European Journal of Soil Biology 47 (2011) 230–235

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

Occurrence and diversity of arbuscular mycorrhizal fungi in trap cultures from El Palmar National Park soils

Silvana Velázquez [a,b,*], Marta Cabello [a,c]

[a] Instituto Spegazzini, FCNyM, UNLP Av. 53 N° 477, CP B1900AVJ, La Plata, Argentina
[b] CONICET, Argentina
[c] CICPBA, Argentina

ARTICLE INFO

Article history:
Received 27 December 2010
Received in revised form
27 April 2011
Accepted 4 May 2011
Available online 25 May 2011
Handling editor: Christoph Tebbe

Keywords:
Arbuscular mycorrhizal fungi
Trap culture
El Palmar National Park

ABSTRACT

The objective of this study was to assess and compare the diversity of arbuscular-mycorrhizal fungi (AMF) obtained from five vegetation types—gallery forest, grassland, marsh, palm forest, and scrubland—within El Palmar National Park (Entre Ríos province, Argentina) through trap cultures with soil as the source of inoculum. Three different plant species—*Lolium perenne* L., *Plantago lanceolata* L., and *Trifolium pratense* L.—were used as trap plants. The experiment, conducted for two years under glasshouse conditions, showed that spore number increased during the second year in all the trap cultures from the five vegetation types, with Glomeraceae being most abundant in the last year. A total of 34 morphospecies were identified at the species level (32 morphospecies during the first year and 26 during the second). The species richness and biodiversity index decreased in the second year and were significantly different between the marsh and the palm forest. The soil-based trap culture isolation procedure indicated the presence of Glomeromycota species not registered from field samples: three belonging to the *Acaulospora* genus, one to the *Glomus* genus, and three to the *Gigaspora* genus. The results of this study confirmed the local competiton of Glomeraceae against other Glomeromycota families under glasshouse conditions.

Published by Elsevier Masson SAS.

1. Introduction

Arbuscular mycorrhizae are an intimate associations between 92% of the plant families [41] and fungi of the phylum Glomeromycota [30]. Arbuscular-mycorrhizal fungi (AMF) probably played a significant historical role in the process of land colonization by plants [28]. AMF not only improve the growth of plants by increasing the uptake of available phosphorus and other nonlabile mineral nutrients essential for plant growth from the soil, but also have other beneficial effects such as alleviating the stress caused by biotic and abiotic conditions [32]. In nature, communities of AMF occurring in differing ecosystems consist in different species [26] and exert diverse symbiotic functions, depending on their particular community structure.

Most studies addressing AMF diversity rely on the morphological identification of AMF spores obtained either directly from the field [10,11,19] or from trap cultures [3]. The propagation of cultures of arbuscular fungi requires growth in association with a living universal host plant (*e. g.*, *Plantago lanceolata* L., *Trifolium pratense* L., *Zea mays* L., *Allium porrum* L.) inoculated with field soil in pot cultures in a glasshouse. Cultures of these fungi are necessary to provide living fungal- and mycorrhizal-root material for research and for practical applications. These cultures are also the method of choice for taxonomic research, where they can be used to provide a sufficient number of viable fungal spores from the field soils previously collected [22,39].

This approach using trap cultures does not reveal the same community composition of AMF species as the direct analysis of spores in the field [15,24,25]. This discrepancy has been attributed to selective effects of the trap-plant species [1,15] or to different growth conditions in the glasshouse, including the time period of cultivation [25]. The present study aimed at assessing the AMF diversity obtained in trap cultures through the use of a consortium of host plants over a 24-month period under glasshouse conditions with soils derived from different vegetation types within a protected area, the El Palmar National Park, as the source of inoculum.

* Corresponding author. Instituto Spegazzini, FCNyM, UNLP Av. 53 N° 477, CP B1900AVJ, La Plata, Argentina. Tel./fax: +54 0221 4219845.
E-mail address: mariasvelazquez@yahoo.com.ar (S. Velázquez).

1164-5563/$ – see front matter Published by Elsevier Masson SAS.
doi:10.1016/j.ejsobi.2011.05.002

2. Methods

2.1. Study site and sampling

Soil was collected in June 2006 from five types of vegetation located in El Palmar National Park, Entre Ríos province, Argentina (31° 50′ S, 58° 17′ W). The climate is temperate with a mean annual temperature of 18.9 °C, a mean annual rainfall of about 1300 mm, and a water deficit frequently occurring during the summertime [14]. The landscape of the park consists in a mosaic of vegetation types; including forests along the rivers and streams, tall grassland on humid alluvial plains, xeric steppes on the sandy outcrops, and scrublands and palm savannahs dominated by *Butia yatay* (Mart.) Becc., an endangered species [8], on the uplands. On the basis of ecophysiological and floristic characteristics, areas with five different vegetation types had been previously identified at that park: a gallery forest (GF), a closed forest frequently flooded along permanent streams and rivers; a grassland (GRA), an area with short grass up to 50 cm tall on sandy soils as vegetation; a marsh (MAR), comprised of tall grasses and sedges on intermittent streams and ponds; a palm forest (PF) with a savanna-like physiognomy containing tall (>12 m height) *B. yatay* palms and sparse trees; and a scrubland (SCR) characterized by open vegetation with a continuous cover of shrubs up to 3 m tall [37].

The sampling design consisted in three replicate samples from each vegetation type. Soil samples were collected by using a composite-random (*i. e.*, serpentine [9]) method of sampling. In those places where each sample was collected, 5 to 6 subsamples were pooled within a square area of *ca.* 3 m^2. Thus, within each site, three representative soil samples (*i. e.*, 15 samples: 5 vegetation types × 3 replicates) were collected and stored in resealable plastic bags for transport to the laboratory, where they were kept under refrigeration at 4 °C until processed.

2.2. Glasshouse study

The experiments were conducted at the Institute Spegazzini, Facultad de Ciencias Naturales y Museo (UNLP), La Plata, Argentina.

Seeds of *Lolium perenne* L., *P. lanceolata* L., and *T. pratense* L. were surface-sterilized (15 min with a 10% [w/v] sodium hypochlorite solution), germinated in autoclaved substrate composed of a mixture of perlite:vermiculite (1:1, w/w), and maintained in an environmentally controlled room at constant moisture and temperatures between 24 ± 1 °C in the daytime and 20 ± 1 °C at night in a 16 h photoperiod provided by incandescent and cool-white lamps until 10 days after shoot emergence.

Trap-culture methodology [25] was used in this study. The AMF inoculum consisted in 180 g of field soil (20 g per trap plant at nine plants per pot) containing resting spores of the fungus, the fungal hyphae in the soil, and root fragments [31]. The inocula were placed in 27 × 17 × 20-cm (length × width × height) pots containing a tyndallized substrate (1 h at 120 °C repeated three times after a 24-h interval) composed of soil:vermiculite (3:1 v/v). Each pot then received a transplant consisting in three *L. perenne*, three *P. lanceolata*, and three *T. pratense* plants per pot. Each transplantation—corresponding to a particular vegetation type—was performed in triplicate (n = nine pots per vegetation type, a total of 45 pot cultures). The plants were grown in a glasshouse for 24 months with the light and temperature controlled as described above, were watered from below by a capillary system, and fed with the nutrient solution: $MgSO_4 \cdot 7H_2O$, 0.75 mM; $NaNO_3$, 1 mM; K_2SO_4, 1 mM; $CaCl_2 \cdot 2H_2O$, 2 mM; $Na_2HPO_4 \cdot 12H_2O$, 3.2 μM; FeNa-EDTA, 0.025 mM; $MnSO_4 \cdot 4H_2O$, 5 μM; $CuSO_4 \cdot 5H_2O$, 0.25 μM; $ZnSO_4 \cdot 7H_2O$, 0.5 μM; H_3BO_3, 0.025 mM, $NaMoO_4 \cdot 2H_2O$, 0.1 μM [7].

2.3. Sampling in the trap cultures

Every two months for two years, 100 g (dry weight) of substrate samples were taken with the aid of a 15-cm^3 core for AMF-spore extraction and identification. After sampling, the holes in the pots were refilled directly with the same sterile substrate used in the experiment.

2.4. AMF spore isolation and identification

The AMF communities in the GF, GRA, MAR, PF, and SCR trap cultures were monitored by spore extraction: 100 g (dry weight) of soil for each sample was wet-sieved and decanted [13] and the supernatant centrifuged in a sucrose gradient [40]. For taxonomic identification, fungal spores were mounted in either polyvinyl-lactic acid–glycerine (PVLG) [16] or PVLG mixed 1:1 (v/v) with Melzer's reagent [6] and examined under a light microscope (Leitz Dialux 20EB, 60×, 250×, 400×, and 1000× magnifications). The specimens obtained were identified following current species descriptions and identification manuals (INVAM: http://invam.caf.wvu.edu; Szczecin University: http://agro.ar.szczecin.pl/~jblaszkowski/index.html). Vouchers were deposited in the Herbarium at the Spegazzini Institute (LPS), La Plata, Argentina.

Soil samples from each pot for each site were screened for AMF spores and the following calculations made: (i) the spore abundance, given as the number of spores of the particular species in a sample; (ii) the spore number, defined as total number of spores found in 100 g dry weight of soil; (iii) the AMF-species richness, measured as the total number of different AMF species occurring in 100 g dry weight of soil; and (iv) the Shannon diversity index (H'). This last parameter was calculated by the equation $H' = -\sum p_i \ln p_i$ where p_i is the relative abundance of the ith species compared with all species identified in a sample [20].

2.5. Statistical analysis

The data for spore number, species richness, and the diversity index were analyzed by means of the one-way analysis of variance

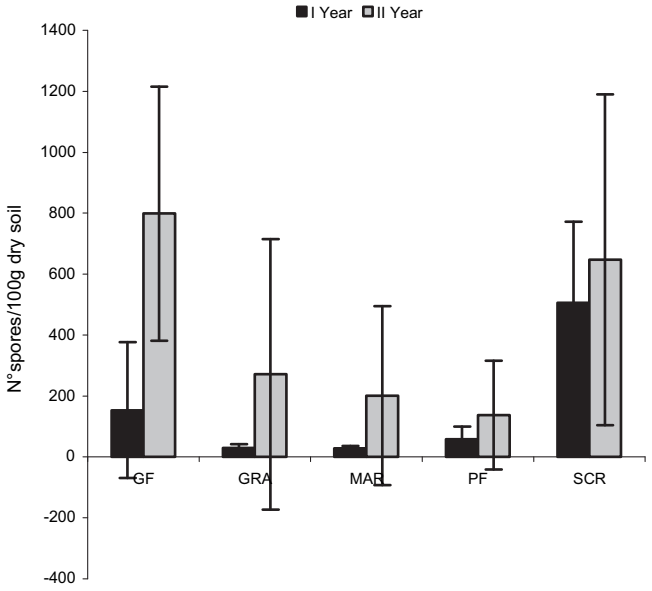

Fig. 1. AMF spore number (means n = 9 and SD) for two year in the trap cultures. GF: gallery forest, GRA: grassland, MAR: marsh, PF: palm forest, and SCR: scrubland. Significant differences were not observed according to Fisher's test (P = 0.01).

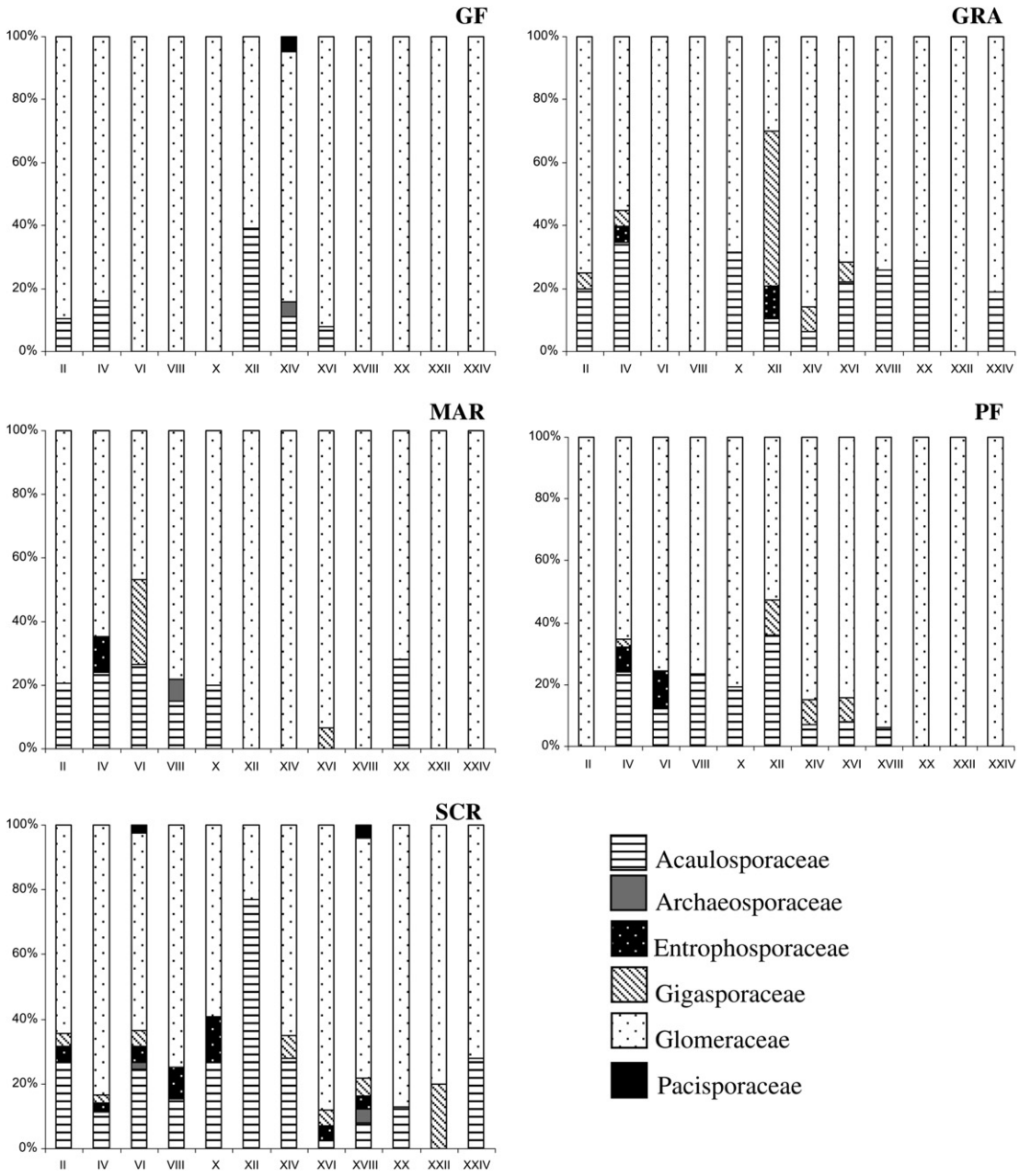

Fig. 2. Number of spores (%) (means $n = 9$) for the members of families Acaulosporaceae, Archaeosporaceae, Entrophosporaceae, Gigasporaceae, Glomeraceae, and Pacisporaceae obtained at two year in the trap cultures. GF, GRA, MAR, PF, and SCR. Some notations as in Fig. 1.

(ANOVA). The Fisher test (LSD) was applied *a posteriori* to locate the differences in treatment among the means [33]. All data were analyzed using InfoStat version 1.1. The percentages of each family of Glomeromycota in trap cultures were transformed used: [($\log_2$ spore number) + 1]. Multivariate analyses were used in order to analyze the AMF-spore composition present in the trap cultures. A Principal Component Analysis (PCA) was carried out with the spore number for each year from the five vegetation types. The PCA was performed with Multivariate Statistical Package (MVSP 3.1).

3. Results

3.1. AMF spore number

The spore number recorded in 100 g of soil was higher in the second year for all sites analyzed (Fig. 1): in the first year, the spore number decreased within the different soil samples in the order SCR > GF > PF > MAR > GRA, while in the second year the order of decreased spore number was GF > SCR > GRA > MAR > PF.

3.2. AMF families in the trap cultures

The percentages of spores belonging to the Acaulosporaceae, Archaeosporaceae, Entrophosporaceae, Gigasporaceae, Glomeraceae, and Pacisporaceae during the two years in the trap cultures were calculated (Fig. 2).

The number of AMF families detected in the pot cultures was always higher during the first year. The percentages of spores belonging to Acaulosporaceae and Glomeraceae were the highest in all the pots; followed by Gigasporaceae, Entrophosporaceae, Archaeosporaceae, and Pacisporaceae in respective decreasing order of abundance.

In the second year the number of Glomeraceae spores increased; while those of the other families decreased—except in the pots with inocula from the marsh, where the number of spores belonging to both the Glomeraceae and Acaulosporaceae families increased.

3.3. Abundance of AMF spores in the trap cultures

A total of 34 AMF morphotaxa were identified at a both generic and species level when possible. Thirteen taxa from *Acaulospora*; ten taxa from *Glomus*; five taxa from *Scutellospora*; three taxa from *Gigaspora*; and one taxon each from *Archaeospora*, *Entrophospora,* and *Pacispora* were recorded. Of the 34 total morphospecies, 32 were recovered in the first year and 26 in the second (94% and 76%, respectively; Table 1).

Spores of *Glomus claroideum, Glomus etunicatum, Glomus microaggregatum*, and *Glomus* sp. were found in all the pots tested during the two years of study; whereas those of *Acaulospora elegans, Acaulospora foveata, Acaulospora spinosa, Acaulospora* sp. 2, *Gigaspora rosea, Glomus constrictum, Scutellospora dipapillosa*, and *Scutellospora* sp. 2 were found only during the first year. During the second year we furthermore recovered two (*Glomus coronatum* and *Glomus intraradices*), species that had not been found initially.

Fig. 3 shows the PCA analysis. The first and second axes of the PCA analysis accounted for 84.3% of the variance. The trap cultures from SCR I, SCR II, and GF I were separated from all the other vegetation types (GF II, GRA I-II, MAR I-II, PF I-II, and SCR I). *Glomus clarum* and *G. etunicatum* were more abundant in the former three and contributed to that grouping. Temporal and vegetation-type effects were not observed.

3.4. AMF-species diversity in the pot cultures

The number of AMF species and the Shannon diversity index (H') in the trap cultures established from the different vegetation types from El Palmar National Park at the end of two years of cultivation were calculated (Fig. 4).

The highest values of species richness and AMF diversity, expressed by the Shannon index, were found in the first year of cultivation in all instances. In the pot cultures with the inocula belonging to the MAR and the PF these differences were significant ($P < 0.05$).

4. Discussion

The effect of cultivation of a consortium of three different trap-plant species on AMF sporulation and diversity was studied with trap-cultures incubated in the glasshouse over a period of two years. Several authors indicated that isolates from trap cultures allowed a recovery of most species of Glomeromycota that had previously been identified from field-collected spores [2,21,23,34]. In this study, the total spore number in all soil environments was higher in the second year of culture, an increase that would be expected since the number of spores includes both newly formed structures and spores remaining from previous period.

This study also demonstrates differences between the two years of cultivation with respect to the relative contribution of different Glomeromycota families. During the first year the Acaulosporaceae and Glomeraceae account for the largest contribution (*e. g.*, 67%), followed by the Gigasporaceae (23%) and, at a much lower representation, the Archaeosporaceae, the Entrophosporaceae, and the

Table 1
Spore abundance ($n = 9$) from each of the arbuscular-micorrhizal fungi (AMF) species distinguished for the two years in the trap cultures. GF, GRA, MAR, PF, and SCR. Some notations as in Fig. 1. Species are ordered according their abundance.

	First year					Second year				
	GF	GRA	MAR	PF	SCR	GF	GRA	MAR	PF	SCR
Glomus etunicatum	20.8	3.7	8.3	32.4	366.2	457.4	170.3	78.3	87.5	405.4
Acaulospora delicata	0.7	2.5	0.5	1.2	55.7	0.5	0.5	–	0.2	0.3
Glomus clarum	111.1	0.6	3.9	0.2	33.2	53.9	0.7	–	22.9	229.9
Glomus sp.	17.8	15.2	11.6	15.7	19.6	7.6	7.8	6	9.4	3.7
Glomus claroideum	0.5	0.1	0.1	0.7	7.6	211.1	116.2	8.4	41.4	15
Glomus microaggregatum	0.6	0.8	0.7	2.3	3.7	0.3	0.4	0.2	0.2	0.2
Glomus mosseae	0.2	0.5	0.1	1.7	1.6	3.1	0.2	0.4	–	4.7
Acaulospora dilatata	0.2	1.6	0.5	1.2	0.7	1	–	46.8	–	–
Acaulospora entreriana	0.2	–	–	0.2	12.6	–	0.4	–	–	4.7
Entrophospora infrequens	–	0.8	0.7	0.7	1.4	–	–	–	–	0.5
Acaulospora sp. 2	0.8	0.4	–	0.5	0.2	–	–	–	–	–
Acaulospora scrobiculata	–	–	0.5	0.2	0.2	0.2	–	–	–	–
Acaulospora mellea	–	–	0.2	0.1	0.2	01	0.3	–	–	0.65
Acaulospora bireticulata	–	–	0.6	–	0.2	–	–	–	–	0.2
Archaeospora sp.	–	–	0.3	–	0.2	0.2	–	–	0.2	0.2
Scutellospora dipapillosa	–	–	0.1	–	0.2	–	–	–	–	–
Scutellospora sp. 2	–	–	–	0.2	0.7	–	–	–	–	–
Scutellospora biornata	–	0.3	–	–	0.1	–	–	0.1	–	–
Gigaspora sp. 2	–	0.6	–	0.2	–	–	0.6	–	0.2	0.6
Scutellospora gilmorei	–	0.6	–	0.1	–	–	–	–	–	0.5
Gigaspora sp. 1	–	–	–	–	0.2	–	–	–	–	0.2
Acaulospora spinosa	–	–	–	–	0.2	–	–	–	–	–
Glomus constrictum	–	–	–	–	0.2	–	–	–	–	–
Pacispora sp. 2	–	–	–	–	0.2	0.2	–	–	–	0.2
Acaulospora rhemii	–	0.6	–	–	–	–	–	–	0.2	–
Glomus tortuosum	–	0.4	–	–	–	–	0.2	–	–	0.2
Acaulospora laevis	–	0.2	–	–	–	–	0.3	–	0.2	–
Acaulospora foveata	–	0.2	–	–	–	–	–	–	–	–
Scutellospora sp. 1	–	0.2	–	–	–	–	–	–	0.2	–
Acaulospora sp. 1	0.2	–	–	–	–	–	–	–	–	3
Glomus intraradices	–	–	–	–	–	120.3	–	–	–	–
Glomus coronatum	–	–	–	–	–	–	–	–	–	4.9
Acaulospora elegans	–	–	–	–	0.1	–	–	–	–	–
Gigaspora rosea	–	–	–	–	0.1	–	–	–	–	–

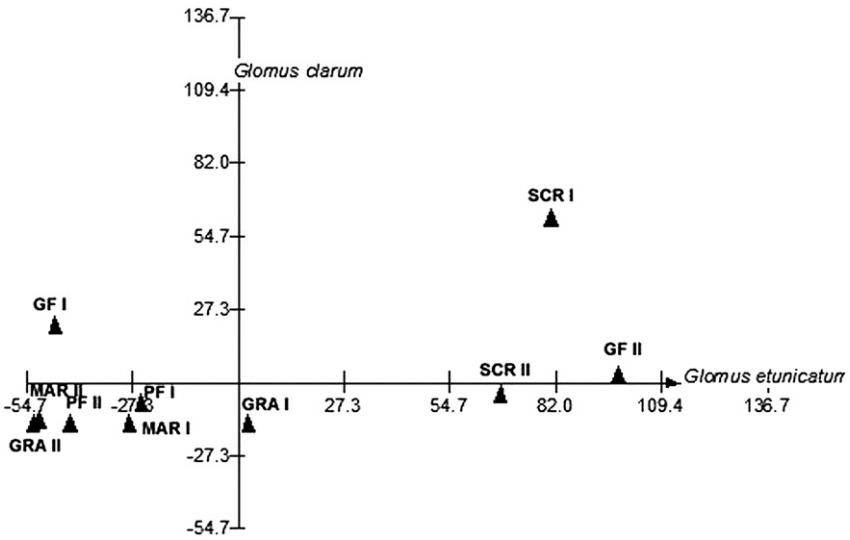

Fig. 3. Principal component analysis (PCA) of the two years in the trap cultures. GF, GRA, MAR, PF, and SCR. Some notations as in Fig. 1.

Pacisporaceae (combined total, 10%). The Glomeraceae, however, become dominant (97%) during the second year of the trap cultures in all pots, while the rest of the families show total spore counts of lower than 3%.

The fungi that sporulate early in a trap culture could potentially be representative of the *r* strategy, where they dominate resource-rich uncolonized habitats in early successive stages of the fungal community [27]. By contrast, a *k* strategist would follow the opposite tactic, characterized by a slow growth under resource-limited conditions and an appearance in the late successive stages. In this experiment, the species of the *Glomus* (considered an *r* strategist) dominated the trap culture during the second year. Other studies indicated that soil-to-trap-culture isolation procedures apparently select the most competitive AMF, or those fungi that can best adapt to the experimental conditions of plant and fungal growth, *e. g.*, chemical fertilization [6,31].

AMF species require different lengths of time to sporulate [12]. Some members of the Glomeromycota are capable of producing spores very early—*i. e.*, from 3 to 4 weeks after the primary root colonization—whereas others require more than 6 months to begin the process [29,31]. *G. etunicatum*, *G. clarum*, and *G. claroideum* were present in all the trap cultures with soil based inoculum from the El Palmar National Park. The higher proportion of *Glomus* species found with soil as the source of inoculum in the present study agrees with previous reports [5,31].

In this investigation the majority of the trap cultures started with soil were dominated by *G. etunicatum* and *G. clarum* throughout the 2 years. *Glomus* species have been seen to be the first to sporulate in soils [25], and were often encountered in arable lands [4,15,17,18,35]. In contrast, in the second year of the trap cultures a decrease in the number of *Acaulospora* and *Gigaspora* species were found. Others reports, however, had indicated that *Acaulospora* species mainly sporulated during the second year of the vegetation period, whereas species of the genus *Scutellospora* sporulated in both years of trap-culturing [23].

In a previous study in El Palmar National Park, 46 AMF morphospecies from field-collected spores were identified [37]. In the present work, trap cultures allowed the recovery of 34 morphospecies, from which seven morphospecies (12%) had not been previously encountered in field samples. Among those newly identified morphospecies, three Acaulosporaceae: *Acaulospora entreriana, A. elegans*, and *Acaulospora* sp. 2 [38]; one Glomeraceae: *Glomus tortuosum*; and three Gigasporaceae: *G. rosea*, *Gigaspora* sp. 1, and *Scutellospora* sp. 2 were identified though the use of the trap culture. Thus, certain AMF that may play a significant role in the natural communities studied cannot be detected by the field-sampling method.

The diversity index and species-richness values evinced a variation over the two years of culture. Higher values in species richness and diversity, as calculated by the Shannon index, were

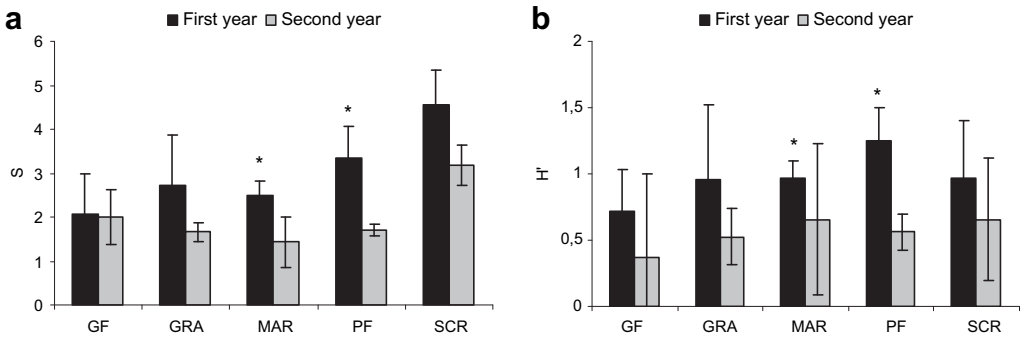

Fig. 4. a) Number of AMF species (S) found in trap cultures. b) Shannon diversity index (*H'*) (mean ± and SD of nine replicates plotted per site). GF, GRA, MAR, PF, and SCR. Some notations as in Fig. 1. Asterix (*) denotes significant differences between two years according to ANOVA and Fisher's LSD test ($P \leq 0.05$).

observed in the first year. Over the two years of trap cultivation, 34 Glomeromycota morphospecies were recovered—32 morphotaxa (94%) in the first year and 26 (76%) in the second. This finding indicated an 18% decrease in the species richness. In contrast, other research had reported a 50% increase in the species registered during the second year [23], or a 25% increase in that same parameter at the end of the first year of culture [34].

In addition, in samples from field soils, a strong AMF-community differential response to different vegetation types in the Park was found [36]. This observation is consistent with the existence of different edaphic conditions and plant-community compositions. In that survey, Gigasporaceae were found to be abundant in PF and GRA in response to a high content of sand. The *Glomus* species were, nevertheless, found to be significantly abundant in and exhibited a high correlation with clay in field samples from the GF, MAR, and SCR. Nevertheless in the present study, the PCA analysis indicated the absence of temporal or vegetation-type effects. This result can be attributed to two main causes: first the homogeneity of the substrate and second the constancy of the glasshouse conditions throughout the entire course of the experiment.

The detection of differences in the mycorrhizal communities among the five vegetation types in the El Palmar National Park investigated here constitutes a significant documentation of the biological variability that has occurred in this protected area. The study has also provided a consequental initial approach in the characterization and conservation of the germplasm of this relevant group of soil microorganisms. In conclusion, among the five different AMF communities, the most diverse was PF, therefore, this vegetation type warrants a priority both in future studies and as a vegetation type to be selected for protection outside the Park.

Acknowledgments

The authors are grateful to Dr. Donald F. Haggerty, a retired career investigator and native English speaker, for editing the final version of the manuscript. Silvana Velázquez is recipient of a scholarship from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Marta Cabello is a researcher from Comisión de Investigaciones Científicas de la provincia de Buenos Aires (CICPBA). This research was supported by grants from UNLP (11/N527) Project, CICPBA, Agencia de Promoción Científica y Tecnológica (PICT 2007-01233). We wish to thank that the Editor Christoph Tebbe and two reviewers for their efforts on the expert reviewer page.

References

[1] E.M. Ahulu, A. Gollote, V. Gianinazzi-Pearson, M. Nonaka, Coocurring plants forming distinct arbuscular mycorrhizal morphologies harbor similar AM fungal species, Mycorrhiza 17 (2006) 37–49.
[2] Z.-Q. An, J.W. Hendrix, D.E. Hershman, G.T. Henson, Evaluation of the «most probable number» (MPN) and wet-sieving methods for determinig soil-borne populations of endogonaceous mycorrhizal fungi, Mycologia 82 (1990) 576–581.
[3] J.D. Bever, J.B. Morton, J. Antonovics, P.A. Schultz, Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland a mown grassland, J. Ecol. 84 (1996) 71–82.
[4] J. Blaszkowski, Comparative studies on the occurrence of arbuscular fungi and mycorrhizae (Glomales) in cultivated and uncultivated soils of Polan, Acta Mycologica 28 (1993) 93–140.
[5] M.C. Brundrett, L.K. Abbot, D.A. Jasper, Glomalean fungi from tropical Australia I. Comparison of the effectiveness of isolation procedures, Mycorrhiza 8 (1999) 305–314.
[6] M.C. Brundrett, L. Melville, R.L. Peterson, Practical Methods in Mycorrhizal Research. Mycologue Publications, Waterloo, Ontario, 1994, pp. 161.
[7] M.N. Cabello, Hydrocarbon pollution: its effect on native arbuscular mycorrhizal fungi (AMF), FEMS Microbiol. Ecol. 22 (1997) 233–236.
[8] J.C. Chebez, Los que se van. Especies Argentinas en peligro. Albatros, Buenos Aires, Argentina, 1994.
[9] R.P. Dick, D.R. Thomas, R.P. Turco, Standarize methods, sampling, and sample treatment. in: J.W. Doran, A.J. Jones (Eds.), Methods for Assessing Soil Quality. Soil Science Society of America, Madison, W.L, 1996, pp. 107–121.
[10] D.D. Douds, P. Millner, Biodiversity of arbuscular mycorrhizal fungi in agroecosystems, Agric. Ecosyst. Environ. 74 (1999) 77–93.
[11] J.P. Gai, P. Christie, G. Feng, X.L. Li, Twenty years of research on biodiversity and distribution of arbuscular mycorrhizal fungi in China: a review, Mycorrhiza 16 (2006) 229–239.
[12] C. Gazey, L.K. Abbot, A.D. Robson, The rate of development of mycorrhizas affects the onset of sporulation and production of external hyphae by two species of *Acaulospora*, Mycol. Res. 96 (1992) 643–650.
[13] J.W. Gerdemann, T.H. Nicolson, Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting, Trans. Br. Mycol. Soc. 46 (1963) 235–244.
[14] L. Goveto, Ocurrencia histórica de fuegos en la sabana del Parque Nacional El Palmar: evidencias climáticas y florísticas. Escuela para graduados Alberto Soriano. Facultad de Agronomía, Universidad de Buenos Aires, 2005, pp. 105.
[15] J. Jansa, A. Mozafar, T. Anken, R. Ruh, I.R. Sanders, E. Frossard, Diversity and structure of AMF communities as affected by tillage in a temperate soil, Mycorrhiza 12 (2002) 225–234.
[16] R.E. Koske, B. Tessier, A convenient, permanent slide mounting medium, Mycol. Soc. Am. Newsl. 34 (1983) 1–59.
[17] J.E. Kurle, F.L. Pfleger, Management influences on arbuscular mycorrhizal fungal species composition in a corn-soybean rotation, Agron. J. 88 (1996) 155–161.
[18] S. Land, H. von Alten, F. Schönbeck, The influence of host plant, nitrogen fertilization and fungicide application on the abundance and seasonal dynamics of vesicular-arbuscular mycorrhizal fungi in arable soils of northern Germany, Mycorrhiza 2 (1993) 157–166.
[19] F.C. Landis, A. Gargas, T.J. Givnish, Relationship among arbuscular mycorrhizal fungi, vascular plants and environmental conditions in oak savannas, New Phytol. 164 (2004) 493–504.
[20] A.E. Magurran, Ecological Diversity and Its Measurement. Croom Helm, London, 1988, pp. 160.
[21] D.D. Miller, P.A. Domoto, C. Walker, Mycorrhizal fungi at eighteen apple rootstock plantings in the United States, New Phytol. 100 (1985) 379–391.
[22] J.B. Morton, Problems and solution for integration of glomalean taxonomy, systematic biology, ansd the study of endomycorrhizal phenomena, Mycorrhiza 2 (1992) 97–109.
[23] F. Oehl, E. Sieverding, P. Mäder, D. Dubois, K. Ineichen, T. Boller, A. Wiemken, Impact of long-term conventional and organic farming on the diversity of arbsucular mycorrhizal fungi, Oecologia 138 (2004) 574–583.
[24] F. Oehl, E. Sieverding, K. Ineichen, P. Mäder, T. Boller, A. Wiemken, Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe, Appl. Environ. Microbiol. 69 (2003) 2816–2824.
[25] F. Oehl, E. Sieverding, K. Ineichen, E.A. Ris, T. Boller, A. Wiemken, Community structure of arbuscular mycorrhizal fungi at different soil dephts in extensively and intensively managed agroecosystems, New Phytol. 165 (2003) 273–283.
[26] M. Ôpik, M. Moora, J. Liira, M. Zobel, Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems Argun the globe, J. Ecol. 94 (2006) 778–790.
[27] E. Pianka, R-selection and K-selection, Am. Nat. 101 (1970) 592–597.
[28] K.A. Pyrozynsky, D.W. Malloch, The origin of land plants: a matter of mycotropism, Biosystems 6 (1975) 153–164.
[29] S. Schalamuk, M. Cabello, Arbuscular mycorrhizal fungal propagules from tillage and non-tillage systems: possible effects on Glomeromycota diversity, Mycologia 102 (2010) 261–268.
[30] A. Schüssler, D. Schwarzott, C. Walker, A new fungal phylum, the Glomeromycota: phylogeny and evolution, Mycol. Res. 105 (2001) 1413–1421.
[31] E. Sieverding, Vesicular-arbuscular Micorrhizal Management in Tropical Agrosystems. Deutche Gesellschaft für Technische Zusammenarbeit, GTZ N° 224 Eschborn (1991) pp. 371.
[32] S.E. Smith, D.J. Read, Mycorrhizal Symbiosis, third ed. Academic, London, 2008, pp. 769.
[33] R.R. Sokal, F.J. Rohlf, Biometry. Freeman & Company, New York, 1998, pp. 507.
[34] J.C. Stutz, J.B. Morton, Successive pot cultures reveal high species richness of arbuscular endomycorrhizal fungi in arid ecosystems, Can. J. Bot. 74 (1996) 1883–1889.
[35] N.C. Talukdar, J.J. Germida, Occurrence and isolation of vesicular-arbuscular mycorrhizae in cropped field soils of Saskatchewan, Canada, Can. J. Microbiol. 39 (1993) 567–575.
[36] M.S. Velázquez. Communities of Arbuscular Micorrhizal Fungi from El Palmar National Park [Doctoral Dissertation]. La Plata, Argentina. Universidad Nacional La Plata, 2010, pp. 157.
[37] M.S. Velázquez, F. Biganzoli, M.N. Cabello, Arbuscular micorrhizal fungi in El Palmar National Park (Entre Rios Province, Argentina) – a protected reserve, Sydowia (2010) 149–163.
[38] M.S. Velázquez, M. Cabello, G. Irrazabal, A. Godeas, Acaulosporaceae from El Palmar National Park, Entre Ríos, Argentina, Mycotaxon 103 (2008) 171–187.
[39] C. Walker, Systematics and taxonomy of the arbuscular mycorrhizal fungi, Agronomie 12 (1992) 887–897.
[40] C. Walker, W. Mize, H.S. McNabb, Populations of endogonaceous fungi at two populations in central Iowa, Can. J. Bot. 60 (1982) 2518–2529.
[41] B. Wang, Y.-L. Qiu, Phylogenetic distribution and evolution of mycorrhizas in lands plants, Mycorrhiza 16 (2006) 299–363.