1 The network structure of plant-arbuscular mycorrhizal fungi

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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 expanding the generality of this pattern to the ubiquitous plant-AMF mutualism.

• Based on certain plant-AMF specificity recently suggested, networks are expected to be nested due to their mutualistic nature, and modular, with certain species interacting more tightly than others. Network analyses were used to 1) test for nestedness and modularity and 2) compare the different contribution of plant and AMF to the overall nestedness.

• Plant-AMF share general network properties with other mutualisms. Plant species with few AMF in their roots tend to associate with those AMF recorded in most plant species. AMF present in 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 plant and AMF show a nested structure, although AMF have lower nestedness than plants.

• Plant-AMF interaction pattern is interpreted in the context of how plant-AMF associations can be underlying mechanisms shaping plant community assemblages.

Keywords: community assemblages, modularity, mutualism, nestedness, network, plant-arbuscular mycorrhizal fungi association.

Introduction

The association between arbuscular mycorrhizal fungi (AMF) 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 (Smith & Read 1997), while the plant provide carbon compounds to the AMF although, in some cases the equitability of resource exchange between plant and AMF could not be mutually beneficial (Johnson et al., 1997). A more efficient nutrient uptake due to 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 on the community (Grime et al., 1987; Van der Heijden et al., 1998a,b; Hartnett & Wilson, 1999) and bottom-up influence of AMF on plant community diversity has also been reported (Grime et al., 1987; Van der Heijden et al. 1998; Hartnett and 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 phosphorus 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 plant-AMF interaction pattern is a first step to explore 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 AMF-plant interaction pattern in a community, it is required 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 of a few -usually the most common- plant species rhizosphere in the community (Daniel et al., 2001; Zaoyong et al., 2006; Kottke et al., 2008; Alguacil et al., 2009; Sonjak et al., 2009; Wilde et al., 2009; see Öpik et al., 2010 for further references) or by exploring the influence of plant community on final AMF composition (Mummey et al., 2005; Hausmann & Hawkes 2009) using artificial (i.e. experimental) communities (Van der Heijden et al., 1998a; 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 non-random plant-AMF interaction patterns.

AMF are constituted by fungi of the phylum Glomeromycota, one of the key taxa inter-connecting 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), fewer than 200 species of these globally important fungi have been described (Morton & Benny, 1990). The apparent low diversity of AMF 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, 2001, Eom et al., 2000) and there is 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 & Rilling 2008; Öpik et al., 2008; Smith & Read 2008; Li et al., 2010; Davison et al., 2011). This shift has been influenced by the larger amounts AMF of diversity revealed by the use of molecular techniques, with higher resolution for distinguishing closely related species (Bever et al., 1996; Eom et al., 2000). However, the different amount of intra-specific genetic variation depending on the family, genus and species prevents the determination of a generalized genetic threshold to delimitate AMF species (Nilsson et al., 2008; Redecker et al., 2003; Rosendahl, 2007). The increasing knowledge about AMF diversity and availability of molecular tools to approach it, offers a unique opportunity to explore plant-AMF interaction pattern in natural communities.

Network analysis is a convenient technique to detect non-random 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 has been rarely applied to fungal communities (but see Vacher et al., 2008 and Peay et al., 2007) 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 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 AMF 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 from a network perspective will provide the opportunity to test two hypotheses. First, if plant-AMF interaction pattern matches the predictions developed by network theory based on other mutualistic systems. Second, if the non-random plant-AMF interactions previously suggested using a low number of species, are also reflected 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 re-analyze 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 cut-off) and, for each of them: 1) describe the network testing for nestedness and modularity and 2) compare the different contribution of plant and AMF to the overall nestedness. Finally we estimate the relative contribution of plant species abundance to the observed interaction pattern. We present 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

157 Study area and plant sampling

158 This study was conducted in the semiarid Valley of Zapotitlán (18º 20´N, 97º

159 28´W), a local basin of the Biosphere reserve of Tehuacán-Cuicatlán Valley in the state

160 of Puebla, Mexico. This region owes its aridity to the rain shadow produced by the

161 Eastern Sierra Madre (Valiente-Banuet et al., 2000). It has an annual average rainfall of

162 380 mm, most of which falls during the summer months, and an annual mean

163 temperature of 21º C with rare frosts (García, 1973). Specifically, the study site is

164 located approximately 30 km south of Tehuacán city in a natural area which vegetation

165 is a xeric shrubland (woody perennial species) dominated by the columnar cactus

166 Neobuxbaumia tetetzo, Agave spp, different Fabaceae and Asteraceae species, among

167 other taxa. The vegetation is characterized by individuals of multiple species spatially

168 associated forming discrete vegetation clumps, although some isolated individuals can

also be found. Vegetation clump areas range from 1 to 5 m

2

169 .

170 Phosphorus concentration in soils at each vegetation clump is very low ranging

171 from 2 to 19 mg/kg, mean= 5.37± 0.44 SE (Lugui Sortibrán, unpublished data).

172

173 Arbuscular mycorrhizal fungi

174 The phylum Glomeromycota (i.e. AMF) is divided in four orders, with most described

175 species belonging to the Glomerales and Diversisporales (Schüßler et al. 2001). Glomus

176 is the largest genus in the phylum, with more than 70 morphospecies (Redecker &

177 Raab, 2006), with Glomus group A accounting for much of this diversity. Glomus

178 dominates AMF communities in many field settings, where 70% of the AMF have been

179 identified as Glomus (range 60%-85%) (Helgason et al., 1998; Öpik et al., 2009;

180 Vandenkoornhuyse et al., 2002; Zaoyong et al., 2006; Alguacil et al., 2009; Sonjak et

181 al., 2009; Wilde et al., 2009; Öpik et al., 2010), and shows the highest root colonization

182 rates among the Glomeromycota taxa (Hart & Reader, 2002). There is come controversy
183 on considering that AMF have dispersal limitation (Lekberg et al., 2007, Dumbrell et
184 al., 2010) or can disperse at the scale of kilometres, recording different dispersal vectors
185 such as animals (Lekberg et al., 2011; Janos & Sahley, 1995; Mangan & Adler, 2000),
186 wind (Warner et al., 1987) and land movements associated with agriculture (Rosendahl
187 et al., 2009). Glomus species are the most common taxa recorded in these dispersal
188 studies.
189
190 Root sampling
191 We performed a plant sampling scheme aimed at including most of the plant
192 species in the community and reflecting the relative abundance of each species sampled.
193 A total of 34 vegetation clumps with 1 to 8 plant species (average 2.7) were sampled
along two transects of 500 m
2
194 each. A total of 130 individuals of 37 plant species
195 representing 66 % of all the species in the community, were sampled (see species in
196 Table 1). Rarely a vegetation clump had more than one individual of the same species;
197 in those cases only one of the individuals was sampled. We have considered relative
198 abundance as an intrinsic characteristic of each plant species in a given community that
199 can influence its interaction pattern with other species. Accordingly plant-AMF
200 interaction matrices should be built from surveys that reflect relative abundance of each
201 species. The root tips were unearthed, cut and dried with silica gel for further DNA
202 extraction.
203
204 DNA extraction
205 The youngest tips of the non-lignified roots were selected from plant samples as they
206 often show a higher proportion of Glomeromycota colonization. Root tips were cut and

207 placed in 2 ml Eppendorf tubes with 2.3 mm stainless steel beads. Then root tissues
208 were pulverized on a Retch MM400 (Biometa) tissue lyser.
209 Total DNA was extracted using the DNeasy plant minikit (Qiagen, Barcelona,
210 Spain) with the addition of 0.33% final concentration of PVP40 to buffer AP1, which
211 facilitated the elimination of some PCR inhibitor compounds and then followed
212 subsequent instructions according to the manufacturer. As these extracts contained a
213 mixture of DNA from fungi and the host plant, DNA quantification was routinely
214 omitted and crude extract were used for subsequent PCRs.
215
216 Glomeromycota Internal Transcribed Spacer (ITS) amplification and sequencing
217 A nested PCR protocol was used for the amplification of the samples. Primary PCR
218 amplified the whole ITS region, including ITS-1, 5.8S and ITS-2. This was conducted
219 in 25 µl volume including 1 × Taq Buffer, (Biotools, Madrid, Spain) 3mM MgCl2, 0.5
220 mM each dNTP, 0.4 mg/ml BSA, 12.5 pmol each of NS5 (forward) and ITS4 (reverse)
221 primers of White et al. (1990), 1U of Taq DNA polymerase and 1 µl of crude DNA
222 extract. The PCR program consisted of an initial DNA melting step of 3 min at 95ºC
223 followed by 30 cycles each of 30 sec at 95ºC, 30 sec at 51ºC for annealing and 2 min at
224 72ºC for extension. After a final extension step of 10 min at 72ºC PCRs were kept at
225 4ºC. One µl of this PCR was used as template for the nested PCR. Four primer-pair
226 combinations were assayed for the nested PCR in an attempt to detect as much diversity
227 as possible of Glomeromycota. PCR cocktail was identical to that of the primary PCR
228 except for the primer-pair used which included Forward/Reverse, Glom1310/ITS4i,
229 (Redecker, 2000; Redecker et al., 2003) for the amplification of Glomus group A
230 (Schübler et al., 2001); LETC1670/ITS4i (Redecker, 2000), for the amplification of
231 Glomus group B (Schübler et al., 2001); NS5/GIGA5.8R (Redecker, 2000), for the
232 amplification of Gigasporaceae and, ACAU1660/ITS4i (Redecker, 2000) for the
233 amplification of Acaulosporaceae. The PCR program consisted of an initial DNA

234 melting step of 3 min at 95ºC followed by 30 cycles each of 45 sec at 95ºC, 50 sec at
235 56ºC for annealing and 1.5 min at 72ºC for extension. After a final extension step of 10
236 min at 72ºC PCRs were kept at 4ºC. PCR products were checked on 1% agarose gels.
237 PCR protocols were optimized for two of these groups of AMF with available axenic
238 cultures of Glomus group A and Gigaspora sp. Of these four primer-pair combinations
239 no amplification was obtained for the families Gigasporaceae and Acaulosporaceae.
240 Less than 30% amplification success was obtained for Glomus group B primer-pair,
241 whereas for the primer-pair of Glomus group A a 78.21% success was achieved
242 suggesting a predominance of this group of Glomus in the AMF communities in the
243 study area. Subsequent sequencing of PCR products was continued only with this
244 monophyletic group of Glomus.
245 Positive amplifications of the expected size were cloned into pGEM-T easy vector
246 (Promega) and transformed onto X-Gal, IPTG Ampicilin, LB Agar plates. Positive
247 colonies were screened with T7 and SP6 vector primers for inserts of appropriate size,
248 then cultured for miniprep plasmid extraction (Roche) and sequenced with the BigDye
249 Terminator Cycle Sequencing Kit (Applied Biosystems). Sequencing was performed by
250 Macrogen Inc., Seoul Korea. Forward and reverse sequences were compared, assembled
and corrected where necessary using SEQUENCHER
®
251 (GeneCodes corp., Michigan,
252 USA), thus establishing the consensus sequence of each sample. BLAST searchers were
253 performed to reliably assign sequences to AMF. BLAST searches were performed on
254 forward, reverse and consensus individual sequences in order to detect possible
255 chimeras (Schechter & Bruns, 2008). Only those that matched a Glomeromycota
256 sequence in both forward and reverse sequences and rendered high bit scores (>1300)
257 and low E values in the consensus sequences were selected for the analysis. This
258 procedure is especially suitable for pair-wise comparisons of sequence from closely

related species. In this 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 cut-off values, which spanned 1%-10% of their sequence being different. This approach seems reasonable since species concepts are difficult to apply in AMF (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 sequence sampled. Rarefaction curves were performed for each cut-off. 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 cut-off, 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 bipartite package for R (Blüthgen et al., 2006).

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 NODF (nested overlap and decreasing fill) (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).

NODF 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 OTUs richness and also in the number of links. Relative NODF is defined as NODFobserved– NODFnull model / NODFnull model , where NODFobserved

is the nestedness of the actual matrix and NODFnull model 313 is the average nestedness of
314 random replicates generated from null model.
315 NODF was also calculated independently for rows (plant species) and columns
316 (AMF OTUs) and the statistical significance assessed comparing against the null model
317 II. Nestedness metrics are influenced by rows and columns order in the matrix. In order
318 to make our results comparable with previous studies, rows and columns were ordered
319 by interaction abundance before the calculation of nestedness metrics. Nestedness
320 metrics were calculated with the help of the software ANINHADO (Guimarães &
321 Guimarães, 2006).
322 Modularity reflects that there are groups of species that tend to interact more
323 within species in the same group than expected by chance. Nodes of a network can be
324 grouped into modules, in such a manner that the number of links within modules is
325 maximized and that between-modules is minimized. A simulated annealing
326 optimization approach was used to detect modules that maximized modularity (i.e.
327 proportion of links within vs. between modules) (Guimerà & Amaral, 2005a,b). Because
328 of its heuristic nature, 10 runs of the algorithm were conducted for each cut-off but the
329 variation in modularity was negligible (SE of the modularity across the 10 runs ranged
330 from 0.0190 to 0.0192 across the different cut-offs). We report the maximum value of
331 modularity obtained in the 10 runs. Although our network is bipartite we used a
332 modularity algorithm for unipartite networks. Because our plant-AMF network is a two
333 party network, one could conclude that an algorithm for two-party network (i.e.
334 bipartite) should be more appropriate. However, this decision depends on the question
335 addressed. For example, algorithms for modularity in a bipartite network search for
336 independent groups of plants (or AMF) that share a similar interaction pattern (i.e. that
337 interact with the same AMF (or plants). Meanwhile, algorithms searching for
338 modularity in an unipartite network, identify mixed groups of plants and AMF tightly
339 interrelated that tend to interact more among them (see Olesen et al., 2007 for a more

340 detailed explanation). As we are interested in the groups of plants and AMF that are
341 highly connected to each other, rather than in groups of plants or/and AMF created as a
342 function of their shared interactions, we used algorithms searching for modularity in
343 unipartite network (see Fortuna et al., 2010 for the appropriateness of this method).
344 However, we also tested for modularity using a bipartite network and the results
345 consistently showed that our networks were composed by modules usually grouping the
346 same species grouped by the modules in the unipartite approach (see Olesen et al., 2007
347 for a similar comparison). Only modularity for unipartite network is reported.
348 Modularity significance was tested by comparing it to the null case of modularity
349 calculated using 100 random graphs with the species ranked according to their degree
350 distribution in the original network (Guimerà et al., 2004). Modularity was calculated
351 and its significance tested using the software Netcarto (Guimerà & Amaral, 2005a,b;
352 Guimerà et al., 2004).
353 In order to provide generalization to our results, we compared them with other
354 studies in which plant-AMF interactions had been intensively surveyed at the
355 community level. After inspecting the 138 studies cited in MaarjAM database (Öpik et
356 al., 2010) and relevant references within them, we only found two studies which aim to
357 survey most of the plant species in the community: Davison et al. (2011) and Öpik et al.
358 (2009). These studies are not independent as they share data from the same site. In these
359 studies 10-11 plant species were used and 40-51 AMF OTUs recorded, belonging to
360 Glomeraceae, Gigasporaceae, Acaulosporaceae and Diversisporaceae. We re-analyzed
361 their data calculating the same nestedness and modularity estimates as described for this
362 study.
363 Although the qualitative analyses described above only consider the presence or
364 absence of an interaction, a species number of interactions can be highly influenced by
365 its abundance, which might have an effect on nestedness (Vázquez, 2005) and
366 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 to interact with a higher number of AMF, because more individuals will be sampled of this plant species. However, a plant species tendency to interact with a given numbers 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 non-random 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 due to 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 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

394 the number of individuals sampled and AMF load per plant species were used as
395 independent variables. The relative contribution of plant relative abundance and AMF
396 load to explain the variance in the number of plant species links in the network was
397 estimated as the ratio of the standard deviations of the two effects, as implemented in
398 the relimp package for R (Silber et al., 1995).

400 Results

401 AMF OTU definition and rarefaction curves

402 Positive amplification for Glomus group A was detected in 103 out of the 130 plants
403 sampled (79.23%) (Table 1). Positive amplification was obtained from at least one of
404 the individuals sampled for each species except for Mammillaria haageana and
405 Jatropha neopauciflora (Table 1).
406 A total of 95 out of the 1909 sequenced clones (4.98%) produced unreadable sequences,
407 251 (13.15%) corresponded to other co-amplified fungi, 40 (2.10%) to chimeric
408 sequences, and 1523 (79.77%) to Glomeromycota with BLAST scores above 1300.
409 Further analyses were based on this subset of 1523 sequences (Genbank numbers
410 JN194215 to JN195737).
411 The number of different AMF OTUs varied depending on the predefined cut-off
412 values of genetic dissimilarity (Fig. S1). Rarefaction curves showed that for the cut-off
413 which grouped together sequences with a genetic difference smaller than 1% (Fig. S1a),
414 163 AMF OTUs were identified out of 1523 sequences. Rarefaction curves did not
415 reach the stabilization until the cut-off value of 5% (Fig. S1e) and more strictly at 8%
416 (Fig. S1h). For the 5% and 8% cut-offs 34 and 23 different OTUs were identified,
417 respectively. The cut-off which grouped together sequences with genetic difference
418 smaller than 10% identified 14 AMF OTUs out of 1523 sequences (Fig. S1j).
419

420 Plant-AMF networks

421 The number of plant species in the network was 35 and the number of AMF OTUs

ranged from 163 to 14 depending on the cut-off value considered (Table 2). Ten networks (i.e. one network per cut-off) were built grouping within an OTU sequences in ten 1% increments in dissimilarity intervals (i.e. 1 to 10%). Approximately 11% of the possible interactions between plant species and AMF OTUs were actually realized (average connectance across different cut-offs: 11.5 ± 0.01, mean ± SE) (Fig. 1, see supporting information for all the other cut-offs Fig. S2-S10).

All ten networks were significantly more nested than expected by chance, both considering the overall network and rows and columns independently (for all cases $p < 0.001$, Table 2). The degree of nestedness was independent of the cut-off 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 higher than 92 (Table 2).

When plant species and AMF OTUs are analyzed 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 twice higher than the AMF OTUs nestedness (Table 2) indicating that this pattern is stronger for plants than for AMF.

All networks except for the cut-off of 10% show a significant modularity (Table 2). For the 10% cut-off 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 less than 50 species it is likely the detection of just one single module. Thus, for all the cut-offs in which modularity was detectable we found significant values of modularity. The number of modules varies from 5 to 9 across the different cut-offs and the average number of nodes within a module range from 9 to 22 (Table 2). Plant and AMF species ascribed to different modules co-exist in the same vegetation clump, with an average of 2.6 (range 1-5) modules per vegetation clump.

447 In this study we only found AMF belonging to Glomeraceae, but the observed
448 pattern of plant AMF interaction might vary in other systems with higher AMF
449 phylogenetic diversity. In order to explore the variability of the observed network
450 pattern across other AMF phylogenetic diversity scenarios, we compared our result with
451 two other studies in which other less-abundant families of AMF have been recorded (i.e.
452 Gigasporaceae, Acauloesporaceae, Diversisporaceae). Both networks presented in Öpik
453 et al., 2009 and Davison et al., 2011 show significant nestedness in the overall network
454 and also for plants and AMF independently (relative NODFt= 0.25-0.28 NODFp= 0.50-
455 0.54 NODFg= 0.26-0.24; all $p < 0.05$)(Fig. S11 supporting information). In addition, in
456 these two networks the nestedness for plants was also stronger than nestedness for
457 AMF. Accordingly, regarding nestedness, our results which have only detected one
458 group of the most abundant AMF were concordant with their results which recorded a
459 broader AMF phylogeneic diversity. However, the connectance in those two networks
460 was higher than in our study (42 % - 41 %) and no significant modularity was observed.
461
462 Relative contribution of plant abundance to plant species number of AMF interactions
463 The mean number of plant individuals per species ranged from 1 to 18 (mean = 2.9 SD
464 = 3.8). Plant species has on average an AMF load of 3.6 AMF OTUs per individual (SD
465 = 1.5; range = 1-7) considering the cut-off of 1% and an average of 1.3 (SD = 0.5; range
466 = 1-3) considering the cut-off of 10%, with the estimates for the rest of the cut-offs
467 contained within the values presented. Dixitalis guatemalensis and Cathestecum
468 brevifolium were consistently the species that had higher AMF load across cut-offs and
469 Thompsonella minutiflora, Echynopterix eglandulosa and Mammillaria casoi the
470 species with the lowest AMF load. Both plant species abundance and AMF load have a
471 significant effect on plant species degree, for every cut-off considered (Table 3). The
472 relative contribution of plant relative abundance and AMF load to plant species degree
473 is similar for cut-offs of 4-10% (Table 3). Only in the lower cut-offs, such as the 1%,

474 plant relative abundance can explain 1.7 times more variation in plant species degree
475 than AMF load (Table 3).
476
477 Discussion
478 In this paper we present the network properties of a plant-AMF mutualistic system. Our
479 results show a non-random interaction pattern in plant-AMF associations with a
480 network with low connectance, highly nested and modular. As expected, 1) the
481 nestedness values observed are concordant with other mutualistic networks (Bascompte
482 & Jordano, 2007) and 2) the modularity detected reinforces the hypothesis that
483 selectivity in plant-AMF interactions can result in emergent patterns at the community
484 level.
485 Our gradient of cut-offs (1-10%) adequately characterizes both intra-specific and
486 inter-specific variation for Glomeromycota. The average of intra-specific ITS variability
487 in this taxon is 7.46 % (SD 4.14), with some examples of intra-specific variation of
488 8.7% and 5.9% variability in Glomus intraradices and in Glomus mosseae (Nilsson et
489 al., 2008). The stabilization of rarefaction curves between cut-offs close to intraspecific
490 variation of the taxa (5%, or more strictly 8%), indicates that our sampling captured a
491 considerable amount of the total diversity of the Glomus A group of AMF present in the
492 area. Finding consistent network properties across cut-offs supports that the network
493 structure is maintained independently of the genetic differentiation threshold considered
494 to define AMF OTUs. Regarding comparisons with other studies re-analyzed in this
495 paper, the presence of nestedness seems to be a consistent pattern although there is high
496 variability in its strength across different plant-AMF communities. Modularity in plant-
497 AMF networks seems to be a less consistent pattern across sites and potentially
498 influenced by the level of connectivity in each community. Interestingly, although both
499 plant and AMF show a nested structure, plants have a stronger pattern of nestedness
500 than AMF. We discuss these results in turn below.

In general, mutualistic networks are characterized by having low connectance and being highly nested (Bascompte et al., 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 present 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 AMF, has been previously reported for plant-AMF systems (Davison et al., 2011). Our reanalysis of this and another, related study (Öpik et al. 2009) indeed show significant nestedness, suggesting that this maybe a general pattern in plant-AMF networks.

Ecological networks with low connectance and that are 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 we have analyzed 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. A greater amount of studies approaching plant-AMF community interactions will be needed to elucidate a general pattern regarding the level of connectance and its influence on modularity in different communities.

The non-random pattern of plant-AMF interactions observed at the community level can be produced by diverse mechanisms. 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.

528 Modularity can emerge from processes such as habitat heterogeneity resulting in
529 non random species spatial distribution (Olesen et al., 2007). However, in this system
530 species from different modules coexist in the same vegetation clumps suggesting that
531 species distribution is not constraining plant-AMF interaction patterns. The modularity
532 found at the community level is consistent with previous studies which have confirmed
533 both qualitative (Ravnskov & Jakobsen, 1995) and quantitative (Bever et al., 1996;
534 Streitwolf-Engel et al., 1997; Eom et al., 2000) selectivity in AMF and plant
535 interactions.
536 In our study we have only detected AMF belonging to the Glomeraceae. However,
537 other orders may be present in the area in a different season or in different habitats such
538 as soil (spores) vs. roots (Camargo-Ricalde et al., 2003). In a more phylogenetically
539 diverse AMF community new interactions will be found, which might affect the
540 network structure. However, our results show that in other communities with higher
541 AMF phylogenetic diversity, nestedness is maintained. This is because the AMF taxa
542 missing in our study, Gigasporaceae, Acaulosporaceae and Diversisporaceae, tend to
543 interact with generalist plant species (Fig. S11 supporting information). However,
544 because our results are based on very few communities, further studies are required to
545 confirm that the observed pattern can be generalized to overall plant-AMF associations.
546 Relative abundance of plant species can also lead to a non-random interaction
547 pattern as AMF OTUs with few interactions will have a higher probability to interact
548 with abundant plant species than with scarce ones. Our results show that although both
549 plants and AMF present a nested structure, plants have a stronger pattern of nestedness
550 than AMF. A potential explanation for this difference might be that sampling is
551 different for plants and AMF. While we can observe plants and account for its relative
552 abundance in our survey, AMF sampling has followed a blind procedure, and it does not
553 necessarily represent relative abundances precisely. Our data support that species
554 relative abundance is significantly contributing to the network structure, however it has

555 been shown that species abundance cannot fully explain the observed interaction
556 pattern, and its relative importance is similar to other ecological processes.
557 An alternative explanation for differences in plant and AMF nestedness is that
558 mutualistic networks can result in a lack of nestedness as a result of a balance between
559 mutualism and competition. Under this particular situation, competition can force
560 generalist species to become more specialists (Ricciardi et al., 2010). A combination of
561 mutualism and competition might well be happening in plant-AMF systems (Husband et
562 al., 2002). However, while AMF compete for root space with other AMF when they
563 interact with a generalist plant species (i.e. with a high AMF load), this is not true for
564 the case of a plant interacting with a generalist AMF taxa.
565 The overall network nestedness suggested a higher importance of mutualism over
566 competition in AMF and plant communities interactions. These mycorrhizal networks
567 established among plants inhabiting multi-specific vegetation clumps could alleviate
568 neighbor plants competition promoting plant-to-plant facilitation (Castillo et al., 2010,
569 Verdú & Valiente-Banuet, 2011). Plant species inhabiting a more diverse phylogenetic
570 neighborhood can benefit from a higher AMF diversity in their rhizosphere (Maherali &
571 Klironomos, 2007). This pattern might help to explain the phylogenetic overdispersion
572 found in plant communities from environments where facilitation is a key process
573 establishing community assemblage (Verdú & Valiente-Banuet, 2008). In support to
574 this idea, nestedness and modularity have been shown to be influenced by species
575 phylogeny (Valiente-Banuet & Verdú 2007; Verdú et al., 2010; Verdú & Valiente-
576 Banuet, 2011; Rezende et al., 2009) and complementary traits (Rezende et al., 2009) in
577 other systems.
578 In conclusion, non-random patterns emerge from analyzing plant-AMF interactions
579 at the community level consistent with previous knowledge. Provided that plant-AMF
580 interactions are organized in certain groups of species within which interactions are
581 more frequent, new biological questions are generated by our results: Do species within

582 a module share certain traits?, phylogeny might be a reasonable proxy for phenotypic
583 variation across species as species with similar ecological relevant traits might be
584 closely related. Do plant and fungi phylogenies explain the observed interaction pattern?
585 Closely related species might tend to interact with the same partners resulting in closely
586 related species sharing membership to a network module. Answering these questions
587 will increase our understanding of the largely unknown potential influence of plant-
588 AMF co-evolutionary history in plant community assemblages.
589
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596

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TABLES

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 gr. A.

Table 2. Descriptive statistics of the Plant-Mycorrhizal fungus network.

				Nestedness								Modularity		
								Relative						
Cut-off %	N	I	C	T	$NODF_t$	$NODF_p$	$NODF_g$	T^a	$NODF_t$	$NODF_p$	$NODF_g$	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)	$0.42^{n.s}$

Cut-off %= percentage of dissimilarity used as cut-off. N= Number of OTUs of *Glomus*. I= Number of interactions. C=Connectance: 100 x I / (N x 35). T= nestedness calculated as matrix temperature. $NODF_t$=Nested overlap and decreasing fill for the overall matrix. $NODF_p$= NODF for plants. $NODF_g$= NODF for *Glomus*. Relative T: $T\text{-}T_{null\ model}/\ T_{null\ model}$ (see "methods" for details about the null model) and $NODF_{t,p,g}$: $NODF_{t,pg}\text{-}NODF_{null\ model}/\ NODF_{null\ model}$. 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 when n.s. is indicated. Number of plant species in every cut-off is 35.

Table 3. GLM testing for the effects of plant species relative abundance and AMF load on the species degree in the network.

Cut off %	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***

The columns heads indicate; cut off %: percentage of genetic sequence dissimilarity; R^2: pseudo-R^2 of the full model; Plant abundance and Individual AMF load effect: mean (standard error) and p-value * $p<0.05$, **$p<0.005$, *** $p<0.0001$ of each effect respectively. The relative importance of plant abundance/ individual AMF: ratio of the standard deviations of the two effects to plant species degree.

Figure legends

Figure 1. Bipartite network showing the interactions between plants (left) and AMF OTUs (right) obtained with the 7% the cut-off. Species are ordered from generalist (up) to specialist (bottom) and colours represent nodes included in the same module, with modules representing subset of species more tightly interconnected.

Supporting information

Figure S1. Rarefaction curves for the Glom1310/ITS4i ITS sequences at increasing dissimilarity levels ranging from 1% (a) to 10% (j) (i.e. cut-offs) based on 1000 randomizations. X axis indicates the level of sampling intensity and Y axis the average number of OTUs observed at a given sampling intensity value. The dashed lines of each plot represent the 95% upper and lower confidence intervals

Figure S2-S10. Bipartite networks for each cut-off (1-10% genetic differentiation, except 7% presented in the main text). Species are ordered from generalist (up) to specialist (bottom) and colours represent nodes included in the same module.

Figure S11. Interaction matrices of our data (cut-off 7%), Davison *et al*. (2011), and Öpik *et al*. (2009). Black and white cells represent presence and absence of the interaction respectively. In Grey: the AMF taxa not present in our study and the plant species in which Davison and Öpik studies differ.

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TABLES

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 gr. A.

Table 2. Descriptive statistics of the Plant-Mycorrhizal fungus network.

				Nestedness								Modularity		
								Relative						
Cut-off %	N	I	C	T	$NODF_t$	$NODF_p$	$NODF_g$	T^a	$NODF_t$	$NODF_p$	$NODF_g$	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)	$0.42^{n.s}$

Cut-off %= percentage of dissimilarity used as cut-off. N= Number of OTUs of *Glomus*. I= Number of interactions. C=Connectance: 100 x I / (N x 35). T= nestedness calculated as matrix temperature. $NODF_t$=Nested overlap and decreasing fill for the overall matrix. $NODF_p$= NODF for plants. $NODF_g$= NODF for *Glomus*. Relative T: $T\text{-}T_{null\ model}/\ T_{null\ model}$ (see "methods" for details about the null model) and $NODF_{t,p,g}$: $NODF_{t,pg}\text{-}NODF_{null\ model}/\ NODF_{null\ model}$. 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 when n.s. is indicated. Number of plant species in every cut-off is 35.

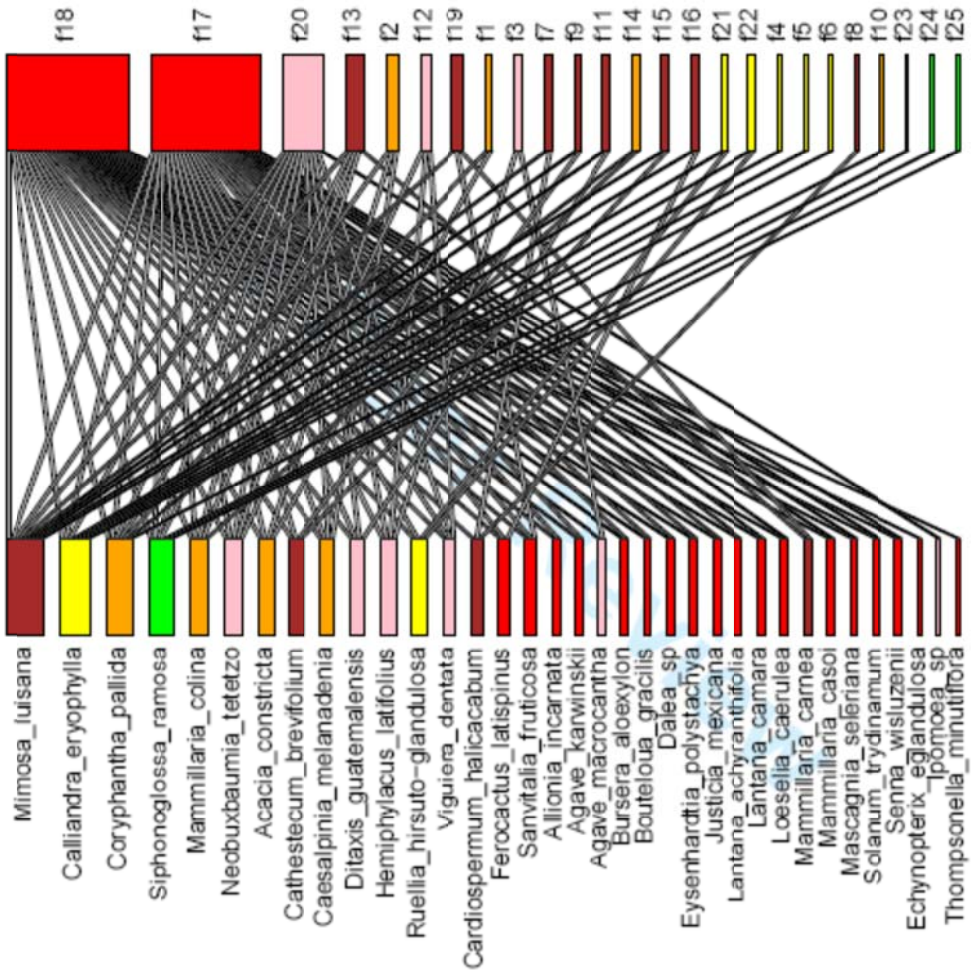
Table 3. GLM testing for the effects of plant species relative abundance and AMF load on the species degree in the network.

Cut off %	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***

The columns heads indicate; cut off %: percentage of genetic sequence dissimilarity; R^2: pseudo-R^2 of the full model; Plant abundance and Individual AMF load effect: mean (standard error) and p-value * $p<0.05$, **$p<0.005$, *** $p<0.0001$ of each effect respectively. The relative importance of plant abundance/ individual AMF: ratio of the standard deviations of the two effects to plant species degree.

Figure legends

Figure 1. Bipartite network showing the interactions between plants (left) and AMF OTUs (right) obtained with the 7% the cut-off. Species are ordered from generalist (up) to specialist (bottom) and colours represent nodes included in the same module, with modules representing subset of species more tightly interconnected.



Supporting information

Figure S1. Rarefaction curves for the Glom1310/ITS4i ITS sequences at increasing dissimilarity levels ranging from 1% (a) to 10% (j) (i.e. cut-offs) based on 1000 randomizations. X axis indicates the level of sampling intensity and Y axis the average

number of OTUs observed at a given sampling intensity value. The dashed lines of each plot represent the 95% upper and lower confidence intervals

Figure S2-S10. Bipartite networks for each cut-off (1-10% genetic differentiation, except 7% presented in the main text). Species are ordered from generalist (up) to specialist (bottom) and colours represent nodes included in the same module.

Figure S11. Interaction matrices of our data (cut-off 7%), Davison *et al*. (2011), and Öpik *et al*. (2009). Black and white cells represent presence and absence of the interaction respectively. In Grey: the AMF taxa not present in our study and the plant species in which Davison and Öpik studies differ.