New Phytologist

The network structure of plant–arbuscular mycorrhizal fungi

A. Montesinos-Navarro[1]*, J. G. Segarra-Moragues[1]*, A. Valiente-Banuet[2,3] and M. Verdú[1]

[1]Centro de Investigaciones sobre Desertificación (CIDE, CSIC-UV-GV), Carretera de Moncada-Náquera Km 4.5 46113 Moncada, Valencia, Spain; [2]Departamento de Ecología de la Biodiversidad, Instituto de Ecología, Universidad Nacional Autónoma de México, A. P. 70-275, C. P. 04510, México, D. F., México; [3]Centro de Ciencias de la Complejidad, Ciudad Universitaria, Universidad Nacional Autónoma de México, México 04510, D. F., México

Author for correspondence:
Alicia Montesinos-Navarro
Tel: +34 96 3424204
Email: ali.montesinos@gmail.com

Received: *1 October 2011*
Accepted: *7 December 2011*

New Phytologist (2012) **194**: 536–547
doi: 10.1111/j.1469-8137.2011.04045.x

Key words: community assemblages, modularity, mutualism, nestedness, network, plant–arbuscular mycorrhizal fungi (AMF) association.

Summary

- Ecological network theory predicts that in mutualistic systems specialists tend to interact with a subset of species with which generalists interact (i.e. nestedness). Approaching plant–arbuscular mycorrhizal fungi (AMF) association using network analyses will allow the generality of this pattern to be expanded to the ubiquitous plant–AMF mutualism.
- Based on certain plant–AMF specificity recently suggested, networks are expected to be nested as a result of their mutualistic nature, and modular, with certain species interacting more tightly than others. Network analyses were used to test for nestedness and modularity and to compare the different contribution of plant and AMF to the overall nestedness.
- Plant–AMF networks share general network properties with other mutualisms. Plant species with few AMFs in their roots tend to associate with those AMFs recorded in most plant species. AMFs present in a few plant species occur in plant species sheltering most AMF (i.e. nestedness). This plant–AMF network presents weakly interlinked subsets of species, strongly connected internally (i.e. modularity). Both plants and AMF show a nested structure, although AMFs have lower nestedness than plants.
- The plant–AMF interaction pattern is interpreted in the context of how plant–AMF associations can be underlying mechanisms shaping plant community assemblages.

Introduction

The association between arbuscular mycorrhizal fungi (AMFs) and plants improves the fitness of both plant and AMF symbionts, constituting a traditionally considered mutualism (Blackwell, 2000). AMF increase plant uptake of soil nutrients, especially phosphorus (P) (Smith & Read, 1997), while the plants provide carbon compounds to the AMF, although, in some cases, the equitability of resource exchange between plants and AMF might not be mutually beneficial (Johnson *et al.*, 1997). A more efficient nutrient uptake as a result of AMF associations can alleviate plant competition for mineral resources (Fitter, 1977; Allen & Allen, 1984; Hetrick *et al.*, 1989; Moora & Zobel, 1996; Bever *et al.*, 1997). An equitable distribution of soil resources among competitively dominant and subdominant host species might promote plant species coexistence (Walter *et al.*, 1996; Malcová *et al.*, 1999). Plant species associations with specific AMF taxa can ultimately influence AMF community composition (Grime *et al.*, 1987; Van der Heijden *et al.*, 1998a,b; Hartnett & Wilson, 1999) and bottom-up influence of AMFs on plant community diversity has also been reported (Grime *et al.*, 1987; Van der Heijden *et al.*, 1998a,b; Hartnett & Wilson, 1999), potentially mediated by plant-to-plant facilitation (Van der Heijden & Horton, 2009). Facilitation is a key process structuring plant communities in semiarid regions, where P soil availability can be limiting (Cross & Schlesinger, 2001; Li *et al.*, 2004). In this P-limiting environment, 97% of the plant species require other plant species to recruit successfully, and 57% of these positive interactions are maintained when the plants reach the adult stage (Verdú & Valiente-Banuet, 2008). Elucidating the plant–AMF interaction pattern is a first step to exploring the potential mechanism underlying plant-to-plant facilitation and its implications in structuring plant community assemblages.

Regardless of the importance of mycorrhizal associations, which form associations with most of land plants (Wang & Qiu, 2006; Smith & Read, 2008; Brundrett, 2009), the pattern of plant–AMF interactions still remains largely unknown in natural communities (Bever, 2003; Van der Heijden & Horton, 2009). In order to evaluate the plant–AMF interaction pattern in a community, it is necessary to sample a representative number of plant species growing in the same area and exposed to the same AMF taxon pool (Davison *et al.*, 2011). The availability of studies presenting representative sampling of plants and AMF communities in a given natural site is scarce. Several studies have characterized the diversity of fungal communities in natural environments by focusing on a few – usually the most common – plant species

*These authors contributed equally to this work.

rhizospheres in the community (Daniell *et al.*, 2001; Zhaoyong *et al.*, 2006; Kottke *et al.*, 2008; Alguacil *et al.*, 2009; Sonjak *et al.*, 2009; Wilde *et al.*, 2009; Öpik *et al.*, 2010 for further references) or by exploring the influence of the plant community on final AMF composition (Mummey *et al.*, 2005; Hausmann & Hawkes, 2009) using artificial (i.e. experimental) communities (Van der Heijden *et al.*, 1998b; Maherali & Klironomos, 2007). However, very few studies aim at sampling most of the plant and AMF communities in natural environments in order to elucidate the pattern of plant–AMF interactions (but see Öpik *et al.*, 2009; Davison *et al.*, 2011). Thus, it is still largely unclear to what extent plant and AMF communities interact in a random way or, alternatively, if biological processes can lead to emerging nonrandom plant–AMF interaction patterns.

Arbuscular mycorrhizal fungi belong to the phylum Glomeromycota, one of the key taxa interconnecting plants into a functional web (Helgason *et al.*, 1998). Despite the fact that Glomales form symbiotic associations with the majority of land plants (65–85%; Wang & Qiu, 2006; Smith & Read, 2008; Brundrett, 2009), < 200 species of these globally important fungi have been described (Morton & Benny, 1990). The apparent low diversity of AMFs compared with their associated plant hosts has led to the historical presumption that plant–AMF associations must have a low specificity (Smith & Read, 1997). Nonetheless, it is becoming increasingly clear that distinct AMF communities are present in the rhizosphere (Bever *et al.*, 1996; Eom *et al.*, 2000) and that there is a certain specificity in the interaction with plant species (Helgason *et al.*, 2002; Vandenkoornhuyse *et al.*, 2002, 2003; Scheublin *et al.*, 2004; Pivato *et al.*, 2007; Santos-González *et al.*, 2007; Mummey & Rillig, 2008; Öpik *et al.*, 2008; Smith & Read, 2008; Li *et al.*, 2010; Davison *et al.*, 2011). This shift has been influenced by the greater AMF diversity revealed by the use of molecular techniques, with higher resolution for distinguishing closely related species. However, the different amount of intraspecific genetic variation depending on the family, genus and species prevents the determination of a generalized genetic threshold to delimitate AMF species (Redecker *et al.*, 2003; Rosendahl, 2007; Nilsson *et al.*, 2008). The increasing knowledge about AMF diversity and availability of molecular tools to measure it, offers a unique opportunity to explore plant–AMF interaction patterns in natural communities.

Network analysis is a convenient technique to detect nonrandom species interaction patterns. This analysis has been used to study different types of mutualisms: plant-pollinators and seed dispersers (Bascompte *et al.*, 2003), marine cleaning mutualisms (Guimarães *et al.*, 2007) or plant-to-plant facilitation (Verdú & Valiente-Banuet, 2008). However, network analyses have been rarely applied to fungal communities (but see Peay *et al.*, 2007 and Vacher *et al.*, 2008) and, as far as we know, have not been previously applied to study plant–AMF interactions at the community level. The wide application of network analyses has led to the development of an ecological network theory based on emerging patterns shared by multiple mutualistic systems. Interestingly, networks representing mutualistic processes have been shown to share a well-defined network structure regardless of the nature of the species involved (Bascompte & Jordano, 2007 but see Joppa *et al.*, 2010 for a methodological critique). Ecological network theory predicts that mutualistic networks are characterized by having a few species much more connected than is expected by chance, in which specialists tend to interact with a subset of the species with which generalists interact (i.e. nestedness) (Bascompte & Jordano, 2007). This particular structure has implications for the robustness of the network and coexistence and stability of species (Bascompte & Jordano, 2007). In addition, if plant–AMF interactions are not as generalist as traditionally thought, it can be expected that any pair of species do not necessarily have the same probability of interacting. Accordingly, a group of plant species will tend to interact predominantly with a given group of AMFs, and vice versa. Network modularity reflects the tendency of a set of species to interact predominantly with species within the set and less frequently with species in other sets. Modularity implies that species can be grouped (i.e. modules) in such a way that weakly interlinked subsets of species are strongly connected internally (Olesen *et al.*, 2007). Approaching the study of plant–AMF interaction from a network perspective will provide the opportunity to test two hypotheses: first, that the plant–AMF interaction pattern matches the predictions developed by network theory based on other mutualistic systems; and second that the nonrandom plant–AMF interactions occur at the community level when most of the plant community is considered.

In this study we characterize the interaction patterns in a plant–AMF mutualistic system. For the sake of generality, we reanalyse, using network analyses, the data from the two available studies which sampled most of the plant community and recorded higher AMF phylogenetic diversity than our study. We define a gradient of AMF genetic differentiation threshold values (hereafter cutoff) and, for each one, first describe the network testing for nestedness and modularity, and then compare the different contributions of plants and AMFs to the overall nestedness. Finally we estimate the relative contribution of plant species abundance to the observed interaction pattern. We show how this mutualism fits into mutualistic network theory and previous knowledge about plant–AMF interactions, discussing the potential implications for plant community structure mediated by plant-to-plant facilitation.

Materials and Methods

Study area and plant sampling

This study was conducted in the semiarid Valley of Zapotitlán (18°20′N, 97°28′W), a local basin of the biosphere reserve of Tehuacán-Cuicatlán Valley in the state of Puebla, Mexico. This region owes its aridity to the rain shadow produced by the Eastern Sierra Madre (Valiente-Banuet *et al.*, 2000). It has an annual average rainfall of 380 mm, most of which falls during the summer months, and an annual mean temperature of 21°C with rare frosts (García, 1973). Specifically, the study site is located *c.* 30 km south of Tehuacán city in a natural area in which vegetation is a xeric shrubland (woody perennial species) dominated by the columnar cactus *Neobuxbaumia tetetzo*, *Agave* spp., different Fabaceae and Asteraceae species, among other taxa. The

vegetation is characterized by individuals of multiple species spatially associated forming discrete vegetation clumps, although some isolated individuals can also be found. Vegetation clump areas range from 1 to 5 m^2.

Phosphorus concentration in soils at each vegetation clump is very low, ranging from 2 to 19 mg kg^{-1}, mean ± SE = 5.37 ± 0.44 (L. Sortibrán, unpublished).

Arbuscular mycorrhizal fungi

The phylum Glomeromycota (i.e. AMF) is divided in four orders, with most described species belonging to the Glomerales and Diversisporales (Schüßler *et al.*, 2001b). *Glomus* is the largest genus in the phylum, with > 70 morphospecies (Redecker & Raab, 2006), with *Glomus* group A accounting for much of this diversity. *Glomus* dominates AMF communities in many field settings, where 70% of the AMF have been identified as *Glomus* (range 60–85%) (Helgason *et al.*, 1998; Vandenkoornhuyse *et al.*, 2002; Zhaoyong *et al.*, 2006; Alguacil *et al.*, 2009; Öpik *et al.*, 2009, 2010; Sonjak *et al.*, 2009; Wilde *et al.*, 2009), and shows the highest root colonization rates among the Glomeromycota taxa (Hart & Reader, 2002). There is some controversy on as to whether AMFs have dispersal limitation (Lekberg *et al.*, 2007; Dumbrell *et al.*, 2010) or whether they can disperse at the scale of km, recording different dispersal vectors such as animals (Janos & Sahley, 1995; Mangan & Adler, 2000; Lekberg *et al.*, 2011), wind (Warner *et al.*, 1987) and land movements associated with agriculture (Rosendahl *et al.*, 2009). *Glomus* species are the most common taxa recorded in these dispersal studies.

Root sampling

We performed a plant sampling scheme aimed at including most of the plant species in the community and reflecting the relative abundance of each species sampled. A total of 37 vegetation clumps, with one to eight plant species (average 2.7), were sampled along two transects of 500 m^2 each. A total of 130 individuals of 37 plant species, representing 66% of all the species in the community, were sampled (see species in Table 1). Rarely, a vegetation clump had more than one individual of the same species; in those cases only one of the individuals was sampled. We have considered relative abundance as an intrinsic characteristic of each plant species in a given community that can influence its interaction pattern with other species. Accordingly plant–AMF interaction matrices should be built from surveys that reflect the relative abundance of each species. The root tips were unearthed, cut and dried with silica gel for further DNA extraction.

DNA extraction

The youngest tips of the nonlignified roots were selected from plant samples, as they often show a higher proportion of Glomeromycota colonization. Root tips were cut and placed in 2 ml Eppendorf tubes with 2.3 mm stainless steel beads. Then root tissues were pulverized in a Retch MM400 (Biometa, Madrid, Spain) tissue lyser.

Table 1 Number of individuals sampled for each plant species

Plant species	Positive	Negative	Total
Neobuxbaumia tetetzo	18	0	18
Mimosa luisana	15	1	16
Mammillaria colina	8	4	12
Coryphantha pallida	7	0	7
Ruellia hirsuto-glandulosa	6	0	6
Siphonoglossa ramosa	4	1	5
Agave macroacantha	3	1	4
Caesalpinia melanadenia	3	4	7
Calliandra eryophylla	3	0	3
Acacia constricta	2	0	2
Cardiospermum halicacabum	2	0	2
Dalea sp.	2	2	4
Justicia mexicana	2	0	2
Mammillaria carnea	2	1	3
Mammillaria casoi	2	0	2
Mascagnia seleriana	2	0	2
Sanvitalia fruticosa	2	0	2
Viguiera dentata	2	0	2
Allionia incarnata	1	2	3
Agave karwinskii	1	0	1
Bouteloua gracilis	1	0	1
Bursera aloexylon	1	3	4
Cathestecum brevifolium	1	0	1
Ditaxis guatemalensis	1	0	1
Echynopterix eglandulosa	1	0	1
Eysenhardtia polystachya	2	1	3
Ferocactus latispinus	1	0	1
Hemiphylacus latifolius	1	1	2
Ipomoea sp.	1	0	1
Lantana achyranthifolia	1	0	1
Lantana camara	1	1	2
Loeselia caerulea	1	0	1
Senna wislizenii	1	2	3
Solanum trydinamum	1	0	1
Thompsonella minutiflora	1	1	2
Mammillaria haageana	0	1	1
Jatropha neopauciflora	0	1	1
Total	103	27	130

Plant species are ranked by their abundance in positive amplification and sequencing of *Glomus* group A.

Total DNA was extracted using the DNeasy Plant Minikit (Qiagen, Las Matas, Madrid, Spain) with the addition of 0.33% final concentration of PVP40 to buffer AP1, which facilitated the elimination of some PCR inhibitor compounds, and we then subsequently followed the manufacturer's instructions. As these extracts contained a mixture of DNA from fungi and the host plant, DNA quantification was routinely omitted and crude extracts were used for subsequent PCRs.

Glomeromycota internal transcribed spacer (ITS) amplification and sequencing

A nested PCR protocol was used for the amplification of the samples. Primary PCR amplified the whole ITS region, including ITS-1, 5.8S and ITS-2. This was conducted in 25 µl volume including 1 × *Taq* Buffer (Biotools, Madrid, Spain), 3 mM $MgCl_2$, 0.5 mM each of dNTP, 0.4 mg ml^{-1} BSA, 12.5 pmol

each of NS5 (forward) and ITS4 (reverse) primers of White *et al.* (1990), 1 U of *Taq* DNA polymerase and 1 μl of crude DNA extract. The PCR program consisted of an initial DNA melting step of 3 min at 95°C followed by 30 cycles each of 30 s at 95°C, 30 s at 51°C for annealing and 2 min at 72°C for extension. After a final extension step of 10 min at 72°C, PCRs were kept at 4°C. One microlitre of this PCR was used as template for the nested PCR. Four primer-pair combinations were assayed for the nested PCR in an attempt to detect as much diversity as possible among Glomeromycota. The PCR cocktail was identical to that of the primary PCR except for the primer-pair used, which included Forward/Reverse, Glom1310/ITS4i (Redecker, 2000; Redecker *et al.*, 2003), for the amplification of *Glomus* group A (Schüβler *et al.*, 2001a); LETC1670/ITS4i (Redecker, 2000) for the amplification of *Glomus* group B (Schüβler *et al.*, 2001a); NS5/GIGA5.8R (Redecker, 2000) for the amplification of Gigasporaceae; and ACAU1660/ITS4i (Redecker, 2000) for the amplification of Acaulosporaceae. The PCR program consisted of an initial DNA melting step of 3 min at 95°C followed by 30 cycles each of 45 s at 95°C, 50 s at 56°C for annealing and 1.5 min at 72°C for extension. After a final extension step of 10 min at 72°C, PCRs were kept at 4°C. PCR products were checked on 1% agarose gels. PCR protocols were optimized for two of these groups of AMFs with available axenic cultures of *Glomus* group A and *Gigaspora* sp. Of these four primer-pair combinations, no amplification was obtained for the families Gigasporaceae and Acaulosporaceae. Less than 30% amplification success was obtained for *Glomus* group B primer-pair, whereas for the primer-pair of *Glomus* group A, 78.21% success was achieved, suggesting a predominance of this group of *Glomus* in the AMF communities in the study area. Subsequent sequencing of PCR products was continued only with this monophyletic group of *Glomus*.

Positive amplifications of the expected size were cloned into pGEM-T easy vector (Promega, Madrid, Spain) and transformed onto X-Gal, IPTG ampicillin, LB agar plates. Positive colonies were screened with T7 and SP6 vector primers for inserts of the appropriate size, then cultured for miniprep plasmid extraction (Roche, Penzberg, Germany) and sequenced with the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequencing was performed by Macrogen Inc. (Seoul, Korea). Forward and reverse sequences were compared, assembled and corrected where necessary using SEQUENCHER® (GeneCodes Corp., Ann Arbor, MI, USA), thus establishing the consensus sequence of each sample. BLAST searchers were performed to reliably assign sequences to AMF. BLAST searches were performed on forward, reverse and consensus individual sequences in order to detect possible chimeras (Schechter & Bruns, 2008). Only those that matched a Glomeromycota sequence in both forward and reverse sequences and rendered high bit scores (> 1300) and low *E* values in the consensus sequences were selected for the analysis. This procedure is especially suitable for pairwise comparisons of sequence from closely related species. In these cases evolutionary processes involving natural recombination and incomplete lineage sorting could be identified as false chimera positives in specific software for the detection of chimeras (Schechter & Bruns, 2008).

DNA sequence alignment and analysis

Sequences were aligned with ClustalW (Thompson *et al.*, 1994) implemented in MEGA4 (Tamura *et al.*, 2007). Sequence alignments were corrected by visual inspection with BioEdit v. 7.0.9 (Hall, available at http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

Pairwise distance matrices were computed using the default values in Dist.seqs implemented in Mothur (Schloss *et al.*, 2009). These served as input for Bin.seqs in order to cluster the sequences into operational taxonomic units (OTUs) of a defined sequence identity. The OTUs were defined according to their sequence dissimilarity at different cutoff values, which spanned 1–10% of their sequence being different. This approach seems reasonable since species concepts are difficult to apply in AMFs (Redecker *et al.*, 2003). Thus, using sequence bins rather than taxonomic assignments based on BLAST analyses is more meaningful for environmental samples without prior information on AMF diversity information, because not all the sequences may match an identified sequence in the database and the use of sequence similarities prevents uncertainties associated with fungal taxonomy and classification.

We used rarefaction curves to illustrate how the number of OTUs increases with the number of sequences sampled. Rarefaction curves were performed for each cutoff. The value of OTUs levels off and reaches an asymptote when more sequences sampled do not reveal more OTUs, indicating sufficient sampling. Confidence intervals for OTUs were calculated based on 1000 randomizations.

Network analyses

For each cutoff, plant–AMF interactions were characterized as bipartite networks consisting of two sets of nodes, plant species, in rows, and AMF OTUs, in columns. Pairs of each type of nodes were considered linked (i.e. interaction) if an AMF OTU was present in a given plant species roots. This qualitative 0/1 matrix was used to calculate network parameters to describe connectance, nestedness and modularity. Connectance is considered as the realized proportion of possible links (Yodzis, 1980), in this case, the proportion of pairs of plant–AMF OTU that directly interact. It was calculated using the bipartite package for R (Blüthgen *et al.*, 2006).

The nestedness concept describes a particular pattern of interaction in which specialists interact with species (or OTUs) that form perfect subsets of the species (or OTUs) with which generalists interact (Bascompte & Jordano, 2007). Nestedness parameters measure how the presence/absence pattern of interactions departs from the perfect nestedness. We used two of the most common metrics to estimate nestedness: temperature index (Atmar & Patterson, 1993) and nested overlap and decreasing fill (NODF) (Almeida-Neto *et al.*, 2008). The significance of nestedness was assessed by comparing the observed nestedness with the frequency distribution of that metric calculated using 1000 replicates of null model II (Bascompte *et al.*, 2003). Null model II uses equal dimension matrices in which each cell of the interaction matrix has a given probability of

being occupied. This probability is the arithmetic mean of the connection probability of the focal plant species and AMF OTU. Accordingly, deviations from this null model result solely from an asymmetric distribution of interactions between species (Vacher *et al.*, 2008).

Nested overlap and decreasing fill values are matrix dimension-dependent and accordingly they are unsuitable to compare across studies. In order to allow cross-network comparisons with other mutualistic systems, the relative nestedness was calculated. This measure corrects for variation in species and OTU richness and also in the number of links. Relative NODF is defined as $(NODF_{observed} - NODF_{null\ model})NODF_{null\ model}$, where $NODF_{observed}$ is the nestedness of the actual matrix, and $NODF_{null\ model}$ is the average nestedness of random replicates generated from the null model.

Nested overlap and decreasing fill was also calculated independently for rows (plant species) and columns (AMF OTUs) and the statistical significance assessed by comparing against the null model II. Nestedness metrics are influenced by order of rows and columns in the matrix. In order to make our results comparable with previous studies, rows and columns were ordered by interaction abundance before the calculation of nestedness metrics. Nestedness metrics were calculated with the help of the software ANINHADO (Guimarães & Guimarães, 2006).

Modularity reflects the fact that there are groups of species that tend to interact more within species in the same group than is expected by chance. Nodes of a network can be grouped into modules, in such a manner that the number of links within the modules is maximized and the number between modules is minimized. A simulated annealing optimization approach was used to detect modules that maximized modularity (i.e. proportion of links within vs between modules) (Guimerà & Amaral, 2005a,b). Because of its heuristic nature, 10 runs of the algorithm were conducted for each cutoff, but the variation in modularity was negligible (SE of the modularity across the 10 runs ranged from 0.0190 to 0.0192 across the different cutoffs). We report the maximum value of modularity obtained in the 10 runs. Although our network is bipartite, we used a modularity algorithm for unipartite networks. Because our plant–AMF network is a two-party network, one could conclude that an algorithm for a two-party network (i.e. bipartite) would be more appropriate. However, this decision depends on the question addressed. For example, algorithms for modularity in a bipartite network search for independent groups of plants (or AMFs) that share a similar interaction pattern (i.e. that interact with the same AMFs (or plants)); whereas algorithms searching for modularity in a unipartite network identify mixed groups of plants and AMFs tightly interrelated (see Olesen *et al.*, 2007 for a more detailed explanation). As we are interested in the groups of plants and AMFs that are highly connected to each other, rather than in groups of plants and/or AMFs created as a function of their shared interactions, we used algorithms searching for modularity in a unipartite network (see Fortuna *et al.*, 2010, for the appropriateness of this method). However, we also tested for modularity using a bipartite network and the results consistently showed that our networks were composed of modules usually grouping the same species grouped by the modules in the unipartite approach (see Olesen *et al.*, 2007 for a similar comparison). Only modularity for a unipartite network is reported. Modularity significance was tested by comparing it to the null case of modularity calculated using 100 random graphs with the species ranked according to their degree distribution in the original network (Guimerà *et al.*, 2004). Modularity was calculated and its significance tested using the software Netcarto (Guimerà *et al.*, 2004; Guimerà & Amaral, 2005a,b).

In order to provide a generalization to our results, we compared them with other studies in which plant–AMF interactions had been intensively surveyed at the community level. After inspecting the 138 studies cited in the Maarj*AM* database (Öpik *et al.*, 2010) and relevant references within, we found only two studies which aim to survey most of the plant species in the community: Davison *et al.* (2011) and Öpik *et al.* (2009). These studies are not independent as they share data from the same site. In these studies 10–11 plant species were used and 40–51 AMF OTUs were recorded, belonging to Glomeraceae, Gigasporaceae, Acaulosporaceae and Diversisporaceae. We reanalysed their data calculating the same nestedness and modularity estimates as described for this study.

Although the qualitative analyses described only consider the presence or absence of an interaction, a species number of interactions can be highly influenced by its abundance, which might have an effect on nestedness (Vázquez, 2005) and modularity. In the next section we use biological information contained in our data to estimate the relative contribution of plant species abundance on plant–AMF interaction pattern.

Relative contribution of plant abundance to plant species number of AMF interactions

A plant species with high relative abundance in the community will have a higher probability of interacting with a higher number of AMFs, because more individuals will be sampled of this plant species. However, a plant species' tendency to interact with a given number of AMF OTUs can also result from other processes independent of its relative abundance. Other biological processes, such as habitat heterogeneity, demographic dynamics, plant–AMF overlapped phenology, AMF competition within the root or specific selectivity in plant–AMF associations, can also produce a nonrandom pattern of plant–AMF interactions independently of the species' relative abundance. While abundance-dependent interaction patterns occur at the species level as an effect of adding up multiple individuals, interaction patterns resulting from abundance-independent processes should be observed at the individual level.

Accordingly, we have calculated plant–AMF interactions at the individual plant level (AMF load) in order to characterize these biological processes independent of species abundance. We define AMF load as the plant species' mean number of AMF OTUs per individual. Several tangled processes can be underlying a given species AMF load, and further experiments can be designed to elucidate these processes. Although we cannot tease apart the specific mechanisms resulting in a given AMF load, this index is

independent of plant species' relative abundance considering that AMF load is calculated as an average of the individuals' trait within a plant species.

We tested if there is a statistically significant relationship between relative plant abundance and AMF load on the number of plant species links in the network, using a generalized linear model with a Poisson distribution of errors. Plant species degree in the network (number of links per plant species) was used as the dependent variable and the number of individuals sampled and AMF load per plant species were used as independent variables. The relative contribution of plant relative abundance and AMF load to explain the variance in the number of plant species links in the network was estimated as the ratio of the standard deviations of the two effects, as implemented in the *relimp* package for R (Silber *et al.*, 1995).

Results

AMF OTU definition and rarefaction curves

Positive amplification for *Glomus* group A was detected in 103 out of the 130 plants sampled (79.23%) (Table 1). Positive amplification was obtained from at least one of the individuals sampled for each species, except for *Mammillaria haageana* and *Jatropha neopauciflora* (Table 1).

A total of 95 out of the 1909 sequenced clones (4.98%) produced unreadable sequences, 251 (13.15%) corresponded to other coamplified fungi, 40 (2.10%) to chimeric sequences, and 1523 (79.77%) to Glomeromycota with BLAST scores above 1300. Further analyses were based on this subset of 1523 sequences (Genbank numbers JN194215 to JN195737).

The number of different AMF OTUs varied depending on the predefined cutoff values of genetic dissimilarity (Supporting Information, Fig. S1). Rarefaction curves showed that for the cutoff which grouped together sequences with a genetic difference smaller than 1% (Fig. S1a), 163 AMF OTUs were identified out of 1523 sequences. Rarefaction curves did not reach the stabilization until the cutoff value of 5% (Fig. S1e) and more strictly at 8% (Fig. S1h). For the 5% and 8% cutoffs, 34 and 23 different OTUs were identified, respectively. The cutoff which grouped together sequences with genetic difference smaller than 10% identified 14 AMF OTUs out of 1523 sequences (Fig. S1j).

Plant–AMF networks

The number of plant species in the network was 35 and the number of AMF OTUs ranged from 163 to 14 depending on the cutoff value considered (Table 2). Ten networks (i.e. one network per cutoff) were built grouping within an OTU all the sequences in 10 increments of 1% in dissimilarity intervals (i.e. 1–10%). Approximately 11% of the possible interactions between plant species and AMF OTUs were actually realized (average connectance across different cutoffs (mean ± SE: 11.5 ± 0.01) (Fig. 1; see Figs S2–S10 for all the other cutoffs).

All 10 networks were significantly more nested than expected by chance, both considering the overall network and considering rows and columns independently (for all cases $P < 0.001$, Table 2). The degree of nestedness was independent of the cutoff considered (Pearson $R^2 = 0.57$, $n = 10$, $P > 0.05$). NODF of the overall network ranged from 14.36 to 54.83, corresponding, in all cases, to a temperature > 92 (Table 2).

When plant species and AMF OTUs are analysed independently (i.e. rows and columns), both showed high NODF values (Table 2) indicating that there is a tendency of specialists to interact with generalists. However, plant species had a relative nestedness more than twofold higher than the AMF OTU nestedness (Table 2), indicating that this pattern is stronger for plants than for AMFs.

All networks except for the cutoff of 10% show a significant modularity (Table 2). For the 10% cutoff, the number of species in the network was 49 (35 plant species and 14 AMF OTUs). According to Olesen *et al.* (2007), when networks are based on < 50 species, it is probably the detection of just one single module. Thus, for all the cutoffs in which modularity was detectable, we found significant values of modularity. The number of modules varies from five to nine across the different cutoffs and the average number of nodes within a module range from nine to 22 (Table 2). Plant and AMF species ascribed to different modules coexist in the same vegetation clump, with an average of 2.6 (range one to five) modules per vegetation clump.

In this study we only found AMFs belonging to Glomeraceae, but the observed pattern of plant–AMF interaction might vary in other systems with higher AMF phylogenetic diversity. In order to explore the variability of the observed network pattern across other AMF phylogenetic diversity scenarios, we compared our results with two other studies in which other less abundant families of AMFs have been recorded (i.e. Gigasporaceae, Acaulosporaceae, Diversisporaceae). Both networks presented in Öpik *et al.* (2009) and Davison *et al.* (2011) show significant nestedness in the overall network, and also for plants and AMFs independently (relative $NODF_t = 0.25–0.28$; $NODF_p = 0.50–0.54$; $NODF_{AMF} = 0.24–0.26$; all $P < 0.05$; $_t$, total; $_p$, plants, $_{AMF}$, arbuscular mycorrhizal fungi) (Fig. S11). In addition, in these two networks, the nestedness for plants was also stronger than nestedness for AMFs. Accordingly, regarding nestedness, our results, which only detected one group of the most abundant AMFs, were in agreement with their results, which recorded a broader AMF phylogenetic diversity. However, the connectance in those two networks was higher than in our study (42–41%) and no significant modularity was observed.

Relative contribution of plant abundance to plant species number of AMF interactions

The mean number of plant individuals per species ranged from one to 18 (mean = 2.9; SD = 3.8). Plant species have, on average, an AMF load of 3.6 AMF OTUs per individual (SD = 1.5; range = 1–7) considering the cutoff of 1%, and an average of 1.3 (SD = 0.5; range = 1–3) considering the cutoff of 10%, with the estimates for the rest of the cutoffs contained within the values

Table 2 Descriptive statistics of the plant–mycorrhizal fungi network

				Nestedness								Modularity		
								Relative						
Cutoff %	*N*	*I*	*C*	*T*	$NODF_t$	$NODF_p$	$NODF_{AMF}$	T	$NODF_t$	$NODF_p$	$NODF_{AMF}$	Modules	Nodes	Modularity
1	163	313	5.5	95	14.4	20.6	14.1	15	0.6	1.4	0.6	9	22 (3–35)	0.57
2	85	232	7.8	94	22.4	31.0	20.9	18	0.8	1.3	0.7	7	17 (7–22)	0.48
3	61	194	9.1	95	28.3	44.1	23.2	20	0.9	1.6	0.6	8	12 (6–18)	0.44
4	45	168	10.1	94	35.0	49.9	26.0	21	0.9	1.5	0.6	6	13 (5–21)	0.42
5	34	146	12.3	92	42.0	55.2	27.9	21	1.0	1.3	0.5	8	9 (4–15)	0.39
6	30	132	12.6	92	46.8	60.4	28.3	21	1.0	1.3	0.5	7	9 (4–14)	0.38
7	25	114	13.0	93	47.9	58.6	26.7	22	0.9	1.1	0.4	6	10 (4–20)	0.40
8	23	111	13.8	93	49.6	58.4	28.9	22	0.9	1.0	0.4	6	10 (7–20)	0.30
9	19	105	15.8	93	52.0	57.4	33.2	26	0.8	0.9	0.4	6	9 (6–19)	0.37
10	14	70	14.3	96	54.8	58.5	30.8	27	1.1	1.1	0.5	5	10 (4–21)	0.42^{ns}

Cutoff %, percentage of dissimilarity used as cutoff; *N*, number of operational taxonomic units (OTUs) of *Glomus*; *I*, number of interactions; *C*, connectance: $(100 \times I)/(N \times 35)$; *T*, nestedness calculated as matrix temperature; $NODF_t$, nested overlap and decreasing fill for the overall matrix; $NODF_p$, NODF for plants; $NODF_{AMF}$, NODF for *Arbuscular mycorrhizal fungi*; Relative *T*, $(T - T_{null\ model})/T_{null\ model}$ (see the Materials and Methods section for details about the null model); $NODF_{t,p,AMF}$, $(NODF_{t,pAMF} - NODF_{null\ model})/NODF_{null\ model}$.
Modules, number of modules; nodes, mean number of nodes per module (range) and modularity values.
For all nestedness and modularity parameters, $P < 0.05$ except where 'ns' is shown. Number of plant species in every cutoff is 35.

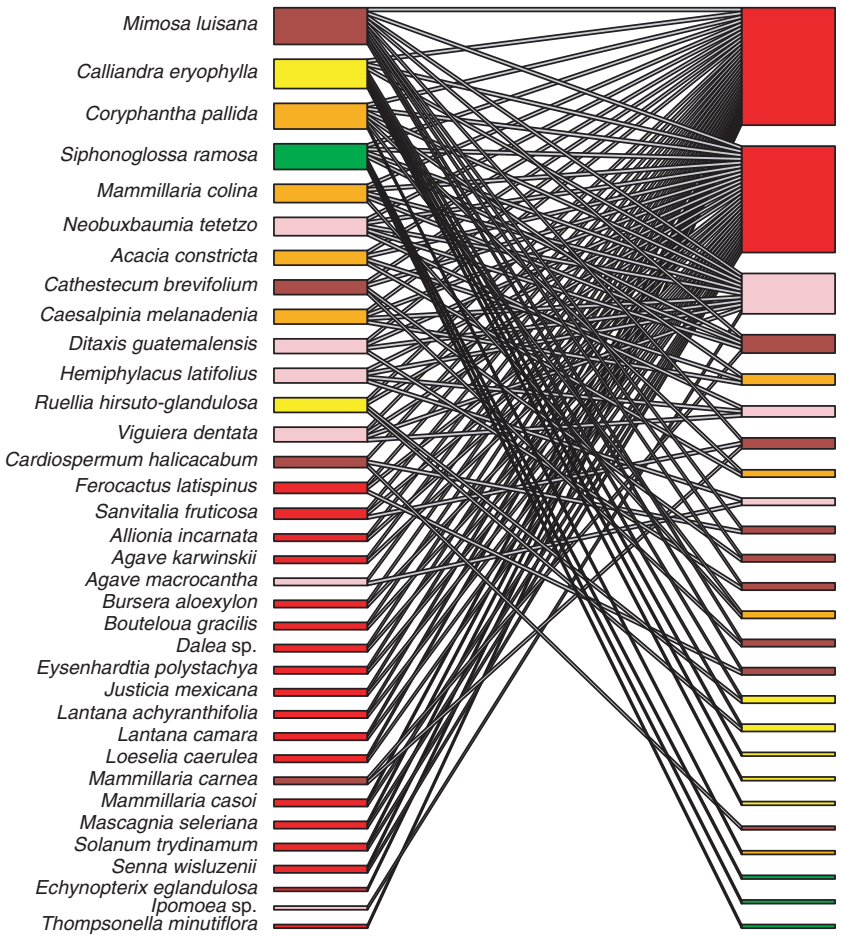

Fig. 1 Bipartite network showing the interactions between plants (left) and arbuscular mycorrhizal fungi (AMF) operational taxonomic units (right) obtained with the 7% cutoff. Species are ordered from generalist (top) to specialist (bottom) and colours represent nodes included in the same module, with modules representing subsets of species more tightly interconnected.

presented. *Dixitalis guatemalensis* and *Cathestecum brevifolium* were consistently the species that had higher AMF load across cutoffs, and *Thompsonella minutiflora*, *Echynopterix eglandulosa* and *Mammillaria casoi* were the species with the lowest AMF load. Both plant species abundance and AMF load have a significant effect on plant species degree, for every cutoff considered (Table 3). The relative contribution of plant relative abundance and AMF load to plant species degree is similar for cutoffs of 4–10% (Table 3). Only in the lower cutoffs, such as the 1%, can plant relative abundance explain 1.7 times more variation in plant species degree than AMF load (Table 3).

Discussion

In this paper we present the network properties of a plant–AMF mutualistic system. Our results show a nonrandom interaction pattern in plant–AMF associations with a network with low connectance, highly nested and modular. As expected, the nestedness values observed are concordant with other mutualistic networks (Bascompte & Jordano, 2007) and the modularity detected reinforces the hypothesis that selectivity in plant–AMF interactions can result in emergent patterns at the community level.

Our gradient of cutoffs (1–10%) adequately characterize both intraspecific and interspecific variation for Glomeromycota. The average of intraspecific ITS variability in this taxon is 7.46% (SD 4.14), with some examples of intraspecific variation of 8.7% and 5.9% variability in *Glomus intraradices* and in *Glomus mosseae* (Nilsson *et al.*, 2008). The stabilization of rarefaction curves between cutoffs close to intraspecific variation of the taxa (5%, or more strictly 8%) indicates that our sampling captured a considerable amount of the total diversity of the *Glomus* A group of AMFs present in the area. Finding consistent network properties across cutoffs supports the idea that the network structure is maintained independently of the genetic differentiation threshold considered to define AMF OTUs. Regarding comparisons with other studies reanalysed in this paper, the presence of nestedness seems to be a consistent pattern, although there is high variability in its strength across different plant–AMF communities. Modularity in plant–AMF networks seems to be a less consistent pattern across sites and potentially influenced by the degree of connectivity in each community. Interestingly, although both plants and AMF show a nested structure, plants have a stronger pattern of nestedness than AMF. We discuss these results in turn in the following.

In general, mutualistic networks are characterized by having low connectance and being highly nested (Bascompte & Jordano 2007). Our plant–AMF network showed similar connectance to that reported for pollination networks (11.89 ± 3.41; Olesen *et al.*, 2006) and plant-to-plant facilitation networks (24.9 ± 2.68; Verdú & Valiente-Banuet, 2008), but other plant–AMF networks show higher connectance (Öpik *et al.*, 2009; Davison *et al.*, 2011). Regarding nestedness, our plant–AMF network showed similar, high values of nestedness (T) than the ones reported for other positive interactions such as plant-to-plant facilitation (89.7 ± 2.7; Verdú & Valiente-Banuet, 2008), seed dispersal (84.3 ± 2.1) and pollination networks (85.3 ± 2.2; Bascompte *et al.*, 2003). A pattern of generalist plants tending to associate with generalist AMFs, has previously been reported for plant–AMF systems (Davison *et al.*, 2011). Our reanalysis of this and another related study (Öpik *et al.*, 2009) does indeed show significant nestedness, suggesting that this may be a general pattern in plant–AMF networks.

Ecological networks with low connectance and highly nested have a tendency to be highly modular (Fortuna *et al.*, 2010). This is also the case of the network presented in this study. However, the other two published networks analysed were nested but not modular. Interestingly, these studies have high connectance and, according to the pattern revealed by Fortuna *et al.* (2010), highly connected networks tend to be either nested or modular, but not both. Further studies looking at plant–AMF community interactions are needed to elucidate a general pattern regarding the degree of connectance and its influence on modularity in different communities.

The nonrandom pattern of plant–AMF interactions observed at the community level can be produced by diverse mechanisms.

Table 3 Generalized linear models (GLM) testing for the effects of plant species' relative abundance and arbuscular mycorrhizal fungal (AMF) load on the species degree in the network

Cutoff %	R^2	Plant abundance	Individual AMF load	Relative importance of plant abundance/individual AMF
1	0.87	0.14 (0.01)***	0.22 (0.04)***	1.7***
2	0.84	0.12 (0.01)***	0.25 (0.05)***	1.4***
3	0.79	0.12 (0.01)***	0.28 (0.06)***	1.3***
4	0.74	0.11 (0.01)***	0.28 (0.06)***	1.1***
5	0.77	0.09 (0.01)***	0.28 (0.07)***	1.1***
6	0.64	0.09 (0.01)***	0.34 (0.11)**	1.2***
7	0.63	0.08 (0.02)***	0.37 (0.11)**	1.1***
8	0.63	0.08 (0.02)***	0.38 (0.11)***	0.96***
9	0.85	0.07 (0.02)***	0.35 (0.12)**	1.05***
10	0.64	0.08 (0.02)***	0.57 (0.19)**	1.08***

Cutoff %, percentage of genetic sequence dissimilarity; R^2, pseudo-R^2 of the full model.
Plant abundance and individual AMF load effect, mean (SE) and P-value of each effect, respectively; relative importance of plant abundance/individual AMF, ratio of the standard deviations of the two effects on plant species degree.
*, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$.

Ecological processes such as habitat heterogeneity, specific selectivity in plant–AMF associations, plant–AMF overlapped phenology, species' relative abundance and phylogenetic diversity or AMF competition within the root can produce modularity and nestedness in plant–AMF networks.

Modularity can emerge from processes such as habitat heterogeneity, resulting in nonrandom species spatial distribution (Olesen *et al.*, 2007). However, in this system, species from different modules coexist in the same vegetation clumps, suggesting that species distribution does not constrain plant–AMF interaction patterns. The modularity found at the community level is consistent with previous studies which have confirmed both qualitative (Ravnskov & Jakobsen, 1995) and quantitative (Bever *et al.*, 1996; Streitwolf-Engel *et al.*, 1997; Eom *et al.*, 2000) selectivity in AMF and plant interactions.

In our study we have detected only AMFs belonging to the Glomeraceae. However, other orders may be present in the area in a different season or in different habitats such as soil (spores) vs roots (Camargo-Ricalde *et al.*, 2003). In a more phylogenetically diverse AMF community, new interactions will be found, which might affect the network structure. However, our results show that in other communities with higher AMF phylogenetic diversity, nestedness is maintained. This is because the AMF taxa missing in our study, Gigasporaceae, Acaulosporaceae and Diversisporaceae, tend to interact with generalist plant species (Fig. S11). However, because our results are based on very few communities, further studies are required to confirm that the observed pattern can be generalized to overall plant–AMF associations.

Relative abundance of plant species can also lead to a nonrandom interaction pattern, as AMF OTUs with few interactions will have a higher probability of interacting with abundant plant species than with scarce ones. Our results show that, although both plants and AMFs present a nested structure, plants have a stronger pattern of nestedness than AMFs. A potential explanation for this difference might be that sampling is different for plants and AMFs. While we can observe plants and account for their relative abundance in our survey, AMF sampling has followed a blind procedure, and it does not necessarily represent relative abundances precisely. Our data support the fact that species' relative abundance contributes significantly to the network structure; however, it has been shown that species abundance cannot fully explain the observed interaction pattern, and its relative importance is similar to other ecological processes.

An alternative explanation for differences in plant and AMF nestedness is that mutualistic networks can result in a lack of nestedness because of a balance between mutualism and competition. In this particular situation, competition can force generalist species to become more specialist (Ricciardi *et al.*, 2010). A combination of mutualism and competition might well be happening in plant–AMF systems (Husband *et al.*, 2002). However, while AMF compete for root space with other AMF when they interact with a generalist plant species (i.e. with a high AMF load), this is not true for the case of a plant interacting with a generalist AMF taxa.

The overall network nestedness suggested a higher importance of mutualism over competition in AMF and plant community interactions. These mycorrhizal networks established among plants inhabiting multispecific vegetation clumps could alleviate competition among neighbouring plants, promoting plant-to-plant facilitation (Castillo *et al.*, 2010; Verdú & Valiente-Banuet, 2011). Plant species inhabiting a more diverse phylogenetic neighbourhood can benefit from a higher AMF diversity in their rhizosphere (Maherali & Klironomos, 2007). This pattern might help to explain the phylogenetic overdispersion found in plant communities from environments where facilitation is a key process establishing community assemblage (Verdú & Valiente-Banuet, 2008). In support of this idea, nestedness and modularity have been shown to be influenced by species phylogeny (Valiente-Banuet & Verdú, 2007; Rezende *et al.*, 2009; Verdú *et al.*, 2010; Verdú & Valiente-Banuet, 2011) and complementary traits (Rezende *et al.*, 2009) in other systems.

In conclusion, nonrandom patterns emerge from analysing plant–AMF interactions at the community level consistent with previous knowledge. Provided that plant–AMF interactions are organized in certain groups of species within which interactions are more frequent, new biological questions are generated by our results. Do species within a module share certain traits? Do plant and fungi phylogenies explain the observed interaction pattern? Closely related species might tend to interact with the same partners, resulting in closely related species sharing membership of a network module. Answering these questions will increase our understanding of the largely unknown potential influence of plant–AMF coevolutionary history in plant community assemblages.

Acknowledgements

We thank J. P. Castillo, M. Morales, C. Silva and L. Sortibrán for help with field samplings and S. Donat and M. Morales for laboratory assistance. This work was funded by AECID (projects A017475/08, A023461/09), DGAPA-UNAM (project IN-224808-3; IN-202811-3), and CYTED (acción 409AC0369). J.G.S-M. was supported by a 'Ramón y Cajal' postdoctoral contract from MICINN.

References

Alguacil MM, Roldán A, Torres MP. 2009. Assessing the diversity of AM fungi in arid gypsophilous plant communities. *Environmental Microbiology* **11**: 2649–2659.

Allen EB, Allen MF. 1984. Competition between plants of different succesional stages: mycorrhizae as regulators. *Canadian Journal of Botany* **62**: 2625–2629.

Almeida-Neto M, Guimarães P, Guimarães PR Jr, Loyola RD, Ulrich W. 2008. A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. *Oikos* **117**: 1227–1239.

Atmar W, Patterson BD. 1993. The measure of order and disorder in the distribution of species in fragmented habitat. *Oecologia* **96**: 373–382.

Bascompte J, Jordano P. 2007. Plant–animal mutualistic networks: the architecture of biodiversity. *Annual Review of Ecology, Evolution, and Systematics* **38**: 567–593.

Bascompte J, Jordano P, Melian CJ, Olesen JM. 2003. The nested assembly of plant–animal mutualistic networks. *Proceedings of the National Academy of Sciences, USA* **100**: 9383–9387.

Bever JD. 2003. Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytologist* **157**: 465–473.

Bever JD, Morton JB, Antonovics J, Schultz PA. 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. *Journal of Ecology* **84**: 71–82.

Bever JD, Westover KM, Antonovics J. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology* **85**: 561–573.

Blackwell M. 2000. Terrestrial life–fungal from the start? *Science* **289**: 1884–1885.

Blüthgen N, Menzel F, Blüthgen N. 2006. Measuring specialization in species interaction networks. *BMC Ecology* **6**: 9.

Brundrett MC. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant and Soil* **320**: 37–77.

Camargo-Ricalde SL, Dhillion SS, Jiménez-González C. 2003. Mycorrhizal perennials of the ''matorral xerófilo'' and the ''selva baja caducifolia'' communities in the semiarid Tehuacán-Cuicatlán Valley, Mexico. *Mycorrhiza* **13**: 77–83.

Castillo JP, Verdú M, Valiente-Banuet A. 2010. Neighborhood phylodiversity affects plant performance. *Ecology* **91**: 3656–3663.

Cross AF, Schlesinger WH. 2001. Biological and geochemical controls on phosphorus fractions in semiarid soils. *Biogeochemistry* **52**: 155–172.

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

Davison J, Öpik M, Daniell T, Moora M, Zobel M. 2011. Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. *FEMS Microbiology Ecology* **78**: 103–115.

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter A. 2010. Relative roles of niche and neutral processes in structuring a soil microbial community. *Multidisciplinary Journal of Microbial Ecology* **4**: 337–345.

Eom AH, Hartnett DC, Wilson GWT. 2000. Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. *Oecologia* **122**: 435–444.

Fitter AH. 1977. Influence of mycorrhizal infection on competition for phosphorus and potassium by two grasses. *New Phytologist* **79**: 119–125.

Fortuna MA, Stouffer DB, Olesen JM, Jordano P, Mouillot D, Krasnov BR, Poulin R, Bascompte J. 2010. Nestedness versus modularity in ecological networks: two sides of the same coin? *Journal of Animal Ecology* **79**: 811–817.

García E. 1973. *Modificaciones al sistema de clasificación climática de Köppen*. México D.F., México: Instituto de Geografía, Universidad Nacional Autónoma de México.

Grime JP, Mackey JML, Hillier SH, Read DJ. 1987. Floristic diversity in a model system using experimental microcosms. *Nature* **328**: 420–422.

Guimarães J, Guimarães P. 2006. Improving the analyses of nestedness for large sets of matrices. *Environmental Modelling & Software* **21**: 1512–1513.

Guimarães PRJ, Sazima C, Furtado dos Reis S, Sazima I. 2007. The nested structure of marine cleaning symbiosis: is it like flowers and bees? *Biology Letters* **3**: 51–54.

Guimerà R, Amaral LAN. 2005a. Functional cartography of complex metabolic networks. *Nature* **433**: 895–900.

Guimerà R, Amaral LAN. 2005b. Cartography of complex networks: modules and universal roles. *Journal of Statistical Mechanics*, theory and experiment. P02001.

Guimerà R, Sales-Pardo M, Amaral LAN. 2004. Modularity from fluctuations in random graphs and complex networks *Physical Review E* **70**: 025101.

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

Hartnett DC, Wilson GWT. 1999. Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology* **80**: 1187–1195.

Hausmann NT, Hawkes CV. 2009. Plant neighborhood control of arbuscular mycorrhizal community composition. *New Phytologist* **183**: 1188–1200.

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW. 1998. Ploughing up the wood-wide web? *Nature* **394**: 431.

Helgason T, Merryweather JW, Denison J, Wilson P, Young JPW, Fitter AH. 2002. Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. *Journal of Ecology* **90**: 371–384.

Hetrick BAD, Wilson GWT, Hartnett DC. 1989. Relationship between mycorrhizal dependence and competitive ability of two tall grass prairie grasses. *Canadian Journal of Botany* **67**: 2608–2615.

Husband R, Herre EA, Turner SL, Gallery R, Young JPW. 2002. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. *Molecular Ecology* **11**: 2669–2678.

Janos DP, Sahley CT. 1995. Rodent dispersal of vesicular–arbuscular mycorrhizal fungi in amazonian Peru. *Ecology* **76**: 1852–1858.

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

Joppa LN, Montoya JM, Solé R, Sanderson J, Pimm SL. 2010. On nestedness in ecological networks. *Evolutionary Ecology Research* **12**: 35–46.

Kottke I, Haug I, Setaro S, Suarez JP, Weiss M, Preussing M, Nebel M, Oberwinkler F. 2008. Guilds of mycorrhizal fungi and their relation to trees, ericads, orchids and liverworts in a neotropical mountain rain forest. *Basic and Applied Ecology* **9**: 13–23.

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

Lekberg Y, Meadow J, Rohr JR, Redecker D, Zabinski CA. 2011. Importance of dispersal and thermal environment for mycorrhizal communities: lessons from Yellowstone National Park. *Ecology* **92**: 1292–1302.

Li LF, Li T, Zhang Y, Zhao ZW. 2010. Molecular diversity of arbuscular mycorrhizal fungi and their distribution patterns related to host-plants and habitats in a hot and arid ecosystem, southwest China. *FEMS Microbiology Ecology* **71**: 418–427.

Li T, Li J, Zhao Z. 2004. Arbuscular mycorrhizas in a valley-type savanna in southwest China. *Mycorrhiza* **14**: 323–327.

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

Malcová R, Vosátka M, Albrechtová J. 1999. Influence of arbuscular mycorrhizal fungi and simulated acid rain on the growth and coexistence of the grasses *Calamagrostis villosa* and *Deschampsia flexuosa*. *Plant and Soil* **207**: 45–57.

Mangan SA, Adler GH. 2000. Consumption of arbuscular mycorrhizal fungi by terrestrial and arboreal small mammals in a Panamanian cloud forest. *Journal of Mammalogy* **81**: 563–570.

Moora M, Zobel M. 1996. Effect of arbuscular mycorrhiza on inter- and intraspecific competition of two grassland species. *Oecologia* **108**: 79–84.

Morton JB, Benny GL. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new sub-orders, Glomineae and Gigasporineae, and two new families Acaulosporaceae and Gigasporaceae, with emendation of Glomaceae. *Mycotaxon* **37**: 471–491.

Mummey DL, Rillig MC. 2008. Spatial characterization of arbuscular mycorrhizal fungal molecular diversity at the submetre scale in a temperate grassland. *FEMS Microbiology Ecology* **64**: 260–270.

Mummey DL, Rillig MC, Holben WE. 2005. Neighboring plant influences on arbuscular mycorrhizal fungal community composition as assessed by T-RFLP analysis. *Plant and Soil* **271**: 83–90.

Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson K. 2008. Intraspecific ITS variability in the kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evolutionary Bioinformatics* **4**: 193–201.

Olesen JM, Bascompte J, Dupont Y, Jordano P. 2006. The smallest of all worlds: pollination networks. *Journal of Theoretical Biology* **240**: 270–276.

Olesen JM, Bascompte J, Dupont YL, Jordano P. 2007. The modularity of pollination networks. *Proceedings of the National Academy of Sciences, USA* **104**: 19891–19896.

Öpik M, Metsis M, Daniell T, Zobel M, Moora M. 2009. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. *New Phytologist* **184**: 424–437.

Öpik M, Moora M, Zobel M, Saks U, Wheatley R, Wright F, Daniell T. 2008. High diversity of arbuscular mycorrhizal fungi in a boreal herb-rich coniferous forest. *New Phytologist* **179**: 867–876.

Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reiler Ü, Zobel M. 2010. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytologist* **188**: 223–241.

Peay KG, Burns TD, Kennedy PG, Bergemann SE, Garbelotto M. 2007. A strong species–area relationship for eukaryotic soil microbes: island size matters for ectomycorrhizal fungi. *Ecology Letters* **10**: 470–480.

Pivato B, Mazurier S, Lemanceau P, Siblot S, Berta G, Mougel C, Van Tuinen D. 2007. *Medicago* species affect the community composition of arbuscular mycorrhizal fungi associated with roots. *New Phytologist* **176**: 197–210.

Ravnskov S, Jakobsen I. 1995. Functional compatibility in arbuscular mycorrhizas measured as hyphal P transport to the plant. *New Phytologist* **129**: 611–618.

Redecker D. 2000. Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. *Mycorrhiza* **10**: 73–80.

Redecker D, Hijri I, Wiemken A. 2003. Molecular identification of arbuscular mycorrhizal fungi in roots: perspectives and problems. *Folia Geobotanica* **38**: 113–124.

Redecker D, Raab P. 2006. Phylogeny of the Glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. *Mycologia* **98**: 885–895.

Rezende EL, Albert EM, Fortuna MA, Bascompte J. 2009. Compartments in a marine food web are associated with phylogeny, body mass, and habitat structure. *Ecology Letters* **12**: 779–788.

Ricciardi F, Boyer M, Ollerton J. 2010. Assemblage and interaction structure of the anemonefish-anemone mutualism across the Manado region of Sulawesi, Indonesia. *Environmental Biology of Fishes* **87**: 333–347.

Rosendahl S. 2007. Communities, populations and individuals of arbuscular mycorrhizal fungi. *New Phytologist* **178**: 253–266.

Rosendahl S, McGee P, Morton JB. 2009. Lack of global population genetic differentiation in the arbuscular mycorrhizal fungus *Glomus mosseae* suggests a recent range expansion which may have coincided with the spread of agriculture. *Molecular Ecology* **18**: 4316–4329.

Santos-González JC, Finlay RD, Tehler A. 2007. Seasonal dynamics of arbuscular mycorrhizal fungal communities in roots in a seminatural grassland. *Applied Environmental Microbiology* **73**: 5613–5623.

Schechter SP, Bruns TD. 2008. Serpentine and non-serpentine ecotypes of *Collinsia sparsiflora* associate with distinct arbuscular mycorrhizal fungal assemblages. *Molecular Ecology* **17**: 3198–3210.

Scheublin TR, Ridgway KP, Young JPW, Van der Heijden MGA. 2004. Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. *Applied Environmental Microbiology* **70**: 6240–6246.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ *et al.* 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75**: 7537–7541.

Schüßler A, Gehrig H, Schwarzott D, Walker C. 2001a. Analysis of partial Glomales SSU rRNA gene sequences: implications for primer design and phylogeny. *Mycological Research* **105**: 5–15.

Schüßler A, Schwarzott D, Walker C. 2001b. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* **105**: 1413–1421.

Shi Z, Zhang L, Feng G, Christie P, Tian C, Li X. 2006. Diversity of arbuscular mycorrhizal fungi associated with desert ephemerals growing under and beyond the canopies of Tamarisk shrubs. *Chinese Science Bulletin* **51**: 132–139.

Silber JH, Rosenbaum PR, Ross RN. 1995. Comparing the contributions of groups of predictors: which outcomes vary with hospital rather than patient characteristics? *Journal of the American Statistical Association* **90**: 7–18.

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

Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis, 3rd edn.* Amsterdam, the Netherlands: Academic Press.

Sonjak S, Beguiristain T, Leyval C, Regvar M. 2009. Temporal temperature gradient gel electrophoresis (TTGE) analysis of arbuscular mycorrhizal fungi associated with selected plants from saline and metal polluted environments. *Plant and Soil* **314**: 25–34.

Streitwolf-Engel R, Boller T, Wiemken A, Sandeers IR. 1997. Clonal growth traits of two *Prunella* species are determined by co-ocurring arbuscular mycorrhizal fungi from a calcareous grassland. *Journal of Ecology* **85**: 181–191.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**: 1596–1599.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**: 4673–4680.

Vacher C, Piou D, Desprez-Loustau ML. 2008. Architecture of an antagonistic tree/fungus network: the asymmetric influence of past evolutionary history. *PLoS ONE* **3**: e1740.

Valiente-Banuet A, Casas A, Alcántara A, Dávila P, Flores-Hernández N, Villaseñor JL, Ortega-Ramírez J. 2000. La vegetación del Valle de Tehuacán-Cuicatlán. *Boletin de la Sociedad Botánica de México* **67**: 25–74.

Valiente-Banuet A, Verdú M. 2007. Facilitation can increase the phylogenetic diversity of plant communities. *Ecology Letters* **10**: 1029–1036.

Van der Heijden MGA, Boller T, Wiemken A, Sanders IR. 1998a. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology* **79**: 2082–2091.

Van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. 1998b. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**: 69–72.

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

Vandenkoornhuyse P, Husband R, Daniell TJ, Watson IJ, Duck JM, Fitter AH, Young JPW. 2002. Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. *Molecular Ecolology* **11**: 1555–1564.

Vandenkoornhuyse P, Ridgway KP, Watson IJ, Fitter AH, Young JPW. 2003. Co-existing grass species have distinctive arbuscular mycorrhizal communities. *Molecular Ecology* **12**: 3085–3095.

Vázquez DP. 2005. Degree distribution in plant–animal mutualistic networks: forbidden links or random interactions? *Oikos* **108**: 421–426.

Verdú M, Jordano P, Valiente-Banuet A. 2010. The phylogenetic structure of plant facilitation networks changes with competition. *Journal of Ecology* **98**: 1454–1461.

Verdú M, Valiente-Banuet A. 2008. The nested assembly of plant facilitation networks prevents species extinctions. *The American Naturalist* **172**: 751–760.

Verdú M, Valiente-Banuet A. 2011. The relative contribution of abundance and phylogeny to the structure of plant facilitation networks. *Oikos* **120**: 1351–1356.

Walter LEF, Harnett DC, Hetrick BAD, Schwab P. 1996. Interspecific nutrient transfer in a tallgrass prairie plant community. *American Journal of Botany* **83**: 180–184.

Wang B, Qiu JL. 2006. Phylogenetic distribution and evolution of micorrhizas in land plants. *Mycorrhiza* **16**: 299–363.

Warner NJ, Allen MF, MacMahon JA. 1987. Dispersal agents of vesicular–arbuscular mycorrhizal fungi in a disturbed arid ecosystem. *Mycologia* **79**: 721–730.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR protocols, a guide to methods and applications.* San Diego, CA, USA: Academic Press, 315–322.

Wilde P, Manal A, Stodden M, Sieverding E, Hildebrandt U, Bothe H. 2009. Biodiversity of arbuscular mycorrhizal fungi in roots and soils of two salt marshes. *Environmental Microbiology* **11**: 1548–1561.

Yodzis P. 1980. The connectance of real ecosystems. *Nature* **284**: 544–545.

Supporting Information

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

Fig. S1 Rarefaction curves for the Glom1310/ITS4i ITS sequences at increasing dissimilarity levels ranging from 1 to 10%.

Figs S2–S10 Bipartite networks for each cutoff (1–10% genetic differentiation, except for the 7% cutoff shown in the main text).

Fig. S11 Interaction matrices of our data (cutoff 7%), Davison *et al.* (2011), and Öpik *et al.* (2009).

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.