Symbiosis
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Hiding in a crowd—does diversity facilitate persistence of a low-quality fungal partner in the mycorrhizal symbiosis?

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Abstract Given that arbuscular mycorrhizal (AM) fungi are not consistently beneficial to their host plants, it is difficult to explain the evolutionary persistence of this relationship. We tested the hypothesis that increasing either fungal or host biodiversity allows an AM fungus to persist on a host where it shows little benefit. We found that growing such a fungus (an isolate of *Glomus custos* associating with *Plantago laceolata*) in combination with certain fungi improved its success as measured by mtLSU DNA abundance. Increasing plant species richness facilitated the spread of this fungus as measured by spore density and fungal colonization; the role of host species richness was not as clear when looking at measures of root abundance. These results indicate that diversity in the AM symbiosis, both plant and fungal, can promote the persistence of low-quality fungi. By existing within a complex mycelial network fungal strains that show little growth benefit to their hosts have a better chance of persisting on that same host. This has the potential to promote selection for heterogeneous AM fungal communities on a small spatial scale.

Keywords Mutualism · Host detection · Cheaters · Arbuscular mycorrhizal fungi · Biodiversity · Common mycelial network

1 Introduction

The relationship between arbuscular mycorrhizal (AM) fungi of the phylum Glomeromycota and their plant hosts is considered as a classic example of a reciprocally beneficial mutualism; both partners benefit from the symbiosis, with plants providing carbohydrates to their fungal partners and fungi providing mineral nutrients, such as nitrogen (N) and phosphorus (P) to their host plants. However, the benefits do not always outweigh the costs of the symbiosis; and not all AM fungi are equally mutualistic under all conditions (Johnson 1993). Many confer few benefits to plant hosts (Kiers et al. 2011; Smith and Smith 2011). As a result, there are numerous examples in which negative effects of AM fungi on host plant growth have been described (Johnson 1993; Klironomos 2003; Jones and Smith 2004; Johnson et al. 1997; Li et al. 2008).

Plants can be colonized on a very small spatial scale (1 cm or smaller) by multiple fungal species differing in mutualistic benefit (Vandenkoornhuyse et al. 2003; Helgason and Fitter 2009). Simultaneously, each fungal individual can interact with multiple host plants or species that differ in the amounts of carbohydrates that they provide (Lekberg et al. 2010;

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Hammer et al. 2011). This complex network of many-to-many interactions constitutes the common mycelial network (CMN) (van der Heijden and Horton 2009) and makes the mycorrhizal mutualism vulnerable to 'low-quality' affiliations (i.e. partners that do not cooperate, but are potentially able to gain the benefit of others cooperating). In the CMN, plant and fungal individuals providing less benefit than their 'fair-share' have a theoretical competitive advantage over high quality affiliations, due to their lower cost to benefit ratio—the ratio of nutrient to carbon exchange (Schwartz and Hoeksema 1998; Douglas 2008; Cowden and Peterson 2009; Kiers et al. 2011). If an individual were able to rely on resources provided by others, why would it cooperate to provide benefits to its partner (West et al. 2007)?

One explanation is that partners in the mycorrhizal mutualism cooperate because they are directly compensated with more resources. This would mean that cooperation leads to a direct fitness benefit for the high quality partner (Denison and Kiers 2011). However, whether plants and fungi can consistently identify the best partners on a small spatial scale, and allocate more resources to these cooperators, has been a subject of debate (Kiers and van der Heijden 2006; Fitter 2006; Bever et al. 2009).

Recent research—in which the cooperative behavior of hosts and fungi was externally manipulated—indicates that plant hosts are able to alter their nutrient distribution, and reward more cooperative fungi even if the root system is also colonized with less-cooperative fungal strains (Kiers et al. 2011). The reciprocal pattern was also found to be true—hosts offering an increased carbon supply were rewarded with more P or N from the fungus (Kiers et al. 2011; Hammer et al. 2011; Fellbaum et al. 2012). In economic terms, this represents an example of a 'biological market' (Noë and Hammerstein 1995; Schwartz and Hoeksema 1998; Kummel and Salant 2006; Cowden and Peterson 2009), because partners offering the best rate of exchange are rewarded, and control is bidirectional. This mechanism, among others, may contribute to the evolutionary stability of the AM symbiosis.

However, it is unknown if the ability to detect less-cooperative partners is absolute or varies depending on host species, ecological context or dynamics within the CMN. For instance, the ability of a host to discriminate may vary according to its mycorrhizal dependence (Hoeksema et al. 2010; Grman 2012) and a host that is highly dependent on AM fungi to meet its nutritional needs may be less discriminatory. A host plant under nutrient limitation may be more likely to reward less-cooperative fungal partners with resources, if these fungi provide access to certain nutrients. Likewise, discrimination effects may depend on the number of plant partners in the CMN, especially if they vary in their punishment strength (Selosse et al. 2006). Variation in discrimination ability may help explain how relatively low-quality fungal strains can persist in nature (Grman 2012), despite accumulating evidence that hosts employ specific mechanisms to detect and exclude less-cooperative symbionts (Denison and Kiers 2011; Friesen et al. 2011).

Here, we test two scenarios that could potentially allow fungi with low benefit to persist in a root system. First, we tested the hypothesis that a relatively low-quality fungal strain will benefit from the presence of additional fungi on the same root system (increase in fungal diversity). Theoretically, increasing the number of simultaneously interacting fungal partners in a CMN could allow a relatively low-quality strain to persist because its contribution to the host partner's resources has a smaller comparable effect on the overall level of circulating resources (West et al. 2002). In addition, it may be more difficult for plants to 'detect' low-quality fungi when fungal partners co-exist as overlapping infections on the root system.

Second, we tested the hypothesis that a relatively low-quality fungus will benefit from an increase in plant diversity. It is known that not all AM fungi are of equal quality, good or bad, for all hosts (Klironomos 2003). Thus a strain that is of low quality for one host may be highly cooperative with another, and maintain ample fitness status in the CMN. Further, it can be assumed that not all plants have the same ability to detect low-quality partners (Kiers and Denison 2008; Grman 2012), similar to what has been shown for leguminous plants interacting with their rhizobia symbionts (Kiers et al. 2007; Friesen et al. 2011). As a result, the ability of a fungus to persist on one host is potentially buffered by interactions with other host plants, because a fungus denied resources by one host plant might still receive resources from another.

To begin to test these hypotheses, we ran separate experiments to investigate the growth of a relatively low-quality fungus under various ecological conditions. In the 'fungal diversity experiment', we examined the effect of increasing fungal diversity on the success of our target fungus (the relatively low-quality isolate). In the 'plant diversity experiment', we analyzed the effect of increasing plant diversity on the growth of our target fungus.

We selected *Glomus custos* (see Krüger et al (2012) for the new taxonomic classification scheme) as our target fungus because it had the potential to behave as a low-quality symbiont with certain hosts in two previous experiments (Kiers et al. 2011; Verbruggen et al. 2012). We tested the quality of *G. custos* on a variety of hosts in a preliminary experiment and found that *G. custos* reduced shoot biomass for *Plantago lanceolata* under the specific conditions of our experiment (data not shown). This was also true in a second experiment when we grew *P. lanceolata* with a variety of AM fungal isolates (Fig. 1). Though all AM fungal isolates suppressed plant growth compared with control plants, this is not uncommon in short-term, pot experiments. For this study, we were interested in comparing the *relative* symbiont quality of

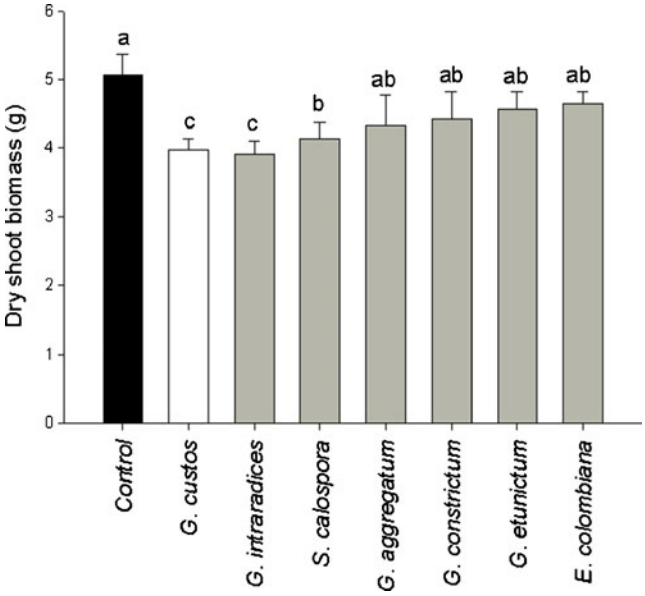

Fig. 1 Results of the experiment to determine the symbiotic quality of our target fungus (*G. custos*) grown with *Plantago lanceolata* in relation to non-mycorrhizal controls and six other AM fungal isolates. Plants were inoculated either with or without with *G. custos* and grown for 12 weeks in greenhouse at the University of Guelph. Shoot biomass was significantly different among AM fungi ($F_{7,74}$=5.684 , p=0.001), and plants grown with *G.custos* were smaller than all other plant*fungal combinations except *G intraradices* (p=0.015). Letters above bars indicate differences between treatments (LSD, p<0.05)

different fungi in our model system. In this case, *G. custos* ranked lowest out of seven isolates tested on shoot biomass of *P. lanceolata,* fulfilling the necessary model system to study the symbiosis when the fungal partner is *low quality*. While it is possible that *G. custos* behaves beneficially with *P. lanceolata* under time periods or different growth conditions, these do not pertain to this study, and so were not included.

2 Methods

2.1 Host plant and target fungus

We selected *Plantago lanceolata* L. as plant host because it is readily colonized, highly responsive to a broad range of AM fungal taxa (Maherali and Klironomos 2007) and shows the lowest response when associated with *G. custos* under our specific growing conditions (Fig. 1). As described above, the AM fungus *G. custos* was chosen as the target species, because this fungus has previously been shown to reduce host plant growth of *P. lanceolata* relative to other fungi of the same genus (Fig. 1). Although most of the fungi we tested suppressed growth of *P. lanceolata, G. custos* was the least beneficial fungus that we tested, and fungi differed in their relative symbiotic quality. We chose to measure host shoot biomass as our indicator of symbiont quality. While it is clear that AM fungi evoke many responses in their host plants, we required general indicator of symbiont quality. Because changes in host biomass result from a suite of physiochemical changes in the plant (i.e. nutrient levels, changes in pathogen load, etc.), we decided that shoot biomass was the simplest and most appropriate measure of symbiont functioning in the AM symbiosis, and is also the most widely used. In a preliminary study, we found that shoot biomass for *P. lanceolata* was correlated with other measures of plant fitness such as inflorescence biomass (r= 0.67, p=0.001) and number of seeds produced per inflorescence (r=0.53, p=0.004)(data not shown).

2.2 Fungal diversity experiment

To test the effect of increasing fungal diversity on a relatively low-quality fungal species, we cultured our target fungus, *G. custos* in three treatments: alone, together with one of six other fungal isolates, or in a mixed community with three randomly selected isolates (SI Table 2) chosen from a larger community of 13 isolates. All fungi originated from the culture collection at the University of Guelph and the International Collection of Vesicular Arbuscular Mycorrhizas (INVAM) (SI Tables 1 and 2). These included: *Entrophospora colombiana, G. aggregatum, G. constrictum, G. etunicatum*, *G. intraradices,* and *Scutellospora calospora* (SI Table 1). For each of the 9 treatments (*G. custos* alone, *G. custos* in combination with *one* out of 6 other fungal strains, *G. custos* with *three* other fungal strains—diversity treatment, and one non-mycorrhizal control) there were 10 replicates, with the exception of the diversity treatment, for which 15 replicates were used. This resulted in a total of 95 experimental units.

Conetainers (750 mL, Stuewe and Sons Inc., Corvallis, OR, USA.) were filled with a sterile sand: Turface (1:2, v:v) (Turface Athletics MVP, Profile Products LLC, Buffalo Grove, IL, USA) mixture. To each conetainer, we added Turface with fungal inoculum that consisted of roots, hyphae, and spores of each isolate, produced with *Allium porrum* (a species generally used to propagate AM fungi) under semi-sterile conditions. Large root pieces were chopped into smaller (<2 cm) fragments before inoculation. Each contetainer received 20 g of inoculum to ensure that the mycorrhizal colonization of the plants was not limited by the availability of fungal propagules. To each conetainer, a microbial solution was added to standardize non-AM microbial communities among the experimental units. For this microbial solution, 10 g inoculum from each fungal culture was pooled and mixed with 300 ml water. This slurry was sieved through a 2 μm mesh filter, and 1 ml of the leachate was added to each pot.

The inoculum was covered with a thin layer of peat to retain moisture during germination. Three seeds were placed on top of the peat and watered daily until germination, and then as needed (every 2–3 days). They were thinned to one

plant per conetainer at 8 weeks, and fertilized biweekly with 1 ml half-strength Hoagland's solution (the full-strength solution contained 2.0 mM $MgSO_4$, 5.0 mM $Ca(NO_3)_2$, 5.0 mM KNO_3, and 1.0 mM $NH_4H_2PO_4$), together with micronutrients and Fe-EDTA.

2.3 Plant diversity experiment

To test whether plant diversity affected the success of a relatively low-quality fungal strain on *P. lanceolata*, *G. custos* was exposed to various levels of plant diversity. For this experiment, we filled 1 L pots with sterile, 1:2 sand: Turface (v:v). The plant number was kept constant at four per pot, allowing us to standardize for possible differences in plant biomass across treatments. Each pot thus contained four plants. These were either all *Plantago* (treatment P+0), three *Plantago* plants plus one other plant species (P+1) or one *Plantago* plant plus three plants of different species (P+3). There were 10 replicates for a total of 30 pots.

The other plant species were chosen randomly for each replicate unit from a collection of seeds (see SI Table 3 for a complete list of plant species) that were collected from 25 locally occurring grassland species at the University of Guelph Long-term Mycorrhizal Research Station and cold stratified over the winter. One *Plantago* plant per pot was randomly selected ('target plant') and later used for measurements of fungal performance in order to control for differences in the colonization among hosts. Biomass for 'non-target' hosts was pooled across species, since we were interested in the effect of host diversity, rather than host-specific effects.

For this experiment we only added one fungus to each pot, *G. custos.* Inoculum and microbial solution was added as described above. Three seeds for each individual plant were placed on top of the peat and watered daily until germination, and then as needed (every 2–3 days). They were thinned to four plants per pot at around 8 weeks, and fertilized biweekly with 1 ml half-strength Hoagland's solution (see above).

2.4 Response variables measured

In both experiments, plants were harvested after 16 weeks. For plants undergoing microbial analysis, the roots were washed from the substrate; one sub-sample of roots was then used to measure the mycorrhizal colonization microscopically (see below) and another sub-sample was used for the molecular quantification assays (see below). All other roots and the shoots were dried at 60 °C for three days and weighed.

P. lanceolata roots (both experiments) were used to measure the abundance of *G. custos* by quantitative real-time PCR (qPCR). For DNA extractions, roots were shaken and washed to remove all adhering soil, chopped into small fragments, frozen in liquid nitrogen, freeze-dried, and stored at room temperature. Approximately 100 mg of dried root material was used to extract DNA using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, CA) following the manufacturer's instructions and stored at −20 °C. The abundance of *G. custos* was measured in the DNA extracts of roots using a TaqMan marker system for the mitochondrial large subunit (mtLSU) of this strain. The specific marker system (primers and a probe) was developed and calibrated according to Kiers et al. (2011). The qPCR amplifications were performed using the 7500 Fast Real-Time PCR System (Applied Biosystems Carlsbad, CA) in 96-well optical plate microtubes. Each reaction contained 10 μL of DyNAmo Master Mix (Thermo Scientific Waltham, MA), 0.25 μL of the forward primer (25 μM), 0.25 μL of the reverse primer (25 μM), 0.2 μL of the specified Taqman probe (25 μM), and 2 μL of DNA, and water to reach a final volume of 20 μL reaction mixture. Cycling conditions were as follows: 95 °C for 15 min and 60 cycles of 95 °C for 15 s and 60 °C for 1 min, followed by incubation at 4 °C infinitely. The quantities of the target DNA obtained from the samples were compared to a standard curve generated from 10^{-1}–10^{-8} dilutions of *G. custos* DNA from monoxenic transformed *Daucus carota* root cultures added to sterile water (see SI Fig. 1). To confirm the fidelity of the *G. custos* probe in the fungal diversity experiment, DNA from all other fungal isolates used in the experiment was tested in cross-amplification qPCR reactions. The presence of fungal DNA in both experiments was additionally confirmed by traditional end-point PCR amplification with the primers AML1 and AML2 (Lee et al. 2008). For information on cycling conditions, see legend of SI Fig. 2.

In the plant diversity experiment, the fraction of root length colonized by *G. custos* of the 'target plant', soil hyphal length density and spore density in the soil were measured. Roots of the 'target plant' were extracted, rinsed and cut into 1 cm fragments and stained with Trypan blue following a modified protocol from Brundrett et al. (1984). Stained roots were mounted on slides and inspected for presence of AM colonization structures (arbuscules, vesicles, hyphae and spores). For each replicate, 100 fields of view were measured to determine abundance. For the fungal diversity experiment, 3 replicates were analyzed to confirm colonization by AM fungi. All other roots were dried at 60 °C for three days and weighed. Fungal hyphae were extracted from soil aliquots following the protocol of Miller et al. (1995) and the hyphal length was measured using the gridline intersect method (Neuman 1966). Spores were counted at 40-fold magnification, following the extraction with the wet-sieving technique of Klironomos et al. (1993).

2.5 Statistical analyses

All statistical analyses were performed using SPSS Statistics 19 software. We used the *t*-test to determine the quality (i.e.

increase in plant biomass) of *G. custos* compared to non-mycorrhizal controls (Fig. 1). For all other analyses, we used one-way analysis of variance (ANOVA) for all response variables to identify differences among the various treatments. Post Hoc LSD tests were then performed to identify significant differences from non-mycorrhizal control plants within groups. Differences were considered as significant at $p \leq 0.05$. Prior to analyses, we conducted descriptive statistics and performed transformations where necessary to satisfy assumptions for normality and homogeneity of variance.

3 Results

3.1 The effect of fungal diversity on *G. custos* abundance

We tested the hypothesis that the abundance of the *G. custos* would be positively affected by the presence of other fungal strains colonizing the same root system. We found that the presence of other AM fungi had a significant effect on the abundance of *G. custos* (ANOVA, $F_{7,32}=2.27$, $p<0.05$), as measured by qPCR. The lowest abundance of *G. custos* was found when grown alone with *P. lanceolata* (Fig. 2). The presence of another AM fungus increased the abundance of *G. custos* in *P. lanceolata* roots, and these differences were highly significant for *E. colombiana (p=0.05)* and *G. etunicatum (p=0.02)* but less so for *S. calospora (p=0.07) and G. intraradices (0.10)* and we were unable to detect a significant difference when we added *G. aggregatum* ($p=0.72$), *G. constrictum* ($p=0.30$), or a diverse cohort of fungi ($p=0.40$). We found evidence of substantial fungal colonization in all mycorrhizal treatments (both visually and using our end-point PCR with general primers, see SI Fig. 2 for a representative gel). In the non-mycorrhizal controls, no mycorrhizal colonization was observed microscopically and the target sequence (mtLSU of *G. custos*) could not be amplified, indicating a lack of contamination among the experimental units.

The shoot biomass of the mycorrhizal *P. lanceolata* plants was significantly lower than that of the non-mycorrhizal controls ($F_{8,86}=4.05$, $p<0.001$, LSD, $p<0.05$, Fig. 3), but there was no difference in the shoot biomass among the various mycorrhizal fungal combinations.

3.2 The effect of plant diversity on *G. custos* abundance and performance

When we kept the fungal identity constant (*G. custos* was the only fungal isolate in all of our treatments) and only manipulated plant diversity, we found mixed support for our original hypothesis that plant diversity facilitated the persistence of our relatively low-quality fungal isolate. Consistent with our hypothesis, root colonization by *G. custos* was significantly lower when *P. lanceolata* was grown alone compared to the treatments to which one or three additional

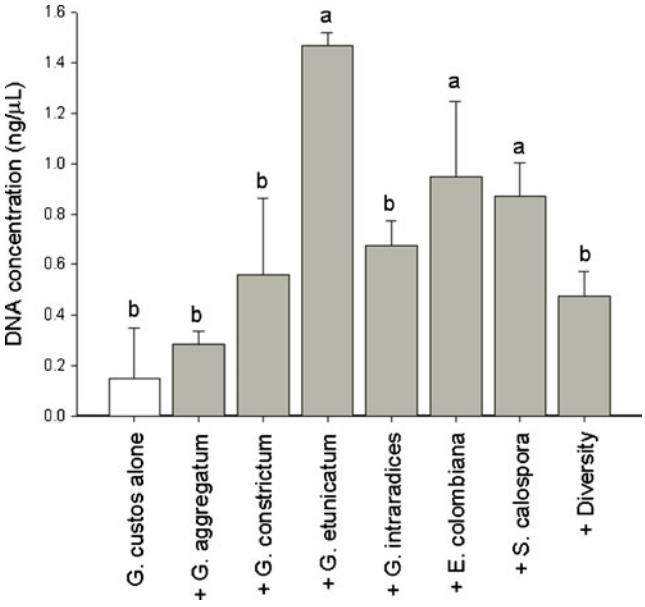

Fig. 2 Concentration of *G. custos* mtLSU in *Plantago* roots. Values were obtained through a quantitative real-time PCR assay using a Taqman probe specific for the mtLSU in *G. custos*, and were calculated using a standard curve given by the equation $y=-12.91Ct+28.05$ ($R^2=0.96$), where Ct is the critical threshold of detection. Values given are average of $n=5$ (SE). White bars indicate *G. custos* grown alone, gray bars are DNA concentration for *G. custos* grown with another AM fungal isolate

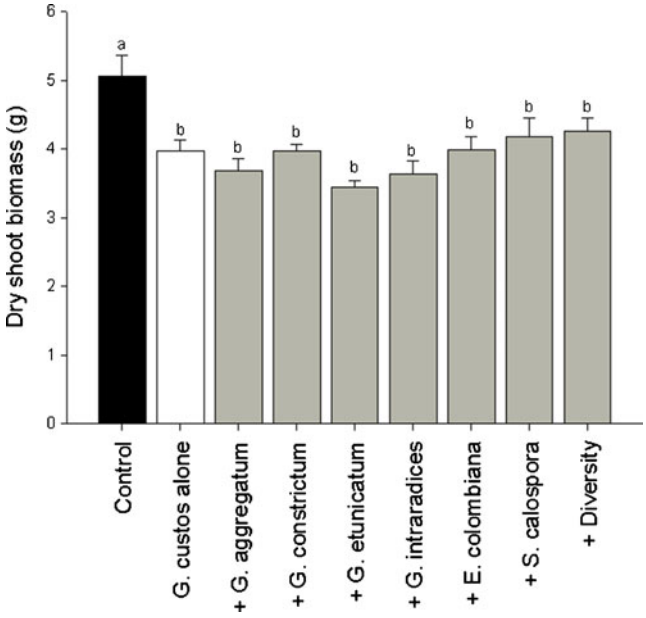

Fig. 3 Shoot biomass for *Plantago* grown with *G. custos* alone or with one of 7 other AMF, plus a non-mycorrhizal control. Values are the average of $n=10$ (SE). Black bars represent nonmycorrhizal controls, white bars stand for plants inoculated with *Glomus custos* alone, and grey bars represent its combination with other AM fungi in the study

plant species were added ($F_{2,12}$=26.7, p<0.001, LSD, p< 0.05 Fig. 4a). However, the treatment with the highest plant diversity (P+3) showed consistently lower levels of root colonization than the intermediate diversity level (P+1; Fig. 4a). This last observation was confirmed by the qPCR analyses, showing a significantly lower abundance of *G. custos* in the roots of the treatment with the highest plant diversity (P+3) compared to the intermediate treatment (P+1, Fig. 4b). In contrast to the microscopic assessment, the qPCR analyses showed a high abundance of the AM fungus in the P+0 treatment (Fig. 4b). When we looked the abundance of *G.custos* in the soil, however, we found support for our hypothesis. Here, we found a positive effect of plant diversity on both hyphal length ($F_{2,12}$=7.85, p=0.007, Fig. 4c) and spore densities ($F_{2,12}$=6.57, p=0.012, Fig. 4d) in the soil. The lowest number of spores and the lowest hyphal colonization of the soil was always found in the P+0 treatment (i.e., *Plantago* alone), but both increased with

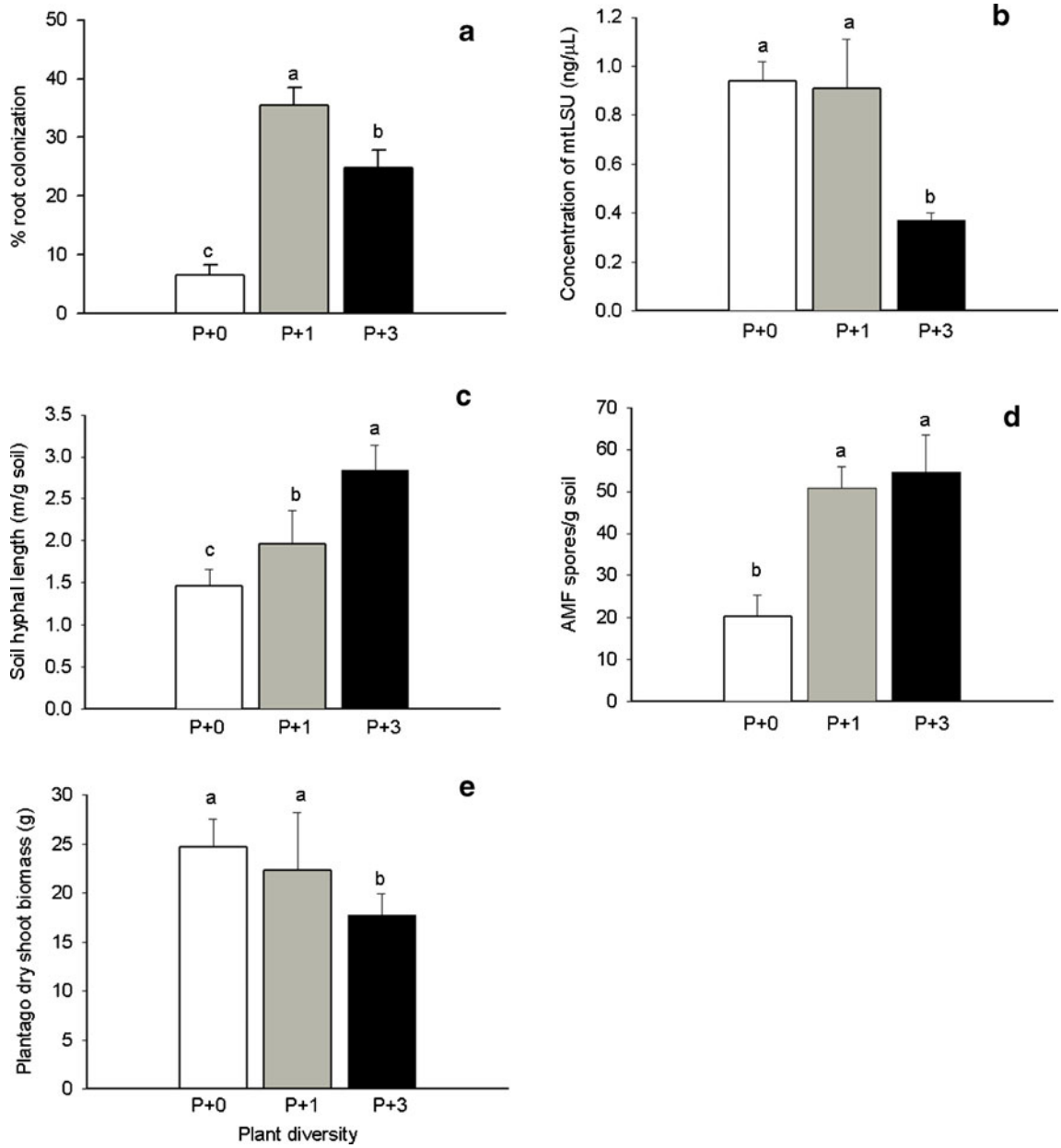

Fig. 4 Percentage of root length colonized (**a**), concentration of mtLSU of *G. custos* (**b**), hyphal length density in the soil (**c**), spore density in the soil (**d**) and shoot biomass (**e**) for *Plantago* grown with 1 or 3 other plant species and inoculated with *G. custos*. Spore density was determined in 10 g soil samples. Measurements of soil hyphal length density reflect total length including both Glomalean and non-Glomalean fungi present in the soil. Values for mtLSU concentration were obtained through a quantitative real-time PCR assay using a Taqman probe specific for the mtLSU in *G. custos*, and were calculated using a standard curve given by the equation y=−13.16Ct+30.24 (R^2=0.94)

increasing plant diversity. Because we inoculated with only *G. custos*, these measurements reflect relative soil colonization of our target fungus.

In the plant diversity experiment, we recorded two measures of shoot biomass: *P. lanceolata* alone and all 'other' (non-*P. lanceolata)* shoots for each pot. When *P. lanceolata* was grown alone or with only one other plant species (P+0 and P+1), only the response of one predetermined *P. lanceolata* plant was measured. The shoot biomass of this plant decreased as the number of plant species increased ($F_{1,24}$=4.40, p=0.047, Fig. 4e). However, there was no significant difference in shoot biomass per pot among treatments, when the shoot biomass of all other plants was included (sum of biomass for each plant in the pot; $F_{2,12}$=1.66, p=0.21). When only the shoot biomass of the 'other' plants was considered, excluding the target *P. lanceolata* (only applicable for the P+1 and P+3 treatments), there was no significant difference between the two treatments (SI Fig. 3). We likewise measured the total root biomass in all pots. Since the roots of *P. lanceolata* could not be distinguished from the roots of the 'other' plants when detached from the shoots, only the total root biomass was recorded for each pot. There was no difference in the root biomass for all plant diversity levels ($F_{2,12}$=1.94, p=0.19, SI Fig. 4).

4 Discussion

Recent research has highlighted potential mechanisms that plant hosts utilize to evaluate and discriminate among their fungal partners (e.g. Bever et al. 2009; Helgason and Fitter 2009; Kiers et al. 2011). Despite these mechanisms, seemingly low-quality AM fungal strains persist in nature, and often lead to significant reductions in plant growth. This suggests that the ecological context in which the mutualism is imbedded plays a large role in the ultimate outcome of the interaction (Hoeksema et al. 2010). We therefore tested two conditions that could potentially allow lower-quality fungal species to persist: fungal diversity and plant diversity.

4.1 A role for fungal diversity

We found some support for our prediction that a relatively low-quality fungal strain would benefit from the presence of additional AM fungi colonizing the same host root. When *G. custos* was grown with *P. lanceolata*, its abundance was positively associated with the presence of other fungi (Fig. 2). However, this effect was not consistent across all AM fungal combinations and the abundance of *G. custos* in the treatment with the highest fungal diversity did not confer additional benefit to our target versus when it was grown alone. So while there was a general trend of other fungi having a positive effect on the abundance of *G. custos*, it appears that the identity of the co-occurring fungi is also important. It is unclear whether this is due to differences in physical colonization patters (i.e., the ability to escape plant detection in a mixed community may be related to how fungal colonization is structured spatially (Bever et al. 2009; Verbruggen et al. 2012)) or due to the phylogenetic distance (and thus functional differences) among the members of the mixed fungal community (Maherali and Klironomos 2007; Powell et al. 2009). Competitive interactions among members of the fungal community are also likely to play a large part in determining the persistence/spread of individual isolates within the root and soil (Alkan et al. 2006; Jansa et al. 2008; Ji et al. 2010).

It is interesting that our 'diversity' treatment did not improve the success of *G. custos* compared to when it was grown alone. It may be that competitive interactions among fungi suppressed the growth of *G. custos* in treatments with 3 other fungi ("diversity" treatments). Plus, the symbiotic quality of other fungi may play a role. In our pairwise interactions, the other fungi were only *relatively* higher quality than *G. custos*, and none benefited *P. lanceolata* to a great extent. But the symbiotic quality of all fungi in our diversity treatments is not known. It may be that these treatments contained fungi that were even lower quality than our target. This may help explain why the presence of other fungi did not have an additive effect on our target.

4.2 A role for plant diversity

We also tested the hypothesis that relatively low-quality fungal strains will benefit from being grown in communities with higher plant diversity because a low-quality fungus might be a better 'fit' with a different host within the CMN. We found that *G. custos,* a low-quality mutualist for *P. lanceolata,* was able to increase soil hyphal length and spore density when it was grown with more than one species of host plant (Fig. 4c,d). These data are in line with our hypothesis that interactions with other host plants will buffer the discrimination effect of *P. lanceolata* on *G. custos*. In our case, it is possible that other hosts benefited from *G. custos* and allocated more resources to this fungus, which allowed it to maintain, and even increase, its belowground abundance. However, this finding can only be confirmed by testing the responsiveness of each host to *G. custos* separately.

We were unable, however, to fully support our hypothesis when looking at measures of root colonization. Both microscopic and qPCR measurements indicated that the roots contained less *G. custos* DNA in plant communities with higher diversity (P+3) than in communities with only one additional plant species (intermediate plant diversity; P+1; Fig. 4a, b). Thus, adding more hosts to the CMN was only advantageous to *G. custos* when one additional plant was added. It is not clear why % root colonization showed a

large increase between P+0 and P+1, whereas DNA concentration—a better reflection of fungal activity as compared to microscopic assessment (Jansa et al. 2008)—did not (Fig. 4b). This may reflect a difference in metabolic activity; *G. custos* grown alone may reduce the allocation of resources to mycelium when grown with a relatively low quality host. We also found that from the P+1 to P+3 treatment, the proportional decrease of fungal DNA concentration in roots was more than 50 %, compared to a 30 % decrease assessed microscopically. These reduced levels of metabolically active fungal colonization (or metabolic activity of the fungus in general), in combination with data demonstrating an increase in hyphal length and spore density in more diverse plant communities (Fig. 4c, d), could reflect a change in fungal strategy with a shift from transport-active interface structures to the host (arbuscules) towards fungal storage (vesicles) or reproduction (spores) structures when different host species are present. Whether the presence of other species is a cue to allocate more energy to spores and hyphae requires further testing.

The maintenance of colonization in the target plant with relatively low physiological activity could represent a competitive advantage for low-quality mutualists such as *G. custos*. Prior colonization potentially reduces the capability of other fungi to colonize the same root system through competitive exclusion (Pearson et al. 1994). It likewise allows the fungus to quickly gain access to new carbon resources if other resources are reduced. This is in agreement with recent findings of Lekberg et al. (2010) who reported that AM fungi maintained colonization rates in poor hosts, despite access to better hosts. They found that when a better host was available, the number of arbuscules in the low quality host was reduced, and these hosts also received less P from the fungus.

Our observations in *P. lanceolata* are in agreement with this work: despite decreasing mycorrhizal colonization rates of roots, *P. lanceolata* shoot biomass also decreased with increasing plant diversity. The fact that we measured increases in soil colonization (soil hyphal length and spore density) but reduced root colonization in *P. lanceolata* indicates that *G. custos* was able to proliferate in roots of other hosts plants. If the other host plants were better partners for *G. custos*, the reduction in *P. lanceolata* shoot biomass could reflect preferential allocation of nutrient resources (P, N) by *G. custos* to higher-quality hosts. In fact, we observed a trend of an increase in shoot biomass of 'other' hosts with increasing plant diversity after inoculation with *G. custos* (SI Fig. 3). The ability of AM fungi to preferentially allocate P and N to host plants providing more benefit has been demonstrated previously (Bücking and Shachar-Hill 2005; Lekberg et al. 2010; Hammer et al. 2011; Kiers et al. 2011; Fellbaum et al. 2012). Overall, these results suggest that, in addition to plant sanctions, fungal control may also help explain the persistence mutualisms where there is variation in symbiont quality.

4.3 An evolutionary role for biodiversity

Research has established that AM fungi can differ greatly in the benefits they confer to their hosts, and that this effect tends to be very context specific (for review see Hoeksema et al. 2010); AM fungi are not unequivocally good or bad. Furthermore, greenhouse studies where plants and fungi are 'forced' to associate under specific growing conditions offer a poor representation of natural AM symbioses (Sýkorová et al. 2007). AM fungi in nature may differ in the amount of benefit they provide to hosts, but it is impossible to cast any single partner as universally 'beneficial' or 'not beneficial' given the great complexity of interactions and conditions implicit in CMN. This is an important difference from other symbiotic interactions.

Despite accumulating evidence that plants employ specific mechanisms to detect and reduce the abundance of relatively low-quality AM fungi (Bever et al. 2009; Kiers et al. 2011), we show here that *G. custos* was never completely excluded. This fungal partner persisted in the symbiosis, and our data suggest that in *P. lanceolata,* this persistence was aided by increases in fungal or plant diversity. These data suggest that host discrimination is not completely effective and/or that discrimination varies with host dependency and ecological context.

Mechanisms allowing plants to direct resource supply solely to high-quality AM fungi may only fully eradicate low-quality symbionts in extreme environments (e.g. where there are fewer hosts or competing fungi available). This leads to the tentative prediction that there will be fewer low-quality species in species-poor environments, and more in species-rich systems (Thrall et al. 2007). This prediction is testable, but contradicts evidence that negative interactions, in terms of soil microbes and plants, are more common in species-poor communities (Johnson 1993; Helgason et al. 1998; Maron et al. 2011; Schnitzer et al. 2011).

Overall, our results indicate that both plant and fungal diversities can help support the persistence of a low-quality symbiont. Despite their obligate biotrophy, AM fungi maintain a modicum of autonomy from the regiment of any individual host by partnering with a variety of plants. Similarly, plants are never at the sole mercy of one single fungus—they cultivate, and support assemblages of fungi, not single strains and it has been suggested, that a diverse AM fungal community improves adaptability and plant productivity in the long term (Kernaghan 2005; Wagg et al. 2011). These characteristics, while helping increase both partners' ability to adapt to various environmental conditions, also potentially allow less-cooperative partners to persist in plant communities. We were able to detect evidence to support

this theory in simplistic pot studies. In natural systems, with diverse assemblages of both plants and fungi, the persistence of relatively low-quality symbionts may be much more pronounced.

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