

Plant facilitation occurs between species differing in their associated arbuscular mycorrhizal fungi

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Summary

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- Complementary beneficial effects of different arbuscular mycorrhizal fungi (AMF) can result in a more efficient exploitation of the soil nutrients available, thus influencing plant communities. Here, we hypothesize that plant–AMF specificity is mediated by phylogenetic constraints defining possible interactions, and that plant–AMF interaction patterns can influence plant–plant facilitation specificity.
- We reanalyzed previous data describing plant–plant and plant–AMF interaction at the community level to specifically test for a phylogenetic signal on plant and AMF interactions and for a relationship between plant–plant facilitation specificity and plant species differences in their AMF associates.
- Closely related AMF operational taxonomical units (OTUs) tend to interact with the same plant species, but there is not a significant signal in the interaction through the plant phylogeny. This indicates that the similarity in the AMF associates of two plant species is independent of their phylogenetic relatedness. Interestingly, plant–AMF interactions match plant facilitation specificity, with pairs of plant species recruiting more frequently under each other tending to have different AMF associates.
- An increment of AMF diversity in the rhizosphere, as a result of plant–AMF and plant–plant selectivity, is suggested as a potential driver of plant–plant facilitation. This study highlights the role of plant–AMF interactions in shaping plant community assemblages.

Introduction

Plant–plant facilitation is an ecological process occurring in communities worldwide (Valiente-Banuet *et al.*, 2006) that is required for the establishment of most woody plant species in semiarid environments (Valiente-Banuet & Verdú, 2008). Plant–plant facilitation is considered as a positive interaction in which the presence of one plant enhances the growth, survival or reproduction of a neighboring plant. Facilitation does not need to be a mutualistic interaction where both participants gain (+,+), but may occur only as a commensalism (+,0) in which only the facilitated species obtains a benefit. However, mere species coexistence without any positive interaction (0,0) is not considered as plant facilitation (Callaway, 2007). Plant facilitation has been experimentally shown to be species-specific, so that benefactor (nurse) species tend to promote the establishment of specific beneficiary (facilitated) species more strongly than others (Valiente-Banuet & Verdú, 2007, 2008; Castillo *et al.*, 2010). Hereafter we will refer to it as plant–plant facilitation specificity. Plant–plant facilitation is patent when adult nurses are facilitating seedlings of the facilitated species. However, even though this positive interaction may turn into competition over time, a high

percentage of specific positive plant–plant interactions remains when facilitated seedlings become adults (Valiente-Banuet & Verdú, 2008). The maintenance of plant–plant facilitation over time implies that the benefits of the association are not only related to germination and seedling establishment, but are also extended to growth and long-term survival. Understanding the mechanisms that promote and maintain plant–plant facilitation will contribute to a better understanding of assembly mechanisms in plant communities.

Mechanisms traditionally considered to underlie plant–plant facilitation involve avoidance of abiotic stresses such as an improved shade or moisture availability (Callaway, 2007). In this case plant–plant facilitation strength will be expected to be independent of ecological interactions (see Fig. 1a for the specific case of plant–arbuscular mycorrhizal fungi (AMF) interactions). However, it is unlikely to explain a stronger facilitation between specific plant species considering only abiotic stress avoidance. Plant–plant facilitation specificity is more likely explained by plant species-specific traits, including traits involved in ecological interactions. Plant–plant facilitation tends to occur among distantly related plant species (Valiente-Banuet & Verdú, 2007). Simultaneously, phylogenetic relatedness could be underlying species similarities in

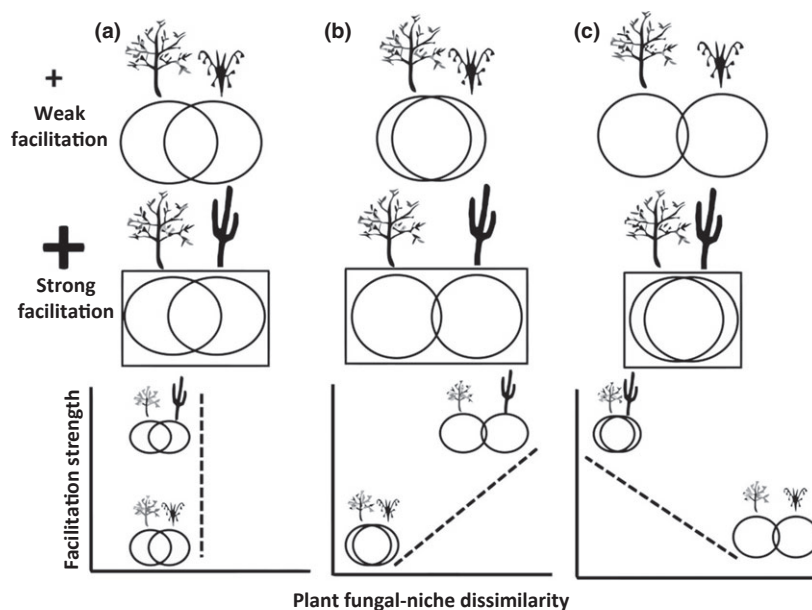


Fig. 1 Schematic representation of the potential relationships between plant–plant facilitation strength and other biotic interactions. Plant fungal-niche dissimilarity represents the differences in the set of arbuscular mycorrhizal fungi (AMF) associated with any pair of plant species. The size of the positive sign indicates the strength of facilitation for a given pair of plant species. Circles represent plant fungal-niche, with high or low overlap indicating similarity or difference in AMF communities, respectively. The rectangle around the circles represents the potential exploitation of soil nutrients. Different scenarios are represented: (a) members of plant pairs with different degree of facilitation strength have a similar plant fungal-niche, leading to an independence of plant–plant facilitation strength from this biotic interaction (plant–AMF association); (b) higher facilitation strength between plant pairs with lower dissimilarity in plant fungal niche (negative relationship), leading to a higher facilitation strength between plant–plant sharing a similar plant fungal-niche; and (c) higher facilitation strength between plant pairs with higher dissimilarity in their plant fungal niche (positive relationship), leading to a higher increment in the potential access to resources (rectangle).

traits influencing ecological interactions with both mutualistic (i.e. AMF) and antagonistic (i.e. pathogens) partners. A phylogenetic conservatism of ecological interactions is a recurrent phenomenon across the entire tree of life (Gómez *et al.*, 2010). The tendency of related species to resemble each other may result in sharing interacting partner species (phylogenetic signal). Recent studies have started to hypothesize that the role of mycorrhizal fungi in interconnecting plants is crucial to understand plant–plant facilitation processes (Van der Heijden & Horton, 2009; Van der Putten, 2009). One potential mechanism by which mycorrhizal fungi can promote plant–plant facilitation is that they can interconnect plant individuals from different species, genera and even families in natural communities (Newman, 1988). Plant interconnections provide pathways for the transference of nutrients such as nitrogen (He *et al.*, 2004, 2005), phosphorus (Smith *et al.*, 2001), arsenic, cesium and rubidium (Meding & Zasoski, 2008). The mutualism between plant and AMF can have considerable importance for seedling establishment, enhancing access to nutrient absorption without requiring completely developed tissue structures (Kytoviita *et al.*, 2003; Van der Heijden, 2004). Later on, the development of plant–plant facilitation could be maintained through the plant interconnections provided by AMF. Plant–plant facilitation may be stronger between plant species with similar AMF if there is an overall benefit as a result of an overlap of the AMF associated with each plant species (hereafter the plant fungal-niche) driven by an increment in the abundance of the shared AMF (Fig. 1b). By contrast, plant–plant facilitation

specificity may be mediated by the degree of complementarity in the interacting plants' fungal-niche. It has been shown in experimental approaches that plant coexistence and productivity increase with increasing numbers of AMF species, as a result of the added beneficial effect of each single AMF species (Van der Heijden *et al.*, 1998; Hartnett & Wilson, 1999; Wagg *et al.*, 2011). Increasing AMF diversity has been suggested to result in a more efficient exploitation of soil nutrients and a better use of the resources available in the system (Van der Heijden *et al.*, 1998). In this sense, plant–plant facilitation may be stronger between plant species that harbor different AMF, leading to an overall benefit as a result of an increase of AMF diversity in the shared rhizosphere (Fig. 1c). Host specificity is likely to be an important driver shaping AMF communities (Hausmann & Hawkes, 2010), and, likewise, AMF communities can influence plant species' performance (Maherali & Klironomos, 2007) through strong bottom-up controls on plant community composition (Grime *et al.*, 1987; Van der Heijden *et al.*, 1998; Hartnett & Wilson, 1999). The role of microorganisms in promoting plant species coexistence was first suggested to be a result of avoidance of below-ground antagonistic interactions (Janzen, 1970; Connell, 1971; Packer & Clay, 2000; Reinhart *et al.*, 2003; Van der Putten, 2009), but the influence of below-ground mutualistic interactions promoting plant–plant facilitation has been less well studied (Dickie *et al.*, 2002, 2005).

Plant–AMF interaction networks have recently been described for a semiarid community (Montesinos-Navarro *et al.*, 2012), showing that there is a nonrandom interaction pattern between

plant and AMF communities as revealed by significant network nestedness and modularity. Nestedness implies that ecological specialists (i.e. with the lower number of links) on one party (e.g. plants) tend to interact with a subset of the ecological-generalist (i.e. with the higher number of links) species on the other party (e.g. AMF). Specifically, in this plant–AMF network there are few AMF ecological-generalists which interact with almost every plant species in the community (Montesinos-Navarro *et al.*, 2012). Modularity, in addition, reflects weakly interlinked subsets of species (modules) that internally consist of strongly connected species (Olesen *et al.*, 2007). Plant and AMF species are grouped within a module on the basis of shared interactions, which means a similar interaction niche (set of species with which they interact), and in some cases this interaction pattern is independent of the spatial association of the species (Montesinos-Navarro *et al.*, 2012). Under this scenario, this compartmentalization of the network can emerge, among other processes, from a phylogenetic conservatism of ecological interactions among taxa (Lewinsohn *et al.*, 2006; Olesen *et al.*, 2007), suggesting that specific plant–AMF association may be limited by phylogenetic constraints. Analyzing the phylogenetic conservatism in module membership is analogous to exploring phylogenetic conservatism in host selectivity (Gómez *et al.*, 2010).

In this paper we test whether plant–AMF interactions are phylogenetically structured. In other words, if there is a nonrandom phylogenetic pattern in plant–AMF interaction; if the modules of the network, previously shown to be independent of species spatial association (Montesinos-Navarro *et al.*, 2012), can emerge from such a phylogenetic pattern (i.e. modules are composed of closely related plant and/or closely related AMF species); and if there is a relationship between facilitation specificity and plant species' fungal-niche. We expect that, as suggested for other ecological networks, there will be a significant phylogenetic signal in the plant–AMF network, resulting in a phylogenetic conservatism of module membership. In addition, we expect that plant–plant facilitation strength between specific species depends on their fungal-niche, potentially influencing the overall nutrient uptake in the plants' shared rhizosphere.

Materials and Methods

Data base

This study is based on available data published by Montesinos-Navarro *et al.* (2012), collected in the semiarid Valley of Zapotitlán, in the state of Puebla, Mexico (18°20' N, 97°28' W). It is a xeric shrubland dominated by the columnar cactus *Neobuxbaumia tetetzo*, *Agave* spp. and different species belonging to the families Fabaceae and Asteraceae. Nonlignified root segments from 130 individuals of 37 plant species, representing the relative abundance of each species, were collected to characterize the AMF community (Montesinos-Navarro *et al.*, 2012). A Glomeromycota 18S (SSU) internal transcribed spacer (SSU-full ITS) was amplified through a nested PCR (as described in detail in Montesinos-Navarro *et al.*, 2012). No amplification was obtained for the families Gigasporaceae and Acaulosporaceae. Less than 30%

amplification success was obtained for *Glomus* group B primer-pair, whereas a 78.21% success was achieved for the primer-pair of *Glomus* group A, suggesting a predominance of *Glomus* A in the AMF communities in the study area. *Glomus* is the most common AMF in many field sites, encompassing 70% of the AMF species identified (range 60–85%) (Helgason *et al.*, 2002; Vandenkoornhuysen *et al.*, 2002; Alguacil *et al.*, 2009; Öpik *et al.*, 2009, 2010; Sonjak *et al.*, 2009; Wilde *et al.*, 2009). Although the subsequent sequencing of PCR products was continued only with the predominant monophyletic group of *Glomus* A, owing to financial constraints, the general pattern of interactions described with this subset of AMF has previously been shown to be largely generalized to other systems with higher AMF diversity (Montesinos-Navarro *et al.*, 2012). The importance of using molecular techniques to prevent an underestimation of AMF richness has been increasingly highlighted. However, there is an unavoidable difficulty in precisely defining AMF species using molecular techniques, because of a lack of precise knowledge about intraspecific genetic variation for multiple species. Some studies have traditionally used a standard cutoff of 3% of genetic dissimilarity to consider AMF species, but the use of this or any other specific cutoff can be controversial. We defined operational taxonomic units (OTUs) for AMF according to their DNA sequence dissimilarity over a wide range of reasonable cutoff values based on the range of intra- and interspecific genetic variation recently described in this genomic region for *Glomus* A (Stockinger *et al.*, 2010; Schoch *et al.*, 2012). Rarefaction curves performed at 1–10% DNA sequence dissimilarity cutoffs reached stabilization between 5 and 8% (Montesinos-Navarro *et al.*, 2012). All the analyses were performed at seven cutoffs ranging from 4 to 10% of DNA sequence dissimilarity. For the analyses regarding module species membership, we used the modules defined by Montesinos-Navarro *et al.* (2012), which have been shown to be independent of spatial species association.

AMF and plant phylogenies

For AMF, the SSU-full ITS sequencing described earlier (Genbank accession numbers in Supporting Information, Table S1) was used to build the AMF phylogenetic tree considering an SSU-fill ITS sequence of *Paraglomus* downloaded from Genbank (accession number FN555285) as the outgroup to root the phylogenetic tree. Phylogenetic analyses of the nuclear ribosomal SSU-full ITS sequences were carried out in the Cyberinfrastructure for Phylogenetic Research (CIPRES) web portal (Miller *et al.*, 2010) using the probabilistic maximum likelihood (ML) method, as implemented in the RAxML blackbox with the default settings (Stamatakis, 2006; Stamatakis *et al.*, 2008). One hundred bootstrap trees were inferred to provide support values for the best-scoring ML tree (Fig. S1). We repeated the analyses to obtain another best-scoring tree and assess the robustness of our results to different phylogenetic trees. As the two best-scoring phylogenetic trees were very similar, with just a few tips showing different placements, the results did not change substantially and thus only those using the first tree will be shown. The tips of this tree that differ by less than a given cutoff (4–10% genetic

dissimilarity) were collapsed in order to obtain the AMF phylogeny for each cutoff.

For plants, the phylogenetic distance matrix was obtained from the community phylogeny generated with Phylocom 4.2 (Webb *et al.*, 2008). This program produces a community phylogeny by matching the family names of our study species with those contained in a backbone phylogeny, which is the megatree of the Angiosperm Phylogeny Group III (Stevens, 2005). We then resolved the phylogenetic relationships at the species level of the Cactaceae and Agavaceae families based on published phylogenies of Good-Avila *et al.* (2006) and Hernández-Hernández *et al.* (2011). Our final tree includes all the plant species in which the amplification of AMF DNA was positive (35 out of the 37 species sampled) and was fully resolved with the exception of two polytomies (Fig. S2). The tree was calibrated with age estimates from Wikstrom *et al.* (2001) plus six ages obtained from the chronograms published by Good-Avila *et al.* (2006) and Arakaki *et al.* (2011) for the nodes of Cactaceae (35 million yr ago (Mya)), *Mammillaria* (6.3 Mya), and the splits between *Mammillaria* and *Coryphantha* (19.7 Mya), *Agave karwinski* and *Agave macroacantha* (6.7 Mya). Calibration was done with the phylocom bladj algorithm, which evenly distributes the undated nodes between dated nodes or between dated nodes and terminals (Webb *et al.*, 2008).

Analyses

Phylogenetic signal of the interaction The phylogenetic signal of plant–AMF interactions was estimated with the estimated generalized-least squares (EGLS) procedure of Ives & Godfray (2006). This procedure is similar to that developed for a single trait (Blomberg *et al.*, 2003), but considers the matrix of interactions as the target trait. The method calculates the strength of the phylogenetic signal in the plant–AMF interactions acting through both the AMF (d_{AMF}) and the plant (d_{plant}) species phylogenies. We used the association rate of plant species k on AMF OTU i (A_{ik}) as a measure of the strength of association between plant and AMF species following eqn 4 in Ives & Godfray (2006):

$$A_{ik} = -\log\left(1 - \frac{F_{ik}}{H_i}\right),$$

where H_i is the number of DNA sequences of the AMF OTU i , and F_{ik} is the number of sequences of OTU i found in the plant species k . Note that there is a typo in the original article, which shows H_i/F_{ik} instead of the correct expression F_{ik}/H_i (A.G. Ives, pers. comm.).

As stated by Ives & Godfray (2006), A_{ik} depends on both the selectivity and abundance of species, two crucial variables explaining the number of interactions occurring in facilitation networks (Verdú & Valiente-Banuet, 2008). In addition to quantitative matrices, we recalculated the strength of association (A_{ik}) by transforming the interaction matrix into a binary matrix where 1/0 denotes the presence or absence of interaction, respectively. Although this approach may have other limitations, it avoids problems derived from assigning abundances on the basis of the number of identical copies of DNA in a given root sample,

potentially coming from the same AMF individual. We perform the analyses with both quantitative and binary matrices to evaluate the robustness of the results.

The procedure estimates an EGLS model to fit A_{ik} in terms of the observed association strength and the separate effects of the plant and AMF species phylogenies. The model is based on the Ornstein–Uhlenbeck model of evolution, which incorporates stabilizing selection and drift, and detects the presence of a phylogenetic signal through the parameter d . This parameter determines the strength of the phylogenetic signal, with $d = 0$ indicating the lack of phylogenetic correlation and $d = 1$ corresponding to the Brownian motion assumption (i.e. pure drift model). The goodness of fit of the different models was estimated by comparing the mean squared error calculated for: the full model (MSE); a ‘star’ phylogeny (MSE_{star}); and a Brownian evolution model (MSE_B). The model minimizing the mean squared error was considered the best fit. We estimated d values for both plant (d_{plant}) and AMF (d_{AMF}) sets of species. Statistical significance was estimated by calculating bootstrap 95% confidence intervals as described in Ives & Godfray (2006). Analyses to assess the phylogenetic signal of the interaction were performed in Matlab (The MathWorks, Inc. Natick, MA, USA), version 7.10.0.499.

Phylogenetic signal of module membership The phylogenetic signal in module membership was determined using the method proposed by Maddison & Slatkin (1991). This test estimates whether the minimum number of evolutionary steps in a character on a phylogenetic tree is lower than expected by chance. It was determined comparing the observed minimum number of steps with a null model in which data were reshuffled 1000 times across the tips of the phylogeny. The character was the module to which the species was ascribed by the annealing algorithm (Guimerà & Amaral, 2005a,b). An annealing algorithm uses a probabilistic function to find the optimum solution based on the ‘locality’ of the preceding solution considering the improvement gained in each move. A module was considered as an unordered, multi-state factor. We mapped the evolution of module membership onto our phylogenetic trees. Analyses to assess the phylogenetic signal in module membership were performed in R, version 2.13.2, using the function ‘phylo.signal.disc’ developed by Enrico Rezende, and the species belonging to each module were extracted from Montesinos-Navarro *et al.* (2012).

Relationship between facilitation and fungal niche of plants Plant–plant facilitation has been corroborated for some species in this system using experimental approaches, showing that seedling establishment is enhanced in the understory of distantly related plant species (Castillo *et al.*, 2010). In addition, studies considering the whole plant community have provided results supporting this facilitation pattern (Valiente-Banuet & Verdú, 2007, 2008; Verdú *et al.*, 2010; Verdú & Valiente-Banuet, 2011). Plant–plant facilitation matrices from Valiente-Banuet & Verdú (2008) and Verdú *et al.* (2010) were used to characterize the strength of the facilitation interaction among each pair of plant species in the community. The strength of plant–plant facilitation species was estimated for each pair of plant species as follows. Contingency

analyses were used to compare the number of individuals on each facilitated species recorded under each nurse species and in open spaces with the expected number of individuals derived from the proportions of area of plant cover vs open space, considering the total cover of perennial plants and open space in four 1000 m² transects (Verdú *et al.*, 2010). This matrix was built considering only the plant–plant facilitation interactions (i.e. seedling plant species recruiting under nurse plant species) that remain with time, resulting in plant–plant facilitation interactions among adult plant species (see Valiente-Banuet & Verdú, 2008 for a deeper description of the matrix). The plant–plant facilitation matrix was reduced to contain only those plant species for which information on their associated AMF was available (Montesinos-Navarro *et al.*, 2012). Dissimilarity in plant species' interaction niche was calculated based on the composition of AMF in each plant species' roots (i.e. a plant fungal-niche). The number of AMF OTUs with which a given plant species interacts (plant species degree) is influenced by the plant species' relative abundance. However, there is a correlation between plant species degree and the mean number of AMF OTUs per individual plant (i.e. AMF load), indicating that plant abundance alone is not enough to explain the number of AMF interactions per species. In fact, both plant abundance and AMF load contribute equally to explain plant species degree (Montesinos-Navarro *et al.*, 2012). Pairwise values of plant species' dissimilarity in their fungal niche were calculated using a Euclidean distance index. The statistical significance of the correlation among plant–plant facilitation strength and plant fungal-niche dissimilarity matrices was tested against a null model based on 1000 randomizations of the plant fungal-niche matrix, using the null model 'frequency' in the PICANTE package implemented in R (Kembel *et al.*, 2010). The correlation between log-transformed plant–plant facilitation and plant fungal-niche matrices was tested for each cutoff using from 4 to 10% of genetic dissimilarity to define AMF OTUs.

Contribution of plant relative abundance to facilitation patterns Plant species' relative abundance can be considered as a species-specific intrinsic characteristic. As many other traits, the relative abundance could influence the species' interaction pattern with other species or it could also be the result of its interaction pattern. In any case, including the relative abundance of species, reflected in the sampling design, is essential to approach species interaction patterns at the community level. An abundant plant species in the community will have a higher probability of interacting with a higher number of species. However, the combined effect of relative abundance with other ecological processes can be a better predictor of the interaction patterns than the neutral process of abundance alone. Previous studies on plant facilitation in this system have shown that the frequency of interactions between a pair of plant species is much better explained by the combined effect of relative abundance and the phylogenetic distance between them than solely by plant relative abundance (Verdú & Valiente-Banuet, 2011). This indicates that there is a tendency of the most abundant plant species to interact with each other, but the final frequency of pairwise interactions is shaped by an additional tendency to interact with distantly related species.

Similarly, we tested whether the dissimilarity in plant fungal-niche combined with plant abundance can explain plant facilitation strength better than plant species' relative abundance alone. We evaluate the ability of abundance and dissimilarity in plant fungal-niche to explain plant–plant facilitation strength by means of the likelihood approach developed by Vázquez *et al.* (2009).

The likelihood of the models including the following matrices to explain the observed matrix of facilitation interactions was estimated: null matrix; plant abundance matrix; plant–fungal niche dissimilarity matrix; and abundance \times plant–fungal niche dissimilarity matrix.

The null matrix was defined as a matrix in which all pairwise interactions had the same probability. The probability matrix derived from plant abundances was constructed by multiplying the vectors of nurse and facilitated plant abundances recorded in 112 vegetation patches in the study area. The resulting matrix was normalized so that the elements added up to one. The probability matrix derived from plant fungal-niches was constructed by normalizing the dissimilarity matrix in plant fungal-niche among plants so that the elements added up to one.

The models' likelihood and Akaike information criteria (AIC) were recalculated considering every cutoff from 4 to 10% to define AMF OTUs and using both quantitative and binary matrices to define the plant–AMF interaction pattern. The model with the lower AIC was selected as the best model. As a rule of thumb, models whose AIC is < 2 units larger than the best model also have substantial support, whereas those models resulting in AIC values > 10 units larger have virtually no support (Burnham & Anderson, 2002). In addition, the likelihood of being a better model than the best model was estimated by means of AIC weights.

Results

Considering the number of modules across cutoffs, our plant–AMF interaction network has, on average, six modules. Focusing, for example, on the representative cutoff of 7%, from the AMF point of view, the two most ecological-generalist AMF OTUs were grouped in the same module with 18 ecological-specialist plants (open diamonds in Fig. 2), whereas from the plant perspective, the most ecological-generalist plants belonged to different modules (open and closed circles and open squares in Fig. 2). In general terms, modules tend to be composed of ecological-generalist species of one party (either plants or AMF) and ecological-specialists of the other party.

A significant phylogenetic signal in the plant–AMF interactions is observed through the AMF phylogeny; the model considering the phylogenetic signal has a better fit than the models considering no phylogenetic covariances or Brownian motion for most of the cutoff values (4–8% considering both quantitative and binary matrices (Table 1). However, the phylogenetic signal of the interaction through plant phylogeny was close to zero and was not significant for any cutoff and for both quantitative and binary matrices (Table 1). In other words, closely related AMF tend to interact with the same set of plant species, but the tendency of plant species to interact with the same set of AMF OTUs is independent of their phylogenetic relatedness.

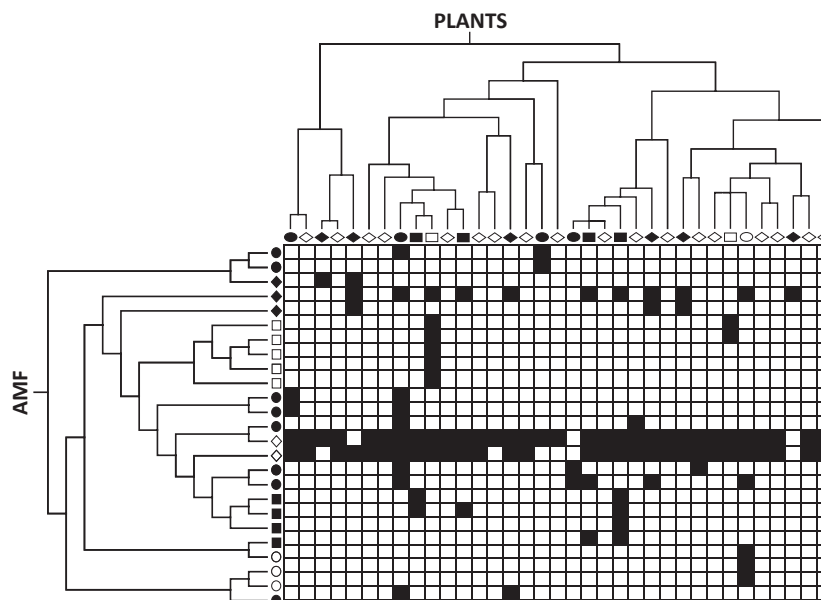


Fig. 2 Plant–arbuscular mycorrhizal fungi (AMF) interaction matrix combined with the phylogenetic topologies, using a cutoff of 7% for AMF. Black and white cells indicate the presence and absence of the interaction, respectively. The six types of icons (open and closed circles, diamonds and squares) on the tips represent the module to which each species belongs, as described in Montesinos-Navarro *et al.* (2012).

Cutoff %	MSE _d	MSE _{Star}	MSE _{Brownian}	d_{AMF} (95% CI)	d_{plant} (95% CI)
Quantitative					
4	0.19	0.22	0.37	0.48 (0.21–0.74)	0 (0–0.05)
5	0.17	0.18	0.35	0.19 (0.002–0.38)	0 (0–0.01)
6	0.16	0.17	0.33	0.44 (0.13–0.82)	0 (0–0.14)
7	0.12	0.14	0.28	0.49 (0.12–0.92)	0 (0–0.14)
8	0.12	0.15	0.31	0.57 (0.1–1)	0 (0–0.1)
9	0.136	0.138	0.359	0.180 (0–0.582)	0 (0–0.21)
10	0.124	0.124	0.417	0.006 (0–0.440)	0 (0–0.336)
Binary					
4	0.18	0.21	0.35	0.46 (0.20–0.74)	0 (0–0.05)
5	0.16	0.17	0.34	0.18 (0.002–0.36)	0 (0–0.06)
6	0.15	0.16	0.31	0.42 (0.09–0.78)	0 (0–0.09)
7	0.09	0.10	0.20	0.49 (0.06–0.94)	0 (0–0.04)
8	0.11	0.14	0.29	0.55 (0.08–1.04)	0 (0–0.20)
9	0.122	0.124	0.334	0.175 (0–0.614)	0 (0–0.199)
10	0.107	0.108	0.383	0.010 (0–0.554)	0 (0–0.358)

Table 1 Phylogenetic signal of the plant–arbuscular mycorrhizal fungi (AMF) interaction using quantitative and binary matrices

MSE_d, mean squared error calculated for the full model; MSE_{star}, a ‘star’ phylogeny; MSE_b, a Brownian evolution model; d , strength of phylogenetic signal for both plant (d_{plant}) and AMF (d_{AMF}).

When an AMF OTU’s membership of a particular module is mapped onto the AMF phylogeny (Fig. 2), a significant phylogenetic signal emerges for every cutoff from 4 to 9% (Table 2), indicating that closely related AMF tend to belong to the same module. In the case of plants (Fig. 2), the membership of a given module does not show a phylogenetic signal (Table 2), indicating that phylogenetically related plant species do not tend to belong to the same module. The convergence of results considering the phylogenetic signal in the plant–AMF interaction and phylogenetic conservatism of a module membership, strengthens the conclusion that AMF phylogeny within Glomeraceae influences their pattern of interaction with plant species but that this is not the case in plants.

The strength of plant–plant facilitation interactions was significantly positively correlated with dissimilarity in their fungal-niche. Pairs of plant species in which facilitation during their adult stage was recorded more frequently, tended to differ in their plant fungal-niche (r range for cutoffs 4–10% = 0.43–0.53 for quantitative interaction matrices; r range = 0.47–0.53 for binary interaction matrices; $P < 0.01$ for every correlation) (Fig. 3). The null matrix was the worst predictor of the observed strength of the plant–plant facilitation matrix (Table 3). Plant relative abundance alone was a better predictor of the strength of plant–plant facilitation than dissimilarity in the plant–fungal niche alone, but, interestingly, the best predictor was the matrix combining the plant relative abundance and dissimilarity in plant–fungal niche probabilities.

Table 2 Phylogenetic signal of the module membership for cutoffs of 4–10% genetic dissimilarity in DNA sequences

Cutoff %	N° of modules	AMF ^a		Plant ^a	
		Observed transitions	Mean null (P-value)	Observed transitions	Mean null (P-value)
4	6	18	27***	19	19 ^{ns}
5	8	14	22***	21	22 ^{ns}
6	7	12	19***	22	22 ^{ns}
7	6	10	15***	17	16 ^{ns}
8	6	11	15***	17	16 ^{ns}
9	6	10	13**	18	17 ^{ns}
10	5	8	9 ^{ns}	16	15 ^{ns}

^aFor both arbuscular mycorrhizal fungi (AMF) and plants, the following are shown: the number of modules detected at each cutoff, the number of observed evolutionary transitions in the module membership, the expected number of transitions under a null model in which data were reshuffled 1000 times across the tips of the phylogeny, and the P-value based on the comparison of observed vs expected values (***, $P < 0.001$; **, $P < 0.01$; ^{ns}, $P > 0.05$).

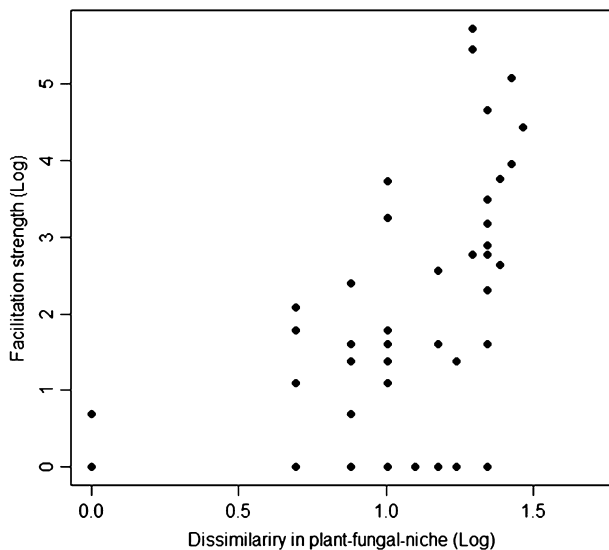


Fig. 3 Relationship between strength of plant–plant among pairwise plant species and their dissimilarity regarding the arbuscular mycorrhizal fungi (AMF) with which they interact. Data are presented for the representative cutoff of 7% of genetic dissimilarity to define AMF operational taxonomical units (OTUs) after log transformation of plant–plant facilitation and dissimilarity plant–fungal-niche matrices. The strength of facilitation among each pair of plant species was estimated as the number of adults on each facilitated species recorded under each nurse plant species (Verdú *et al.*, 2010), and the dissimilarity in plant fungal niche was calculated based on the composition of AMF interacting with each plant species. The correlation coefficient between matrices was calculated against a null model, randomizing plant–fungal-niche dissimilarities among plant species ($r = 0.48$; $P < 0.001$).

The combined effect of plant relative abundance and dissimilarity in plant–fungal niche was significantly better than abundance alone at every cutoff when quantitative plant–fungal matrices were considered and also when binary interaction matrices were used, except for the cutoff of 10% (Table 3). The combined matrix

Table 3 The effect of plant abundance, dissimilarity in fungal-niche and their interaction on plant facilitation using quantitative and binary fungal niche matrices

		Likelihood		AIC	
(a) Observed (1)		45.72		93.44	
(b) Null (1)		169.20		340.41	
(c) Abundance (1)		139.69		281.37	
(d) Plant–fungal niche (1)		(e) Abundance × plant–fungal niche (2)			
Cutoff %	Likelihood	AIC	Cutoff %	Likelihood	AIC
Quantitative					
4	150.86	303.73	4	126.51	257.02
5	150.43	302.86	5	126.15	256.31
6	149.86	301.73	6	125.73	255.47
7	145.45	292.91	7	122.55	249.09
8	145.47	292.94	8	122.56	249.11
9	145.40	292.79	9	122.50	249.00
10	140.77	283.54	10	120.61	245.22
Binary					
4	147.19	296.38	4	122.74	249.47
5	147.74	297.49	5	123.26	250.51
6	144.17	290.34	6	120.98	245.97
7	148.43	298.87	7	128.53	261.07
8	149.10	300.21	8	129.10	262.20
9	146.06	294.12	9	127.39	258.77
10	191.92	385.84	10	176.33	356.66

Matrices were log-transformed and the likelihood and Akaike information criteria (AIC) are presented for: (a) an observed model, using the same matrix as a predictor; (b) a null model in which all pairwise interactions were equally probable; (c) interaction probability determined solely by relative species abundance, or (d) solely fungal niche, for each cutoff; and (e) the interaction of abundance and fungal niche matrices, for each cutoff. The number of parameters of each model is presented in parentheses. The AIC weights of each candidate model with respect to the best-fit model are < 0.05 except for abundance at the cutoff 10% (AIC weight = 0.31).

represents the interaction probabilities expected if the species interact proportionally to both their relative abundance and dissimilarity in plant fungal-niche. Although this combined matrix was the best predictor, it should be noted that much variation still remains unexplained, as the differences in AICs compared with the observed model suggest, indicating that other factors besides these two are contributing to the strength of plant–plant facilitation.

Discussion

Our results show that closely related AMF OTUs tend to interact with the same set of plant species while the similarity in plant fungal-niches of two plant species is independent of their phylogenetic relatedness. We further show that, although facilitation is more frequent among the most abundant plant species, this trend is significantly modulated by plant species' fungal-niche. There is a tendency of plant–plant facilitation specificity to occur among plant species that differ in their fungal-niche, resulting in stronger facilitation between pairs of plant species with different associated AMF. We argue in the following that this might be a potential mechanism to increase AMF diversity in the shared

rhizosphere which, by means of complementary beneficial effects of each AMF, can provide a more efficient exploitation of soil nutrients.

Previous studies have looked at conservatism in plant–fungal interactions, considering mainly ectomycorrhizal associations within a particular plant phylogenetic clade, orchids (Shefferson *et al.*, 2007, 2010; Jacquemyn *et al.*, 2011; Martos *et al.*, 2012). In this context, closely related plant species tend to interact with the same fungi, but closely related fungal species either do not share the same plant hosts (Jacquemyn *et al.*, 2011) or their phylogenetic signal is weaker than the plant's phylogenetic signal (Martos *et al.*, 2012). Although our results seem to challenge these previous results, it is important to note that these studies focused on a particular plant phylogenetic clade, considering species that might not be co-occurring. Our study approaches, for the first time, plant–fungal interactions at the community level, resulting in a wider range of plant phylogenetic diversity by considering most of the coexisting plant species. In this framework, interestingly, closely related AMF tend to interact with the same plant species, and a plant phylogenetic signal is not detected. This suggests that, although the pattern of interactions between plant and mycorrhizal fungi is evolutionarily conserved within a particular plant clade, this pattern does not scale when broader plant phylogenetic diversity is considered. This could potentially be the result of convergent patterns of plant–fungal interaction across clades, but further studies with a community perspective, considering jointly a phylogenetic signal in the plant–AMF interactions, will be required to confirm this hypothesis. Furthermore, this study is based on a group of fungal species within the genus *Glomus*. Although plant–AMF interaction patterns described for this group of fungi can be generalized to a wider AMF phylogenetic diversity (Montesinos-Navarro *et al.*, 2012), further studies considering the phylogenetic community interactions of other groups of AMF will shed light on the understanding of coevolutionary patterns of plants and AMF. Nevertheless, the generality of host specificity in AMF remains speculative, with some plants showing repeatable AMF communities (Vandenkoornhuysen *et al.*, 2002) and others showing variation with habitat and environmental conditions (Aldrich-Wolfe, 2007). In addition, the taxonomic delimitation of AMF species is controversial and the AMF phylogenetic signal will be influenced by this limitation. Considering lower cutoffs to define OTUs can result in a phylogenetic signal either as a result of biological processes occurring at lower taxonomic levels or as an artifact of considering intraspecific variation as different OTUs interacting with the same plant species. Similarly, the AMF phylogenetic signal can disappear at higher cutoffs that could potentially merge different genera or families within a given OTU, which might avoid the detection of plant–AMF specificity occurring at lower taxonomic levels. Our results report a decrease in AMF phylogenetic signal at the highest cutoff (10%) and a consistent significant phylogenetic signal over the rest of the cutoff range. This suggests that the range of cutoffs considered might be covering a biologically meaningful range of interspecific variation.

It has previously been reported that phylogenetic conservatism in AMF traits can promote competition among closely related AMF species leading to a low contribution to plant biomass. Accordingly,

plant performance decreases with the phylogenetic relatedness of the AMF species in their roots (Maherali & Klironomos, 2007). In our system, the two most generalist AMF – those interacting with almost all plant species – are closely related, which, according to Maherali & Klironomos (2007), could result in fewer benefits to the plant. Species-specific patterns of plant–plant facilitation could compensate for this effect, increasing AMF diversity in the shared rhizosphere. One possible mechanism may be by promoting associations with plant species that differ in their fungal-niche, potentially affecting ecosystem properties such as productivity (Cadotte *et al.*, 2008). In this study we present correlative evidence to support the idea that plant–AMF interactions might be one of the underlying mechanisms influencing plant–plant facilitation. Functional complementarity of AMF promoting plant productivity and plant–plant interactions have so far been reported for AMF belonging to different families (Hart & Reader, 2002; Maherali & Klironomos, 2007; Powell *et al.*, 2009). Our results suggest, for the first time, that AMF phylogenetic diversity within the genus *Glomus* can also result in complementary functionality.

There is a tendency, supported by experimental (Castillo *et al.*, 2010) and comparative evidence (Valiente-Banuet & Verdú, 2007), for plant–plant facilitation to occur among distantly related plant species. Interestingly, our results show that the similarity in the associated AMF between a pair of plant species is independent of the phylogenetic distance between them. Accordingly, the observed match between plant–plant facilitation strength and fungal niche cannot be attributed to a passive process in which distantly related plant species tend to differentiate in their plant–fungal niche. Furthermore, the combination of relative abundance and dissimilarity in plant fungal-niche substantially improves the prediction of facilitation specificity compared with plant species' relative abundance alone. Taking all this together, it suggests that, among the possible plant–plant facilitation interactions (i.e. most abundant and distantly related species), the strength of facilitation increases when the involved plant species have a higher fungal-niche dissimilarity. Consequently, facilitation also occurs between distantly related plant species with similar plant fungal-niche, but these interactions present weak facilitation strength. Specific plant–plant facilitation may be the result of facilitating species which differ in their overall fungal niche, increasing AMF diversity and adding the beneficial effects of each AMF species (Van der Heijden *et al.*, 1998; Hartnett & Wilson, 1999; Wagg *et al.*, 2011). At the same time, plant species differing in their plant–fungal niche might be weaker resource competitors, and natural selection might positively select for these plant–plant interactions. Previous studies support the idea that both AMF host selectivity and plant fungal-niches can influence the emergent pattern of species-specificity in plant–AMF interaction, potentially influencing plant–plant facilitation. AMF community composition can be highly influenced by the initial establishment of certain plant species (Hausmann & Hawkes, 2010), and seedling success can be affected by the presence of established AMF networks (Kytoviita *et al.*, 2003; Van der Heijden, 2004). Besides plant–AMF interactions, other mechanisms can be underlying the observed correlation between plant facilitation strength and plant fungal niche. For example, an increase in AMF diversity in the rhizosphere can decrease the

presence of plant pathogens (Van der Putten, 2009), resulting in more complex indirect effects underlying plant community assemblages.

Although our results are in agreement with previous information on species-specificity in plant facilitation, experimental studies will be required to ultimately test for the specific biotic below-ground mechanisms underlying plant–plant facilitation. Novel research lines are derived from our results exploring the potential implications of AMF networks in structuring plant community assemblages. Plants and AMF can regulate the resource allocation to the partner, depending on the benefit received (Kiers *et al.*, 2011). If specific plant–plant facilitation allows more efficient nutrient uptake (Van der Heijden *et al.*, 1998; Hartnett & Wilson, 1999; Wagg *et al.*, 2011) through an increase in AMF diversity, plants may increase their contribution to the plant–AMF mutualism, resulting in a higher resource allocation from AMF to the plants sharing a specific rhizosphere. Exploring the role of plant–AMF interaction as a potential mechanism promoting plant–plant facilitation specificity will contribute to a better understanding of the assembly rules in plant communities.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 The best-scoring maximum likelihood arbuscular mycorrhizal fungi tree inferred from 100 bootstrap trees.

Fig. S2 Phylogeny of the plant species based on the megatree of the Angiosperm Phylogeny Group 3 (Stevens, 2005) as the backbone phylogeny.

Table S1 Genbank accession numbers of all sequences used in the analyses

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