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Competition and facilitation in synthetic communities of arbuscular mycorrhizal fungi

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Abstract

Interactions between arbuscular mycorrhizal fungal (AMF) species cocolonizing the same host plant are still little understood in spite of major ecological significance of mycorrhizal symbiosis and widespread occurrence of these fungi in communities rather than alone. Furthermore, shifting the composition of AMF communities has demonstrated consequences for the provision of symbiotic benefits to the host as well as for the qualities of ecosystem services. Therefore, here we addressed the nature and strength of interactions between three different AMF species in all possible two-species combinations on a gradient of inoculation densities. Fungal communities were established in pots with *Medicago truncatula* plants, and their composition was assessed with taxon-specific real-time PCR markers. Nature of interactions between the fungi was varying from competition to facilitation and was influenced by both the identity and relative abundance of the coinoculated fungi. Plants coinoculated with *Claroideoglomus* and *Rhizophagus* grew bigger and contained more phosphorus than with any of these two fungi separately, although these fungi obviously competed for root colonization. On the other hand, plants coinoculated with *Gigaspora* and *Rhizophagus*, which facilitated each other's root colonization, grew smaller than with any of these fungi separately. Our results point to as yet little understood complexity of interactions in plant-associated symbiotic fungal communities, which, depending on their composition, can induce significant changes in plant host growth and/or phosphorus acquisition in either direction.

Keywords: barrel medic (*Medicago truncatula*), *Claroideoglomus claroideum*, functional complementarity, *Gigaspora margarita*, quantitative real-time PCR, *Rhizophagus irregularis*

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Introduction

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that colonize roots of a wide range of host plants (Smith & Read 2008). Apart from their well-documented role in providing nutritional benefits to their hosts, these fungi are strongly implicated in plant's tolerance to a variety of other abiotic and biotic stresses (Newsham *et al.* 1995); they affect composition and diversity of plant communities (van der Heijden *et al.* 1998; Wagg *et al.* 2011b) and play role in stabilization of soil aggregates (Rillig 2004). There are numerous pieces of evidence that different AMF species and/or isolates largely differ with respect to their growth and physiological traits as well as with respect to the nutritional benefits conferred to their host plants (Jakobsen *et al.* 1992; Jansa *et al.* 2005; Lendenmann *et al.* 2011). This phenomenon is often referred to as functional diversity (Feddermann *et al.* 2010). Almost all the data gathered on functional diversity of AMF have, however, been obtained in experiments where plants have been inoculated with single AMF isolates. Although these experiments provided timely insights into functioning of arbuscular mycorrhizal

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(AM) symbiosis, they only had limited relevance to field situations, where AMF are present as a multispecies community in the roots and in the soil (e.g. Jansa *et al.* 2002; Mathimaran *et al.* 2005; Pivato *et al.* 2007; Rosendahl & Matzen 2008).

Several of the earlier descriptive studies demonstrated that a single root system and even individual roots can be cocolonized by different AMF species (Daft & Hogarth 1983; Jansa *et al.* 2003b). This observed co-occupation of roots by different fungal taxa is still poorly understood and so is its meaning for the host plants. Until now, only few studies have experimentally addressed whether such multiple colonizations are a result of competitive, synergistic or antagonist interactions (Alkan *et al.* 2006; Maherali & Klironomos 2007; Jansa *et al.* 2008). The lack of specific tools to detect and quantify different AMF species in roots and/or in soils was the reason for a slow development in this area, although some early studies attempted to address these very questions using microscopy or other tools (Daft & Hogarth 1983; Wilson 1984; Hepper *et al.* 1988). Nevertheless, recent introduction of quantitative real-time PCR largely alleviated this problem and opened new avenues in research of AMF communities (Kiers *et al.* 2011; Thonar *et al.* 2012). With this technique, quantification of the interactions between different AMF species is now possible, and thus, it is also possible to specifically address the validity of the hypothesis of functional complementarity in AMF communities. This suggests that AMF species with complementary functions (e.g. uptake of nutrients from different soil pools, specialization on provision of different services such as nutrient acquisition or pathogen protection) inoculated in mixtures may bring more benefits to the plant than any of the species separately (Koide 2000; Jansa *et al.* 2008). Real-time PCR then allows linking realized community composition of the AMF with their ecosystem functions (Wagg *et al.* 2011a).

When different AMF taxa (genera, species or isolates) are present in the same inoculum, they will face several events of interactions that may result in a varying degree of root and soil colonization by each of the taxa (Wilson & Trinick 1983; Hart *et al.* 2001; Golubski 2002). The first event of interaction will be that upon primary infection of the roots. Its outcome will mainly depend on the density, distribution and state of activation of infective propagules in the inoculum and on their rate of root interception. Data of Wilson (1984) suggest that in subterranean clover, competition for penetration sites does exist and can occur even between different members of the same population. Inside the root, the different fungal taxa will further interact and most probably compete for space and/or carbon resources (Pearson *et al.* 1993; Lekberg *et al.* 2007). If certain fungus can tap on newly developing roots faster than others, this could provide it with exclusive access to plant carbon, resulting in positive feedback and local overdominance by a single taxon (Dumbrell *et al.* 2010). Besides competition, some studies have also outlined facilitation between AMF colonizing the same root systems. This is the case of a study published by van Tuinen *et al.* (1998), where it has been shown that *Gigaspora rosea* and *Scutellospora castanea* occurred more frequently in the roots when in the presence of other fungi. This earlier work was only carried out with semiquantitative tools, but the framework was largely confirmed with more recent studies based on real-time PCR. These studies provided evidence for both competition and facilitation, depending on the AMF species, time of harvest and environmental conditions (Alkan *et al.* 2006; Jansa *et al.* 2008). Interacting AMF may also compete for soil nutrients such as nitrogen and phosphorus as well as micronutrients such as zinc and (probably to a lesser extent) for other resources such as water and oxygen (Clark 1965). Other studies (Pearson *et al.* 1993, 1994; Kiers *et al.* 2011) suggested that availability of sugars in the roots and the phosphorus status of the host plant both influenced the outcome of AMF interactions occurring in the same root system. The various carbon sink imposed by the different AMF, whose strength depends on the identity of both the plant and the fungus as well as on the environmental conditions, will result in a differential susceptibility of the root to further colonizations by the other AMF (Lerat *et al.* 2003; Heinemeyer *et al.* 2006). It seems that systemic regulation is at least partly implicated in these processes (Vierheilig 2004b).

The main objective of this study was to assess and characterize the nature of interactions occurring between three different and naturally co-occurring AMF species, when colonizing the same root system of a medic (*Medicago truncatula*) plant. Further, we addressed the consequences of these multiple colonizations for the host plant in terms of plant biomass production and phosphorus uptake. To achieve these objectives, synthetic communities, i.e. assemblages consisting of two AMF species, were established with three AMF isolates belonging to different species (*Rhizophagus irregularis*, *Claroideoglomus claroideum* and *Gigaspora margarita*), all isolated from a single field site in Switzerland (Jansa *et al.* 2002). These fungi have been shown to differ in their strategies to acquire phosphorus from the soil (Thonar *et al.* 2011) and in their carbon costs (Lendenmann *et al.* 2011). Phylogenetic distance of *Gigaspora* from both *Rhizophagus* and *Claroideoglomus* is about twice as high as that between *Rhizophagus* and *Claroideoglomus* (Krüger *et al.* 2012). The inoculum density of the competing fungi varied in an additive experimental design (Weigelt & Jolliffe 2003) to allow conclusions about fungal competition and/or facilitation

along a range of inoculation densities (Snaydon 1991). The quantification of the root mycorrhizal community, using the molecular assay described earlier (Thonar *et al.* 2012), has enabled assessment of interactions between the AMF species when colonizing the same host root system.

Material and methods

Plants and AMF

Medicago truncatula Gaertn. (barrel medic) genotype J5 was used as the host plant in the experiment described later. The seeds were produced on plants generated from a certified seedstock kindly provided by Dr. Gérard Duc, INRA Dijon. To break seed dormancy and synchronize germination, seeds were treated with concentrated (98%) sulphuric acid for 10 min (Massoumou *et al.* 2007) and then washed with sterile water five times and germinated for 3 days on a moistened filter paper.

Three AMF isolates, all obtained from a single field site in Switzerland (Jansa *et al.* 2002), were used in this study. These were *Rhizophagus irregularis* isolate number BEG 158, *Claroideoglomus claroideum* BEG 155 and *Gigaspora margarita* BEG 152. The inoculum was produced with leek (*Allium porrum* L.) in 1-kg pots for 8 months and consisted of colonized roots and substrate. The substrate was a mixture of soil collected at the field of fungal origin, sterilized by γ-irradiation and autoclaved coarse quartz sand (grain diameter 0.7–1.2 mm) and fine quartz sand (grain diameter 0.08–0.2 mm). The components of the substrate were mixed in a ratio of 1:3:1 (v:v:v), respectively. Between 50 and 100 spores of the respective fungal strain were added into each inoculum production pot upon starting the cultures. After the cultivation period of 8 months, leek shoots were removed; roots were chopped to fragments 5–8 mm long and returned to the substrate, which was then thoroughly mixed to achieve homogeneous mycorrhizal inoculum used in the plant experiment. The density of the AMF spores was assessed in each inoculum (using five analytical replicates of 20 g each) and then diluted with the sterile potting substrate so as to reach comparable infectivity. The aim was to reach ~50% medic root length colonized after 6 weeks of cultivation. The infectivity assay was carried out prior to the experiment described here, using a range of inoculum densities of the same AMF isolates as here and the *M. truncatula* J5 as a host plant (see Supporting information Appendix S1 for details). Based on the infectivity assay, we used potting substrate enriched with the AMF inocula so as to contain 40, 80 and 1200 spores per 80 mL substrate for *R. irregularis*, *C. claroideum* and *G. margarita*, respectively. These doses are further referred to as 100% inoculum density for each of the fungal species. Lower inoculum densities were reached by further diluting the inoculum with substrate from pots, where nonmycorrhizal (NM) leek was grown for previous 8 months (so-called mock inoculum).

Experimental growth conditions

The experiment was carried out in small plastic containers (80 mL each) arranged in plates 8 × 12 units. These containers were filled with a substrate that has been homogeneously mixed with the mycorrhizal inoculum. Only containers bordering with other units from all four sides were used for growing experimental plants, and edge rows were planted but not included in the experiment so as to minimize the border effect. The substrate consisted of sterilized soil, coarse quartz sand (grain diameter 0.7–1.2 mm) and fine quartz sand (grain diameter 0.08–0.2 mm) mixed in the ratio 1:3:1 (v:v:v). The soil was collected from a cropped field in Tänikon, Switzerland, from which also the AMF cultures used in this study originated. The soil was air-dried, passed through 5-mm sieve and γ-irradiated at LEONI Studer Hard (Däniken, Switzerland), applying a dose of 25–75 kGy with ^{60}Co source. The available P content of the substrate was 21.9 ± 0.4 mg/kg (ammonium acetate–EDTA extraction, 1:10 w:v, 16 h), and the readily available P pool ($E_{1\ min}$) was 1.73 ± 0.06 mg/kg as assessed by the isotope exchange kinetics approach (Frossard & Sinaj 1997). Substrate C and N contents were 2.2 ± 0.1 and 0.24 ± 0.01 g/kg, respectively.

The growing plants were watered daily with deionized water and received 15 mL/plant/week of a full-strength Hoagland nutrient solution (Hoagland & Arnon 1950) containing no phosphorus throughout the duration of the experiment. The experiment was conducted in a growth chamber (Conviron PGV36; Winnipeg, MB, Canada) under the following conditions: temperature 22/18 °C and relative aerial humidity 75/90% (day/night, respectively); photoperiod 16 h; and combined fluorescent and incandescent light 330 μmol photons/m^2/s.

Experimental design

The experiment included a number of single- and two-species (i.e. dual) inoculations of the experimental plants. There was also a NM treatment included in the design, where the plants were inoculated by the mock inoculum (5 g per container). Each AMF species was inoculated singly at five different inoculum densities (100%, 40%, 10%, 2.5% and 1%), totalling 15 independent treatments. In the dually inoculated treatments, inoculum density of one (so-called target) species was maintained constant at 100% inoculum density and the

density of the other (competitor) species varied similarly as for the single species inoculations. Full treatment combination would result in 30 independent treatments. However, because the treatment combining 100% inoculum density of both species would effectively be duplicated in the full design, we only established one set of these treatments and corrected for multiple testing thereafter. Thus, there were 27 two-species treatments. For all 43 treatments outlined above, we established five individual containers (replicates) per treatment. Further, there were 10 additional containers inoculated by each AMF singly at the 100% inoculum density species or by the mock inoculum (i.e. NM treatment) for checking the time-course of root colonization at 14 and 28 days (five replicates per treatment and time point). Thus, there were 255 independent containers included in this study, each planted with a single medic plantlet.

The five replicate containers of each treatment were kept together in blocks on the seedling plates for practical reasons (minimizing cross-contamination), but the plates were frequently moved and rotated to minimize space effects. Therefore, the individual containers were regarded as independent replicates of the same inoculation treatment and handled accordingly during the statistical evaluation of the results.

Harvest and measurements

The experiment was harvested 42 days after sowing. Shoots were cut and dried at 105 °C for 48 h and weighed. Roots were washed from the substrate under tap water and then rinsed with deionized water, weighed, cut to 1-cm pieces and mixed. Subsamples were taken from the AMF monocultures for drying, DNA extraction and root staining. From the dually inoculated treatments, subsamples of roots were taken for drying and DNA extraction only. Subsamples for drying were weighed before and after drying at 105 °C for 48 h. The root staining procedure followed the protocol described by Phillips & Hayman (1970) and Brundrett *et al.* (1984). Briefly, roots were macerated in 10% (w:v) KOH at 90 °C for 25 min, rinsed with water, incubated in 1% HCl at room temperature for 1 h and briefly rinsed with water before transfer to 0.5% Trypan blue in lactic acid/glycerol/water (1:1:1; v:v:v). In this solution, the roots were stained at 90 °C for 2 h and then at room temperature overnight. Finally, the roots were destained for at least 24 h in water at room temperature. The extent of root length colonized by mycorrhizal hyphae, arbuscules and vesicles was determined on stained roots according to the method of McGonigle *et al.* (1990), recording 50 intersections per sample. Dried root and shoot biomass samples were incinerated at 550 °C for 4 h and ashes dissolved in 2 mL 65% HNO_3, made up to 100 mL with distilled water and filtered through a paper filter (Whatman No. 40). The concentration of P in the extracts was determined by flow injection analysis using colorimetric reaction after Boltz & Mellon (1948).

The concentration of large ribosomal subunit (LSU) gene copies of each AMF species was estimated in the roots by real-time PCR with taxon-specific primers and hydrolysis probes as described earlier (Thonar *et al.* 2012). In brief, DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hombrechtikon, Switzerland) from lyophilized root subsamples (100–150 mg fresh weight, recorded before lyophilization) after homogenization by bead disruption (Mini Bead Beater; BioSpec Products, Bartlesville, OK, USA), following the manufacturer's recommendations. Before homogenization, all samples were spiked with a known number (5 billion copies) of an internal standard, which consisted of a cassava mosaic virus DNA fragment (GenBank Accession no. AJ427910) carried in a pUC19 plasmid. Quantification of internal standard recovery in the DNA extracts, using a specific primers pair and a hydrolysis probe, allowed for a correction of both the DNA fraction lost during the extraction and the presence of unspecific PCR inhibitors in the DNA extracts (Thonar *et al.* 2012). All the real-time PCRs were performed in LIGHTCYCLER 2.0 (Roche Diagnostics, Rotkreuz, Switzerland) using Roche chemistry with specific primers and probes labelled with fluorescein and BHQ-1 quencher as described earlier (Thonar *et al.* 2012). The DNA samples used as templates were diluted five times before the real-time PCR. Readings below the detection limits of the individual real-time PCR assays were handled as zeroes for statistical purposes.

Calculations and data analysis

The percentage of root length colonized by the AMF is given as the ratio of intersections containing any of the mycorrhizal structures to all observed root intersections per sample ×100. Phosphorus concentrations in shoot and root extracts were used to calculate the P content in the shoots and roots, respectively. The LSU gene copy numbers per unit of root dry mass were calculated according to the equations presented in Thonar *et al.* (2012), taking into account the internal standard recoveries in the individual DNA extracts, and the dry-to-fresh weight ratios estimated on the root samples subjected to drying.

Regarding the AMF species abundances (i.e. LSU gene concentrations) in roots, we asked the following specific questions:

1 How does AMF species (inoculated at a constant density) abundance change on a gradient of inoculum

density of a competitor species (or the realized abundance of the competitor)?

2 How does the abundance of an AMF species in competition compare with its abundance in monoculture under the same inoculation density?

3 How does AMF species abundance change with increasing density of its inoculum in the presence of another species inoculated at a constant density?

4 How does the abundance of an AMF species depend on its inoculum density with and without a competing AMF species inoculated at a constant density?

The questions 1 and 3 are answered only with the data from dually inoculated containers, whereas the questions 2 and 4 include comparisons of dually and singly inoculated plants. To answer these questions, we have fitted generalized linear models (Fox 2008) with LSU gene copy numbers of one of the three compared AMF species as a response variable and the competitor species presence or abundance (expressed either as its relative inoculation density or as its abundance expressed in log-transformed LSU counts) as predictors. The LSU gene copy numbers exhibit, as a response variable, a strong overdispersion, and therefore, quasi-likelihood estimates of the model parameters and significance tests based on the *F*–statistic were used (Fox 2008, p. 391). Because all the models contain inoculation density of one of the competing species as a predictor and the inoculation density of the other species is held constant within the data subset used for model estimation, the effect of the total inoculation density is collinear and therefore absent in the models. We have compared the models of monotonous change of AMF species abundance with inoculum density with models where the effect of inoculum density was expressed as a second-order polynomial, to test for a possibility of optimum performance at intermediate inoculum densities. When fitting models corresponding to question 4, we have tested for possible opposite effects of inoculum density in monocultures and under competition, by comparing a model with additive effects of density and competitor presence with another one, including interaction between these two predictors.

Each of the models was fitted separately for different combinations of the three studied AMF species, used as a target or a competitor species in dual cultures. The models corresponding to questions 2 and 4 reuse the same subset of observations from monocultures for each AMF species, and we have therefore adjusted the computed significance levels using Holm correction (Holm 1979). Similarly, Holm correction was also applied to significances estimated for models addressing questions 1 and 3 for the same AMF species combination and therefore reusing the same subset of observations.

The relation between LSU counts and (log-transformed) percentage of colonized root length in the monocultures of the three AMF species, as well as the differences among the species was tested with a single generalized linear model, assuming overdispersed Poisson distribution for the stochastic variation.

Regarding the biomass and P content data, the differences among various AMF species combinations (including both monocultures and all possible dual cultures of AMF species), as well as the effect of total inoculation density were evaluated using generalized linear models assuming gamma distribution for its stochastic component and using logarithmic link function, using an *F*-ratio-based tests of significance. When a significant difference among species combinations was found for a particular response variable, post hoc multiple-comparison tests were performed using MULTCOMP package. All statistical models were fitted using program R, version 3.0 (R Core Team 2013).

Results

Root colonization in singly inoculated treatments

Inocula of all three AMF isolates were infective. When administered singly and in the 100% inoculum density, the percentage of root length colonized by the AMF reached on average 30% for *Gigaspora* and 43% for both *Rhizophagus* and *Claroideoglomus* at 42 days after sowing (Fig. S1, Supporting information). Time-course data indicated an earlier colonization of roots by *Claroideoglomus* as compared to the other two fungi (Fig. S1, Supporting information). No mycorrhizal structures were observed in the NM treatment at any time point (data not shown). We have found a highly significant relation between root colonization estimates obtained by microscopy and by real-time PCR ($F_{1,72} = 432$, $P < 0.001$, Fig. S2, Supporting information). When compared across the fungi, the slopes of regression lines did not differ significantly ($F_{2,68} = 0.85$, n.s.), but the number of LSU copies differed ($F_{2,70} = 66.5$, $P < 0.001$), with *Rhizophagus* showing significantly more LSU gene copies per unit of root colonization than the two other fungi, whereas *Claroideoglomus* and *Gigaspora* did not differ significantly from each other ($z = -0.769$, n.s. – see Fig. S2, Supporting information). Both staining and real-time PCR also showed that both *Rhizophagus* and *Gigaspora* inoculated singly only colonized roots at detectable levels when the inoculum was provided at 100% and 40% levels. If more diluted, the inoculation did not result in any detectable colonization (Fig. 1 for qPCR results, microscopy data not shown). In contrast, detectable levels of colonization were measured for all inoculation densities of *Claroideoglomus* inoculated singly at 42 days

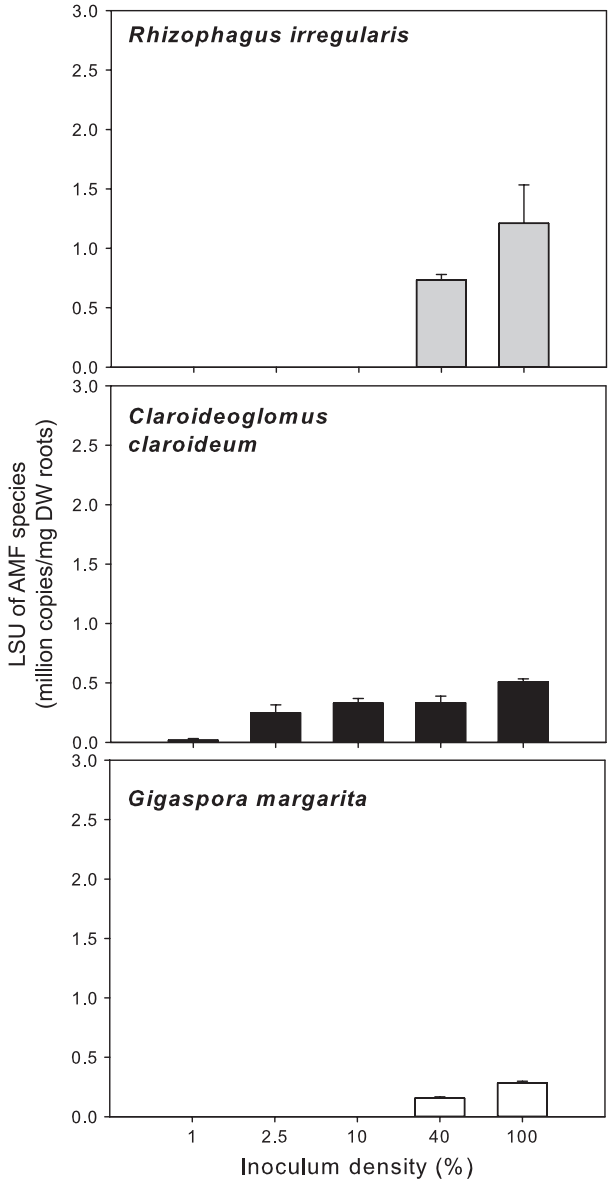

Fig. 1 Abundance in the *Medicago truncatula* roots of three arbuscular mycorrhizal fungi inoculated singly and at varying inoculum densities, as assessed by quantitative real-time PCR at 42 days after sowing. Mean values of five independent replicates +1 standard error of mean are shown. LSU, large ribosomal subunit gene.

after sowing, although the values for the lowest inoculum density remained low (Fig. 1).

Fungal interactions in dually inoculated treatments

Response of target species (100% inoculum density) to a competitor. Abundance in roots of *Rhizophagus* inoculated at a constant density (100%) and combined with *Claroideoglomus* significantly decreased as compared with the *Rhizophagus* monoculture and further declined with increasing inoculum density of *Claroideoglomus* (Fig. 2, Tables 1 and 2). This gradual decline was, however, not explained by the realized abundance of *Claroideoglomus* in the mixed cultures ($F_{1,23}$ = 0.24, n.s.). Abundance of *Rhizophagus* when inoculated at 100% density was not significantly affected by coinoculation with *Gigaspora* in any respect (Tables 1 and 2), although the absolute levels of root colonization by *Rhizophagus* were seemingly higher in the presence of *Gigaspora* (Figs 1 and 2). The response of *Rhizophagus* was not significant even when nonlinear model was employed ($F_{1,25}$ = 0.11, n.s.).

When *Claroideoglomus* was inoculated at a constant density of 100%, its abundance was not affected by coinoculation with *Gigaspora* provided at different inoculum densities (Tables 1 and 2, Fig. 2). When exposed to *Rhizophagus*, however, there was a clear negative relationship between *Claroideoglomus* abundance and inoculum density of *Rhizophagus* (Table 1), although this trend was not visible when realized abundance of *Rhizophagus* was used as a predictor of the effect ($F_{1,23}$ = 0.22, n.s.).

Abundance of *Gigaspora* in roots following inoculation at 100% density was systematically increased by coinoculation with either of the other fungi as compared to *Gigaspora* monoculture (Table 2). There was a gradual decline in this positive effect with increasing inoculum density of *Rhizophagus* (Table 1, Fig. 2), but there was obviously no interference whatsoever with *Claroideoglomus* (Table 1).

Response of the competitor species (inoculated at variable densities). The most straightforward case was that of *Gigaspora*. Its abundance increased by increasing its inoculum density in dually inoculated treatments (Table 3). Further, there was a systematic positive effect on the abundance of *Gigaspora* of the coexistence with other AMF, as compared with *Gigaspora* monocultures (Table 2, Figs 1 and 2). However, the pace of increase in abundance with increasing inoculum densities was not affected by coinoculation with the other fungi, because of the insignificant interaction between the main effects (Table 4).

Rhizophagus abundance did not show any trivial relationships to its inoculum densities when exposed to either *Claroideoglomus* or *Gigaspora* (Table 3). However, when monocultures were included in the model, slight yet significant increase in abundance with increasing inoculum densities showed up, independent of the presence of *Gigaspora* (Table 4, Fig. 2). There was no overall change in abundance of *Rhizophagus* with or without the *Gigaspora*, and there was also no significant change in the pace of increase in its abundance along the inoculum density gradient between monocultures and the dual cultures with *Gigaspora* (Table 4). When

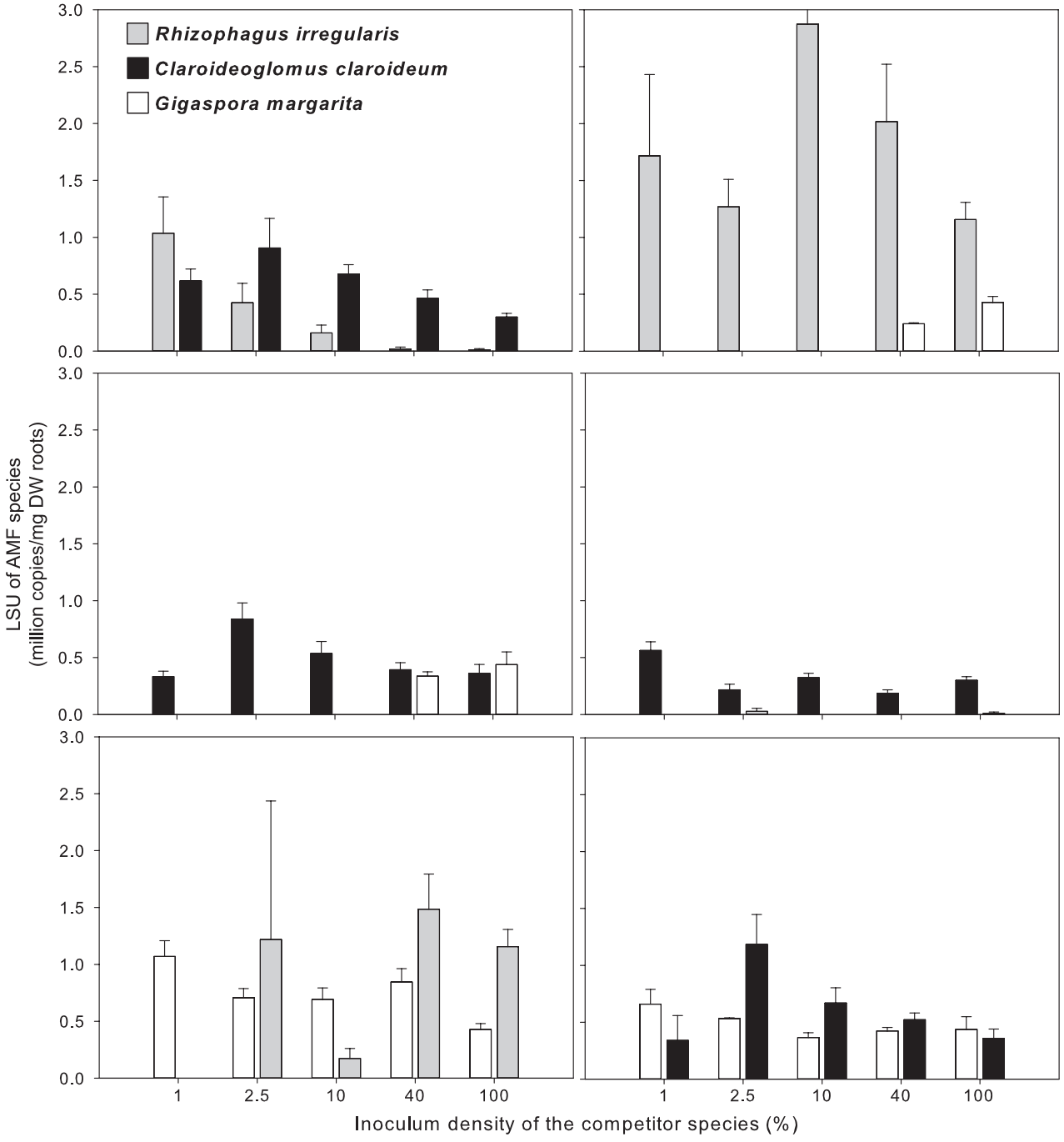

Fig. 2 Abundance in the *Medicago truncatula* roots of three arbuscular mycorrhizal fungi, coinoculated in dual mixtures, as assessed by quantitative real-time PCR at 42 days after sowing. Inoculum density of one of the species (so-called target species, shown always as the left bar of the bar pairs) was kept constant at the 100% inoculum density, whereas the inoculum density of the other (so-called competitor) species, whose abundance is always shown as the right bar of the bar pairs, varied according to the *x*-axis. First row of graphs shows results of combinations of *Rhizophagus* as the target species with the two other fungal taxa. Second row shows results of competition of *Claroideoglomus* as target with the two others, and the last row that of *Gigaspora* as the target species with *Rhizophagus* and *Claroideoglomus* as competitor species. Mean values of five independent replicates +1 standard error of mean are shown. LSU, large ribosomal subunit gene.

Rhizophagus was exposed to coinoculation with *Claroideoglomus*, its abundance significantly decreased in dual cultures as compared to monocultures (Table 4). Significant interaction term between the main factors then explained that the increase in *Rhizophagus* abundance along its inoculation density gradient was only

Table 1 Change in the abundance in *Medicago truncatula* roots of the target arbuscular mycorrhizal fungal species, inoculated at a constant inoculum density (100%), with the inoculum density of the other (competitor) species. Results of GLM-based test assuming quasi-Poisson distribution are shown with *P*-values corrected for multiple testing

Target species	Competitor species	$F_{1,23}$	*P*-value	Regression coefficient (significant change)
Rhizophagus irregularis	*Claroideoglomus claroideum*	48.9	<0.001	−0.802
R. irregularis	*Gigaspora margarita*	0.005	n.s.	n.a.
C. claroideum	*G. margarita*	1.55	n.s.	n.a.
C. claroideum	*R. irregularis*	20.7	<0.001	−0.205
G. margarita	*R. irregularis*	8.49	<0.05	−0.120
G. margarita	*C. claroideum*	4.85	n.s.	n.a.

n.s. $P \geq 0.05$, n.a. not applicable.

Table 2 Differences in the abundance in *Medicago truncatula* roots of the target AMF species inoculated at a constant density (100%) due to coinoculation with another (competitor) AMF species and due to the inoculation density of the competitor species. Results of GLM-based test assuming quasi-Poisson distribution are shown with *P*-values corrected for multiple testing

Target species	Competitor species	Presence of competitor AMF species, $F_{1,27}$	Inoculation density of competitor AMF species, $F_{1,26}$
Rhizophagus irregularis	*Claroideoglomus claroideum*	14.9**	21.1***
R. irregularis	*Gigaspora margarita*	0.24 n.s.	0.93 n.s.
C. claroideum	*G. margarita*	0.02 n.s.	3.80 n.s.
C. claroideum	*R. irregularis*	5.00 n.s.	0.18 n.s.
G. margarita	*R. irregularis*	21.6***	10.5**
G. margarita	*C. claroideum*	6.26 *	1.82 n.s.

AMF, arbuscular mycorrhizal fungal.
n.s. $P \geq 0.05$, *$0.05 > P \geq 0.01$, **$0.01 > P \geq 0.001$, ***$P < 0.001$.

detected in monocultures, but actually vanished in the dual cultures (Table 3, Fig. 2).

The effects recorded with *Claroideoglomus* were the most complex ones. Coinoculation with *Rhizophagus* indicated a monotonous, yet not a very strong decrease in abundance with increasing inoculum density of *Claroideoglomus* (Table 3). Further analyses including comparisons with the relevant monocultures showed that the abundance of *Claroideoglomus* systematically increased when coinoculated with *Rhizophagus* (Fig. 2,

Table 3 Change in the abundance in *Medicago truncatula* roots of the 'competitor' AMF species with its inoculation density, when coinoculated with another AMF species (i.e. a target species) at a constant inoculum density. Results of GLM-based test assuming quasi-Poisson distribution are shown with *P*-values corrected for multiple testing

Target species	Competitor species	$F_{1,23}$	*P*-value	Regression coefficient (significant change)
Rhizophagus irregularis	*Claroideoglomus claroideum*	6.97	0.015	−0.158
R. irregularis	*Gigaspora margarita*	222	<0.001	1.29
C. claroideum	*G. margarita*	89.4	<0.001	1.14
C. claroideum	*R. irregularis*	0.61	n.s.	n.a.
G. margarita	*R. irregularis*	1.24	n.s.	n.a.
G. margarita	*C. claroideum*	1.31	n.s.	n.a.

AMF, arbuscular mycorrhizal fungal. n.s. $P \geq 0.05$, n.a. not applicable.

Table 4). Significant interaction between the main factors (Table 4) then explained why the effect of inoculum density on *Claroideoglomus* abundance was not significant across all the treatments (Table 4): whereas *Claroideoglomus* abundance increased with increasing inoculum densities in monocultures, it did decrease when exposed to *Rhizophagus* (Table 3). When *Claroideoglomus* was exposed to *Gigaspora*, its abundance strongly increased as compared to the monocultures (Table 4). The trend of increasing *Claroideoglomus* abundance with inoculum density in monocultures (Fig. 1), however, vanished when exposed to *Gigaspora*. This differential response of *Claroideoglomus* abundance to its own inoculum density with and without *Gigaspora* resulted in highly significant interaction term in Table 4 and was also the reason for the absence of overall significance of the effect of inoculum density on *Claroideoglomus* abundance in roots across the singly and dually inoculated treatments.

AMF communities, plant growth and P nutrition

Growth and nutritional responses of plants to inoculation with the different AMF communities were mainly determined by the combination of fungi rather than their inoculum densities (Table S2, Fig. S4, Supporting information). Compared with the NM treatment, single AMF inoculations did not lead to any significant effects on plant growth (Fig. 3). Mixed inoculations, however, showed more stratified responses. On the one hand, coinoculation with *Rhizophagus* and *Claroideoglomus* showed a significantly higher plant biomass as compared to the NM and to the AMF monocultures. This

Table 4 Change in the abundance in *Medicago truncatula* roots of AMF species inoculated at variable inoculum densities (i.e. competitor species) due to coinoculation with another AMF species at a constant inoculum density (i.e. target AMF species), and with its own inoculation density. Results of GLM-based test assuming quasi-Poisson distribution are shown with *P*-values corrected for multiple testing

Target species	Competitor species	Coinoculation, $F_{1,48}$	Inoculum density, $F_{1,47}$	Interaction, $F_{1,46}$
Rhizophagus irregularis	*Claroideoglomus claroideum*	16.4***	0.02 n.s.	26.5***
R. irregularis	*Gigaspora margarita*	12.1**	479***	0.01 n.s.
C. claroideum	*G. margarita*	11.5**	241***	0.39 n.s.
C. claroideum	*R. irregularis*	9.48*	13.9**	8.10*
G. margarita	*R. irregularis*	1.29 n.s.	5.80*	4.62 n.s.
G. margarita	*C. claroideum*	12.1**	0.23 n.s.	11.7**

AMF, arbuscular mycorrhizal fungal.
n.s. $P \geq 0.05$, *$0.05 > P \geq 0.01$, **$0.01 > P \geq 0.001$, ***$P < 0.001$.

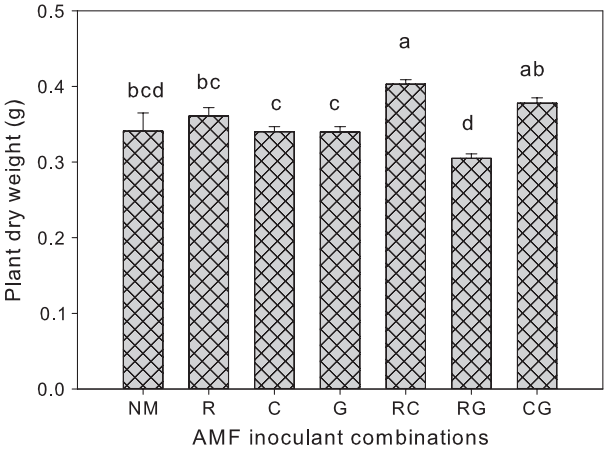

Fig. 3 Biomass (shoots and roots combined) of the *Medicago truncatula* plants inoculated with mycorrhizal fungi or not and harvested 42 days after sowing. Data were pooled for each fungal combination irrespective of the inoculum densities of the different fungal taxa. Mean values +1 standard error of means are shown. NM, nonmycorrhizal treatment; R, *Rhizophagus irregularis*; C, *Claroideoglomus claroideum*; G, *Gigaspora margarita*; RC, *Rhizophagus* combined with *Claroideoglomus*; RG, *Rhizophagus* combined with *Gigaspora*, CG, *Claroideoglomus* combined with *Gigaspora*. Different letters indicate significant differences between treatment means ($P < 0.05$), according to post hoc test of a significant inoculation type term, and assuming gamma distribution of the stochastic component.

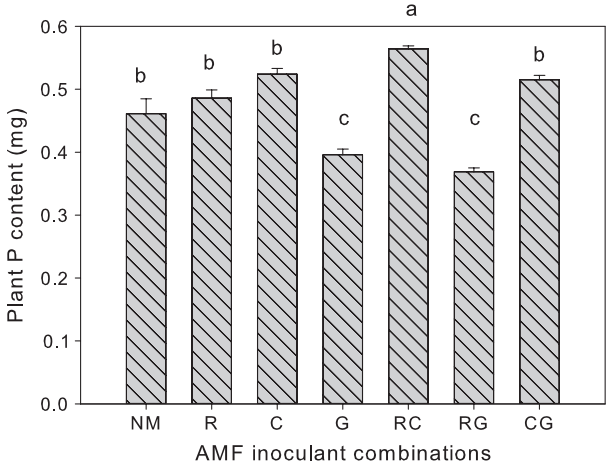

Fig. 4 Phosphorus content (shoots and roots combined) of the *Medicago truncatula* plants inoculated with mycorrhizal fungi or not and harvested 42 days after sowing. Data were pooled for each fungal combination irrespective of the inoculum densities of the different fungal taxa. Mean values +1 standard error of means are shown. Treatment abbreviations are as in Fig. 3. Different letters indicate significant differences between treatment means ($P < 0.05$), according to post hoc test of a significant inoculation type term, and assuming gamma distribution of the stochastic component.

effect was also accompanied by significant shift in partitioning of biomass between shoots and roots (Fig. S3, Supporting information). On the other hand, coinoculation with *Rhizophagus* and *Gigaspora* resulted in the lowest plant biomass, significantly different from any of the monocultures, although not from the NM treatment (Fig. 3). Even more contrastive were the effects on plant P content (Fig. 4). Consistent with the positive growth responses, the plants coinoculated with *Rhizophagus* and *Claroideoglomus* contained more P than any other fungal combinations or the NM treatment. On the other hand, inoculation with *Gigaspora* alone or coinoculation with *Gigaspora* and *Rhizophagus* resulted in the lowest P content of plants among all the treatments (Fig. 4).

Discussion

Our experiment allowed novel insights into the nature of interactions between different AMF species cocolonizing the same plant root system. Several lessons could be learnt from the results presented above:

1 The identity of co-occurring mycorrhizal fungi and their inoculation densities determine whether they

compete or facilitate each other's development during colonization of host plant roots;

2 Realized colonization measured at the end of the experiment is telling a different story from inoculation density in explaining the competitive interactions;

3 Plant responses in terms of growth and/or P nutrition depend on AMF community composition and can vary from positive to negative.

Here, we try to explain and reconcile our results, confront them with published literature and propose underlying mechanisms of the observed effects.

Interspecies interactions in AMF community

The strongest competition was recorded between *Rhizophagus* and *Claroideoglomus*, whereas the strongest facilitation was between *Gigaspora* and the other fungi. Another recurring observation was that the higher was the overall inoculum density in our experiment (i.e. the closer was the ratio of the different inocula to 1:1, or to 100%:100%), the weaker was the facilitation (if any) and the stronger the competition. The fact that more closely related AMF compete for root colonization more strongly than distantly related ones is consistent with previous research (Maherali & Klironomos 2007, 2012; Mummey *et al.* 2009). This seems related to a general trend that more phylogenetically diverse organisms are likely to be more functionally different (Munkemuller *et al.* 2012). By hosting functionally different AMF communities in its roots, plant could benefit from functional complementarity of their symbionts or insure its survival or maximize symbiotic benefits under changing environmental conditions (Maherali & Klironomos 2007). But how is this promotion of diverse communities achieved mechanistically? If the fungi simply competed for root space and plant carbon resources, facilitation is unlikely to be easily explained, whereas explanation for competition is fairly straightforward. Facilitation could probably only be achieved if strong plant sanctions active against one fungus (e.g. *Gigaspora*) would be suppressed by the presence of another unrelated fungus, by directly or indirectly affecting the signalling pathways (e.g. through manipulating the immunity response of the plant or through improving nutritional status of the host). There is no experimental support for this scenario; rather, it seems that plant may not control the initial stages of symbiosis establishment and root colonization very strongly (David-Schwartz *et al.* 2003; Akiyama *et al.* 2005) but rather promotes the more beneficial symbionts at later stages of symbiosis through preferential allocation of carbon (Kiers *et al.* 2011). Alternative scenarios to achieve facilitation would be creating more supportive environment for infective propagule activation and root penetration by one of the fungi through the presence of the other. This view is, however, challenged by the fact that the fungus creating more conducive environment for the other should be active earlier. This is unlikely in case of *Rhizophagus* facilitating *Gigaspora*, as *Rhizophagus* was probably slower in establishing root colonization than the *Gigaspora* under the conditions of our experiment (Fig. S1, Supporting information). So, the last possibility is that the plant, by as yet unknown mechanism, actively promotes the diversity of its root symbionts. Although there is very little mechanistic understanding of the processes involved, there is an indirect evidence for such a mechanism: In spite of preferential allocation of carbon to the most beneficial symbiont (Kiers *et al.* 2011), and in spite of strong overdominance recorded in AM communities at small spatial scales, which was assigned to positive feedback following early root colonization by one fungus (Dumbrell *et al.* 2010), the AMF communities, even at spatially small scale, are rarely monospecific, but the diversity is the rule (Jansa *et al.* 2003b; Kiers *et al.* 2011; Verbruggen *et al.* 2012). Possibly, induced changes in chemical defence such as those described for three-way interactions between plants, mycorrhiza and lepidopteran herbivores (Bennett *et al.* 2009) could contribute to explanation of the observed trends, but the nature of compounds involved in shaping AMF communities in plant roots still remains to be uncovered.

The competition for root colonization between different AMF species could be explained by different mechanisms or their combination, with the host plant as the sole carbon source for the fungi being strongly involved in any of the scenarios (Pearson *et al.* 1993). This is because there is as yet no reported experimental evidence for direct antagonism between the hyphae of different AMF species, although there is a possibility that signalling compounds produced by the hyphae or their associated microbes (Jansa *et al.* 2013) could potentially play some role. Certainly, the timing of root colonization will play a very important role as the first colonizer will have a major competitive advantage over a latecomer sharing the same root niche (Dumbrell *et al.* 2010). This is partly because the primary colonizer of roots will tap early on the root carbon resources, allowing it to occupy the available niche, and partly because it will induce mechanisms active at preinfection (root exudate) level or root colonization stages, limiting further colonization of the roots by the AMF (Pinior *et al.* 1999; Vierheilig 2004a,b; Herrera-Medina *et al.* 2008). As this is a plausible scenario for competition encountered between *Rhizophagus* and *Claroideoglomus*, it does not really explain why *Gigaspora* colonization gets stimulated by *Claroideoglomus*, which is colonizing roots

earlier than *Gigaspora* under our conditions (Fig. S1, Supporting information). An intriguing idea is whether the different fungi would specifically colonize distinct root zones to reduce the competitive pressure. For example, carbon cost of the symbiosis between medic plants and *Gigaspora* has been shown to be higher than with the other two species (Lendenmann *et al.* 2011; Thonar *et al.* 2011). At the same time, colonization of roots by *Gigaspora* has been shown to enhance the underground carbon 'sink' (Lerat *et al.* 2003). This could have resulted in a preferential spread of *Gigaspora* towards the root zones close to the shoot where the concentration of photosynthates is possibly higher. This mechanism of avoidance of competition through spatial separation within the root system could explain the lack of competition between this and the other species. Yet, the theory of occupation of different root zones by different AMF species remains to be confirmed experimentally and so is its importance in facilitating colonization of one fungus through another fungus.

The fact that facilitation vanishes and competition gets stronger by increasing the overall inoculum density of AMF community indicates that a mechanism to reduce further colonization/root colonization is activated. As our experiment was designed so that the highest inoculum densities of the different fungi would alone result in occupation of ~50% of the root length of the experimental plants, combining two such inoculum doses could, under the assumption of absolutely neutral interaction between the fungi and the colonization intensity of the colonized root sections being at maximum, fill the roots completely. Under such situations, plants activate nonspecific regulatory pathways to suppress further root colonization (Vierheilig 2004a), which is also well in line with our results. The backside part of the story is that to achieve comparable levels of root colonization at a certain time point by the different AMF, we had to strongly manipulate the infectivity of our inocula, getting different trajectories of root colonization by the different AMF species (Fig. S1, Supporting information). For the lowest inoculum doses, the inoculum sometimes contained, on average, <1 spore of the fungus per container. This is not necessarily a problem for colonization establishment as root colonization can also be initiated from colonized roots or mycelium fragments in soil (Klironomos & Hart 2002), which were included in our inocula. However, these inoculation levels are unusually low as compared to most previous studies (Jansa *et al.* 2005, 2008; Thonar *et al.* 2011). In these previous studies, root colonization levels were nearing 100% of the root length colonized for the *Rhizophagus*, which was much higher than the numbers encountered here. Therefore, the results of this study must be regarded cautiously with respect to other (e.g. field) systems.

Explanatory power of inoculum density and realized abundance of the AMF

Although there is certainly a causal relationship between inoculum density and realized abundance of the AMF at any time point, this relationship is not trivial – it will be a product of interactions between initial inoculum infectivity, root growth, soil and climate conditions and interactions with other fungi that will all potentially affect the realized AMF abundance at any time point (Wilson & Trinick 1983; Wilson 1984). Given all these influential factors, and considering that only a single harvest was included in our study to address the interactions between different AMF species, it is not surprising that it was the inoculum density rather than the realized abundance of one of the fungal species explaining the interaction with the other species. More work is certainly needed to follow the developing AMF community throughout time and to specifically address the importance of prior root occupancy (Dumbrell *et al.* 2010) in shaping AMF communities, as well as the role of other microbes supplied with the different inocula in changing the competitive environment.

AMF communities and plant responses

The case of *Claroideoglomus* and *Rhizophagus* being particularly beneficial combination for growth and P uptake of the medic plants as observed here is consistent with our previous observations (Jansa *et al.* 2008). This is further documented by the fact that this was the only fungal combination where root-to-shoot biomass ratio was significantly lower than in the NM treatment (Fig. S3, Supporting information). Both of these fungi are known to support P nutrition and growth of this model plant (Lendenmann *et al.* 2011), although their strategies to gather soil P differ (Thonar *et al.* 2011). Such a functional partitioning would substantiate functional complementarity, leading to the observed overyielding of dually inoculated plants as compared to the monocultures (Koide 2000; Jansa *et al.* 2008). What remains unclear, however, is how the previously established differences in soil exploitation by hyphae of these two AMF species, stretching over centimetres from the roots (Thonar *et al.* 2011), could come to significant play in small containers used here. Most likely, the fungi could still partition their contribution even in these small containers, possibly due to dynamic adaptation of their mycelium networks (Jansa *et al.* 2003a). Alternatively, it is possible that physiological activity of one of the fungi (e.g. *Claroideoglomus*) was stimulated in the presence of the other AMF species (e.g. *Rhizophagus*). The fact that the abundance of *Rhizophagus* in some of the mixed treatments was very low, questioning its possible direct impact on host nutrition, would speak

for this alternative scenario. However, because the biomass of *Claroideoglomus* did not increase (at least not in the roots) in the mixed treatments, it provides a strong counterargument and leaves this option open to further studies.

The low P content of plants colonized by *Gigaspora* is consistent with the recent observation (Thonar *et al.* 2011) for this AMF species to store P temporarily in the mycorrhizal hyphae before being delivered to the plants. Together with described down-regulation of plant's own P acquisition mechanism upon AMF symbiosis establishment (Facelli *et al.* 2009), this combination of effects possibly led to the strong decline in overall P uptake in *Gigaspora* monocultures as well as in dual cultures of *Gigaspora* and *Rhizophagus*. In these dual cultures, the abundance of *Gigaspora* was further stimulated and so were most likely amplified also the negative effects on plant P uptake and, consequently, the growth.

Together, our results demonstrate a discrepancy between the finding that (i) in the mixed treatments, medic plants would promote root co-occupancy of *Gigaspora* and *Rhizophagus* or *Gigaspora* and *Claroideoglomus* although this brings no obvious returns to the plants and that (ii) coinoculations of species that are competing for colonization (e.g. *Rhizophagus* and *Claroideoglomus*) could still result in the greatest benefits for the plants. This apparent contradiction, however, could easily turn into a consistent picture if we assume that plant would generally support AMF communities with the greatest functional complementarity (Maherali & Klironomos 2007) to optimize the benefits in spatially or temporarily heterogeneous environments, possibly through yet unknown mechanisms of carbon trading (Kiers *et al.* 2011) or chemical defence (Bennett *et al.* 2009). Setting up experiments with spatially and/or temporarily heterogeneous resources is, however, remaining a major challenge for the future experimentation as is including more isolates from each fungal species to test whether the observed functional consequences of mixing AMF species are consistent among isolates belonging to the same species or are due to unique properties of each isolate without a significant phylogenetic signal.

Conclusions and outlook

The principal message from this study is that root colonization by a particular mycorrhizal fungus is depending not only on its initial inoculum density/infectivity, but also on the fungal community context, namely on the identity of the co-occurring fungi and their inoculum density/infectivity. Within the AMF community, both competitive suppression and facilitation can occur. For thorough understanding of the factors determining AMF community assembly, we now need to improve our understanding of the AMF community development through time, on different plant species and in different soil and climatic conditions. Some individual factors have been tested previously (Daft & Hogarth 1983; Alkan *et al.* 2006; Jansa *et al.* 2008), but the knowledge still remains fragmented. Manipulative experiments of the similar kind as presented here are critically important yet rarely performed to uncover the mechanisms determining the composition of organismal communities across a competitive landscape. Such experiments are also urgently needed for the selection of industrial microbial inoculants that not only need to prove beneficial when inoculated alone, but also need to persist and successfully establish in competition with the native microbes sharing their ecological niche.

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References

Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature*, **435**, 824–827.

Alkan N, Gadkar V, Yarden O, Kapulnik Y (2006) Analysis of quantitative interactions between two species of arbuscular mycorrhizal fungi, *Glomus mosseae* and *G. intraradices*, by real-time PCR. *Applied and Environmental Microbiology*, **72**, 4192–4199.

Bennett AE, Bever JD, Bowers MD (2009) Arbuscular mycorrhizal fungal species suppress inducible plant responses and alter defensive strategies following herbivory. *Oecologia*, **160**, 771–779.

Boltz DF, Mellon MG (1948) Spectrophotometric determination of phosphorus as molybdiphosphoric acid. *Analytical Chemistry*, **20**, 749–751.

Brundrett MC, Piché Y, Peterson RL (1984) A new method for observing the morphology of vesicular-arbuscular mycorrhizae. *Canadian Journal of Botany*, **62**, 2128–2134.

Clark FE (1965) The concept of competition in microbial ecology. In: *Ecology of Soil-Borne Plant Pathogens* (eds Baker KF, Snyder WC), pp. 339–347. University of California Press, Berkeley, CA.

Daft MJ, Hogarth BG (1983) Competitive interactions amongst 4 species of *Glomus* on maize and onion. *Transactions of the British Mycological Society*, **80**, 339–345.

David-Schwartz R, Gadkar V, Wininger S *et al.* (2003) Isolation of a premycorrhizal infection (pmi2) mutant of tomato, resistant to arbuscular mycorrhizal fungal colonization. *Molecular Plant-Microbe Interactions*, **16**, 382–388.

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH (2010) Idiosyncrasy and overdominance in the structure of natural communities of arbuscular mycorrhizal fungi: is there a role for stochastic processes? *Journal of Ecology*, **98**, 419–428.

Facelli E, Smith SE, Smith FA (2009) Mycorrhizal symbiosis—overview and new insights into roles of arbuscular mycorrhizas in agro- and natural ecosystems. *Australasian Plant Pathology*, **38**, 338–344.

Feddermann N, Finlay R, Boller T, Elfstrand M (2010) Functional diversity in arbuscular mycorrhiza—the role of gene expression, phosphorous nutrition and symbiotic efficiency. *Fungal Ecology*, **3**, 1–8.

Fox J (2008) *Applied Regression Analysis and Generalized Linear Models*, 2nd edn. Sage Publications, Los Angeles, California.

Frossard E, Sinaj S (1997) The isotope exchange kinetic technique: a method to describe the availability of inorganic nutrients. Applications to K, P, S and Zn. *Isotopes in Environmental and Health Studies*, **33**, 61–77.

Golubski AJ (2002) Potential impacts of multiple partners on mycorrhizal community dynamics. *Theoretical Population Biology*, **62**, 47–62.

Hart MM, Reader RJ, Klironomos JN (2001) Life-history strategies of arbuscular mycorrhizal fungi in relation to their successional dynamics. *Mycologia*, **93**, 1186–1194.

van der Heijden MGA, Klironomos JN, Ursic M *et al.* (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, **396**, 69–72.

Heinemeyer A, Ineson P, Ostle N, Fitter AH (2006) Respiration of the external mycelium in the arbuscular mycorrhizal symbiosis shows strong dependence on recent photosynthates and acclimation to temperature. *New Phytologist*, **171**, 159–170.

Hepper CM, Azcón-Aguilar C, Rosendahl S, Sen R (1988) Competition between 3 species of *Glomus* used as spatially separated introduced and indigenous mycorrhizal inocula for leek (*Allium porrum* L). *New Phytologist*, **110**, 207–215.

Herrera-Medina MJ, Tamayo MI, Vierheilig H, Ocampo JA, Garcia-Garrido JM (2008) The jasmonic acid signalling pathway restricts the development of the arbuscular mycorrhizal association in tomato. *Journal of Plant Growth Regulation*, **27**, 221–230.

Hoagland D, Arnon D (1950) *The Water-Culture Method for Growing Plants Without Soil*. pp. 32. Circular 347, California Agricultural Experimental Station, Berkeley, California.

Holm S (1979) A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, **6**, 65–70.

Jakobsen I, Abbott LK, Robson AD (1992) External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. *New Phytologist*, **120**, 371–380.

Jansa J, Mozafar A, Anken T *et al.* (2002) Diversity and structure of AMF communities as affected by tillage in a temperate soil. *Mycorrhiza*, **12**, 225–234.

Jansa J, Mozafar A, Frossard E (2003a) Long-distance transport of P and Zn through the hyphae of an arbuscular mycorrhizal fungus in symbiosis with maize. *Agronomie*, **23**, 481–488.

Jansa J, Mozafar A, Kuhn G *et al.* (2003b) Soil tillage affects the community structure of mycorrhizal fungi in maize roots. *Ecological Applications*, **13**, 1164–1176.

Jansa J, Mozafar A, Frossard E (2005) Phosphorus acquisition strategies within arbuscular mycorrhizal fungal community of a single field site. *Plant and Soil*, **276**, 163–176.

Jansa J, Smith FA, Smith SE (2008) Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? *New Phytologist*, **177**, 779–789.

Jansa J, Bukovská P, Gryndler M (2013) Mycorrhizal hyphae as ecological niche for highly specialized hypersymbionts—or just soil free-riders? *Frontiers in Plant Science*, **4**, 134.

Kiers ET, Duhamel M, Beesetty Y *et al.* (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science*, **333**, 880–882.

Klironomos JN, Hart MM (2002) Colonization of roots by arbuscular mycorrhizal fungi using different sources of inoculum. *Mycorrhiza*, **12**, 181–184.

Koide RT (2000) Functional complementarity in the arbuscular mycorrhizal symbiosis. *New Phytologist*, **147**, 233–235.

Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A (2012) Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. *New Phytologist*, **193**, 970–984.

Lekberg Y, Koide RT, Rohr JR, Aldrich-Wolfe L, Morton JB (2007) Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. *Journal of Ecology*, **95**, 95–105.

Lendenmann M, Thonar C, Barnard R *et al.* (2011) Symbiont identity matters: carbon and phosphorus fluxes between *Medicago truncatula* and different arbuscular mycorrhizal fungi. *Mycorrhiza*, **21**, 689–702.

Lerat S, Lapointe L, Gutjahr S, Piché Y, Vierheilig H (2003) Carbon partitioning in a split-root system of arbuscular mycorrhizal plants is fungal and plant species dependent. *New Phytologist*, **157**, 589–595.

Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science*, **316**, 1746–1748.

Maherali H, Klironomos JN (2012) Phylogenetic and trait-based assembly of arbuscular mycorrhizal fungal communities. *PLoS ONE*, **7**, e36695.

Massoumou M, van Tuinen D, Chatagnier O *et al.* (2007) *Medicago truncatula* gene responses specific to arbuscular mycorrhiza interactions with different species and genera of Glomeromycota. *Mycorrhiza*, **17**, 223–234.

Mathimaran N, Ruh R, Vullioud P, Frossard E, Jansa J (2005) *Glomus intraradices* dominates arbuscular mycorrhizal communities in a heavy textured agricultural soil. *Mycorrhiza*, **16**, 61–66.

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective-measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. *New Phytologist*, **115**, 495–501.

Mummey DL, Antunes PM, Rillig MC (2009) Arbuscular mycorrhizal fungi pre-inoculant identity determines community composition in roots. *Soil Biology and Biochemistry*, **41**, 1173–1179.

Munkemuller T, de Bello F, Meynard CN *et al.* (2012) From diversity indices to community assembly processes: a test with simulated data. *Ecography*, **35**, 468–480.

Newsham K, Fitter AH, Watkinson AR (1995) Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *Journal of Ecology*, **83**, 991–1000.

Pearson JN, Abbott LK, Jasper DA (1993) Mediation of competition between 2 colonizing VA mycorrhizal fungi by the host plant. *New Phytologist*, **123**, 93–98.

Pearson JN, Abbott LK, Jasper DA (1994) Phosphorus, soluble carbohydrates and the competition between 2 arbuscular mycorrhizal fungi colonizing subterranean clover. *New Phytologist*, **127**, 101–106.

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, **55**, 158–161.

Pinior A, Wyss U, Piché Y, Vierheilig H (1999) Plants colonized by AM fungi regulate further root colonization by AM fungi through altered root exudation. *Canadian Journal of Botany*, **77**, 891–897.

Pivato B, Mazurier S, Lemanceau P *et al.* (2007) *Medicago* species affect the community composition of arbuscular mycorrhizal fungi associated with roots. *New Phytologist*, **176**, 197–210.

R Core Team (2013) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.r-project.org.

Rillig MC (2004) Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecology Letters*, **7**, 740–754.

Rosendahl S, Matzen HB (2008) Genetic structure of arbuscular mycorrhizal populations in fallow and cultivated soils. *New Phytologist*, **179**, 1154–1161.

Smith S, Read D (2008) *Mycorrhizal Symbiosis*, 3rd edn. Academic Press, Amsterdam.

Snaydon RW (1991) Replacement or additive designs for competition studies. *Journal of Applied Ecology*, **28**, 930–946.

Thonar C, Schnepf A, Frossard E, Roose T, Jansa J (2011) Traits related to differences in function among three arbuscular mycorrhizal fungi. *Plant and Soil*, **339**, 231–245.

Thonar C, Erb A, Jansa J (2012) Real-time PCR to quantify composition of arbuscular mycorrhizal fungal communities—marker design, verification, calibration and field validation. *Molecular Ecology Resources*, **12**, 219–232.

van Tuinen D, Jacquot E, Zhao B, Gollotte A, Gianinazzi-Pearson V (1998) Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. *Molecular Ecology*, **7**, 879–887.

Verbruggen E, El Mouden C, Jansa J *et al.* (2012) Spatial structure and interspecific cooperation: theory and an empirical test using the mycorrhizal mutualism. *American Naturalist*, **179**, E133–E146.

Vierheilig H (2004a) Further root colonization by arbuscular mycorrhizal fungi in already mycorrhizal plants is suppressed after a critical level of root colonization. *Journal of Plant Physiology*, **161**, 339–341.

Vierheilig H (2004b) Regulatory mechanisms during the plant-arbuscular mycorrhizal fungus interaction. *Canadian Journal of Botany*, **82**, 1166–1176.

Wagg C, Jansa J, Schmid B, van der Heijden MGA (2011a) Belowground biodiversity effects of plant symbionts support aboveground productivity. *Ecology Letters*, **14**, 1001–1009.

Wagg C, Jansa J, Stadler M, Schmid B, van der Heijden MGA (2011b) Mycorrhizal fungal identity and diversity relaxes plant-plant competition. *Ecology*, **92**, 1303–1313.

Weigelt A, Jolliffe P (2003) Indices of plant competition. *Journal of Ecology*, **91**, 707–720.

Wilson JM (1984) Competition for infection between vesicular arbuscular mycorrhizal fungi. *New Phytologist*, **97**, 427–435.

Wilson JM, Trinick MJ (1983) Infection development and interactions between vesicular-arbuscular mycorrhizal fungi. *New Phytologist*, **93**, 543–553.

The plant experiment was carried out in the Group of Plant Nutrition, ETH Zurich. C.T. designed and performed the research, analysed the data, wrote the paper and contributed to the revisions. E.F. contributed to the design of the research, data analyses and paper writing. P.S. carried out statistical analysis of the data and contributed to paper writing. J.J. designed the research, analysed the data, wrote the paper and contributed to the revisions.

Data accessibility

The primary data are available as a part of Supporting information (Table S1) to this paper. Details of the quantitative real-time PCR quantification of the three AMF taxa along with extensive discussion have been published previously: Thonar *et al.* (2012).

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Development of colonization of *Medicago truncatula* roots by the three arbuscular mycorrhizal fungi inoculated singly, as revealed by Trypan blue staining and microscopy.

Fig. S2 Relationship between root colonization levels by arbuscular mycorrhizal fungi estimated by Trypan blue staining coupled with microscopy and quantitative real-time PCR with taxon-specific markers.

Fig. S3 Root-to-shoot biomass ratio in the *Medicago truncatula* plants inoculated with mycorrhizal fungi or not and harvested 42 days after sowing.

Fig. S4 Dry weight and phosphorus content of *Medicago truncatula* plants (shoots and roots combined) 42 days after sowing, as depends on the different fungal inoculum combinations and inoculum densities.

Table S1 Primary data on growth, phosphorus content and root colonization levels by the different arbuscular mycorrhizal fungal taxa of the *Medicago truncatula* plants.

Table S2 Influence of fungal community compositions (i.e. species combination, including the nonmycorrhizal treatment) and overall inoculum density (i.e. sums of inoculum densities of fungi added per pot) on plant growth and phosphorus content.

Appendix S1 Infectivity assay for adjusting inoculum densities of arbuscular mycorrhizal fungal strains in a competition experiment.