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The composition of arbuscular mycorrhizal fungal communities in the roots of a ruderal forb is not related to the forest fragmentation process

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Summary

Land-use changes and forest fragmentation have strong impact on biodiversity. However, little is known about the influence of new landscape configurations on arbuscular mycorrhizal fungal (AMF) community composition. We used 454 pyrosequencing to assess AMF diversity in plant roots from a fragmented forest. We detected 59 virtual taxa (VT; phylogenetically defined operational taxonomic units) of AMF – including 10 new VT – in the roots of *Euphorbia acerensis.* AMF communities were mainly composed of members of family Glomeraceae and were similar throughout the fragmented landscape, despite variation in forest fragment size (i.e. small, medium and large) and isolation (i.e. varying pairwise distances). AMF communities in forest fragments were phylogenetically clustered compared with the global, but not regional and local AMF taxon pools. This indicates that non-random community assembly processes possibly related to dispersal limitation at a large scale, rather than habitat filtering or biotic interactions, may be important in structuring the AMF communities. In this system, forest fragmentation did not appear to influence AMF community composition in the roots of the ruderal plant. Whether this is true for AMF communities in soil and the roots of other ecological groups of host plants or in other habitats deserves further study.

Introduction

Arbuscular mycorrhizal fungi are ubiquitous fungal symbionts that colonize the roots of nearly 90% of vascular plants (Wang and Qiu, 2006; Brundrett, 2009). The mycorrhizal symbiosis is principally involved in the transport of nutrients to the plant and of carbon compounds to the fungus (Smith and Read, 2008). Arbuscular mycorrhizal fungal (AMF) have also been shown to promote plant resistance to drought and protection against pathogens, among other functions (Newsham *et al.*, 1995; Azcón-Aguilar *et al.*, 2002). However, the outcomes of the mycorrhizal symbiosis on plant performance depend on the identity of the plant host and on the composition of the local mycorrhizal fungal community (Öpik *et al.*, 2009; Uibopuu *et al.*, 2012). Thus, AMF communities have been shown to play an important and dynamic role in the structure and functioning of plant communities (van der Heijden *et al.*, 1998; Moora *et al.*, 2004). Consequently, there is increasing interest in describing AMF diversity around the world (Öpik *et al.*, 2010; 2013).

The molecular diversity of AMF communities is not randomly distributed among plant roots. One line of investigation has identified an association between particular fungi and plant ecological groups – i.e. habitat generalists versus forest specialists – rather than particular plant species (Öpik *et al.*, 2009; Davison *et al.*, 2011; Öpik and Moora, 2012). Grime's C-S-R (Competitor-Stress Tolerator-Ruderal) framework has also proven useful for predicting AMF–plant associations in natural ecosystems (Chagnon *et al.*, 2013). This is based on the assumption that functional traits in Glomeromycota families correspond to certain life history strategies (Powell *et al.*, 2009). For instance, the family Glomeraceae (formerly Group A, Schüssler and Walker, 2010) exhibit traits – high growth rate, rapid recovery after disturbance of hyphal networks and spore production – that might correspond to a ruderal life strategy (de la Providencia *et al.*, 2005; Powell *et al.*, 2009). Therefore, if the AMF–plant

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association reflects a symbiosis between organisms with similar functional traits – for example, similar responses to environmental perturbation – we might expect ruderal plants to be associated with ruderal AMF.

Land-use changes are a major threat to biodiversity worldwide (Sala *et al*., 2000). The Chaco forest in Central Argentina has one of the highest rates of deforestation in the world because of the conversion of natural forest to agricultural land (Zak *et al*., 2004). Moreover, remaining areas of forest are highly fragmented, with patches of different size and degrees of isolation persisting in a matrix of agricultural land (e.g. Grilli *et al*., 2013). New landscape configurations such as these can cause alterations to the ecological patterns and processes that exist in continuous forest (Fischer and Lindenmayer, 2007). Several studies have shown that forest fragment size and isolation, or a combination of both, can determine species richness in fragmented landscapes (Fahrig, 2013). Indeed, mycorrhizal fungi have been shown to be indicators of the forest fragmentation process (Peay *et al*., 2007; Grilli *et al*., 2012; 2013). AMF spore diversity in soil is known to be positively related to island and forest fragment size in fragmented landscapes and inversely related to nutrient availability (Mangan *et al*., 2004; Grilli *et al*., 2012). However, AMF spore diversity in soil does not necessarily reflect root-colonizing AMF community composition (Clapp *et al*., 1995; Hempel *et al*., 2007; Saks *et al*., 2014). Therefore, the responses to forest fragmentation of AMF communities in plant roots remain largely unknown.

The assembly rules that govern the composition of plant communities have long been studied (reviewed by Götzenberger *et al*., 2012), but less is known about the forces that structure AMF communities. Assuming that functional traits of species are to some degree phylogenetically constrained, communities might be phylogenetically more distant than expected by chance (i.e. phylogenetic overdispersion) if species exclusion by inter-specific competition is important in structuring the community. In such a scenario, niche differentiation might be an important process structuring AMF communities irrespective of forest fragment size or isolation. Conversely, organisms might be phylogenetically more closely related than expected by chance (i.e. phylogenetically clustered) if habitat filtering or dispersal limitation selects species with similar functional traits or from a subset of genetic lineages respectively. Recent studies of assembly rules in AMF communities have suggested that dispersal limitation, abiotic filtering or management intensity in fields are important (Dumbrell *et al*., 2010; Kivlin *et al*., 2011; Verbruggen *et al*., 2012). Although the effect of the forest fragmentation process on AMF community assembly is unknown, it is plausible to hypothesize that the isolation of forest fragments might represent a barrier to organism dispersal (Collinge, 2009). Moreover, larger forest fragments might be more diverse and structurally complex than smaller ones (Magnago *et al*., 2014). In addition, habitat filtering of AMF communities might occur if abiotic conditions, such as nutrient availability in soils, are related to forest fragment size (Grilli *et al*., 2012; 2013). Thus, we might expect evidence of phylogenetic clustering due to dispersal limitation or habitat filtering to be present in the AMF communities inhabiting ruderal plants in forest fragments.

The aims of this study were to assess (i) rDNA sequence-based AMF diversity in the roots of a native ruderal forb (*Euphorbia acerensis* Boiss.), (ii) the spatial variation of AMF communities in forest fragments of different size in a fragmented landscape in central Argentina and (iii) the phylogenetic dispersion of AMF communities in forest fragments compared with local, regional and global AMF species pools. We hypothesized that AMF community composition in roots would vary in relation to forest fragment size and isolation and that AMF community composition would be phylogenetically clustered as a result of habitat filtering due to variations in abiotic conditions (i.e. nutrient availability) related to forest fragment size and due to dispersal limitation of AMF between forest fragments.

Results

AMF diversity in plant roots

Analysis of 45 *E. acerensis* root samples using 454 pyrosequencing yielded a total of 25 307 cleaned reads, varying between 170 and 520 nucleotides in length (median = 517.8). After removing 364 (1.4%) chimeric reads, a total of 12 676 (i.e. 52%) reads were assigned to Glomeromycota. Two samples were omitted from further analysis because they yielded fewer than 10 Glomeromycota reads. Numbers of reads per sample ranged from 47 to 671 (median 260), and after standardizing to the median number of reads, from 47 to 260. The 12 267 potentially non-AMF reads were subjected to a BLAST against INSD (International Nucleotide Sequence Database): 86% belonged to Viridiplantae, 7.5% to Metazoa, 2.9% to non-Glomeromycota sequences and 2.5% to Glomeromycota (at 90% sequence identity level).

Glomeromycota sequence reads (i.e. 12 916 reads) could be assigned to 59 non-singleton virtual taxa (VT) of AMF (including 10 new VT): 57 Glomeraceae, 1 Paraglomeraceae and 1 Claroideoglomeraceae (Fig. S1). In addition, 13 singleton VT were detected, but these were omitted from further analyses. Ten new VT (17% of total VT, 10.1 % of AMF reads) were recognized among sequences with no match to existing VT. All novel VT belonged to the family Glomeraceae. Only 11 detected

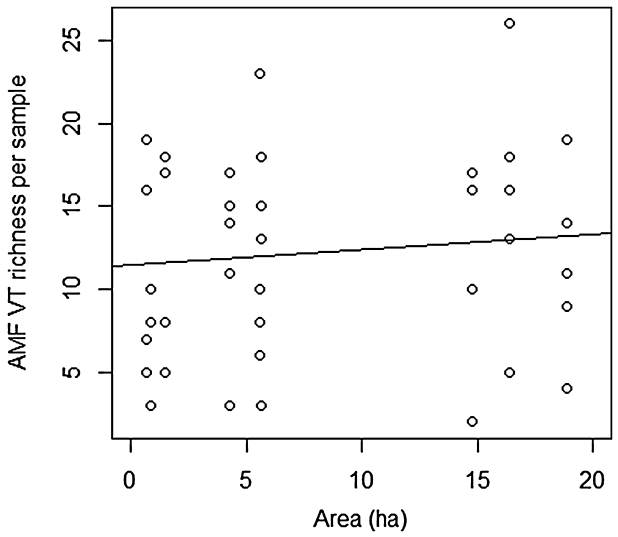

Fig. 1. The relationship between AMF richness per sample and forest fragment size (ha) in a fragmented Chaco forest landscape ($n = 43$).

VT (19% of total VT) corresponded to Glomeromycota morphospecies (Table S1).

AMF richness

Species accumulation curves suggested that sampling effort and sequencing depth were sufficient to adequately reach asymptotic VT richness estimates per forest fragment size (Fig. S2). AMF VT richness was not related to sequence count in root samples. In addition, when compared with data source in Grilli and colleagues (2013), AMF reads number was not related to mycorrhizal colonization (Fig. S3). AMF VT richness was not related to nutrient availability in forest fragments (i.e. ammonium, nitrate and phosphorus; data source Grilli *et al.*, 2013; Fig. S4). Also, AMF richness in samples was not related to forest fragment size (Pearson $r = 0.17$, $P = 0.26$; Fig. 1). Average VT richness per forest fragment size was 12.7 for large, 11.5 for medium and 11.6 for small fragments (Fig. 2) and did not differ significantly between forest fragment sizes (Negative binomial generalized linear mixed model (GLMM): $\chi^2 = 0.46$; $P = 0.79$).

Indicator species analysis detected three indicator VT: *Glomus* GCL9 (Indicator value: 0.52; $P = 0.02$) and GCL10 (Indicator value: 0.35; $P = 0.02$) were associated with large forest fragments; and *Glomus* VT156 was associated with small forest fragments (Indicator value: 0.46; $P = 0.046$). Sequence similarity with known AMF morphospecies for GCL10 and GCL9 was < 95% and for VT156 was 97% with *Glomus iranicum*. Among the total of 59 VT, 8 VT (13.56 % VT, 0.19% reads) were found only in large forest fragments, 3 VT (5.08% VT, 0.09% reads) in medium size and 3 VT (5.08% VT, 0.61% reads) in small forest fragments. A total of 27 VT (45.76% VT, 95.24% reads) were found in all three forest fragment sizes (Fig. 3).

AMF community composition in forest fragments

AMF community composition in the roots of *E. acerensis* did not differ significantly between forest fragments of different size (PerManova; relative abundance: Pseudo-F = 0.83, $P = 0.6$; presence/absence: Pseudo-F = 0.76,

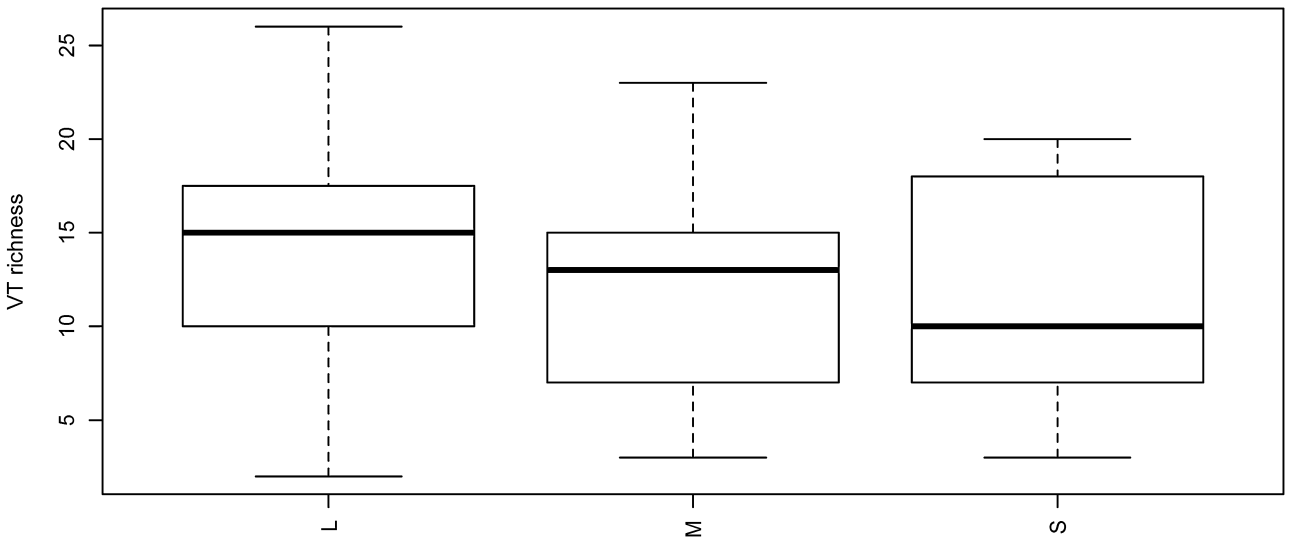

Fig. 2. AMF richness in forest fragments of three different sizes (i.e. L, large; M, medium and S, small). Boxplots represent the median, the first and the third quartile. Results of negative binomial GLMM ($\chi^2 = 0.46$; $P = 0.79$) are shown.

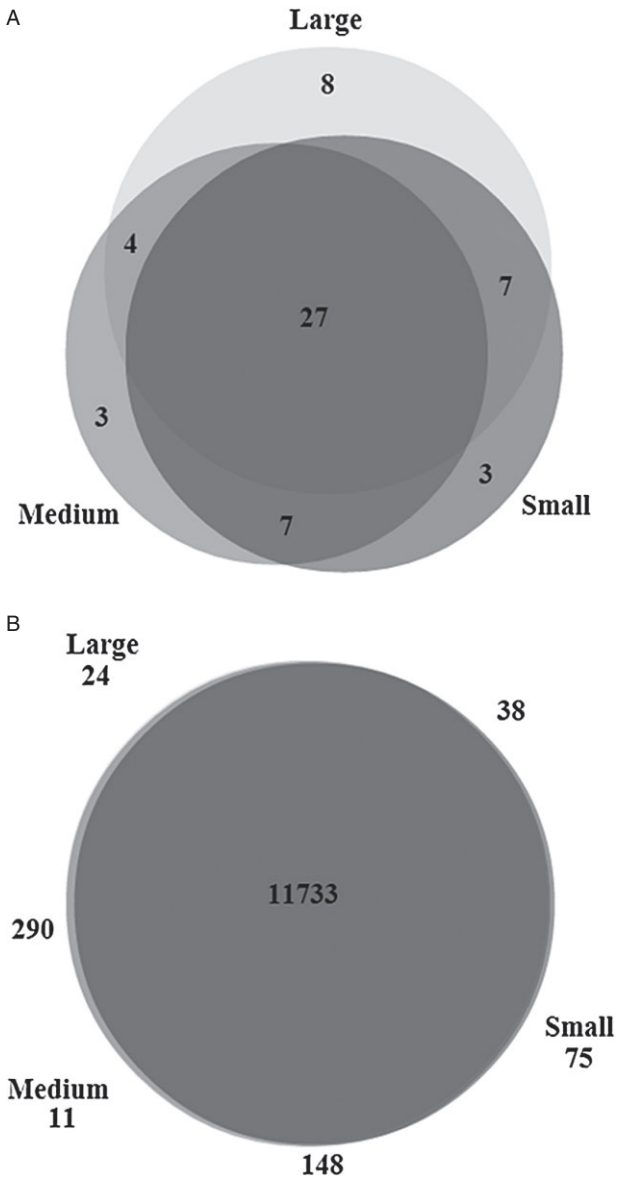

Fig. 3. The occurrence of AMF VT in the roots of *Euphorbia acerensis* in forest fragments of different sizes. (A) Number of AMF VT and (B) number of 454-sequencing reads of respective VT unique to and shared between forest fragment sizes.

$P = 0.82$; Fig. 4). In addition, the similarity of AMF communities was not related to the distance between forest fragments (Mantel test, 999 permutations: $r = 0.009$, $P = 0.41$).

Phylogenetic dispersion of AMF communities in forest fragments

The phylogenetic composition of AMF communities associating with the roots of *E. acerensis* was similar between forest fragments of different size (i.e. small, medium and large, PerManova: Pseudo-F = 0.69, $P = 0.81$, Fig. S5). The number of VT and sequence reads recorded per fragment size was 47 VT – 5120 reads in large forest fragments, 42 VT – 4179 reads in medium and 45 VT – 3617 reads in small forest fragments (Table S1). Families Paraglomeraceae (VT001) and Claroideoglomeraceae (VT193) were present in the three forest fragment sizes. The VT members of the family Glomeraceae were evaluated separately and seven AMF VT showed differences between forest fragment sizes (Fig. S6). In addition, the mpd (mean phylogenetic distance between recorded sequences) of AMF communities in forest fragments did not differ significantly from a random selection of taxa from the phylogeny of central Argentina (using records of VT in the Maarj*AM* database; Table 1, Fig. S6). However, the mpd of AMF communities in the roots of *E. acerensis* from medium forest fragments was lower than expected from a random selection of taxa from a phylogeny of South America (Table 1, Fig. S7); and the mpd observed in all three sizes of forest fragment was lower than expected from a random selection of AMF taxa from the global phylogeny (Table 1, Fig. 5).

Discussion

AMF diversity in the roots of *E. acerensis* (59 VT) in a fragmented Chaco forest was lower than that described from the most diverse forest ecosystems (83 VT) (Davison *et al*., 2012; Saks *et al*., 2014). However, those studies described AMF diversity in both soil and the roots of multiple plant species, and it is known that soil AMF diversity can be higher, lower or equal to that present in plant roots (Pivato *et al*., 2007; Torrecillas *et al*., 2011; Busby *et al*., 2013). In the context of local AMF diversity in Córdoba province, the present study (59 VT) shares 16 VT and increases by 43 taxa the number of VT previously described in the roots of five local plant species (35 VT; Öpik *et al*., 2013). Although the molecular approach used in this study and in Öpik and colleagues' (2013) was the same (454 pyrosequencing), the difference in AMF richness could be due to variation in the temperature, altitudinal ranges and plant community composition of the study sites – montane grassland (1600 m asl; Öpik *et al*., 2013) versus Chaco forest (500 m asl; this study). Also, we cannot discard the potential effect of higher sampling intensity in our study (i.e. number of samples: 45 versus 16).

The similar composition of AMF communities in the roots of *E. acerensis* in forest fragments of different size (i.e. small, medium and large) in this study contrasts with the positive relationships found between AMF spore morphospecies richness in the rhizosphere of these plants with decreasing nutrient availability and increasing forest fragment size (Grilli *et al*., 2012). Moreover, AMF VT richness was not related to soil nutrient content in

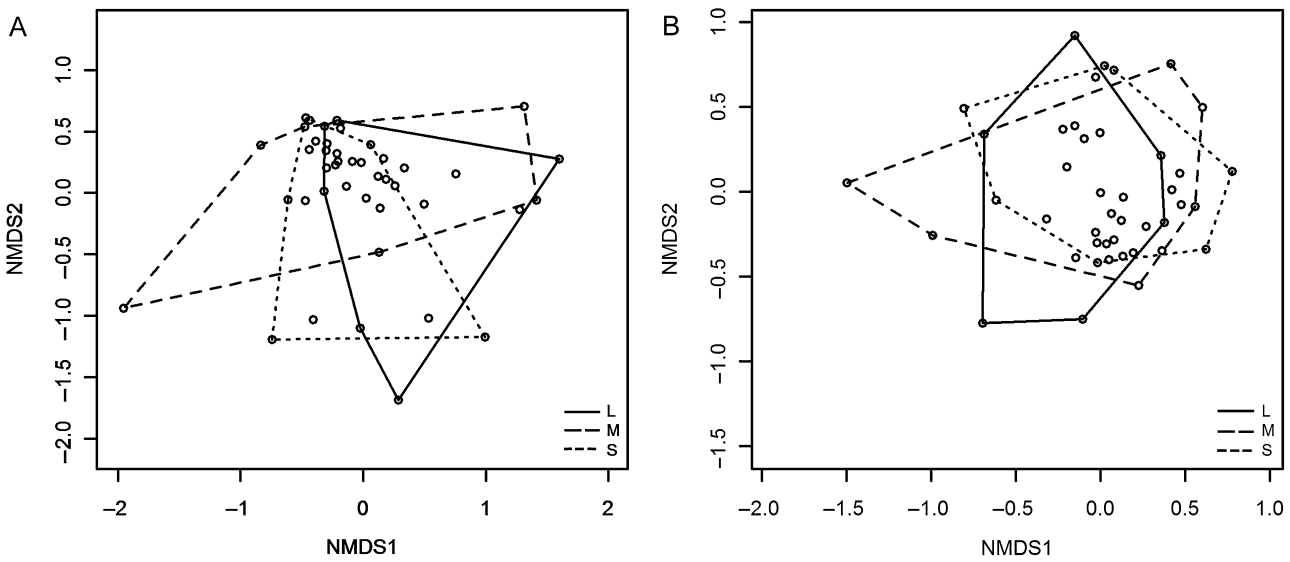

Fig. 4. (A) Qualitative (presence–absence based) and (B) quantitative (relative abundance based) non-metric multi-dimensional scaling (NMDS) plots of variation in AMF community composition in the roots of *E. acerensis* in forest fragments of three different sizes. L, large; M, medium and S, small. (A) Stress: 0.16; (B) Stress: 0.16.

forest fragments, although the nutrient availability was spatially and temporally heterogeneous (Grilli *et al*., 2012; 2013). The finding is however consistent with several studies showing differences between the composition of AMF communities in soil and plant roots (Saks *et al*., 2014 and references therein) and between spore-based and molecular approaches (Hempel *et al*., 2007). Also, contrary to our hypothesis that forest fragmentation affects the composition of AMF communities, there is evidence that belowground organisms may be generally less responsive to and/or respond more slowly to changes in vegetation than aboveground organisms (Wardle, 2006; Urcelay *et al*., 2009). Therefore, small and isolated forest fragments might exhibit a delay in the loss of AMF diversity and thus have extinction debt in their AMF communities (Tilman *et al*., 1994). In addition, the reduction of AMF colonization (i.e. lower AMF biomass) in the roots of *E. acerensis* with decreasing forest fragment size and increasing soil nutrient availability (Grilli *et al*., 2012; 2013) was not reflected as a reduction in the abundance of AMF 454 reads. Therefore, the level of AMF biomass reduction due to lower colonization of *E. acerensis* roots might not be reflected in a lower AMF DNA concentration possibly due to known variations in distribution of nuclei in AMF mycelium (Gamper *et al*., 2008).

Although these data might indicate that forest size reduction does not constitute an extinction risk to AMF communities inhabiting plant roots, it should be recalled that the effects of fragmentation on species diversity may be species-specific (Hanski *et al*., 2013). In addition, the possibility exists that a plant host effect (i.e. the host ecological group specificity of AMF) structures the

Table 1. Mean pairwise phylogenetic distance (mpd) between VT in the AMF communities found in forest fragments of three sizes.

		Reference sets in Maarj*AM* database														
		VT of Cordoba Argentina (78 VT)					VT of South America (158 VT)					VT of Worldwide (358 VT)				
Forest size	VT	Observed mpd	Randomized mpd Mean	SD	Z	*P*	Observed mpd	Randomized mpd Mean	SD	z	*P*	Observed mpd	Randomized mpd Mean	SD	z	*P*
Large	46	0.082	0.10	0.02	−0.71	0.241	0.082	0.12	0.03	−1.31	0.072	0.082	0.14	0.03	−2.23	*0.008*
Medium	41	0.085	0.10	0.02	−0.75	0.224	0.085	0.12	0.03	−1.45	*0.042*	0.085	0.15	0.03	−2.37	*0.005*
Small	44	0.076	0.09	0.02	−0.64	0.267	0.076	0.10	0.03	−1.19	0.069	0.076	0.13	0.03	−2.04	*0.009*

Significant *P*-values indicating differences in mpd between data sets are highlighted in italic type. Observed mpd values were compared with random mpd values derived from local (Cordoba), regional (South America) and global (all *MaarjAM* database) phylogenies. SD, standard deviation.

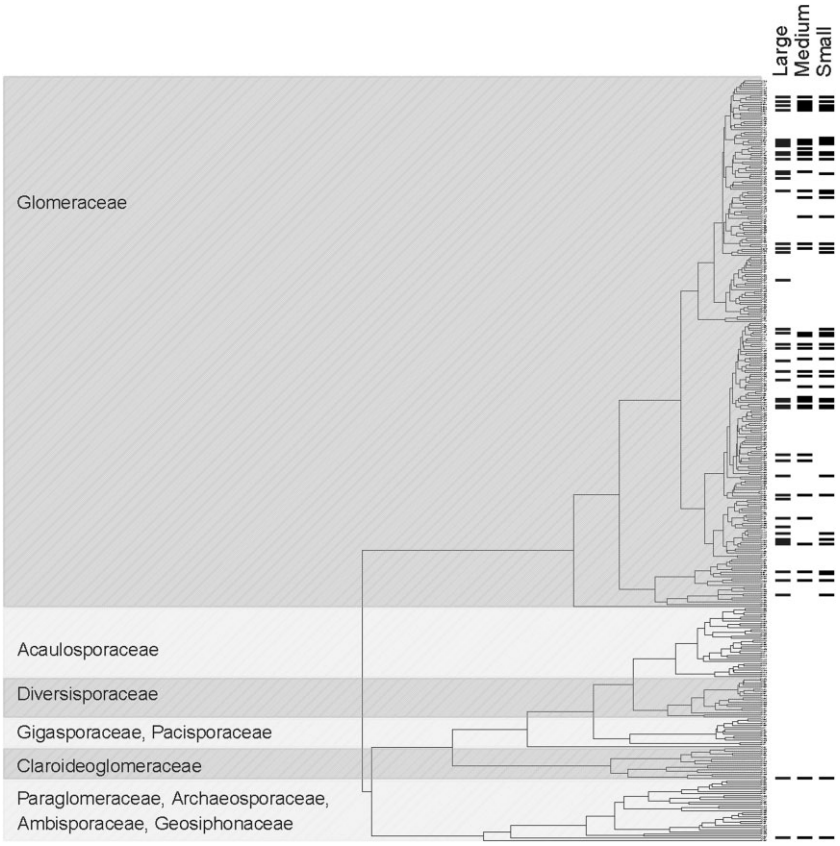

Fig. 5. Phylogenetic tree of AMF VT type sequences from the global AMF diversity Maarj*AM* database. Symbols at the right of the tree indicate the VT found in forest fragments of three different sizes (i.e. small, medium and large).

composition of AMF communities, in particular selecting for generalist species that are relatively unaffected by disturbances such as forest fragmentation (Öpik *et al*., 2009). AMF diversity in the roots of the ruderal *E. acerensis* mainly consisted of the members of family Glomeraceae (57 of 59 VT). This is consistent with the suggestion of Chagnon and colleagues (2013) that AMF–plant associations, according to Grime's C-S-R framework, might share functional traits between symbionts. A ruderal plant with a short life cycle, low growth cost and quick reproduction might not be able to afford AMF partners with slow growth rates, thick hyphal walls and extensive extraradical mycelia, such as members of the Gigasporaceae or Acaulosporaceae families, that represent a drain on plant carbon resources in the short term (Chapin, 1980; Lerat *et al*., 2003). Although, linking functional traits to AMF community composition is problematic because of the lack of a species concept in AMF and the unknown functional significance of uncultured AMF types (van der Heijden and Scheublin, 2007; Ohsowski *et al*., 2014), it has the potential to contribute to our understanding of AMF–plant association patterns.

As with forest fragment size, AMF community composition in roots of *E. acerensis* remained similar irrespective of the distances between forest fragments. This is in contrast with studies that show spatial variation in the composition of AMF communities at the local (Helgason *et al*., 1999; Dumbrell *et al*., 2010; Davison *et al*., 2012) and regional scale (Lekberg *et al*., 2007; van der Gast *et al*., 2011). However, it matches the findings of a recent study at the landscape scale where geographical distances and changes in land use did not correspond with variation in AMF community composition (Hazard *et al*., 2013). Therefore, local environment or plant host ecological group might be more important in structuring AMF community composition in plant roots.

AMF communities in forest fragments were phylogenetically more closely related than expected from random subsets of the global AMF phylogeny. Therefore, habitat filtering and/or dispersal limitation at the global scale might be structuring AMF communities in plant roots of this fragmented landscape when compared with a global phylogeny (Kivlin *et al*., 2011; Saks *et al*., 2014). In addition, phylogenetic distance in AMF communities were not consistent between forest fragments: when compared with random subsets of the South America phylogeny the AMF community in medium fragments was significantly clustered, whereas the communities in small and large fragments displayed no significant deviation from random South America phylogeny. However, it should be noted that the South American AMF phylogeny diversity is one of the least well-studied ones on the global scale (Öpik

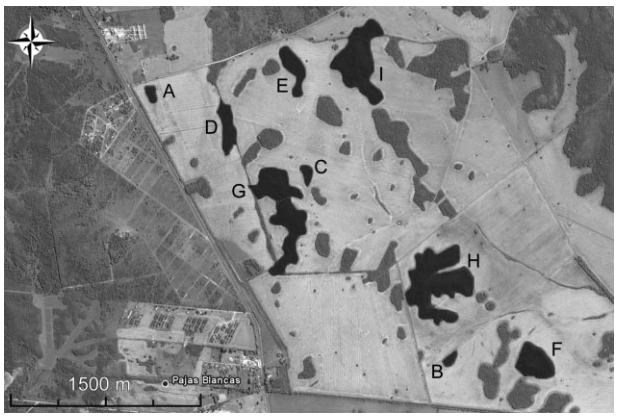

Fig. 6. Study sites in the Chaco forest fragmented landscape in Cordoba Province, Argentina. Nine forest fragments were sampled: small (A, B, C), medium (D, E, F) and large (G, H, I).

et al., 2013). Therefore, we may expect the pattern to change as more data about AMF species pool of this region become available. The comparison against random subsets of a local phylogeny suggested that habitat filtering and dispersal limitation of AMF communities in plant roots are not important at this scale. This contrasts with previous findings of local AMF communities being randomly assembled in soils and non-randomly assembled in plant roots (Davison *et al.*, 2011; Saks *et al.*, 2014). Therefore, future studies that include AMF diversity in soil, more plant species belonging to different ecological groups and other habitat elements of the landscape might provide a more complete understanding of the forces structuring AMF communities in this fragmented landscape.

In summary, this study presents the first molecular survey of AMF diversity in the Chaco forest. Our results demonstrate a tight association between a ruderal plant host and mainly ruderal AMF community members (i.e. mostly Glomeraceae). AMF community composition was similar in all forest fragments, suggesting that the forest fragmentation process might not be affecting mycorrhizal fungal communities inside the roots of this plant species. In addition, we found significant phylogenetic clustering of AMF communities in forest fragments compared with a global phylogeny, indicating that non-random assembly processes – most likely dispersal limitation – generate the AMF communities occurring in the roots of this plant species; however, neither dispersal limitation nor habitat filtering appear to influence community composition at the local scale. A more conclusive understanding of AMF diversity in this fragmented landscape requires assessment of AMF diversity in soils and additional host plant ecological groups.

Experimental procedures

Study site

The study was conducted in a fragmented Chaco forest in Cordoba Province, central Argentina. The study area lies approximately between 31°11′19″ S; 64°16′02″ W and 31°13′05″ S; 64°15′55″ W. A detailed description of the study area and Chaco forest composition is provided in Grilli and colleagues (2012). The Chaco xerophilic forests in Cordoba Province have been subject to high rates of deforestation and fragmentation (Zak *et al.*, 2004). Forest fragments in the landscape can be characterized as relict forests because of the vast modification of the landscape that has occurred and the disappearance of most of the original vegetation (Fischer and Lindenmayer, 2007).

Root sampling

We sampled nine forest fragments corresponding to three replicates each of ‘small’, ‘medium’ and ‘large’ fragments (Fig. 6; Table 2). Differences in forest fragment size and border length to forest size ratio were calculated and compared between each of the three fragment classes (Fig. S8). A higher border length : area ratio in small than medium and large forest fragments might be indicative of an edge effect in this forest fragment class. Therefore, plant individuals were collected in the core of forest fragments to avoid major edge effects. Distances between forest fragments were calculated in metres between the centre points of forest

Table 2. Size of sampled forest fragments and pairwise distances between them.

			Distance (m)							
Fragment size	ID	Area (ha)	A	B	C	D	E	F	G	H
Small	A	0.69								
Small	B	0.86	3153.78							
Small	C	1.48	1389.75	1848.60						
Medium	D	5.63	655.26	2543.00	751.20					
Medium	E	4.27	1170.65	2641.62	863.63	699.260				
Medium	F	5.59	3714.76	646.13	2336.63	3045.470	3013.99			
Large	G	14.78	1385.17	1704.28	380.46	868.558	1213.74	2251.70		
Large	H	18.88	2666.02	618.66	1314.93	2050.010	2051.93	1011.16	1248.65	
Large	I	16.37	1631.43	2453.42	948.13	112.730	475.44	2757.54	1317.53	1859.11

Coding of the forest fragments is the same as in Fig. 6.

fragments (using QUANTUM GIS VERSION 1.8.0; Quantum GIS Development Team, 2012). Five *E. acerensis* individuals were sampled in approximately 30 m^2 of plots lacking visible soil disturbance in the core of each forest fragment in April 2011. *Euphorbia acerensis* is an annual ruderal forb that is native to the region. It is patchily distributed in the forest and has shown high levels of arbuscular mycorrhizal root colonization (Grilli *et al*., 2012; 2013). Entire plants were excavated, placed into plastic bags and transferred to the laboratory. Roots were cut from shoots, carefully washed within a day, dried with silica gel and stored in airtight plastic bags at room temperature.

Molecular analyses

DNA was extracted from an average (i.e. because of the varying size of root systems) of 30 (10–70) mg of dried roots of each plant individual (sample) with the PowerSoil 96 Well Soil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) with the following modifications (as in Öpik *et al*., 2013). Glomeromycota sequences were amplified using the nuclear SSU rRNA gene primers NS31 and AML2 (Simon *et al*., 1992; Lee *et al*., 2008), linked to sequencing primers A and B, respectively, following Öpik and colleagues (2013). In order to identify sequences from different root samples, we used barcodes (8 bp long) designed following (Parameswaran *et al*., 2007). The barcodes were inserted between A and NS31 primer sequences and between the B primer and AML2 primer sequences. Polymerase chain reaction (PCR) was conducted in two steps to ensure proper amplification of long primer sequences. In the first PCR, reaction PCR primers were linked to barcodes and partial 454-sequencing adaptors A and B; in the second reaction, the full A and B adaptors were used as PCR primers, completing the full 454-adaptor + barcode + PCR primer construct. Thus, the composite forward primer in the first PCR reaction was: 5′ GTCTCCGACTCAG (NNNNNNNN) *TTGGAGGGCAAGTCT GGTGCC 3′*; and the reverse primer: 5′ TTGGCAGTCTCAG (NNNNNNNN) *GAACCCAAACACTTTGGTTTCC 3′*, where the A and B adaptors are underlined, the barcode is indicated by Ns in parentheses, and primers NS31 and AML2 are shown in italics. In the second PCR, 10 × diluted product of the first PCR was used as template with complete 454-adaptors A (5′CCATCTCATCCCTGCGTGTCTCCGACTCAG 3′) and B (5′ CCTATCCCCTGTGTGCCTTGGCAGTCTCAG 3′) serving as primers. The reaction mix contained 5 μl of Smart-Taq Hot Red 2 × PCR Mix (0.1 U μl^{-1} of Smart Tag Hot Red Thermostable DNA Polymerase, 4 mM $MgCl_2$, 0.4 mM each of dNTPs; Naxo OÜ, Estonia), 0.2 μM each primer and 1 μl of template DNA, in a total volume of 10 μl. The reactions were run on a Thermal cycler 2720 (Applied Biosystems, Foster City, CA, USA) following the conditions in Davison and colleagues (2012). PCR products were separated by electrophoresis through a 1.5% agarose gel in 0.5 TBE, and the PCR products were purified from the gel using the Qiagen QIAquick Gel Extraction kit (Qiagen Gmbh, Germany) and further purified with Agencourt® AMPureXP® PCR purification system (Agencourt Bioscience, Beverly, MA, USA). A total of 1.7 μg of the resulting DNA mix was sequenced on a Genome Sequencer FLX System, using Titanium Series reagents (Roche Applied Science, Mannheim, Germany) at GATC Biotech AG (Constanz, Germany). Preparatory procedures for 454 sequencing (barcoded PCRs and PCR product purification) were performed by BiotaP LLC (Tallinn, Estonia).

Bioinformatics and phylogenetic analysis

454-sequencing reads were retained only if they carried the correct barcode and forward primer sequences, an average quality score > 25 and length ≥ 170 nucleotides. Chimeric sequences were detected and removed using UCHIME (Edgar *et al*., 2011) in reference database mode (using Maarj*AM* – see below) using the default settings. After stripping the primers and barcodes, the remaining reads were identified against taxa in the Maarj*AM* database of Glomeromycota (Öpik *et al*., 2010) using a closed reference operational taxonomic unit picking approach (Bik *et al*., 2012). Sequence reads were assigned to VT (i.e., sequences clustered based on the neighborg joining (NJ) tree at ≥ 97% similarity; as in Öpik *et al*., 2010) by conducting a BLAST search (soft masking with the DUST filter) with the following criteria required for a match: sequence similarity ≥ 97%; the alignment length not differing from the length of the shorter of the query (454 read) and subject (reference sequences in Maarj*AM* database) by more than 10% of the length; and a BLAST e-value of < 1e-50.

Sequences not receiving a match against Maarj*AM* were compared against the INSD database using the same parameters, except a similarity threshold of 90% and an alignment length not differing from the shorter of the query and subject by 10 nucleotides. Phylogenetic analysis was performed to determine whether putatively Glomeromycotan sequences represent new VT. Sequences shorter than 450 nucleotides were removed, and the remaining sequences were clustered at 100% similarity level using JALVIEW version 2.8 (Waterhouse *et al*., 2009). Sequences in clusters of > 2 sequences were aligned with all sequences available in Maarj*AM* database (status 03/2013) using the MAFFT multiple sequence alignment web service in JALVIEW version 2.8 and subjected to a neighbor-joining analysis in TOPALI V2.5 (Milne *et al*., 2009). Novel VT sequences were added to the reference sequence set consisting of sequences from the Maarj*AM* database, and a second BLAST with the original sequence set was performed against this updated reference sequence set. Read filtering, removal of primer and barcode sequences and parsing of BLAST output was carried out using a series of PYTHON and JAVA scripts developed in house as in Davison and colleagues (2012). Novel VT were identified on the basis of sequence similarity and tree topology, following the approach of Öpik and colleagues (2010). We used phylogenetically well supported and distinguishable genus and species ID on the background of environmental sequences that were recently mapped onto the phylogeny of all Glomeromycota VT (Öpik *et al*., 2013). Global singleton VT (represented once in the data set) and samples yielding fewer than 10 sequences were removed from the final data set. The non-AMF : AMF reads ratio was higher in the samples with small sequencing depth, suggesting that AMF DNA was scarce in those samples (Table S2). Representative sequences of each VT at each forest fragment size were submitted to EMBL database under accession numbers HG969426- HG969650.

Phylogenetic dispersion

In order to assess phylogenetic dispersion, a Bayesian phylogenetic tree was generated using type sequences in the Maarj*AM* database plus new VT described in this study (using BEAST version 2.0; Drummond and Rambaut, 2007). The GTR + I + G nucleotide substitution model was chosen on the basis of Akaike´s Information Criterion (AIC) (JMODELTEST; Darriba *et al*., 2012). Posterior parameter estimates were drawn every 1000 steps from three separate 10 000 000 step Markov Chain Monte Carlo (MCMC) runs, with the first 10% of steps discarded as burn-in. Posterior clade probabilities were summarized on a maximum clade credibility tree. The tree was trimmed to include only VT type sequences using drop.tip() function of package APE in R (Paradis *et al*., 2004; R Core Team 2014). Samples from different forest fragment sizes were pooled to define communities associating with each of the size categories (i.e. small, medium and large). We then calculated the mpd between VT in each community using the ses.mpd() function in package PICANTE (Kembel *et al*., 2010). The observed mpd was compared with 999 mpd values calculated using randomization of a reference phylogenetic tree representing the available taxon pool. Randomizations of the reference tree were conducted as in Saks and colleagues (2014). We used three reference trees: (i) a local tree containing the type sequences of VT recorded in Cordoba, (ii) a regional tree containing the type sequences of VT recorded anywhere in South America and (iii) a global tree containing all VT type sequences from the Maarj*AM* database (Table S3).

Statistical analyses

AMF read number per sample varied, but was not correlated with root mass used for DNA extraction ($n = 43$, r_spearman = –0.012, $P = 0.94$; Fig. S9) showing that root mass did not affect sequencing success. We conducted correlations to test whether mean AMF reads per forest fragment were related to mycorrhizal colonization in the roots of *E. acerensis*. Also, the relationships of ammonium, nitrate and phosphorus availability with AMF VT richness were assessed using correlations. Mycorrhizal colonization and soil nutrient availability data were published in Grilli and colleagues (2013). Sampling design and analysis of soil nutrient availability together with root colonization are described in Grilli and colleagues (2013). All correlations were tested with function cor.test() in the R package STATS. After these analyses, the data matrix was standardized by rarefaction to the median read count per sample (260). This approach, which consists of randomly selecting reads in each sample until the median read count is reached, has been shown to represent an optimal approach for reducing bias due to differences in sample size while retaining information (de Cárcer *et al*., 2011). Samples with fewer reads than the median remain unchanged. The relative abundance of different VT per sample was calculated by dividing the read counts of individual VT by the total read count per sample. Species accumulation curves were assessed using the function speccacum() in the R package VEGAN (Oksanen *et al*., 2011). AMF richness between forest fragment sizes were assessed using a negative binomial generalized linear mixed model, implemented using the glmmadmb() function from the GLMMADMB package (Skaug *et al*., 2014). 'Forest Size' was included as a fixed factor, while forest fragment ID was included as a random intercept. Indicator species analysis using function indval() from R package LABDSV was used to investigate pairwise associations between forest fragment size and AMF VT (Roberts, 2012). Variation in AM fungal community composition, both presence-absence and relative abundance data were visualized using non-metric multidimensional scaling (NMDS; implemented using R package VEGAN). PERMANOVA (multivariate ANOVA) with 100 permutations using function nested.npmanova() from R package BIODIVERSITYR (Kindt and Coe, 2005) was then used to test whether forest fragment size (with forest fragment ID nested within fragment size) affected AMF community composition, with Bray–Curtis dissimilarity (BC) used as a measure of distance between pairs of AMF communities. We assessed whether multivariate dispersion differed between groups using function betadisper() from VEGAN. The possibility of significant effects arising due to differences in multivariate dispersion rather than compositional change in AMF communities was excluded on the basis of beta diversity measurements within and between groups (using function betadisper() from VEGAN; in relation to forest fragment size: linear mixed effects model, fragment ID as a random intercept; $F = 0.19$, $P = 0.83$).

The relationship between AMF community similarity and the spatial distances separating forest fragments was assessed using a Mantel test.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Neighbour-joining phylogeny of AMF VT present in forest fragments at the Chaco study site. Indicated in black: new virtual taxa and VT corresponding to morphospecies.

Fig. S2. Sampling efficacy of root AMF samples from the Chaco forest fragmented landscape ($n = 43$). Species accumulation curves from the three forest fragment sizes.

Fig. S3. Correlation of (A) VT richness with sequence read count per sample (***n* = 43**) and (B) mean AMF reads with mean mycorrhizal colonization (data source Grilli *et al.*, 2013) per forest fragments ($n = 9$).

Fig. S4. Pearson correlations between AMF VT richness and (A) ammonium, (B) nitrate and (C) phosphorus in soil samples (data published in Grilli *et al.*, 2013) from forest fragments ($n = 9$).

Fig. S5. Non-metric multi-dimensional scaling (NMDS) plot of AMF community phylogenetic composition in forest fragments of three different sizes. L-large, M-medium and S-small. Solid lines show dispersion ellipses (1 standard deviation) around groups of samples. Stress = 0.14.

Fig. S6. Phylogenetic tree of type sequences for AMF VT present in Cordoba Province – Argentina- taken from the Maarj*AM* database. Coloured symbols at the right of the tree indicate VT found in forest fragments of three different sizes (i.e. Small, Medium and Large). Differences in AMF VT abundance between forest fragments were tested with negative binomial GLM. *P*-values: *0.01; **0.001; ***0.0001.

Fig. S7. Phylogenetic tree of type sequences for AMF VT present in South America – taken from the Maarj*AM* database. Coloured symbols at the right of the tree indicate VT found in forest fragments of three different sizes (i.e. small, medium and large).

Fig. S8. Linear models (ANOVA) were used to test differences between (A) area in hectares ($F = 117.5$, $P < 0.0001$) and (B) border length/fragment size ratio ($F = 10.5$, $P = 0.01$) in three forest fragment size classes (L = Large, M = Medium and S = Small; $n = 9$ forest fragments).

Fig. S9. Spearman correlation between obtained AMF reads per sample and dry root mass used for DNA extraction ($n = 43$; r_spearman = –0.012, $P = 0.94$).

Table S1. List of detected AMF VT and morphospecies in forest fragments of three different sizes (small, medium and large; $n = 9$) in a fragmented Chaco forest in central Argentina.

Table S2. AMF read counts per sample in the five *E. acerensis* root samples with highest and smallest sequencing depths.

Table S3. Details of VT present in the Maarj*AM* database used to construct the local 'Cordoba' and Regional 'South America' data sets.