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1. Title: Plant traits determine the phylogenetic structure of arbuscular mycorrhizal fungal communities.

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6. Running title: Plant determines AMF phylogenetic structure

Abstract

Functional diversity in ecosystems has traditionally been studied using aboveground plant traits. Despite the known effect of plant traits on the microbial community composition, their effects on the microbial functional diversity are only starting to be assessed. In this study, the phylogenetic structure of arbuscular mycorrhizal (AM) fungal communities associated to plant species differing in life cycle and growth form, i.e. plant life forms, was determined to unravel the effect of plant traits on the functional diversity of this fungal group. The results of the 454-pyrosequencing showed that the AM fungal community composition differed across plant life forms and this effect was dependent on the soil collection date. Plants with ruderal characteristics tended to associate with phylogenetically clustered AM fungal communities. By contrast, plants with resource-conservative traits associated with phylogenetically over-dispersed AM fungal communities. Additionally, the soil collected in different seasons yielded AM fungal communities with different phylogenetic dispersion. In summary, we found that the phylogenetic structure, and hence the functional diversity, of AM fungal communities is dependent on plant traits. This finding adds value to the use of plant traits for the evaluation of belowground ecosystem diversity, functions and processes.

Introduction

The study of ecosystem functioning is receiving an increasing attention due to the need to understand the consequences of global change and assess the impact of land management on key ecosystem functions. For terrestrial ecosystems, the analysis of plant traits has proven to be a key tool to reveal the ecological processes determining plant community composition, species diversity and ecosystem functioning (Adler, Fajardo, Kleinhesselink, & Kraft, 2013; Cadotte, Carscadden, & Mirotchnick, 2011; Lavorel & Garnier, 2002; Suding et al., 2008). Indeed, plant traits at the community level are affected by changes in the abiotic environment and these changes have the potential of altering biogeochemical cycles such as those of C and N (Lavorel et al., 2011), the rate of litter decomposition (Fortunel et al., 2009; Garnier et al., 2004) or the nitrification potential (Laughlin, 2011) in the ecosystems. Growing evidence points out that these effects can be mediated by changes in soil microbial communities (Bardgett & Wardle, 2010). Thus, De Vries et al. (2012), for example, found that bacterial-dominated microbial communities in soil were associated with exploitative plant traits whilst fungal-dominated communities associated with resource-conservative plant traits.

In this context, little is known about the relationship of plant traits and root-associated mycorrhizal fungal communities. At the ecosystem level, the type of mycorrhizal association of plants, either ectomycorrhizal or arbuscular mycorrhizal, can influence C cycling (Phillips, Brzostek, & Midgley, 2013; Soudzilovskaia, van der Heijden, Cornelissen, & Mikhail, 2015). Only few studies have addressed the effect of plant traits on the composition and functioning of arbuscular mycorrhizal (AM) fungal communities. The preference for partners in AM symbioses is being increasingly appreciated due to the findings of non-random associations between plant species and AM fungal communities (Öpik, Metsis, Daniell, Zobel, & Moora,

2009; Varela-Cervero et al., 2015). Indeed, experiments under controlled conditions and spatially explicit studies have demonstrated not only certain preferences in the plant-fungus association but also the potential of plant traits to determine the AM fungal community composition (see Chagnon, Bradley, & Klironomos, 2015; Koorem et al., 2017; López-García, Azcón-Aguilar, & Barea, 2014a).

In this context, it has been hypothesized that plant traits could also determine the traits of their associated AM fungal partners (Chagnon, Bradley, Maherali, & Klironomos, 2013). AM fungi differ in fundamental traits, both inter- and intra-specifically (Hart & Reader, 2002; Munkvold, Kjølter, Vestberg, Rosendahl, & Jakobsen, 2004), and some of these attributes, e.g. the colonization pattern, have been seen to be phylogenetically conserved (Koch, Antunes, Maherali, & Klironomos, 2017; Powell et al., 2009). Thus, the phylogeny of AM fungal communities has been used by several authors to trace ecological processes such as coexistence mechanisms (Lekberg, Gibbons, Rosendahl, & Ramsey, 2013; Maherali & Klironomos, 2007; 2012) or dispersal (García de León et al., 2016; Horn, Caruso, Verbruggen, Rillig, & Hempel, 2014; Nielsen, Kjølter, Bruun, Schnoor, & Rosendahl, 2016). The link between plant and AM fungal traits has been partly supported using these phylogenetic approaches: Chagnon et al. (2015) found that plants with similar functional traits associated with groups of AM fungi that are phylogenetically clustered. However, the molecular approach they used is known to bias towards certain families of AM fungi (Lee, Lee, & Young, 2008) and hence a more complete characterization of the AM fungal community is still needed to disentangle the relationship between plant traits and AM fungal community phylogeny.

We hypothesized that the phylogenetic structure of associated AM fungal communities can vary in a gradient from more ruderal plants (herbaceous annuals) towards plants with more conservative traits (semiwoody perennials). This variation can be the result of changes in the dominant AM fungal family, as suggested by Chagnon et al. (2013), or due to different levels of phylogenetic diversity of AM fungal communities. Additionally, we expected to find different AM fungal communities associated to different plant functional groups, i.e. legumes and non-legumes, and a certain phylogenetic clustering due to the particular environmental conditions of the nitrogen fixing roots of legumes. Thus, we aimed to determine to what extent plant traits determine the phylogenetic structure of AM fungal communities. Due to the known functional differences among AM fungal families, we aimed to: i) assess the phylogenetic diversity of AM fungal communities, as a proxy of functionality, across plant life forms, i.e. groups of plants sharing life cycle and growth form; ii) determine whether AM fungal families preferentially associate with different plant life forms; iii) determine the effect of plant life form traits and other plant functional traits (nitrogen fixing ability) on the phylogenetic structure of AM fungal communities.

Material and Methods

Study site and sampling

The study site was located in the Sierra de Baza Natural Park (Granada province, Southeast Spain), a protected area with a Mediterranean semi-arid climate. The climatic conditions are characterized by low and irregular precipitation with an annual mean of 385 mm, and a temperature of 25 °C in summer and 6 °C in winter, on average. The soil is a Calcaric Cambisol. The vegetation is an open autochthonous sclerophyll woodland combined with naturalized *Pinus pinaster* and *P. halepensis* from earlier restoration tasks. The most abundant

woody species are *Quercus ilex* subsp. *ballota* (Desf.) Samp., *Pinus* sp., *Juniperus oxycedrus* L., *Rosmarinus officinalis* L., *Berberis vulgaris* subsp. *australis* (Boiss.) Heywood and *Rosa* sp. accompanied with herbaceous plant species. An area of 15,000 m² was selected using a geographic information system (ArcGIS v9.3.1, ESRI, Redlands, CA) that keeps a constant slope (15–20%) and orientation (east).

A representative AM fungal inoculum of the target area (37°24'1.01"N, 2°50'43.60"W) was obtained by sampling 20 randomly selected points. After removing the first soil layer, approximately 7 l of soil in each point was collected between 2- and 20-cm depths. Since the phylogenetic structure of AM fungal communities can fluctuate in response to seasonality (Dumbrell et al., 2011; López-García, Palenzuela, Barea, & Azcón-Aguilar, 2014b) the sampling was repeated twice: December (autumn) 2010 and June (spring) 2011. All soil samples were pooled and mixed in a concrete mixer by each sampling date. Big stones were removed by hand and the soil was not sieved so that big soil aggregates were still present. The soil was used to fill 1 l pots which were placed in a greenhouse.

Experimental set up

Six plant species with different life forms were selected regarding two plant traits: growth form –either herbaceous or semi-woody- and life cycle –annual vs. perennial. Following the species list of the Sierra de Baza Natural Park by Blanca and Morales (1991) two plant families in the area (Asteraceae and Fabaceae) harbor three of the four combinations of these plant traits (herbaceous annual, herbaceous perennial and semi-woody perennial), therefore the study site was surveyed to locate members of these families and life forms. The following members of the Asteraceae family were found in the area and selected for the study:

Xeranthemum inapertum (L.) Mill (herbaceous annual), *Inula montana* L. (herbaceous perennial) and *Santolina canescens* Lag. (semi-woody). From the Fabaceae family the selected species were: *Trifolium angustifolium* L. (herbaceous annual), *Medicago sativa* L. (herbaceous perennial) and *Ononis natrix* L. (semi-woody). The presence of these plant species in the area ensures that they share habitat with the local AM fungi and potentially can be colonized by these. Seeds from Andalusian natural populations of these plant species were provided by the Real Jardín Botánico (CSIC, Madrid) and the Banco de Germoplasma Vegetal Andaluz (Junta de Andalucía, Córdoba).

The seeds were pre-germinated on sterile filter paper one week before soil sampling. Immediately after the soil sampling and its processing in the lab, the germinated seeds were planted into the 1 l pots filled with the natural soil. The experiment was repeated twice using natural soil collected in two different times as described above. Four replicate pots per plant species and soil collection date were set up. Growing conditions in the greenhouse were 16/8-h day-night light cycle and 24–18 °C. After a growth period of 6 months the entire plants were harvested. The root system was extracted by hand and cleaned using running tap water. Roots were cut into 5 mm pieces, mixed by hand and frozen at -80°C in aliquots of 100 mg fresh weight.

Molecular analyses

A total of 48 root samples (4 replicates x 6 species x 2 collection dates) were subjected to 454 pyrosequencing. Individual root samples (100 mg fresh weight) were crushed with a sterile mortar and pestle using liquid nitrogen. Genomic DNA was extracted using DNeasy Plant Mini Kit (Qiagen Inc., Mississauga, ON, Canada), following manufacturer's instructions. The

glomeromycotinan specific primer pair NS31 and AML2 (Simon, Lalonde, & Bruns, 1992; Lee et al., 2008) was used to amplify the nuclear SSU rRNA gene. The 454 sequencing primers A and B were attached to them respectively as well as an 8 bp barcode as previously described in Varela-Cervero et al. (2015). A two-step PCR was conducted to ensure proper amplification of the long primer sequences composed by 454-adaptor+Barcode+PCR primer. The reactions were set up in a final volume of 10 µl that contained 1 µl of template DNA, primers with 0.2 µM final concentrations and Smart-Taq Hot Red 2x PCR Mix (0.1 U/µL Smart Taq Hot Red Thermostable DNA Polymerase, 4 mM MgCl₂, 0.4 mM dATP, 0.4 mM dCTP, 0.4 mM dGTP, 0.4 mM dTTP; Naxo OU, Estonia). The reactions were run on a Thermal cycler 2720 (Applied Biosystems) under the following conditions: 95 °C for 15 min, five cycles of 92 °C for 45 s, 42 °C for 30 s, 72 °C for 90 s; 35 cycles (first PCR) or 20 cycles (second PCR) of 92 °C for 45 s, 65 °C for 30 s, 72 °C for 90 s; followed by 65 °C for 30 s and 72 °C for 10 min. Final PCR products were separated by electrophoresis in a 1.5 % agarose gel in 0.5x TBE, bands were excised and subsequently purified using Agencourt AMPureXP Kit (Beckman Coulter Inc.). DNA concentration of purified amplicons was measured using Appliskan fluorescence-based microplate reader (Thermo Scientific) and PicoGreen® dsDNA Quantitation Reagent (Quant-iT dsDNA Broad Range Assay Kit, Invitrogen). A pool of purified PCR products was set up and its final concentration measured on Qubit™ fluorometer. One hundred µl of DNA library, 20.7 ng/µl concentration, was analyzed by Roche 454 FLX next generation sequencing platform in Microsynth AG (Balgach, Switzerland). Preparatory procedures for 454 sequencing (barcoded PCRs and PCR product purification) were performed by Biotap LLC (Tallinn, Estonia).

Bioinformatic analyses

Read filtering, removal of primer and barcode sequences and parsing of BLAST output were carried out using a series of Python and Java programs developed at the Department of Botany, University of Tartu (Davison et al., 2012). This work was partially carried out in the High Performance Computing Center of the University of Tartu.

The following criteria were applied to consider 454-sequencing reads in the analysis: (i) a barcode matching one used in this study, (ii) to carry the correct forward primer sequence, (iii) to be at least 170 nucleotides in length (excluding the barcode and primer sequences (see Fig. S1 for read length distribution in the study) and (iv) an average quality score ≥ 25 . Reads were trimmed to 520 nucleotides to exclude reverse primer sequences. UCHIME algorithm (v7.0.1090, Edgar, Haas, Clemente, Quince, & Knight, 2011) was used to search and exclude a total of 3,438 chimeric sequences, resulting in a total of 138,726 quality-filtered sequences.

The MaarjAM database of published Glomeromycotina SSU rRNA gene sequences (Öpik et al., 2010) was used to identify obtained reads. An open-reference OTU (operational taxonomic unit) picking approach (Bik et al., 2012) was used for the taxonomic identification of reads. Sequence reads were assigned to VT via BLAST (v2.5.0+, Camacho et al., 2009) against the MaarjAM database with the following criteria: sequence similarity ≥ 97 %, query coverage 95 %, and a BLAST e-value $< 1e-50$.

A total of 86,219 (60.65 %) sequences were identified as Glomeromycotina in a first BLAST against the MaarjAM database. Sequences that did not find a match against the MaarjAM database were then subjected to a further BLAST search against INSDC with the same criteria as before, except that the sequence similarity threshold was set to ≥ 90 % and alignment

length at least 90 % of the shorter sequence. We further identified 3,319 sequences being potential Glomeromycotina. To assess whether these sequences were novel VT, a phylogenetic analysis was performed including all VT type sequences from the MaarjAM database and 2,338 previously detected AM fungal sequences from Sierra de Baza Natural Park (Varela-Cervero et al., 2015). Potential glomeromycotinan sequences were clustered at 97 % similarity using BLASTclust (v2.2.26, Altschul, Gish, Miller, Myers, & Lipman, 1990) and clusters containing just one sequence were removed. Up to 4 sequences from each cluster were aligned together with the MaarjAM VT type sequences and the previously recorded sequences from Sierra de Baza using MAFFT (v6.940b, Katoh, Misawa, Kuma, & Miyata, 2002) multiple sequence alignment web service implemented in JALVIEW v2.8 (Waterhouse, Procter, Martin, Clamp, & Barton, 2009). A neighbor-joining phylogenetic analysis was carried out in TOPALi v2.5 (Milne et al., 2009). The longest high-quality sequence of each novel VT was chosen as the representative and included in the reference dataset (i.e. MaarjAM database) to perform a second BLAST with additional VT. This yielded a total of 86,519 AM fungal reads identified as Glomeromycotina. The Glomeromycotina nomenclature used here follows Redecker et al. (2013) except that genus *Glomus* is treated *sensu lato* for the reasons provided in Öpik et al. (2013).

Statistical analyses

In order to investigate taxon accumulation as a function of sequencing depth per sample, rarefaction curves were constructed using the function *rarefy()* from the R package *vegan* (Oksanen et al., 2013). Due to the similar completeness of capturing the total VT richness among samples (Fig. S2), number of sequence reads per sample were not normalized. Sequence reads per VT were used as a proxy of relative abundance of the VT in the sample

and they were used to construct an abundance matrix. Prior to statistical analyses this matrix was Hellinger transformed.

Diversity indices (richness and Simpson index, 1-D) were calculated using the *diversity()* function from R package *vegan* (Oksanen et al., 2013).

The phylogenetic structure of the AM fungal community was addressed by calculating the standardized effect size of mean pair wise distance (SES-MPD) using the *picante* package in R (Kembel et al., 2010) calculated with abundance-weighted data. This index compares the mean phylogenetic distance between pairs of species in a sample with that obtained in a series of generated random communities. The index was calculated by using a phylogenetic distance matrix between representative sequences of each VT in the experiment (local pool) calculated using the function *dist.seqs* in *mothur* (Schloss et al., 2009) plus the abundance matrix of OTUs. The sequence alignment was carried out in MAFFT (Katoh et al., 2002) and trimmed to the length of the shortest sequence in the dataset (507 bp). The calculations of SES-MPD were repeated using a phylogenetic distance matrix including representative sequences of every VT in MaarjAM database (global pool). The *independentswap* algorithm was used to generate 999 randomized null communities to obtain the standardized effect size of mean pairwise distance. The mean value of the SES-MPD per group was then used to judge the phylogenetic clustering or segregation of the AM fungal community. Negative values are correlated with clustering, positive values with over-dispersion (Webb, Ackerly, McPeck, & Donoghue, 2002). The statistical significance of the calculated SES-MPD was tested using t-test.

The effect of plant traits, taxonomy -plant family and species- and soil collection date on the diversity and phylogenetic structure of AM fungal communities was analyzed by performing generalized linear models with Gaussian error structure (function *glm()* from R package MASS; Ripley et al., 2014). Tukey's honest significant difference (HSD) tests were carried out to determine significant differences among groups.

The effect of plant traits, taxonomy –species and family levels- and soil collection date on the community composition of the AM fungal community was addressed by means of permutational multivariate analysis of variance (PERMANOVA) using Euclidean distance as a measure of dissimilarity, which, since the abundance matrix was Hellinger-transformed, must be considered a Hellinger-based dissimilarity (Legendre & Gallagher, 2001). Since PERMANOVA is sensitive to changes in multivariate dispersion among samples, the *betadisper()* function of vegan package was used to assess differences in multivariate dispersion. A Non-metric Multidimensional Scaling (NMDS) ordination was carried out to allow for a visualization of the AM fungal community variation.

AM fungal species indicative of particular groups of samples were identified by using Dufrêne–Legendre indicator species analysis (Dufrêne & Legendre, 1997), implemented with the *indval()* function from the R package labdsv (Roberts, 2014).

Results

454 sequencing data

A total of 86,503 SSU rRNA gene sequences identified as Glomeromycotina were assigned to 84 AM fungal VT, including four novel VT (Table S1): 2 Acaulosporaceae, 1

Ambisporaceae, 3 Archaeosporaceae, 5 Claroideoglomeraceae, 10 Diversisporaceae, 2 Gigasporaceae, 59 Glomeraceae and 2 Paraglomeraceae. The novel VT represented 1 Archaeosporaceae and 3 Glomeraceae (Fig. S3, Table S1). From the 113 VT registered in the MaarjAM database from Sierra de Baza, 57 were detected in this study and 27 were found for the first time in the area (Fig. S4). From the initial 48 samples, the PCR amplification failed for seven of them: two in the autumn collection date (*X. inapertum* and *M. sativa*) and five in spring (one per each: *T. angustifolium*, *M. sativa*, *S. canescens*, and two for *X. inapertum*).

AM fungal diversity

Plant life form had a marginally significant effect on VT richness (Table S2), probably due to the lower average richness of AM fungal VT in semi-woody plants in autumn (Table 1). In general, a lower VT richness per sample was also found for the soil collected in autumn (Table 1, Table S2). Asteraceae plants showed a higher number of VT per sample than Fabaceae (Table 1, Table S2). The strongest effect was found for the interaction between plant life form and soil collection date (Table S2): semi-woody plants grown in autumn soil had lower number of VT in roots (26.6 ± 1.6 standard error) than other plant life form and collection date combinations (Table 1). Simpson (1-D) diversity index only varied according to plant family (Table S2): Asteraceae plants had a more even AM fungal community (0.81 ± 0.02) than Fabaceae (0.72 ± 0.04). When comparing with the previous study using pyrosequencing on field samples of Sierra de Baza Natural Park (Varela-Cervero et al., 2015), the richness in the present study was overall higher than in the previous study, which ranged from 16 to 22 VT per sample in average.

AM fungal community composition

All tested variables (plant family, plant life form and soil collection date) affected the composition of AM fungal communities (Table 2, Fig. 1). Except for plant family, none of the significant differences were due to different multivariate dispersion between treatments (Table 2). The separate PERMANOVA for each soil collection date confirms the interaction term found when all samples were analyzed together (“All samples”) (Table 2, Fig. 1). In autumn, plants having different life cycles (annual vs. perennial) differed more than plants having different growth forms (herbaceous vs. semiwoody) (pairwise PERMANOVAs: herbaceous annual-herbaceous perennial: PseudoF= 2.40, $P= 0.065$; herbaceous annual-semiwoody: PseudoF= 5.51, $P= 0.001$; herbaceous perennial-semiwoody: PseudoF= 1.44, $P= 0.157$).

AM fungal community phylogenetic structure

Due to the similarity of results, we only reported those using the local pool of species in the calculation of the standardized effect size of mean pairwise distance (SES.MPD). For the whole dataset, AM fungal communities associated to semiwoody plants had higher phylogenetic dispersion (over-dispersion) than annual and perennial herbs (Table S2, Table 3). The phylogenetic structure of herbaceous perennial plants did not differ from random expectations and phylogenetic clustering was detected for herbaceous annuals (Table 3). The same pattern for each life form was found when analyzing the subset of samples from the autumn soil collection (Table 3, Fig. 2a). Finally, when analyzing the subset of samples from spring, the phylogenetic structure of AM fungal communities of any plant life form did not differ from random expectations (Fig. 2a).

Overall, AM fungal community data resulting from soil collected in spring showed phylogenetic clustering, while those obtained using soil collected in autumn did not differ from random expectations (Table 3). Differences between collection dates were confirmed by the GLM analysis (Table S2). On the other hand, no difference between the AM fungal community phylogenetic structure in Fabaceae and Asteraceae was detected (Table S2).

Distribution of AM fungal families

In general, all treatments were dominated by Glomeraceae (Fig. 2b). However, an increase in the relative abundance of Claroideoglomeraceae, Paraglomeraceae, Archaeosporaceae and Diversisporaceae in plant roots (Fig. 2b) was seen in the direction of herbaceous annuals>herbaceous perennials>semiwoody plants and also for the comparison between spring and autumn. Indicator species analysis (Table 4, Fig. 3) revealed that Glomeraceae VT were the predominant indicators for the soil collected in spring and the herbaceous life forms (either annuals or perennials), whereas for semiwoody plants and, in general, for the plants grown in the soil sampled in autumn, indicator VT belonged to the Paraglomeraceae and Claroideoglomeraceae.

Discussion

In this study we expected to link plant traits related with life history strategies with AM fungal communities both in terms of species composition and their phylogenetic structure, as a proxy of their functionality. To our knowledge, this is the first study focusing in an explicit gradient of plant host traits, from ruderal to conservative. The homogenization of the starting mycorrhizal fungal inoculum, together with its local nature, ensures that stochastic processes were minimized, e.g. dispersal or priority effects, letting plant species as the only source of

selection for the AM fungal community. Even taking into account the study system, i.e. a greenhouse pot experiment, the diversity of VT was not much lower than in the group of studies in Sierra de Baza. In addition, several new VT were described for the area belonging to different AM fungal families. In general, we found that certain plant traits and times act as filters in the community assembly process of AM fungi, selecting for a subset of similar AM fungal species. Interestingly, we could not detect a similar phylogenetic selection in Fabaceae plants which was expected due to their inherent special characteristics. The revealed phylogenetic patterns have the potential of explaining how functional diversity of AM fungal communities varies spatially and temporally across ecosystems.

Effect of plant life forms

Despite of the already known effect of plant host species (Martínez-García, Richardson, Tylianakis, Peltzer, & Dickie, 2015; Varela-Cervero et al., 2015) and their characteristics (Chagnon et al., 2015; López-García et al., 2014a; Öpik et al., 2009) on the AM fungal community composition, the most remarkable effect of plant life form in the present study was the promotion of AM fungal communities with different phylogenetic structures. Thus, in the order annual herbs-perennial herbs-semiwoody plant species, there was a transition from phylogenetic clustered to over-dispersed AM fungal communities. This trend is consistent with a transition from environmentally filtered communities to communities where competition among species dominates the community assembly processes (Götzenberg et al., 2012). A similar pattern has been observed previously in gradients of fertilization (Liu et al., 2015) and soil heavy metal contamination (Montiel-Rozas, López-García, Kjølner, Madejón, & Rosendahl, 2016). In both cases the authors attributed phylogenetic over-dispersion to a stronger role of limiting similarity due to competition between species when a certain

environmental filter is relaxed. Competition between AM fungal individuals is difficult to assess (Cano & Bago 2005; Hepper, Azcón-Aguilar, Rosendahl, & Sen, 1988); however some studies have revealed certain interactions between AM fungal species which can imprint the phylogenetic structure of their communities (Engelmoer, Behm, & Kiers, 2014; Maherali & Klironomos, 2012; Mummey, Antunes, & Rillig, 2009). The phylogenetic clustering of AM fungal communities associated to annual plants is in agreement with the predictions made by Chagnon et al. (2013), who proposed that plants with ruderal life history strategies preferentially associate with Glomeraceae. This hypothesis is based in the similar ruderal life style of the plant and fungal partner where they develop short life cycles and invest in less costly biological structures. Indeed, most of the AM fungal VT preferentially associated to annual plants belonged to Glomeraceae. Phylogenetic clustering has been previously recorded for AM fungal assemblages, possibly resulting from processes acting at wide spatial scales such as dispersal or environmental filtering (e.g. Horn et al., 2014; Davison et al., 2016). In contrast, phylogenetic over-dispersion has been less commonly found (but see Liu et al., 2015; Maherali & Klironomos, 2012).

Effect of plant functional group

It is noteworthy that an effect of plant functional group (legumes vs. non-legumes) on AM fungal community phylogenetic structure was not detected. However Fabaceae plants harbored a different AM fungal community composition and lower taxonomic diversity compared to Asteraceae. Previous studies had identified similar trends (López-García et al., 2014a; Scheublin, Ridgway, Young, & van der Heijden, 2004; Vandenkoornhuyse et al., 2002), proposed to be caused by strong restrictions to the fungal life style where the plant requires big amounts of phosphorus to maintain N-fixing nodules or due to particular

physicochemical conditions (Scheublin et al., 2004). These special conditions for AM fungi living in Fabaceae roots must imply the selection of fungi with specific traits. Our results showed that in contrast to other fungal life-history traits that have been seen to drive community assembly of AM fungi (Maherali & Klironomos, 2012) and are phylogenetically conserved (Koch et al., 2017; Powell et al., 2009), the potential adaptations needed to associate to N-fixing plants are not phylogenetically conserved.

Effect of soil collection date

Other clear effect on the phylogenetic structure of the AM fungal community was the collection time of the soil used as inoculum. The strong phylogenetic clustering detected when using the soil collected in spring is in agreement with the findings of Dumbrell, Nelson, Helgason, Dytham and Fitter (2010) who found an over-dominance of single AM fungal species in spring, when C supply is increased. In our study, this increase in clustering was driven exclusively by Glomeraceae species, which were the most abundant family in the roots. Usually, an increase in a limiting resource benefits competitors more adapted to capture disproportionate amounts of resources leading to over-dominance (Tilman, 1987). In the case of AM fungi, Glomeraceae species are known to be the best competitors when colonizing plant roots (Hart & Reader, 2002; López-García et al., 2014b) and hence they usually are the most frequent AM fungal family found into plant roots (Varela-Cervero et al., 2015). In the present study, they were found to mainly dominate in the plants grown in the soil collected in spring, which is probably the main reason of the phylogenetic clustering. In contrast, the relative limitation of carbon availability during autumn, which stems from a previous stressful period with high temperatures and drought in summer, would imply lower rates of photosynthesis and may derive, in agreement with our results, in a more even AM fungal

community (Dumbrell et al., 2011) and more diverse in terms of species and families. The higher phylogenetic diversity in the soil collected in autumn has probably led the differences detected regarding AM fungal community composition and phylogenetic structure across plant life forms in that time. Davison et al. (2011) found a similar pattern when recorded that main differences in AM fungal community composition between plant ecological groups occurred later in the growing season.

Conclusion

The forces that shape the communities of AM fungi are of wide interest among soil microbial researchers (Vályi, Mardhiah, Rillig, & Hempel, 2016). Our results point out the role of plant traits to shape the phylogenetic structure of AM fungal communities into their roots, either by filtering or promoting their phylogenetic, i.e. functional, diversity. Despite our clear results, the limited number of plant species studied points out that future research on community phylogenetic patterns of AMF should include a wider spectrum of plant species and traits. Previous studies have highlighted the importance of maintaining a high functional diversity of AM fungi, for example to keep high plant productivity (Maherali & Klironomos, 2007). Our results show that ecological processes shifting the physiognomy of plant communities have the potential of altering such hidden diversity in soils. However, trait-based ecology of AM fungi needs to be developed to fully understand the functional significance of the recorded patterns, not only inside plant roots but also in soil rhizosphere. Further research in this line will allow us to know if more fungal traits, with potential to affect ecosystem functioning, are really phylogenetically conserved and thus support the value of the analysis of AM fungal phylogenetic diversity.

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744 **Data accessibility**

745 Representative sequences maximum of two from each VT per each plant species and soil
746 collection date were deposited in the GenBank nucleotide collection under accession numbers
747 KU246403 - KU247479.

748

749 **Author contribution statement**

750 A.L.G., C.A.A. and J.M.B. designed the study. A.L.G. conducted the experiments and
751 laboratory analyses. S.V.C., M.O. and M.V. conducted the molecular and bioinformatics
752 analyses. A.L.G. conducted the statistical analyses and wrote the manuscript with
753 contributions from all the authors.

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Tables and figures

Table 1. Average Virtual Taxa (VT) richness ($\pm$ standard error) shown for the different species and plant life forms according to soil collection date. Means sharing a letter in common are not significantly different from each other according to the Tukey's honest significant difference test ($P < 0.05$).

	<i>Trifolium angustifolium</i> (Fabaceae)	<i>Xeranthemum inapertum</i> (Asteraceae)	<i>Medicago sativa</i> (Fabaceae)	<i>Inula montana</i> (Asteraceae)	<i>Ononis natrix</i> (Fabaceae)	<i>Santolina canescens</i> (Asteraceae)	Collection date average
Autumn	31.8 $\pm$ 0.6 abc	42.7 $\pm$ 0.9 a	34.3 $\pm$ 5.6 abc	34.8 $\pm$ 2.2 ab	23.5 $\pm$ 1.8 c	29.8 $\pm$ 1.3 bc	32.3 $\pm$ 1.5 b
Spring	32.3 $\pm$ 4.7 abc	38.5 $\pm$ 2.5 ab	36.3 $\pm$ 3.5 ab	35.8 $\pm$ 0.8 ab	37.5 $\pm$ 3.0 ab	38.3 $\pm$ 0.9 ab	36.4 $\pm$ 1.1 a
	Herbaceous annual		Herbaceous perennial		Semiwoody		
Autumn	36.4 $\pm$ 2.3 a		34.6 $\pm$ 2.4 a		26.6 $\pm$ 1.6 b		
Spring	34.8 $\pm$ 3.1 ab		36.0 $\pm$ 1.4 a		37.9 $\pm$ 1.7 a		

Table 2. Variation in arbuscular mycorrhizal fungal community composition (PERMANOVA and multivariate dispersion analyses) in relation to plant life form, plant family and soil collection date for all samples (a), and effect of plant life form for autumn (b) and spring (c) subsets. Values of Degrees of Freedom (DF), Sum of Squares (SS) and Mean Squares (MS) are shown.

Model/parameter	DF	SS	MS	PseudoF/F	R2	P
(a) All samples						
PERMANOVA						
Plant life form	2	0.91	0.46	2.68	0.09	0.002
Soil collection date	1	1.04	1.04	6.10	0.11	0.001
Plant family	1	0.96	0.96	5.62	0.10	0.002
Plant life form $\times$ Soil collection date	2	0.80	0.40	2.35	0.08	0.010
Plant life form $\times$ Plant family	2	0.46	0.23	1.36	0.05	0.165
Soil collection date $\times$ Plant family	1	0.14	0.14	0.84	0.01	0.541
Life form $\times$ Soil collection date $\times$ Family	2	0.45	0.23	1.33	0.05	0.165
Residuals	29	4.94	0.17		0.51	
Total	40	9.71			1.00	
Multivariate dispersion						
Plant life form	2	0.00	0.00	0.14		0.871
Residuals	38	0.64	0.02			
Soil collection date	1	0.00	0.00	0.42		0.523
Residuals	39	0.45	0.01			
Plant family	1	0.07	0.07	5.71		0.022
Residuals	39	0.46	0.01			
(b) Autumn						
PERMANOVA						
Plant life form	2	1.06	0.53	2.98	0.24	0.002
Residuals	19	3.37	0.18		0.76	
Total	21	4.43			1.00	
Multivariate dispersion						
Plant life form	2	0.04	0.02	2.36		0.121
Residuals	19	0.18	0.01			
(c) Spring						
PERMANOVA						
Plant life form	2	0.68	0.34	1.52	0.16	0.114
Residuals	16	3.56	0.22		0.84	
Total	18	4.24			1.00	
Multivariate dispersion						
Plant life form	2	0.03	0.02	0.91		0.421
Residuals	16	0.28	0.02			

Table 3. Phylogenetic dispersion of arbuscular mycorrhizal fungal communities related to plant life form and soil collection date, expressed as standardized effect size of phylogenetic mean pairwise distance (SES.MPD). Bold letters indicate significance ($P < 0.05$).

	Average SES.MPD	t	df	P
Plant life form				
Herbaceous annuals	-1.044±0.212	-4.9331	11	0.041
Herbaceous perennials	-0.348±0.387	-0.897	13	0.386
Semiwoody	0.597±0.178	3.352	14	0.005
Soil collection date				
Autumn	0.153±0.234	0.656	21	0.519
Spring	-0.621±0.282	-2.207	18	0.041
Plant life form x Soil collection date				
Herb annual: Autumn	-1.074±0.207	-5.18	6	0.002
Herb perennial: Autumn	0.481±0.320	1.503	6	0.184
Semiwoody: Autumn	0.939±0.219	4.277	7	0.004
Herb annual: Spring	-1.003±0.452	-2.2163	4	0.091
Herb perennial: Spring	-1.176±0.565	-2.0817	6	0.083
Semiwoody: Spring	0.206±0.215	0.95436	6	0.377

777 **Table 4.** Indicator virtual taxa for the different soil collection dates and plant life forms.
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	Corresponding morphospecies	Group	Indicator value	<i>P</i>
<i>Claroideoglomus</i> VT56		Autumn soil	0.699	0.002
<i>Glomus</i> VT69	<i>Glomus sinuosum</i> (AJ133706)	Autumn soil	0.273	0.029
<i>Glomus</i> VT113	<i>Glomus intraradices</i> (FJ009595)	Autumn soil	0.617	0.006
<i>Paraglomus</i> VT335	<i>Paraglomus majewskii</i> (JN131587)	Autumn soil	0.636	0.002
<i>Paraglomus</i> VT351		Autumn soil	0.433	0.042
<i>Ambispora</i> VT283	<i>Ambispora fennica</i> (AM268193)	Spring soil	0.316	0.005
<i>Diversispora</i> VT62		Spring soil	0.602	0.007
<i>Glomus</i> VT BG3		Spring soil	0.389	0.046
<i>Glomus</i> VT BG8		Spring soil	0.368	0.003
<i>Glomus</i> VT BiG2		Spring soil	0.577	0.028
<i>Glomus</i> VT64	<i>Glomus constrictum</i> (AJ506090)	Spring soil	0.624	0.002
<i>Glomus</i> VT65	<i>Glomus caledonium</i> (Y17635)	Spring soil	0.211	0.041
<i>Glomus</i> VT67	<i>Glomus mosseae</i> (AJ306438)	Spring soil	0.647	0.021
<i>Glomus</i> VT108		Spring soil	0.740	0.001
<i>Glomus</i> VT125		Spring soil	0.648	0.002
<i>Glomus</i> VT129		Spring soil	0.627	0.001
<i>Glomus</i> VT143		Spring soil	0.630	0.004
<i>Glomus</i> VT195		Spring soil	0.362	0.011
<i>Glomus</i> VT199	<i>Glomus macrocarpum</i> (FR750376)	Spring soil	0.896	0.001
<i>Glomus</i> VT222	<i>Glomus indicum</i> (GU059535)	Spring soil	0.591	0.044
<i>Glomus</i> VT342		Spring soil	0.575	0.025
<i>Glomus</i> VT69	<i>Glomus sinuosum</i> (AJ133706)	Herbaceous annual	0.386	0.004
<i>Glomus</i> VT72		Herbaceous annual	0.430	0.049
<i>Glomus</i> VT108		Herbaceous annual	0.410	0.049
<i>Glomus</i> VT160		Herbaceous annual	0.404	0.030
<i>Glomus</i> VT295		Herbaceous annual	0.429	0.030
<i>Glomus</i> VT67	<i>Glomus mosseae</i> (AJ306438)	Herbaceous perennial	0.478	0.040
<i>Claroideoglomus</i> VT56		Semiwoody	0.512	0.004
<i>Claroideoglomus</i> VT193	<i>Claroideoglomus lamellosum</i> (AJ276087)	Semiwoody	0.490	0.002

779
780
781

Table S1. Recorded virtual taxa and their sequence read abundance per treatment in the study. Red, bold letters indicate new virtual taxa.

Table S2. Generalized lineal model analyses of arbuscular mycorrhizal virtual taxa (VT) richness, Simpson dominance and phylogenetic structure (as standardized effect size of mean pairwise phylogenetic distance) responses to soil collection date, plant family and plant life form. Bold letters indicate significance ($P < 0.05$).

Fig. 1. Non-metric multidimensional scaling ordination of AM fungal communities (k=2, stress= 0.162). a) Plants grown in soil collected in autumn; b) Plants grown in soil collected in spring. Lines show dispersion ellipses (1 standard deviation) around centroid of groups of samples. Dotted line: herbaceous annual, dashed line: herbaceous perennial, solid line: perennial semiwoody.

Fig. 2. Phylogenetic structure (a) and abundance of glomeromycotinan families (b). a) Standardized effect size of mean pair wise distance (SES-MPD) of AM fungal communities in relation to life form of plants inoculated with soils sampled at different dates. Means of boxes sharing a letter in common are not significantly different from each other ($P<0.05$). Asterisks indicate significant deviation from phylogenetic dispersion random expectations ($P<0.01$). b) Abundance distribution of glomeromycotinan families.

Fig. 3. Relative abundance of most abundant arbuscular mycorrhizal virtual taxa (VT) across plant life forms and soil collection dates. Only VT present in more than 5% of any of the groups are shown. Bars indicate mean percentage of sequence reads with standard error.

Fig. S1. Size distribution of pyrosequenced reads for the fragment NS31-AML2 of the small subunit ribosomal DNA of arbuscular mycorrhizal communities.

Fig. S2. Rarefaction analyses of root samples of different plant life forms and families grown in soil collected at two soil collection dates.

Fig. S3. Neighbor-joining tree of a fragment of the small subunit rRNA gene showing phylogenetic relationships between the arbuscular mycorrhizal fungal sequences found in the study, including representative virtual taxa (VT) sequence types from the MaarjAM database (Öpik et al., 2010). Bootstrap support values higher than 50 % are shown (100 replicates). Glomeromycota nomenclature follows the consensus classification proposed by Redecker et al. (2013), except that genus *Glomus* is treated as *sensu lato*. Novel VT found in the study are coded as BiG (for those belonging to *Glomus*) and BiA (for those belonging to *Archaeospora*). Those coded by BG or BGi, corresponded to novel VT described in Varela-Cervero et al. (2015). Accession numbers of sequences are followed by the name of the genus, the virtual taxon code and the origin of the sequence (plant species and date of soil collection). Type sequences of existing VT are indicated with “TYPE”. Two sequences per VT in each plant species and collection date were aligned using MAFFT multiple alignment web service implemented in JALVIEW v2.8 (Waterhouse et al., 2009). The tree was implemented in MEGA v7 (Kumar, Stecher, & Tamura, 2016).

Fig. S4. Shared Virtual Taxa detected in previous studies in Sierra de Baza Natural Park (extracted from MaarjAM database) and the present study.