Funnelformis mosseae*) and *Glomus etunicatum* (GE, currently *Claroideoglomus etunicatum*) isolated from the Inner Mongolia steppe and provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences. Both AMF species are widespread in China, have a worldwide distribution and have been found in a wide range of ecosystems (Öpik, Moora, Liira, & Zobel, 2006). The two fungi originated from single spores that were extracted from field soil using sucrose centrifugation. In our study, pure inocula of GM and GE were propagated separately in a mixture of autoclaved sand and zeolite (1:1) with *Sorghum bicolor* (L.) Moench (Broomcorn) for 12 weeks. The mycorrhizal inoculum was composed of fungal spores, hyphae and root fragments. In our previous study, we found that the effects of GM and GE colonization on the growth of *A. sibiricum* varied. Specifically, GE colonization had beneficial effects on plant growth. In contrast, GM colonization had detrimental effects on the plants (Zhou et al., 2016). The AMF treatment groups (either GM or GE) received 100 g mycorrhizal inoculum in each pot. The non-AMF treatment group (NM) was inoculated with the same amount of autoclaved inoculum; 50 ml of non-autoclaved inoculum filtrate (passed through a 10 µm sieve) was added to the non-AMF treatment to correct for possible differences in the microbial community and nutrients between AMF and non-AMF treatments.

### 2.2 | Experimental design

The experiment was set up as a randomized block design with three factors. The first factor was AMF inoculation treatment (GM, GE or a non-mycorrhizal (NM) control). The second factor was plant mixture type (monoculture and mixture). The third factor was endophyte infection status of *A. sibiricum* (E+ and E– *A. sibiricum*). A DeWit-type replacement series (de Wit, 1960) with equal plant density (eight plant individuals per plant species for monocultures and four + four individuals of each plant species in mixtures) was used to assess competitive interactions between *A. sibiricum* and *S. grandis*. Both E+ and E– *A. sibiricum* were grown

alone as monoculture or in interspecific mixtures with *S. grandis*. This resulted in five groups: monoculture of E+ *A. sibiricum*, monoculture of E- *A. sibiricum*, monoculture of *S. grandis*, mixtures of E+ *A. sibiricum* and *S. grandis* (E+S), and mixtures of E- *A. sibiricum* and *S. grandis* (E-S). Together with the three AMF groups, this resulted in 15 combinations. Note that *S. grandis* is not colonized by endophytes; as such, there are no E+ and E- treatments for *S. grandis*. Each combination was replicated five times, yielding a total of 75 pots. The experiment began on 20 May 2014 and was conducted in the campus experimental field at Nankai University, Tianjin, China. During the experiment, each pot was watered two-three times a week. Nutrients were supplied by the addition of Hoagland nutrient solution once per week to ensure the normal growth of plants. The experiment lasted 4 months.

At the end of the experiment, shoots were harvested from each species by clipping 1 cm above the soil surface, and roots were collected by gently washing and separating each species. Root subsamples were set aside for assessing AMF colonization rate. The total shoot and root biomass was measured after drying in an oven at 80°C for 24 hr. Additionally, subsamples of shoot and root material were ground into a powder and the P content was determined using the molybdenum antimony colorimetric method (Bao, 2000).

### 2.3 | AMF colonization rate

Fresh roots were washed free of soil and cut into small pieces, and root fragments were randomly collected to obtain approximately 1 g roots. The sampled roots were cleared with 10% KOH in a water bath of 90°C for 60 min and then stained with aniline blue for 30 min. AMF colonization rate was recorded using the cross-hair eyepiece method (McGonigle, Miller, Evans, Fairchild, & Swan, 1990), with a minimum of 100 intersections per sample.

### 2.4 | Mycorrhizal growth response

Mycorrhizal growth response (MGR) of GM and GE was calculated according to the following equations based on van der Heijden (2002): If  $M > NM_{\text{mean}}$ , then  $MGR (\%) = 100 \times (1 - NM_{\text{mean}}/M)$ , but if  $M < NM_{\text{mean}}$ , then  $MGR (\%) = 100 \times (-1 + M/NM_{\text{mean}})$ , where  $M$  was the plant total dry weight in the given replicate of the mycorrhizal treatment, and  $NM_{\text{mean}}$  was the mean total dry weight in the corresponding NM treatment. Positive values for MGR indicated that plant growth was promoted by AMF, and negative values indicate that plant growth was suppressed by AMF.

### 2.5 | Competitiveness

The competitive ability of *A. sibiricum* and *S. grandis* in a plant mixture was assessed using the aggressivity index (AGR) and relative interaction intensity index (RII), both of which were calculated using the total biomass of plant species. The AGR of species  $i$  relative to species  $j$  was measured as McGilchrist and Trenbath (1971):

$$AGR_{ij} = RY_i - RY_j = (DM_{ij}/DM_{ii}) - (DM_{ji}/DM_{jj})$$

where RY is the relative yield of species  $i$  or  $j$ , defined as the dry matter yield of a species grown in mixture ( $DM_{ij}$  or  $DM_{ji}$ ) relative to the dry matter in the respective monoculture ( $DM_{ii}$  or  $DM_{jj}$ ). Species  $i$  and  $j$  have the same competitive ability if  $AGR_{ij}$  value is zero. An  $AGR_{ij}$  value greater than zero indicated higher competitive ability of species  $i$  than that of species  $j$ . An  $AGR_{ij}$  value less than zero indicated the opposite.

The RII provides a simple comparison of interaction strength across species and treatments (Armas, Ordiales, & Pugnaire, 2004). The RII is expressed as

$$RII = (DM_{ij} - DM_{ji}) / (DM_{ij} + DM_{ji})$$

The RII is a measure of the strength of interaction between species and is centred on zero with negative interactions (competition) indicated by values between 0 and -1, and positive interactions (facilitation) indicated by values between 0 and +1.

## 2.6 | Statistical analyses

All statistical analyses were performed with SPSS software (Version 19.0, SPSS, Chicago, USA). A three-way analysis of variance (ANOVA) was used to analyse the effects of endophyte infection (E), mycorrhizal colonization (M) and plant mixture type (C) on the mycorrhizal colonization rate, mycorrhizal growth response, plant biomass and P content of *A. sibiricum*, while the effects on *S. grandis* were analysed by means of a two-way ANOVA considering the factors C and M. The effects of E and M on the AGR and RII were analysed using a two-way ANOVA. The differences between the means among different factors were compared using Duncan's multiple-range tests at  $p < .05$ .

## 3 | RESULTS

### 3.1 | Plant growth performance

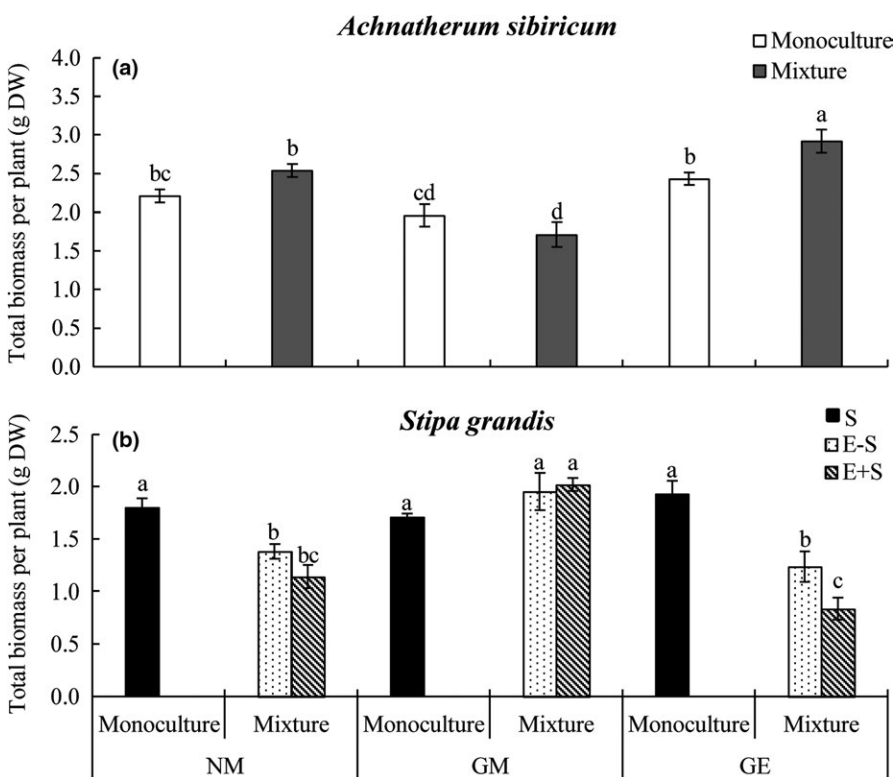
The total biomass of *A. sibiricum* was significantly affected by endophyte infection, with endophyte infection significantly increasing the total biomass of *A. sibiricum* (Table 1, E+ =  $2.49 \pm 0.09$  g, E- =  $2.09 \pm 0.09$  g). The interaction between mycorrhizal colonization and plant mixture type significantly affected the plant total biomass (Table 1): In mixture with *S. grandis*, GM inoculation decreased the total biomass, while GE inoculation increased the total biomass compared to the NM treatment. GE inoculation in mixtures also improved the total biomass of *A. sibiricum* compared with the other treatments, indicating that GE infection had a positive effect on the growth of *A. sibiricum* when competing with *S. grandis* (Figure 1a). However, there was no significant interaction among endophyte infection, mycorrhizal colonization and plant mixture type (Table 1).

There was a significant interaction between mycorrhizal colonization and plant mixture type for the total biomass of *S. grandis* (Table 1). In NM and GE treatments, the total biomass of *S. grandis* was lower in

**TABLE 1** Analyses of variance (ANOVA) for plant biomass, phosphorus (P) content, mycorrhizal colonization rate and mycorrhizal growth response (MGR) of *Achnatherum sibiricum* and *Stipa grandis*

	df	Total biomass		Shoot P content		Root P content		Mycorrhizal colonization rate		MGR		
		F	p	F	p	F	p	F	p	F	p	
<i>Achnatherum sibiricum</i>												
Endophyte(E)	1	24.240	<.001	39.451	<.001	12.522	.001	1	14.058	.001	6.518	.016
Mycorrhiza(M)	2	36.687	<.001	140.079	<.001	3.938	.026	1	13.778	.001	65.584	<.001
Competition(C)	1	5.617	.022	38.144	<.001	0.119	.731	1	0.680	.416	4.814	.036
E × M	2	2.127	.130	7.008	.002	0.868	.426	1	1.439	.239	4.158	.050
E × C	1	3.507	.067	11.255	.002	0.927	.340	1	0.492	.488	1.002	.324
M × C	2	7.595	.001	12.602	<.001	0.797	.457	1	1.285	.265	9.038	.005
E × M × C	2	0.388	.681	2.795	.071	0.101	.905	1	1.464	.235	0.062	.806
<i>Stipa grandis</i>												
Mycorrhiza(M)	2	21.547	<.001	42.334	<.001	20.690	<.001	1	7.675	.011	37.186	<.001
Competition(C)	2	14.304	<.001	17.308	<.001	6.484	.004	2	5.731	.009	0.957	.398
M × C	4	10.889	<.001	9.889	<.001	1.352	.270	2	3.456	.048	18.977	<.001

Significant *p*-values (*p* < .05) are in bold.

**FIGURE 1** Effects of mycorrhizal colonization and plant mixture type on the total biomass per plant in monocultures and mixtures of *Achnatherum sibiricum* (a) and *Stipa grandis* (b) when inoculated with *Glomus mosseae* (GM) or *Glomus etunicatum* (GE). Bars represent means  $\pm$  SE (*n* = 5). Different letters above bars denote means that are significantly different (*p* < .05). Monoculture, *A. sibiricum* and *S. grandis* grow separately in pots; mixture, *A. sibiricum* and *S. grandis* grow together in pots; S, the monoculture of *S. grandis*; E+S, *S. grandis* grown with E+ *A. sibiricum*; E-S, *S. grandis* grown with E- *A. sibiricum*; NM, non-AMF treatment. For statistical details, see Table 1

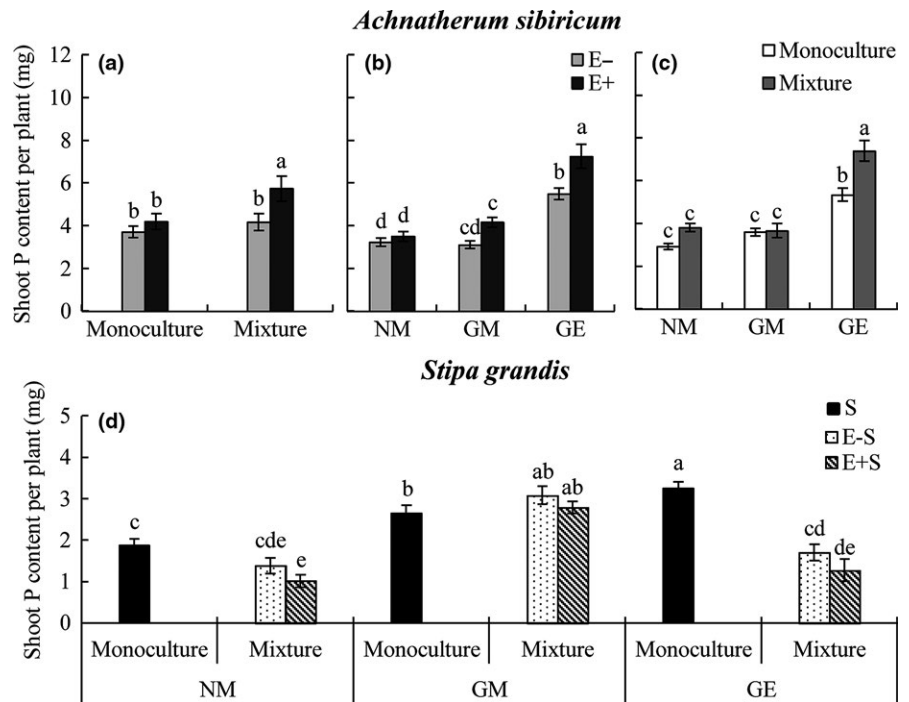
mixtures than those in monocultures (Figure 1b). AMF did not affect the total biomass of *S. grandis* when the plants were grown in monocultures. However, in mixtures, *S. grandis* produced more biomass when grown with E- than E+ *A. sibiricum* plants in GE treatments. The total biomass of *S. grandis* was increased by GM colonization regardless of the endophyte infection status of *A. sibiricum* compared to the NM and GE treatments (Figure 1b). These results showed that GM colonization alleviated the detrimental effect of *A. sibiricum* on the growth

of *S. grandis*, but GE colonization did not contribute to the inhibitory effect of *A. sibiricum* on *S. grandis*, especially the inhibitory effect of E+ plants.

### 3.2 | Phosphorus content in plants

The shoot P content of *A. sibiricum* was significantly affected by endophyte infection, mycorrhizal colonization, plant mixture type

**FIGURE 2** Effects of the interaction between endophyte infection (E) × plant mixture type (C) (a), E × mycorrhizal colonization (M) (b), and M × C (c) on the shoot P content per plant of *Achnatherum sibiricum*, and effect of the interaction between M × C on the shoot P content per plant of *Stipa grandis* (d). Bars represent  $M \pm SE$  ( $n = 5$ ). Different letters above bars denote means that are significantly different ( $p < .05$ ). Monoculture, *A. sibiricum* and *S. grandis* grow separately in pots; mixture, *A. sibiricum* and *S. grandis* grow together in pots; E+, endophyte-infected plants; E-, endophyte-free plants; S, the monoculture of *S. grandis*; E+S, *S. grandis* grown with E+ *A. sibiricum*; E-S, *S. grandis* grown with E- *A. sibiricum*; NM, non-AMF treatment; GM, *Glomus mosseae*; GE, *Glomus etunicatum*. For statistical details, see Table 1



and the interaction of any two factors (Table 1). Unlike the result in monoculture, endophyte infection significantly increased the shoot P content of *A. sibiricum* in mixture (Figure 2a). GM colonization significantly increased the shoot P content of E+ *A. sibiricum* compared to the E+ plants in NM treatment (Figure 2b). When inoculated with GE, in either E+ or E- plants, monoculture or mixture, mycorrhizal colonization increased the shoot P content of *A. sibiricum* compared to NM and GM treatments (Figure 2b,c). Meanwhile, simultaneous infections of endophytes and GE had a significantly higher shoot P content than single infection of GE in plants without endophytes (Figure 2b), and shoot P content of *A. sibiricum* was also higher in mixture than that in monoculture when colonized by GE (Figure 2c).

The shoot P content of *S. grandis* was significantly affected by the interaction between mycorrhizal colonization and plant mixture type (Table 1). GM and GE colonization significantly increased the shoot P content of *S. grandis* in monoculture compared to NM treatment (Figure 2d). In mixtures, the shoot P content of *S. grandis* was significantly increased by GM colonization, but there was no significant difference between NM and GE treatments. Endophyte infection did not significantly affect the shoot P content of *S. grandis* under mixture conditions (Figure 2d).

### 3.3 | AMF colonization

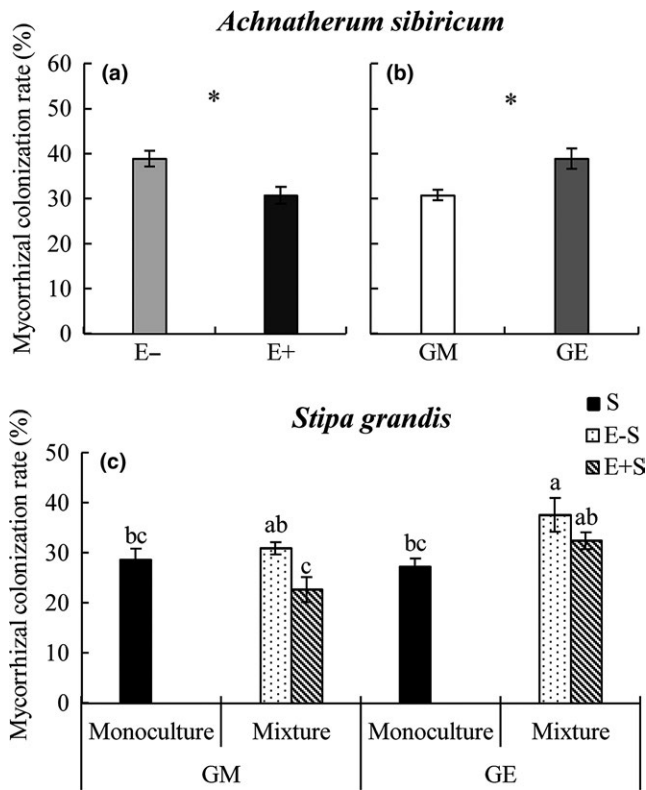
In the AMF treatment group, endophyte infection significantly reduced mycorrhizal colonization of *A. sibiricum* (Table 1, Figure 3a). The mean mycorrhizal colonization rate of E+ *A. sibiricum* was 30.8% compared to 38.9% for that of E- *A. sibiricum* plants. Mycorrhizal colonization rates of *A. sibiricum* were also significantly affected by the AMF species identity, with a significantly higher colonization rate in the roots of GE-inoculated plants than that of GM-inoculated

plants (Figure 3b). Interestingly, root colonization levels of *S. grandis*, which does not associate with endophytes, were lower when grown in mixture with E+ *A. sibiricum* compared to E- *A. sibiricum* (Figure 3c). Roots of *A. sibiricum* and *S. grandis* in the non-AMF treatment were not colonized by AMF.

### 3.4 | Mycorrhizal growth responses

The MGRs of *A. sibiricum* were significantly affected by the interaction between mycorrhizal colonization and plant mixture type (Table 1). When inoculated with GM, the MGRs of *A. sibiricum* were negative in monocultures and mixtures, and the MGRs in mixtures were significantly less negative than those in monocultures, indicating that the growth of host plants was suppressed by GM colonization, especially under mixture conditions (Figure 4a). When inoculated with GE, the MGRs of *A. sibiricum* were positive under both monoculture and mixture conditions, indicating that the growth of host plants was improved by GE colonization, but there was no significant difference between these treatments (Figure 4a).

There was also a significant interaction between mycorrhizal colonization and plant mixture type for the MGRs of *S. grandis* (Table 1). The addition of GM had an approximately neutral effect on *S. grandis* when it was grown in monoculture, but the effects differed when the species were grown in mixture, as the MGRs were positive in the mixture. The magnitude of the positive effect of GM on *S. grandis* increased when grown in mixture with E+ *A. sibiricum* plants compared to E- plants, indicating that the responsiveness of *S. grandis* to GM was increased by endophyte infection (Figure 4b). When inoculated with GE, the MGRs of *S. grandis* were positive under monoculture conditions, but negative when grown in mixture with *A. sibiricum*. Compared to grown in monoculture, the MGRs were significantly lower when grown in

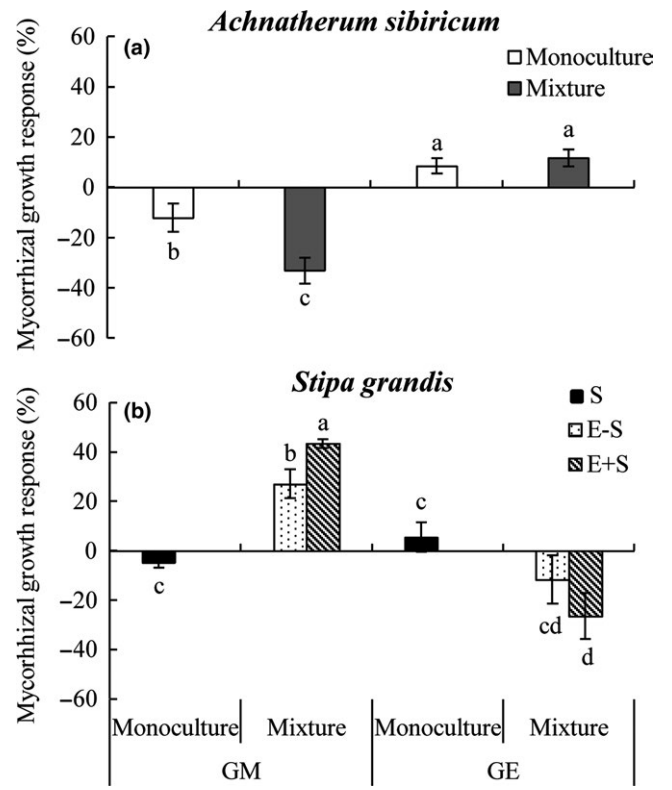


**FIGURE 3** Effects of endophyte infection (a) and arbuscular mycorrhizal fungi (AMF) species identify (b) on the mycorrhizal colonization rates of *Achnatherum sibiricum* and effect of the interaction between mycorrhizal colonization and plant mixture type on the mycorrhizal colonization rates of *Stipa grandis* (c). Bars represent  $M \pm SE$  ( $n = 5$ ). The asterisk and different letters above bars denote means that are significantly different ( $p < .05$ ). E+, endophyte-infected plants; E-, endophyte-free plants; GM, *Glomus mosseae*; GE, *Glomus etunicatum*; S, the monoculture of *S. grandis*; E+S, *S. grandis* grown with E+ *A. sibiricum*; E-S, *S. grandis* grown with E- *A. sibiricum*; Monoculture, *A. sibiricum* and *S. grandis* grow separately in pots; mixture, *A. sibiricum* and *S. grandis* grow together in pots. For statistical details, see Table 1

mixture with E+ *A. sibiricum*, while no significant difference occurred when grown in mixture with E- *A. sibiricum* (Figure 4b).

### 3.5 | Competitiveness

The AGR was used to measure the competitive ability of *A. sibiricum* relative to *S. grandis*. The results showed that the AGR was significantly affected by the main effects of endophyte infection and mycorrhizal colonization (Table 2). The AGR of E+ *A. sibiricum* plants was higher



**FIGURE 4** Effects of the interaction between mycorrhizal colonization and plant mixture type on the mycorrhizal growth responses of *Achnatherum sibiricum* (a) and *Stipa grandis* (b). Bars represent  $M \pm SE$  ( $n = 5$ ). Different letters above bars denote means that are significantly different ( $p < .05$ ). Monoculture, *A. sibiricum* and *S. grandis* grow separately in pots; mixture, *A. sibiricum* and *S. grandis* grow together in pots; S, the monoculture of *S. grandis*; E+S, *S. grandis* grown with E+ *A. sibiricum*; E-S, *S. grandis* grown with E- *A. sibiricum*; GM, *Glomus mosseae*; GE, *Glomus etunicatum*. For statistical details, see Table 1

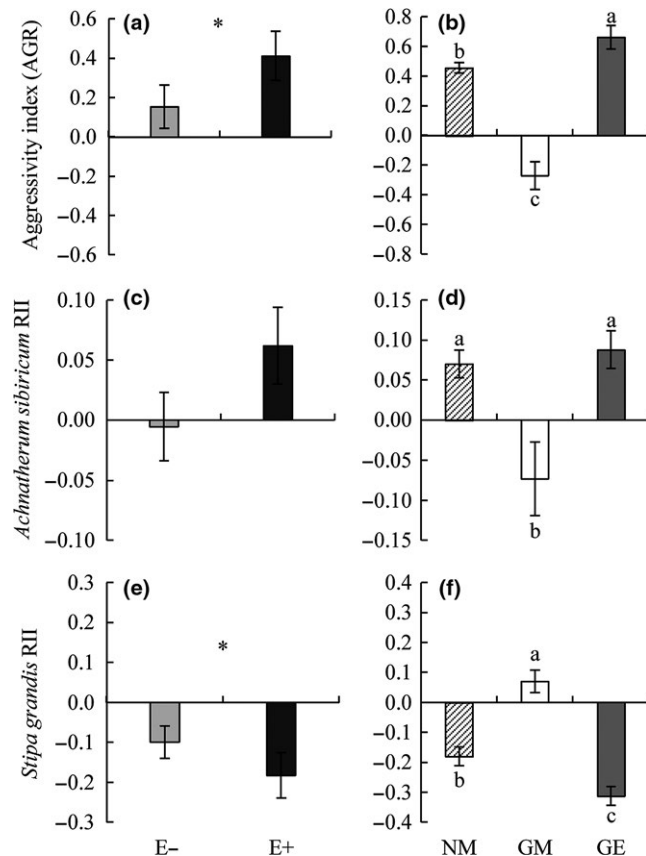
than E- plants regardless of AMF inoculation treatment, indicating a greater competitive ability of E+ plants (Figure 5a). When inoculated with GM, the AGR of *A. sibiricum* was significantly lower than that in NM treatments, indicating that GM colonization reduced the competitive ability of *A. sibiricum*. In contrast, the competitive ability of *A. sibiricum* was increased by GE colonization, with the significantly higher AGR when infected by GE compared to the NM treatments (Figure 5b).

As for the response of *A. sibiricum* to the competition with *S. grandis*, *S. grandis* facilitated the growth of E+ *A. sibiricum* plants ( $RII > 0$ ), while E- *A. sibiricum* plants were not affected by *S. grandis* (Figure 5c), but no significant endophyte main effect was observed

	df	AGR		<i>A. sibiricum</i> RII		<i>S. grandis</i> RII	
		F	p	F	p	F	p
Endophyte (E)	1	13.683	<b>.001</b>	3.704	.066	5.644	<b>.026</b>
Mycorrhiza (M)	2	66.352	<b>&lt;.001</b>	8.465	<b>.002</b>	40.898	<b>&lt;.001</b>
E × M	2	1.229	.310	0.376	.690	2.773	.082

Significant  $p$ -values ( $p < .05$ ) are in bold.

**TABLE 2** Effects of endophyte infection and mycorrhizal colonization on the aggressivity index (AGR) for *Achnatherum sibiricum* and the relative interaction intensity indices (RII) for *A. sibiricum* and *Stipa grandis*



**FIGURE 5** Effects of endophyte infection and mycorrhizal colonization on plant competition between *Achnatherum sibiricum* and *Stipa grandis*, evaluated using the aggressivity index (AGR) for *A. sibiricum* (a, b) or the relative interaction intensity (RII) for *A. sibiricum* (c, d) or *S. grandis* (e, f). Bars represent  $M \pm SE$  ( $n = 5$ ). The asterisk and different letters above bars denote means that are significantly different ( $p < .05$ ). E+, endophyte-infected plants; E-, endophyte-free plants; NM, non-AMF treatment; GM, *Glomus mosseae*; GE, *Glomus etunicatum*. For statistical details, see Table 2

(Table 2). The growth of *A. sibiricum* plants was significantly suppressed by *S. grandis* when inoculated with GM compared to the NM and GE treatments (Figure 5d). The RII of *S. grandis* was significantly affected by the main effect of endophyte infection (Table 2), with the values were significantly lower when grown in mixture with E+ *A. sibiricum* plants than E- plants (Figure 5e). Plant growth was suppressed by *A. sibiricum* in NM and GE treatments (RII < 0), with the competitive suppression being highest in the presence of GE. Unlike the effects of GE, when *S. grandis* was inoculated by GM, the plant growth was facilitated by *A. sibiricum* plants (RII > 0) (Figure 5f). There was no significant interaction between endophytes and AMF on the RII of *A. sibiricum* and *S. grandis* (Table 2).

## 4 | DISCUSSION

The results of this study demonstrate that endophytes can directly alter competitive interaction between its host plant, *A. sibiricum*, and another plant species, *S. grandis*, which lacks endophytes. So far, few

studies have tested the effects of endophyte infection on the competitive ability of native grasses (Brem & Leuchtman, 2002; Craig et al., 2011; Faeth et al., 2004; Vázquez-de-Aldana et al., 2013). Our results show that endophyte infection can increase the competitive ability of the host plant *A. sibiricum*, with higher biomass and aggressivity index than the endophyte-free plants. The similar results were reported by Craig et al. (2011) and Vázquez-de-Aldana et al. (2013) who found positive effects of endophyte infection on the interspecific competitive ability of host plants. Our results further suggest that endophytes facilitate coexistence between a subordinate plant species (*A. sibiricum*) and a dominant species (*S. grandis*) of the Inner Mongolian steppe vegetation. While earlier work showed that endophytes contribute to grass dominance and reduce plant diversity in North American grasslands (Clay & Holah, 1999; Rudgers & Clay, 2007), this work suggests that the opposite can also occur, where the presence of endophytes facilitates the competitive ability of a relatively rare species against the dominant plant that lacks endophytes.

Existing studies indicate that endophyte infection affects the interspecific competitive ability of host plants not only directly (Aschehoug, Metlen, Callaway, & Newcombe, 2012; Malinowski, Belesky, & Fedders, 1999; Vázquez-de-Aldana et al., 2013) but also indirectly by changing the community of soil micro-organisms associated with plant roots (Cripps et al., 2013; Rudgers & Orr, 2009). Similar to endophytes, AMF can influence plant growth and nutrition; AMF have been shown to alter plant diversity (van der Heijden et al., 1998) and competitive relationships between plants (Bever, 2003; Facelli, Smith, Facelli, Christophersen, & Andrew Smith, 2010; Mariotte et al., 2013). Several studies also indicate that the effects of AMF on plant competition and plant diversity depend on AMF species identity and AMF diversity (Scheublin, van Logtestijn, & van der Heijden, 2007; Wagg, Jansa, Stadler, Schmid, & van der Heijden, 2011). Our previous study with *A. sibiricum* showed that the effects of simultaneous infection by endophytes and AMF on host plant growth depended on AMF species identity: endophyte infection alleviated the detrimental effects of GM colonization on host growth under sufficient nutrient supply conditions, while no significant interaction was observed between endophytes and GE on host growth (Zhou et al., 2016). So far, no study has investigated whether dual infection of a host plant by endophytes and AMF altered the plant's interspecific competitive ability. Interestingly, this study showed that the competitive ability of a plant not only depends on the presence of AMF and endophytes, but also that it varies depending on which AMF colonizes the plant roots. When inoculated with GM, the detrimental effect of *A. sibiricum* on the growth of *S. grandis* was alleviated by GM colonization, but no significant difference was occurred between the two endophyte infection statuses, suggesting that the positive effect of GM colonization on the competitive ability of *S. grandis* was not influenced by endophytes. When inoculated with GE, the presence of endophytes increased the inhibitory effect of *A. sibiricum* on the growth of *S. grandis*, indicating that co-infection by both endophytes and GE had an additive effect on the competitive ability of *A. sibiricum*.

At present, the exact mechanisms for the effects of AMF colonization on the competitive ability of endophyte-infected plants have



not been identified. Possible explanations for our observations are (1) endophyte infection can change the mycorrhizal colonization of the host plant and companion plant. Several studies have reported that suppression of AMF colonization can affect the growth of host plants, resulting in altered plant interactions (Bray, Kitajima, & Sylvia, 2003) and plant community structure (Callaway, Thelen, Barth, Ramsey, & Gannon, 2004; Hartnett & Wilson, 1999), especially for non-beneficial AMF (Grman, 2012). (2) Endophyte infection changes the mycorrhizal growth responses of the companion plant *S. grandis*. The mycorrhizal growth responses of individual plant species can determine the effects of AMF on the competitive outcome among plant species (Lin, McCormack, & Guo, 2015). Several studies have reported that a greater competitive ability is related to the higher mycorrhizal growth responses of competitive plants (Scheublin et al., 2007; Wagg et al., 2011). (3) The simultaneous infection by endophytes and AMF can influence plant nutrient uptake. Earlier studies showed that endophytes and AMF enhanced the competitive ability of host plants by increasing the P content of host plants (Mariotte et al., 2013; Omacini et al., 2006; Vázquez-de-Aldana et al., 2013). Thus, the change of plant P uptake and storage in our study may be one reason why the simultaneous infection by endophytes and AMF increased the interspecific competitive ability of *A. sibiricum*.

In conclusion, this study demonstrates that endophyte infection has a positive effect on the interspecific competitive ability of its host plant *A. sibiricum*. This work further shows that competitive interactions between plants depend on the presence of endophytes and are also affected by AMF species identity. Interestingly, while earlier work indicated that endophytes contribute to the dominance of its host plant in North American grassland, this work indicates that the presence of endophytes facilitates coexistence of a relatively rare species with a dominant plant that lacks endophytes. Further studies are needed to investigate how communities of endophytes and AMF interact and whether AMF and endophyte diversity influence host plant performance and plant community structure.

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## AUTHORS' CONTRIBUTIONS

Y.Z., Y.-B.G. and A.-Z.R. conceived the ideas and designed methodology; Y.Z., X.L., Y.G. and H.L. collected the data; Y.Z. and X.L. analysed the data; Y.Z. and M.G.A.H. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## DATA ACCESSIBILITY

Raw data used in this manuscript are deposited at figshare.com <https://doi.org/10.6084/m9.figshare.5418655.v2> (Zhou et al., 2017).

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