

## 1 The network structure of plant-arbuscular mycorrhizal fungi

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Word counts: Introduction: 1320, Materials and Methods: 2827, Results: 925, Discussion: 1302, Acknowledgements: 54, Figures: 1, Tables: 3, Supporting information: 11 Figures.

## 22 Summary

23 • Ecological network theory predicts that in mutualistic systems specialists tend to  
24 interact with a subset of species with which generalists interact (i.e. nestedness).

25 Approaching plant-arbuscular mycorrhizal fungi (AMF) association using  
26 network analyses will allow expanding the generality of this pattern to the  
27 ubiquitous plant-AMF mutualism.

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

33 • Plant-AMF share general network properties with other mutualisms. Plant  
34 species with few AMF in their roots tend to associate with those AMF recorded  
35 in most plant species. AMF present in few plant species occur in plant species  
36 sheltering most AMF (i.e. nestedness). This plant-AMF network presents  
37 weakly interlinked subsets of species, strongly connected internally (i.e.  
38 modularity). Both plant and AMF show a nested structure, although AMF have  
39 lower nestedness than plants.

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

43

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

46

## 47 Introduction

48 The association between arbuscular mycorrhizal fungi (AMF) and plants improves the

49 fitness of both plant and AMF symbionts, constituting a traditionally considered  
50 mutualism (Blackwell, 2000). AMF increase plant uptake of soil nutrients, especially  
51 phosphorus (Smith & Read 1997), while the plant provide carbon compounds to the  
52 AMF although, in some cases the equitability of resource exchange between plant and  
53 AMF could not be mutually beneficial (Johnson et al., 1997). A more efficient nutrient  
54 uptake due to AMF associations can alleviate plant competition for mineral resources  
55 (Fitter, 1977; Allen & Allen, 1984; Hetrick et al., 1989; Moora & Zobel, 1996; Bever et  
56 al., 1997). An equitable distribution of soil resources among competitively dominant  
57 and subdominant host species might promote plant species coexistence (Walter et al.,  
58 1996; Malcová et al., 1999). Plant species associations with specific AMF taxa can  
59 ultimately influence AMF community composition on the community (Grime et al.,  
60 1987; Van der Heijden et al., 1998a,b; Hartnett & Wilson, 1999) and bottom-up  
61 influence of AMF on plant community diversity has also been reported (Grime et al.,  
62 1987; Van der Heijden et al. 1998; Hartnett and Wilson 1999) potentially mediated by  
63 plant-to-plant facilitation (Van der Heijden & Horton, 2009). Facilitation is a key  
64 process structuring plant communities in semiarid regions, where phosphorus soil  
65 availability can be limiting (Cross & Schlesinger, 2001; Li et al., 2004). In this P-  
66 limiting environment, 97% of the plant species require other plant species to recruit  
67 successfully, and 57% of these positive interactions are maintained when the plants  
68 reach the adult stage (Verdú & Valiente-Banuet, 2008). Elucidating plant-AMF  
69 interaction pattern is a first step to explore the potential mechanism underlying plant-to-  
70 plant facilitation and its implications in structuring plant community assemblages.  
71 Regardless of the importance of mycorrhizal associations, which form  
72 associations with most of land plants (Wang & Qiu, 2006; Smith & Read, 2008;  
73 Brundrett, 2009), the pattern of plant-AMF interactions still remains largely unknown in  
74 natural communities (Bever, 2003; Van der Heijden & Horton, 2009). In order to  
75 evaluate AMF-plant interaction pattern in a community, it is required to sample a

76 representative number of plant species growing in the same area and exposed to the  
77 same AMF taxon pool (Davison et al., 2011). The availability of studies presenting  
78 representative sampling of plants and AMF communities in a given natural site is  
79 scarce. Several studies have characterized the diversity of fungal communities in natural  
80 environments by focusing on of a few -usually the most common- plant species  
81 rhizosphere in the community (Daniel et al., 2001; Zaoyong et al., 2006; Kottke et al.,  
82 2008; Alguacil et al., 2009; Sonjak et al., 2009; Wilde et al., 2009; see Öpik et al., 2010  
83 for further references) or by exploring the influence of plant community on final AMF  
84 composition (Mummey et al., 2005; Hausmann & Hawkes 2009) using artificial (i.e.  
85 experimental) communities (Van der Heijden et al., 1998a; Maherali & Klironomos,  
86 2007). However, very few studies aim at sampling most of the plant and AMF  
87 communities in natural environments in order to elucidate the pattern of plant-AMF  
88 interactions (but see Öpik et al., 2009; Davison et al., 2011). Thus, it is still largely  
89 unclear to what extent plant and AMF communities interact in a random way or  
90 alternatively, if biological processes can lead to emerging non-random plant-AMF  
91 interaction patterns.

92 AMF are constituted by fungi of the phylum Glomeromycota, one of the key  
93 taxa inter-connecting plants into a functional web (Helgason et al., 1998). Despite the  
94 fact that Glomales form symbiotic associations with the majority of land plants (65%-  
95 85%: Wang & Qiu, 2006; Smith & Read, 2008; Brundrett, 2009), fewer than 200  
96 species of these globally important fungi have been described (Morton & Benny, 1990).  
97 The apparent low diversity of AMF compared with their associated plant hosts has led  
98 to the historical presumption that plant-AMF associations must have a low specificity  
99 (Smith & Read, 1997). Nonetheless, it is becoming increasingly clear that distinct AMF  
100 communities are present in the rhizosphere (Bever et al., 1996, 2001, Eom et al., 2000)  
101 and there is certain specificity in the interaction with plant species (Helgason et al.,  
102 2002; Vandenkoornhuyse et al., 2002, 2003, Scheublin et al., 2004; Pivato et al., 2007;

103 Santos-González et al., 2007; Mummey & Rilling 2008; Öpik et al., 2008; Smith &  
104 Read 2008; Li et al., 2010; Davison et al., 2011). This shift has been influenced by the  
105 larger amounts AMF of diversity revealed by the use of molecular techniques, with  
106 higher resolution for distinguishing closely related species (Bever et al., 1996; Eom et  
107 al., 2000). However, the different amount of intra-specific genetic variation depending  
108 on the family, genus and species prevents the determination of a generalized genetic  
109 threshold to delimitate AMF species (Nilsson et al., 2008; Redecker et al., 2003;  
110 Rosendahl, 2007). The increasing knowledge about AMF diversity and availability of  
111 molecular tools to approach it, offers a unique opportunity to explore plant-AMF  
112 interaction pattern in natural communities.

113 Network analysis is a convenient technique to detect non-random species  
114 interaction patterns. This analysis has been used to study different types of mutualisms:  
115 plant-pollinators and seed dispersers (Bascompte et al., 2003), marine cleaning  
116 mutualisms (Guimarães et al., 2007) or plant-to-plant facilitation (Verdú & Valiente-  
117 Banuet, 2008). However, network analyses has been rarely applied to fungal  
118 communities (but see Vacher et al., 2008 and Peay et al., 2007) and, as far as we know,  
119 have not been previously applied to study plant-AMF interactions at the community  
120 level. The wide application of network analyses has led to the development of an  
121 ecological network theory based on emerging patterns shared by multiple mutualistic  
122 systems. Interestingly, networks representing mutualistic processes have been shown to  
123 share a well-defined network structure regardless of the nature of the species involved  
124 (Bascompte & Jordano, 2007 but see Joppa et al., 2010 for a methodological critique).  
125 Ecological network theory predicts that mutualistic networks are characterized by  
126 having a few species much more connected than expected by chance, in which  
127 specialists tend to interact with a subset of the species with which generalists interact  
128 (i.e. nestedness) (Bascompte & Jordano, 2007). This particular structure has  
129 implications for the robustness of the network and coexistence and stability of species

130 (Bascompte & Jordano, 2007). In addition, if plant-AMF interactions are not as  
131 generalist as traditionally thought, it can be expected that any pair of species do not  
132 necessarily have the same probability of interacting. Accordingly, a group of plant  
133 species will tend to interact predominantly with a given group of AMF and vice versa.  
134 Network modularity reflects the tendency of a set of species to interact predominantly  
135 with species within the set and less frequently with species in other sets. Modularity  
136 implies that species can be grouped (i.e. modules) in such a way that weakly interlinked  
137 subsets of species, are strongly connected internally (Olesen et al., 2007). Approaching  
138 the study of plant-AMF from a network perspective will provide the opportunity to test  
139 two hypotheses. First, if plant-AMF interaction pattern matches the predictions  
140 developed by network theory based on other mutualistic systems. Second, if the non-  
141 random plant-AMF interactions previously suggested using a low number of species,  
142 are also reflected at the community level when most of the plant community is  
143 considered.

144 In this study we characterize the interaction patterns in a plant-AMF mutualistic  
145 system. For the sake of generality, we re-analyze using network analyses the data from  
146 the two available studies which sampled most of the plant community and recorded  
147 higher AMF phylogenetic diversity than our study. We define a gradient of AMF  
148 genetic differentiation threshold values (hereafter cut-off) and, for each of them: 1)  
149 describe the network testing for nestedness and modularity and 2) compare the different  
150 contribution of plant and AMF to the overall nestedness. Finally we estimate the  
151 relative contribution of plant species abundance to the observed interaction pattern. We  
152 present how this mutualism fits into mutualistic network theory and previous knowledge  
153 about plant-AMF interactions, discussing the potential implications for plant  
154 community structure mediated by plant-to-plant facilitation.

155

156 Materials and Methods

157 Study area and plant sampling

158 This study was conducted in the semiarid Valley of Zapotitlán (18° 20'N, 97°  
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 21° 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

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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 Vandenkoornhuysen 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 some 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 MgCl<sub>2</sub>, 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 95°C  
223 followed by 30 cycles each of 30 sec at 95°C, 30 sec at 51°C for annealing and 2 min at  
224 72°C for extension. After a final extension step of 10 min at 72°C PCRs were kept at  
225 4°C. 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 95°C followed by 30 cycles each of 45 sec at 95°C, 50 sec at  
235 56°C for annealing and 1.5 min at 72°C for extension. After a final extension step of 10  
236 min at 72°C 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

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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

259 related species. In this cases evolutionary processes involving natural recombination  
260 and incomplete lineage sorting, could be identified as false chimera positives in specific  
261 software for the detection of chimeras (Schechter & Bruns, 2008).

262

263 DNA Sequence alignment and analysis

264 Sequences were aligned with CLUSTALW (Thompson et al., 1994) implemented in

265 MEGA4 (Tamura et al., 2007). Sequence alignments were corrected by visual

266 inspection with BioEdit v. 7.0.9 (Hall, available at

267 <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>).

268 Pairwise distance matrices were computed using the default values in Dist.seqs

269 implemented in Mothur (Schloss et al., 2009). These served as input for Bin.seqs in

270 order to cluster the sequences into operational taxonomic units (OTUs) of a defined

271 sequence identity. The OTUs were defined according to their sequence dissimilarity at

272 different cut-off values, which spanned 1%-10% of their sequence being different. This

273 approach seems reasonable since species concepts are difficult to apply in AMF

274 (Redecker et al., 2003). Thus, using sequence bins rather than taxonomic assignments

275 based on BLAST analyses is more meaningful for environmental samples without prior

276 information on AMF diversity information because not all the sequences may match an

277 identified sequence in the database and the use of sequence similarities prevents

278 uncertainties associated with fungal taxonomy and classification.

279 We used rarefaction curves to illustrate how the number of OTUs increases with

280 the number of sequence sampled. Rarefaction curves were performed for each cut-off.

281 The value of OTUs levels off and reaches an asymptote when more sequences sampled

282 do not reveal more OTUs, indicating sufficient sampling. Confidence intervals for

283 OTUs were calculated based on 1000 randomizations.

284

285 Network analyses

286 For each cut-off, plant-AMF interactions were characterized as bipartite networks  
287 consisting of two sets of nodes, plant species, in rows, and AMF OTUs, in columns.  
288 Pairs of each type of nodes were considered linked (i.e. interaction) if an AMF OTU  
289 was present in a given plant species roots. This qualitative 0/1 matrix was used to  
290 calculate network parameters to describe connectance, nestedness and modularity.  
291 Connectance is considered as the realized proportion of possible links (Yodzis 1980), in  
292 this case, the proportion of pairs of plant-AMF OTU that directly interact. It was  
293 calculated using bipartite package for R (Blüthgen et al., 2006).  
294 Nestedness concept describes a particular pattern of interaction in which  
295 specialists interact with species (or OTUs) that form perfect subsets of the species (or  
296 OTUs) with which generalists interact (Bascompte & Jordano, 2007). Nestedness  
297 parameters measure how the presence/absence pattern of interactions departs from the  
298 perfect nestedness. We used two of the most common metrics to estimate nestedness:  
299 temperature index (Atmar & Patterson, 1993) and NODF (nested overlap and  
300 decreasing fill) (Almeida-Neto et al., 2008). The significance of nestedness was  
301 assessed by comparing the observed nestedness with the frequency distribution of that  
302 metric calculated using 1000 replicates of Null model II (Bascompte et al., 2003). Null  
303 model II uses equal dimension matrices in which each cell of the interaction matrix has  
304 a given probability of being occupied. This probability is the arithmetic mean of the  
305 connection probability of the focal plant species and AMF OTU. Accordingly,  
306 deviations from this null model result solely from an asymmetric distribution of  
307 interactions between species (Vacher et al., 2008).  
308 NODF values are matrix-dimension dependent and accordingly they are  
309 unsuitable to compare across studies. In order to allow cross-network comparisons with  
310 other mutualistic systems, the relative nestedness was calculated. This measure corrects  
311 for variation in species and OTUs richness and also in the number of links. Relative  
NODF is defined as  $NODF_{observed} - NODF_{null\ model} / NODF_{null\ model}$  312 , where  
NODF<sub>observed</sub>

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

367 estimate the relative contribution of plant species abundance on plant-AMF interaction  
368 pattern.

369

370 Relative contribution of plant abundance to plant species number of AMF interactions  
371 A plant species with high relative abundance in the community will have a  
372 higher probability to interact with a higher number of AMF, because more individuals  
373 will be sampled of this plant species. However, a plant species tendency to interact with  
374 a given numbers of AMF OTUs can also result from other processes independent of its  
375 relative abundance. Other biological processes such as habitat heterogeneity,  
376 demographic dynamics, plant-AMF overlapped phenology, AMF competition within  
377 the root or specific selectivity in plant-AMF associations can also produce a non-  
378 random pattern of plant-AMF interactions independently of the species relative  
379 abundance. While abundance-dependent interaction patterns occur at the species level  
380 as an effect of adding up multiple individuals, interaction patterns due to abundance-  
381 independent processes should be observed at the individual level.

382 Accordingly, we have calculated plant-AMF interactions at the individual plant  
383 level (AMF load) in order to characterize these biological processes independent of  
384 species abundance. We define AMF load as the plant species mean number of AMF  
385 OTUs per individual. Several tangled processes can be underlying a given species AMF  
386 load, and further experiments can be designed to elucidate these processes. Although we  
387 cannot tease apart the specific mechanisms resulting in a given AMF load, this index is  
388 independent of plant species relative abundance considering that AMF load is calculated  
389 as an average of individuals' trait within a plant species.

390 We tested if there is a statistically significant relationship between relative plant  
391 abundance and AMF load on the number of plant species links in the network, using a  
392 generalized linear model with a Poisson distribution of errors. Plant species degree in  
393 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



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

428 All ten networks were significantly more nested than expected by chance, both  
429 considering the overall network and rows and columns independently (for all cases  $p <$   
430  $0.001$ , Table 2). The degree of nestedness was independent of the cut-off considered  
(Pearson R  
2  
431 =  $0.57$ ,  $n = 10$ ,  $p > 0.05$ ). NODF of the overall network ranged from  $14.36$   
432 to  $54.83$  corresponding, in all cases, to a temperature higher than  $92$  (Table 2).

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

438 All networks except for the cut-off of 10% show a significant modularity (Table  
439 2). For the 10% cut-off the number of species in the network was 49 (35 plant species  
440 and 14 AMF OTUs). According to Olesen et al. (2007), when networks are based on  
441 less than 50 species it is likely the detection of just one single module. Thus, for all the  
442 cut-offs in which modularity was detectable we found significant values of modularity.

443 The number of modules varies from 5 to 9 across the different cut-offs and the average  
444 number of nodes within a module range from 9 to 22 (Table 2). Plant and AMF species  
445 ascribed to different modules co-exist in the same vegetation clump, with an average of  
446  $2.6$  (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 NODF<sub>t</sub>= 0.25-0.28 NODF<sub>p</sub>= 0.50-  
455 0.54 NODF<sub>g</sub>= 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 phylogenetic 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. *Digitalis 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.

501 In general, mutualistic networks are characterized by having low connectance  
502 and being highly nested (Bascompte et al., 2007). Our plant-AMF network showed  
503 similar connectance to that reported for pollination networks ( $11.89 \pm 3.41$ ; Olesen et  
504 al., 2006) and plant-to-plant facilitation networks ( $24.9 \pm 2.68$ ; Verdú & Valiente-  
505 Banuet, 2008), but other plant-AMF networks present higher connectance (Öpik et al.,  
506 2009; Davison et al., 2011). Regarding nestedness, our plant-AMF network showed  
507 similar, high values of nestedness (T) than the ones reported for other positive  
508 interactions such as plant-to-plant facilitation ( $89.7 \pm 2.7$ ) (Verdú & Valiente-Banuet,  
509 2008), seed dispersal ( $84.3 \pm 2.1$ ) and pollination networks ( $85.3 \pm 2.2$ ) (Bascompte et  
510 al., 2003). A pattern of generalist plants tending to associate with generalist AMF, has  
511 been previously reported for plant-AMF systems (Davison et al., 2011). Our reanalysis  
512 of this and another, related study (Öpik et al. 2009) indeed show significant nestedness,  
513 suggesting that this maybe a general pattern in plant-AMF networks.

514 Ecological networks with low connectance and that are highly nested have a  
515 tendency to be highly modular (Fortuna et al., 2010). This is also the case of the  
516 network presented in this study. However, the other two published networks we have  
517 analyzed were nested but not modular. Interestingly, these studies have high  
518 connectance and, according to the pattern revealed by Fortuna et al. (2010), highly  
519 connected networks tend to be either nested or modular, but not both. A greater amount  
520 of studies approaching plant-AMF community interactions will be needed to elucidate a  
521 general pattern regarding the level of connectance and its influence on modularity in  
522 different communities.

523 The non-random pattern of plant-AMF interactions observed at the community level  
524 can be produced by diverse mechanisms. Ecological processes such as habitat  
525 heterogeneity, specific selectivity in plant-AMF associations, plant-AMF overlapped  
526 phenology, species relative abundance and phylogenetic diversity or AMF competition  
527 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

590 Acknowledgements

591 We thank J.P. Castillo, M. Morales, C. Silva and L. Sortibrán for help with field  
592 samplings and S. Donat and M. Morales for laboratory assistance. This work was  
593 funded by AECID (Projects A017475/08, A023461/09), DGAPA-UNAM (Project IN-  
594 224808-3; IN-202811-3), and CYTED (Acción 409AC0369). JGS-M was supported by  
595 a “Ramón y Cajal” postdoctoral contract from MICINN.

596

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TABLES

**Table 1.** Number of individuals sampled for each plant species.

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

Cut-off %	Nestedness											Modularity		
	N	I	C	T	NODF <sub>t</sub>	NODF <sub>p</sub>	NODF <sub>g</sub>	Relative				Modules	Nodes	Modularity
								T <sup>a</sup>	NODF <sub>t</sub>	NODF <sub>p</sub>	NODF <sub>g</sub>			
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 <sup>n.s</sup>

Cut-off %= percentage of dissimilarity used as cut-off. N= Number of OTUs of *Glomus*. I= Number of interactions. C=Connectance:  $100 \times I / (N \times 35)$ . T= nestedness calculated as matrix temperature. NODF<sub>t</sub>=Nested overlap and decreasing fill for the overall matrix. NODF<sub>p</sub>= NODF for plants. NODF<sub>g</sub>= NODF for *Glomus*. Relative T:  $T - T_{\text{null model}} / T_{\text{null model}}$  (see “methods” for details about the null model) and NODF<sub>t,p,g</sub>:  $\text{NODF}_{t,p,g} - \text{NODF}_{\text{null model}} / \text{NODF}_{\text{null model}}$ . 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.

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

Cut off %	R <sup>2</sup>	Plant abundance	Individual load	AMF	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***

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

8  
 9

10

11 Figure legends

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

16

17 Supporting information

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

23

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

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

31

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304 TABLES  
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**Table 1.** Number of individuals sampled for each plant species.

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

307 Plant species are ranked by their abundance in positive amplification and sequencing of  
 308 *Glomus* gr. A.  
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**Table 2.** Descriptive statistics of the Plant-Mycorrhizal fungus network.

Cut-off %	Nestedness											Modularity		
	N	I	C	T	NODF <sub>t</sub>	NODF <sub>p</sub>	NODF <sub>g</sub>	Relative				Modules	Nodes	Modularity
								T <sup>a</sup>	NODF <sub>t</sub>	NODF <sub>p</sub>	NODF <sub>g</sub>			
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 <sup>n.s</sup>

Cut-off %= percentage of dissimilarity used as cut-off. N= Number of OTUs of *Glomus*. I= Number of interactions. C=Connectance:  $100 \times I / (N \times 35)$ . T= nestedness calculated as matrix temperature. NODF<sub>t</sub>=Nested overlap and decreasing fill for the overall matrix. NODF<sub>p</sub>= NODF for plants. NODF<sub>g</sub>= NODF for *Glomus*. Relative T:  $T - T_{\text{null model}} / T_{\text{null model}}$  (see “methods” for details about the null model) and NODF<sub>t,p,g</sub>:  $\text{NODF}_{t,p,g} - \text{NODF}_{\text{null model}} / \text{NODF}_{\text{null model}}$ . 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.

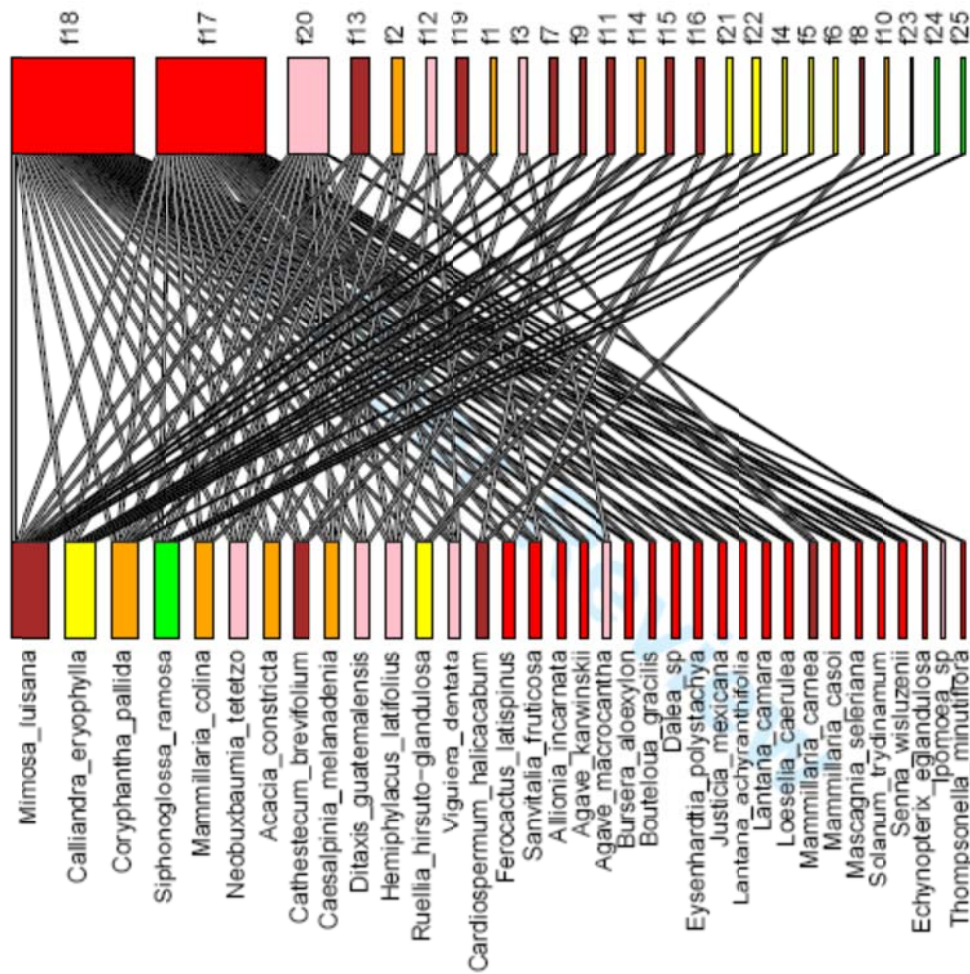
**Table 3.** GLM testing for the effects of plant species relative abundance and AMF load on the species degree in the network.

Cut off %	R <sup>2</sup>	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<sup>2</sup>: pseudo-R<sup>2</sup> 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.