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Reduced mycorrhizal responsiveness leads to increased competitive tolerance in an invasive exotic plant

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Summary

1. Arbuscular mycorrhizal (AM) fungi can exert a powerful influence on the outcome of plant–plant competition. Since some exotic plants interact differently with soil biota such as AM fungi in their new range, range-based shifts in AM responsiveness could shift competitive interactions between exotic and resident plants, although this remains poorly studied.

2. We explored whether genotypes of the annual exotic *Centaurea solstitialis* (yellow starthistle), collected from populations across the native and non-native ranges, differed in responsiveness to AM fungi in the introduced range and whether range-based differences in mycorrhizal responsiveness affected how strongly *C. solstitialis* tolerated competition with the North American native bunchgrass, *Stipa pulchra*.

3. Grown alone, *C. solstitialis* from both ranges derived only weak benefits from AM fungi. However, association with AM fungi was costly to plants when grown in competition with *S. pulchra*. The magnitude of the suppressive effect of AM fungi was greater for genotypes from native versus introduced populations.

4. *Synthesis.* Many exotic invasive species are known to associate weakly with AM fungi, which may be beneficial in disturbed habitats where competition for resources is low. Our results indicate that reduced mycorrhizal associations may also benefit invaders in a competitive environment. *Centaurea solstitialis* were more strongly suppressed by established *S. pulchra* plants in the presence versus absence of AM fungi, but exotic genotypes were less suppressed than native genotypes. This suggests that AM fungi may contribute to invasion resistance in established native communities, but range-based shifts in the way exotic genotypes respond to AM fungal partners may counter such biotic resistance.

Key-words: Arbuscular mycorrhizal fungi, *Centaurea solstitialis*, competition, invasion, mycorrhizal responsiveness, *Stipa pulchra*

Introduction

Exotic, invasive plant species often attain much higher densities in their introduced range compared to their native range (Parker *et al.* 2013). Yet the factors that explain exotic plant success are poorly understood. A variety of evolutionary forces have been thought to play an important role in enabling introduced species to colonize and succeed in novel habitats. For example, novel selection pressures may lead to adaptive change in introduced genotypes that can enhance their performance in the new range (Blossey & Nötzold 1995; Weber & Schmid 1998; Mooney & Cleland 2001; Reznick & Ghalambor 2001; Uesugi & Kessler 2013). Hybridization events following introduction can produce novel phenotypes (Ellstrand & Schierenbeck 2000; Campbell, Snow & Ridley 2006). Finally, genetic drift in small founder populations could fix advantageous alleles that lead to differences in phenotypes between introduced and native genotypes (Barrett &

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Husband 1990). Novel genotypes of an introduced species may interact differently with mutualists and/or antagonists in recipient communities (Willis 1999; Siemann & Rogers 2001; Wolfe 2002; Maron, Vilà & Arnason 2004b; Maron *et al.* 2004a; Zangerl & Berenbaum 2005; Agrawal *et al.* 2012), which can in turn affect the way they compete with native plants. A lesser-explored topic is whether introduced genotypes may interact differently with mutualists in their new range, and whether that might affect subsequent interactions with native species.

Enhanced performance by some invasive plants can be attributed to reduced dependency on generalized mutualists in their introduced range (Pendleton & Smith 1983; Richardson *et al.* 2000; Seifert, Bever & Maron 2009; Bunn, Ramsey & Lekberg 2015). For example, Seifert, Bever & Maron (2009) showed that introduced genotypes of the invasive plant, *Hypericum perforatum*, did not respond as positively to AM fungi as did native genotypes and also had finer root architecture, a morphology consistent with reduced mycorrhizal dependence. AM fungi are ubiquitous (Brundrett 1991) and can elicit a wide range of responses from their hosts (Klironomos 2003), as well as mediating interactions between plants (Fitter 1977; Grime *et al.* 1987; Allen & Allen 1990; Hartnett & Wilson 1999; Smith, Hartnett & Wilson 1999; Scheublin, Van Logtestijn & Van Der Heijden 2007). However, other than Seifert, Bever & Maron (2009), few studies have examined whether exotic plant genotypes differ from native genotypes in AM fungal responsiveness, and no work has tested whether reduced AM responsiveness might enhance the competitive performance of exotic invaders in their new range. Thus, it is unclear how widespread this phenomenon is, or whether altered interactions with AM fungi by exotic genotypes provide any competitive advantages to exotic invaders in recipient communities.

The benefits of association with AM fungi can be quite context specific (Hoeksema *et al.* 2010), which may influence when or where reduced mycorrhizal responsiveness is favourable for exotic species. For example, exotics that are introduced to disturbed areas free of competitors, where resources are readily available, and where AM fungal abundance may be reduced (Moorman & Reeves 1979; Stahl, Williams & Christenses 1988; Jasper, Abbott & Robson 1989) may gain few nutritional benefits from the symbiosis. Thus, reduced associations with AM fungi may be beneficial in those habitats if reduced allocation to symbionts translates into faster growth for invaders. Alternatively, if growth is constrained by nutritional limitations (i.e. in competitive environments), reduced associations with AM fungi may be unfavourable. Indeed, correlative results support the idea that many exotic species have low mycorrhizal dependency (Pendleton & Smith 1983; Richardson *et al.* 2000; Pringle *et al.* 2009; Vogelsang & Bever 2009; Bunn, Ramsey & Lekberg 2015), yet it is unclear whether reduced mycorrhizal dependency can actually benefit invaders when competing with more mycorrhizal neighbours. Many studies investigating the effects of AM fungi on competitive interactions between plants typically demonstrate that the competitive advantage favours the plant that can derive the greatest growth benefit from the symbiont (Fitter 1977; Grime *et al.* 1987; Allen & Allen 1990; Hartnett & Wilson 1999; Smith, Hartnett & Wilson 1999; Scheublin, Van Logtestijn & Van Der Heijden 2007). Thus, where reduced responsiveness may benefit invaders in disturbed environments where competition for resources is low, reduced responsiveness may instead be less beneficial in competitive environments.

To explore these ideas, we conducted a common garden experiment where we grew native genotypes from Europe and introduced genotypes from North America of the annual forb, *Centaurea solstitialis* (yellow starthistle). In North America *C. solstitialis* is a potent invader. Our goal was to quantify whether the relative advantages or disadvantages of associating with AM fungi differed for native versus exotic *C. solstitialis* genotypes, and whether altered responsiveness influenced competitive tolerance when plants were forced to compete with a heterospecific. We manipulated competition and the presence of AM fungi to determine whether (i) introduced versus native genotypes differed in their genetically based growth responses to AM fungi, (ii) AM fungi influences competitive tolerance to the native perennial bunchgrass, *Stipa pulchra*, and (iii) whether the influence of AM fungi on competitive tolerance differs between native and introduced genotypes.

Materials and methods

Centaurea solstitialis was originally introduced in the mid-nineteenth century into disturbed grasslands that had been previously invaded by annual exotic grasses (Sun 1997). While *C. solstitialis* continues to be a problematic invader in those habitats, it is less successful in undisturbed grasslands, where native, perennial bunchgrasses appear to resist invasion to some extent (Dukes 2002; Morghan, Kimberly & Rice 2005). *S. pulchra* is a perennial bunchgrass native to North America and is the dominant native competitor occurring in grasslands in the invaded range of *C. solstitialis*.

In April of 2009, we collected seeds of *C. solstitialis* from a minimum of 10 maternal plants in each of 12 populations in North America and Europe, respectively (Table S1 in Supporting Information). Soil was collected during the summer of 2008 from ten native grassland sites across the invaded range in western North America (Table S2). We chose sites where neither *C. solstitialis* nor other strong invaders (as defined in Ortega & Pearson 2005) were present, but in typical habitat for *C. solstitialis*. This reduced the probability of collecting soils containing specialist pathogens of *C. solstitialis*, while still making it likely that collected soils contained generalist AM fungal species that these plants likely encounter. We grew plants only in soil from the introduced range because we were interested in making inferences about the impacts of North American AM fungi on *C. solstitialis* populations. At each site, we took a minimum of 15 soil cores with a bulb planter (10×6 cm^2) at randomly chosen locations covering approximately 0.75 ha and then pooled these samples to provide us with 80 L of live soil. We shipped soil to Guelph University where we extracted the AM fungal spores by filtering the soil through fine mesh filters (45 μm) and collecting what remained on the top of the filter (see Klironomos 2002 for additional methodological details). These spores were mixed with autoclaved field soil

to provide 10 L of inoculum, which was then shipped to the University of Montana campus in Missoula, MT, and stored at 4 °C for one week.

Because of the large number of pots required for our experiments, we increased the AM fungal spore abundance from field-collected soil in the glasshouse prior to the main experiment. We accomplished this by cultivating these AM fungal spores with two plant species, *Sorghum bicolor* and *Allium porrum,* which are known to be good hosts for AM fungi (Wilson & Hartnett 1998) and are commonly used as trap plants for AM fungi. We mixed together the AM fungal inoculum from the ten populations and added this inoculum to 15 L of Turface®, Buffalo Grove, IL, USA (a calcite clay medium) and 15 L of silica sand in a large, shallow tub ($66 \times 40 \times 17.5$ cm^3) to produce 40 L of mixed soil AM fungal inoculum. We surface-sterilized *S. bicolor* and *A. porrum* seeds in a 1% sodium hypochlorite solution and sowed the seeds across the surface of the soil and fertilized every 3 weeks with a half strength 20-2-20 (N-P-K) fertilizer. After 6 weeks of growth, we harvested a small sample of plants and stained a subsample of roots (Brundrett, Piche & Peterson 1984) to confirm mycorrhizal colonization using standard procedures (McGonigle *et al.* 1990). After 12 weeks of growth, we stopped watering, to allow plants to die back naturally, and then, we cut the *S. bicolor* and *A. porrum* roots into small pieces and mixed them back into the sand-Turface® mix to use as our inoculum. This and all subsequent steps of this experiment were conducted in the glasshouses on the University of Montana campus, Missoula, MT.

To test for differences in growth responses to AM fungi between native (European) and introduced (North American) genotypes of *C. solstitialis,* we compared the growth of these genotypes in soils with and without AM fungi. To accomplish this, we mixed and triple autoclaved a potting medium of 20 parts sand, 40 parts Turface® and 40 parts soil gathered locally in Missoula, MT. We added 425 mL of this sterile potting mix to each of 480 (500 mL) pots. To half of these pots, we added an additional 75 mL of our AM fungal inocula. To the other half, we added 75 mL of the sand-Turface®-soil mix without AM fungal spores. To each pot with and without the inoculum, we added two seeds from a single maternal *C. solstitialis* and then thinned to one individual per pot. In total, we established ten replicates (each representing a single maternal plant) from each population in each AM fungi treatment (2 ranges $\times$ 12 populations $\times$ 10 replicates/population $\times \pm$ AM fungi = 480 pots). Daytime temperatures ranged between 18–26 °C and 12–18 °C at night. A constant photoperiod was maintained throughout the experiment with supplemental light that ensured 14 h of light and 10 h of dark each day.

To determine how variation in growth responses to AM fungi might interact with competitive tolerance, we also grew native and introduced genotypes of *C. solstitialis* in competition with the C_3 native North American bunchgrass, *Stipa pulchra*. We sowed four or five *S. pulchra* seeds (Larner Seeds, Bolinas, CA, USA) into each of 500 additional pots, half containing sterile soil and the other half containing the AM fungal inoculum. Pots were watered daily and thinned to one individual per pot. After letting *S. pulchra* grow alone for 3 weeks, we added *C. solstitialis* seeds to each of 480 pots, using the same methods and amount of replication as above. To measure the mycorrhizal responsiveness of *S. pulchra* when grown alone, we also prepared 20 pots (ten with the fungal inoculum and ten in sterile soil) with only a single *S. pulchra* individual.

Plants grew for a total of 18 weeks, after which we harvested all plants, separating shoots from roots. Harvested plants were dried at 65 °C for three days, and roots and shoots were then weighed. We retained a subset of roots from each population, kept separate by treatment, to measure AM fungal colonization using the standard gridline intersect method (McGonigle *et al.* 1990). We measured differences in biomass between plants grown with and without AM fungi to compare the relative costs and benefits of association with AM fungi. Mycorrhizal growth responses are commonly measured in experiments such as these to approximate mycorrhizal dependency (Hoeksema *et al.* 2010), which is a measure of a non-mycorrhizal plant's inability to grow in the absence of phosphorus supplementation (Janos 2007). We chose to measure mycorrhizal growth responses over dependency primarily because the latter requires a much more complicated experimental design.

ANALYSES

We used the GLIMMIX module within SAS 9.2 (SAS Institute 2003) to analyse our data. To determine whether introduced *C. solstitialis* populations from North America differed in responsiveness to AM fungi compared to native populations from Europe, whether AM fungi influences competitive tolerance, and to explore whether AM fungi affects the competitive tolerance of introduced versus natives differently, we used a generalized linear mixed model with a log-normal distribution. In this model, we tested the effects of range, AM fungi, competition and their interactions on total biomass and on root-to-shoot ratios. We also used a generalized linear mixed model with a normal distribution to test for the effects of range, AM fungi, competition and their interactions on the total biomass of *S. pulchra*. To test for differences in AM fungal colonization, measured as the proportion of roots colonized with hyphae, arbuscules and vesicles per population, we used a generalized linear model with a beta distribution to determine the effects of range, competition and their interactions on plants grown with AM fungi. In these and other models testing for the effect of range, we treated population and individual (i.e. each maternal plant) and individuals as repeated measures within each population as random factors.

To test for proportional differences in mycorrhizal responses between ranges, we used a modified Relative Interaction Intensity (RII) Index (after Armas, Ordiales & Pugnaire 2004) to determine how native and introduced *C. solstitialis* populations responded to AM fungi. We defined RII with AM fungi as (Biomass+AMF−Biomass−AMF)/(Biomass+AMF + Biomass−AMF). This index was chosen because (i) it is bounded between +1 and −1, so it controls for extreme values and (ii) it is symmetrical around zero, so values on either side of zero provide a comparable effect of AM fungi, reflecting either a positive or negative interaction depending on the sign. For each maternal plant from each population, we calculated the mean RII with AM fungi by comparing the biomass of one maternal offspring grown with AM fungi with the biomass of its sibling when grown in sterile soil, calculated separately for each competition treatment. We performed a generalized linear mixed model with a normal distribution to test for the effect of range, competition and their interactions on the responsiveness of *C. solstitialis* to AM fungi. We treated the same random factors as above in this model.

Results

MYCORRHIZAL COLONIZATION

Inoculation with AM fungi resulted in colonization of *C. solstitialis* roots, whereas uninoculated plants were not

colonized. There was no significant difference in root colonization between native (European) and introduced (North American) genotypes inoculated with AM fungi ($F_{1,16} = 0.09$, $P = 0.64$, Table 2). However, *C. solstitialis* roots were over twice as colonized by AM fungi when grown in competition (81 ± 3%) than when grown alone (37 ± 3%, $F_{1,16} = 73.45$, $P < 0.0001$) and colonized *C. solstitialis* plants had a far higher percentage of vesicles in their roots when grown in competition (58 ± 7%) than when grown alone (7 ± 3%). We found no arbuscules in any of our roots.

EFFECTS OF AM FUNGI ON PLANT GROWTH

We examined whether there were differences in how AM fungi affected the absolute biomass of native versus introduced genotypes of *C. solstitialis*, and whether the effects of AM fungi differed when plants were grown alone versus in competition. AM fungi ($F_{1,66} = 6.36$, $P < 0.01$, Table 1) and competition by the North American bunchgrass *S. pulchra* ($F_{1,66} = 1019.77$, $P < 0.001$) each significantly reduced the total biomass of *C. solstitialis* (Fig. 1). However, there was a significant interactive effect of AM fungi and competition (AM fungi × competition; $F_{1,67} = 16.35$, $P < 0.001$). AM fungi had a weak but positive effect on plants grown alone, but competition shifted the plant response to AM fungi from positive to negative (Fig. 1).

We examined whether the effects of AM fungi on the competitive tolerance of *C. solstitialis* differed between native and introduced genotypes. Although the total plant biomass did not differ between populations from the native and non-native ranges (when averaged across AM fungi and competition treatments, $F_{1,22} = 1.93$, $P = 0.18$), the negative effect of AM fungi on plant biomass in competition was stronger in populations originating in the native range (range × AM fungi × competition; $F_{1,67} = 4.03$, $P < 0.05$). Hence, AM fungi increased the negative effect of competition by *S. pulchra* on plants from native *C. solstitialis* populations, whereas AM fungi had relatively small effects on the competitive tolerance of introduced genotypes of *C. solstitialis*.

We also used the Relative Interaction Intensity (RII) Index to compare the proportional biomass response of native versus introduced populations to AM fungi when grown alone and in competition (Table 2, Fig. 2). Similar to the results presented above, there was no difference in the proportional

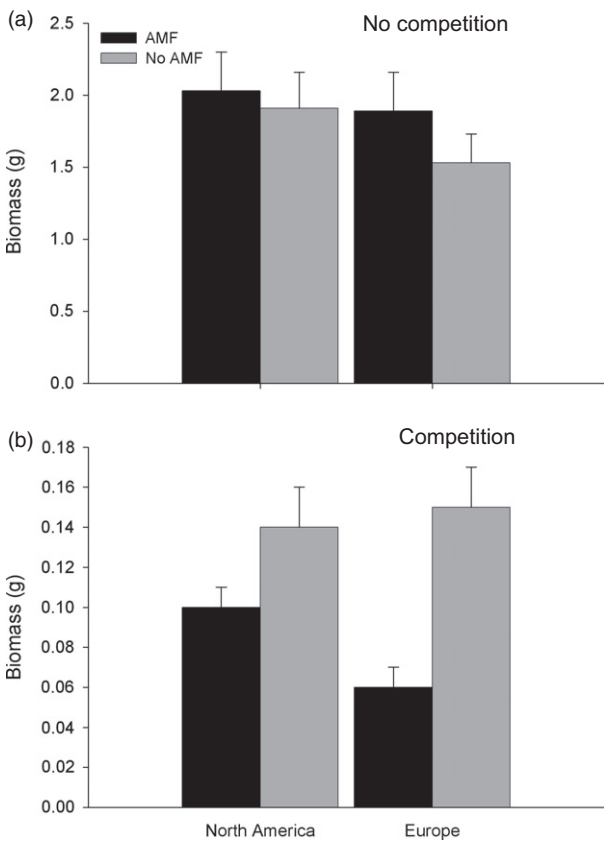

Fig. 1. Mean total biomass (+SE) of non-native (North American) and native (European) genotypes of *Centaurea solstitialis* plants grown (a) alone and (b) with the North American native bunchgrass, *Stipa pulchra*. Bars represent the mean of population means ($n = 12$ North American populations and 12 European populations).

Table 1. Generalized linear mixed-model ANOVA with a log-normal distribution testing the effects of range (North America versus Europe), AM fungi, competition and their interactions on total biomass and root-to-shoot ratios. Population and individual (i.e. each maternal plant) were treated as random factors

	Biomass			Root to shoot		
Factors	Num. d.f.	Den. d.f.	*F*-value	Num. d.f.	Den. d.f.	*F*-value
Range	1	22	1.84^{ns}	1	22	1.47^{ns}
AMF	1	66	6.13*	1	64	12.15**
Competition	1	66	1014.4***	1	64	245.16***
Range × AMF	1	66	1.34^{ns}	1	64	3.76(*)
AMF × Comp	1	67	15.91***	1	64	4.13*
Range × Comp	1	66	0.01^{ns}	1	64	4.11*
Range × AMF × Comp	1	67	3.84*	1	64	0.02^{ns}

$^{ns}P > 0.06$; *$0.05 \geq P > 0.01$; **$0.01 \geq P > 0.001$; ***$P \leq 0.001$ (ANOVA *F*-values).
$^{(*)}P = 0.057$.

Table 2. Generalized linear mixed-model ANOVA with a normal distribution to test for the effect of range, competition and their interactions on the proportional responsiveness of *C. solstitialis* to AM fungi. Population and individual (i.e. each maternal plant) were treated as random factors

	Colonization rate			Relative Interaction Intensity (RII)		
Factors	Num. d.f.	Den. d.f.	*F*-value	Num. d.f.	Den. d.f.	*F*-value
Range	1	16	0.09^{ns}	1	39	1.50^{ns}
Competition	1	16	73.45***	1	39	18.41***
Range × Comp	1	16	0.17^{ns}	1	39	4.32*

$^{ns}P > 0.05$; *$0.05 \geq P > 0.01$; **$0.01 \geq P > 0.001$; ***$P \leq 0.001$ (ANOVA *F*-values).

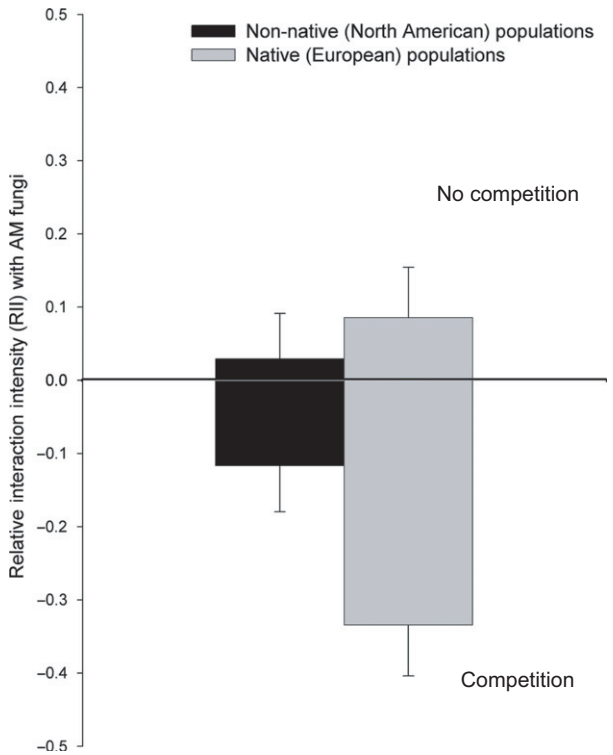

Fig. 2. Proportional response of *Centaurea solstitialis* to AM fungi, measured by the Relative Interaction Intensity (RII) Index (+SE) of non-native (North American, black bars) or native (European, grey bars) populations of *C. solstitialis* to AM fungi from North America, with and without competition from the North American native bunchgrass, *Stipa pulchra*. Bars represent the mean of population means (n = 12 North American populations and 12 European populations). Positive values represent plants grown alone, which received a net benefit from AM fungi. Negative values represent plants grown in competition, which received a net negative interaction with AM fungi.

effects of AM fungi between ranges when averaged across competition treatments (range $F_{1,39} = 1.50$, $P = 0.23$), but the response did differ between ranges in the presence of competition (range × competition $F_{1,39} = 4.32$, $P < 0.05$). Plants from native populations were significantly more suppressed by AM fungi than plants from introduced populations (post hoc comparison, $t = 2.32$, d.f. = 39, $P < 0.05$). In contrast, when grown alone, there was no significant difference in the proportional effect of AM fungi on plants from the native versus non-native range (post hoc comparison, $t = -0.61$, d.f. = 39, $P = 0.55$).

We also examined the effects of AM fungi and competition by native and introduced genotypes of *C. solstitialis* on the biomass of *S. pulchra*. Neither range ($F_{2,35} = 0.81$, $P = 0.45$), AM fungi ($F_{1,33} = 0.01$, $P = 0.94$), nor their interaction ($F_{2,35} = 0.35$, $P = 0.88$) significantly affected *S. pulchra* biomass.

ROOT-TO-SHOOT RATIOS

We examined the effects of AM fungi, competition and their interaction on biomass allocation to roots versus shoots by native versus introduced populations of *C. solstitialis*. Overall, *C. solstitialis* plants allocated more biomass to roots when grown with AM fungi ($F_{1,64} = 12.15$, $P < 0.001$, Table 1), but more to shoots when grown in competition ($F_{1,64} = 245.16$, $P < 0.001$). However, the AM fungi effect varied depending on whether *C. solstitialis* was grown in competition or not (AM fungi × competition; $F_{1,64} = 4.13$, $P < 0.05$). When grown alone, AM fungi had no significant effect on the root-to-shoot ratio of *C. solstitialis*. However, when grown in competition, mycorrhizal *C. solstitialis* allocated significantly more biomass to shoots than non-mycorrhizal plants (contrast, $t = 3.86$, d.f. = 64, $P < 0.01$).

There was no difference in root-to-shoot ratios between native and introduced populations of *C. solstitialis* overall ($F_{1,22} = 1.47$, $P = 0.24$). The effect of range was only apparent when plants were grown alone (range × competition; $F_{1,64} = 4.11$, $P < 0.05$, Fig. 3). Plants from introduced populations tended to allocate more to roots than native populations when grown alone, but there was no difference between ranges when grown in competition. Range also had a marginally significant interaction with AM fungi (range × AM fungi; $F_{1,64} = 3.76$, $P = 0.06$), as the positive shift in allocation to roots in response to AM fungi tended to be greater for native compared to introduced populations of *C. solstitialis*. This pattern did not vary significantly with competition (range × AM fungi × competition; $F_{1,64} = 0.02$, $P = 0.90$), but the AM fungal-induced shift in root allocation was most apparent for native populations of *C. solstitialis* in the presence of competition.

Discussion

Our experiments comparing the effects of AM fungi on the growth of native (European) and introduced (North American)

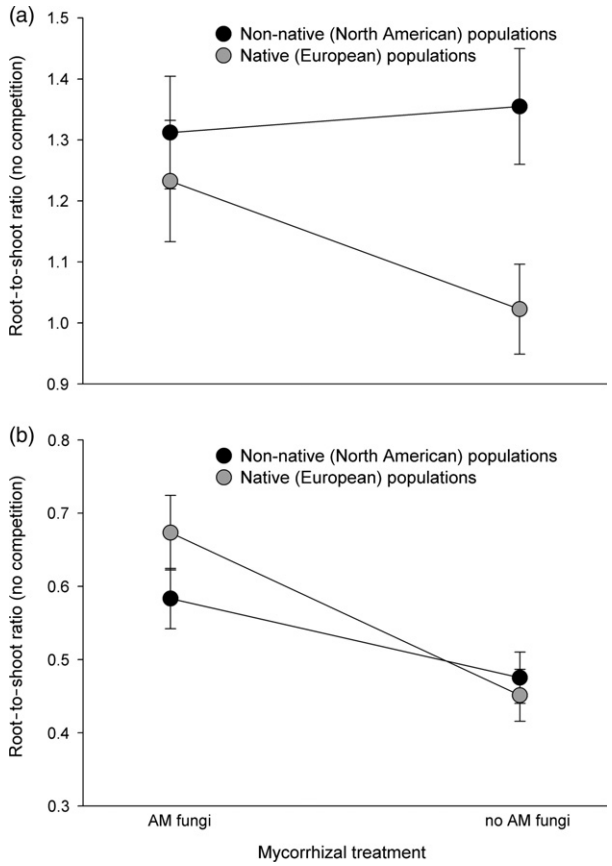

Fig. 3. Root-to-shoot biomass ratio (±SE) of non-native North American (black circles) or native European (grey circles circles) populations of *C. solstitialis*, grown (a) alone and (b) in competition with *S. pulchra*, with and without AM fungi.

genotypes of *C. solstitialis* provide novel insights into the ecological implications of altered interactions with a ubiquitous soil mutualist by an exotic plant. When grown alone, plants from both ranges derived weak benefits from AM fungi, but introduced genotypes tended to be less responsive to AM fungi than native genotypes. When grown with the strong competitor *S. pulchra* in the absence of AM fungi, there was no difference in competitive tolerance between introduced and native genotypes. However, AM fungi suppressed the biomass of *C. solstitialis* when grown in competition, and the suppressive effect of AM fungi was much stronger on native as opposed to introduced genotypes. These results imply that invasive genotypes pay a lower cost of association with AM fungi than do native genotypes, and these reduced costs are particularly manifest when plants are grown in competition.

This is the first study to our knowledge that both compares the differential responses of native and invasive genotypes to AM fungi and then examines how these responses may be fundamentally changed in a competitive context. *Centaurea solstitialis* was originally introduced to and experiences its greatest success in disturbed grasslands dominated by weakly mycorrhizal annual exotic grasses (Sun 1997). Other studies have shown that low dependency on AM fungi may benefit invasive plants (Pendleton & Smith 1983; Richardson *et al.* 2000; Pringle *et al.* 2009; Vogelsang & Bever 2009), particularly in disturbed environments where weakly mycotrophic exotic annuals such as *Bromus tectorum* have already degraded AM fungal communities (Stinson *et al.* 2006; Vogelsang & Bever 2009; Lekberg *et al.* 2013). Here, the weak responsiveness to AM fungi when plants from both ranges were grown alone may explain their success in disturbed, competition-free environments, where AM fungal communities may be compromised (Moorman & Reeves 1979; Stahl, Williams & Christenses 1988; Jasper, Abbott & Robson 1989).

In contrast, *C. solstitalis* experiences more limited success where bunchgrasses such as *S. pulchra* are large and well-established, compared to where they are small (Morghan, Kimberly & Rice 2005). We found that *S. pulchra* had a strong negative effect on *C. solstitialis*, and the competitive effect did not differ between native and introduced genotypes in the absence of AM fungi. However, AM fungi suppressed the biomass of *C. solstitialis* plants when grown in competition, even though this effect was much weaker for introduced genotypes compared to native genotypes. Thus, our results suggest a role for AM fungi in the apparent biotic resistance offered by *S. pulchra* and potentially by other native bunchgrasses in native-dominated habitats.

What explains the strong suppression of *C. solstitialis* by *S. pulchra* that was particularly evident when AM fungi were present? Growth depressions are not uncommon in plants infected with AM fungi (Klironomos 2003; Hoeksema *et al.* 2010), particularly at low light levels (Hayman 1974; Tester, Smith & Smith 1985; Olsson, Rahm & Aliasgharzad 2010). *Centaurea solstitialis* plants were heavily shaded by *S. pulchra*. Shaded *C. solstitialis* plants allocated more biomass above-ground than plants that were grown alone. This is a common plant response when light becomes limiting (Poorter & Nagel 2000). Thus, the growth depressions observed in shaded *C. solstitialis* plants are unsurprising. In addition to directly acquiring limiting C from their hosts, AM fungi can also increase asymmetric competition between seedlings and adult plants connected into a common mycorrhizal network (CMN, Newman 1988; Zabinski, Quinn & Callaway 2002; Kytöviita, Vestberg & Tuomi 2003; Merrild *et al.* 2013) by increasing nutrient pre-emption by strong C-delivering hosts to a greater extent than C-limited hosts (Lekberg, Hammer & Olsson 2010; Kiers *et al.* 2011). We speculate that AM fungi exchanged more P for C with *S. pulchra* than with C-limited *C. solstitialis* plants, which created stronger P-limitations for *C. solstitialis* in mycorrhizal treatments.

Interestingly, mycorrhizal *C. solstitialis* plants grown in competition with *S. pulchra* had significantly higher colonization by AM fungi than plants grown alone. This is unusual, as mycorrhizal colonization in roots is often reduced at low irradiance (Graham, Leonard & Menge 1982; Tester, Smith & Smith 1985; Olsson, Rahm & Aliasgharzad 2010), and plants grown with *S. pulchra* were heavily shaded. However, the difference in colonization was largely the result of a greater proportion of vesicles, the carbon storage structures for AM

fungi. Furthermore, we observed no arbuscules, the active site of resource exchange between plant and fungus in *C. solstitialis* plants. Thus, it is unlikely that the higher colonization in *C. solstitialis* reflects an active symbiosis. Alternatively, the carbon stored in vesicles in *C. solstitialis* roots likely derived from *S. pulchra* and was transferred via the CMN, since carbon transfer within a CMN is source/sink-driven (Francis & Read 1984; Waters & Borowicz 1994; Nakano, Takahashi & Kimura 2001; Lerat *et al.* 2002; Lekberg, Hammer & Olsson 2010). We are not suggesting that this carbon was transferred to nurse *C. solstitialis* seedlings. On the contrary, experimental evidence has shown that carbon transferred within CMNs is unlikely to be available to plants, but remains locked in fungal tissue (Pfeffer *et al.* 2004; Voets *et al.* 2008; Lekberg, Hammer & Olsson 2010). Our results support the mycocentric view of the symbiosis, where C-limited hosts are used as storage units for fungi that are supported by a stronger C-delivering host (Lekberg, Hammer & Olsson 2010). Indeed, the growth reduction in plants with high vesicle colonization clearly suggests that *C. solstitialis* seedlings derived no benefit from the C stored in their roots since high vesicle colonization was correlated with growth *reductions* in those plants.

Not only did AM fungi increase the suppressive effects of competition by *S. pulchra* on *C. solstitialis* plants, but also the magnitude of the response by *C. solstitialis* depended on plant origin. Plant species that are more responsive to AM fungi may have less control over exploitation by AM fungi than plant species that are less responsive to AM fungi (Grman 2012). Thus, when conditions do not favour mycorrhizal mutualisms, species that are more responsive are more likely to experience costs of hosting AM fungi. The stronger suppressive effect of AM fungi on native genotypes may suggest that they are more responsive to, and more prone to exploitation by AM fungi. Although range-based differences in total biomass between mycorrhizal and non-mycorrhizal plants were weak when plants were grown alone, we did observe differences in allocation to roots versus shoots between native and exotic genotypes that suggest greater control over allocation by invasive, compared to native genotypes. When grown alone, native non-mycorrhizal genotypes decreased their allocation to roots, whereas introduced genotypes did not shift their biomass allocation in response to AM fungi. This also suggests that introduced genotypes can exploit a greater soil volume for nutrient acquisition without depending on the nutritional services delivered by AM fungi. This might benefit non-native more than native genotypes when nutrients and AM fungi are limiting.

Although we are unsure of the evolutionary drivers of variation in mycorrhizal responsiveness between native and introduced populations, it is unlikely that our results were due to strong founder effects. Molecular analysis on the neutral genetic variation in 22 *C. solstitialis* populations sampled from the southernmost to the northernmost extent of the invaded range revealed high genetic diversity within populations, counter to what one would expect if only a few genotypes were introduced into North America (Sun 1997). Thus, our common garden experiment suggests that introduced genotypes of the invader *C. solstitialis* have undergone an evolutionary change whereby they do not pay a cost of associating with AM fungi when facing strong competition for light.

Many studies have demonstrated that AM fungi can amplify the effects of competition by strongly AM responsive plants (Fitter 1977; Grime *et al.* 1987; Hartnett *et al.* 1993; Hartnett & Wilson 1999; Smith, Smith & Jakobsen 2003; Scheublin, Van Logtestijn & Van Der Heijden 2007) and that different plant species can differ in the magnitude of these effects (Hartnett *et al.* 1993; Hartnett & Wilson 1999). However, this is the first work to our knowledge to demonstrate genotypic differences in response to AM fungi when grown in interspecific competition. Of particular, future interest is elucidating the drivers behind these differences. More broadly, we advocate for additional studies to assess how commonly native and invasive plants demonstrate genetically based differences in the way they interact with soil-borne mutualists in recipient communities, and importantly, how these may benefit invaders across different environmental contexts.

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Data accessibility

Data associated with this study are deposited in the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.ph02r (Waller *et al.* 2016).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Sites in North America and Europe where seeds were collected.

Table S2. Sites where soil was collected.