Functional diversity of arbuscular mycorrhizal fungal isolates in relation to extraradical mycelial networks

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Summary

• We investigated the functional significance of extraradical mycorrhizal networks produced by geographically different isolates of the arbuscular mycorrhizal fungal (AMF) species *Glomus mosseae* and *Glomus intraradices*.

• A two-dimensional experimental system was used to visualize and quantify intact extraradical mycelium (ERM) spreading from *Medicago sativa* roots. Growth, phosphorus (P) and nitrogen (N) nutrition were assessed in *M. sativa* plants grown in microcosms.

• The AMF isolates were characterized by differences in extent and interconnectedness of ERM. Phenotypic fungal variables, such as total hyphal length, hyphal density, hyphal length per mm of total or colonized root length, were positively correlated with *M. sativa* growth response variables, such as total shoot biomass and plant P content.

• The utilization of an experimental system in which size, growth rate, viability and interconnectedness of ERM extending from mycorrhizal roots are easily quantified under realistic conditions allows the simultaneous evaluation of different isolates and provides data with a predictive value for selection of efficient AMF.

Key words: anastomosis, arbuscular mycorrhizas, functional diversity, *Glomus intraradices*, *Glomus mosseae*, *Medicago sativa*, mycorrhizal networks.

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Introduction

Arbuscular mycorrhizas are symbiotic associations established between soilborne fungi (Glomeromycota) and the roots of most terrestrial plant species. They play a fundamental role in soil fertility and plant nutrition, since the two partners of the symbiosis interact, producing mutual benefits: fungi obtain carbon compounds from colonized roots and plants receive mineral nutrients, such as phosphorus (P), nitrogen (N), sulphur (S), potassium (K), calcium (Ca), iron (Fe), copper (Cu) and zinc (Zn), absorbed and translocated by extraradical fungal hyphae which spread from mycorrhizal roots into the surrounding soil (Smith & Read, 1997).

Arbuscular mycorrhizal (AM) fungi show varying degrees of host specificity, in terms of both colonization ability and functioning, since the outcome of the symbiotic interaction is affected by factors related to host plant and fungal symbiont genotypes (Jakobsen *et al.*, 1992; van der Heijden *et al.*, 1998; Smith *et al.*, 2000).

The symbiotic performance of AM fungal isolates depends on two main parameters, colonization ability and efficiency. The rate of colonization is influenced by the ability of AM fungi (AMF) to spread rapidly and extensively in plant roots, and is affected by factors linked to spore germination, presymbiotic mycelial growth and appressorium formation (Giovannetti, 2000). Efficiency is correlated with the ability of different isolates to promote plant growth by improving mineral nutrition and increasing tolerance to biotic and abiotic stresses (Giovannetti & Avio, 2002; Jakobsen *et al.*, 2002).

The study of parameters affecting fungal colonization ability and efficiency is essential for predicting the outcome of

the symbiosis, and for further utilization of AMF in the field, since a large functional variability occurs among different isolates, even those belonging to the same species (Smith *et al.*, 2000; Munkvold *et al.*, 2004). The inter- and intraspecific differences in AM fungal efficiency and the differential increases in P and N supply to host plants have been attributed to phenotypic and functional properties of extraradical mycorrhizal mycelium (ERM) (Ames *et al.*, 1983; Jakobsen *et al.*, 1992; Mäder *et al.*, 2000; Hodge *et al.*, 2001; Smith *et al.*, 2004), some of which depend on the occurrence and differential expression of P transporter and N assimilation fungal genes (Harrison & van Buuren, 1995; Maldonado-Mendoza *et al.*, 2001; Govindarajulu *et al.*, 2005).

The ability to develop extensive and highly interconnected extraradical mycorrhizal networks could represent an important feature of efficient AMF. Some studies provided data on the large diversity among different AM fungal isolates in the extension, viability, structure and anastomosis formation ability of ERM originating from mycorrhizal roots (Sanders *et al.*, 1977; Abbott & Robson, 1985; Hamel *et al.*, 1990; Friese & Allen, 1991; Jones *et al.*, 1998; Giovannetti *et al.*, 2001). Although some studies show that ERM is of critical importance for P uptake (van der Heijden *et al.*, 1998; Schweiger & Jakobsen, 2000), a question remains as to whether the differential growth responses obtained in plants inoculated with different AMF may be correlated with the interconnectedness of extraradical mycelium, through which mineral nutrients flow from the soil to plant roots.

Investigations on AM networks have utilized microcosm systems involving destructive extraction from soil (Jakobsen *et al.*, 1992; Jones *et al.*, 1998), whereas a few nondestructive observations of the architecture and developmental dynamics of AM mycelium have been carried out using root observation chambers (Friese & Allen, 1991; Hart & Reader, 2005) and *in vitro* dual systems (Bago *et al.*, 1998; Koch *et al.*, 2004). Recently we employed an *in vivo* two-dimensional experimental model system to visualize and quantify intact AM networks spreading from mycorrhizal roots to the surrounding environment (Giovannetti *et al.*, 2001, 2004).

Here we applied the same experimental system to assess the extent and interconnectedness of ERM of geographically different isolates of two globally distributed AM fungal species, *Glomus mosseae* and *G. intraradices.* We investigated the relationships between mycorrhizal network characteristics, plant growth responses, and P and N uptake in *Medicago sativa*, a mycotrophic plant species highly dependent on mycorrhizal inoculation, particularly in nutrient-poor soils.

Materials and Methods

Plant and fungal material

Arbuscular mycorrhizal fungi used were *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, isolate IMA1 from UK (collector B. Mosse) and isolate AZ225C from USA (collector J. C. Stutz), and *G. intraradices* Schenck & Smith, isolate IMA5 from Italy (collector M. Giovannetti) and isolate IMA6 from France (collector V. Gianinazzi-Pearson). They were obtained from pot cultures maintained in the collection of the Soil Microbiology Laboratory of the Department of Crop Plant Biology, University of Pisa, Italy. The plant species used was the forage legume *Medicago sativa* cv. Messe.

Experiment 1: extent and interconnectedness of ERM produced by four geographically different *Glomus* isolates in an *in vivo* two-dimensional model system

This experiment was aimed at evaluating the extent and interconnectedness of ERM produced by four geographically different *Glomus* isolates using an *in vivo* two-dimensional model system (Giovannetti *et al.*, 2001).

Spore collection and germination. Sporocarps and spores were extracted from pot-culture soil by wet-sieving and decanting, down to a mesh size of 100 μm, flushed into Petri dishes and manually collected with forceps under a Wild dissecting microscope (Leica, Milano, Italy). They were washed by vortexing in sterile distilled water (SDW) for 20 s, rinsed three times in SDW and germinated in the dark at 24°C between two 47-mm-diameter cellulose nitrate Millipore™ membranes (pore diameter 0.45 μm) placed on acid-washed, autoclaved quartz grit (2–5 mm diameter) in 14-cm-diameter Petri dishes (Logi *et al.*, 1998). Possible differences in germination and hyphal growth of the different isolates were balanced by using a high number of sporocarps or spore clusters (20 sporocarps for *G. mosseae* isolates and 20 spore clusters for *G. intraradices* isolates) in each membrane sandwich. Germination and hyphal growth were monitored once a week on a subset of samples.

Experimental system. Surface-sterilized seeds of *M. sativa* were germinated in moist sterile grit. After 21 d the root system of each seedling was sandwiched between the Millipore membranes containing germinated propagules showing homogeneous hyphal lengths. Plants were individually placed into 10-cm-diameter pots and the sandwiched roots were buried in sterile quartz grit. Pots were closed in Sun Transparent Bags (Sigma Aldrich s.r.l., Milano, Italy), maintained in a growth chamber under controlled conditions (24°C day and 21°C night temperature, 16 : 8 h light : dark cycle, 200 μmol photons m^{-2} s^{-1}). Plants were harvested after 8 wk of growth. Roots were removed from the sandwich system by immersion in water and checked for the presence of extraradical hyphae, spores and sporocarps, which were carefully plucked with forceps under the dissecting microscope. Mycorrhizal colonization was assessed on sample plants by clearing and staining, using lactic acid instead of phenol (Phillips & Hayman, 1970).

Plant roots were then sandwiched between two halves of 140-mm-diameter cellulose nitrate Millipore membranes,

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transferred into 14-cm-diameter Petri dishes containing sterile quartz grit and maintained in a growth chamber as described. Once a week, a subset of membranes was opened, and the development of ERM spreading from the roots on the membranes was checked, to detect harvest time, that is when the mycorrhizal network spread at least 1–2 cm from the root system onto the membranes.

Viability, extent and interconnectedness of the mycorrhizal network. After 4 wk, membranes were opened and ERM was tested for viability by staining for succinate dehydrogenase activity (SDH) (Smith & Gianinazzi-Pearson, 1990). After SDH test, the same ERM was stained with Trypan blue in lactic acid (0.05%) to assess hyphal density within mycorrhizal networks (hyphal length mm^{-2}), which was estimated under a dissecting microscope with the gridline intersect method (Giovannetti & Mosse, 1980) by measuring, using a grid eyepiece, the length of hyphae in five areas of 64 mm^2, on at least six replicate sandwiches for each isolate. Numbers of hyphal contacts and anastomoses were counted on five replicate sandwiches for *G. mosseae* isolates and three replicate sandwiches for *G. intradices* isolates. For each replicate, three areas of 133 mm^2 were cut, mounted on microscope slides and examined under a Reichert-Jung (Vienna, Austria) Polyvar microscope. Hyphal anastomoses were counted at magnifications of ×125–500 and verified at a magnification of ×1250. Frequency of anastomosis was calculated by determining the proportion of hyphal contacts that had anastomosed. Findings are based on at least 366 hyphal contacts per replicate.

The area covered by ERM was measured by an image analyser (Quantimet 500, Leica). Total hyphal length was calculated, multiplying hyphal density by the area covered by the mycelial network. Total root length and percentage of AM colonization were assessed after root staining, by using the gridline intersect method (Giovannetti & Mosse, 1980).

Experiment 2: evaluation of growth responses and nutrient uptake of *M. sativa* inoculated with four geographically different *Glomus* isolates

This glasshouse experiment was aimed at evaluating growth and nutritional responses of *M. sativa* inoculated with the *Glomus* isolates tested in experiment 1.

Experimental setup. Seeds (20) of *M. sativa* were sown in 600 ml plastic pots containing a mixture (1 : 1, by volume) of soil and Terragreen (calcinated clay, OILDRI, Chicago, IL, USA). The soil was a sandy loam collected near S. Piero (Pisa). Chemical and physical characteristics of the soil were as follows: pH (H_2O), 8.0; clay, 15.3%; silt, 30.1%; sand, 54.5%; organic matter, 2.24%; total N, 1.27%; total P, 469.5 p.p.m.; extractable P, 17.6 p.p.m. (Olsen); extractable K, 149.6 p.p.m. The main characteristics of terragreen were: pH 5.1; extractable P 3.3 p.p.m. The mixture was steam-sterilized (121°C for 25 min, on two consecutive days), to kill naturally occurring AMF. Pots were inoculated either with 90 ml of crude inoculum (mycorrhizal roots and soil containing spores and extraradical mycelium) of one of the four fungal isolates or with 90 ml of a sterilized mixture of them (nonmycorrhizal control). In this way, possible differences in AM fungal colonization ability of the four isolates were balanced by using such high amounts of inoculum (15% by volume). All the pots received 120 ml of a filtrate, obtained by sieving a mixture of the four inocula and of agricultural soil from a *M. sativa* field, through a sieve with pore diameter 50 µm, to ensure a common microflora for all treatments. After emergence, seeds of *M. sativa* were thinned to 10 to reproduce field densities. Plants were grown in a glasshouse, supplied with tap water as needed and with a weekly fertilization of half-strength Hoagland's solution (10 ml per pot). The experiment was a completely randomized design with five inoculum treatments (each fungal isolate and the control), five replicates and two harvests. Three months after emergence, plant shoots were harvested by cutting 1 cm above soil level. To evaluate the effect of AM inoculation on regrowth ability of *M. sativa*, a perennial legume subject to several cuts per year, a second harvest was carried out after 1 month's regrowth.

Measurements. At both harvests, stems and leaves of *M. sativa* plants were separated and dry weights determined after drying at 95°C for 48 h. At the second harvest, root systems were removed and dry weights determined on a subsample (half of each root system). A sample of dry roots was weighed and used to assess root nutritional status. Percentage of AM colonization and total root length were assessed on half of each root system after root staining, by using the gridline intersect method (Giovannetti & Mosse, 1980).

P concentrations of shoots and roots were measured after sulphuric/perchloric acid digestion using the photometric method (Jones *et al.*, 1991). Tissue N concentrations of shoots were assessed using the Kjeldahl method (Jones *et al.*, 1991). The total P and N contents were calculated by multiplying P and N concentration values by dry weights. At the second harvest, the number of stems regrown from each plant was also determined.

Statistics and data analyses. Analysis of variance (ANOVA) was performed on SPSS 11.0 software (SPSS Inc., Chicago, IL, USA), after the necessary transformations, and differences between means were determined by appropriate test. Tukey B procedure was used for comparing means of experiment 1, and orthogonal contrasts were used to test inter- and intraspecific differences in experiment 2. Data of percentage colonization were arcsine-transformed. Analysis of covariance was used to separate the effects of different AMF isolates on colonization rate from those on hyphal growth, using colonization rate (colonized root length) as a covariate. Regression of selected plant parameters

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vs ERM parameters was performed by GLM procedures on SPSS software.

Results

Extent and interconnectedness of ERM produced by four geographically different *Glomus* isolates in an *in vivo* two-dimensional model system (experiment 1)

The experimental system allowed the visualization of intact extraradical mycorrhizal networks produced by the different *Glomus* isolates, spreading from colonized roots of *M. sativa* (Fig. 1). The four *Glomus* isolates showed large differences in patterns of ERM development, measured as total hyphal length, area covered by ERM and number of anastomoses (Table 1). *G. intraradices* IMA6 produced the largest hyphal length (41.11 m), and density (5.74 mm mm^{-2}) and covered the largest area of the membrane surface (7208 mm^2). Moreover, the highest values of hyphal length per total or per colonized root length were always associated with *G. intraradices* IMA6 (21.28 mm mm^{-1}, 30.36 mm mm^{-1}). *G. mosseae* isolates showed the lowest values for all the parameters, except for IMA1 hyphal density, which was similar to that of IMA5.

The different AMF isolates showed different values of mycorrhizal colonization, which ranged from 25.9 to 43.0% in *G. mosseae* AZ225C and IMA1, respectively, and from 59.3 to 70.1% in *G. intraradices* IMA5 and IMA6, respectively ($P < 0.001$). Covariance analysis showed that the effects of

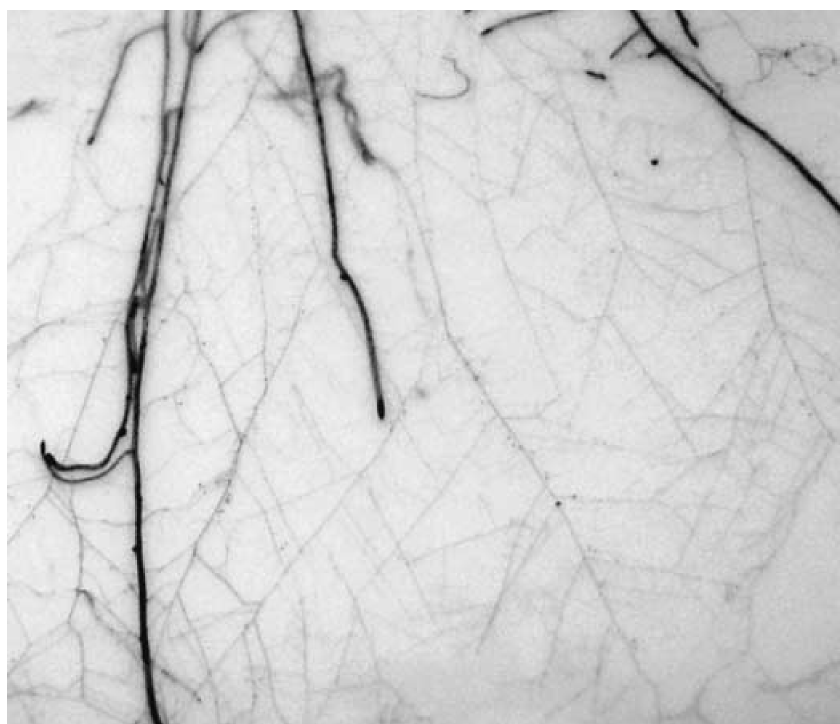

Fig. 1 Visualization of the extraradical network produced by the arbuscular mycorrhizal fungal isolate *Glomus mosseae* IMA1, spreading from mycorrhizal roots of *Medicago sativa* and uniformly colonizing the surrounding environment.

Table 1 Extent, structure and interconnectedness of extraradical mycelium (ERM) produced by four geographical isolates of the arbuscular mycorrhizal fungal species *Glomus mosseae* (IMA1 and AZ225) and *Glomus intraradices* (IMA6 and IMA5) in symbiosis with *Medicago sativa*

	AZ225C	IMA1	IMA5	IMA6
Total hyphal length (m)	11.67[a]	14.22[a]	21.27[a]	41.11[b]
Area covered by ERM (mm^2)	4471.71[a]	3787.21[a]	5292.17[ab]	7208.00[b]
Hyphal length per root length (mm mm^{-1})	3.81[a]	5.22[a]	9.42[a]	21.28[b]
Hyphal length per colonized root length (mm mm^{-1})	14.72[a]	12.14[a]	15.88[a]	30.36[b]
Hyphal density (mm mm^{-2})	2.50[a]	3.70[b]	3.99[b]	5.74[c]
Number of anastomoses mm^{-2}	0.77[a]	1.11[a]	3.82[b]	3.47[b]
Number of anastomoses mm^{-1} of hyphal length	0.28[a]	0.29[a]	0.82[b]	0.61[b]
Number of anastomoses per hyphal contact (%)	29.7[a]	46.3[ab]	47.0[ab]	66.6[b]

Values in each row not followed by the same letters are significantly different ($P < 0.05$).

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different AMF isolates on hyphal length were independent of the effects of root colonization extent ($P < 0.001$).

G. intraradices and *G. mosseae* isolates showed different patterns of mycelial growth, as measured by the number of anastomoses mm^{-2}, and mm^{-1} of hyphal length, parameters which are considered indicators of ERM interconnectedness. The two *G. mosseae* isolates showed lower values than *G. intraradices* isolates (Table 1). However, when the number of anastomoses per hyphal contact (anastomosis formation ability) was considered, the two *G. intraradices* isolates and *G. mosseae* IMA1 showed similar values, and *G. intraradices* IMA6 showed significantly higher values than *G. mosseae* AZ225C.

Succinate dehydrogenase activity staining of the mycorrhizal network allowed us to verify ERM viability, which was 100%. Moreover, all the observed anastomoses showed protoplasmic continuity in hyphal bridges, while no hyphal incompatibility reactions were found.

Evaluation of growth responses and nutrient uptake of *M. sativa* inoculated with four geographically different *Glomus* isolates (experiment 2)

Mycorrhizal colonization. The four *Glomus* isolates successfully established mycorrhizal symbioses with *M. sativa*, showing percentages of colonized root length ranging from 54.1 to 61.4% (in *G. mosseae* IMA1 and AZ225C, respectively), to 69.0 to 79.7% (in *G. intraradices* IMA5 and IMA6, respectively), 4 months after inoculation. No colonization was observed in uninoculated plants.

Rhizobial populations naturally occurring in the inoculum filtrate supplied to all plants ensured that the plants produced nodules in all treatments.

Plant growth. Shoot biomass production was significantly affected by mycorrhizal symbiosis at each harvest (Table 2). Host benefits, calculated for each fungal species as ((dry weight mycorrhizal plant – dry weight nonmycorrhizal plant)/dry weight nonmycorrhizal plant) × 100, were 100, 96, 117 and 106% for *G. mosseae* IMA1, *G. mosseae* AZ225C, *G. intraradices* IMA6 and *G. intraradices* IMA5, respectively, based on the total shoot weights obtained from both harvests. Total shoot biomass correlated well with the percentage of colonized root length ($R^2 = 0.882$, $P = 0.06$).

Plants inoculated with *G. intraradices* isolates showed significantly larger shoot dry weights than those colonized by *G. mosseae* isolates ($P = 0.008$) at the first harvest, while no intraspecific differences were observed. At the second harvest there were no differences among mycorrhizal treatments ($P = 0.644$) (Table 2).

However, a different resource allocation was observed during plant regrowth, depending on the inoculated fungal species: at the second harvest, plants inoculated with *G. mosseae* isolates showed a larger production of foliar biomass than those inoculated with *G. intraradices* ($P = 0.049$), which in contrast had a larger production of stem biomass ($P = 0.03$). No intraspecific differences were observed, as shown by orthogonal contrasts (*P*-values ranging between 0.24 and 0.72; Table 2).

The different partitioning of shoot biomass was confirmed by the different numbers of stems produced during regrowth, which were significantly higher ($P < 0.001$) in plants inoculated with *G. intraradices* isolates than in those inoculated with *G. mosseae* isolates. In addition, highly significant intraspecific differences were observed between *G. mosseae* isolates ($P = 0.001$).

Root biomass production and length were significantly affected by mycorrhizal symbiosis ($P < 0.001$) (Table 3). Interestingly, although root dry weights did not vary among the different *Glomus* isolates, except for *G. mosseae* IMA1 vs AZ225C ($P = 0.034$), highly significant differences in root length were observed at the species level, between *G. mosseae* and *G. intraradices* ($P < 0.001$), and at the intraspecific level, between *G. intraradices* isolates ($P = 0.018$) (Table 3).

Table 2 *P*-values of linear orthogonal contrasts for shoot, leaves and stem dry weights and for shoot P and N content of *Medicago sativa* plants at first and second harvests

Treatments compared	Shoot dry matter	Stem dry matter	Leaves dry matter	Shoot P content	Shoot N content
First harvest					
NM vs M	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Glomus mosseae vs *G. intraradices*	**0.008**	0.108	**0.005**	**0.002**	**0.003**
IMA1 vs AZ225C	0.832	0.731	0.647	**0.014**	0.159
IMA5 vs IMA6	0.165	0.874	0.076	**0.017**	0.361
Second harvest					
NM vs M	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
G. mosseae vs *G. intraradices*	0.644	**0.030**	**0.049**	0.358	0.796
IMA1 vs AZ225C	0.370	0.720	0.240	0.500	0.744
IMA5 vs IMA6	0.877	0.249	0.535	0.070	0.302

M, mychorrhizal; NM, nonmycorrhizal; In bold, statistically significant values ($P < 0.05$).
IMA1 and AZ225, *Glomus mosseae*; IMA6 and IMA5, *Glomus intraradices*.

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Table 3 Root response variables of *Medicago sativa* plants inoculated with four geographical isolates of the arbuscular mycorrhizal fungal species *Glomus mosseae* (IMA1 and AZ225) and *Glomus intraradices* (IMA6 and IMA5) or not inoculated (NM)

	Root dry weight (g per pot)	Root length (m per pot)	Root P concentration (%)	Root P content (mg per pot)
NM	1.34 ± 0.11[a]	61.01 ± 2.51	0.11 ± 0.01	1.52 ± 0.17
IMA1	2.42 ± 0.14	111.59 ± 4.26	0.15 ± 0.00	3.66 ± 0.19
AZ225C	2.00 ± 0.16	110.13 ± 5.81	0.15 ± 0.01	3.03 ± 0.28
IMA6	2.35 ± 0.07	79.27 ± 5.83	0.17 ± 0.01	3.95 ± 0.11
IMA5	2.29 ± 0.15	95.46 ± 2.40	0.15 ± 0.01	3.45 ± 0.19
Treatments compared (P-values of linear orthogonal contrasts)				
NM vs M	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
G. mosseae vs *G. intraradices*	0.388	**< 0.001**	0.323	0.083
IMA1 vs AZ225C	**0.034**	0.818	0.957	**0.032**
IMA5 vs IMA6	0.746	**0.018**	0.134	0.085

[a]Values are means ± SE of means of five replicate pots for each treatment; In bold, statistically significant values ($P < 0.05$).

P and N uptake. All mycorrhizal *M. sativa* plants showed significantly higher P and N contents compared with nonmycorrhizal controls. Shoot P content of plants colonized by *G. intraradices* was higher than that of plants colonized by *G. mosseae* at the first harvest ($P = 0.002$) (Table 2). Within each species, *G. mosseae* AZ225C and *G. intraradices* IMA6 mycorrhizal plants showed the highest shoot P content ($P = 0.014$ and $P = 0.017$, respectively) (Table 2). Shoot N content followed the same pattern as dry matter, that is, a larger N content was found in *G. intraradices*- than in *G. mosseae*-inoculated plants, only at the first harvest (Table 2).

P and N shoot concentrations in mycorrhizal plants were significantly ($P < 0.001$) higher than in controls and were not affected by AM fungal species ($P > 0.05$) (Table 4).

Table 4 Shoot N and P concentrations at first and second harvest in *Medicago sativa* plants inoculated with four geographical isolates of the arbuscular mycorrhizal fungal species *Glomus mosseae* (IMA1 and AZ225) and *Glomus intraradices* (IMA6 and IMA5) or not inoculated (NM)

	Shoot P concentration (%)	Shoot N concentration (%)
First harvest		
NM	0.12 ± 0.01[a]	2.45 ± 0.03
IMA1	0.16 ± 0.01	2.79 ± 0.05
AZ225C	0.21 ± 0.01	3.08 ± 0.12
IMA6	0.21 ± 0.01	2.96 ± 0.06
IMA5	0.19 ± 0.00	3.05 ± 0.03
Second harvest		
NM	0.18 ± 0.01	2.67 ± 0.04
IMA1	0.21 ± 0.01	2.82 ± 0.13
AZ225C	0.24 ± 0.01	2.91 ± 0.10
IMA6	0.26 ± 0.01	3.09 ± 0.12
IMA5	0.22 ± 0.01	2.83 ± 0.12

	P-values of linear orthogonal contrasts			
	Shoot P concentration (%)		Shoot N concentration (%)	
Treatments compared	First harvest	Second harvest	First harvest	Second harvest
NM vs M	**< 0.001**	**< 0.001**	**< 0.001**	0.051
G. mosseae vs *G. intraradices*	0.072	0.084	0.332	0.384
IMA1 vs AZ225C	**0.002**	0.057	**0.007**	0.545
IMA5 vs IMA6	0.076	**0.013**	0.407	0.097

M, mychorrhizal; NM, nonmycorrhizal; In bold, statistically significant values ($P < 0.05$).
[a]Values are means ± SE of means of five replicate pots of each treatment.

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Interestingly, intraspecific differences were shown at the first harvest by *G. mosseae*-inoculated plants ($P = 0.007$ and $P = 0.002$, for N and P concentrations, respectively), and at the second harvest by *G. intraradices*-inoculated plants, for P concentration ($P = 0.013$) (Table 4).

Relationships between host benefit and ERM extent and interconnectedness

Plant growth response variables, such as shoot biomass and P and N contents, obtained in experiment 2, were analysed for correlation with fungal variables, such as total hyphal length, area covered by ERM, hyphal density, hyphal length per mm of root length and hyphal length per mm of colonized root length, determined in experiment 1. Generally, positive correlations were found between all the plant and fungal variables analysed. In particular, total shoot biomass was highly correlated with total hyphal length and density ($R^2 = 0.980$, $P = 0.01$ and $R^2 = 0.957$, $P = 0.02$), and with hyphal length per mm of root length ($R^2 = 0.978$, $P = 0.01$) (Fig. 2a–c). A very good correlation was also found between P content of all plant tissues and hyphal length per mm of colonized root ($R^2 = 0.980$, $P = 0.01$) (Fig. 2d).

Plant response variables were also analysed for correlation with fungal variables associated with ERM interconnectedness, such as anastomosis frequency, total number of anastomoses and the number of anastomoses, both per mm^2 and per mm of hyphal length. Good correlations were found between total shoot biomass and anastomosis frequency ($R^2 = 0.911$, $P = 0.045$) or total number of anastomoses ($R^2 = 0.920$, $P = 0.041$), and between root P content and anastomosis frequency ($R^2 = 0.918$, $P = 0.042$) (Fig. 3a–c).

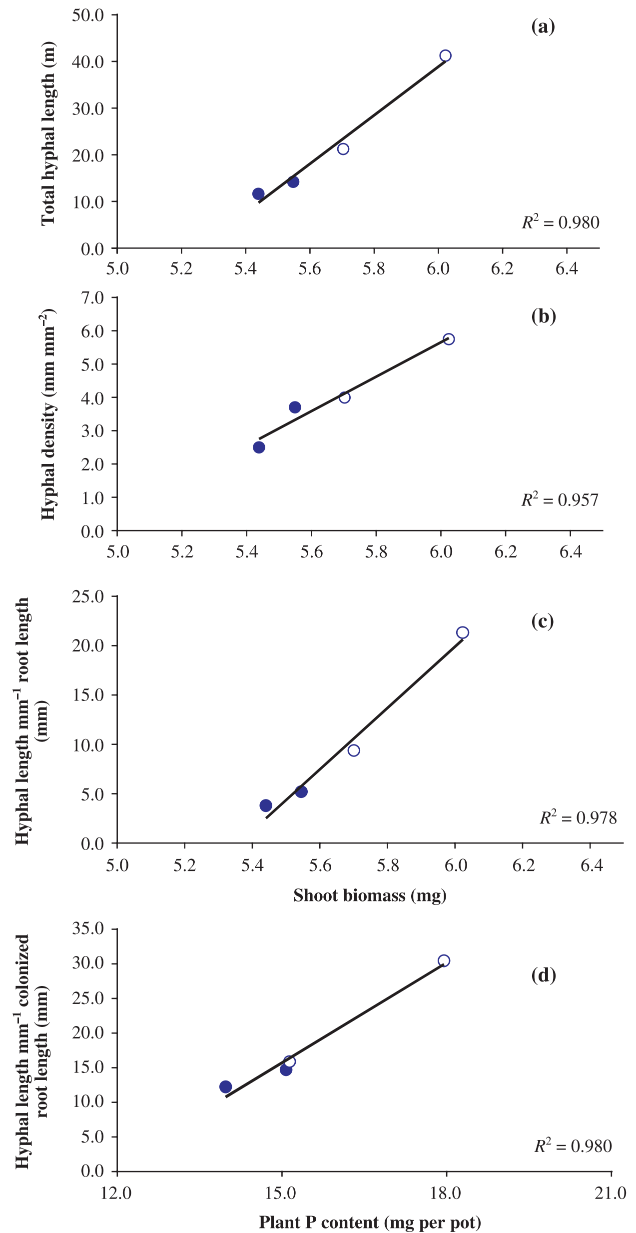

Fig. 2 Relationships between *Medicago sativa* shoot biomass and total hyphal length (a), hyphal density (b) and hyphal length mm^{-1} root length (c), and between total plant P content and hyphal length mm^{-1} colonized root length (d), for geographically different isolates of *Glomus mosseae* (IMA1 and AZ225C) (closed circles) and *Glomus intraradices* (IMA6 and IMA5, open circles). Slopes are significant (a, $P = 0.01$; b, 0.02; c, 0.01; d, 0.01).

Discussion

Visualization and quantification of ERM produced by four geographically different *Glomus* isolates (experiment 1)

To our knowledge this is the first *in vivo* study, in which plants and AMF are grown under realistic conditions, showing differences in both extent and interconnectedness of ERM in different AM fungal isolates. We studied intact AM mycelium extending from *M. sativa* mycorrhizal roots by means of a bidimensional culture system developed to study extraradical AM hyphae (Giovannetti *et al.*, 2001). Here, total lengths of hyphae ranged, in *G. mosseae* isolates, from 11.7 to 14.2 m, values lower than those found in *G. intraradices* isolate IMA6, which produced not only the largest amount of ERM (41.1 m) but also the largest hyphal density (5.7 mm mm^{-2}) and hyphal length per total root length (21.3 mm mm^{-1}). The latter data compare favourably with figures obtained using destructive extraction from soil, which ranged from 1.6 to 1420 mm of hyphal length per mm of root (Tisdall & Oades, 1979; Abbott & Robson, 1985). Moreover, some differences between the present data and those previously reported may be the result of the different experimental systems used, but also of a host plant effect on the development of ERM, as already suggested by other studies with *G. mosseae* IMA1: for example, hyphal density in cotton was much higher than in lettuce, parsley and eggplant (Giovannetti *et al.*, 2004, 2006).

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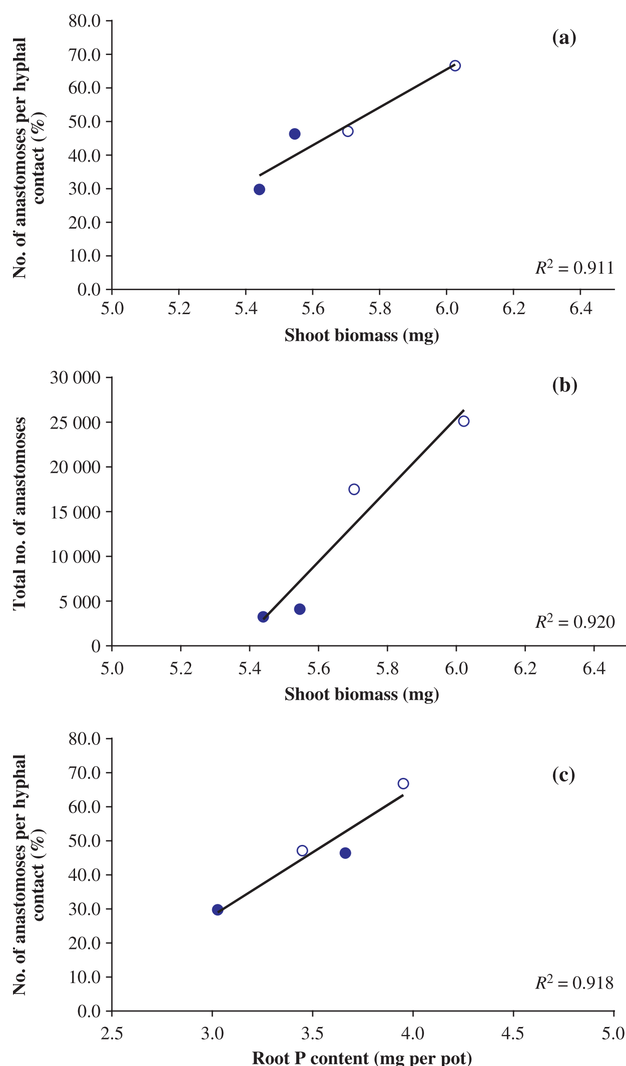

Fig. 3 Relationships between *Medicago sativa* shoot biomass and number of anastomosis per hyphal contact (anastomosis frequency) (a) and total number of anastomosis (b), and between root P content and anastomosis frequency (c), for geographically different isolates of *Glomus mosseae* (IMA1 and AZ225C, closed circles) and *Glomus intraradices* (IMA6 and IMA5, open circles). Slopes are significant (a, $P = 0.045$; b, 0.041; c, 0.042).

Average growth rates of ERM are consistent with figures obtained in works carried out using the same *in vivo* experimental system: it ranged from 417 to 1468 mm d^{-1} in *G. mosseae* and *G. intraradices*, comparing well with values of 738–1067 mm d^{-1}, reported for *G. mosseae* mycelium originating from three different host plants (Giovannetti *et al.*, 2001). It is interesting to note that hyphal growth rates obtained in our bidimensional system (6.2–14.3 cm cm^{-2} wk^{-1}) are very similar to those reported for *G. intraradices* growing *in vitro* associated with Ri T-DNA-transformed carrot roots (2–9 cm cm^{-2} wk^{-1}) (Koch *et al.*, 2004). Moreover, the average rates of hyphal spread obtained in three-dimensional soil microcosms ranged from 0.7 mm d^{-1} in *Glomus* sp. and *Scutellospora calospora*, to 3.1 mm d^{-1} in *Acaulospora laevis* (Jakobsen *et al.*, 1992), values that are similar to our data calculated using the mean distance of hyphal tips from the root: 1.1–1.4 mm d^{-1}.

Some nondestructive investigations on the structure of ERM of AMF have provided qualitative information on its architecture and development before and after symbiosis establishment (Friese & Allen, 1991; Bago *et al.*, 1998), but they did not study anastomosis formation, which represents the mechanism underlying the establishment of extensive and highly integrated extraradical mycelial networks through which nutrients flow from soil to host plants (Giovannetti *et al.*, 2001, 2004). Here, data representing the interconnectedeness of ERM produced by *G. mosseae* isolates in symbiosis with *M. sativa* are consistent with those reported in other works: for example, the number of anastomoses mm^{-2}, ranging from 0.8 to 1.1, are similar to data obtained with different host plants, that is, 0.8–1.3 (Giovannetti *et al.*, 2001, 2004). By contrast, *G. intraradices* isolates showed significantly higher values, 3.5 and 3.8 anastomoses mm^{-2}, which, very interestingly, are comparable with the figures obtained in cotton colonized by *G. mosseae*: 4.2 anastomoses mm^{-2}. These findings, together with those of total hyphal length, showing that host plant species may affect ERM structure and length, confirm that a systematic approach aimed at detecting the best performing AMF should be based on the screening of different host plant–fungal symbiont combinations (Giovannetti & Avio, 2002).

Our data on the interconnectedness of ERM show a large interspecific variability, which was not observed in a previous study carried out in monoxenic cultures of *Glomus hoi*, *G. intraradices*, *Glomus proliferum* in association with Ri T-DNA-transformed carrot roots (de la Providencia *et al.*, 2005). This difference may be the result of the experimental model system, which yielded much lower numbers of anastomoses per hyphal length (0.05–0.07 compared with 4.6–5.1 cm^{-1} of hyphae). However, intraspecific differences within *G. intraradices* were not detected, in agreement with our results.

An important parameter of fungal efficiency, determining the actual fungal biomass actively functioning in the soil for nutrient uptake and translocation, is represented by the viability of ERM. In our experimental system, the viability of the mycorrhizal network, verified by means of SDH staining, was 100% in 4-wk-old *G. mosseae* and *G. intraradices* hyphae, confirming previous data on metabolic activity of hyphae growing in the soil (Hamel *et al.*, 1990). Other authors reported that the length of vital extraradical hyphae ranged from 20 to 74 m m^{-1} of colonized root in *Allium porrum*, *Eucalyptus coccifera*, *Prunus cerasifera* and *Thymus vulgaris* inoculated with four different AMF (Jones *et al.*, 1998; Giovannetti *et al.*, 2001). These results are in contrast to previous data suggesting a high turnover rate of C in ERM (Staddon *et al.*, 2003).

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Growth responses and P and N uptake of *M. sativa* colonized by four geographically different *Glomus* isolates

Medicago sativa is considered a highly mycotrophic legume (Barea & Azcón-Aguilar, 1983), although the outcome of the mycorrhizal symbiosis may depend on host varieties (Lambert *et al.*, 1980; O'Bannon *et al.*, 1980) and fungal species (Azcón *et al.*, 1991; Vazquez *et al.*, 2001). Our work confirms the high mycorrhizal dependency of *M. sativa*, since all fungal treatments improved shoot biomass, P and N contents and concentrations.

We detected a large functional diversity between the two AM fungal species utilized, since *G. intraradices* isolates were generally more effective than *G. mosseae* isolates. Our results are similar to previous data obtained with a different *M. sativa* variety inoculated with the same isolates of *G. mosseae* (Rothamsted isolate = IMA1) and *G. intraradices* (LPA 8 = IMA6) (Vazquez *et al.*, 2001). Interestingly, the better symbiotic performance of *G. intraradices* was restricted to the first harvest, suggesting that different fungal endophytes may affect the regrowth ability of *M. sativa* plants differently. Such effects have not been reported previously, although several studies have reported that AMF occurrence and species diversity are affected by defoliation or grazing (Klironomos *et al.*, 2004).

We detected not only interspecific but also intraspecific functional diversity, both in *G. mosseae* and in *G. intraradices*. The two isolates within each species promoted plant P content and/or P and N concentration differently, with *G. mosseae* isolates showing more variability than *G. intraradices* isolates. Up until now, no information has been available on the effects of different isolates of the same AMF species on *M. sativa* plants. A large functional diversity of geographically different isolates of *G. mosseae* and *G. intraradices* was reported in *Cucumis sativus*, *Plantago* spp. and *Poa* spp. (Hart & Reader, 2002; Munkvold *et al.*, 2004). In contrast, little variation in ^{33}P uptake by ERM was detected among isolates of *G. mosseae* and *G. intraradices* originating from the same field site (Jansa *et al.*, 2005).

Relationship between fungal variables and plant growth and nutrient uptake

In this study, phenotypic fungal variables such as the extent and interconnectedness of ERM obtained in the *in vivo* two-dimensional experimental system (experiment 1) were positively correlated with *M. sativa* growth response variables obtained in the microcosm experiment (experiment 2). In particular, AM fungal isolates producing higher total hyphal lengths and densities yielded larger increases in total shoot biomass ($R^2 = 0.980$ and 0.957, respectively), confirming that the growth ability and developmental pattern of ERM are important factors of fungal efficiency (Sanders *et al.*, 1977; Jakobsen *et al.*, 1992). Interestingly, plant P content correlated well with hyphal length, in accordance with recent data obtained with a compartmented experimental system, where a good correlation was found between shoot ^{33}P content and hyphal length measured in a root-free chamber (Munkvold *et al.*, 2004). Previous works did not report significant correlations between ERM size and P uptake and/or plant growth responses, when comparing AM fungal isolates belonging to different genera and families (Dodd *et al.*, 2000; Smith *et al.*, 2000; Hart & Reader, 2002). However, highly significant correlations are usually found between ^{33}P uptake and ERM size when comparing different isolates of the same species, suggesting that the efficiency of hyphal P uptake and transport is a species-specific fungal variable (Munkvold *et al.*, 2004; Smith *et al.*, 2004; Jansa *et al.*, 2005; Poulsen *et al.*, 2005).

To our knowledge this is the first study showing a correlation between phenotypic fungal variables affecting ERM interconnectedness (anastomosis frequency and number) and plant growth variables (total shoot biomass) ($R^2 = 0.911$ and 0.920, respectively). A good correlation was also found between anastomosis frequency and root P content ($R^2 = 0.918$). Although good correlations were not found between anastomosis density and plant response variables, the functional importance of fungal parameters related to ERM interconnectedness may still be of greatest importance in the field, where hyphal networks are subject to mechanical disruption such as Collembolan grazing or ploughing in arable sites (Daniell *et al.*, 2001; Johnson *et al.*, 2005). Further work should be aimed at investigating the relationship between ERM structure and host benefit in AMF belonging to different species, genera and families, since some genera, such as *Gigaspora* and *Scutellospora*, show a null or very low capability of developing a structured mycorrhizal network (Giovannetti *et al.*, 1999; de la Providencia *et al.*, 2005).

The utilization of a bidimensional model system, where intact AM fungal networks are visualized, and ERM actually spreading from colonized roots is easily quantified, offers evident advantages. First, there is the possibility of measuring size, growth rate, viability and interconnectedness of ERM while avoiding interference by hyphae from original inoculum and soil contaminants. Secondly, the system, allowing the simultaneous evaluation of different isolates in the same conditions, may provide data with a predictive value useful to select efficient AM fungal endophytes.

References

Abbott LK, Robson AD. 1985. Formation of external hyphae in soil by four species of vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **99**: 245–255.

Ames RN, Reid CPP, Porter LK, Cambardella C. 1983. Hyphal uptake and transport of nitrogen from two ^{15}N-labelled sources by *Glomus mosseae*, a vesicular-arbuscular mycorrhizal fungus. *New Phytologist* **95**: 381–396.

Azcón R, Rubio R, Barea JM. 1991. Selective interactions between different species of mycorrhizal fungi and *Rhizobium meliloti* strains, and their effects on growth, N_2-fixation (^{15}N) and nutrition of *Medicago sativa*. *New Phytologist* **117**: 399–404.

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Bago B, Azcón-Aguilar C, Piché Y. 1998. Architecture and developmental dynamics of the external mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown under monoxenic conditions. *Mycologia* **90**: 2–62.

Barea JM, Azcón-Aguilar C. 1983. Mycorrhizas and their significance in nodulating nitrogen-fixing plants. *Advances in Agronomy* **36**: 1–54.

Daniell TJ, Husband R, Fitter AH, Young JPW. 2001. Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops. *FEMS Microbiology Ecology* **36**: 203–209.

de la Providencia IE, de Souza FA, Fernandez F, Delmas NS, Declerck S. 2005. Arbuscular mycorrhizal fungi reveal distinct patterns of anastomosis formation and hyphal healing mechanisms between different phylogenic groups. *New Phytologist* **165**: 261–271.

Dodd JC, Boddington CL, Rodriguez A, Gonzales-Chavez C, Mansur I. 2000. Mycelium of arbuscular mycorrhizal fungi (AMF) from different genera: form, function and detection. *Plant and Soil* **226**: 131–151.

Friese C, Allen MF. 1991. The spread of VA mycorrhizal fungal hyphae in the soil: Inoculum types and external hyphal architecture. *Mycologia* **83**: 409–418.

Giovannetti M. 2000. Spore germination and pre-symbiotic mycelial growth. In: Kapulnick Y, Douds DD, eds. *Arbuscular mycorrhizas: physiology and function*. Dordrecht, the Netherlands: Kluwer Academic Publishers, 47–68.

Giovannetti M, Avio L. 2002. Biotechnology of Arbuscular Mycorrhizas. In: Khachatourians GG, Arora DK, eds. *Applied mycology and biotechnology*, **Vol. 2**. Agriculture and food production. Amsterdam, the Netherlands: Elsevier, 275–310.

Giovannetti M, Avio L, Fortuna P, Pellegrino E, Sbrana C, Strani P. 2006. At the root of the wood wide web: self recognition and non-self incompatibility in mycorrhizal networks. *Plant Signaling and Behavior* **1**: 1–5.

Giovannetti M, Azzolini D, Citernesi AS. 1999. Anastomosis formation and nuclear and protoplasmic exchange in arbuscular mycorrhizal fungi. *Applied and Environmental Microbiology* **65**: 5571–5575.

Giovannetti M, Fortuna P, Citernesi AS, Morini S, Nuti MP. 2001. The occurrence of anastomosis formation and nuclear exchange in intact arbuscular mycorrhizal networks. *New Phytologist* **151**: 717–724.

Giovannetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist* **84**: 489–500.

Giovannetti M, Sbrana C, Avio L, Strani P. 2004. Patterns of below-ground plant interconnections established by means of arbuscular mycorrhizal networks. *New Phytologist* **164**: 175–181.

Govindarajulu M, Pfeffer PE, Hairu J, Abubaker J, Douds DD, Allen JW, Bücking H, Lammers PJ, Shachar-Hill Y. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature* **435**: 819–823.

Hamel C, Fyles H, Smith DL. 1990. Measurement of development of endomycorrhizal mycelium using three vital stains. *New Phytologist* **115**: 297–302.

Harrison MJ, van Buuren ML. 1995. A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. *Nature* **378**: 626–629.

Hart MM, Reader RJ. 2002. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist* **153**: 335–344.

Hart MM, Reader RJ. 2005. The role of the external mycelium in early colonization for three arbuscular mycorrhizal fungal species with different colonization strategies. *Pedobiologia* **49**: 269–279.

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**: 72–75.

Hodge A, Campbell CD, Fitter AH. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* **413**: 297–299.

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

Jakobsen I, Smith SE, Smith FA. 2002. Function and diversity of arbuscular mycorrhizae in carbon and mineral nutrition. In: van der Heijden MGA, Sanders IR, eds. *Mycorrhizal ecology*. Berlin, Germany: Springer, 157: 75–92.

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

Johnson D, Krsek M, Wellington EMH, Stott AW, Cole L, Bardgett RD, Read DJ, Leake JR. 2005. Soil invertebrates disrupt carbon flow through fungal networks. *Science* **309**: 1047.

Jones Jr, Benton J, Wolf B, Mills HA. 1991. *Plant analysis handbook – a practical sampling, preparation, analysis, and interpretation guide*. Athens, USA: Micro-Macro Publishing, Inc.

Jones MD, Durall DM, Tinker PB. 1998. Comparison of arbuscular and ectomycorrhizal *Eucalyptus coccifera*: growth response, phosphorus uptake efficiency and external hyphal production. *New Phytologist* **140**: 125–134.

Klironomos JN, McCune J, Moutoglis P. 2004. Species of arbuscular mycorrhizal fungi affect mycorrhizal responses to simulated herbivory. *Applied Soil Ecology* **26**: 133–141.

Koch AM, Kuhn G, Fontanillas P, Fumagalli L, Goudet J, Sanders I. 2004. High genetic variability and low local diversity in a population of arbuscular mycorrhizal fungi. *Proceedings of the National Academy of Sciences, USA* **101**: 2369–2374.

Lambert DH, Cole H Jr, Baker DE. 1980. Variation in the response of alfalfa clones and cultivars to mycorrhizae and phosphorus. *Crop Science* **20**: 615–618.

Logi C, Sbrana C, Giovannetti M. 1998. Cellular events involved in survival of individual arbuscular mycorrhizal symbionts growing in the absence of the host. *Applied and Environmental Microbiology* **64**: 3473–3479.

Mäder P, Vierheilig H, Streitwolf-Engel R, Boller T, Frey B, Christie P, Wiemken A. 2000. Transport of ^{15}N from a soil compartment separated by a polytetra uoroethylene membrane to plant roots via the hyphae of arbuscular mycorrhizal fungi. *New Phytologist* **146**: 155–161.

Maldonado-Mendoza IE, Dewbre GR, Harrison MJ. 2001. A phosphate transporter gene from the extraradical mycelium of an arbuscular mycorrhizal fungus *Glomus intraradices* is regulated in response to phosphate in the environment. *Molecular Plant–Microbe Interaction* **14**: 1140–1148.

Munkvold L, Kjøller R, Vestberg M, Rosendahl S, Jakobsen I. 2004. High functional diversity within species of arbuscular mycorrhizal fungi. *New Phytologist* **164**: 357–364.

O'Bannon JH, Evans DW, Peaden RN. 1980. Alfafa varietal response to seven isolates of vesicular arbuscular mycorrhizal fungi. *Canadian Journal of Plant Science* **60**: 859–863.

Phillips JM, Hayman DS. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assesment of infection. *Transactions of the British Mycological Society* **55**: 158–160.

Poulsen KH, Nagy R, Gao LL, Smith SE, Bucher M, Smith FA, Jakobsen I. 2005. Physiological and molecular evidence for Pi uptake via the symbiotic pathway in a reduced mycorrhizal colonization mutant in tomato associated with a compatible fungus. *New Phytologist* **168**: 445–454.

Sanders FE, Tinker PB, Black RLB, Palmerley SM. 1977. The development of endomycorrhizal root systems. I. Spread of infection and growth-promoting effects with four species of vesicular-arbuscular endophyte. *New Phytologist* **78**: 257–268.

Schweiger PF, Jakobsen I. 2000. Laboratory and field methods for measurement of hyphal uptake of nutrients in soil. *Plant Soil* **226**: 237–244.

Smith SE, Gianinazzi-Pearson V. 1990. Phosphate uptake and arbuscular activity in mycorrhizal *Allium cepa* L. effects of photon irradiance and phosphate nutrition. *Australian Journal of Plant Physiology* **17**: 177–188.

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Smith FA, Jakobsen I, Smith SE. 2000. Spatial differences in acquisition of soil phosphate between two arbuscular mycorrhizal fungi in symbiosis with *Medicago truncatula*. *New Phytologist* **147**: 357–366.

Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*, 2nd edn. San Diego, USA: Academic Press.

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.

Staddon PL, Bronk Ramsey C, Ostle N, Ineson P, Fitter AH. 2003. Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of ^{14}C. *Science* **300**: 1138.

Tisdall JM, Oades JM. 1979. Stabilization of soil aggregates by the root system of ryegrass. *Australian Journal of Soil Research* **17**: 429–441.

Vazquez MM, Barea JM, Azcon R. 2001. Impact of soil nitrogen concentration on *Glomus* spp–*Sinorhizobium* interactions as affecting growth, nitrate reductase activity and protein content of *Medicago sativa*. *Biology and Fertility of Soils* **34**: 57–63.

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