Research

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Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants

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Summary

- Common mycorrhizal networks (CMNs) of arbuscular mycorrhizal (AM) fungi in the soil simultaneously provide multiple host plants with nutrients, but the mechanisms by which the nutrient transport to individual host plants within one CMN is controlled are unknown.
- Using radioactive and stable isotopes, we followed the transport of phosphorus (P) and nitrogen (N) in the CMNs of two fungal species to plants that differed in their carbon (C) source strength, and correlated the transport to the expression of mycorrhiza-inducible plant P (*MtPt4*) and ammonium (*1723.m00046*) transporters in mycorrhizal roots.
- AM fungi discriminated between host plants that shared a CMN and preferentially allocated nutrients to high-quality (nonshaded) hosts. However, the fungus also supplied low-quality (shaded) hosts with nutrients and maintained a high colonization rate in these plants. Fungal P transport was correlated to the expression of *MtPt4*. The expression of the putative ammonium transporter *1723.m00046* was dependent on the fungal nutrient supply and was induced when the CMN had access to N.
- Biological market theory has emerged as a tool with which the strategic investment of competing partners in trading networks can be studied. Our work demonstrates how fungal partners are able to retain bargaining power, despite being obligately dependent on their hosts.

Introduction

The 450-million-yr-old arbuscular mycorrhizal (AM) symbiosis is among the world's most widespread mutualisms and is formed by *c.* 65% of all known land plant species (Wang & Qiu, 2006). The extraradical mycelium (ERM) of the fungus forms an extensive network in the soil and provides the host plant with access to nutrient resources beyond the root depletion zone. The ERM of the AM fungus takes up phosphorus (P), nitrogen (N), sulfur and various trace elements from the soil, and transfers these nutrients to the intraradical mycelium (IRM), where the nutrients are exchanged for carbon (C) from the host (Marschner & Dell, 1994; Smith *et al.*, 2009). The plant transfers up to 20% of its photosynthetically fixed C to the AM fungus (Wright *et al.*, 1998) and the fungus uses these C resources to maintain and to extend its hyphal network in the soil.

The maintenance of cooperation in the mycorrhizal partnership has long posed a paradox for evolutionary theory. Cooperation between different species is hard to explain because selfish individuals can exploit mutualisms, reaping benefits while paying no costs (Leigh, 2010). Sanctions – or other feedback mechanisms that allow a host to control the fitness of its partners – play a key role in stabilizing cooperation in many mutualisms (West *et al.*, 2007). However, in the mycorrhizal symbiosis, neither plant nor fungal partner is truly 'in control'. Both partners in the mycorrhizal symbiosis interact with multiple partners simultaneously: a single plant host is colonized by multiple fungal species, and fungal 'individuals' interact with multiple plant hosts and species, interconnected by a common mycorrhizal network (CMN). This complex system of many-to-many interactions means that neither partner can be 'enslaved', because both plant and fungus can choose among multiple trading partners (Kiers *et al.*, 2011; Walder *et al.*, 2012).

Biological market theory is a useful framework to study how cooperation can be stabilized in many-to-many interactions. The theory argues that resource trade can be analyzed from an economic vantage point: partners on both sides of the interaction compete and those offering the best 'rate of exchange' will be favored (Noë & Hammerstein, 1995; Werner *et al.*, 2014). However, for market dynamics to emerge, individuals must be able to discriminate among competing partners. Recently, a series of manipulative experiments demonstrated that mycorrhizal plants are able to detect, discriminate, and reward the best fungal partners with more carbohydrates (Kiers *et al.*, 2011). There is evidence that fungal partners are also able to discriminate and preferentially allocate P and N to roots grown under

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high carbohydrate conditions (Bücking & Shachar-Hill, 2005; Hammer *et al.*, 2011; Kiers *et al.*, 2011; Fellbaum *et al.*, 2012a). However, these experiments have so far only been conducted in *in vitro* root organ cultures, and it has been questioned whether these artificial systems function with enough ecological realism to capture the dynamics of the complex underground fungal networks that form among different plants in natural ecosystems (Smith & Smith, 2011).

CMNs can be formed by one individual fungus or when several conspecific fungal individuals connect by hyphal anastomoses (Mikkelsen *et al.*, 2008). In both cases, CMNs can transfer nutrients to several host plants simultaneously (van der Heijden & Horton, 2009; Lekberg *et al.*, 2010; Merrild *et al.*, 2013). However, the mechanisms that determine how an AM fungus allocates nutrients among competing plants connected by one CMN are currently unknown. Recently, Walder *et al.* (2012) demonstrated that plant species differ in their C investment into the CMN. They suggested that this contribution was unrelated to the amount of nutrients they receive. They found that the C_3 plant flax (*Linum usitatissimum*), despite its smaller measured contribution to the C pool, still received the majority of nutrients from the CMN compared with the competing C_4 plant sorghum (*Sorghum bicolor*). While this could be interpreted as evidence that AM fungi cannot discriminate among hosts of differing quality, this trade asymmetry can also be explained by differences in host plant compatibility, C to nutrient exchange ratios, or other physiological differences (e.g. C_3 versus C_4 photosynthesis). For example, sorghum showed lower levels of fungal colonization in roots and soil when grown in mixed cultures with flax, prohibiting standardized measurements of nutrient allocation to competing plants (Walder *et al.*, 2012).

To test for fungal discrimination processes, we varied photosynthetically active radiation by shading one of two *Medicago truncatula* plants and tracked fungal P and N allocation patterns of the CMNs of two fungal strains. We hypothesized that AM fungi could discriminate between high- and low-quality partners in a CMN and would preferentially transfer more N and P to high-quality (nonshaded) partners. Considering the key role that AM fungi play in the nutrient uptake of land plants, plant community composition and C sequestration in ecosystems, it is crucial to better understand how C and nutrient resources are allocated in the CMNs of the most important and ancient symbiosis of land plants.

Materials and Methods

Plant and fungal material

We scarified and germinated seeds of *Medicago truncatula* Gaertn. variety cv Jemalong, A17 according to Salzer *et al.* (1999). After 5 d, we transferred two plants each into custom-made multicompartment systems filled with a sterilized (2 h at 121°C) growth substrate of 20% organic soil, 40% perlite, and 40% sand that contained 3.9 mg kg^{-1} NO_3^-, 40.3 mg kg^{-1} NH_4^+, and 1 mg kg^{-1} P (Olsen extraction; v/v/v; Fig. 1). These systems were constructed of a 4-cm (internal diameter) PVC pipe and matching fittings. The root compartment was divided into two halves by a 50-µm nylon mesh (BioDesign Inc., New York, NY, USA) to prevent intermingling of roots, but allowed fungal crossover into both root compartments (RCs). Fungal compartments (FCs) were made out of a cap fitting joined by a 6-cm-long PVC pipe, and separated from the RC by a double layer of a 50-µm nylon mesh, which was divided by a 30-cm-long piece of wire (0.9 mm) wrapped into a spiral to prevent ion diffusion from the FC into the RC.

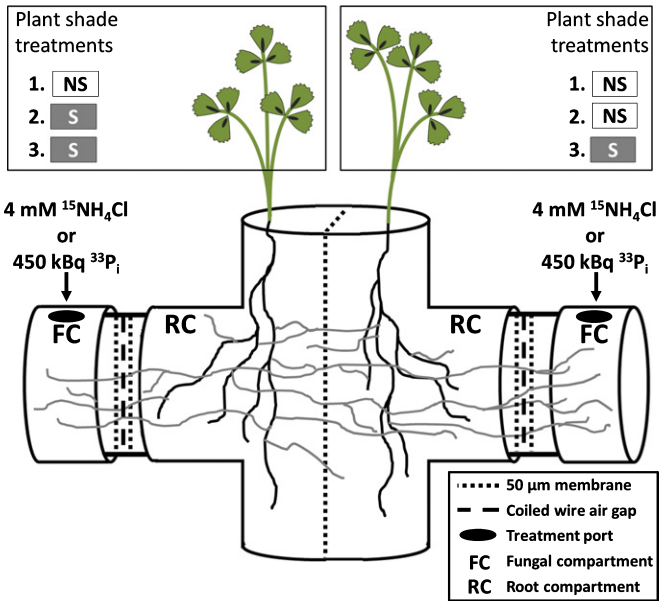

Fig. 1 The custom-made growth system. A double membrane with an air gap (two sheets of 50-µm nylon mesh divided by a 30-cm-long wire spiral) prevented the diffusion of nutrients from the fungal compartment (FC) to the root compartment (RC), but allowed fungal hyphae to cross from the RCs into the FCs. Three different shade treatments were applied to the plants: (1) both nonshaded (NS/NS), (2) one nonshaded and one shaded (NS/S), and (3) both shaded. To the FCs, 4 mM $^{15}NH_4Cl$ or 450 kBq ^{33}P-orthophosphate was added.

Twenty-four days after sowing, both plants were inoculated with 350–450 spores of either *Rhizophagus irregularis* (Blaszk., Wubet, Renker & Buscot; Walker & Schüßler, 2010; isolate 09 collected from southwest Spain by Mycovitro S.L. Biotechnología ecológica, Granada, Spain) or *Glomus aggregatum* (N.C. Schenck & G.S. Sm.; isolate 0165 collected from the Long Term Mycorrhizal Research Site, University of Guelph, Guelph, Canada). We selected these two fungal isolates because both fungi previously exhibited different levels of symbiont quality. Based on plant growth responses, and costs of C per unit P transferred, *R. irregularis* tends to be a more cooperative strain than *G. aggregatum* for *M. truncatula* (Kiers *et al.*, 2011). The inoculum was produced in axenic Ri T-DNA transformed carrot (*Daucus carota* clone DCI) root organ cultures in Petri dishes filled with mineral medium (St-Arnaud *et al.*, 1996). After 8 wk of growth, we isolated the spores by blending the medium in 10 mM citrate buffer (pH 6.0).

The plants were grown in a growth chamber (model TC30; Conviron, Winnipeg, MB, Canada) under the following conditions: 14 h photoperiod, 25°C : 20°C, day : night cycle, photosynthetically active radiation of 225 µmol m^{-2} s^{-1}, and 30% humidity. We watered the RC with 40 ml of distilled water every

4 d, and the FC when needed. The plants were fertilized once halfway through the growing period by adding a modified Ingestad solution with 250 μM NH_4NO_3 and 100 μM KH_2PO_4 to the RC of each growth chamber (Ingestad, 1960). The P and N concentrations of the fertilizer were reduced to maintain a low nutrient status and a high mycorrhizal colonization rate of the plants. The plants exhibited signs of nutrient stress such as stunted growth and yellowish leaves at the time of P and N labeling.

Experimental design

We conducted two experiments (N or P addition) to test the effect of C availability on resource allocation, and reduced the photosynthetically active radiation by applying a shade treatment to neither plant (nonshaded/nonshaded (NS/NS)), one plant (nonshaded/shaded (NS/S)) or both plants (shaded/shaded (S/S)) in each growth system by covering the entire plant with a sheath made out of 12 cm × 14 cm 50% black shade cloth (Growers Solution, Cookeville, TN, USA). The shading reduced the photosynthetically active radiation by 60% from 222.75 to 89.1 μmol m^{-2} s^{-1} as measured with a Li-Cor LI-185b light meter (Li-Cor, Lincoln, NE, USA). One day after the plants were shaded, we injected 17.1 mg (leading to a concentration of 4 mM) of 99% enriched $^{15}NH_4Cl$ (Cambridge Isotope Laboratories, Tewksbury, MA, USA) dissolved in 6 ml or 450 kBq (0.078 ng) ^{33}P-orthophosphate (Perkin Elmer, Waltham, MA, USA) dissolved in 3 ml of purified H_2O through a port to the FCs. Previous tests indicated that these labeling volumes homogenously wet the substrate in the FC without saturation. There were five biological replicates per shade treatment and nutrient supply and each treatment was inoculated with either *R. irregularis* or *G. aggregatum*. In total, 30 systems each were supplied with N or with P. Additionally, three systems each with noninoculated controls were labeled to confirm that there was no significant leakage of $^{15}NH_4^+$ or of ^{33}P from the FC into the RC.

As the detectability of the radioactive label is higher but time sensitive (the ^{33}P half-life is 25.3 d), we harvested the plants 5 d after P addition. As the ^{15}N analysis is not as sensitive, and in preliminary experiments the ^{15}N labeling of the plants was too low after 14 d, the plants were harvested 23 d after N was supplied to the FC. At harvest, the roots were cleaned and the total fresh weight was taken. The roots were then divided into three aliquots. The first aliquot was weighed and dried at 70°C for 2 d, and the dry to fresh weight ratio was used to determine the total root dry weight. This aliquot was later used for ^{33}P and ^{15}N analysis. The second aliquot was cryofixed in liquid nitrogen and stored at −80°C for RNA extraction, and the third aliquot was stored in 50% ethanol until the mycorrhizal colonization assays were performed. The shoots were stored at −80°C until they were ground in a mortar cooled with liquid nitrogen, lyophilized, weighed and prepared for elemental analysis.

Analysis of mycorrhizal colonization and ERM development

We extracted ERM from the FC as described previously (Miller *et al.*, 1995) with slight modifications. The ERM was collected with a 50-μm nylon mesh (BioDesign Inc.) and stained for 30 min. The nylon mesh with the ERM was rinsed with 2 ml of ultrapure water (EMD Millipore) and collected on a 0.45-μm gridded membrane (EMD Millipore), air-dried and mounted with 30% glycerol. The length of the ERM was quantified according to Brundrett *et al.* (1994). We also determined the percentage of the total root length colonized in a minimum of 50 root segments by the grid intersection technique (McGonigle *et al.*, 1990).

Analysis of ^{33}P labeling and extraction of various P pools

We analyzed the soil of the RC to make sure that there was no diffusion of nutrients from the FC into the RC. Aliquots of the soil were dried at 70°C, and the P content was extracted with 0.5 M $NaHCO_3$ at pH 8.5 according to Olsen *et al.* (1954). The samples were vortexed, allowed to sit for 30 min and then centrifuged. An aliquot of the supernatant was taken and the ^{33}P content was measured by liquid scintillation counting.

After homogenization of the root and shoot samples, an aliquot was taken, dried at 70°C, weighed and digested by adding 500 μl of tissue solubilizer to the sample (TS-2; Research Product International, Mount Prospect, IL, USA). After digestion, 150 μl of glacial acetic acid and 2 ml of scintillation cocktail (Biosafe II; Research Product International) were added. We also analyzed in mycorrhizal root samples the allocation of P into different P pools according to Aitchison & Butt (1973). The samples were dried at 70°C and homogenized, and the following P pools were analyzed: ortho-phosphate (P_i) and acid-soluble or short-chain polyphosphates (polyP) with a chain length of ≤ 20 phosphate residues after extraction with ice-cold 10% TCA; phospholipids after extraction with 100% ethanol and ethanol/ether (3 : 1, v/v); acid-insoluble polyP with a chain length of > 20 phosphate residues after extraction with 1 M KOH; and DNA-, RNA-, and protein-phosphates as residue after extraction of all other pools. All samples were measured with a Wallac scintillation counter (Perkin Elmer) and the data were corrected for differences in the counting efficiency by use of an internal standard.

Analysis of ^{15}N labeling

We digested 10–15-mg aliquots of homogenized and freeze-dried root and shoot material in 750 μl of concentrated H_2SO_4 and heated the samples for 2 h at 225°C followed by an addition of 36 drops of 30% H_2O_2 (three drops at a time every 30 s) as described previously (Fellbaum *et al.*, 2012a). The solution was then heated for an additional 3 h at 225°C to remove any traces of water and allowed to cool. Forty microliters of the resulting clear H_2SO_4 solution of $(NH_4)_2SO_4$ was dissolved in 600 μl of 99.9% d_6 dimethylsulfoxide (DMSO) containing 0.05% (v/v) tetramethylsilane (TMS) reference. The ^{1}H spectrum was obtained in a 5-mm *z*-axis pulsed field gradient dual broad-band probe on a 9.2 Tesla Varian Inova spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) operating at 400 MHz. The spectra were acquired using *c.* 1400 transients with a 90° (10.8-μs) pulse width, a spectral width of 5042 Hz, a pulse delay of 1.0 s, and an acquisition time of 1.6 s at 25°C. The T_1

relaxation time of the NH_4 protons was measured to be 0.4 s. The triplet resonance of the ^{1}H-^{14}N and doublet resonance of the ^{1}H-^{15}N were observed centered at 7.2 ppm relative to the TMS resonance 0.0 ppm with observed ^{1}H-^{15}N couplings of 53 and 74 Hz, respectively. The percentage of total N labeled with ^{15}N in the tissue was determined by dividing the integrated area of the ^{1}H-^{15}N doublet resonances by the sum of the integrated doublet and triplet resonance areas (Supporting Information Fig. S1).

Quantitative real-time PCR of genes involved in nitrogen and phosphate transport

Using quantitative real-time PCR (qPCR), we studied the transcript levels of genes encoding the mycorrhiza-inducible plant P transporter *MtPt4* (Chiou *et al.*, 2001; Harrison *et al.*, 2002; Javot *et al.*, 2007), and of *1723.m00046*, a mycorrhiza-inducible plant ammonium transporter that is induced in cortical cells harboring arbuscules (Gomez *et al.*, 2009). All steps were performed according to the manufacturer's instructions unless otherwise stated. We homogenized the root samples with a mortar and pestle cooled with liquid nitrogen, and extracted total RNA using TRIzol Reagent (Invitrogen, Grand Island, NY, USA). The supernatant was treated with an RNeasy MinElute Cleanup Kit (Qiagen, Valencia, CA, USA) and the RNA was eluted into 1 μl of an RNase inhibitor (Murine; New England Biolabs, Ipswich, MA, USA). The extracted RNA was treated using RQ1 RNase-Free DNase (Promega, Madison, WI, USA) and quantified by a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). cDNA was synthesized using 0.15 μg μl^{-1} DNase-treated RNA, Moloney Murine Leukemia Virus reverse transcriptase (Promega), Random Primer 6 (New England Biolabs), and dNTPs (Qiagen). qPCR was performed using the QuantiTect SYBR Green PCR Kit (Qiagen), 2 μl of 1 : 5 diluted cDNA, and 0.625 μM forward and reverse primers (NCBI: *MtPt4*: Pr010288303; *1723.m00046*: Pr010288319; Elongation factor 1-alpha (*EF1α*): Pr010288292; Gomez *et al.*, 2009) in a 20-μl reaction using an ABI 7900HT thermal cycler (Applied Biosystems, Grand Island, NY, USA). The PCR conditions were as follows: 50°C for 2 min; 95°C for 15 min; 40 cycles at 95°C for 10 s, 60°C for 15 s, and 72°C for 10 s; dissociation at 95°C for 15 s; 60°C for 15 s; and 95°C for 15 s. Changes in gene expression (*MtPt4*: NCBI AY116211; *1723.m00046*) were compared with nonmycorrhizal control plants and by using *EF1α* (TC106485) as a reference gene (Gomez *et al.*, 2009) and the $\Delta\Delta C_t$ method (Winer *et al.*, 1999). The results are based on three to 12 biological replicates and two technical replicates.

Statistical treatment

If not otherwise stated, we only discuss treatment effects when they were statistically significant according to two-way ANOVA with inoculation (*G. aggregatum* or *R. intraradices*), or shade treatment (various shade treatments) or three-way ANOVA with inoculation (*G. aggregatum* or *R. intraradices*), shade treatment (various shade treatments), and nutrient supply to the FC (^{15}N or ^{33}P) as factors. Paired *t*-tests were used to compare shaded and nonshaded plants in NS/S systems and treatment effects were tested by Fisher's least significant difference (LSD) test ($P \leq 0.05$). If the within-treatment variability was too high, we log-transformed the data before analysis. If a normal distribution of the data could not be guaranteed, we used the nonparametric Mann–Whitney *U*-test (when the *U*-test was used, this is given in the text). Correlations and computed *P*-values were analyzed using Pearson's correlation coefficient. All tests were conducted with JMP 10 (SAS, Cary, NC, USA) or UNISTAT 6.0 (Unistad Ltd, London, UK).

Results

Host plant growth and mycorrhizal colonization by fungal partners

First, we analyzed host plant biomass to determine the effect of shading and fungal inoculation (Fig. S2). There was a high variability in the root and shoot biomass of all treatments and, even though some statistical differences were observed (Fig. S2a,d), there were no consistent effects related to shading or fungal inoculation. The analysis of the hyphal length in each FC demonstrated that in growth systems with one shaded and one nonshaded plant (NS/S) the ERM development did not differ between the two FCs, and that both fungal species successfully established a CMN in the growth systems (Table S1). While the hyphal lengths of *G. aggregatum* were slightly greater (78.4 ± 17.8 g g^{-1} dry soil) than those of *R. irregularis* (53.8 ± 4.4 g g^{-1} dry soil), there was no statistically significant difference between the two fungal species (Table S1). However, consistent with the slightly higher ERM development, we found a higher labeling with ^{33}P or ^{15}N in roots colonized with *G. aggregatum* (see later, Tables S2, S3). Shading had a significant effect on the mycorrhizal colonization. While the colonization levels of both fungi were high for NS/NS and NS/S systems (> 93.8%), these levels decreased in S/S systems to 73.7% for *G. aggregatum* and 77.5% for *R. irregularis* (Fig. S3).

Phosphate and nitrogen allocation in common mycorrhizal networks

When the fungus had access to a shaded and a nonshaded host plant, both fungi preferentially transferred more of the P and N taken up to nonshaded hosts (Fig. 2, Tables S2, S3). This suggests that both fungal strains were able to discriminate between host plants, and preferentially allocated resources to nonshaded plants.

This preferential allocation by *G. aggregatum* led to significantly higher P concentrations in the shoots (but not roots) of nonshaded hosts of NS/S systems (Fig. 3a,b). In *G. aggregatum* growth systems with a shaded and a nonshaded plant (NS/S), the shoots of nonshaded plants had higher P contents but the shoots of shaded plants had lower P contents than in systems in which the fungus had only access to shaded or to nonshaded plants (S/S or NS/NS; significant according to *U*-test; $P = 0.0152$; Fig. 3b). In *R. irregularis* inoculated plants, the preferential allocation resulted in higher P concentrations in both the roots and the shoots of nonshaded plants, independently of whether systems in which both plants were shaded or nonshaded (S/S versus NS/NS)

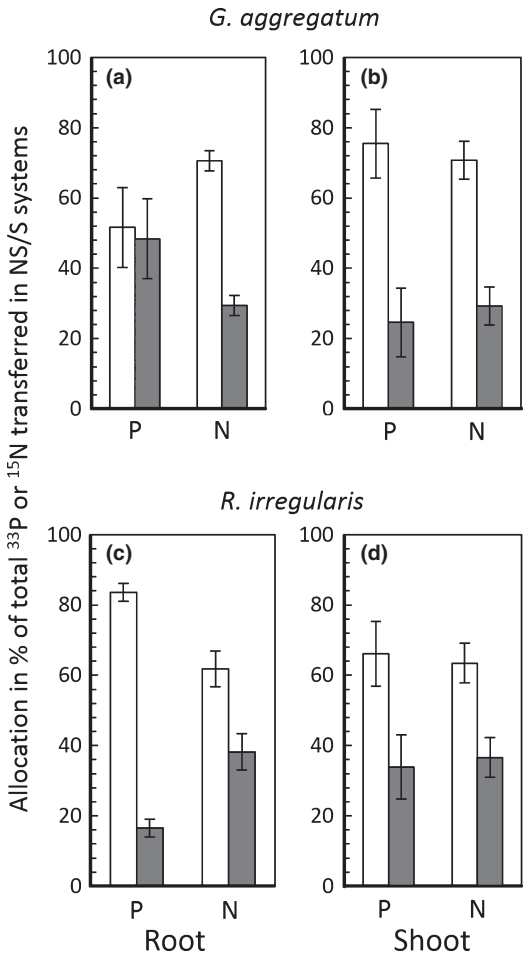

Fig. 2 Allocation of the total ^{33}P or ^{15}N taken up by the common mycorrhizal network (CMN) of *Glomus aggregatum* (a, b) and *Rhizophagus irregularis* (c, d) and transferred to the roots (a, c) or shoots (b, d) of nonshaded (open bars) and shaded (closed bars) *Medicago truncatula* plants in NS/S systems (calculated as a percentage based on the nitrogen (N) or phosphate (P) content in shaded (S) and nonshaded (NS) plants). Shown is the average of $n = 5 \pm$ SE.

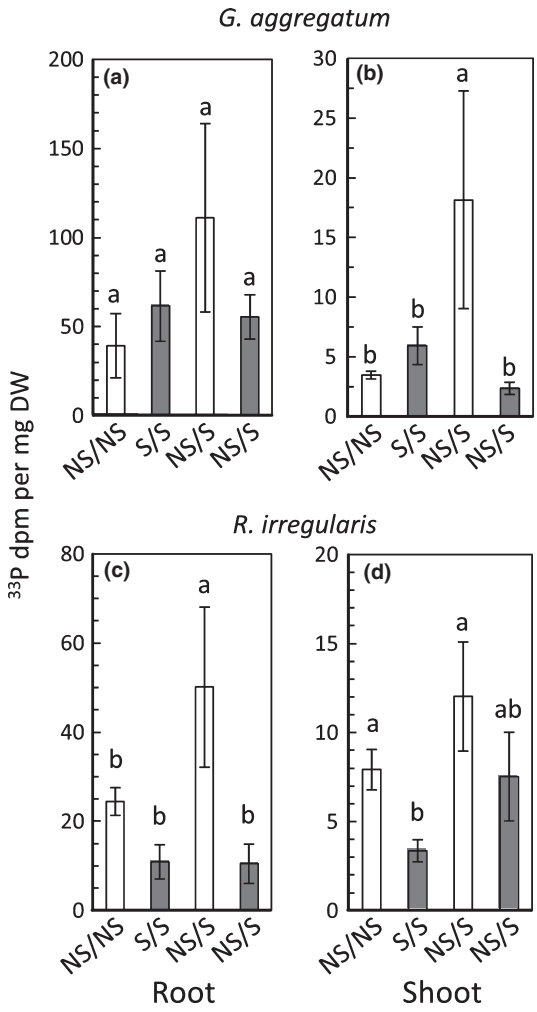

Fig. 3 Phosphate transport (in dpm per mg DW) of the common mycorrhizal network (CMN) of *Glomus aggregatum* (a, b) and *Rhizophagus irregularis* (c, d) from the fungal compartment to the root (a, c) or shoot (b, d) of *Medicago truncatula* plants dependent on their photosynthetic capability (nonshaded (NS), open bars; shaded (S), closed bars). Systems with two nonshaded plants (NS/NS), two shaded plants (S/S) or one nonshaded and one shaded plant (NS/S) were used. Shown is the average of $n = 5$–$12 \pm$ SE. Different letters on the bars indicate statistically significant differences within each graph according to the least significant difference (LSD) test ($P \leq 0.05$). The results of the two-way ANOVA are shown in Supporting Information Table S2.

were compared, or systems in which the fungus had access to both a shaded and a nonshaded plant (NS/S; Fig. 3c,d).

Between 5.5 and 17.3% of the total P transferred to the mycorrhizal roots was stored in the form of long-chain or short-chain polyphosphates (poly-P). Fungal strain and shading did not have a significant effect on the total poly-P content, and in roots colonized with *G. aggregatum*, the proportion of long-chain to short-chain poly-P was unaffected by the shading treatment. However, we observed a shift in the ratio between long-chain and short-chain poly-P in roots that were colonized with *R. irregularis*: a significantly higher proportion of the poly-P in the roots of shaded plants was stored in the form of long-chain poly-P (Fig. S4).

Both fungi transferred N from the fungal compartment to their host and up to 24.4% of the N in the root and up to 38.0% of the N in the shoot became labeled with ^{15}N (Figs 4a,b, S1). The shading had a significant effect on the N labeling in roots and shoots of *G. aggregatum* but not in *R. irregularis* (Fig. 4). The CMN of *G. aggregatum* transferred significantly more N to roots and shoots of nonshaded host plants (Fig. 4a,b). When shaded plants in NS/S or S/S systems were compared, *G. aggregatum* transferred more N to the shoots of shaded plants when only shaded plants were available as hosts (S/S); the difference in the ^{15}N labeling on a dry weight basis was not significant on the 5% level ($P = 0.0743$; Fig. 4b). There was a high variability in the ^{15}N labeling in the plants that were colonized with *R. irregularis*, and shading did not lead to a significant reduction in the ^{15}N labeling of the plants (Fig. 4c,d).

Expression of plant P and N transporters in mycorrhizal roots

MtPt4 expression was induced in mycorrhizal roots colonized by both fungi. However, the fold induction depended on the fungal

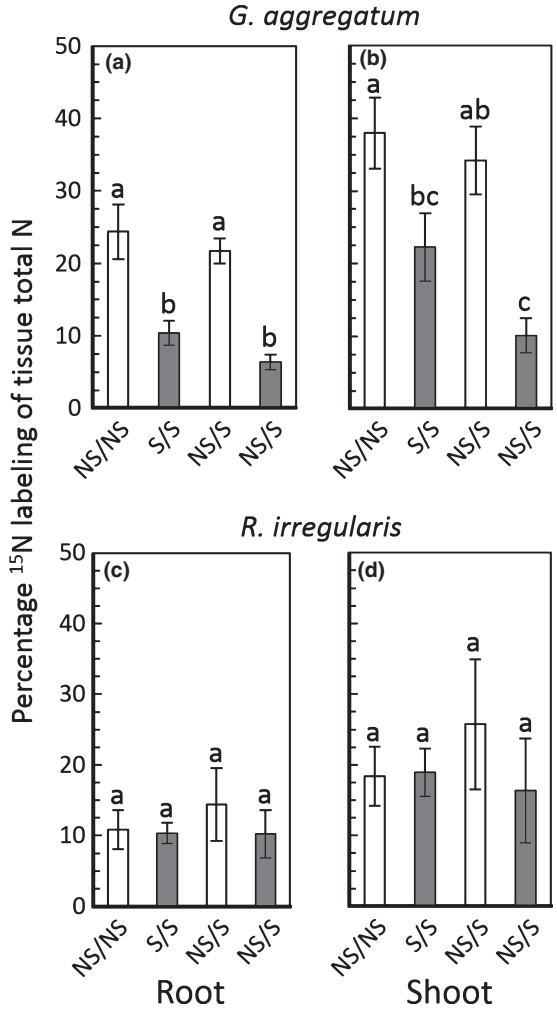

Fig. 4 Nitrogen (N) transport (in per cent ^{15}N labeling of total N) of the common mycorrhizal network (CMN) of *Glomus aggregatum* (a, b) or *Rhizophagus irregularis* (c, d) from the fungal compartment to the root (a, c) or shoot (b, d) of *Medicago truncatula* plants dependent on their photosynthetic capability (nonshaded (NS), open bars; shaded (S), closed bars). Systems with two nonshaded plants (NS/NS), two shaded plants (S/S) or one nonshaded and one shaded plant (NS/S) were used. Shown is the average of $n = 5–8 \pm$ SE. Different letters on the bars indicate statistically significant differences within each graph according to the least significant difference (LSD) test ($P \leq 0.05$). The results of the two-way ANOVA are shown in Supporting Information Table S3.

species colonizing the root; while *G. aggregatum* increased the expression of *MtPt4* by up to 90-fold compared with the nonmycorrhizal controls (particularly under nonshaded conditions), we only found inductions of up to 25-fold in roots colonized with *R. irregularis* (Fig. 5). Consistent with the reduction in the P transport to the shaded hosts, we found that shading reduced *MtPt4* expression in mycorrhizal roots (Fig. 5a,b) independently of whether the fungus had access to both nonshaded and shaded plants or only to shaded or nonshaded plants. The decrease in the *MtPt4* transcript levels in shaded plants was particularly pronounced in systems colonized by *R. irregularis*; the transcript level of the nonshaded plants was four to five times higher than of the shaded plants in NS/S systems. The expression of *MtPt4* was proportional to the P transport to the colonized roots and was correlated to the P tissue concentration in dpm per mg DW and to the total P content in the mycorrhizal roots (Fig. 6a,b).

1723.m00046, which encodes a putative ammonium transporter with a 99% sequence similarity to *Medicago truncatula* ammonium transporter MTR_8g074750 (NCBI XM_00362 9175.1), was induced in roots colonized with *G. aggregatum*, and the expression level was dependent on whether N or P was supplied to the CMN (Fig. 5c). When the CMN of *G. aggregatum* was supplied with N, *1723.m00046* was up-regulated up to 50-fold (compared with nonmycorrhizal roots), whereas, when supplied with P, only a two- to three-fold induction was observed. We found no effect of the shading treatment on the transcript levels of *1723.m00046*. By contrast, the transcript levels of *1723.m00046* were comparatively low in roots that were colonized with *R. irregularis* and the transcript levels in shaded and nonshaded plants of NS/S systems were lower than in nonmycorrhizal control plants (fold induction < 1; Fig. 5d). We only found a five- to six-fold induction of *1723.m00046* in systems, in which two shaded (S/S) or nonshaded (NS/NS) plants shared one CMN that was supplied with N.

Discussion

Underground, plants compete with other plants for nutrients provided by fungal CMNs, but the mechanisms that control the allocation patterns among plants are only poorly understood. We examined how nutrients supplied to the CMN were allocated between two host plants that differed in their ability to provide C resources. Specifically, we asked if fungal partners were able to discriminate among hosts interconnected by a CMN. We found that, when fungi were given a choice, they consistently allocated a higher percentage of both P and N to nonshaded hosts (Fig. 2), and that host plants that were restricted in their photosynthetic capability (here by shading) became relatively weak competitors for nutrient resources that were available from the CMN.

We varied the C source strength of the host plants by reducing the photosynthetically active radiation of half of the plants via shading. In systems in which the fungus had only access to shaded host plants (S/S), the mycorrhizal colonization was significantly reduced (N supply experiment; Fig. S3). This suggests that the shading treatment reduced the C supply for the CMN and that the AM fungus was unable to maintain its high colonization rate. *Medicago sativa* for example has been shown to respond very sensitively to shading, and even short-term shading can reduce the C allocation to the root system, and lead to a higher C allocation, particularly to the shoot meristems, to compensate for the decrease in the photosynthetic activity (Schmitt *et al.*, 2013). Shading for 1–2 wk has been shown to reduce the mycorrhizal colonization of plants, but not to lower the C costs relative to nutrient benefit for the host plant (Heinemeyer *et al.*, 2004; Olsson *et al.*, 2010).

Interestingly, we found that shading did not reduce the mycorrhizal colonization rate of shaded plants when these plants shared a CMN with nonshaded host plants (NS/S). This suggests that the fungus used parts of the C derived from nonshaded host plants (or from its own reservoir in storage lipids) to maintain a

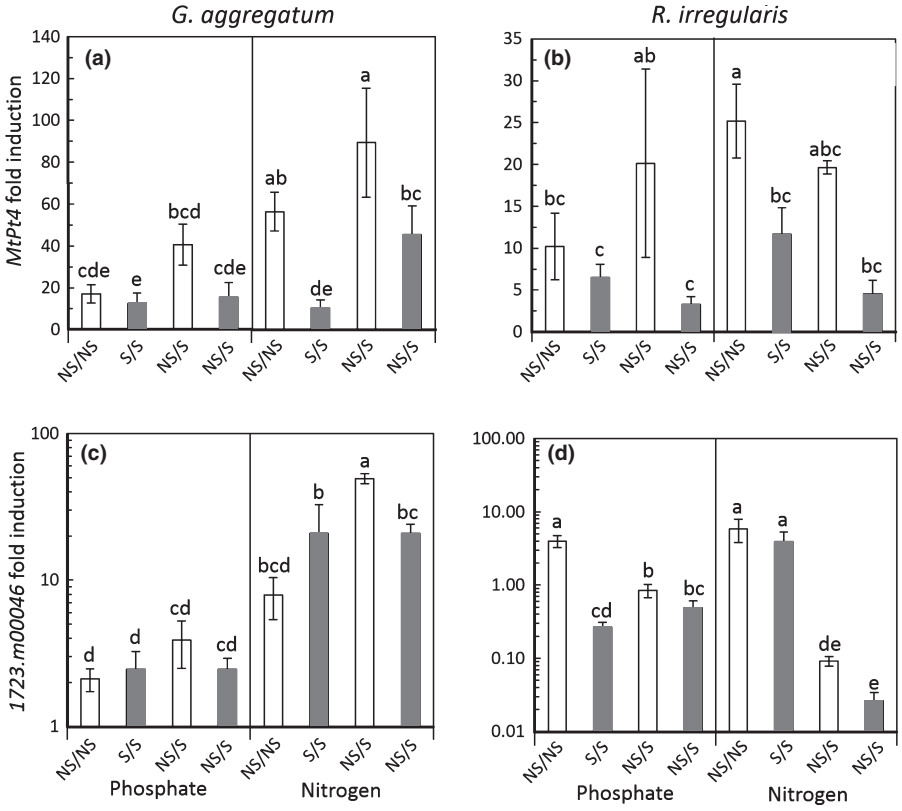

Fig. 5 Expression of the mycorrhiza-inducible plant phosphate transporter *MtPt4* and plant ammonium transporter *1723.m00046* (c, d) in the roots of *Medicago truncatula* plants that were colonized by *Glomus aggregatum* (a, c) or *Rhizophagus irregularis* (b, d). Shown in the figure are systems with two nonshaded plants (NS/NS), two shaded plants (S/S), or one nonshaded and one shaded plant (NS/S); nonshaded plants (open bars) and shaded plants (closed bars), or systems to which ^{33}P (phosphate) or ^{15}N (nitrogen) was added to the fungal compartments. Shown is the average of $n = 3–12 \pm$ SE. Different letters on the bars indicate statistically significant differences according to the least significant difference (LSD) test ($P \leq 0.05$). Results of a three-way ANOVA are shown in Table S4.

high colonization rate in low-quality hosts. This strategy potentially allows the obligate biotrophic fungus to maintain access to additional C sources, ensuring that the loss of a high-quality host (e.g. by pathogen or herbivore damage) would be less detrimental. In the P supply experiment, the shading treatment was much shorter (6 d in total), but still the resource allocation patterns indicated that both fungi were discriminating among the hosts. These data suggest that, even before a significant reduction in the mycorrhizal colonization is expected (the whole arbuscular life cycle lasts *c.* 8 d, and the functionality for 2–3 d; Kobae & Hata, 2010), the fungus is able to change its nutrient allocation strategy in response to the shading treatment.

Previous studies using root organ cultures have shown that the C supply of the host acts as an important trigger for P and N transport in the AM symbiosis (Bücking & Shachar-Hill, 2005; Hammer *et al.*, 2011; Kiers *et al.*, 2011; Fellbaum *et al.*, 2012a). However, these systems have often been criticized for their artificial nature, most notably because the lack of a shoot in these systems prevents shoot-associated effects on nutrient uptake and sink strength (Smith & Smith, 2011). Another important difference is that, in nature, CMNs can connect host plants of different ages and of multiple species (van der Heijden & Horton, 2009; Walder *et al.*, 2012). When large plants were grown with small seedlings, several studies showed that the inter-connectedness to a large plant via a CMN can have a positive, negative or neutral effect on the growth of a smaller seedling (van der Heijden & Horton, 2009). However, consistent with our results, Pietikäinen & Kytöviita (2007) reported that the mycorrhizal benefit for the seedling was low when the seedling shared a CMN with a nondefoliated adult plant (i.e. high-quality host), but the benefit to the seedling began to increase when the C source strength of the adult plant was reduced by defoliation.

One could argue that the observed reduction in nutrient transport to shaded plants was the result of a lower plant nutrient demand. Low photosynthetic rates and the subsequent reduction in plant growth are expected to decrease the nutrient demand of the host (Cui & Caldwell, 1997). This is, however, unlikely in our experiment because the plants were grown under both P and N limitation before the shading treatment was started, and we found a preferential allocation of P to nonshaded host plants already after 6 d, when substantial differences in the growth between shaded and nonshaded plants were not expected. Even after shading for 24 d (N supply experiment), we found no significant difference in the plant biomass (Fig. S2), suggesting that differences in the nutrient demand of the host did not play a large role in the observed nutrient allocation pattern.

We also analyzed the expression of the AM-inducible P transporter gene *MtPt4* in the roots, and found that both fungi induced *MtPt4*, but that the induction by *G. aggregatum* was stronger than that by *R. irregularis*. MtPt4 is localized in the periarbuscular membrane (Pumplin *et al.*, 2012), and is involved in the P uptake from the mycorrhizal interface. *MtPt4* has been shown to be essential for the AM symbiosis, and in mutants in which this transporter gene was not expressed, arbuscules were prematurely degraded (Javot *et al.*, 2007). Mycorrhiza-inducible P transporters have been identified in several plant species, and high P availabilities for the host have been shown to reduce the transcript levels (Xu *et al.*, 2007; Breullin *et al.*, 2010). We found that the

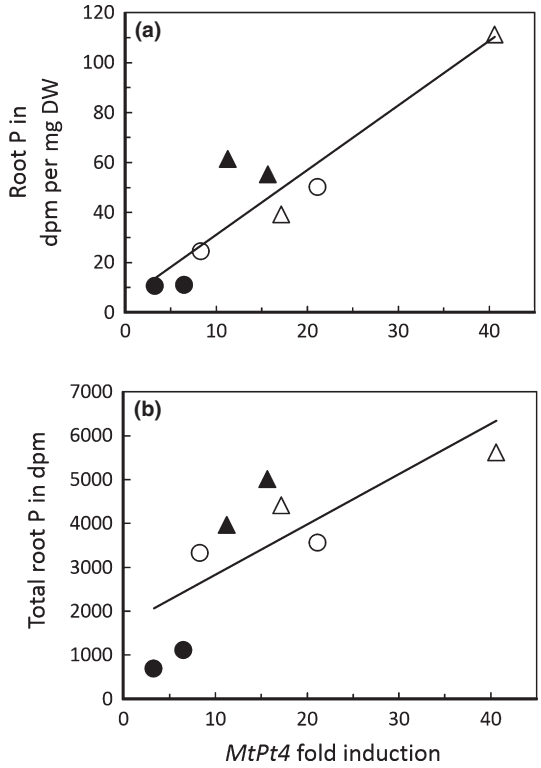

Fig. 6 Correlation between the expression of the mycorrhiza inducible plant phosphate transporter *MtPt4* in mycorrhizal roots and the phosphate (P) transport to the roots of *Medicago truncatula* plants (a) in dpm per milligram DW and (b) in terms of the total dpm content of the roots. The *MtPt4* expression is shown as fold induction according to the $\Delta\Delta C_t$ method (Winer *et al.*, 1999). *MtPt4* expression levels in roots colonized with *Glomus aggregatum* (triangles) or *Rhizophagus irregularis* (circles) for nonshaded plants (open triangles or circles) or shaded plants (closed triangles or circles) are shown. Computed *P*-values of the correlation analysis are (a) $P = 0.0002$; $R^2 = 0.84535$; (b) $P = 0.0140$; $R^2 = 0.58762$.

transcript levels of *MtPt4* were positively correlated to the P content in mycorrhizal roots, and also indicated a higher P transport activity across the mycorrhizal interface. While a correlation also between *MtPt4* expression and arbuscular colonization cannot be completely excluded, the consistent colonization but differential transcript levels of *MtPt4* in shaded and nonshaded plants in NS/S systems suggests that there is a direct correlation between *MtPt4* expression and transport activity. That higher P transport to the root can also be coupled to up-regulation of *MtPt4* was also shown by Fiorilli *et al.* (2013); the authors suggested that the P flux to the mycorrhizal host requires high expression levels.

The fact that shading reduced the expression of *MtPt4* is consistent with the predicted lower C allocation to the roots, and a reduction in the P transport across the mycorrhizal interface to low-quality hosts. This supports the hypothesis that P and C transports across the mycorrhizal interface are tightly linked (Bücking & Shachar-Hill, 2005; Kiers *et al.*, 2011) and is consistent with the finding of Helber *et al.* (2011) that the expression of a fungal monosaccaride transporter gene of *R. irregularis* (formerly *G. intraradices*; *GintMST2*), suggested to be involved in C uptake from the mycorrhizal interface, was positively correlated to the expression of *MtPt4*.

In contrast to the expression of *MtPt4*, shading did not result in reduced expression of *1723.m00046*. This is consistent with the relatively high transport of N to shaded plants we observed. However, our results demonstrate that both fungi can transfer substantial amounts of N to the host and that, 23 d after $^{15}NH_4Cl$ was supplied to the CMN, a significant proportion of the N in the shoots was labeled. In light of the high mobility of N in the soil, the significance of the AM symbiosis for the N nutrition of the plant is still a matter of debate (for a review, see Smith & Smith, 2011). The present work and that of others (Toussaint *et al.*, 2004; Tanaka & Yano, 2005) demonstrate that AM fungi can contribute substantially to the N nutrition of plants. It is thought that the fungus transfers N in the form of ammonium across the mycorrhizal interface to the host (Tian *et al.*, 2010; Fellbaum *et al.*, 2012a). We found here that fungal N transport was coupled to an induction of *1723.m00046*, a gene encoding a putative ammonium transporter of *M. truncatula*. *1723.m00046* was first described by Gomez *et al.* (2009) and has been shown to be induced in the cortical cells of roots that were colonized with *R. irregularis*. We found that the transcript levels of *1723.m00046* in roots that were colonized with *R. irregularis* were lower than in roots that were colonized with *G. aggregatum*, but the four- to six-fold induction level in some of the *R. irregularis* treatments was consistent with the up-regulation observed by Gomez *et al.* (2009).

Our finding that this transporter was particularly up-regulated in roots that were associated with a CMN supplied with NH_4^+ supports the view that this transporter is potentially involved in the N uptake from the mycorrhizal interface. AM-inducible ammonium transporters that are localized in the periarbuscular membrane have been identified in several plant species (Kobae *et al.*, 2010; Koegel *et al.*, 2013). The AM-inducible ammonium transporter of *Lotus japonicus*, *LjAMT2;2*, has been shown to transport NH_3 instead of NH_4^+, and it has been suggested that the protons from the NH_4^+ deprotonation remain in the interfacial apoplast and contribute to the H^+ gradient that facilitates proton-dependent transport processes across the mycorrhizal interface (Guether *et al.*, 2009).

Fungi can only preferentially allocate resources when there is a choice of high- versus low-quality plant hosts. We found that, in *G. aggregatum* colonized systems, shaded plants connected with other shaded plants (S/S) received more resources from the CMN than shaded plants that had to compete with nonshaded host plants (NS/S). Following biological market dynamics (Kiers *et al.*, 2011), this finding suggests that, in the absence of choice, *G. aggregatum* transfers more resources per unit C to low-quality hosts, and that the higher C demand of the fungus shifts the cost-to-benefit ratio in favor of the host (under the assumption that C transport of shaded plants to the CMN did not differ between S/S and NS/S systems). As the mycorrhizal colonization was reduced in systems with two shaded plants (S/S), this indicates that the P and N transport rate *per unit interface* increased under these conditions. This supports the findings of Treseder (2013), who reported that mycorrhizal growth responses depend not only on the mycorrhizal colonization, but also on the mycorrhizal benefits provided per unit root length colonized.

Our findings support the hypothesis that the fungus is more in control than previously thought, despite its obligate dependence on the host. Often, the plant host is considered to be more in control of mycorrhizal outcomes. This is because, in contrast to the AM fungus, many plant species are not obligately dependent on the symbiosis (Smith & Smith, 2012), and reduce their mycorrhizal colonization rate actively by a premature degeneration of arbuscules. This has particularly been demonstrated in cases where the P availability was high, or where the plant was unable to benefit from the P transport across the mycorrhizal interface (Javot *et al.*, 2007; Breullin *et al.*, 2010). It has been suggested, however, that the fungus can actively control the transport of P and N into the mycorrhizal interface by the regulation of poly-P formation and/or remobilization in the IRM (Bücking & Shachar-Hill, 2005; Ohtomo & Saito, 2005; Takanishi *et al.*, 2009). This is consistent with our finding that, in the roots of shaded plant hosts colonized by *R. irregularis* (but not *G. aggregatum*), a significantly higher proportion of the total poly-P was stored in the form of long-chain poly-P (Fig. S4). Long-chain poly-P better represents the long-term storage capacity of P in AM fungal hyphae, whereas short-chain poly-P is seen as a good indicator for P transport to the host (Takanishi *et al.*, 2009; Kiers *et al.*, 2011). The fungus could also potentially control its nutrient transport to the host via differential expression of transporters in the arbuscular membrane. The expression of fungal phosphate and ammonium transporters in the arbuscular membrane suggests that both partners, plant and fungus, compete for P and N that becomes available in the interfacial apoplast (Balestrini *et al.*, 2007; Pérez-Tienda *et al.*, 2011).

While we found strong evidence that both fungal partners successfully discriminated among hosts of different quality, both fungi still transferred substantial amounts (*c.* 20–40%) of P and N to low-quality hosts. Detailed studies on the arbuscular lifespan in roots are limited, but fungal arbuscules undergo in host cells a cycle of growth, maturity, senescence and recurrent growth; it has been suggested that the turnover of arbuscules potentially provides the host plant with an instrument 'to penalize' inefficient fungal symbionts (Javot *et al.*, 2007; Parniske, 2008). A low but continuous flux of nutrients to low-quality hosts would allow the fungus to escape arbuscular degradation. This, in turn, decreases the dependence of the fungus on a specific host. Multiple host plants that contribute to the C supply and compete for limited resources available for the CMN will probably shift the cost-to-benefit ratio in favor of the fungus, as increasing the number of hosts would give the fungus more bargaining power.

Conclusions

AM associations are a perfect illustration of mutualisms involving many-to-many interactions: plants are typically colonized by AM fungal communities of multiple species, and fungal 'individuals' form a CMN and simultaneously colonize multiple host plants and species. Understanding the trading and distribution of resources is a key goal in elucidating the AM symbiosis, and mutualisms in general. We examined here how plants compete for limited resources that become available for the CMN, and how fungal symbionts regulate the nutrient allocation to multiple host plants. Our current understanding of resource exchange and cost-to-benefit relationships in the AM symbiosis is mainly based on experiments with *in vitro* root organ cultures or studies that were performed with single plants lacking mycelial inter-connections to other plants. These only poorly represent nutrient and resource allocation under natural conditions when multiple plants compete for resources from the CMN (van der Heijden & Horton, 2009). We demonstrate here in a whole-plant system that both fungi preferentially allocated nutrient resources to host plants that were able to provide more benefit. This is consistent with previous reports from *in vitro* root organ cultures, in which the C supply of the host was shown to act as an important trigger that stimulates fungal P and N transport (Bücking & Shachar-Hill, 2005; Hammer *et al.*, 2011; Kiers *et al.*, 2011; Fellbaum *et al.*, 2012a,b).

Our results suggest that the fungal partner, although an obligate biotroph, still retains power via its ability to change nutrient allocation patterns. However, we also found that, in the absence of choice, fungi (e.g. *G. aggregatum*) transfer more resources per unit C to low-quality hosts, shifting the cost-to-benefit ratio in favor of the host. Our studies also support the hypothesis that C to nutrient exchange ratios at the mycorrhizal interface follow biological market dynamics, which depend on the compatibility between the plant and fungal species involved (Smith *et al.*, 2004), and resource supply and demand conditions (Kiers *et al.*, 2011; Fellbaum *et al.*, 2012a). As we demonstrated the importance of both N and P allocation patterns, future studies should track both resources simultaneously to understand the market dynamics of multiple nutrient commodities and how costs and benefits of the symbiosis (Johnson *et al.*, 1997; Johnson & Graham, 2013) manifest within complex CMNs.

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References

Aitchison PA, Butt VS. 1973. The relation between the synthesis of inorganic polyphosphate and phosphate uptake by *Chlorella vulgaris*. *Journal of Experimental Botany* **24**: 497–510.

Balestrini R, Gómez-Ariza J, Lanfranco L, Bonfante P. 2007. Laser microdissection reveals that transcripts for five plant and one fungal phosphate transporter genes are contemporaneously present in arbusculated cells. *Molecular Plant Microbe Interactions* **20**: 1055–1062.

Breullin F, Schramm J, Hajrezaei M, Favre P, Druege U, Hause B, Bucher M, Kretzschmar T, Bossolini E, Kuhlermeier C *et al.* 2010. Phosphate systematically inhibits development of arbuscular mycorrhiza in *Petunia hybrida* and represses genes involved in mycorrhizal functioning. *Plant Journal* **64**: 1002–1017.

Brundrett M, Peterson L, Melville L, Addy H, McGonigle T, Schaffer G, Bougher N, Massicotte H. 1994. *Practical methods in mycorrhizal research.* Sidney, BC, Canada: Mycologue Publications.

Bücking H, Shachar-Hill Y. 2005. Phosphate uptake, transport and transfer by the arbuscular mycorrhizal fungus *Glomus intraradices* is stimulated by increased carbohydrate availability. *New Phytologist* **165**: 899–912.

Chiou TJ, Liu H, Harrison MJ. 2001. The spatial expression patterns of a phosphate transporter (*MtPT1*) from *Medicago truncatula* indicate a role in phosphate transport at the root/soil interface. *Plant Journal* **25**: 281–293.

Cui M, Caldwell MM. 1997. Shading reduces exploitation of soil nitrate and phosphate by *Agropyron desertorum* and *Artemisia tridentata* from soils with patchy and uniform nutrient distributions. *Oecologia* **109**: 177–183.

Fellbaum CR, Gachomo EW, Beesetty Y, Choudhari S, Strahan GD, Pfeffer PE, Kiers ET, Bücking H. 2012a. Carbon availability triggers fungal nitrogen uptake and transport in the arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences, USA* **109**: 2666–2671.

Fellbaum CR, Mensah JA, Pfeffer PE, Kiers ET, Bücking H. 2012b. The role of carbon in fungal nutrient uptake and transport: implications for resource exchange in the arbuscular mycorrhizal symbiosis. *Plant Signaling and Behavior* **7**: 1509–1512.

Fiorilli V, Lanfranco L, Bonfante P. 2013. The expression of *GintPT*, the phosphate transporter of *Rhizophagus irregularis*, depends on the symbiotic status and phosphate availability. *Planta* **237**: 1267–1277.

Gomez SK, Javot H, Deewatthanawong P, Torres-Jerez I, Tang Y, Blancaflor E, Udvardi M, Harrison M. 2009. *Medicago truncatula* and *Glomus intraradices* gene expression in cortical cells harboring arbuscules in the arbuscular mycorrhizal symbiosis. *BMC Plant Biology* **9**: 10.

Guether M, Neuhauser B, Balestrini R, Dynowski M, Ludewig U, Bonfante P. 2009. A mycorrhizal-specific ammonium transporter from *Lotus japonicus* acquires nitrogen released by arbuscular mycorrhizal fungi. *Plant Physiology* **150**: 73–83.

Hammer EC, Pallon J, Wallander H, Olsson PA. 2011. Tit for tat? A mycorrhizal fungus accumulates phosphorus under low plant carbon availability. *FEMS Microbiology Ecology* **76**: 236–244.

Harrison MJ, Dewbre GR, Liu J. 2002. A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. *The Plant Cell* **14**: 2413–2429.

van der Heijden MGA, Horton TR. 2009. Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *Journal of Ecology* **97**: 1139–1150.

Heinemeyer A, Ridgway KP, Edwards EJ, Benham DG, Young JPW, Fitter AH. 2004. Impact of soil warming and shading on colonization and community structure of arbuscular mycorrhizal fungi in roots of a native grassland community. *Global Change Biology* **10**: 52–64.

Helber N, Wippel K, Sauer N, Schaarschmidt S, Hause B, Requena N. 2011. A versatile monosaccharide transporter that operates in the arbuscular mycorrhizal fungus *Glomus* sp. is crucial for the symbiotic relationship with plants. *Plant Cell* **23**: 3812–3823.

Ingestad T. 1960. Studies on the nutrition of forest tree seedlings. III Mineral nutrition of pine. *Physiologia Plantarum* **13**: 513–532.

Javot H, Penmetsa RV, Terzaghi N, Cook DR, Harrison MJ. 2007. A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences, USA* **104**: 1720–1725.

Johnson NC, Graham JH. 2013. The continuum concept remains a useful framework for studying mycorrhizal functioning. *Plant and Soil* **363**: 411–419.

Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist* **135**: 575–585.

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A *et al.* 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* **333**: 880–882.

Kobae Y, Hata S. 2010. Dynamics of periarbuscular membranes visualized with a fluorescent phosphate transporter in arbuscular mycorrhizal roots of rice. *Plant and Cell Physiology* **51**: 341–353.

Kobae Y, Tamura Y, Takai S, Banba M, Hata S. 2010. Localized expression of arbuscular mycorrhiza-inducible ammonium transporters in soybean. *Plant and Cell Physiology* **51**: 1411–1415.

Koegel S, Lahmidi NA, Arnould C, Chatagnier O, Walder F, Ineichen K, Boller T, Wipf D, Wiemken A, Courty PE. 2013. The family of ammonium transporters (AMT) in *Sorghum bicolor*: two AMT members are induced locally, but not systemically in roots colonized by arbuscular mycorrhizal fungi. *New Phytologist* **198**: 853–865.

Leigh EG. 2010. The evolution of mutualism. *Journal of Evolutionary Biology* **23**: 2507–2528.

Lekberg Y, Hammer E, Olsson PA. 2010. Plants as resource islands and storage units – adopting the mycocentric view of arbuscular mycorrhizal networks. *FEMS Microbiology Ecology* **74**: 336–345.

Marschner H, Dell B. 1994. Nutrient uptake and mycorrhizal symbiosis. *Plant and Soil* **159**: 89–102.

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.

Merrild MP, Ambus P, Rosendahl S, Jakobsen I. 2013. Common arbuscular mycorrhizal networks amplify competition for phosphorus between seedlings and established plants. *New Phytologist* **200**: 229–240.

Mikkelsen BL, Rosendahl S, Jakobsen I. 2008. Underground resource allocation between individual networks of mycorrhizal fungi. *New Phytologist* **180**: 890–898.

Miller RM, Reinhardt DR, Jastrow JD. 1995. External hyphal production of vesicular-arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. *Oecologia* **103**: 17–23.

Noë R, Hammerstein P. 1995. Biological markets. *Trends in Ecology & Evolution* **10**: 336–339.

Ohtomo R, Saito M. 2005. Polyphosphate dynamics in mycorrhizal roots during colonization of an arbuscular mycorrhizal fungus. *New Phytologist* **167**: 571–578.

Olsen SR, Cole CV, Watanabe FS, Dean LA. 1954. *Estimation of available phosphorus in soils by extraction with sodium bicarbonate.* USDA Circular 939. Washington, DC: Government Printing Office.

Olsson PA, Rahm J, Aliasgharzad N. 2010. Carbon dynamics in mycorrhizal symbioses is linked to carbon costs and phosphorus benefits. *FEMS Microbiology Ecology* **72**: 123–131.

Parniske M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology* **6**: 763–775.

Pérez-Tienda J, TP S, Balestrini R, Fiorilli V, Azcón-Aguilar C, Ferrol N. 2011. *GintAMT2*, a new member of the ammonium transporter family in the arbuscular mycorrhizal fungus *Glomus intraradices*. *Fungal Genetics and Biology* **48**: 1044–1055.

Pietikäinen A, Kytöviita MM. 2007. Defoliation changes mycorrhizal benefit and competitive interactions between seedlings and adult plants. *Journal of Ecology* **95**: 639–647.

Pumplin N, Zhang XC, Noar RD, Harrison MJ. 2012. Polar localization of a symbiosis-specific phosphate transporter is mediated by a transient reorientation of secretion. *Proceedings of the National Academy of Sciences, USA* **109**: E665–E672.

Salzer P, Corbiere H, Boller T. 1999. Hydrogen peroxide accumulation in *Medicago truncatula* roots colonized by the arbuscular-forming fungus *Glomus intraradices*. *Planta* **208**: 319–325.

Schmitt A, Pausch J, Kuzyakov Y. 2013. C and N allocation in soil under ryegrass and alfalfa estimated by ^{13}C and ^{15}N labelling. *Plant and Soil* **368**: 581–590.

Smith FA, Grace EJ, Smith SE. 2009. More than a carbon economy: nutrient trade and ecological sustainability in facultative arbuscular mycorrhizal symbioses. *New Phytologist* **182**: 347–358.

Smith SE, Smith FA. 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. *Annual Review Plant Biology* **62**: 227–250.

Smith SE, Smith FA. 2012. Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. *Mycologia* **104**: 1–13.

Smith SE, Smith FA, Jakobsen I. 2004. Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake

pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytologist* **162**: 511–524.

St-Arnaud M, Hamel C, Vimard B, Caron M, Fortin JA. 1996. Enhanced hyphal growth and spore production of the arbuscular mycorrhizal fungus *Glomus intraradices* in an *in vitro* system in the absence of host roots. *Mycological Research* **100**: 328–332.

Takanishi I, Ohtomo R, Hayatsu M, Saito M. 2009. Short-chain polyphosphate in arbuscular mycorrhizal roots colonized by *Glomus* spp.: a possible phosphate pool for host plants. *Soil Biology & Biochemistry* **41**: 1571–1573.

Tanaka Y, Yano K. 2005. Nitrogen delivery to maize via mycorrhizal hyphae depends on the form of N supplied. *Plant, Cell & Environment* **28**: 1247–1254.

Tian C, Kasiborski B, Koul R, Lammers PJ, Bücking H, Shachar-Hill Y. 2010. Regulation of the nitrogen transfer pathway in the arbuscular mycorrhizal symbiosis: gene characterization and the coordination of expression with nitrogen flux. *Plant Physiology* **153**: 1175–1187.

Toussaint JP, St-Arnaud M, Charest C. 2004. Nitrogen transfer and assimilation between the arbuscular mycorrhizal fungus *Glomus intraradices* Schenck & Smith and Ri T-DNA roots of *Daucus carota* L. in an *in vitro* compartmented system. *Canadian Journal of Microbiology* **50**: 251–260.

Treseder KK. 2013. The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. *Plant and Soil* **371**: 1–13.

Walder F, Niemann H, Natarajan M, Lehmann MF, Boller T, Wiemken A. 2012. Mycorrhizal networks: common goods of plants shared under unequal terms of trade. *Plant Physiology* **159**: 789–797.

Wang B, Qiu Y-L. 2006. Phylogenetic distribution and evolution of mycorrhizae in land plants. *Mycorrhiza* **16**: 299–363.

Werner GDA, Strassmann JE, Ivens ABF, Engelmoer DJP, Verbruggen E, Queller DC, Noë R, Johnson NC, Hammerstein P, Kiers ET. 2014. Evolution of microbial markets. *Proceedings of the National Academy of Sciences, USA* **111**: 1237–1244.

West SA, Griffin AS, Gardner A. 2007. Evolutionary explanations for cooperation. *Current Biology* **17**: R661–R672.

Winer J, Jung CKS, Shackel I, Williams PM. 1999. Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocetes *in vitro*. *Analytical Biochemistry* **270**: 41–49.

Wright DP, Read DJ, Scholes JD. 1998. Mycorrhizal sink strength influences whole plant carbon balance of *Trifolium repens* L. *Plant, Cell & Environment* **21**: 881–891.

Xu GH, Chague V, Melamed-Bessudo C, Kapulnik Y, Jain A, Raghothama KG, Levy AA, Silber A. 2007. Functional characterization of LePT4: a phosphate transporter in tomato with mycorrhiza-enhanced expression. *Journal of Experimental Botany* **58**: 2491–2501.

Supporting Information

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

Fig. S1 400-MHz ^{1}H NMR spectra of Kjeldahl degraded tissue of nonmycorrhizal roots, mycorrhizal roots of shaded plants, or mycorrhizal roots of nonshaded plants.

Fig. S2 Effect of mycorrhizal colonization and shading treatment on shoot and root biomass.

Fig. S3 Mycorrhizal colonization with *G. aggregatum* or *R. irregularis* of nonshaded or shaded plants.

Fig. S4 Percentage of long-chain poly-P of the total poly-P in mycorrhizal roots colonized with *G. aggregatum* or *R. irregularis*.

Table S1 Results of two-way ANOVA: effect of shade treatment or fungal species on ERM development in the fungal compartment of the growth systems

Table S2 Results of two-way ANOVA: effect of shade treatment or fungal species on ^{33}P contents in dpm per milligram DW in roots and shoots of plants interconnected by a CMN (see also Fig. 3)

Table S3 Results of two-way ANOVA: effect of shade treatment or fungal species on ^{15}N contents in % of total N in roots and shoots of plants interconnected by a CMN (see also Fig. 4)

Table S4 Results of three-way ANOVA: effect of shade treatment, fungal species, or nutrient addition to the FC on the expression of *MtPt4* or *1723.m00046* (see also Fig. 5)

Table S5 Results of one-way ANOVA: biomass of root and shoots of nonmycorrhizal and mycorrhizal plants of the ^{15}N or ^{33}P labeling experiment (see also Fig. S2)

Table S6 Results of two-way ANOVA: effect of shade treatment and fungal species on mycorrhizal root colonization (see also Fig. S3)

Table S7 Results of two-way ANOVA: effect of shade treatment and fungal species on the percentage of long-chain polyP of the total root polyP (see also Fig. S4)

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