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Root colonizing and soil borne communities of arbuscular mycorrhizal fungi differ among soybean fields with contrasting historical land use

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are a key component of soil microbiota in natural and anthropogenic ecosystems. Even though soil type and climate conditioned land uses in the past, soybean cultivation has overrode such limitations and replaced the earlier diverse agro- and natural ecosystems in many countries of South America. We investigated whether actual diversity patterns of local AMF communities were determined by previous land uses and their intrinsic environmental conditions. We sequenced AMF DNA from root and soil samples collected from current soybean fields with three historical land use situations (HLU): agricultural fields, livestock farming and forest sites. We detected overall high AMF richness: 87 virtual taxa (VT) in soil and 69 VT in soybean roots. Mean number of VT per sample ranged from 8.1 to 19.2; it was not affected by HLU nor type of sample, but correlated with soil texture, pH, and plant density. Conversely, AMF community composition did significantly diverge among HLU and type of sample. A distinctive community composition was observed in roots of historical agricultural fields which differed from any other soil and root sample evaluated in this study. We attribute this finding to variations in the abundance pattern of predominant AMF taxa (*Glomeraceae* and *Gigasporaceae*). Our results indicate that soybean cultivation supports relatively high AMF diversity, with apparent legacies from earlier management and natural habitats in the composition of resident AMF communities.

1. Introduction

Arbuscular mycorrhizal fungi (AMF, Glomeromycota; Schüßler et al., 2001) are a key component of the soil microbiota forming obligate symbiosis with roots of *ca.* 80% of terrestrial plant species (Smith and Read, 2010). It is one of most common and widespread terrestrial plant symbioses and contributes towards plant nutrition, soil structure and other ecosystem services (van der Heijden et al., 2015). AMF diversity and functioning is affected by environmental conditions (Powell and Rillig, 2018) such as altitude and habitat type (Kotilínek et al., 2017), and edaphic and climatic properties (Alguacil et al., 2016). Furthermore, anthropogenic activities have considerable influence on AMF (García de León et al., 2018b). In agroecosystems, soil disturbance (e.g., ploughing), chemical inputs (fertilization, use of pesticides), and limitations on host availability (e.g., continuous monoculture) can negatively affect diversity of AMF (Druille et al., 2013; Säle et al., 2015; Williams et al., 2017). In addition, grazing and cattle induced changes in soil properties can also have negative impact on AMF diversity in livestock farms (van der Heyde et al., 2017).

Several studies have demonstrated that symbiotic associations between resident AMF species and plant host are related to the identity of both partners (Vályi et al., 2016). Although AMF generally exhibit low host specificity some mutual preferences exist (Davison et al., 2015; Werner and Kiers, 2015; Horn et al., 2014; Martínez-Garcia et al., 2015; Vandenkoornhuyse et al., 2002). Sepp et al. (2018) observed that AMF communities in different habitat types were more similar in the roots of a single host plant species than in soil samples, suggesting a non-random pattern in host-fungal interaction. Further, López-García et al. (2017) found that plants with ruderal traits tended to associate with phylogenetically clustered AMF communities. Therefore, the identity of

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Table 1
Soil, crop, and climatic variables and root AMF colonization levels in the studied soybean fields with different historical land uses (HLU). Mean values and standard deviation (± SD) are shown (n = 10). Different letters indicate significant differences among HLU according to Tukey test ($p \leq 0.05$).

HLU	Soil									Soybean		Geographical	
	USDA Classif.	P mg kg^{-1}	N	C %	pH	EC µS cm^{-1}	Water %	Sand	Clay	Root AMF %	Plant density pl m^{-2}	MAP mm	Altitude m.a.s.l.
Agricultural	Argiudoll	14.6 **b** (± 6.0)	44.6 (± 22.8)	3.1 **a** (± 0.4)	6.0 **b** (± 0.2)	186.5 (± 265.5)	6.9 **a** (± 0.77)	24.0 **a** (± 8.2)	76.0 **a** (± 8.2)	67 **a** (± 16)	29.3 (± 6.4)	900	119.8 **b** (± 14.9)
Livestock	Haplustoll	37.9 **a** (± 23.4)	29.7 (± 23.0)	1.6 **b** (± 0.6)	6.5 **a** (± 0.2)	80.0 (± 20.5)	1.5 **b** (± 0.34)	66.0 **c** (± 12.4)	34.0 **c** (± 12.4)	57 **ab** (± 15)	27.7 (± 7.9)	700	203.3 **b** (± 53.4)
Forest	Haplustoll	22.0 **ab** (± 19.7)	55.6 (± 47.1)	3.2 **a** (± 1.0)	6.4 **ab** (± 0.6)	155.0 (± 164.8)	6.5 **a** (± 0.84)	41.2 **b** (± 14.2)	58.8 **b** (± 14.2)	48 **b** (± 18)	37.9 (± 14.4)	800	638.6 **a** (± 135.8)

MAP: mean annual precipitation.

the crop species may result in the predominance of certain AMF species, leading to the decline or the loss of other species and decrease in overall diversity and mycorrhizal functioning.

During the last decades, many American countries have undergone a drastic expansion of agricultural lands. Soybean (*Glycine max* (L.) Merr.), a legume oilseed crop originating in South Asia and currently one of the most important food crops of the world, is adapted to successful cropping. Indeed, Americas' harvest 84.5% of worldwide soybean production every year (FAO, 2015). In Argentina, the area under soybean cultivation soared from 9.8 mln ha in 1995 to 26 mln in 2016 (SAGPyA, 2017). Concurrently, during the same period, the area used for livestock farms has decreased by 10 mln ha, and forest and shrubland areas' annual decrease has reached 1% (297,000 ha per year; FAO, 2015; SAGPyA, 2017). Subsequently, former livestock farms and natural areas have been gradually converted to soybean fields, and the historical agricultural lands have experienced a strong intensification in management.

The change in land use towards soybean cultivation brings about consequences for above and belowground organisms. Even though no-tillage is the dominant sowing practice in Argentina, and it is less disturbing than conventional ploughing (Albertengo et al., 2013), the use of pesticides and fertilizers coupled with low crop rotation constitute the basis of the intensification of soybean production. In such scenario, local surveys have revealed declining diversity of several groups of soil biota, including ants, prostigmatid mites, earthworms and collembolans (Bedano et al., 2016), bacteria (Ferrari et al., 2015; Figuerola et al., 2015), AMF (Cofré et al., 2017), and saprophytic fungi (Ferrari et al., 2015). In addition, a recent study from Argentinean soybean fields shows somewhat lower AMF diversity and shifted community composition compared to native Espinal vegetation (Garcia de Leon et al., 2018a). Such alteration of biodiversity can alter ecosystem level processes and the ecosystem resilience to further environmental changes (Powell and Rillig, 2018).

In contrast to the developed countries where agriculture was established hundreds of years ago, the change in land use to agriculture in Americas is only a few decades old and its impacts on AMF diversity and functioning are little understood. For example, González-Cortés et al. (2012) observed that the conversion of natural/native forests to avocado plantations and maize fields in Mexico generates a shift in the composition but do not alter the richness of AMF. However, to the best of our knowledge, in Argentina there is a lack of local empirical evidence regarding the consequences of change in land use as well as the intensification of existing agricultural fields on AMF diversity patterns. Surveys based on root-colonizing and soil-borne communities might allow revealing the potential impact of soybean cultivation on AMF communities.

Here we explored AMF diversity in three areas in Argentina that differ in terms of time since land use conversion towards soybean cultivation, and have different historical land uses (HLU), namely, Agricultural (more than 28 years of intensive soybean cropping), Livestock (28-18 years of soybean cropping in rotation with pastures) and Forest (less than 18 years of intensive soybean cultivation after clearing mountainous native shrub-lands). In order to disentangle the effect of soybean cropping on resident AMF, we addressed AMF both in soil and root samples. While the first allows to gather fungal spores and hyphae contributing towards locally available AMF diversity, intraradical AMF represent the fungi involved in active symbiosis with the plant host (Varela-Cervero et al., 2015; Osborne et al., 2018; Sepp et al., 2018). We expected to find different AMF community composition in soil samples from each HLU because of the combined effect of local conditions and the legacy of earlier land uses. We also expected that AMF communities in soybean roots represent a subset of locally available AMF because of mutual partner preferences.

2. Materials and methods

2.1. Field sites and sampling

The study sites were located in Cordoba province, central Argentina. We targeted current soybean fields in three areas with different edaphic and climatic conditions, which had dictated the predominant historical land uses (HLU): agricultural, livestock farming and forest. Agricultural sites have been under intensive agricultural production for more than 60 years, and 28 under soybean cultivation. The predominant soil type is Argiudoll (USDA, 2014) with high fertility and productivity (INTA, 1978, 1985) (Table S1). Livestock farming sites had been under crop production in rotation with perennial pastures, with a gradual inclusion of soybean cultivation since 1990, and a continuous cultivation since early 2000´s. Soil type is mainly Haplustoll with moderate fertility (INTA, 2004). Forest sites had been native shrub forests that were turned into soybean fields during the last 18 years after clearing the native vegetation (*Prosopis* spp., *Acacia* spp., *Zanthoxylum coco* Engl., *Schinopsis marginata* Engl., *Festuca* spp, *Stipa* spp., *Setaria* spp.). They are located in a mountainous area at 500–900 m a.s.l., mainly on Haplustoll soils with high fertility (INTA, 1996, 2004) (Tables 1, S1). Ten sites were sampled in each of the three areas (total of 30 sites). The sites within each HLU were located 10 km (min) to 30 km (max) from each other; HLU regions were located 320 km from each other.

Sampling was conducted when soybean crops were at vegetative or initial reproductive stage (from V3 to R4). We collected roots, rhizosphere soil and bulk soil. Roots were sampled from a set of 20 plants per site and pooled to form one composite sample per site. Briefly, after removing shoots, we collected roots by loosening the soil on the sides of the sowing line with a shovel from 0 to 20 cm of depth. This was possible because soybean root system consists of a coarse primary root with abundant, and superficial secondary roots. Collected root samples were stored on ice until processed within 24 h. In the laboratory, roots were cleaned with tap water, then finest and not lignified secondary roots

were individually selected, separated from the main root, washed with ddH_2O and cut into 1 cm length pieces. One set of 10 g fresh weight (FW) of roots was stored at -20 °C for DNA extraction. A separate 1 g FW was used for assessing AMF root colonization. After removing the roots, rhizosphere soil samples, i.e. soil immediately surrounding soybean roots in the sowing line, were sampled with a garden trowel from a depth of approximately 0–10 cm, bulked to form one sample per site, sieved (2 mm mesh) and stored at -20 °C for DNA extraction. Finally, in the same area of root sampling, 10 bulk soil samples from depth of 0–20 cm were taken randomly with a 2 cm diameter soil corer for chemical and physical analyses and pooled to form one sample per site.

2.2. Mycorrhizal colonization

Immediately after sampling, 1 g FW of washed roots were cleared (10% KOH, 60 °C, 30 min) and stained (50 °C, 15 min) with trypan blue (0.05%) in a lacto-glycerol solution (lactic acid: glycerol: distilled water 1:1:1: ratio) according to the modified method described by Phillips and Hayman (1970). Root mycorrhizal colonization level was assessed microscopically from 120 fields of view per sample at 400x magnification (McGonigle et al., 1990).

2.3. Soil properties

The following properties were measured for bulk soil samples: extractable phosphorus (Bray and Kurtz, 1945); nitrate nitrogen (Bremmer, 1965); organic carbon (Walkey and Black, 1934); pH; electrical conductivity at 25 °C; and textural fractions corresponding to particles of clay and silt (< 53 µm) and sand (> 53 µm), respectively (Gee and Bauder, 1986).

2.4. Molecular analyses

2.4.1. DNA extraction

Rhizosphere soil samples (50 g FW) were wet sieved and decanted (Gerdemann and Nicolson, 1963), followed by 80% sucrose gradient centrifugation (Walker, 1992), and the material retained on the sieve of 74-µm mesh was recovered for DNA extraction. Collected material contained AMF hyphae and spores, debris, and soil particles. Still on the sieve, the material was thoroughly rinsed with sterile water and gently dried with a tissue paper from underneath the mesh. DNA was extracted from a subsample of 200 ± 20 mg of the frozen material which was crushed with a sterile micro-pestle in a 1.5 ml Eppendorf tube in the Lysis Buffer, provided by the Power Soil DNA isolation kit (MO BIO Lab. Inc., USA). From this step onwards, manufacturer's instructions were followed.

Soybean root samples (10 g FW) were homogenized in liquid N_2 with a mortar and pestle, and genomic DNA extraction was performed with NucleoSpin® Plant II kit (Macherey-Nagel GmbH and Co, Düren, Germany) with modifications of the original protocol in order to obtain more uniform DNA from AMF: a) the root sample aliquot was increased from 100 mg to 200 mg ± 10 mg FW; b) because of the larger volume of root material, volumes of PL1 Buffer (cell lysis), RNase A, and PC (binding buffer) were two times larger; c) lysis incubation at 65 °C was conducted for 30 min instead of 10 min.

2.4.2. PCR and 454-sequencing of AMF

The identification of Glomeromycota in root and soil samples was performed using PCR with SSU rRNA gene primers followed by 454 sequencing. A semi-nested PCR approach was carried out in order to increase the yield of target AMF amplicon. The first PCR reaction was conducted with the AMF specific primers AML1 and AML2 yielding an approximately 800bp fragment of the 18S rRNA gene (Lee et al., 2008). The PCR reactions were run under the following conditions: 2.5 units Taq DNA polymerase (Promega), 200 µM each of the primers, 200 µM each of the four deoxynucleoside triphosphates (dNPTs), 1.8 mM $MgCl_2$ buffer, 1 µl dimethyl sulfoxide (DMSO), and 3 µl of DNA extract in a volume of 25 µl. The reactions were run on a T9600-G thermal cycler (Applied Biosystems, NY, USA) as follows: 4 min initial denaturation at 94 °C, 40 cycles of 95 °C for 30 s, 58 °C for 40 s; and 72 °C for 1 min, followed by 72 °C for 5 min. PCR products were checked by electrophoresis on a 1.5% agarose gel in 0.5 x TBE using GelRed™ (Biotium) stain.

The semi-nested PCR reaction was conducted with primers NS31 (Simon et al., 1992) and AML2 yielding an amplicon of *c*. 560 bp. This amplicon is widely used in ecological studies of AMF, and therefore provided us with a larger dataset of environmental sequences than other marker regions (Öpik and Davison, 2016). According to the guidelines for sequencing on a Roche GS-LX 454 platform, the fusion primers called universal tail A (*Univ-A*) and universal tail B (*Univ-B*) were linked to each of the specific primers. Thus, the composite forward primer was: 5′-CACGACGTTGTAAAACGAC*TTGGAGGGCAAGTCTGGT GCC*-3′; and the reverse primer was: 5′- CAGGAAACAGCTATGACC*GA ACCCAAACACTTTGGTTTCC*-3′, where *Univ-A* and *Univ-B* are underlined and the specific primers NS31 and AML2 are shown in italic. Each PCR reaction contained 1 µl of 1:10 diluted product of the first PCR, 2.5 units Taq DNA polymerase (GoTaq, Promega), 200 µM each of the four dNTPs, 1.8 mM $MgCl_2$ buffer, 1 µl DMSO, and 200 µM of each of the composite primers in a volume of 20 µl. The reactions were run on a T9600-G thermal cycler (Applied Biosystems, NY, USA) as follows: 95 °C for 15 min; five cycles of 42 °C for 30 s, 72 °C for 90 s, and 92 °C for 45 s; 35 cycles of 65 °C for 30 s, 72 °C for 90 s, and 92 °C for 45 s; followed by 65 °C for 30 s and additional extension at 72 °C for 10 min. Amplification products were checked on agarose gel as above and purified with Fast Pure PCR Product Purification Kit (Roche). Amplicon library preparation and subsequent steps to sequencing were carried out in INDEAR (Instituto de Agrobiotecnologia de Rosario, Argentina) following the Roche's instructions for *Universal Tailed Amplicon Sequencing* (Sections 4 and 8 in the *Method Manual,* http://454.com/my454/). During this process, a MID sequence (multiplex identifier) was attached to the forward primer of each sample as a barcode.

2.5. Bioinformatical analyses

Analysis of 454 sequence reads was performed using a series of Java programs as in Davison et al. (2012).Briefly, it consisted of successive steps of filtering and cleaning the sequences before performing further analyses. First, filtering with exact match was applied to retain sequences with the correct MID tag and forward primer. MID tags and primer sequences were removed and the reads were cleaned with following parameters: average quality score ≥25; minimum length ≥170 bp; and longer sequences trimmed to 520 bp to remove reverse primer. Potential chimeras were detected and removed using UCHIME v7.0.1090 (Edgar, 2010) in reference database mode (Maarj*AM*, http://www.maarjam.botany.ut.ee/, Öpik et al., 2010) with the default settings. Maarj*AM* database contains 6115 sequences (status February 2015, 352 VT) covering the NS31/AML2 amplicon from identified spores and environmental samples. These sequences have been clustered with blastclust v2.2.26 (Altschul et al., 1990) on the basis of phylogenetic similarity threshold ≥97% (frequently 99%), in order that each cluster would roughly correspond to species level taxon (Öpik et al., 2010). Maarj*AM* database was used for taxonomic assignment of the obtained reads using BLAST v2.5.0+ search (Camacho et al., 2009) with following parameters for a match: sequence similarity ≥97%; alignment length not differing from the length of the shorter of the query (454-read) and subject (reference sequence) by more than 5%; and BLAST e-value $\leq 1e^{-50}$. Sequences not finding a match in the Maarj*AM* database according to these criteria (so-called no-hits; 21038 reads) were subjected to a further BLAST against the INSDC database (sequence similarity ≥90%, alignment length not differing from the shorter query and subject by 10% of the length, and BLAST e-value $\leq 1e^{-50}$). Putative Glomeromycota sequences represented by more

than 100 reads were aligned with Maarj*AM* sequences using MAFFT (version 7) multiple sequence alignment web service in Jalview v2.8 (Waterhouse et al., 2009) and subjected to a neighbor joining analysis in TOPALi v2.5 (Milne et al., 2009). Novel VTs were defined on the basis on sequence similarity and phylogenetic clustering (Öpik et al., 2010). Finally, one representative sequence from each novel VT was included in the reference database and a new BLAST was conducted with 454-reads against the updated set. Representative sequences of novel VT and hits against Maarj*AM* database were deposited in the NCBI nucleotide collection under accession numbers KU708516-KU708528 and KY588141-KY588335.

2.6. Statistical analyses

Sequencing efficacy was assessed with rarefaction analysis using rarefy () function, and sampling efficacy using specaccum () function from R package VEGAN (Oksanen, 2013). For further analyses, the data matrix was standardized by rarefaction to the median read count per sample (3644). 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). Afterwards, the proportion of VT reads in a sample was estimated with decostand () function (standardization method ="total") from R package VEGAN (Oksanen, 2013).Linear models were used to test for differences in AMF VT richness per sample among HLU and type of samples using lm () function from R package STATS (R Development Core Team, 2018).

The effect of HLU on AMF community composition in root and soil samples was assessed by permutational multivariate analysis of variance (Anderson, 2001) using Adonis () function with 9999 permutations. Variation in AMF taxonomic community composition was visualized by non-metric multidimensional scaling (three-dimensional NMDS, with 50 iterations) using metaMDS () function and Bray-Curtis distance from R package VEGAN (Oksanen, 2013). We plotted ellipses representing communities belonging to the different HLU and type of sample with ordiellipse () function using the standard deviations of weighted averages. Linear correlations between ordination and environmental variables were estimated with envfit () function from VEGAN, and vectors of the most significant variables (p.max = 0.05, Oksanen, 2013) were overlapped onto NMDS ordination diagram.

To identify AMF taxa associated with particular HLU we used indicator species analysis (Dufrene and Legendre, 1997) as implemented by function indval () from R package LABDSV (Robert, 2012). Only those VT with an indicator value of at least 0.25 were considered. To identify abundant taxa we detected those VT with ≥ 0.10 of relative abundance per sample.

3. Results

3.1. Soil properties

Sites with contrasting HLU differed in soil texture, with higher sand content in Livestock soils than in Forest and Agricultural sites (Table 1). Hitherto, our defined HLU situations were resultant of distinct soil and climate properties at a regional scale (Table S1). Soil P, C and pH differed according to HLU, with higher P, lower C and higher pH in Livestock soils (Table 1). Average root mycorrhizal colonization levels ranged from 48 to 67% in Agricultural and Forest sites, respectively (Table 1).

3.2. 454 sequencing

454 sequencing yielded 227327 quality filtered reads from 30 soil and 28 root samples (2 root samples failed to yield AMF reads). A total of 204131 reads (90%) could be assigned to Glomeromycota against Maarj*AM* database, 100130 reads from soil and 100481 from root samples. Altogether, 103 AMF VT from 8 families were detected (Table S2): *Acaulosporaceae* (3), Archaeosporaceae (3), *Claroideoglomeraceae* (4), *Diversisporaceae* (13), *Gigasporaceae* (8), *Glomeraceae* (57), *Pacisporaceae* (1), and *Paraglomeraceae* (6). Thirteen VT were novel and accounted for 30% of sequences with no hit against Maarj*AM* database. Eight VT were singletons (i.e. taxa represented by only one sequence) and were excluded from further analysis, leaving 95 V T in the dataset. Of the remaining reads, 28% (6937 reads) were identified as non-AMF sequences against INSD database at 90% sequence identity level: 72% were Streptophyta (Fabaceae), 1% Chlorophyta, 23.5% Chytridiomycota, 2% Ascomycota, and 1.5% Basidiomycota.

3.3. AMF richness

Rarefaction analysis suggested that the number of AMF reads per sample was in general sufficient to produce asymptotic estimates of VT richness per sample (Fig. S1). VT accumulation curves indicated that the overall number of VT was slightly higher in soil than root samples, and that additional sampling may have resulted in the detection of additional VT in roots (Fig. S1). A total of 87 V T were detected in soil samples and a subset of 69 V T were recorded in soybean roots. Sixty one VT were common between both types of samples while 26 V T were exclusively found in soil, and 8 V T in roots samples (Table S2). Altogether, contrasting HLU shared 25.3% of VT in soil (22 V T), and 34.3% in root samples (24 V T), corresponding to 78.2% and 85.6% of reads in the respective sample types.

VT richness per sample did not differ among HLU (ANOVA lm, $p = 0.79$) nor type of sample (ANOVA lm, $p = 0.06$). The average AMF richness was 12.9 V T (± 6.5) per sample, ranging from 1 to 26 V T (Table 2). There was a significant interaction between the type of sample and HLU (ANOVA lm, $p < 0.004$), particularly in Livestock, where the number of VT in roots (mean = 8.1) was lower than in soil samples (mean = 19.2).

VT richness was correlated with soil textural components in soil and root samples, but in opposite trends (Table 3). Plant density and pH were positively correlated with AMF VT richness in soil, but not in root samples (Table 3).

3.4. AMF community composition

PERMANOVA indicated that there were significant differences in AMF community composition among HLU, and between types of samples (Table 4). Using pairwise comparisons we found a distinctive community composition in roots of Agriculture sites which significantly differed from any other soil and root sample evaluated in this study (Table 5). This finding is further illustrated in the NMDS plots where Agricultural root samples formed a separate and less dispersed group on the diagram (Fig. 1a). However, when considering soil samples, fungal communities from Livestock sites were located separately on the NMDS

Table 2
AMF virtual taxon richness (VT) and sequencing intensity of root and soil samples collected from soybean fields of contrasting historical land uses (HLU). Ten sites were sampled per each HLU, with one pooled sample per site.

HLU	Type of sample	Total no. of VT	Mean no. of VT per sample (range)	Mean no. of AMF reads per sample (range)
Agricultural	root	48	16.6 (7-26)	3845.6 (3653-4121)
	soil	43	14 (4-21)	3377.3 (2291-4326)
Livestock	root	37	8.1 (3-12)	3903.7 (1932-5931)
	soil	61	19.2 (8-26)	3682.5 (2483-5143)
Forest	root	51	14 (3-24)	4663.3 (1998-8133)
	soil	54	14.6 (5-24)	2953.2 (2046-4126)

Table 3
Significant Spearman correlations ($p < 0.05$) between virtual taxon richness per sample and soybean field properties.

Type of sample (n)	Variable	Spearman Coefficient	*p-value*
Root (28)	Sand (%)	−0.49	< 0.01
	Clay (%)	0.50	< 0.01
Soil (30)	Sand (%)	0.39	0.04
	Clay (%)	−0.39	0.04
	pH	0.37	0.04
	Plant density (pl m^{-2})	0.44	0.02

Table 4
Variation in the AMF community composition (PERMANOVA analysis) in relation to historical land use (HLU) and type of sample (soil compared with root samples) in current soybean fields.

Model	Df	SS	MS	Pseudo F	R^2	P *value*
HLU	2	2.350	1.175	3.335	0.115	**0.001**
Residuals	51	17.971	0.352			
Total	53	20.322				
Soil vs roots	1	1.048	1.048	2.829	0.051	**0.002**
Residuals	52	19.273	0.370			
Total	53	20.322				

Table 5
Pairwise comparison (PERMANOVA analysis) between AMF community composition in root and soil samples from different historical land use (HLU) based on relative abundance data.

Permanova		Agriculture		Forest		Livestock	
p (adjusted)		Root	Soil	Root	Soil	Root	Soil
Agriculture	Root	–	**0.001**	**0.001**	**0.0001**	**0.001**	**0.0001**
	Soil		–	**0.05**	0.16	**0.001**	**0.01**
Forest	Root			–	**0.05**	0.07	**0.001**
	Soil				–	**0.001**	**0.02**
Livestock	Root					–	0.20
	Soil						–

plot (Fig. 1b, Table 5). Using linear correlations between ordination and environmental variables, we found that soil properties (texture, C) significantly correlated with AMF community composition in both root and soil samples, but plant density only correlated with colonizing communities (Fig. 1ab)

In general, *Glomus* and *Gigaspora* VT were most abundant in this study (Fig. 2). Eleven VT were present with a relative abundance greater than 10%, 8 in roots and 6 in soil. They represented 11.4% and 6.8% of identified VT, and accounted for 61% and 59.8% of total AMF reads, in root and soil samples. Only three VT were predominant in both root and soil samples: *Gigaspora* VT39 (related to *G. albida, G. decipiens, G. gigantea, G. margarita, G. rosea*), *Glomus* VT280, and *Glomus* VT63 (related to *Gl. viscosum*). Indicator species analysis detected 25 indicator VT in this study (Table 6). They corresponded to 26.3% of identified VT and 55.3% of AMF sequences. Root samples were dominated by *Glomus* and *Paraglomus* as indicator VT. Soil samples, however, were characterized by a broader range of indicator VT. Forest sites were characterised by indicator VT from a larger number of AMF families than other HLU.

4. Discussion

This survey of AMF diversity in current soybean fields converted from earlier agricultural fields, livestock farming areas, and forests

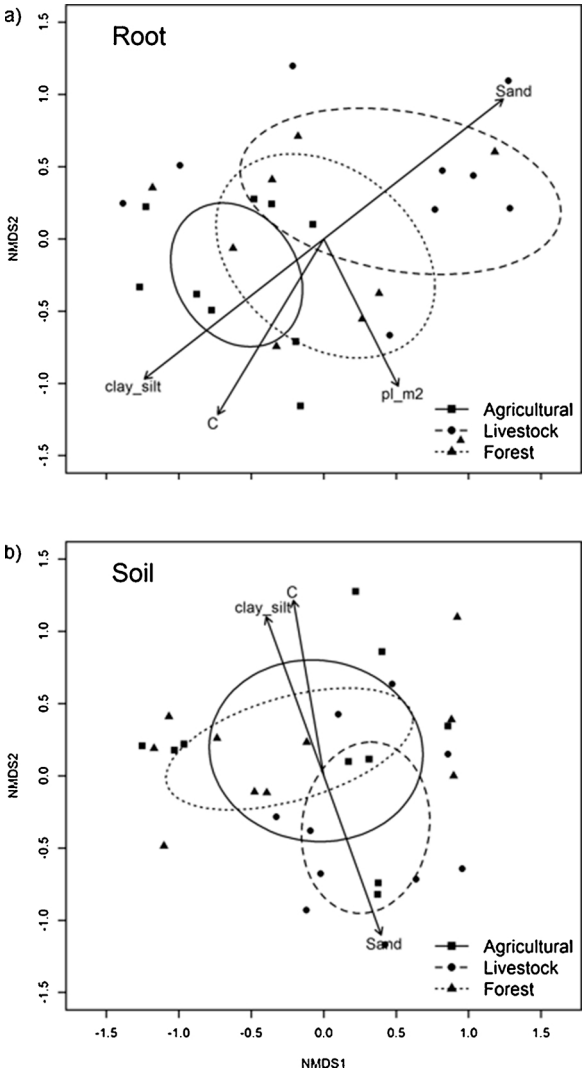

Fig. 1. NMDS of AMF taxonomic community composition among root (a) and soil (b) samples in current soybean fields of contrasting historical land use – agricultural fields, livestock areas and forests - ($k = 3$, soil: root stress 0.10; stress 0.17). Ellipses indicate one SD around group centroids of each HLU. Arrows represent statistically significant fitted vectors ($p < 0.05$) of soil and crop related variables onto ordination plot where arrow points indicate the direction of the gradient, and the length represents the correlation between ordination and the variable.

shows that soybean cropping maintained a relatively high AMF diversity in the study area. AMF richness did not differ among historical land use (HLU) situations nor sample types (soil vs roots), but AMF community composition showed a clear shift depending on HLU, attributable to changing abundances of dominant AMF. Admittedly, the HLU types of this study coincide with different edaphic and climatic (altitudinal) conditions, but this reflects availability of sites and historical land uses in the focal landscapes, and thus represents real landscapes of the region.

4.1. Richness of AMF

Overall, we detected a high VT richness along the study area under soybean cultivation. Similar pattern was observed by García de Leon et al. (2018a) and Colombo et al. (2014) from different locations of Argentina. Interestingly, AMF richness did not differ between soybean fields and their respective pristine condition in either case. In our study, the absence of difference in VT richness among HLU might be due to the

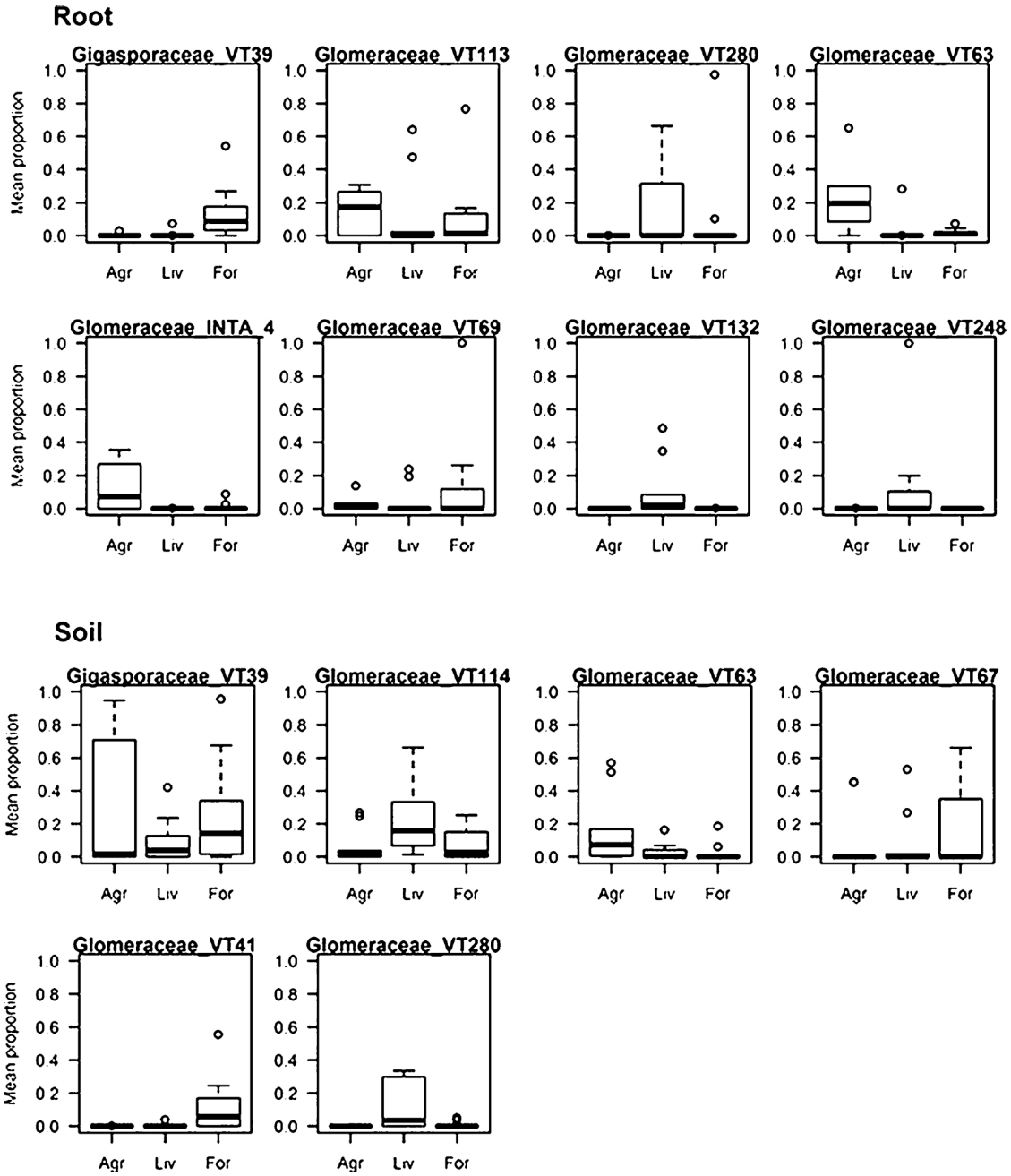

Fig. 2. Proportion of reads of most abundant virtual taxa (VT, > 0.10 of relative abundance) in root (upper panel) and soil samples (lower panel) from different historical land use – agricultural fields (Agr.), livestock areas (Liv.) and forests (For). Solid lines indicate medians; boxes and whiskers indicate quartiles and ranges, respectively.

widespread use of no-tillage practices associated with soybean cultivation. This conservative soil management has been well documented as positive in the maintenance of AMF richness (Alguacil et al., 2008; Brito et al., 2012; Säle et al., 2015; Zhang et al., 2015). Besides that, roots of Livestock sites exhibited a reduced number of colonizing AMF taxa. Considering that Livestock sites were on predominantly sandy soils located along the driest region of our study, the observed trend can be a consequence of different textural preferences of AMF species (Lekberg et al., 2007) as well as the negative effect of the scarcity of water on key stages of AMF colonization and crop development. Finally, we found that plant density was significantly and positively correlated with VT richness. Larger density of plant roots might improve resource availability for AMF because more carbohydrates would be available to support the symbiosis (Lekberg et al., 2010). In addition, roots and the associated fungal network might explore higher soil volume and contact propagules of rare and infrequent AMF species. Therefore, our results reveal that appropriate plant density management is a promising agronomical parameter for the maintenance of suitable AMF diversity, abundance and activity in agroecosystems.

4.2. AMF community composition

We found that AMF communities significantly differed among HLU. In soil samples, Livestock sites revealed different taxon composition than Agricultural and Forest sites. This pattern may be related to the aforementioned dry and sandy conditions of Livestock sites coupled with their history under cattle production (van der Heyde et al., 2017). On the other hand, the lack of differences between Agricultural and

Table 6
Indicator species analysis showing characteristic virtual taxa (VT, indicator value > 0.25) of contrasting historical land use (HLU) from root and soil samples of current soybean fields.

HLU	Source	VT	Group	Species	Indicator value	prob.
Agriculture	Soil	INTA_2	*Gigasporaceae*	*Scutellospora sp.*	0.400	0.004
		INTA_7	*Archaeosporaceae*	*Archaeospora sp.*	0.283	0.019
	Root	INTA_4	*Glomeraceae*	*Glomus sp.*	0.536	0.002
		VT63	*Glomeraceae*	*Glomus viscosum; Diversispora sp.*	0.354	0.044
		VT113	*Glomeraceae*	*Glomus fasciculatum, intraradices*	0.356	0.048
		VT140	*Glomeraceae*	*Glomus sp.*	0.585	0.001
		VT310	*Glomeraceae*	*Glomus sp.*	0.560	0.002
		VT423	*Glomeraceae*	*Glomus sp.*	0.455	0.003
Livestock	Soil	INTA_5	*Glomeraceae*	*Glomus sp.*	0.884	0.001
		VT92	*Glomeraceae*	*Glomus sp.*	0.315	0.014
		VT114	*Glomeraceae*	*Glomus intraradices, irregulare*	0.421	0.011
		VT312	*Glomeraceae*	*Glomus sp.*	0.278	0.041
Forest	Soil	VT41	*Gigasporaceae*	*Scutellospora castanea, gilmorei, gregaria; Racocetra tropicana*	0.443	0.009
		VT60	*Diversisporaceae*	*Diversispora celata, eburnea; Entrophospora nevadensis*	0.382	0.007
		VT61	*Diversisporaceae*	*Diversispora sp; Glomus versiforme*	0.364	0.007
		VT67	*Glomeraceae*	*Glomus coronatum, mosseae*	0.344	0.019
		VT263	*Diversisporaceae*	*Diversispora spurca*	0.499	0.002
		VT380	*Diversisporaceae*	*Diversispora sp.*	0.337	0.017
	Root	MO.P2	*Paraglomeraceae*	*Paraglomus sp.*	0.475	0.001
		LH.Pg01	*Paraglomeraceae*	*Paraglomus sp.*	0.285	0.024
		VT64	*Glomeraceae*	*Glomus constrictum, africanum, Septoglomus furcatum, fuscum, xanthium*	0.284	0.026
		VT99	*Glomeraceae*	*Glomus sp.*	0.252	0.036
		VT222	*Glomeraceae*	*Glomus indicum*	0.337	0.031

Forest soil samples was surprising because we expected to find different communities in fields historically intended for anthropogenic use than in recently deforested areas (Moora et al., 2014). However, in our survey, AMF communities in soil were more strongly influenced by soil texture. Indeed, it has been also shown that community composition of AMF is mostly driven by environmental filtering at the regional scale (Kivlin et al., 2014), including impact of soil properties, altitude and geographical distance, and that management may explain a smaller proportion of community variation than soil and geography (Jansa et al., 2014). As the sites explored in our study differed significantly in soil properties as well as climatic conditions and land uses in the past, the environmental conditions could drive the assembly of AMF communities, overriding the impact of contemporary common practices of soybean cultivation.

The composition of AMF communities significantly differed between root and soil samples, suggesting that the host plant is selectively associating with locally available AMF (Davison et al., 2016). Host identity has been identified as an important driver of AMF community structure in several case studies (Alguacil et al., 2016; Martinez-García et al., 2015). In addition, Senés-Guerrero and Schüßler (2016) confronted highly contrasting environments cultivated with potato and observed a core-species community of colonizing AMF. Moora et al. (2011) also studied the AMF composition in a single host species along different environments and found that roots were mostly associated with generalist AMF. The impact of host on AMF community composition depends on the scale of the study, and the interaction between both symbionts and habitat conditions (Davison et al., 2016; Vályi et al., 2015). In our study, the effect of soybean roots was substantial enough to significantly shape AMF community structure even in the dissimilar environmental conditions. For example, although soil AMF community composition did not differ between Agricultural and Forest sites, colonizing community composition strongly differed. Indeed, roots of Agricultural sites were highly predominated by *Glomeraceae* (96.1% relative abundance) compared with Forest sites (72.3%). Meanwhile, the over dominance of *Glomeraceae* taxa generated a greater homogeneity between root samples. Phylogenetic similarity is expected to indicate a degree of functional similarity (Maherali and Klironomos, 2007). Therefore, it can involve the loss of functional traits that are crucial for the sustainability of agroecosystems.

Among AMF, the high abundance of *Gigasporaceae* in soil samples across the studied gradient of HLU was a somewhat unexpected. First, although *Gigaspora* VT39 is a geographically widespread taxon, *Gigaspora* species have been usually more related to undisturbed environments than anthropogenic soils (e.g. Brito et al., 2012; Hiiesalu et al., 2014; Vályi et al., 2014). Second, this genus was previously reported to be negatively affected by soil clay content (Lekberg et al., 2007), but in our study it was abundant even with 76% of clay and silt particles. On the other hand, as the propagation of *Gigasporaceae* depends on spores, because hyphae are not infective (Biermann and Linderman, 1983), the absence of ploughing practices might allow the spores formed during one crop season remain protected from environmental exposition. In addition, many previous studies carried out in soybean fields reported the presence of *Gigaspora* spp. spores (An et al., 1993; Hendrix et al., 1995; Johnson et al., 1991; Schenk and Kinloch, 1980; Saito and Vargas, 1991), and *Gigaspora* phylotypes from either soil or root samples (Beauregard et al., 2013; Cheng et al., 2013; Higo et al., 2013, 2014, 2015). Hence, the observed abundance of *Gigaspora* could rely on both no-tillage seeding and soybean cultivation that are broadly adopted in the study area.

Indicator taxon analysis revealed the presence of AMF phylotypes descriptive of each HLU condition. In general, root samples were dominated by *Glomeraceae* and *Paraglomeraceae* while soil samples exhibited particularities according to the HLU. The sites with longer anthropogenic history (i.e. Agricultural and Livestock) presented as indicator taxa four VT which did not have previous reports in Maarj*AM* dataset (INTA_2, 4, 5, and 7) suggesting that they might be particularly associated with long term soybean cultivation. On the other hand, the greater number of families of indicator taxa detected in Forest sites compared with Agricultural and Livestock sites, can be interpreted as a clear evidence of the negative impact of anthropogenic practices on the community structure of resident AMF.

5. Conclusions

Information about soil microbial communities in Latin America remains scarce. Our study is the first regional survey comparing root and soil AMF communities from agricultural fields differing in the time since soybean cultivation was introduced. Overall, our results suggest

that soil AMF communities differ according to local conditions and the legacy of past land uses, and that soybean acts as a biotic filter which drives the structure of root-colonizing AMF communities. We found that while soils presented a high abundance of *Gigasporaceae*, roots were predominantly colonized by *Glomeraceae*. The conservation of biodiversity guarantees the provision of services provided by AMF, therefore, further studies should investigate how changes in community structure are related with the alteration in functional diversity.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2018.10.002.

References

Albertengo, J., Belloso, C., Giraudo, M.B., Peiretti, R., Permingeat, H., Wall, L., 2013. 15 Conservation Agriculture in Argentina. Conservation Agriculture: Global Prospects and Challenges. pp. 352.

Alguacil, M., Lumini, E., Roldan, A., Salinas-García, J.R., Bonfante, P., Bianciotto, V., 2008. The impact of tillage practices on arbuscular mycorrhizal fungal diversity in subtropical crops. Ecol. Appl. 18, 527–536.

Alguacil, M., Torres, P., Montesinos-Navarro, A., Rodan, A., 2016. Soil characteristics driving arbuscular mycorrhizal fungal communities in semiarid mediterranean soils. Appl. Environ. Microbiol. 82, 3348–3356. https://doi.org/10.1128/AEM.03982-15. Editor.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.

An, Z., Hendrix, J.W., Hershman, D.E., Ferris, R.S., Henson, G.T., 1993. The influence of crop-rotation and soil fumigation on a mycorrhizal fungal community associated with soybean. Mycorrhiza 3, 171–182.

Anderson, M.J.M., 2001. A new method for non-parametric multivariate analysis of variance. Aust. Ecol. 26, 32–46. https://doi.org/10.1111/j.1442-9993.2001.tb00081.x.

Beauregard, M.S., Gauthier, M.P., Hamel, C., Zhang, T., Welacky, T., Tan, C.S., St-Arnaud, M., 2013. Various forms of organic and inorganic P fertilizers did not negatively affect soil- and root-inhabiting AM fungi in a maize-soybean rotation system. Mycorrhiza 23, 143–154. https://doi.org/10.1007/s00572-012-0459-6.

Bedano, J.C., Domínguez, A., Arolfo, R., Wall, L.G., 2016. Effect of Good Agricultural Practices under no-till on litter and soil invertebrates in areas with different soil types. Soil Tillage Res. 158, 100–109. https://doi.org/10.1016/J.STILL.2015.12.005.

Biermann, B., Linderman, R.G., 1983. Use of vesicular -arbuscular mycorrhizal roots, intraradical vesicles and extraradical vesicles as inoculum. New Phytol. 95, 97–105.

Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Sci. 59, 39–46.

Bremmer, J.M., 1965. Nitrogen availability indexes. Methods Soil Anal. Part 2, 1324–1345.

Brito, I., Goss, M.J., de Carvalho, M., Chatagnier, O., van Tuinen, D., 2012. Impact of tillage system on arbuscular mycorrhiza fungal communities in the soil under Mediterranean conditions. Soil Tillage Res. 121, 63–67. https://doi.org/10.1016/j.still.2012.01.012.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. BLAST+: architecture and applications. BMC Bioinf. 10, 421.

Cheng, Y., Ishimoto, K., Kuriyama, Y., Osaki, M., Ezawa, T., 2013. Ninety-year-, but not single, application of phosphorus fertilizer has a major impact on arbuscular mycorrhizal fungal communities. Plant Soil 365, 397–407. https://doi.org/10.1007/s11104-012-1398-x.

Cofré, M., Ferrari, A.E., Becerra, A., Domínguez, L., Wall, L.G., Urcelay, C., 2017. Effects of cropping systems under no-till agriculture on arbuscular mycorrhizal fungi in Argentinean Pampas. Soil Use Manage. 33, 364–378.

Colombo, R.P., Fernández Bidondo, L., Silvani, V.A., Carbonetto, M.B., Rascovan, N., Bompadre, M.J., Pérgola, M., Cuenca, G., Godeas, A.M., 2014. Diversity of arbuscular mycorrhizal fungi in soil from the Pampa Ondulada, Argentina, assessed by pyrosequencing and morphological techniques. Can. J. Microbiol. 60, 819–827.

Davison, J., Öpik, M., Zobel, M., Vasar, M., Metsis, M., Moora, M., 2012. Communities of arbuscular mycorrhizal fungi detected in forest soil are spatially heterogeneous but do not vary throughout the growing season. PLoS One 7. https://doi.org/10.1371/journal.pone.0041938.

Davison, J., Moora, M., Öpik, M., Adholeya, A., Ainsaar, L., Bâ, A., Burla, S., Diedhiou, A.G., Hiiesalu, I., Jairus, T., Johnson, N.C., Kane, A., Koorem, K., Kochar, M., Ndiaye, C., Pärtel, M., Reier, Ü., Saks, Ü., Singh, R., Vasar, M., Zobel, M., 2015. Global assessment of arbuscular mycorrhizal fungus diversity reveals very lowendemism. Science 127, 970–973. https://doi.org/10.5061/dryad.2m15n.

Davison, J., Moora, M., Jairus, T., Vasar, M., Öpik, M., Zobel, M., 2016. Hierarchical assembly rules in arbuscular mycorrhizal (AM) fungal communities. Soil Biol. Biochem. 97, 63–70. https://doi.org/10.1016/j.soilbio.2016.03.003.

de Cárcer, D.A., Denman, S.E., McSweeney, C., Morrison, M., 2011. Evaluation of subsampling-based normalization strategies for tagged high-throughput sequencing data sets from gut microbiomes. Appl. Environ. Microbiol. 77, 8795–8798.

Druille, M., Cabello, M.N., Omacini, M., Golluscio, R.A., 2013. Glyphosate reduces spore viability and root colonization of arbuscular mycorrhizal fungi. Appl. Soil Ecol. 64, 99–103. https://doi.org/10.1016/j.apsoil.2012.10.007.

Dufrêne, M., Legendre, P., 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67, 345–366.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

FAO, 2015. FAOSTAT: Food and Agriculture Organization of the United Nations - Statistics Division. http://faostat3.fao.org/browse/Q/QC/E.

Ferrari, A., Ravnskov, L., Larsen, J., Tønnersen, T., Maronna, R., Wall, L., 2015. Crop rotation and seasonal effects on fatty acid profiles of neutral and phospholipids extracted from no-till agricultural soils. Soil Use Manage. 31, 165–175. https://doi.org/10.1111/sum.12165.

Figuerola, E.L.M., Guerrero, L.D., Türkowsky, D., Wall, L.G., Erijman, L., 2015. Crop monoculture rather than agriculture reduces the spatial turnover of soil bacterial communities at a regional scale. Environ. Microbiol. 17, 678–688. https://doi.org/10.1111/1462-2920.12497.

García de León, D., Cantero, J.J., Moora, M., Öpik, M., Davison, J., Vasar, M., Jairus, T., Zobel, M., 2018a. Soybean cultivation supports a diverse arbuscular mycorrhizal fungal community in central Argentina. Appl. Soil Ecol. 124, 289–297.

García de León, D., Davison, J., Moora, M., Öpik, M., Feng, H., Hiiesalu, I., Jairus, T., Koorem, K., Liu, Y., Phosri, C., 2018b. Anthropogenic disturbance equalizes diversity levels in arbuscular mycorrhizal fungal communities. Glob. Change Biol. 24 (6), 2649–2659. https://doi.org/10.1111/gcb.14131.

Gee, G.W., Bauder, J.W., 1986. 15-Particle Size Analyses, Methods in Soil Analyses. ASA and SSSA, Madison.

Gerdemann, J.W., Nicolson, T.H., 1963. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc. 46, 235–244. https://doi.org/10.1016/S0007-1536(63)80079-0.

González-Cortés, J.C., Vega-Fraga, M., Varela-Fregoso, L., Martínez-Trujillo, M., Carreón-Abud, Y., Gavito, M.E., 2012. Arbuscular mycorrhizal fungal (AMF) communities and land use change: the conversion of temperate forests to avocado plantations and maize fields in central Mexico. Fungal Ecol. 5, 16–23.

Hendrix, J.W., Guo, B.Z., An, Z.-Q., 1995. Divergence of mycorrhizal fungal communities in crop production systems. Plant Soil 170, 131–140. https://doi.org/10.1007/BF02183061.

Higo, M., Isobe, K., Yamaguchi, M., Drijber, R.A., Jeske, E.S., Ishii, R., 2013. Diversity and vertical distribution of indigenous arbuscular mycorrhizal fungi under two soybean rotational systems. Biol. Fertil. Soils 49, 1085–1096. https://doi.org/10.1007/s00374-013-0807-5.

Higo, M., Isobe, K., Drijber, R.A., Kondo, T., Yamaguchi, M., Takeyama, S., Suzuki, Y., Niijima, D., Matsuda, Y., Ishii, R., Torigoe, Y., 2014. Impact of a 5-year winter cover crop rotational system on the molecular diversity of arbuscular mycorrhizal fungi colonizing roots of subsequent soybean. Biol. Fertil. Soils 50, 913–926. https://doi.org/10.1007/s00374-014-0912-0.

Higo, M., Isobe, K., Matsuda, Y., Ichida, M., Torigoe, Y., 2015. Influence of sowing season and host crop identity on the community structure of arbuscular mycorrhizal fungi colonizing roots of two different gramineous and leguminous crop species. Adv. Microbiol. 5, 107–116. https://doi.org/10.4236/aim.2015.52011.

Hiiesalu, I., Pärtel, M., Davison, J., Gerhold, P., Metsis, M., Moora, M., Öpik, M., Vasar, M., Zobel, M., Wilson, S.D., 2014. Species richness of arbuscular mycorrhizal fungi: associations with grassland plant richness and biomass. New Phytol. 203, 233–244. https://doi.org/10.1111/nph.12765.

Horn, S., Caruso, T., Verbruggen, E., Rillig, M.C., Hempel, S., 2014. Arbuscular mycorrhizal fungal communities are phylogenetically clustered at small scales. ISME J. 8, 2231–2242. https://doi.org/10.1038/ismej.2014.72.

INTA, 1978. Carta de Suelos de la Republica Argentina. Instituto Nacional de Tecnologia Agropecuaria – Argentina Hoja, pp. 3363.

INTA, 1985. Carta de Suelos de la Republica Argentina. Instituto Nacional de Tecnologia Agropecuaria – Argentina Hoja, pp. 3363.

INTA, 1996. Carta de Suelos de la Republica Argentina. Instituto Nacional de Tecnologia Agropecuaria – Argentina Hojas, pp. 3366.

INTA, 2004. Carta de Suelos de la Republica Argentina. Instituto Nacional de Tecnologia Agropecuaria – Argentina Hojas, pp. 3563.

Jansa, J., Erb, A., Oberholzer, H.R., Šmilauer, P., Egli, S., 2014. Soil and geography are more important determinants of indigenous arbuscular mycorrhizal communities than management practices in Swiss agricultural soils. Mol. Ecol. 23, 2118–2135. https://doi.org/10.1111/mec.12706.

Johnson, N.C., Pfleger, F.L., Crookston, R., Simmons, S.R., Copeland, P.J., 1991. Vesicular–arbuscular mycorrhizas respond to corn and soybean cropping history. New Phytol. 117, 657–663.

Kivlin, S.N., Winston, G.C., Goulden, M.L., Treseder, K.K., 2014. Environmental filtering

affects soil fungal community composition more than dispersal limitation at regional scales. Fungal Ecol. 12, 14–25. https://doi.org/10.1016/j.funeco.2014.04.004.
Kotilínek, M., Hiiesalu, I., Košnar, J., Šmilauerová, M., Šmilauer, P., Altman, J., Dvorský, M., Kopecký, M., Doležal, J., 2017. Fungal root symbionts of high-altitude vascular plants in the Himalayas. Sci. Rep. 7, 6562. https://doi.org/10.1038/s41598-017-06938-x.
Lee, J., Lee, S., Young, J.P., 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiol. Ecol. 65, 339–349. https://doi.org/10.1111/j.1574-6941.2008.00531.x.
Lekberg, Y., Koide, R.T., Rohr, J.R., Aldrich-Wolfe, L., Morton, J.B., 2007. Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. J. Ecol. 95, 95–105. https://doi.org/10.1111/j.1365-2745.2006.01193.x.
Lekberg, Y., Hammer, E.C., Olsson, P.A., 2010. Plants as resource islands and storage units - adopting the mycocentric view of arbuscular mycorrhizal networks. FEMS Microbiol. Ecol. 74, 336–345. https://doi.org/10.1111/j.1574-6941.2010.00956.x.
López-García, Á., Varela-Cervero, S., Vasar, M., Öpik, M., Barea, J.M., Azcón-Aguilar, C., 2017. Plant traits determine the phylogenetic structure of arbuscular mycorrhizal fungal communities. Mol. Ecol. 26, 6948–6959.
Maherali, H., Klironomos, J.N., 2007. Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316, 1746–1748. https://doi.org/10.1126/science.1143082.
Martínez-García, L.B., Richardson, S.J., Tylianakis, J.M., Peltzer, D.A., Dickie, I.A., 2015. Host identity is a dominant driver of mycorrhizal fungal community composition during ecosystem development. New Phytol. 205, 1565–1576. https://doi.org/10.1111/nph.13226.
McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A new method which gives an objective measure of colonization of roots by vesicular—-arbuscular mycorrhizal fungi. New Phytol. 115, 495–501.
Milne, I., Lindner, D., Bayer, M., Husmeier, D., Mcguire, G., Marshall, D.F., Wright, F., 2009. TOPALi v2: a rich graphical interface for evolutionary analyses of multiple alignments on HPC clusters and multi-core desktops. Bioinformatics 25, 126–127. https://doi.org/10.1093/bioinformatics/btn575.
Moora, M., Berger, S., Davison, J., Öpik, M., Bommarco, R., Bruelheide, H., Kühn, I., Kunin, W.E., Metsis, M., Rortais, A., Vanatoa, A., Vanatoa, E., Stout, J.C., Truusa, M., Westphal, C., Zobel, M., Walther, G.R., 2011. Alien plants associate with widespread generalist arbuscular mycorrhizal fungal taxa: evidence from a continental-scale study using massively parallel 454 sequencing. J. Biogeogr. 38, 1305–1317. https://doi.org/10.1111/j.1365-2699.2011.02478.x.
Moora, M., Davison, J., Öpik, M., Metsis, M., Saks, U., Jairus, T., Vasar, M., Zobel, M., 2014. Anthropogenic land use shapes the composition and phylogenetic structure of soil arbuscular mycorrhizal fungal communities. FEMS Microbiol. Ecol. 90, 609–621. https://doi.org/10.1111/1574-6941.12420.
Oksanen, J., 2013. Multivariate Analysis of Ecological Communities in R: Vegan Tutorial. [WWW Document]. .
Öpik, M., Davison, J., 2016. Uniting species- and community-oriented approaches to understand arbuscular mycorrhizal fungal diversity. Fungal Ecol. 24, 106–113. https://doi.org/10.1016/j.funeco.2016.07.005.
Öpik, M., Vanatoa, A., Vanatoa, E., Moora, M., Davison, J., Kalwij, J.M., Reier, Ü., Zobel, M., 2010. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol. 188, 223–241. https://doi.org/10.1111/j.1469-8137.2010.03334.x.
Osborne, O.G., De-Kayne, R., Bidartondo, M.I., Hutton, I., Baker, W.J., Turnbull, C.G.N., Savolainen, V., 2018. Arbuscular mycorrhizal fungi promote coexistence and niche divergence of sympatric palm species on a remote oceanic island. New Phytol. 217, 1254–1266.
Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55https://doi.org/10.1016/S0007-1536(70)80110-3. 158–IN18.
Powell, J.R., Rillig, M.C., 2018. Biodiversity of arbuscular mycorrhizal fungi and ecosystem function. New Phytol. https://doi.org/10.1111/nph.15119.
R Development Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
Robert, D.W., 2012. Labdsv: Ordination and Multivariate Analysis for Ecology. R Package Version 1.5-0.
SAGPyA, 2017. Secretaria de Agricultura Ganaderia, Pesca y Alimentación. [WWW Document]. http://www.siia.gov.ar/.
Säle, V., Aguilera, P., Laczko, E., Mäder, P., Berner, A., Zihlmann, U., van der Heijden, M.G.A., Oehl, F., 2015. Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. Soil Biol. Biochem. 84, 38–52. https://doi.org/10.1016/j.soilbio.2015.02.005.
Schenck, N.C., Kinloch, R.A., 1980. Incidence of mycorrhizal fungi on six Field crops in monoculture on a newly cleared woodland site. Mycologia 72, 445–456. https://doi.org/10.2307/3759518.
Schüßler, A., Schwarzott, D., Walker, C., 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol. Res. 105, 1413–1421.
Senés-Guerrero, C., Schüßler, A., 2016. A conserved arbuscular mycorrhizal fungal core-species community colonizes potato roots in the Andes. Fungal Diversity 77, 317–333. https://doi.org/10.1007/s13225-015-0328-7.
Sepp, S.-K., Jairus, T., Vasar, M., Zobel, M., Öpik, M., 2018. Effects of land use on arbuscular mycorrhizal fungal communities in Estonia. Mycorrhiza 28, 259–268.
Simon, L., Lalonde, M., Bruns, T.D., 1992. Specific amplification of 18S fungal ribosomal genes from VA endomycorrhizal fungi colonizing roots. Appl. Environ. Microbiol. 58, 291–295.
Smith, S.E., Read, D.J., 2010. Mycorrhizal Symbiosis. Academic press, Cambridge, UK.
USDA, 2014. USDA Soil Classification. Soil Survey Staff. Keys to Soil Taxonomy, 12th ed. USDA-Natural Resources Conservation Service, Washington, DC.
Vályi, K., Rillig, M.C., Hempel, S., 2015. Land-use intensity and host plant identity interactively shape communities of arbuscular mycorrhizal fungi in roots of grassland plants. New Phytol. 205, 1577–1586. https://doi.org/10.1111/nph.13236.
Vályi, K., Mardhiah, U., Rillig, M.C., Hempel, S., 2016. Community assembly and coexistence in communities of arbuscular mycorrhizal fungi. ISME J. 10, 2341–2351. https://doi.org/10.1038/ismej.2016.46.
van der Heijden, M.G., Martin, F.M., Selosse, M.A., Sanders, I.R., 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytol. 205, 1406–1423. https://doi.org/10.1111/nph.13288.
van der Heyde, M., Ohsowski, B., Abbott, L.K., Hart, M., 2017. Arbuscular mycorrhizal fungus responses to disturbance are context-dependent. Mycorrhiza 27, 431–440. https://doi.org/10.1007/s00572-016-0759-3.
Vandenkoornhuyse, P., Husband, R., Daniell, T.J., Watson, C.A., Duck, J.M., Fitter, A.H., Young, J.P., 2002. Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. Mol. Ecol. 11, 1555–1564.
Varela-Cervero, S., Vasar, M., Davison, J., Barea, J.M., Öpik, M., Azcón-Aguilar, C., 2015. The composition of arbuscular mycorrhizal fungal communities differs among the roots, spores and extraradical mycelia associated with five Mediterranean plant species. Environ. Microbiol. 17, 2882–2895. https://doi.org/10.1111/1462-2920.12810.
Walker, C., 1992. Systematics and taxonomy of the arbuscuar endomycorrhizal fungi (glomales) - a possible way forward. Agronomie 12, 887–897.
Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37, 29–38.
Waterhouse, A.M., Procter, J.B., Martin, D.M.A., Clamp, M., Barton, G.J., 2009. Jalview Version 2-A multiple sequence alignment editor and analysis workbench. Bioinformatics 25, 1189–1191. https://doi.org/10.1093/bioinformatics/btp033.
Werner, G.D.A., Kiers, E.T., 2015. Partner selection in the mycorrhizal mutualism. New Phytol. 205, 1437–1442.
Williams, A., Manoharan, L., Rosenstock, N.P., Olsson, P.A., Hedlund, K., 2017. Long-term agricultural fertilization alters arbuscular mycorrhizal fungal community composition and barley (Hordeum vulgare) mycorrhizal carbon and phosphorus exchange. New Phytol. 213, 874–885. https://doi.org/10.1111/nph.14196.
Zhang, S., Li, Q., Lü, Y., Sun, X., Jia, S., Zhang, X., Liang, W., 2015. Conservation tillage positively influences the microflora and microfauna in the black soil of Northeast China. Soil Tillage Res. 149, 46–52. https://doi.org/10.1016/j.still.2015.01.001.