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Interactions between functionally diverse fungal mutualists inconsistently affect plant performance and competition

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Plants form mutualistic relationship with a variety of belowground fungal species. Such a mutualistic relationship can enhance plant growth and resistance to pathogens. Yet, we know little about how interactions between functionally diverse groups of fungal mutualists affect plant performance and competition. We experimentally determined the effects of interaction between two functional groups of belowground fungi that form mutualistic relationship with plants, arbuscular mycorrhizal (AM) fungi and *Trichoderma*, on interspecific competition between pairs of closely related plant species from four different genera. We hypothesized that the combination of two functionally diverse belowground fungal species would allow plants and fungi to partition their symbiotic relationships and relax plant–plant competition. Our results show that: 1) the AM fungal species consistently outcompeted the *Trichoderma* species independent of plant combinations; 2) the fungal species generally had limited effects on competitive interactions between plants; 3) however, the combination of fungal species relaxed interspecific competition in one of the four instances of plant–plant competition, despite the general competitive superiority of AM fungi over *Trichoderma*. We highlight that the competitive outcome between functionally diverse fungal species may show high consistency across a broad range of host plants and their combinations. However, despite this consistent competitive hierarchy, the consequences of their interaction for plant performance and competition can strongly vary among plant communities.

Keywords: arbuscular mycorrhizal fungi, fungal symbionts, fungal–fungal interactions, plant–fungal interactions, plant–plant interactions, *Trichoderma*

Introduction

Species interactions within one trophic or functional group may affect species interactions at other levels or organization (Menge and Sutherland 1976, Agrawal et al. 2007, Bascompte and Jordano 2007, Tylianakis et al. 2008, Valiente-Banuet et al. 2015). For instance, competition among multiple herbivores or pollinators could potentially

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affect interactions among their host plants, and thus local plant diversity (Olff and Ritchie 1998, Biesmeijer et al. 2006, Bagchi et al. 2014, Temeles et al. 2016). Moreover, it has often been shown that negative interactions among herbivores can weaken their net effects on the dominant plant species within a community, which then reduce the evenness within that plant community (Hulme 1996, Allan and Crawley 2011, Young et al. 2013, Mortensen et al. 2018). Currently, we lack an understanding of whether competitive interactions among plant mutualists have similar consequences for plant community structure.

Plants associate with a variety of mutualist organisms both above- and belowground. These associations can increase plant fitness by increasing their ability to acquire resources and boosting their resistance to herbivores (Bronstein et al. 2006, Van Dam and Heil 2011, Philippot et al. 2013). A significant proportion of plant mutualists are microorganisms residing belowground that can increase plant access to vital nutrients as well as help plants to overcome herbivore and pathogen pressure both aboveground and in the soil (Pineda et al. 2010, Biere and Bennett 2013, Vandenkoornhuyse et al. 2015). In turn, plants provide carbon-based compounds as resources to these belowground microorganisms. Among the vast diversity of belowground mutualists, two fungal groups are widely recognized for their symbiotic associations with plants: arbuscular mycorrhizal (AM) fungi (Bonfante and Genre 2010, van der Heijden et al. 2015) and fungi in the genus *Trichoderma* (Harman et al. 2004, Martinez-Medina et al. 2016).

The symbiosis between AM fungi and plants mainly involves transfer of photosynthetic carbon from plants to AM fungi and nutrients (e.g. nitrogen and phosphorus) acquired by AM fungi from soil to plants (Hodge and Storer 2014, van der Heijden et al. 2015). The *Trichoderma* symbiosis with plants is widely known for the suppression of several antagonistic microbial agents of plants by *Trichoderma*, which subsequently benefits by obtaining carbon-related resources excreted by plants in the soil (Harman et al. 2004, Martinez-Medina et al. 2016). While AM fungi establish symbioses with most terrestrial plants, *Trichoderma* are among the most commonly isolated saprophytic fungi from natural and agricultural soils. A key difference between AM fungi and *Trichoderma* relates to their dependence on host plants. That is, AM fungi cannot complete their life cycle without a host plant (obligate symbionts) (Verbruggen and Kiers 2010, van der Heijden et al. 2015), whereas *Trichoderma* can (facultative symbionts) (Harman et al. 2004, Martinez-Medina et al. 2016). Another distinction between the two fungi is that AM fungi and plants form symbiotic structures within plant roots, hence AM fungi obtain carbon in intimate contact with plant roots (van der Heijden et al. 2015), whereas *Trichoderma* obtain resources mostly from the root exudates (Martinez-Medina et al. 2016). Interestingly, previous studies have shown that AM fungi can suppress the performance of *Trichoderma* in plant monocultures mainly through the alteration of the rhizosphere environment that constrains the proliferation of *Trichoderma* (Martínez-Medina et al. 2009, 2011).

The presence of multiple belowground fungal mutualists can relax interspecific competition between plants (Wagg et al. 2011a). Such a relaxed competition usually occurs due to a greater niche differentiation among fungal mutualists, given that there is a choice of host plants (Wagg et al. 2011a). As a result, plants are able to partition symbiotic partners, which in turn could relax plant competition. Most of the evidence for effects of fungal mutualists on plant–plant interactions comes from plant competition experiments using multiple species of AM fungi (Hart et al. 2003, Wagg et al. 2011a, b, Powell and Rillig 2018). Currently it is unknown whether the interaction between functionally diverse groups of fungal species can also foster niche differentiation between plants in terms of partitioning fungal mutualist partners and thereby affecting plant–plant competition. While AM fungi have been shown to suppress the growth of *Trichoderma* in plant monocultures (Martínez-Medina et al. 2009, 2011), we hypothesize that the presence of multiple plant species could foster niche differentiation between these two fungi. Alternatively, we may even expect stronger competition between these two fungal groups for acquiring the most favourable host plant, for instance the one with larger carbon supply (Green et al. 1999, Harman et al. 2004). Whether niche differentiation between these two functionally different fungal mutualists takes place in the presence of multiple host plants will thus be key for their net effects on interspecific plant competition.

In this study, we investigate the effects of interactions between an AM fungal species and a *Trichoderma* species on the interaction between several pairs of congeneric grassland plant species that co-occur in nature. The congener plants were chosen because we assume that closely related plant species are likely to exert greater competitive effects on each other than more distantly related species. We hypothesize that the relative strength of interspecific competition between the pairs of congeneric plants will be relaxed in the presence of two functionally diverse fungal mutualists, particularly when the presence of multiple host plants also enhances niche differentiation between these two fungal species.

Material and methods

Plant and fungal species

In our experiment, we used four congeneric pairs of common forb species: 1) *Centaurea jacea* and *Centaurea scabiosa* (Asteraceae); 2) *Leontodon autumnalis* (now *Scorzoneroides autumnalis*) and *Leontodon hispidus* (Asteraceae); 3) *Plantago lanceolata* and *Plantago media* (Plantaginaceae), and 4) *Prunella grandiflora* and *Prunella vulgaris* (Lamiaceae). These congeneric plant pairs often co-occur in semi-natural grasslands of the Molinio–Arrhenatheretea or Festuco–Brometea type in Germany (Jäger and Werner 2011). All these plants are known hosts of AM fungi (Harley and Harley 1987).

Seeds of these plants were purchased from Rieger-Hofmann GmbH, Germany. We used *Rhizoglomus irregulare* (previously known as *Rhizophagus irregularis*) as AM fungus in this experiment. *Rhizoglomus irregulare* soild inoculum (INOQ-Sprint) was purchased from INOQ GmbH, Germany (<https://inoq.de>) with 220 mycorrhizal units per ml in sand. The *Trichoderma* species used in this study was *Trichoderma harzianum* isolate T-78 (CECT 20714, Spanish Type Culture Collection; Martínez-Medina et al. 2009). *Trichoderma harzianum* was cultured on a solid medium containing commercial oat and vermiculite (Martínez-Medina et al. 2009).

Experimental set-up

Plant seeds were surface sterilized in 10% sodium hypochlorite (for about a minute), rinsed thoroughly with water and germinated in a 1:1 (v:v) sterilized (autoclaved) sand: vermiculite mixture at 16 h daylight (20°C) and 8 h darkness (16°C). Seeds were germinated for two to three weeks until the seedlings reached a height between 3 and 5 cm and thereby considered ready for transplantation.

Seedlings were transplanted to 1 l PVC pots (top width = 11 cm, bottom width = 8.3 cm, height: 12 cm) that were filled with ~800 g of a 1:1 sterilized (autoclaved) sand:vermiculite mixture. Substrate inoculation with the fungal inoculum was done as four treatments: no fungi (substrate without addition of any fungal inoculum), AM fungi only (substrate inoculated with *R. irregulare* inoculum), *Trichoderma* species only (substrate inoculated with *T. harzianum*), and both fungi together (substrate inoculated with both *R. irregulare* and *T. harzianum*). Inoculation with the AM fungi was achieved by mixing 10% of the mycorrhizal inoculum with the substrate before transplanting according to Fernández et al. (2014). Please note that the AM fungal inoculum may also contain other microbial communities as they were obtained from open pot cultures (ex-vitro), and thus AM fungal effects are the net effects of the complex inoculum. Inoculation with *T. harzianum* was achieved by mixing the *T. harzianum* inoculum through the substrate to a final density of 1×10^6 conidia g^{-1} before transplanting (Martínez-Medina et al. 2009). We constituted a substitutive design for the fungal mixtures, that is, in the treatment with both fungi, the amount of each of the fungi was half the amount of their respective monocultures.

Half-strength Hoagland nutrient solution (Hoagland and Arnon 1950) with 25% of the standard phosphorus concentration (Fernández et al. 2014) was added to all treatments (250 ml kg^{-1} of soil) at the beginning of the experiment. We placed the PVC pots on petri dishes to allow individual water/nutrient supply and to prevent cross-contamination between the different treatments. For each plant genus, we established three plant communities across the four substrates: two monocultures and one mixed plant community. Plant monocultures were assembled with four plant individuals from the same species, whereas mixed plant communities also contained four individuals, but assembled with two individuals from each of the species from the same genus. In total, we established 12 plant communities (8 monocultures + 4 mixed communities with the pairs of plant congeners) in four different substrates (control + three fungal treatments) and replicated each treatment combinations five times (n = 240 pots).

To minimize water stress for plants at the beginning of the experiment, we sprinkled tap water from the top for the first two weeks. After week 2, we watered the plants three times a week, alternatively with tap water (50 ml) and low phosphorus half-strength Hoagland solution (50 ml) on the petri-dishes (Supplementary material Appendix 1 Fig. A1). After week 7, we doubled the amount of water and nutrient solution to 100 ml. Plants were grown for 13 weeks in a climate chamber using a 16 h light (20°C) and 8 h dark (16°C) cycle and 60% relative humidity. The light intensity in the climate chamber (during the 16 h day light) in the range of 450–500 μmol m^{-2} s^{-1}.

Plant measurements

At the end of the experiment, we harvested plant shoots by clipping plants at the soil surface. We assessed species-specific dry shoot biomass after drying the fresh biomass at 70°C for 72 h. We also collected the combined roots (a fraction of which were taken for the measurement of fungal colonization; details below) from each pot to estimate community level root biomass. Remaining soil was removed by washing the roots thoroughly with tap water before the roots were dried at 70°C for 72 h.

Fungal colonization

For the estimation of AM fungal colonization in roots, we gently collected species-specific roots right beneath the given plant individual. The AM fungal colonization of individual plant roots was estimated after clearing washed roots in 10% KOH and subsequent staining of fungal structures with 5% ink (Koh-I-Noor) and 2% acetic acid (Vierheilig et al. 2005). To calculate the percentage of total root colonization, we used the gridline intersection method (Giovannetti and Mosse 1980) using a binocular stereo microscope.

To quantify the amount of colony forming units (CFU) of *T. harzianum*, we sampled substrate from the bulk rhizosphere at the end of the experiment, because the presence of *Trichoderma* is higher in the rhizosphere than on the roots (Martinez-Medina et al. 2016). Thus, the estimation of *Trichoderma* was possible only at the plant community level. To quantify *Trichoderma*, we made a series of dilutions of the substrate in sterile water. We used the plate count technique using potato dextrose agar (PDA), amended with 50 mg l^{-1} rose bengal and 100 mg l^{-1} streptomycin. Plates were incubated at 28°C in darkness, and CFU were counted after 5 days. The CFU were calculated per gram of dry (1 week, 50°C) soil (Martínez-Medina et al. 2011).

Calculation of the plant's competitiveness

The strength of inter- versus intraspecific competition ('competitiveness') of the two plant species within each genus was calculated as:

$$\text{Competitiveness}_n = (\text{Bmix}_n - \text{Bmono}_n)/\text{Bmono}_n$$

where Bmix is the total shoot biomass (per pot) of a given species when grown in mixture, and Bmono is the total shoot biomass (per pot) when the same species is grown in its monoculture, for n replicates (Kaisermann et al. 2017). The larger the difference in competitiveness the greater is competitive dissimilarity between the congeners. Effects of fungal mutualists on this competitive dissimilarity are interpreted as effects of the fungal mutualists on the extent of interspecific competition between the plants.

Statistical analysis

We analyzed the effects of fungal treatments on plant performance (species-specific shoot biomass per plant) using two-way ANOVA mixed-effects models with fungal treatments (control, AM fungi only, *Trichoderma* only, AM fungi and *Trichoderma* together) and plant community type (monoculture and mixtures) as explanatory variables for each plant species separately. As our shoot biomass measurements were on the plant individual level, we adopted a mixed-effect model approach using the pot identity as the random intercept to avoid pseudo-replication of individual plants at the pot level. This analysis allowed us to estimate whether the performance of plants is affected by the presence of multiple fungal mutualists and whether they are grown in monoculture or with their congener. Effects of plant species within a genus and fungal treatments on plant competitiveness were also analysed using two-way ANOVAs with fungal treatments, plant species identity (the two species within each genus) and their interaction as explanatory variables, for each plant genus separately. Since roots from pots containing plant species mixtures could not be separated for plant species, effects of plant community and fungal treatments on root biomass was analyzed using two-way ANOVAs in which plant community had three levels (two monocultures and one mixture).

We then analyzed the effects of fungal treatments and plant communities on the outcome of the interaction between AM fungi and *Trichoderma* (colonization rate of hosts) using two-way ANOVAs with fungal treatment (fungal monocultures or mixtures), plant community treatment (plant monocultures or mixtures) and their interactions as explanatory variables, for each plant congeneric pair separately. As AM fungal colonization rates were measured at plant species level (by aggregating the plant individuals), analyses of AM fungal colonization rates were run for each plant species separately. But since *Trichoderma* could only be quantified at pot level, *Trichoderma* colonization was analyzed separately for each plant genus, using three levels of plant community type (two monocultures and one mixture). The models for *Trichoderma* colonization were analyzed using negative binomial errors as *Trichoderma* CFU counts were overdispersed and homogeneity of variance was lacking when modelled with Gaussian and Poisson errors. Post hoc multiple comparisons (Tukey HSD) were carried out for significant treatment effects (p-value < 0.05) in all cases. For all mixed-effects models, we estimated marginal (only fixed effects) and conditional (fixed + random effects) R^2 as explained by Nakagawa and Schielzeth (2013). For models without random terms, the effect size for each treatment and their interactions were estimated using partial-omega squared (ω^2) (Olejnik and Algina 2003). Greater ω^2 values represent larger effect sizes.

All final statistical models used in this study met linear model assumptions. All statistical analyses were carried out in R statistical software (<www.r-project.org>). Mixed-effects models were run with the lme4 package (Bates et al. 2015), whereas post hoc tests were performed using the multcomp package (Hothorn et al. 2008). The MuMIn package was used to estimate marginal and conditional R^2 (Barton 2018). Model diagnostics were performed using the DHARMa package (Hartig 2019).

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.5hs7h37> (Thakur et al. 2019).

Results

Fungal responses

In general, AM fungal colonization of roots was not strongly affected by the presence or absence of *Trichoderma*, nor by the identity of plant competitors, i.e. whether plants were grown in monocultures with conspecifics or in mixtures with heterospecific congeners. Only in two instances, plant-specific AM fungal colonization of roots (%) was significantly affected by treatment (Table 1). First, root colonization of *Centaurea jacea* by AM fungi was higher when it grew in mixture with *C. stoebe* than when it grew in *C. jacea* monoculture (Fig. 1). This was, however, only true when plants were inoculated with both AM fungi and with *Trichoderma*, and not when inoculated with AM fungi only (Fig. 1). Second, we found that *P. media* had lower root colonization by AM fungi when grown in mixture with *Plantago lanceolata* than in *P. media* monoculture (Table 1, Fig. 1). This result was true irrespective of whether it was inoculated with AM fungi alone or in combination with *Trichoderma*.

By contrast, the number of *Trichoderma* CFUs was strongly suppressed by the presence of AM fungi (Fig. 2, Supplementary material Appendix 1 Table A1). Only in two out of the twelve plant communities, the negative effect of AM fungi on *Trichoderma* was not statistically significant (*Plantago* mixtures and *P. grandiflora* monocultures (Fig. 2), resulting in significant interactions between plant

Table 1. Results of two-way ANOVA on AM fungal colonization for eight plant species affected by plant community composition (monocultures versus mixtures) and belowground mutualists (AM fungi alone versus AM fungi with *Trichoderma*). F-values and p-values are in bold when statistically significant ($p < 0.05$). The ω^2 values are partial-ω^2 as a measure of effect size of the treatments and their interactions.

		Plant community (P)			Belowground mutualists (B)			P×B		
Genera	Plant species	F-value	p-value	ω^2	F-value	p-value	ω^2	F-value	p-value	ω^2
Centaurea	*C. jacea*	$4.12_{1,16}$	0.05	0.13	$0.83_{1,16}$	0.37	<0.01	$\mathbf{8.79_{1,16}}$	**<0.01**	0.28
	C. scabiosa	$0.30_{1,16}$	0.58	0.03	$2.13_{1,16}$	0.16	0.05	$0.08_{1,16}$	0.77	0.04
Leontodon	*L. autmnalis*	$0.28_{1,15}$	0.28	0.03	$1.23_{1,15}$	0.59	0.01	$3.62_{1,15}$	0.07	0.12
	L. hispidus	$0.39_{1,15}$	0.54	0.03	$<0.01_{1,15}$	0.93	0.05	$3.39_{1,15}$	0.08	0.11
Plantago	*P. lanceolata*	$0.01_{1,16}$	0.89	0.05	$0.68_{1,16}$	0.42	0.01	$0.74_{1,16}$	0.40	0.01
	P. media	$\mathbf{22.46_{1,16}}$	**<0.001**	0.51	$<0.01_{1,16}$	0.94	0.05	$0.97_{1,16}$	0.33	<0.01
Prunella	*P. grandiflora*	$4.10_{1,16}$	0.05	0.13	$0.06_{1,16}$	0.79	0.04	$0.79_{1,16}$	0.38	0.01
	P. vulgaris	$0.16_{1,14}$	0.68	0.04	$<0.01_{1,14}$	0.94	0.05	$2.52_{1,14}$	0.13	0.07

community type (monoculture versus mixture) and the presence or absence of AM fungi for these two plant genera (Supplementary material Appendix 1 Table A1). The strength of the suppression of *Trichoderma* by AM fungi was largely independent of plant community combinations (Supplementary material Appendix 1 Table A1).

Plant responses

We found stronger effects of plant community composition (plant monocultures versus mixtures) than of fungal treatments on plant shoot biomass for most of the plant species (Table 2). In particular, the shoot biomass of *Leondoton* and *Plantago* species strongly depended on whether their competitors were conspecifics or heterospecific congeners. For instance, *P. lanceolata* produced more shoot biomass in the presence of *P. media*, than in its monoculture. Conversely, *P. media* produced significantly less shoot biomass when grown with *P. lanceolata* than when grown with conspecifics (Fig. 3), implying a competitive superiority of *P. lanceolata* in our study. Similarly, *L. autumnalis* produced more shoot biomass in the presence of *L. hispidus* than in its monoculture, and *L. hispidus* performed worse in the presence of *L. autumnalis* than in its monoculture. In the *Prunella* species pair, the outcome of competition was more asymmetrical. *Prunella grandiflora* performed worse when growing with *P. vulgaris* than in its monoculture, but *P. vulgaris* did not have a higher shoot biomass in the presence of *P. grandiflora* than in its monoculture.

The effects of fungal treatments (*Trichoderma*, AM fungi, or their combination) on plant shoot biomass were significant in only few cases (Table 2, Fig. 3). Shoot biomass of *C. jacea* was higher in the presence of *Trichoderma* than in the presence of AM fungi when these were singly inoculated (Table 2, belowground mutualist effect, $p < 0.01$; Fig. 3). Similarly, *Trichoderma* tended to enhance the performance of *P. lanceolata* whereas AM fungi tended to reduce their performance (Table 2, belowground mutualist effect, $p < 0.05$; Fig. 3). Furthermore, the extent to which shoot biomass of *Leontodon hispidus* was reduced in mixtures with its congener *L. autumnalis* was stronger in the absence of *Trichoderma* than in its presence (Table 2; plant community type × belowground mutualist significant; Fig. 3).

The community root biomass was consistently lower in AM fungal treatments than in control and *Trichoderma* only treatments (Supplementary material Appendix 1 Fig. A2, Table A2). Interestingly, in each of the plant species pairs, root biomass significantly differed between the plant monocultures, but the root biomass of the mixtures was not significantly different from either of the monocultures, except for the *Prunella* species pair (Supplementary material Appendix 1 Fig. A2). In the *Prunella* species, the community root biomass was similar between monocultures, but was lower in their mixture (Supplementary material Appendix 1 Fig. A2).

The competitive dissimilarity (difference in competitiveness) between plant pairs was most pronounced for the *Plantago* and *Leontodon* species. For the *Plantago* species, the extent of competitive dissimilarity was independent of the belowground fungal treatment (Table 3, no interaction PxB; Fig. 4). However, for the two *Leontodon* species, the extent of competitive dissimilarity was significantly affected by the belowground fungal treatment (Fig. 4). Specifically, the AM fungal treatment enhanced the competitive dissimilarity between the two *Leontodon* species compared to the control (no fungal inoculation), but this effect was negated in the presence of *Trichoderma* (Table 3, P × B interaction, $p < 0.01$). Interestingly, this indicates that plant–plant competition was intensified in the presence of AM fungi, but that in the mixed inoculum (in which AM fungi and Trichoderma could interact), the presence of *Trichoderma* resulted in relaxation of AM fungal-induced intensification of plant–plant competition. This underscores the impact of single versus multiple fungal mutualist in interspecific plant competition.

Discussion

Understanding the roles of species interactions in shaping community structure is central for advancing predictive ecology (Petchey et al. 2015). Toward this end, we studied how interactions between two common fungal symbionts of plant that form mutualistic relationships with plants affect the performance of and competition between several pairs of closely related plant species. While our results show a consistent negative effect of one fungal species (AM fungi) on

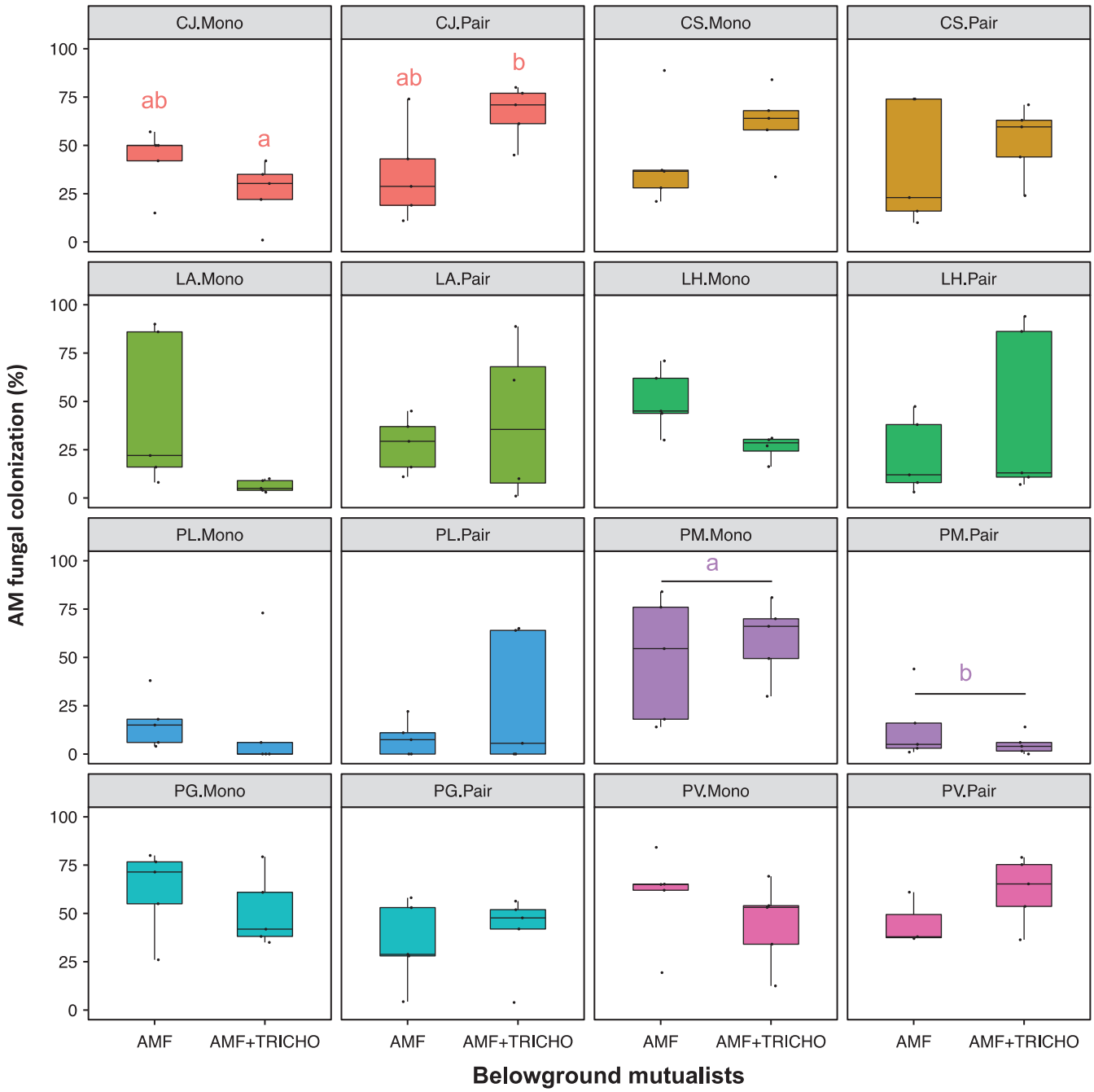

Figure 1. Effects of belowground mutualist combinations on arbuscular mycorrhizal (AM) fungal colonizations (%) in plant monocultures and mixtures with congeneres for all plant species. Letters above the boxes are based on post hoc Tukey multiple comparison tests ($p < 0.05$). CJ: *Centaurea jacea*, CS: *Centaurea scabiosa*, LA: *Leontodon autumnalis*, LH: *Leontodon hispidus*, PL: *Plantago lanceolata*, PM: *Plantago media*, PG: *Prunella grandiflora*, PV: *Prunella vulgaris*. Belowground mutualist combinations are AMF: AM fungi monocultures, and AMF + TRICHO: AM fungi together with *Trichoderma*.

the other (*Trichoderma*) independent of host plant communities, the net effects of such competitive fungal interactions on plant performance and competition were inconsistent. Among the four congeneric pairs, we found that in three cases competitive interactions between the plants were not affected by fungal mutualist species, but that in one case they were. In that case, competitive dissimilarity between the two plant congeners (*Leondoton* species) was enhanced in the presence of AM fungi, but this effect was relaxed when AM fungi and *Trichoderma* were together, indicating that effects of fungal mutualists on plant–plant competition can be affected by the functional diversity of belowground fungal mutualists.

One of the key results of our study is a consistent negative effect of AM fungi on *Trichoderma* independent of plant

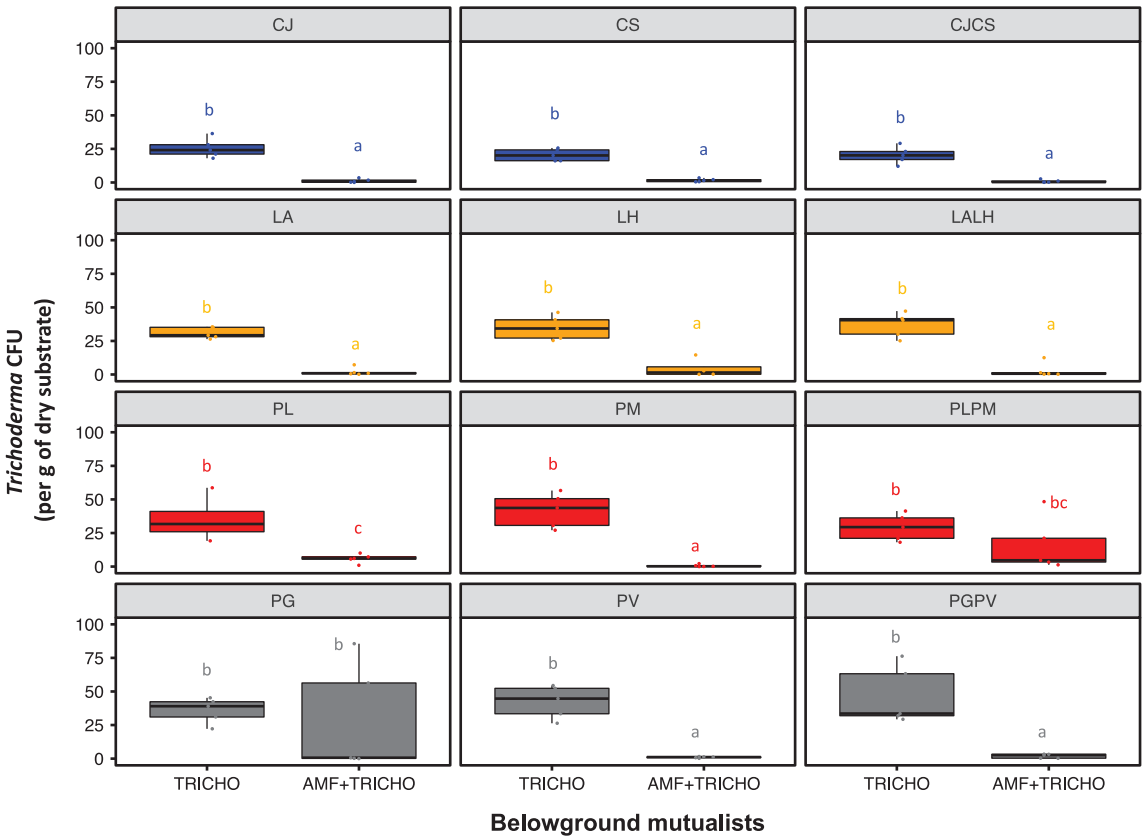

Figure 2. Effects of belowground mutualist combinations on the number of *Trichoderma* colonies (colony forming units, CFU) in plant monocultures and plant mixtures across all plants. Letters above the boxes are based on post hoc Tukey multiple comparison tests ($p < 0.05$). Belowground mutualist combinations are TRICHO: *Trichoderma* monocultures, and AMF + TRICHO: AM fungi together with *Trichoderma*.

communities (Fig. 2). Although the success of AM fungi in colonizing specific plant species varied to a certain degree, all plants used in this study were colonized by the AM fungal species in both plant monocultures and in mixed plant communities independent of the presence of *Trichoderma* (Fig. 1). For niche differentiation to occur between the two fungal species, one of them, the AM fungus in our study (since their plant-specific colonization could be assessed), should have shown higher colonization rates of one of the plant species in the plant pairs in the presence of *Trichoderma*. In contrast, we observed comparable colonization rates of the AM fungal species in either of the congeneric plants when they grew together in the presence of *Trichoderma*, indicating an overall lack of niche differentiation between the two fungi in mixed plant communities.

Negative effects of AM fungi on *Trichoderma* have been observed in previous studies mainly with monocultures of crop species (Martínez-Medina et al. 2009, 2011). Although

Table 2. Results of two-way ANOVA mixed-effects models of the effects of plant community (monocultures versus mixtures) and belowground mutualists (control, only AM fungi, only *Trichoderma*, or AM fungi + *Trichoderma*) on plant shoot biomass. F-value and p-value are bold when statistically significant ($p < 0.05$). The marginal R^2 (fixed effects) and conditional R^2 (random + fixed effects) are shown for each mixed-effect model. The degrees of freedom are based on the Satterthwaite's approximation method from the lmerTest package (Kuznetsova et al. 2017).

		Plant community (P)		Belowground mutualists (B)		P × B		R^2	
Genera	Plant species	F-value	p-value	F-value	p-value	F-value	p-value	Marginal	Conditional
Centaurea	*C. jacea*	$1.78_{1,85.13}$	0.18	$\mathbf{5.70_{3,107.19}}$	**<0.01**	$1.28_{3,107.19}$	0.28	0.13	0.18
	C. scabiosa	$0.26_{1,112}$	0.60	$0.50_{3,112}$	0.68	$0.39_{3,112}$	0.75	0.25	0.25
Leontodon	*L. autmnalis*	$\mathbf{11.11_{1,106}}$	**<0.01**	$0.23_{3,106}$	0.87	$0.36_{3,106}$	0.77	0.10	0.10
	L. hispidus	$\mathbf{30.82_{1,100}}$	**<0.001**	$0.18_{3,100}$	0.90	$\mathbf{2.77_{3,100}}$	**0.04**	0.29	0.29
Plantago	*P. lanceolata*	$\mathbf{20.94_{1,69.88}}$	**<0.001**	$\mathbf{2.76_{3,103.84}}$	**0.04**	$0.75_{3,103.84}$	0.51	0.21	0.23
	P. media	$\mathbf{17.93_{1,82.22}}$	**<0.001**	$0.76_{3,88.05}$	0.51	$0.35_{3,87.92}$	0.78	0.20	0.25
Prunella	*P. grandiflora*	$\mathbf{5.60_{1,85}}$	**0.02**	$0.27_{3,85}$	0.84	$1.71_{3,85}$	0.16	0.11	0.11
	P. vulgaris	$1.92_{1,72}$	0.17	$1.58_{3,72}$	0.20	$0.25_{3,72}$	0.85	0.08	0.08

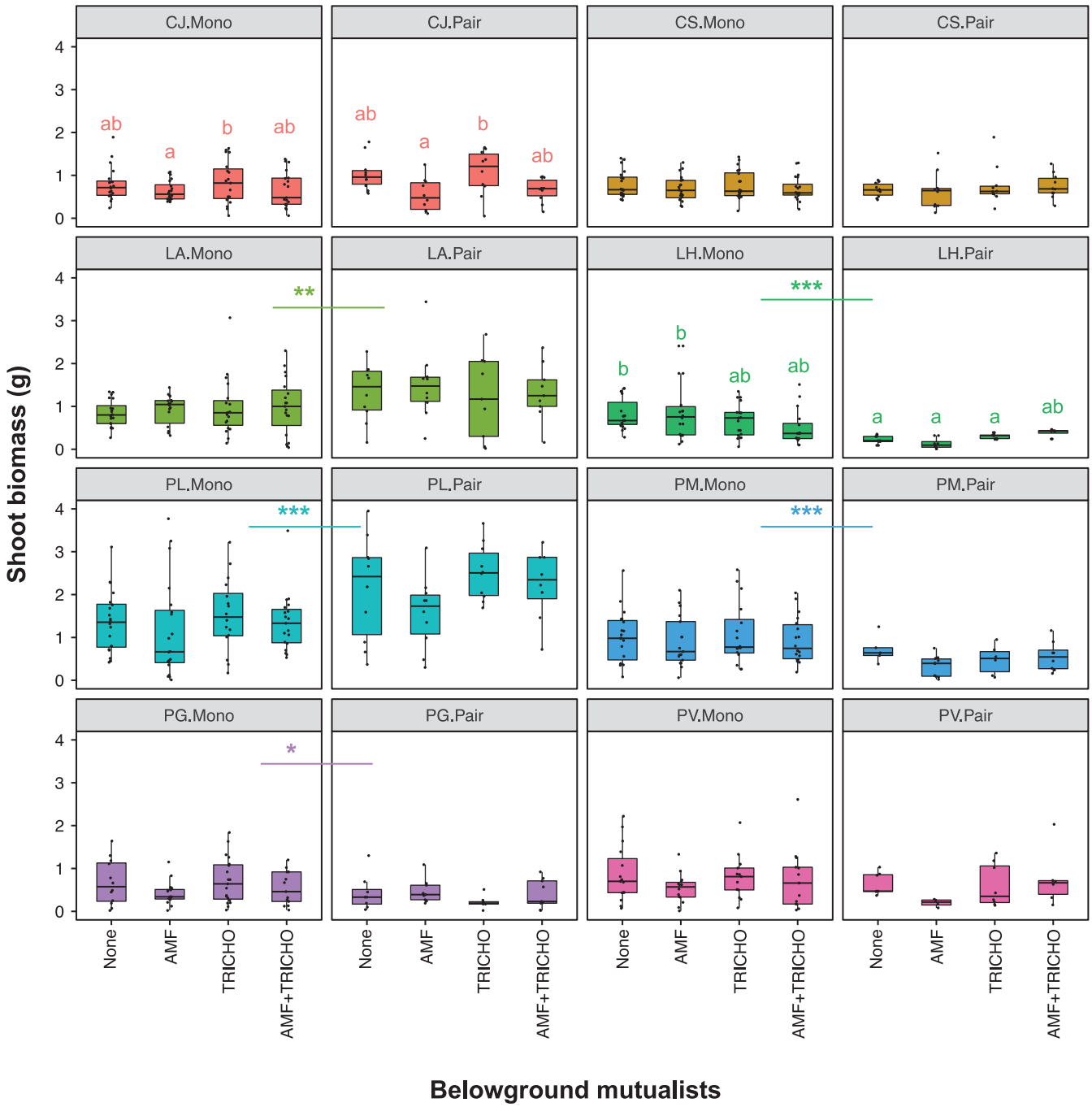

Figure 3. Plant-specific shoot biomass of plants grown in monocultures and in mixture with congeners in four belowground mutualist fungal treatments. Different letters above the boxplots, based on post hoc Tukey multiple comparison tests, indicate significant differences between belowground mutualist treatments (Table 1), and asterisks indicate differences in performance between plants growth in monoculture and in mixture with congeners. The figure panels for LH mono and LH mixture contain the letters from the post hoc comparisons on the interaction term between plant and mutualist community (Table 1). CJ: *Centaurea jacea*, CS: *Centaurea scabiosa*, LA: *Leontodon autumnalis*, LH: *Leontodon hispidus*, PL: *Plantago lanceolata*, PM: *Plantago media*, PG: *Prunella grandiflora*, PV: *Prunella vulgaris*.

the specific reasons for such effects are not yet well-known, it is assumed that the rhizosphere environment after colonization by AM fungi becomes detrimental for the growth of *Trichoderma* (Martínez-Medina et al. 2009, 2011). It has been further shown that despite the preference of *Trichoderma* for plant rhizosphere soil over the bulk soil environments, they can be quite vulnerable to changes in nutrient availability in the rhizosphere (Green et al. 1999, Druzhinina et al. 2011). We suspect that the successful root colonization of almost all the host plants by AM fungi may have suppressed the

Table 3. Results of two-way ANOVAs of the effects of plant species (within genera) and belowground mutualists on plant competitiveness, shown for four plant genera. F-values and p-values are in bold when statistically significant ($p < 0.05$). The ω^2 values are partial-ω^2 as a measure of effect size of the treatments and their interactions.

	Plant community (P)			Belowground mutualist (B)			P×B		
Genera	F-value	p-value	ω^2	F-value	p-value	ω^2	F-value	p-value	ω^2
Centaurea	$2.83_{1,32}$	0.10	0.04	$0.88_{3,32}$	0.45	<0.01	$1.15_{3,32}$	0.34	0.01
Leontodon	$\mathbf{63.73_{1,31}}$	**<0.001**	0.61	$0.27_{3,31}$	0.84	0.07	$\mathbf{4.28_{3,32}}$	**0.01**	0.20
Plantago	$\mathbf{94.33_{1,29}}$	**<0.001**	0.71	$0.50_{3,29}$	0.68	0.03	$0.42_{3,29}$	0.73	0.04
Prunella	$<0.01_{1,27}$	0.95	0.02	$0.80_{3,27}$	0.49	0.01	$2.10_{3,27}$	0.12	0.08

nutrient availability in the rhizosphere, and thus constrained the growth of *Trichoderma* (Fig. 1, 2). This speculation is supported by the pattern of *Trichoderma* CFUs in *Plantago* pairs (Fig. 2). That is, *Trichoderma* was more successful in pots with *P. media* when this species grew in mixtures with its congener *P. lanceolata* than when it grew in monoculture (in which AM fungi had higher colonization rates) (Fig. 1, 2).

One of the main objectives of our study was to examine whether the interaction between two functionally distinct belowground fungal mutualists of plants (e.g. different modes of symbiosis with host plants) affect interspecific plant interactions. We expected that the strength of plant–plant competition would be diminished in the presence of multiple, functionally dissimilar, fungal mutualists compared to the situation where there would be only a single mutualist species (Wagg et al. 2011a). However, in most of the cases, fungal interactions neither enhanced competitive dissimilarity between the four plant pairs, nor did they reduce it. The only exception to this was the competition between the two *Leontodon* species, in which we observed that AM fungi enhanced the competitive dissimilarity between the species, but that fungal–fungal interactions reduced the competitive difference between the two congeners (Fig. 4). One potential reason for such a pattern could be that only in the presence of AM fungi alone *L. hispidus* produced less shoot biomass when grown with its congener *L. autumnalis* than in its monocultures (Fig. 3). Interestingly, the reduction in competitive dissimilarity between the two Leontodon species was true despite the reduction of *Trichoderma* colonies by AM fungi (Fig. 2, 4). We thus suspect that *Trichoderma*, even when lower in count, may have benefitted the competitively inferior *L. hispidus* relative to its superior competitor *L. autumnalis* (Fig. 3, 4). However, this was not the case for *Plantago* pairs, where we observed that *Trichoderma* was not

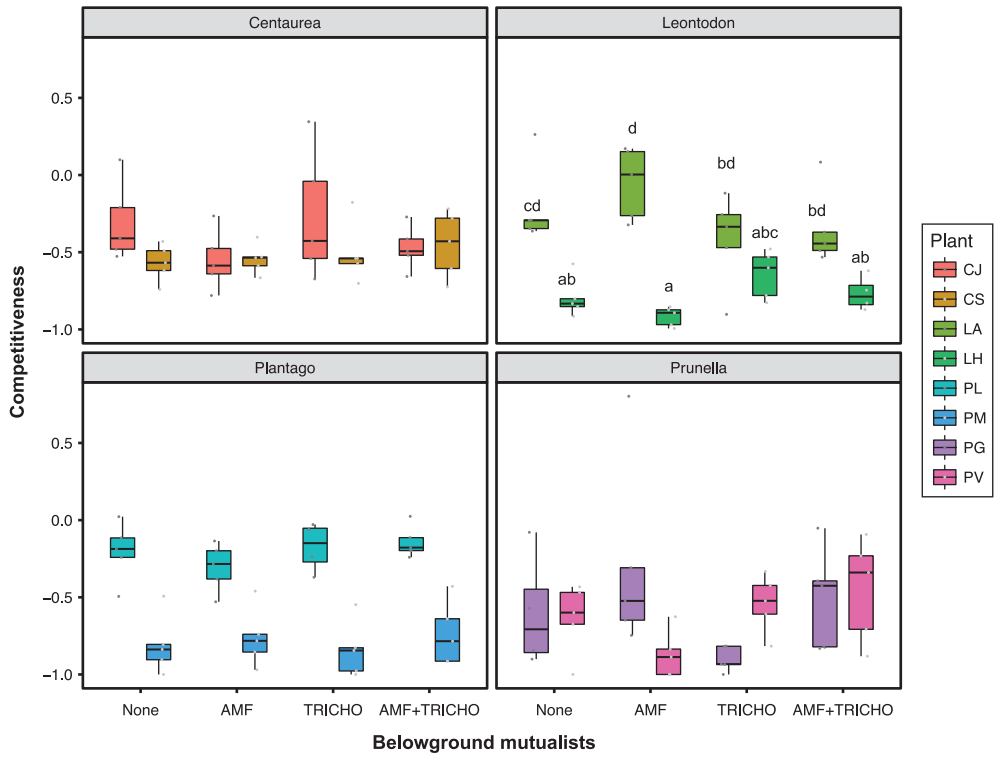

Figure 4. Effects of belowground fungal mutualists on the competitiveness of eight plant species grouped per genus. Different letters above the boxplots, based on post hoc Tukey multiple comparison tests, indicate significant differences between belowground mutualist treatments.

significantly suppressed by AM fungi in the plant species mixture (Fig. 2), yet competitive dissimilarity between the two plant species in the presence of AM fungi was unchanged when two fungi were together (Fig. 4). Hence, we speculate that the competitive interaction between congeneric plants are likely to be more plant context-dependent than dependent on their interactions with plant–beneficial fungal species in the soil.

The ability of ecologically similar plants (e.g. congeners) to partition some of their key resources (Tilman 1988) or their consumers (Kim et al. 2013, Bever et al. 2015) is one of the key machineries for their coexistence (Grime 1977, Tilman 1988, Silvertown 2004). In line with this, we expected that the presence of two functionally different fungal mutualists of plants in the soil would also help closely related plants to partition their niches (by forming separate mutualistic partnerships) and eventually relax plant competition. Our results showed only limited support for this assumption as competition between closely related plants was only relaxed in one out of four plant competition scenarios by the presence of two fungal species. Moreover, we show that the consequences of a consistently negative effect of AM fungi on *Trichoderma* for both plant performance and competition varied among plants. Although previous studies have shown that the diversity of belowground mutualists can relax plant competition, these studies used fungal mutualists from the same functional group (AM fungi only) and plants of different functional groups (Wagg et al. 2011a, b). We reiterate that our experimental design did not allow for disentangling the effects of other soil microorganisms that could have been present in our mycorrhizal inoculum, however, a recent study pointed that no proper control is feasible when using complex mycorrhizal inoculum (Gryndler et al. 2018). Nevertheless, we highlight that the effect of fungal–fungal interactions on plant communities are likely to be moderated by the functional identity of mutualist fungi and how they form partnerships with the co-occurring closely-related plants.

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Supplementary material (available online as Appendix oik-06138 at <www.oikosjournal.org/appendix/oik-06138>). Appendix 1.