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Can soil properties and agricultural land use affect arbuscular mycorrhizal fungal communities indigenous from the Argentinean Pampas soils?

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ABSTRACT

In order to determine if intensive agricultural land use and/or edaphic characteristics modify the community of the arbuscular mycorrhizal fungi (AMF) indigenous of soils from the Argentinean Pampa; mycorrhizal colonization, sporulation, glomalin (GRSP) and genetic diversity were assessed. Soils were collected from seven localities from Buenos Aires Province (Argentina) under contrasting land use intensity (at each site both pristine and agricultural soils). The capacity for root colonization, sporulation and glomalin content of trap plants and growth substrate were considered as measure of AMF activity. Analysis of diversity was conducted using PCR-single strand conformation polymorphism (SSCP). Profiles were generated by using primers to amplify the 28S rDNA of AMF from rDNA extracted directly from the substrate of trap plant or AMF spore. Five bands of the SSCP gel were sequenced and showed similarity with the genus *Glomus*. Analysis of colonization and GRSP content, were not clearly related to the land use intensity but sporulation capacity was, in general, lower in agricultural soils than in pristine ones. Soil phosphorus, ferrum and zinc contents, were negatively related to the root colonization and sporulation capacities. High genetic diversity was found both from soil and spore samples. Although similar diversity was found under agricultural and pristine soils, in most sites SSCP-diversity was separately clustered by land use. Still remains clarify if soil characteristics, resulting from agricultural management; exert selection pressure on the AMF and whether they could be vectors of interest to select potential plant growth promoting microorganisms.

1. Introduction

In recent decades, several strategies for improving soil fertility which range from changes in management practices to use plant growth promoting microorganisms have been developed. Some strategies include the use of arbuscular mycorrhizal fungi (AMF), belonging to the phylum Glomeromycota (Schüßler and Schwarzott, 2001), which form mutualistic symbiotic associations with roots of 80–90% higher plants and extends over all habitats on Earth (Kivlin et al., 2011). Their importance is mainly related to cycling and transfers to the plant of low mobile nutrients in soils like phosphorus and zinc, among others (Bucher, 2007). In addition to increases in plant nutrition (Cardoso and Kuyper, 2006), a proper selection and application of AMF can increase plants resistance of against abiotic or biotic stress (Linderman, 2000; Morte et al., 2001) and also contributes to increasing the soil aggregates stability (Purin and Rillig, 2007).

Most known AMF activity function on soil is production of a glycoprotein component of the hyphal wall (Driver et al., 2005) called glomalin (Wright and Upadhyaya, 1996) which is operationally quantified from soil as easily extractable-glomalin-related soil protein (GRSP). However, evidence linking GRSP to soil aggregation (Rillig and Mummey, 2006) is evidenced only by the correlation between variables, but involved mechanisms are still unclear. Even

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so, because soil glomalin has lower turnover (from 6 to 42 years) and remains in the soil longer than the hyphal wall glomalin (Steinberg and Rillig, 2003) the inclusion of soil GRSP quantification for monitoring AMF from environmental samples could be considered a good indicator of soil quality and AMF activity (Purin and Rillig, 2007).

Some evidences suggest that AMF activity and/or diversity are affected by soil land use intensity (Oehl et al., 2003), soil type and characteristics (Lekberg et al., 2007; Covacevich et al., 2012), host specificities (Bever et al., 2001), spatial and temporal climatic and latitude gradients (Lovelock and Ewel, 2005), among others. Although diversity of AMF have been traditionally assessed by taxonomical identification of their spores (Stürmer, 2012), sometimes it becomes difficult because: (i) spore production and characteristics are highly dependent on AMF physiology and environment (climatic and edaphic conditions), (ii) some AMF are non-sporulating species under controlled conditions and (iii) the AMF spores in the field could be parasitized or degraded (Redecker et al., 2003). These problems can be partially circumvented by setting up "AMF trap cultures" which allows to propagate AMF occurring in the field and to obtain fresh spores of all developmental stages under controlled conditions (Redecker et al., 2003; Tchabi et al., 2008). In recent decades the use of molecular techniques complemented and attended classical taxonomy both in the identification of the AMF as well in ecological studies. A potential strategy to analyze changes in genetic diversity of microorganisms indigenous from several environmental samples may be the single stranded conformation polymorphism (SSCP) technique, which was introduced for Schwieger and Tebbe (1998) for soil microbial community analysis. The SSCP has proven to be a suitable strategy also for diversity studies of AMF (Simon et al., 1993; Kjøller and Rosendahl, 2000).

The Buenos Aires Province, of Pampas region (Argentina), is the main area of agricultural production of Argentina. About 12.54 million hectares are destined for extensive agricultural production that represents 41% of the total area of the province and 29% of the area devoted to agriculture in the country (SIIA, 2015). In the last decades, the agricultural frontier has practically forward pristine soils and currently few sites have remained in the unaltered state. Depending on local soils and climate characteristics, the same agricultural field is continuously cultivated with crops such as soybean (*Glycine max*), wheat (*Triticum aestivum*), maize (*Zea mays*), barley (*Hordeum vulgare*), and sorghum (*Sorghum* spp.). The crops are generally rotated and cultivated in mean/large-scale and is practically nil the time in which the soil remains uncultivated. Some results indicate that some microbial consortia with AMF indigenous of Buenos Aires Province could be potentially efficient to increase maize and tomato growth (Astiz Imaz et al., 2014; Thougnon Islas et al., 2014). Preliminary results indicate that mycotrophic capacity (as an indicator of the AMF activity) is more sensitive to edaphic characteristics than affected by agricultural land use (Covacevich et al., 2012). However, the impact of agricultural land use and soil characteristics on some AMF activity parameters and genetic diversity of AMF communities has not yet been investigated. We hypothesize that, intensive agricultural land use of last decades modified soil natural properties which undoubtedly may be lead to root colonization, sporulation, soil glomalin content and diversity decreases of AMF communities indigenous from Argentinean Pampas.

Under such preconditions, the multiple benefits of the mycorrhizal symbiosis are likely to play a pivotal role for maintaining natural soil fertility by enhancing plant nutrient use efficiency, plant health, and stabilization of a favourable soil structure. Thus, it is important to explore the impact of the commonly applied agricultural practices on the native AMF community. Therefore, we expected that AMF diversity and activity vary both in relation to land use and edaphic characteristics. To achieve an understanding of dynamics of AMF associated to land use and/or edaphic characteristics in different agricultural zones, some AMF activity (spore density, mycotrophic capacity and GRSP) parameters and molecular SSCP-diversity were compared at seven sites of Buenos Aires Province each under contrasting land use intensity (pristine and agricultural).

2. Materials and methods

2.1. Soil sampling and analysis of chemical properties of soils

The samples were collected from 7 soils distributed in different fields with agricultural aptitude of the Buenos Aires Province, Argentina (Fig. 1). The selected sites were located at south-east (Benito Juárez (BJ), Coronel Dorrego (CD), Lobería (L) and Madariaga (M)), and at north-west (Junín (J), Carlos Casares (CC) and Trenque Lauquen (TL)) of the province (Fig. 1). Soil samples were collected both in agricultural soils (A) and in soils without agriculture or near-pristine (P) conditions, resulting 14 sampled

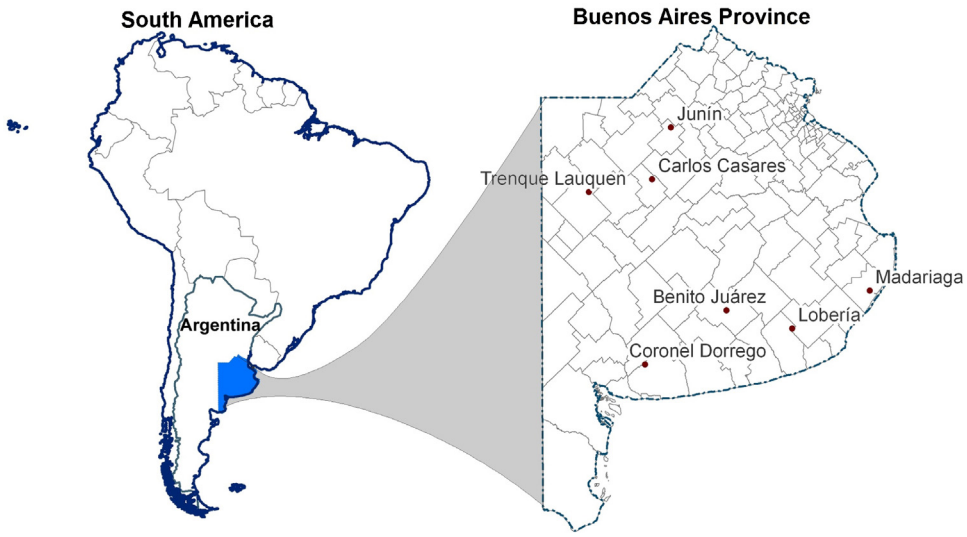

Fig. 1. Location of the 7 sites selected from the Buenos Aires Province, Argentina.

sites (7 locations and 2 soil land use per each). Each sample (about 2 kg soil) consisted in ten geo-referenced soil sub-samples collected on a 50-m grid at 20 cm of deep. All samples were bulked, mixed and analyzed for pH (Mc Lean, 1982); organic matter (OM; Walkley and Black, 1934); available P (Bray and Kurtz, 1945); exchangeable cations: Ca, Mg, Na and K (Chapman, 1965); and available Fe and Zn (Lindsay and Norvell, 1978).

2.2. Trap plants and AMF related-activity parameters

All soil samples were used as substrate for performing trap cultures (in four replicates for each site) that consisted in pots (100 mL) containing field soil (soil inoculum) and autoclaved sand (ratio: 2:1 soil:sand). Ryegrass (*Lolium multiflorum* Lam.) was sown (surface-sterilized seeds by rinsing with distilled H_2O, ethanol 90% 3 min, rinsing with distilled H_2O, NaClO 3.5%, 3 min and a final rinsing with distilled H_2O) as host (trap) plant. The treatments were arranged in a completely randomized design with 4 repetitions. Plants were maintained under controlled conditions (12 h light/12 h dark; 23 °C), in a growth chamber. The trap plants were watered once every 2 days and Hoagland's nutrient solution (with P 50%: 1 mL KH_2PO_4/L solution) was supplied every 2 weeks. The substrate was maintained at water holding capacity (65% w/s).

Seventy days after emergency, trap plants were harvested, the washed roots were cleared (10% KOH, 60 °C, 30 min) and stained (50 °C, 15 min) with trypan blue (0.05%) in a lactoglycerol solution (lactic acid: glycerol: destilled water 1:1:1: ratio) according to the modified method described by Phillips and Hayman (1970). Arbuscular mycorrhizal colonization (AM%) and arbuscule content (Ar%) were microscopically assessed (Brundrett et al., 1984).

As a measure of AMF sporulation ability, the AMF spore occurrence was quantified both from the field soil samples and substrates of trap plants. Briefly, the soils were wet sieved through 450, 124, 53 and 30 μm sieves and centrifuged in a sucrose gradient 60% (Gendermann and Nicholson, 1963). Spores with evident nuclei inside were quantified by microscopic examination (20X).

Substrates of trap cultures were also used for glomalin-related soil protein (GRSP) quantification (Wright and Upadhyaya, 1996) by mixing substrate (1 g) with sodium citrate (citric acid, trisodium salt dehydrate, 20 mM pH 7.00), autoclaving (121 °C, 30 min) and centrifugation (3500 rpm, 15 min). Protein concentrations in the supernatant were determined spectrophotometrically by measuring absorbance at 595 nm with bovine serum albumin (BSA) as standard (Gadkar and Rillig 2006; Rosier et al., 2006).

2.3. DNA extraction and AMF diversity analysis

The genomic DNA was extracted, both from the substrate of trap plants and the AMF spores, by using the *ZR Soil Microbe DNA MiniPrep*™ DNA isolation kit. In the first case, genomic DNA of 500 mg of mixed, homogenized freeze–dried substrate of the trap plants was extracted following manufacturer's instructions. In the second case one a modification of the protocol was included: frozen spores were crushed in the Lysis Buffer, provided by the kit, by using a sterile micropestle. Later, manufacturer's instructions were followed. Isolated DNAs were dissolved in 100 μL of TE buffer and quantified by using Epoch (Biotech). The DNA quality was checked on 1% agarose gel stained with GelRed®.

The genetic diversity of AMF was assessed by a genotypic fingerprinting approach using the polymerase chain reaction-single stranded conformation polymorphism (PCR–SSCP). A nested PCR was performed for amplifying a fragment of the large subunit of ribosomal DNA gene (28S) (Kjøller and Rosendahl, 2000). Briefly, each 30 μL of PCR reactions contained 3 μL of template, 1.5 mM $MgCl_2$ Buffer, 0.25 mM dNTPs, 0.02 μM forward and reverse primers and 1 U GoTaq® DNA Polymerase (*Promega*). In the first PCR reaction, approximately 700 bp were amplified using the fungal specific primer pair LSU0061/LSU0599 (5′-AGCATATCAA-TAAGCGGAGGA-3′/5′-TGGTCCGTGTTTCAAGACG-3′). The DNAs from substrates and spores were used as template and the program carried out consisted of an initial denaturation for 2 min at 94 °C followed by 30 cycles of 1 min at 94 °C; 1 min at 53 °C and 5 min at 72 °C and finally an extension during 7 min at 72 °C (van Tuinen et al., 1998). On the second PCR reaction, the products resulting of the first PCR reaction (diluted 1:50) were used as template for amplifying an approximately 400 bp fragment, corresponding to the 28S rDNA gene of AMF belonging the genus *Glomus* (*Funneliformis mosseae = Glomus mosseae; Glomus caledonium* and *Glomus geosporum*) (Kjøller and Rosendahl, 2000; Rosendahl and Stukenbrock, 2004). The specific primers LSUrk4f/LSUrk7r (5′-GGGAGGTAAATTTCTCCTAAGGC-3′/5′-ATC-GAAGCTACATTCCTCC-3′) were used and thermo-cycling conditions consisted of an initial denaturation 2 min at 94 °C; 25 cycles (1 min at 94 °C; 1 min at 60 °C; 1 min at 72 °C) and the final extension (7 min at 72 °C). All PCR reactions were performed in an Eppendorf Thermal Cycler (Bio-Rad, USA) and all amplicons were checked by agarose gel electrophoresis (1.0% w/v agarose; 100 V, 45 min) using Gel Red® staining and stored at −20 °C for subsequent SSCP analysis.

Nested PCR products generated by LSUrk4f and LSUrk7r primer pairs were subsequently analyzed by SSCP using a Dcode Universal Mutation Detection System (Bio-Rad, USA). 5 μL of the PCR product added to 3 uL of denaturing loading mixture (95% deionized formamide, 0.05% bromophenol blue, 0.05% xylene cyanol FF and EDTA20 mM) were denatured at 95 °C for 5 min, and immediately plunged into ice. Each PCR product was loaded into the 1 mm-thick gel 0.5X MDE® (Cambrex, Rockland ME, USA), 1X TBE (0.045 M Tris/borate, 0.001 MEDTA) and run at 15 °C, 8 W, 300 V for 4 h. The SSCP gels were silver stained according to Benbouza et al. (2006), scanned and finally analyzed. The SSCP banding patterns obtained both from substrate of host plants and isolated spores were separately compared by using the Phoretix 1D PRO software (Nonlinear Dynamics, United Kingdom). The gels were run twice with the rearrangement of the samples to check the repeatability of the analysis.

The Shannon-Weaver diversity H Index of AMF was calculated based in the SSCP pattern according to Minggui et al. (2012). The pattern was first analysed by the Phoretix 1D PRO software and then a dendrogram of Weighted Neighbor-Joining (100 bootstraps) were constructed by using the DARwin software v.6.0.10 (Perrier and Jacquemoud-Collet, 2006). Ten isolated bands both of pristine and agricultural sites were excised from the SSCP gel, resuspended in 30 μL of sterile molecular grade water, maintained 1 hour at 60 °C; frozen at −20 °C and maintained 1 h at 60 °C again. The amplicons were recovered by centrifugation (5 min at 10000 rpm) and re-amplified by using the LSU Rk4f/LSU Rk7r primers pair as previously described. Five samples could be re-amplified, and were purified with the Wizard® SV Gel and PCR Clean-up System (Promega). The sequencing was carried out by capillary electrophoresis in a Genetic Analyzer 3500xl (Applied Biosystems) sequencer by using Big Dye Terminator v3.1 chemistry. All AMF generated sequences were deposited in the EMBL Nucleotide Sequence Database under the accession numbers KJ920202 and KM262654 to KM262657 (available online). Obtained sequences were analyzed and aligned using the Bioedit Sequence Alignment Editor (Hall, 1999) software and compared to known sequences in GenBank using the BLAST program at BLASTn (Basic Local Alignment Search Tool) program (://blast.ncbi.nlm.nih.gov/Blast.cg). Known sequences yielding the greatest percent similarity to submitted sequences were chosen for identification. Sequences of *Acaulospora lacunosa* (AJ510230), *Gigaspora margarita* (AJ852014)

and *Scutellospora calospora* (AJ510231) were also included. The sequence alignments and dendrograms were achieved by using the program MEGA version 6 (Tamura et al., 2013). A maximum parsimony tree was obtained using the Kimura two-parameter method (subtree-pruning-regrafting (SPR) algorithm) (Nei and Kumar, 2000).

2.4. Statistical data analysis

Variance analyses (ANOVA) on AMF mycorrhizal colonization, spore number and GRSP were assessed. Differences among means of each variable were compared ($p < 0.05$; Tukey's test) both among sites and land use (R Core Team, 2012). Instead interactions, all studied parameters are shown at each site and separated by land use, while mean differences were analyzed both between land use for each site and among sites for each land use. Regressions were performed to test the relationship of edaphic properties (pH, OM, P, Fe and V) on the GRSP content (R Core Team, 2012). Principal component analysis (PCA) was performed among soil and land use characteristics of sites and AMF mycorrhizal colonization, spore number, GRSP and diversity by using the CANOCO (CANOCO for Windows v4.0; Microcomputer Power, Ithaca, NY).

3. Results and discussion

3.1. Arbuscular mycorrhizae related-activity parameters

Mycorrhizal fungal structures such as spore, vesicles, arbuscules, mycelium, and even colonized roots were found in the roots of host plants indicating an active sporulation and mycotrophic capacity of the indigenous AMF of all tested sites. We use the trap plant strategy to assess, among others parameters, the ability of root colonization of AMF propagules of soils because, in part, field host roots where not always of the same specie (Table 1). There are different opinions regarding the use of trap plants, however, some reports agree with the idea that AMF are generalist with respect to the ability to form association with host plants (Schwartz et al., 2006). The statistical analysis for mycotrophic capacity (AM and Ar) and GRSP showed that there were not individual effect of site and land use but some interactions were statistically significant (data not shown). Mycorrhizal colonization (AM) ranged from 4.8% to 56.7% while the percentages of arbuscules (Ar) varied from 0% to 35%, being the highest value found in Carlos Casares, while roots of trap plants growing in pristine soils from Madariaga did not form arbuscules (Table 1).

When we analyzed differences between land uses (management) at each site, we found significant differences between agricultural and pristine soils at Benito Juarez and Junín for AM and at Carlos Casares for Ar where roots of trap plants grown of agricultural soils showed higher AMF colonization capacity than the pristine ones. The analysis among sites at the same management (pristine or agricultural) showed highest percentages of both AM and Ar at Carlos Casares and Lobería for agricultural soils and at Benito Juarez and Lobería for pristine soils (Table 1). Similar values of mycotrophic capacity of indigenous AMF were found by Covacevich et al. (2012) at this region. Also Liu and Wang (2003) reported similar mycotrophic capacity values at three different soils from China (Coalmine spoil, Island forest and Saline soils). They found differences in mycorrhizal colonization status associated to host plants (corn, white clover, tobacco, silverweed and cinquefoil) and indigenous soil inocula. As mentioned, we use trap plants to avoid any host plant interaction on colonization capacity. Tchabi et al. (2008) reported low or even zero root colonization after 24 months of multiplication in trap cultures of cultivated and pristine soils from different ecological zones in sub-Saharan savannas, which was probably associated to an unsuccessful AMF propagation.

Low (<5 spores 100 g soil^{-1}) or any nucleated (like viable) AMF spore were obtained from soil samples collected directly from the field (data not shown). After multiplication in trap plants, the abundance of AMF spores in the substrate increased from 80 to 1175 spores 100 g soil^{-1} and high mycotrophic capacity was found. Troeh and Loynachan (2009) also reported low spore number (<1 spore g soil^{-1}) in soil samples collected in different agricultural

Table 1
Location of sampling sites, land use history, soil chemical characteristics, AMF activity and diversity. A: agricultural (crops in agriculture management = B: barley; M: maize; S: soybean; S-lp: soybean-late planted; W: wheat); *P*: pristine; Lat.: latitude; Long.: longitude; C.E.C: cation exchange capacity; OM: organic matter; *V*: base saturation, AM: arbuscular mycorrhizal colonization; Ar: arbuscule content; GRSP: Glomalin-Related Soil Protein; H': Shannon–Weaver diversity Index; ds: dry soil.

Site	Land use	Lat.	Long.	C.E.C	*V*	pH	Zn	Fe	*P*	OM	AM	Ar	Spore	GRSP	H' diversity Index	
		Decimal degrees		$mmol_c\ kg^{-1}$	%		mg kg ds^{-1}			%	%	%	n° 100 g ds^{-1}	mg g ds^{-1}	Spore	Soil
Junín	A (S/M)	34.8	61	14.2	59	6.3	0.7	60.8	8.8	3	20.6 Ab'	5.2 Abc'	130	0.469 Ad'	1.5	2.8
	P	34.8	61	15.2	83.1	6.5	8.0	126.4	96	3.4	6.7 Bc	0.6 Acd	163	0.958 Bbc	1.9	2.7
Carlos Casares	A (W/S-lp/S/M)	35.6	61.3	15.2	64	6.3	1.0	89.2	13.6	3.1	56.7 Aa'	35.0 Aa'	370	0.624 Acd'	2.4	3.0
	P	35.6	61.3	14.8	83.3	6.2	6.0	105.9	161.4	4	23.9 Ab	11.7 Bb	1175	0.862 Abc	2.0	3.1
Trenque Lauquen	A (M/S/M)	35.8	62.5	15.2	65.4	6.4	1.2	117.5	16.1	3.3	4.8 Ac'	1.4 Ac'	120	0.573 Acd'	1.7	2.6
	P	35.8	62.5	19	103.1	6.6	8.3	147	219.1	6.6	14.3 Abc	3.1 Abcd	80	0.850 Abc	2.4	2.4
Benito	A (S/W)	37.6	59.9	28.4	71.3	6.2	0.7	124.5	12.4	5.9	26.7 Bb'	13.3 Ab'	180	1.061Abc'	3.0	2.3
Juárez	P	37.6	59.9	21.4	97.8	7.6	1.3	105.8	80	6.6	53.9 Aa	22.2 Aa	518	0.845 Abc	2.7	2.7
Madariaga	A (S/M)	37.2	57.1	24.8	59.2	6.1	2.8	245.3	16.5	6.8	16.7 Abc'	7.8 Abc'	163	1.648 Aa'	2.5	2.9
	P	37.2	57.1	23.4	98.8	6.8	12.5	189.4	286.4	6.5	7.5 Ac	0.0 Ad	530	1.532 Aa	2.8	2.5
Lobería	A (S/S)	37.8	58.6	29.2	54.2	5.9	2.0	178.4	31	6.1	42.5 Aa'	27.5 Aa'	320	1.307 Aab'	2.7	2.8
	P	37.8	58.6	23.6	69.7	6.1	8.5	156.3	58.1	6.0	47.9 Aa	25.4 Aa	900	1.006 Ab	2.4	2.1
Coronel	A (W/B/W)	38.5	61.4	20.6	57.3	5.9	0.3	63.5	28.2	2.6	20.6 Ab'	14.4 Ab'	325	0.383 Ad'	2.1	3.2
Dorrego	P	38.5	61.4	16.8	96.7	7.5	0.3	16.5	11.8	2.9	22.8 Ab	10.6 Abc	358	0.457 Ac	2.9	2.7
Mean	A			21.1	61.5	6.2	1.2	125.6	18.1	4.4	26.9	14.9	229.7	0.867	2.3	2.8
Std.	A			6.5	5.8	0.2	0.9	66.5	8.3	1.8	17.3	12.2	104.7	0.479	0.5	0.3
Mean	P			19.2	90.4	6.8	6.4	121	130.4	5.2	25.3	10.5	532	0.93	2.4	2.6
Std	P			3.7	12	0.6	4.3	54.8	96.8	1.6	18.8	10.2	391.5	0.319	0.4	0.3

Indications for statistical analysis: values followed by different capital letters indicate significant differences between land use for each location ($p < 0.05$); different lowercase letters indicate significant differences between locations for each land use ($p < 0.05$); letter with apostrophe: differences for agricultural land use; letter without apostrophe: differences for pristine condition. Mean values and standard deviations (Std.) calculated for global agricultural and pristine are shown in the last two rows.

sites in Iowa, USA. In our study, field soil multiplication in trap plants allowed increasing spore number as compared to the field soil, which was consistent with previous reports (Troeh and Loynachan, 2009; Lugo and Cabello, 2002). Thus, it is likely that AMF propagules in the analyzed soil samples consisted mainly in AMF hyphal segments and colonized roots, rather than spores. Lugo and Cabello (2002) reported similar AMF spore abundance (50–2000 spores' 100 g soil^{-1}) from soil samples of prairie grasses at the south of Cordoba, Argentina. However, our results are lower than reported by Zangaro et al. (2012) who found 4000–8000 spores 100 g soil^{-1} in forest soils at the southern Brazil. Differences in the ecosystem (mainly soil and host plants characteristics of each environment) could explain disparity in the results. Because sporulation may occur between 4 and 8 weeks after the start of AMF colonization, some authors suggested that more than 10 weeks of multiplication are required for spore production from AMF viable propagules (Liu and Wang, 2003; Schalamuk and Cabello, 2010) which was the time of our experiment. At 6 of the 7 tested sites, higher AMF spore numbers were found in the pristine soils than in agricultural and differences were three fold higher at Benito Juárez, Carlos Casares, Lobería and Madariaga. Although no obvious higher mycotrophic capacity was found under pristine conditions, the higher sporulation capacity in pristine soils emphasize the importance of soil conservation of agricultural aptitude soils for maintaining the activity and genetic diversity of AMF. We did not find any correlation between increases of spore number and root mycorrhizal colonization ($r = 0.37$; $p = 0.197$) which was most evident in pristine soils from Carlos Casares and Madariaga (Table 1). This was in accordance with reported by Becerra et al. (2009) and Tommerup (1983), who associated the lack of correlation to that AMF hyphae grow faster than spore, so that colonization is attained in less time than it belonging of spore germination. These results might also indicate that a large proportion of the spores recovered in that areas were dormant (Tchabi et al., 2008). Also Lopes Leal et al. (2009) reported that AMF indigenous of Amazon (Brazil) under contrasting land uses sporulate more abundantly in trap cultures than in the field. They speculate that high sporulation in trap culture might reflect an r strategy of some species that allocate most of the carbon to sporulation while a k strategy might be represented by those species that are not prolific sporulators and allocate resource to vegetative growth. We agree with this idea and more studies about sporulation dynamics could help answer this hypothesis.

Glomalin is produced by AMF as a component of hyphal and spore walls (Driver et al., 2005). Burrows (2014) stated that the ability to assess production of glomalin adds one more tool for use in characterizing patterns and relationships of AMF hyphal activity. We also quantified the soil GRSP content as an indicator, among others, of AMF activity. However, any consistent pattern was found of GRSP content between soil land uses; and the GRSP content was significantly lower only in Junin under pristine land use in comparison to the agricultural one, even having higher mycotrophic capacity under the agricultural condition (Table 1). Other inconsistencies were found between the mycotrophic capacity and the GRSP content. For example, the highest GRSP content was found in Madariaga soils under both managements, even having shown low mycotrophic capacity. Another inconsistency was found in Coronel Dorrego that showed, both under pristine or agricultural land uses, intermediate-low mycotrophic capacity and spore abundance but the lowest GRSP content. Also Burrows (2014) reported any relationship between mycotrophic capacity and glomalin content and emphasized the importance of using a variety of parameters to characterize AMF communities, as we have done in our study.

Because these are the first results of glomalin content in agricultural and pristine soils from an area of the Argentina Pampa, it is difficult to make a comparison and say whether if they were low or high. The GRSP content ranged from 0.383 to 1.648 mg g soil^{-1}. The GRSP contents found at this study were within the range reported by Carrizo et al. (2015) for Typic Hapludoll and Argiudoll soils of the Santa Fe Province (centre of Argentina). However, they were higher than reported (0.13–0.2 mg g dry soil^{-1}) by Perez-Brandán et al. (2014) for maize and soybean cultivated Ustocrepte Cerrillos series soils from Salta Province (north of Argentina). Disparities in the GRSP contents may be associated with differences in soil types, differential organic matter content, chemical and microbiological characteristics among others.

Regression analyzes showed a significant positive relation between GRSP with OM and Fe contents. In both cases, the intercept and the slope differed from 0 (GRSP = 0.1843 OM + 0.0189 and GRSP = 0.0062 Fe + 0.1356) and the model explained the 63% and 85% of the variability on GRSP content for OM and Fe, respectively. Strong correlations between total glomaline content (TG) and soil carbon were previously found both in pristine and cultivated soils (Nichols and Wright 2005), suggesting that carbon and TG soil content are probably subjected to similar deposition and decomposition dynamics. We did not find a significant relation between GRSP and mycorrhizal parameters (spore number and AM%) (data not shown). Also Bedini et al. (2007) did not find any relation between glomalin content and AMF biomass (hyphal length) and Burrows (2014) stated that AMF species that produce higher amounts of glomalin may not be prolific spore producers, which would account for our lack of relationship between glomalin and sporulation capacity. Rillig and Mummey (2006) suggested that glomalin may be a member of the hydrophobin group of proteins, produced by all filamentous fungi, including both endomycorrhizal and ectomycorrhizal fungi. Besides, the quantification of soil GRSP protein may be underestimated by up to six fold because of positive interference of non-proteins and the negative interference in the Bradford assay caused by humic substances co-extracted and complexes with proteins (Jorge-Araújo et al., 2015). Despite these limitations, all samples were processed in the same way and GRSP by using the Bradford method could be considered a useful test (fast and inexpensive tool) to estimate the AMF activity (Wang et al., 2014; Wu et al., 2015).

3.2. AMF molecular analysis for diversity evaluation

Most studies on diversity of AMF have solely analysed AMF spore collected from soil samples. However, the spore production rate and proportions of AMF mycelium in roots and soils could vary substantially. So that, we analysed AMF communities from seven different areas and two land uses based on the SSCP profiles of 28S rDNA gene fragments coming from soil-substrate of trap plant or collected AMF spores. The SSCP patterns, including band number, position and average relative intensity, were analyzed (Fig. 2). The SSCP gel showed that the diversity of 28S rDNA fragments amplified from the AMF community (some represents of the *Glomus* genus) of both DNA from substrate of trap plant or spores was high (Fig. 2). For most localities and land uses we found about 12–30 SSCP bands for soil-DNA (corresponding the lowest to Benito Juarez-agricultural and the highest to Carlos Casares-pristine) and 5–28 SSCP bands for spore-DNA (being the lowest for Junin and the highest to Benito Juarez both under agricultural land use).

As mentioned, we use the trap plant strategy to multiply the native AMF before analyses were performed. We used the trap plant strategy, in part, because low or inexistent viable spore were collected of field samples. Because our goal was to analyze changes in the activity and diversity of AMF in relation to land use and soil characteristics, we use trap plants also to standardize the

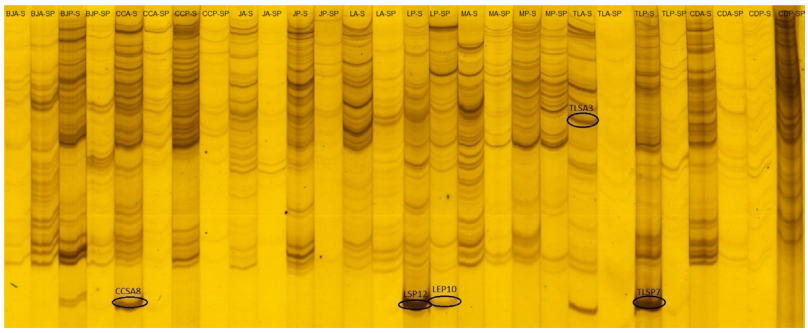

Fig. 2. PCR-SSCP profiles of AM fungal 28S-rDNA gene from products soil (S) or spores (SP) from seven sites and two management of Buenos Aires Province (Argentina). Locations: Benito Juárez (BJ), Carlos Casares (CC), Coronel Dorrego (CD), Junín (J), Lobería (L), Madariaga (M) and Trenque Lauquen (TL). Land use: agricultural (A); pristine (P). Source of DNA: soil (S), spore (SP). Ovals labelled bands were cut and sequenced.

conditions in relation to the host plant. However, some reports indicate decreasing of genetic diversity of AMF when trap plants are used due to some specificity with the host plant. Burrows and Pfleger (2002) demonstrated that after five cycles of trap sub-culturing; none of the species present in the field soil were found in the trap cultures. Trejo-Aguilar et al. (2013) reported decreases in AMF species after five cycles of trap cultures (of 3 month each), as compared to the native soil sample. However, we conducted the evaluations after one multiplication cycle at trap plant and evaluations of AMF diversity were performed on some members of the genus *Glomus*, which is considered a generalist (Torrecillas et al., 2014).

The similarity dendrogram constructed from the SSCP patterns of DNA extracted from soil-substrate samples did not show a pattern among sites or between soil lands uses (Fig. 3a). Dendrogram analysis of DNA extracted from spores showed that 13 from the 14 samples were grouped in three distinct clusters (Fig. 3b); an exception was Trenque Lauquen-agricultural which was not clustered, being an out layer. Two clusters contained only two sites each (Cluster 1 for sites of the centre and southeast of the province and Cluster 2 for sites of the southeast of the province) and the Cluster 3 grouped three sites of western of the province. Sites of cluster 2 and 3 were grouped according the land use, leaving aside the pristine soil samples separately to the agricultural soil. Also Oehl et al. (2003) showed that the AMF communities changed with land use intensity. Ours results also agree with reported by Li et al. (2010), who found that clusters constructed of RFLP patters of AMF communities for DNA's extracted of roots were grouped according the land use. The grouping was attributed by the authors to that AMF community composition changed according the land use, which might caused changes in soil factors and plant community composition. Our results indicate that

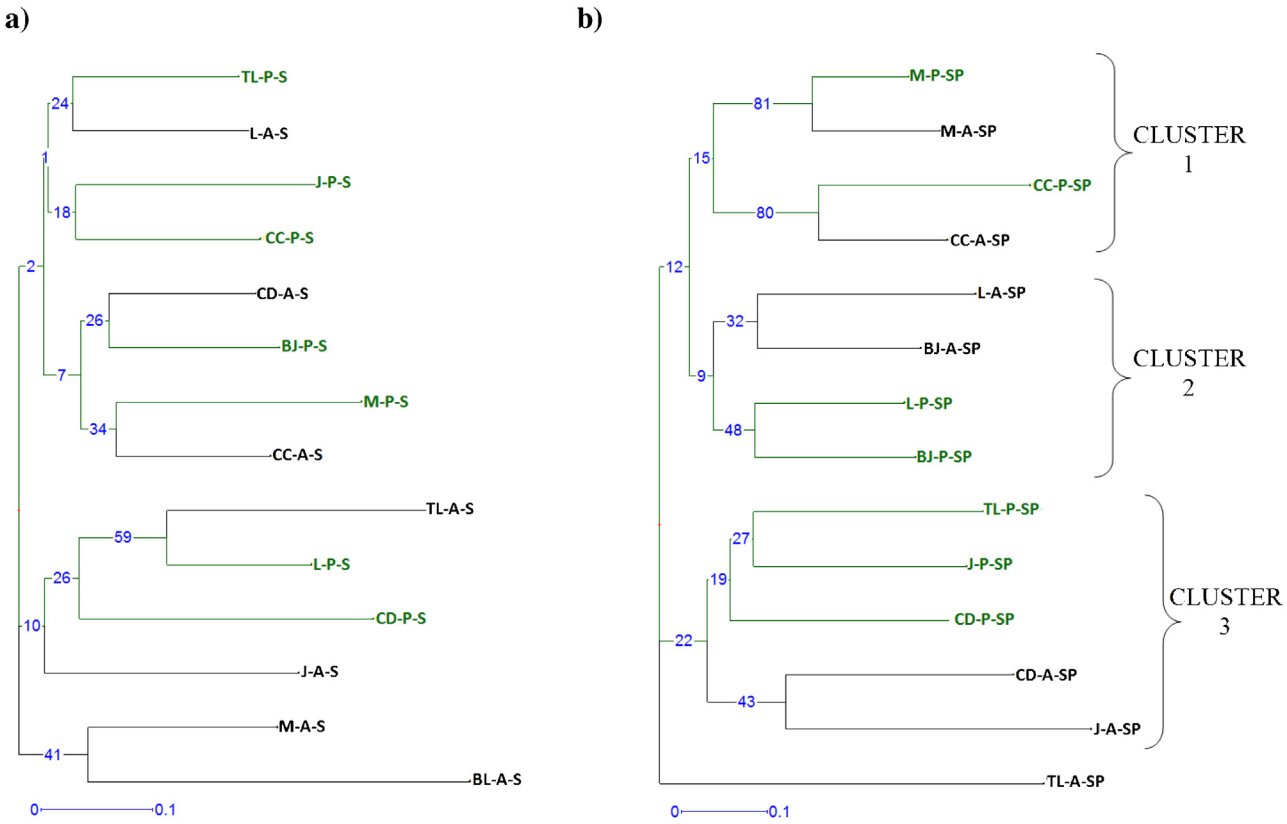

Fig. 3. Similarity dendrogram constructed from the patterns obtained by PCR-SSCP of DNA of soils and AMF spores from trap plants substrate. Figure (a) DNA from soil-S-; figure (b) DNA from spores-SP-). Land use: agricultural (A); pristine (P). Sites references described in Fig. 2.

contrasting land use systems in the centre of Argentinean Pampas region harbours a diverse community of AMF species that could be measured by the SSCP strategy after multiplication in trap cultures. In Cluster 1, however, Madariaga and Carlos Casares regrouped with the two land uses together. The fact that each site had the two land uses in the same cluster could create the idea that communities would be grouped probably for some geographical-soil characteristics regardless land use. Soil factors, such as pH, nutrient content, total soil C and N, moisture and temperature, are known to influence AMF spore distribution (Husband et al., 2002). However we did not find any soil chemical property that justifies such grouping.

The Shannon–Weaver' diversity index (H) is commonly used to characterize species diversity in a community and ranges from 0 for communities with only one taxonomic group, to higher values for communities with various taxonomic groups. At this study, the analysis of AMF diversity was performed based on the position and the average relative intensity of bands of the SSCP profile. Highest H value (3.2) was obtained for the SSCP pattern of soil DNA from Coronel Dorrego while lowest diversity index (1.5) was obtained for the SSCP pattern of spore DNA from Junin, both under agricultural land use (Table 1). As mentioned for glomalin content, these are the first results about AMF diversity assessed by the SSCP strategy of some representatives of the *Glomus* genus indigenous of agricultural and pristine soils of an area of global agricultural importance from the Argentinean Pampas. So that, it is difficult to compare and say whether they were low or high. Our H diversity indices were lower than some H diversity index reported of a DGGE assay (Minggui et al., 2012) for 5 soil samples from China which ranged from 3.67 to 4.15. However, our results were higher than reported (about 1.8) by Sánchez-Castro et al. (2012) that were computed for the SSCP strategy for AMF communities indigenous of roots of different host plants and also found low correlation between AMF taxa of roots, spore and soils. The diversity indices were calculated based on the patterns obtained by SSCP, but it is probably that an overestimation could occurred because the AMF spore are multinucleated and sometimes with different genetic makeup, and the same specie could result in more than one band. This has been described by De Souza et al. (2004) for isolates of AMF analyzed by the DGGE methodology by which genetic patterns more than a solely band was obtained for isolated AMF. When comparing both the average value of the H index calculated from SSCP pattern obtained for DNA of spores and soil we did not find, contrarily to expected, any difference when source of DNA was the soil or AMF spore. This may be due to that there are AMF species with low or null sporulation capacity (Clapp et al., 1995), which could explain why the AMF diversity from DNA obtained of spores was not higher than the soil, as expected. Some hypotheses have been proposed to explain the inconsistencies in the diversity of AMF quantified from different sources (spores or soil). An explanation could be that although the processed samples of soil for DNA extraction had 100 fold lower material than spores (0.5 g and 500 g for spore and soil of trap plant) they had both AMF spores plus propagules. However, we do not agree with this because the biomass of extra-radical AMF mycelium generally exceeds (about in 10^2 and 10^3) to the spores in soils (Johnson et al., 2003a; Gryndler et al., 2006). Hempel et al. (2007) showed remarkable differences in the composition of AMF taxa between the spore and both intra-radical and extra-radical mycelium. Johnson et al. (2003b) found that roots recruit only a fraction of the AMF taxa present as spore's pool in soils. It is clear that we can not generalize nor suggest the most appropriate or most representative AMF source in order to analyze the diversity in some environment. Therefore it is recommended that studies are carried out with a range of sources and strategies that allows analyzing the situations holistically. Irrespective of this, main differences in diversity among sites were obtained for diversity calculated from the DNA of spore, while diversity from soil was almost similar among sites. The diversity quantified from spores or soil did not show any pattern that allowed generalizing in relation to the diversity of AMF associated with soil land use or soil chemical characteristics. There was a tendency, however, that in general, higher diversity indices quantified from spores were recorded at sites from the south and southeast of the province (Benito Juarez, Coronel Dorrego, Loberia and Madariaga; located closest to the sea) while the lowest at sites from the north and northwest of the province (Carlos Casares, Junín and Trenque Lauquen located farthest from sea). However, this would be just a trend not supported statistically and more research is needed to reach a conclusion with geographic scope.

To confirm the identity of some indigenous AMF of the area of study, we proceeded to cut some 'isolated' bands from the SSCP gel for sequencing. Although 10 bands were cut and processed for DNA extraction, we had a recovery of 50% and only 5 could be re-amplified and sequenced (see oval labelled bands at Fig. 2). Obtained sequences were deposited under the following accession numbers: KJ920202 and from KM262654 to KM262657 (available online http://www.ncbi.nlm.nih.gov/). Maximum Parsimony analysis of taxa (Fig. 4) grouped the sequenced band together with representatives of the *Glomus* genus and, as expected, they were separated from other genera of AMF (*Acaulospora. Gigaspora* or *Scutellospora*) because we used specific primers in the PCR reaction that amplified part of the 28 rDNA region of *Glomus*. Although the bands CCSA8, TLSP7 and LSP12 seemed to be located in a similar position of the SSCP profile, sequencing resulted in different sequences that were grouped into different positions on the phylogenetic tree. This would indicate, in part, a high susceptibility of the SSCP method to separate different genetic sequences that look in similar location in gel by the human eye. On the other hand, this could indicate some weakness of the fingerprinting method. Still, the method used allowed to detect a high diversity of native AMF and both contributed to progress in the genetic identification of native AMF from an important agro-ecological region.

3.3. Soil chemical characteristics and land used related to AMF related-activity and diversity parameters

Chemical soil characteristics of analyzed sites are shown in Table 1. In general, soils under agricultural land use showed decrease in nutrient concentrations with respect to pristine soils being the Zn which showed the greatest decrease. An exception was the Fe content, which remained without significant changes. The Zn was, in general, higher at pristine soils than at agricultural ones. Ours results agree with reported by Sainz Rozas et al. (2015) who stated that soils of the Argentina Pampas region had high fertility under pristine condition but intensification of agriculture, increasing grain yields, and poor or no Zn fertilization reduced soil available Zn. Also the OM content decreased, in general, under agriculture, which could evidence a negative carbon balance associated with land use. Particulate or active OM is the first pool to be affected by changes in soil management (Galantini and Rosell, 2006), accounting for most of the early losses when cultivation of virgin soil begins. Acidification along with the decline in OM and Zn concentration in soil evidences the current degradation of the soil with loss of the original good soil properties of these soils as a result of the highly extractive agricultural land use of this region (Sainz Rozas et al., 2011). The available soil P content showed differences of up to 15 fold among sites and it was, in general, higher for pristine sites than for agricultural ones. This is because the P balance in the soil at this region is currently negative, because in general P removal for crop production exceeds P inputs through fertilization or others by 45–50%, particularly in agricultural

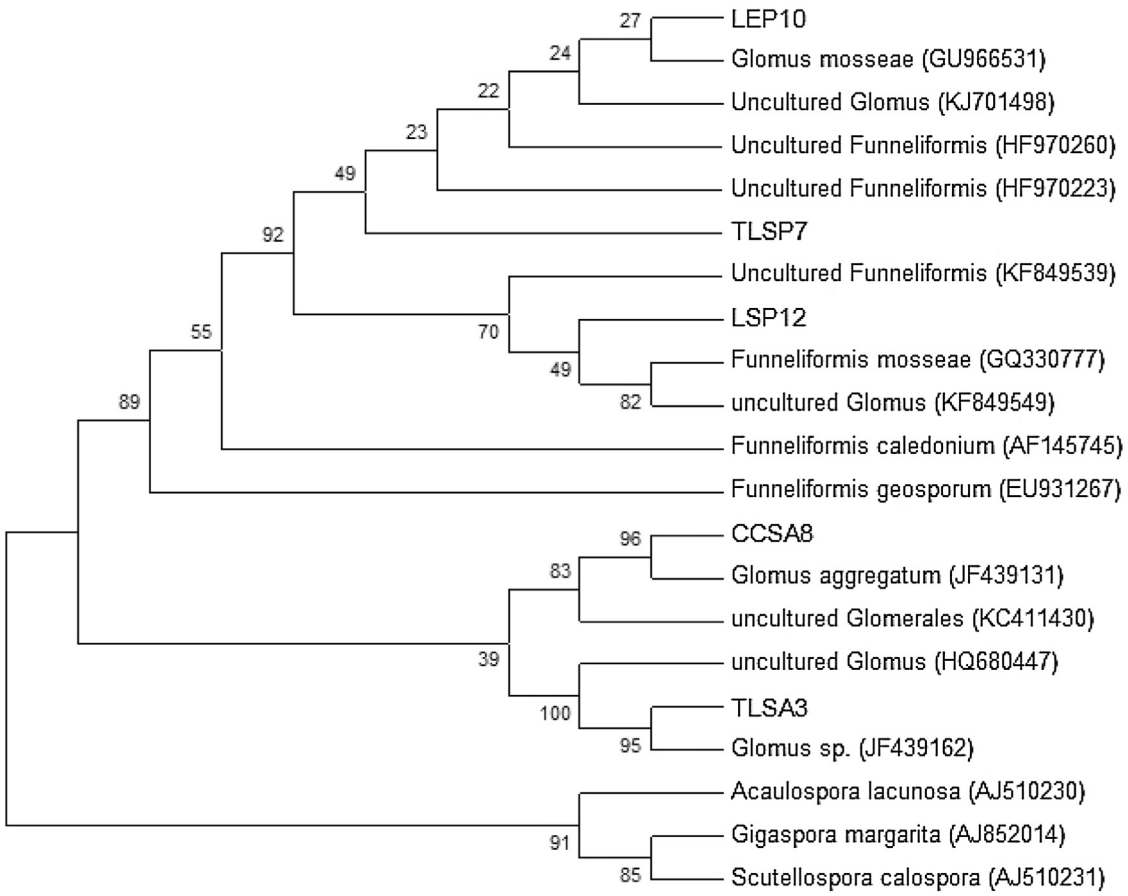

Fig. 4. Phylogenetic tree constructed as paximum parsimony analysis of taxa with obtained SSCP sequences of their closely related species. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) is shown next to the branches. References accession of each sequenced band: LEP10: KM262656; TLSP7: KM262654; LSP12: KM262657; CCSA8: KM262655; TLSA3: KJ920202 (sequences available at http://www.ncbi.nlm.nih.gov/).

production systems of the northwest and west of the Pampean Region (Sainz Rozas et al., 2012). An exception was Coronel Dorrego when no differences between land uses were detected for the Zn content and P was higher at agricultural condition. The pHs were generally slightly acid, characteristic of the study area and showed light acidification in agricultural management. This can be attributed to the negative balance between extraction of base cations of grains and its application by fertilizers. Also, the increased consumption of nitrogen and phosphate fertilizers in the last twenty years increased soil acidity (Sainz Rozas et al., 2012).

The principal component analysis (PCA) revealed clear relationships between the structures of AMF community, its activity and soil environmental properties. The cumulative percentage variance of data showed that the first two PCA axes explained the 78.9% of the data variability, being the 60.6% of the variance explained by the Principal Component 1 (PC1) (Fig. 5). Diversity indices were pooled together regardless whether they were calculated from DNA of soil or spores and showed negative association with the P content. The PC1 also showed positive association (right) between diversity indices with the GRSP, OM and Fe contents, the former mainly explained by the pristine soils. The analysis shows that the AMF root colonization and spore production, but no GRSP, were high under low available soil P, Zn and Fe contents as well as V. Contrary to expected, the CP2 suggested some negative association between parameters of AMF activity (colonization and spore production) and OM or GRSP contents. The negative association between mycotrophic capacity (AMF colonization) and Fe or P confirmed previous reports of Covacevich et al. (2012) for others

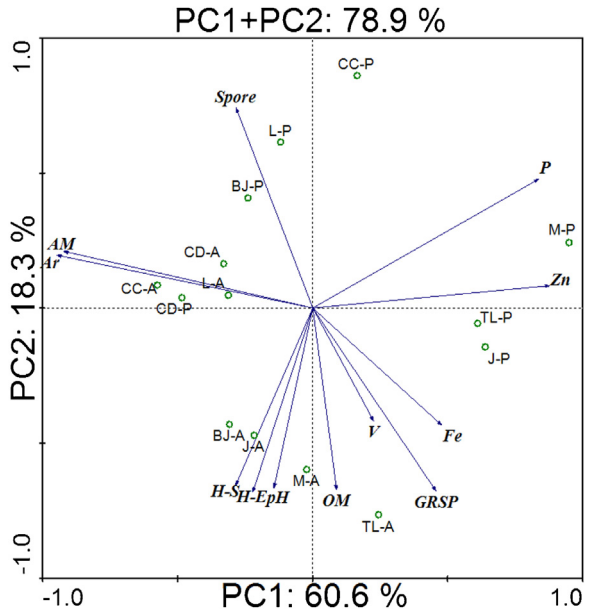

Fig. 5. Principal component analysis (PCA) showing the relation among chemical properties of pristine (-P) and agricultural (A) soils of 7 locations of Buenos Aires Province (Argentina), and mycotrophic capacity (AM: mycorrhizal colonization and Ar: arbuscule content), abundance of AMF spores (Spore), glomaline content (GRSP) and diversity indices from SSCP profiles (H: Shannon-Weaver index; S: DNA of soil; SP: from DNA of spores). Sites references described in Fig2.

agricultural and pristine sites of Buenos Aires Province (Argentina) and for Yoshimura et al. (2013) for soils from China. Also negative association between AMF spore number and soil Fe content confirmed previous studies by Thougnon Islas et al. (2014) under similar conditions. In general, we found some grouping of the sites associated by land use. An exception was the site Coronel Dorrego-pristine, which was grouped with agricultural land use.

4. Conclusions

In this study, we reported for the first time activity and occurrence of AMF species associated with pristine and agricultural land use systems at the Argentinean Pampas. The study describes relationships between soil chemical parameters and activity (colonization, sporulation and glomalin) and diversity of AMF (particularly for some representatives of the genus *Glomus*) indigenous of agricultural aptitude soils from the Buenos Aires Province collected under contrasting land use conditions. We confirmed negative relationships between indigenous AMF and available soil P. In addition, we found negative relationships also among soil Fe and Zn contents with AMF mycotrophic and sporulation abilities but no with glomaline content. Genetic diversity of AMF assessed by the SSCP strategy was not clearly related to chemical soil parameters, but some geographical-spatial association was found; however, this needs future confirmation. Although no expected decreases in activity and AMF diversity in sites under agricultural management was evident, in most sites diversity was separately grouped by land use. It is probably that reduced availability of key nutrients at agricultural soils associated with high nutrient extraction without replacement reduced the soil disturbance effect. High sporulation capacity was found in undisturbed (pristine) soils which could be considered a reserve pool of diversity and activity of indigenous AMF. The diversity of AMF assessed by the SSCP strategy from spore was not as higher as expected than the assessed from soil. This would indicate that in addition to spore, large amount of propagules (probably hyphae) were the main inoculums' source of the AMF. This fact was confirmed by low or no relationship between spore number with the mycotrophic capacity (AMF colonization) and/or glomalin content. Moreover, we confirmed the identity by the PCR-SSCP technique of AMF sequences, which were grouped together with representatives of the genus *Glomus*. Still remains clarify if soil characteristics, resulting from agricultural land use; could exert some selection pressure on the AMF and whether they could be vectors of interest to select potential promoters of plant growth.

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