# Modularity reveals a tendency of arbuscular mycorrhizal fungi to interact differently with generalist and specialist plant species in gypsum soils.

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**Running title:** AMF diversity and modularity in gypsum plants

**Abstract**

Patterns in plant-soil biota interactions could be influenced by the spatial distribution of species due to soil conditions or by functional traits of species. Gypsum environments usually constitute a mosaic of heterogeneous soils where gypsum and non-gypsum soils are imbricated at a local scale. Plant-arbuscular mycorrhizal fungi (AMF) interactions occurring in gypsum environments can be an illustrative case study for the detection of patterns in biotic interactions. We hypothesized that a) soil characteristics might affect the AM fungal community, and b) there are differences between the AMF communities (modules) associated with plants exclusive to gypsum soils (gypsophytes) and plants which show facultative behaviour on gypsum and/or marly limestone soils (gypsovags). We used indicator species and network analyses to test for differences between the AM fungal communities harbored in gypsophyte and gypsovag plants. We recorded 46 OTUs belonging to nine genera of *Glomeromycota*. The indicator species analysis showed two OTUs preferentially associating with gypsum soils and three OTUs preferentially associating with marly limestone soils. The modularity revealed that soil type can be a major factor shaping AMF communities, and there were some AM fungal groups with a tendency to interact differently with plants having distinct ecological strategies (gypsophytes and gypsovags). The characterization of the ecological networks can be a valuable tool to ascertain the potential influence of above-below ground biotic interactions (plant-AMF) on plant community composition.

## Introduction

The close relationship between some plant species and particular geological substrates has long been recognized. Certain soil types can impose stressful conditions to plants due to low water and nutrient availability, or high levels of heavy metals (dolomites, serpentines, gypsum) inducing adaptive syndromes in the plants growing on them (1). Plants species specifically adapted to particular stressful substrates are called "specialist" plants. Other plant species, called "generalist" plants, usually inhabit non-stressful substrates but can also inhabit stressful substrates. Plant communities from gypsum soils are composed by plant species defined as gypsophytes or specialists, when they occur only in soils with gypsum, and gypsovags or generalists, when they can occur in both gypsum and other soil types (2). Physiological and edaphic factors are responsible for the occurrence of gypsophytes and gypsovags, but there is no generalized adaptive strategy (3). Among these factors, the ability to accumulate sulfate (1) and mineral nutrients (N, P, Ca, M, K), aminoacids, and proteins (4), and a better water balance have been proposed. However, factors controlling the distribution and occurrence of gypsophytes and gypsovags are still not fully understood (5), and specially the differences of above-below ground biotic interactions on the coexistance of gypsophytes-gypsovags have been largerly unexplored.

Arbuscular mycorrhizal fungi (AMF) are responsible for establishing mycorrhizal symbioses with most vascular plants in all environments, including semi-arid ecosystems where they help plants to cope with nutrient deficiency, drought, salinity, and other stresses (6). There are case studies for the general abiotic versus biotic affectors of AMF community assembly (7, 8), Differences in soil type have been reported to be key factors determining the AMF species and community composition (9), and this is particularly relevant in stressed environments like serpentine soils (10, 11, 12), thermal soils (13), heavy metal soils and

73 saline soils (14, 15). However, some authors pointed out that AMF communities might be
74 more determined by biotic factors such as the host plant (16, 17) or even can be more specific
75 to plant functional groups than to individual plant species (18).

76 The evidence of the importance of AMF diversity for ecosystem functioning (19) has
77 resulted in a growing interest to identify the species that colonize plants in natural
78 ecosystems. Specifically, the plant-AM fungal symbioses and AM fungal diversity have been
79 studied recently in gypsum ecosystems, where it has been found that the AM fungal
80 community is host plant-dependent (20, 21) resulting in different AM fungal communities
81 colonizing perennial and annual plant species (22) and presenting a higher AMF infection in
82 gypsovags than in gypsophytes (5). Despite these previous findings, it still remains unknown
83 whether the community compositions of the AMF colonizing the roots of gypsovags differ
84 markedly from those of gypsophytes when both are growing in gypsum soils.

85 Recently, network analysis has been applied to characterize AMF communities and
86 plant-AMF interactions (23, 24). Network analyses allow the detection of generalized patterns
87 within the structure of biotic interactions at the community level. Network modularity reflects
88 the tendency of a set of species to interact predominantly with species within the set and less
89 frequently with species in other sets. Modularity in these networks implies that there are
90 distinct communities of plants and AMF (modules) that interact more among themselves than
91 with other plants or AMF.

92 In this study, we used indicator species analysis and network analyses to characterize
93 and test for differences in AM fungal communities harbored in gypsophytes in gypsum soils
94 and gypsovags in gypsum and non-gypsum soils. Afterwards, we explored the factors that
95 best explain the grouping of species within a module (communities). We hypothesized that
96 soil characteristics might affect the AM fungal community that can inhabit gypsum soils,

97 resulting in different AMF communities (modules) in gypsum and non-gypsum (marly-
98 limestone) soils. Also, focusing only on gypsum soils, we hypothesized that there are
99 differences between the AMF communities (modules) associated with gypsophyte and
100 gypsovag plants. This study considers the potential influence of above-below ground biotic
101 interactions (plant-AMF) on plant community composition.
102
103
104 **Materials and Methods**
105
106 *Study area and plant sampling*
107 The study area was located in Sierra de Las Ventanas, Albatera (Alicante), in
108 southeastern Spain (38º14' N, 0º55' W, 320 m altitude). The climate is semiarid, with annual
109 evapotranspiration (ETP) of 954 mm, annual average rainfall of 290 mm, a pronounced dry
110 season from June to September, and a mean annual temperature of 18.4ºC. It is a natural zone
111 with gypsum outcrops and marly-limestone soils in a patchy distribution; the vegetation is
112 scrubland (woody perennial species) dominated by gypsophytes in the gypsum areas and by
113 typical scrub species of semiarid plant communities in the adjacent soils.
114 The soils are classified as Petrogypsid, (25), with a gypsic and petrogypsic horizon
115 within 100 cm of the surface, developed on gypsum parental rocks, and as Typic Torriorthent,
116 with low organic matter content (Table 1).
117 The sampled plant species were classified into two groups according to their
118 ecological strategy. On the one hand, three of the most-widely-distributed gypsophytes (26)
119 were considered: *Ononis tridentata* L. (OT), *Helianthemum squamatum* (L.) Dum. Cours.
120 (HS), and *Launaea pumila* (Cav.) O. Kuntze (LP). On the other hand, three species common

throughout the Mediterranean semiarid areas were considered as gypsovags: *Globularia alypum* L. (GA), *Helichrysum stoechas* (L.) Moench (ST), and *Anthyllis terniflora* (Lag.) Pau (AT).

Ten plots (2x5m=10 $m^2$) were established randomly in the experimental area (at least 5 m between plots), five with gypsum outcrops and five with marly-limestone outcrops. Within each plot, three individual plants for each selected plant species were sampled. A total of 90 plants (45 gypsophytes and 45 gypsovags) were sampled in the gypsum area and a total of 45 plants (gypsovags) outside the gypsum area, resulting in 135 root samples. At the same time, one soil sample per plot (0-20 cm depth) was collected for soil characterization.

All samples were collected in the second half of May (late spring). Plants, including root systems, were collected, placed in polyethylene bags, and transported to the laboratory, where fine roots were separated from the soil. The roots were then briefly rinsed, quickly dried on paper, and used for molecular analysis.

*Soil analysis*

Alkaline phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) (Fluka) as substrate. The *p*-nitrophenol (PNP) formed was determined by spectrophotometry at 398 nm (27).

The β-glucosidase was determined using p-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M) as substrate. This assay is based on the release and detection of PNP. The amount of PNP was determined at 398 nm (28).

Dehydrogenase activity was determined according to Garcia et al (29) and Trevors (30).

144 The N-benzoyl-L-argininamide (BAA) hydrolyzing protease activity was determined
145 in 0.1 M phosphate (Panreac) buffer at pH 7; 0.03 M BAA (MP Biomedicals) was used as
146 substrate. The activity was determined as the $NH_4^+$ released in the hydrolysis reaction (31).
147 Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous solution. In
148 soil aqueous extracts, water soluble carbon was determined in an automatic carbon analyzer
149 for liquid samples (TOC-V CSN Analyzer, Shimadzu).
150 Glomalin related soil protein (GRSP) was determined in the easily extractable
151 glomalin form, according to Wright and Anderson (32).
152 The total N and total organic carbon were measured with a FLASH EA 1112 SERIES
153 THERMO elemental analyzer.
154 Available phosphorus was extracted with 0.5 M $NaHCO_3$ (1:10, w/v) for 30 minutes
155 and measured colorimetrically.
156 The carbonate content was determined by Bernard calcimetry, and the percentage of
157 gypsum in the soil was estimated by measuring total S with an elemental analyzer (LECO).
158
159 *Root DNA extraction and PCR*
160 For each root sample, 0.1 g fresh root material was frozen with liquid nitrogen, placed
161 into a 2-ml, screw-cap propylene tube, together with two tungsten carbide balls (3 mm), and
162 ground (3 min, 13000 r.p.m.) using a mixer mill (MM 400, Retsch, Haan, Germany). Total
163 DNA was extracted using a DNeasy Plant Mini Kit, following the manufacturer's
164 recommendations (Qiagen). The extracted DNA was resuspended in 20 µl of water.
165 Extractions of DNA from 135 root samples were carried out. The three DNA
166 extractions from each plant species within a plot were pooled into one composite sample,
167 resulting in a total of 45 samples. This procedure was performed to assure maximum coverage

of the inter and intraspecific variation within each replicated plot.

Several dilutions of the extracted DNA (1/10, 1/50, 1/100) were prepared and 2 µl were used as a template. Partial small subunit (SSU) ribosomal RNA gene fragments were amplified using nested PCR, with the universal eukaryotic primers NS1 and NS4 (33). PCR was carried out in a final volume of 25 µl, using "ready to go" PCR beads (Amersham Pharmacia Biotech), 0.2 µM dNTPs, and 0.5 µM of each primer (PCR conditions: 94 °C for 3 min, then 30 cycles at 94 °C for 30 s, 40 °C for 1 min, 72 °C for 1 min, followed by a final extension period at 72 °C for 10 min).

Two microliters of 1/10 dilution from the first PCR were used as template DNA in a second PCR reaction performed using the specific primers AML1 and AML2 (34). The PCR reactions were carried out in a final volume of 25 µl, using PuReTaq Ready-To-Go PCR beads (Amersham Pharmacia Biotech), 0.2 µM dNTPs, and 0.5 µM of each primer (PCR conditions: 94 °C for 3 min, then 30 cycles of 1 min denaturation at 94 °C, 1 min primer annealing at 50 °C, and 1 min extension at 72 °C, followed by a final extension period of 10 min at 72 °C). Positive and negative controls, using PCR positive products and sterile water, respectively, were also included in all amplifications. All the PCR reactions were run on a Perkin Elmer Cetus DNA Thermal Cycler. Reaction yields were estimated by using a 1.2%-agarose gel containing *GelRed*™ (Biotium).

*Cloning and sequencing*

The PCR products of the expected band, approximately 795 bp, were purified using a Gel Extraction Kit (Qiagen), cloned into pGEM-T Easy (Promega), and transformed into *Escherichia coli* (Xl1 blue). From the 45 clone libraries, a total of 1440 clones were screened by PCR. Thirty-two positive transformants were screened in each resulting SSU rRNA gene

192 library, using 0.7 units of RedTaq DNA polymerase (Sigma) and a re-amplification with the
193 AML1 and AML2 primers - with the same conditions described above. Product quality and
194 size were checked in agarose gels as described above. Insert-containing clones (1019) were
195 sequenced.
196 Clones were grown in liquid culture and the plasmid extracted using the QIAprep Spin
197 Miniprep Kit (Qiagen). The sequencing was done by the Laboratory of Sistemas Genómicos
198 (Valencia, Spain), using the universal primers SP6 and T7. The sequence editing was done
199 using the program Sequencer version 4.1.4 (Gene Codes Corporation). The 135 unique
200 sequences of the clones generated in this study have been deposited at the National Center for
201 Biotechnology Information (NCBI) GenBank (http://www.ncbi.nlm.nih.gov) under the
202 accession numbers HF559237-HF559371.
203
204 *Phylogenetical analysis*
205 Sequence similarities were determined using the Basic Local Alignment Search Tool
206 (BLASTn) sequence similarity search tool (35), provided by NCBI. Phylogenetic analysis
207 was carried out on the sequences obtained in this study and those corresponding to the closest
208 matches from the GenBank and MaarjAM databases (36), as well as on sequences from
209 cultured AMF taxa - including representatives of the major groups of *Glomeromycota* from
210 GenBank. Sequences were aligned using the program ClustalX (37) and the alignment was
211 adjusted manually with BioEdit software version 7.0.4.1 (38). Neighbor-joining (NJ) (39) and
212 maximum likelihood (ML) phylogenetic analyses were performed with the programs
213 PAUP4.08b (40) and RAxML v.7.0.4 (41), respectively. Distances for the NJ tree were
214 computed using the default parameters. For the ML analysis, a GTR-GAMMA model of
215 evolution was used. A total of 200 independent bootstrap analyses were performed to provide

nodal support. The ML bootstrap values were calculated with 1000 replicates, using the same substitution model. *Endogone pisiformis* Link and *Mortierella polycephala* Coem were used as the out-groups.

Different sequence types, or OTUs, were defined as groups of closely related sequences, usually with a high level of bootstrap support in the phylogenetic analyses (higher than 80%) and with pairwise similarity (higher than 97%). The pairwise analysis within clusters was carried out using BioEdit software version 7.0.4.1. (38).

*Statistical analysis*

For each plant species in each plot, the number of sequences and the number of samples for each AM fungal OTU was used to construct the sampling effort curves (with 95 % confidence intervals), using the software EstimateS 8.00 (42). The sample order was randomized by 100 replications.

We used indicator species analyses to generate a numerical classification of OTUs (43). This method uses a reciprocal averaging ordination to classify the OTUs, according to apparently important environmental properties (44). We performed two indicator species analyses, using soil type and plant species, respectively, as the ordination properties.

The indicator value (IndVal) index (45) was used to measure the association between a species and a site group. Finally, the statistical significance of this relationship was tested using a permutation test with 999 permutations. We performed these analyses using the "indcspecies" package implemented in R (46).

*Modularity*

239 Plant–AMF interactions were characterized as considering that there is a link between
240 a plant and AMF taxa (i.e. interaction) if an AMF OTU was present in the roots a given plant
241 species. This qualitative 0/1 matrix was used to calculate network modularity.

242 The nodes (i.e., plant species and AMF OTUs) of a network can be grouped into
243 modules, in such a way that the number of links (i.e., the presence of an interaction) within
244 the modules is maximized and the number of links between modules is minimized. We used a
245 modularity algorithm for unipartite networks to search for independent groups of AMFs that
246 tend to be harbored in the same plants (i.e., that share a similar interaction pattern) (see
247 Olesen et al (47) for a more detailed explanation). In this type of network an interaction
248 between two AMF OTUs will be present if they share at least one host (i.e., plant). A
249 simulated annealing optimization approach was used to detect modules that maximized
250 modularity (i.e., the proportion of links within vs. between modules) and test if the pattern
251 observed differed significantly from random, using 100 randomizations (48, 49). Because of
252 its heuristic nature, 10 runs of the algorithm were conducted, but the variation in modularity
253 was negligible (the SE of the modularity across the 10 runs ranged from 0.0104 to 0.0106
254 when all interactions were considered and from 0.0113 to 0.0115 when only plants growing in
255 gypsum soil were considered). We report the maximum value of modularity obtained in the
256 10 runs. The modularity was calculated and its significance tested using the software Netcarto
257 (50, 48, 49).

258 Firstly, we tested for the contribution of soil type (i.e., gypsum and non-gypsum soil),
259 to explain the assignment of each AMF OTU to a given module. Each OTU-plant interaction
260 was classified based on the module to which the OTU was ascribed and the soil in which the
261 plant was growing. We used multinomial regression models with module as the dependent
262 variable and soil type as the independent variable. The model was compared with a null

model in which the independent variable was a constant. As a rule of thumb, if the proposed model's Akaike information criteria index (AIC) is more than 2 units lower than that of the null model it has some support and if the AIC value is more than 10 units lower it has substantial support (51). Secondly, we arranged the dataset in subsets, selected the interactions that occur only in the gypsum area, and recalculated the modularity. Using the same methodology described above, we built multinomial regression models to explore the relative contribution of plant ecological strategy (gypsophytes or gypsovags) to the ascription of each OTU to a given module, considering only the plant-AM fungal associations occurring within the gypsum soil.

## Results

### *Soil analysis*

The gypsum and marly limestone soils had similar fertility levels, except for the available phosphorus content, which was significantly higher in the marly limestone soil. Biological properties (enzymatic activities) did not differ significantly between the two soil types. The most marked differences were the significantly higher amounts of calcium sulfate in the gypsum soil and of calcium carbonate in the marly limestone soil (Table 1).

### *PCR and sequence analysis*

The BLAST search revealed that 734 sequences (72%) had a high degree of similarity (94-100% similarity) to sequences from taxa belonging to the phylum *Glomeromycota*. The rest of the sequences were attributed to *Ascomycota* and plants.

287

288 *Phylogenetic analysis of AMF OTUs*

289 The phylogenetic analyses of 734 glomalean sequences obtained in this study from
290 plant roots, and 50 sequences downloaded from GenBank, made possible the recognition of
291 46 OTUs as separate clades, on the basis of bootstrap values ≥ 80%. According to Schüßler
292 and Walker (52), the sequence groups or OTUs covered six families of the *Glomeromycota*:
293 the *Glomeraceae, Claroideoglomeraceae, Diversisporaceae, Paraglomeraceae,*
294 *Gigasporaceae*, and *Archaeosporaceae*. The pairwise sequence similarities within the clades
295 ranged from 97.5 to 100%. The 135 clones that produced unique sequences were represented
296 in the NJ and ML tree. Of all the AMF OTUs, 26 belonged to the genus *Glomus*, two to
297 *Funneliformis*, six to *Claroideoglomus*, one to *Sclerocystis*, one to *Rhizophagus*, two to
298 *Diversispora*, one to *Redeckera*, one to *Scutellospora*, one to *Archaeospora*, and five to
299 *Paraglomus* (Fig. 1).

300

301 *AMF OTUs distribution*

302 To determine whether the number of clones sequenced and the number of samples
303 taken were sufficient to represent the diversity of the AMF in the roots, sampling effort curves
304 were constructed (Fig. S1 and S2). The results indicate that the number of sequences analysed
305 and the number of samples were sufficient to provide coverage of the AM fungal diversity in
306 roots from the six plant species studied. In fact, all the curves reached the plateau.

307 The OTUs richness found in the roots of the different plant species in each soil type is
308 shown in Table 2.

309 The most abundant OTUs in this study were *Glomus* G24 (20.1% of the clones
310 belonged to this OTU), *Glomus* G25 (17.7%), and *Glomus* G1 (11.7%), which occurred in all

311 the plant species, followed by *Glomus* G8 (5.7%) and *Paraglomus* P5 (5.7%), present in the
312 roots of all the plant species growing outside the gypsum soil.

313 An Indicator Species Analysis was conducted to find specific OTUs for the host and
314 soil types. Two OTUs tended to occur in gypsum soils, G10 and G9 (IndVal: 0.66 (p-value
315 0.007) and 0.58 (p-value 0.013), respectively), and three in non-gypsum (marly limestone)
316 soils, G8, Cl5, and P5 (IndVal: 0.64 (p-value 0.003), 0.57 (p-value 0.023), and 0.57 (p-value
317 0.035), respectively). Regarding plant species, just the OTU G20 was significantly associated
318 with *O. tridentata* (IndVal 0.78 (p-value 0.003)) (Table 3 A and B).

319

320 *Modularity*

321 The networks showed a significant modularity, considering all interactions
322 (Modularity=0.152, confidence interval of the null model CIN=[0.105-0.107]) or only plants
323 growing in the gypsum area (Modularity=0.185, CIN=[0.092-0.137]) (Table 4). Four modules
324 were detected in both networks, the one considering all the interactions and the other
325 considering only interactions occurring in gypsum soil (Table 4). When all the interactions
326 were considered, the adscription of OTUs to different modules was explained significantly by
327 the soil type (AIC Null model: 632.6; using soil type as explanatory factor: 613.8). The AMF
328 OTUs ascribed to module B (Table 4) tended to prefer the gypsum area (Multinomial
329 regression coefficient 0.13) and the OTUs ascribed to modules C and D tended to strongly
330 avoid the gypsum area (i.e., they preferred the non-gypsum area), compared to a reference
331 module (A; Table 4) (multinomial regression coefficients -1.43 and -1.06).

332 Focusing on the gypsum area data set, plant ecological strategy contributed
333 significantly to the explanation of the ascription of AMF OTUs to different modules (AIC
334 Null model: 418.10; using plant ecological strategy as explanatory factor: 408.90). The OTUs

ascribed to module G (Table 4) tended to avoid gypsovags (Multinomial regression coefficient -1.22) (i.e., they prefer gypsophytes) more than the OTUs ascribed to the reference module (E; Table 4). Meanwhile, the OTUs ascribed to modules F and H (Table 4) tended to associate more with gypsovags (Multinomial regression coefficients 0.98 and 0.31, respectively).

**Discussion**

This study recorded 46 OTUs, belonging to nine genera (*Archaeospora*, *Glomus*, *Diversispora*, *Claroideoglomus*, *Funneliformis*, *Sclerocystis, Rhizophagus, Redeckera, Scutellospora*, and *Paraglomus*). This level of richness is higher than that of a previous study in different gypsum areas (21). However, the results may not be directly comparable since different primers were used. In the previous study, the NS31/AM1-3 primers developed by Santos-González et al (53) were used; these cover a shorter SSU rRNA gene fragment than the AML1/AML2 primers of Lee et al (34). In fact, no sequences from the present trial showed neither identical nor similar (97%) homology with sequences obtained in the earlier study. This greater richness can also be attributed to the number of plant species sampled: six, three of them in two different soil types.

The most abundant OTUs in this study were *Glomus* G24 (20.1% of the clones belonged to this OTU), *Glomus* G25 (17.7%), and *Glomus* G1 (11.7%), which occurred in all the plant species. *They* clustered with root derived sequences of uncultured AMF species and they have been recorded in other semiarid ecosystems (20, 21). OTUs G24 and G25 are closely related to the *Rhizophagus intraradices* group (sequence homology 96%) which represent a ubiquitous generalist fungus, since it is one of the most common taxon that has

359 been found in a broad range of environments (54, 36); Öpik et al (54) proposed that some
360 AMF species occur globally showing high local abundance and low specificity, and *R.*
361 *intraradices* group clearly falls into this category as a generalist species. The presence of
362 "potential specialist" AMF in gypsum ecosystems has been suggested (21). In the present
363 work, the indicator species analysis showed two OTUs (G10 and G9) which tended to occur
364 in gypsum soils, and three OTUs (G8, Cl5, and P5) which tended to occur in marly limestone.
365 However, further studies are required to be able to assert that certain AMF tend to occur in
366 these types of soil. Furthermore, OTUs G10 and G9 have been previously cited by Wubet et
367 al (55, 56) in non-gypsum soils from tropical montane forests in Ethiopia, in spite of, these
368 OTUs may represent peculiar ecotypes adapted to the particular environmental conditions of
369 gypsum soils.

370 A major environmental factor producing differences in the AMF community
371 composition in our work was related to the soil type. The modularity analysis supported this
372 idea. There are indications that the AMF diversity and distribution in soils depend on soil
373 properties such as water availability (57), soil texture (58), or soil chemistry (59, 14, 15). Oehl
374 et al (9), in an extensive study, found that soil type is a key factor determining the
375 composition of the AMF community. Recent AMF diversity studies have revealed that the
376 AMF community composition is host plant-dependent (21, 22, 16, 17, 60, 61, 62, 63, 64,65).
377 Interestingly, when we focused on the plant and AMF community inhabiting gypsum soils,
378 we also found differences in the AMF harbored in gypsophyte and gypsovag plants.
379 Similarly, Öpik et al (18) found, in a boreal ecosystem, that AMF communities are more
380 specific to plant functional groups than to individual plant species. The modularity and
381 multinomial regression model results support the hypothesis that soil type structures the AM
382 fungal community composition. We found OTUs ascribed to modules preferring plants in the

383 gypsum areas (module B; Table 4) and others ascribed to modules preferring plants in the
384 non-gypsum areas (modules C and D; Table 4). However, when we focused on the gypsum
385 areas, particular patterns emerged. Within the gypsum areas, there were OTUs ascribed to
386 modules with a tendency to interact with gypsophytes (module G; Table 4), other OTUs
387 ascribed to modules with a tendency to interact with gypsovags (modules F and H; Table 4),
388 and others that did not show a preference to interact with either of the two plant ecological
389 strategies (module E; Table 4). Chagnon et al (23) derived a significantly modular
390 mycorrhizal network from the data of the mycorrhizal community described by Öpik et al
391 (18); they concluded that members of the genera *Acaulospora* and *Scutellospora* were mostly
392 confined to a single module associated with "forest specialist plants", whereas members of the
393 genus *Glomus* were more generalist in their partner choice and were found mainly in the
394 module comprising the common or "generalist" plant species.

395 Referring to our network, in the gypsum soil, where gypsophytes and gypsovags grow
396 together, modules that interacted with gypsophytes were formed mostly by OTUs ascribed to
397 the genus *Glomus*. However, the modules that interacted with gypsovags contained OTUs
398 belonging to *Glomus, Claroideoglomus*, *Paraglomus, Rhizophagus, Sclerocystis, Redeckera*,
399 and *Diversispora*. Interacting with a more phylogenetically diverse community of AMF can
400 increase plant growth and potentially plant coexistence (19, 66, 67), mainly due to plant stress
401 amelioration as a result of a high AMF functional complementarity in resource acquisition
402 and delivery to the mutualistic association. Our results suggest that gypsophyte plant species,
403 which might have specific adaptations to live in gypsum, are less dependent on the benefits of
404 associating with a wide phylogenetic diversity of AMF. However gypsovags, which
405 presumably do not have specific adaptations to gypsum soils, might require the benefits of
406 associating with a wide diversity of AMF to survive. This result opens new perspectives in

our understanding of the influence of biotic interactions on plant community assembly rules, but future research in other ecological conditions will be required to ascertain whether this result is a general trend.

In conclusion, characterization of the ecological networks can be a valuable tool to ascertain the potential influence of above-below ground biotic interactions (plant-AMF) on plant community composition. Our case study revealed that soil type can be a major factor shaping AMF communities, and there were some AM fungal groups with a tendency to interact differently with plants having distinct ecological strategies (gypsophytes and gypsovags).

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**Table 1**. Biological, chemical and physical characteristics of the soil in the two areas sampled (n=5).

| | Gypsum area | Marly limestone area |
|---|---|---|
| Alkaline Phosphatase (μmol PNP $g^{-1}h^{-1}$) | 0.89±0.12a | 0.31±0.06b |
| Dehydrogenase (μg INTF $g^{-1}$ soil) | 30.66±2.60a | 37.66±4.85a |
| Protease (μmol $NH_3$ $g^{-1}$ $h^{-1}$) | 0.09±0.02a | 0.14±0.03a |
| β-glucosidase (μmol PNP $g^{-1}$ $h^{-1}$) | 0.18±0.02a | 0.13±0.03a |
| Available P (mg $kg^{-1}$) | 1.27±0.4b | 4.96±0.3a |
| EC (μS $cm^{-1}$) | 2.400±100a | 2.380±100a |
| pH | 7.9±0.3a | 8.3±0.2a |
| TOC (g $kg^{-1}$) | 5.5±5.0a | 4.2±5.0a |
| Total N (g $kg^{-1}$) | 0.7±0.2a | 0.6±0.2a |
| WSC(μg$g^{-1}$) | 68±6a | 67±4a |
| GRSP(μg$g^{-1}$ soil) | 31.66±12.46b | 85.96±8.15a |
| $CO_3Ca$ (g $kg^{-1}$) | 216±53b | 506±72a |
| $SO_4Ca{\cdot}2H_2O$(g $kg^{-1}$) | 434±35a | 21.8±8b |

Mean ± SE. [1]Values in columns followed by the same letter do not differ significantly ($P<0.05$) as determined by the Duncan test. TOC: total organic carbon. WSC: Water soluble C; GRSP: glomalin related soil protein.

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624 **Table 2.** Number of sequences of each OTU and richness per plant species and per soi ltype.

| | Gypsum Soil | | | | | | Marly-limestone Soil | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gypsophytes | | | Gypsovags | | | | | | | |
| | *Launaea pumila* | *Ononis tridentata* | *Anthyllis terniflora* | *Globularia alypum* | *Helianthemum squamatum* | *Helichrysum stoechas* | *Anthyllis terniflora* | *Globularia alypum* | *Helichrysum stoechas* | *n* | % |
| P1 | - | - | 2 | - | - | - | - | - | - | 2 | 0,27 |
| P2 | - | - | - | - | - | - | 2 | - | - | 2 | 0,27 |
| P3 | - | - | - | - | - | - | 2 | - | - | 2 | 0,27 |
| P4 | - | - | 2 | - | - | - | - | - | - | 2 | 0,27 |
| P5 | - | 2 | 2 | 6 | - | - | 14 | 10 | 8 | 42 | 5,72 |
| Ar1 | - | - | - | - | 2 | - | - | - | - | 2 | 0,27 |
| Scu1 | - | - | - | 2 | - | - | - | - | - | 2 | 0,27 |
| Re1 | - | - | - | - | - | 2 | - | - | - | 2 | 0,27 |
| Cl1 | - | - | - | - | - | 2 | - | - | - | 2 | 0,27 |
| Cl2 | - | 2 | 2 | - | - | - | 2 | - | 2 | 8 | 1,09 |
| Cl3 | - | - | 2 | - | - | - | - | - | - | 2 | 0,27 |
| Cl4 | - | - | - | - | - | - | - | 2 | - | 2 | 0,27 |
| Cl5 | - | - | 2 | 2 | 2 | - | - | 2 | 9 | 17 | 2,32 |
| Cl6 | - | 2 | - | - | - | - | 2 | - | - | 4 | 0,54 |
| G1 | 14 | 6 | 5 | 13 | 7 | 2 | 10 | 3 | 26 | 86 | 11,72 |
| G2 | - | - | - | 2 | - | - | 2 | 2 | - | 6 | 0,82 |
| G3 | 2 | - | - | - | - | - | - | - | - | 2 | 0,27 |
| G4 | - | - | - | 2 | - | - | - | - | - | 2 | 0,27 |
| G5 | - | - | - | - | 2 | - | - | - | - | 2 | 0,27 |
| G6 | - | - | - | 2 | 2 | - | - | 2 | 2 | 8 | 1,09 |
| G7 | - | - | - | - | - | - | - | - | 3 | 3 | 0,41 |
| G8 | - | 3 | - | - | - | - | 12 | 14 | 13 | 42 | 5,72 |
| G9 | 8 | 7 | 4 | 2 | 2 | - | - | - | - | 23 | 3,13 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G10 | - | - | 2 | 18 | 6 | 7 | - | - | - | 33 | 4,5 |
| G11 | - | - | - | 2 | - | - | - | - | - | 2 | 0,27 |
| G12 | 2 | - | - | 2 | - | - | 3 | - | 2 | 9 | 1,23 |
| G13 | - | 2 | - | - | - | - | - | - | 2 | 4 | 0,54 |
| G14 | 2 | - | 2 | - | - | 3 | 2 | - | - | 9 | 1,23 |
| G15 | - | 5 | 2 | 2 | 2 | - | - | 7 | 3 | 21 | 2,86 |
| G16 | - | - | - | - | - | - | - | - | 2 | 2 | 0,27 |
| G17 | 4 | - | 2 | - | - | - | - | - | - | 6 | 0,82 |
| G18 | 2 | 5 | - | - | - | 2 | - | 4 | 5 | 18 | 2,45 |
| G19 | - | - | 2 | 2 | - | - | - | - | - | 4 | 0,54 |
| G20 | - | 6 | - | - | - | - | - | - | - | 6 | 0,82 |
| G21 | - | - | - | - | 2 | - | - | - | - | 2 | 0,27 |
| G22 | - | 4 | 2 | 3 | - | - | 2 | - | 2 | 13 | 1,77 |
| G23 | - | - | - | 2 | - | - | - | - | - | 2 | 0,27 |
| G24 | 47 | 6 | 14 | 16 | 11 | 24 | 4 | 2 | 24 | 148 | 20,16 |
| G25 | 16 | 13 | 34 | 5 | 15 | 26 | 6 | 2 | 13 | 130 | 17,71 |
| G26 | - | - | - | - | 2 | - | - | - | - | 2 | 0,27 |
| D1 | - | - | 2 | - | - | - | - | - | - | 2 | 0,27 |
| D2 | - | - | - | 2 | - | - | - | 2 | 2 | 6 | 0,82 |
| Fu 1 | - | - | 2 | - | - | - | 2 | - | - | 4 | 0,54 |
| Fu2 | 2 | 2 | 7 | - | 2 | - | 5 | - | 5 | 23 | 3,13 |
| S1 | - | - | - | 2 | - | - | - | - | - | 2 | 0,27 |
| Rh1 | - | 2 | 10 | - | - | - | 4 | 2 | 3 | 21 | 2,86 |
| Total | 99 | 67 | 102 | 87 | 57 | 68 | 74 | 54 | 126 | 734 | 100 |
| Richness | 10 | 15 | 20 | 19 | 13 | 8 | 16 | 13 | 18 | | |

**Table 3 (A).** Indicator Species Analysis

| **Gypsum soil Group** #sps. 24 | | | **Marly limestone soil Group** #sps. 22 | | |
|---|---|---|---|---|---|
| OTUs | Indicator Value Index | p-value | OTUs | Indicator Value Index | p-value |
| G10 | 0.658 | 0.007 ** | G8 | 0.639 | 0.003 ** |
| G9 | 0.577 | 0.013 * | Cl5 | 0.566 | 0.023 * |
| G24 | 0.689 | 0.107 | P5 | 0.566 | 0.035 * |
| G25 | 0.648 | 0.189 | G7 | 0.365 | 0.108 |
| G17 | 0.316 | 0.520 | D2 | 0.414 | 0.109 |
| G20 | 0.316 | 0.536 | Fu1 | 0.516 | 0.169 |
| Re1 | 0.258 | 0.551 | G2 | 0.327 | 0.263 |
| G19 | 0.258 | 0.575 | Fu2 | 0.327 | 0.264 |
| G14 | 0.304 | 1.000 | P2 | 0.258 | 0.301 |
| G3 | 0.183 | 1.000 | Cl4 | 0.258 | 0.340 |
| G4 | 0.183 | 1.000 | P3 | 0.258 | 0.343 |
| G5 | 0.183 | 1.000 | G1 | 0.617 | 0.348 |
| G12 | 0.183 | 1.000 | G16 | 0.258 | 0.371 |
| G21 | 0.183 | 1.000 | Rh1 | 0.405 | 0.442 |
| G23 | 0.183 | 1.000 | G6 | 0.298 | 0.564 |
| G26 | 0.183 | 1.000 | G11 | 0.298 | 0.580 |
| Cl1 | 0.183 | 1.000 | Cl2 | 0.298 | 0.611 |
| Cl3 | 0.183 | 1.000 | G15 | 0.390 | 0.716 |
| S1 | 0.183 | 1.000 | G18 | 0.330 | 1.000 |
| D1 | 0.183 | 1.000 | G22 | 0.276 | 1.000 |
| Ar1 | 0.183 | 1.000 | G13 | 0.211 | 1.000 |
| D1 | 0.183 | 1.000 | Cl6 | 0.211 | 1.000 |
| P1 | 0.183 | 1.000 | | | |
| P4 | 0.183 | 1.000 | | | |

631 **p<0.01 *p<0.5

**Table 3 (B).** Indicator Species Analysis

| Gypsophytes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Anthyllis cytisoides* Group** #sps. 13 | | | ***Globularia alypum* Group** #sps. 8 | | | ***Helichrysum stoechas* Group** #sps. 6 | | |
| OTUs | Indicator Value Index | p-value | OTUs | Indicator Value Index | p-value | OTUs | Indicator Value Index | p-value |
| Fu2 | 0.548 | 0.112 | G2 | 0.365 | 0.288 | Cl1 | 0.500 | 0.153 |
| G14 | 0.447 | 0.125 | D2 | 0.316 | 0.391 | G7 | 0.447 | 0.164 |
| Rh1 | 0.500 | 0.142 | G4 | 0.316 | 1.000 | Re1 | 0.447 | 0.173 |
| P5 | 0.400 | 0.245 | G12 | 0.316 | 1.000 | G1 | 0.391 | 0.853 |
| G22 | 0.283 | 0.447 | G23 | 0.316 | 1.000 | G16 | 0.316 | 1.000 |
| G14 | 0.316 | 0.865 | Cl4 | 0.316 | 1.000 | Cl1 | 0.316 | 1.000 |
| Cl3 | 0.316 | 1.000 | S1 | 0.316 | 1.000 | | | |
| D1 | 0.316 | 1.000 | D1 | 0.316 | 1.000 | | | |
| P1 | 0.316 | 1.000 | | | | | | |
| P2 | 0.316 | 1.000 | | | | | | |
| P3 | 0.316 | 1.000 | | | | | | |
| P4 | 0.316 | 1.000 | | | | | | |
| G19 | 0.224 | 1.000 | | | | | | |
| | | | | | | | | |
| Gypsovags | | | | | | | | |
| ***Helianthemum squamatum* Group** #sps. 6 | | | ***Launaea pumila* Group** #sps. 4 | | | ***Ononis tridentata* Group** #sps. 9 | | |
| OTUs | Indicator Value Index | p-value | OTUs | Indicator Value Index | p-value | OTUs | Indicator Value Index | p-value |
| G10 | 0.474 | 0.118 | G17 | 0.566 | 0.073 | G20 | 0.775 | 0.003 ** |
| G5 | 0.447 | 0.326 | G3 | 0.447 | 0.328 | G15 | 0.507 | 0.106 |
| G21 | 0.447 | 0.326 | G24 | 0.416 | 0.681 | G9 | 0.474 | 0.233 |
| G26 | 0.447 | 0.326 | G11 | 0.283 | 0.742 | G8 | 0.381 | 0.367 |
| Ar1 | 0.447 | 0.326 | | | | G18 | 0.365 | 0.569 |
| G6 | 0.283 | 0.758 | | | | Cl6 | 0.365 | 0.605 |
| | | | | | | G13 | 0.365 | 0.615 |
| | | | | | | G30 | 0.283 | 0.773 |
| | | | | | | G25 | 0.405 | 0.929 |

632 **p<0.01 *p<

**Table 4**. Modularity values of the unipartite Arbuscular mycorrhizal fungi (AMF) network.

| Networks Modules | Modularity |
|---|---|
| All interactions | |
| Module A: G24 G12 G19 P4 S1 P2 G25 Re1 G2 Cl1 D1 G3 G4 De1<br>Module B:G10 G23 G9 G6 Ar1 Fu1 G5 G26 G21<br>Module C:G11 G14 Cl5 G17 P1 Cl3 G22 Fu2<br>Module D:G7 G16 G18 G13 D2 G8 G15 Cl4 Cl6 P3 G2 Cl2 P5 Rh1 G1 G20 | 0.152<br>[0.105-0.107] |
| Gypsum area | |
| Module E: D2 G11 G9 G23 G1 G10 G21 G26 G5 Ar1 G6<br>Module F: Fu2 Cl3 Rh1 P1 G22 P5 G17 G14 Cl5<br>Module G: Cl2 Cl6 G12 G15 G20 G8 Fu1 G18 G13<br>Module H: G24 Re1 Cl1 S1 P4 G19 G4 G2 De1 D1 G3 G25 | 0.185<br>[0.092, 0.137] |

Modularity values and confidence intervals, in brackets, are presented for each subset of data: considering: a) all interactions and b) only plants growing in the gypsum area. The AMF OTUs' ascription to each module is presented for each analysis. Four modules (A to D and E to H) were detected in both datasets.

656 **Figure 1.** Neighbour-Joining (NJ) phylogenetic tree showing AM fungal sequences isolated
657 from roots of *Ononis tridentata, Helianthemum squamatum, Launaea pumila, Globularia*
658 *alypum, Helichrysum stoechas* and *Anthyllis terniflora,* and reference sequences from
659 GeneBank. All bootstrap values > 80% are shown (1000 replicates). Numbers above branches
660 indicate the bootstrap values of the maximum likelihood analysis. Sequences obtained in the
661 present study are shown in bold type. They are labelled with the host plant from which they
662 were obtained and the clone identity number. *O. tridentata* (OT)*, H. squamatum* (HS), *L.*
663 *pumila* (LP), *G. alypum* (GA)*, H. stoechas* (ST) and *A. terniflora* (AT). The letter G before
664 the gypsovags species name means that they were obtained from the gypsum soil. Group
665 identifiers (for example G1) are OTUs found in our study. See the supplemental material for a
666 detailed description of the all clones obtained in the present study for each group. *Endogone*
667 *pisiformis* and *Mortierella polycephala* were used as out-groups.
668