Research Paper

Arbuscular mycorrhizal fungi in saline soils: Vertical distribution at different soil depth

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Abstract

Arbuscular mycorrhizal fungi (AMF) colonize land plants in every ecosystem, even extreme conditions such as saline soils. In the present work we report for the first time the mycorrhizal status and the vertical fungal distribution of AMF spores present in the rhizospheric soil samples of four species of Chenopodiaceae (*Allenrolfea patagonica*, *Atriplex argentina*, *Heterostachys ritteriana* and *Suaeda divaricata*) at five different depths in two saline of central Argentina. Roots showed medium, low or no colonization (0-50%). Nineteen morphologically distinctive AMF species were recovered. The number of AMF spores ranged between 3 and 1162 per 100 g dry soil, and AMF spore number decreased as depth increased at both sites. The highest spore number was recorded in the upper soil depth (0-10 cm) and in *S. divaricata*. Depending of the host plant, some AMF species sporulated mainly in the deep soil layers (*Glomus magnicaule* in *Allenrolfea patagonica*, *Septoglomus* aff. *constrictum* in *Atriplex argentina*), others mainly in the top layers (*G. brohultti* in *Atriplex argentina* and *Septoglomus* aff. *constrictum* in *Allenrolfea patagonica*). Although the low percentages of colonization or lack of it, our results show a moderate diversity of AMF associated to the species of Chenopodiaceae investigated in this study. The taxonomical diversity reveals that AMF are adapted to extreme environmental conditions from saline soils of central Argentina.

Key words: arbuscular mycorrhiza, saline environments, soil profile, vertical distribution, mycorrhizal status.

Introduction

In central Argentina 9% of the area is occupied by halophytic vegetation (Cabido and Zak, 1999). This type of vegetation grows in habitats that are rare worldwide, since only approximately 7% of the global land surface is covered with saline habitats (Ruíz-Lozano and Azcón, 2000). Central Argentina presents some conspicuous salt flats: the Salinas Grandes and the Salinas de Ambargasta, which together occupies an area of approximately 600,000 hectares. The environmental isolation, the harsh climatic conditions, the characteristics of marginal lands for agriculture and livestock and no stable human population within it, have facilitated the preservation of this pristine ecosystem. Within these saline habitats, the distribution patterns of plant communities are defined by the salt gradient, with plant cover inversely proportional to the presence of salt. At sites where plant life is still possible, the most characteristic plant community is the halophytic shrub or jumeal, composed of species of the Chenopodiaceae family (Cabido and Zak, 1999).

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According to Juniper and Abbott (1993), high salinity in soils has adverse effects on plant colonization by arbuscular mycorrhizal fungi (AMF). However, there are reports in the literature from all over the world that plants of salt marshes can be colonized by AMF (Hildebrandt *et al.*, 2001; Juniper and Abbott, 1993; Landwehr *et al.*, 2002; Smith and Read, 2008; Wang *et al.*, 2004). Even in families that are generally considered non-mycorrhizal, such as Chenopodiaceae (Gerdemann, 1968; Hirrel *et al.*, 1978; Mohankumar and Mahadevan, 1987; Peterson *et al.*, 1985), the most salt-tolerant *Salicornia* sp. and *Suaeda maritima* can be colonized (Kim and Weber, 1985; Rozema *et al.*, 1986; Sengupta and Chaudhuri, 1990).

Ecological studies on the community structure of AMF are generally restricted to the top 20 cm of soil, where most of the root biomass is concentrated (Brundrett, 1991). Only a few studies included the subsoil. Mycorrhizal colonization (Jakobsen and Nielsen, 1983; Rillig and Field, 2003), infective propagules (Ann *et al.*, 1990), extra-radical mycelium (Kabir *et al.*, 1998) and AMF spores (Oehl *et al.*, 2005) decrease with increasing soil depth. Few studies have documented what happens with AMF diversity along soil profile. Cooke *et al.* (1993), Oehl *et al.* (2005), Cuenca and Lovera (2010) and Wang *et al.* (2004) have published the species diversity and distribution across the soil profile in salt marsh grasses in the United States, in cultivated soils of Central Europe, tropical soils of Venezuela and in the Yellow River Delta of China, respectively. Until now, nothing has been reported about vertical distribution of AMF communities in natural saline soils of Central Argentina.

In the present work we report for the first time the mycorrhizal status and the vertical fungal spores in four species of Chenopodiaceae (*Allenrolfea patagonica* (Moq.) Kuntze, *Atriplex argentina* Speg., *Heterostachys ritteriana* (Moq.) Moq. and *Suaeda divaricata* Moq.) in two saline soil of central Argentina. Species of Chenopodiaceae are the only plants able to growth in such extremophilus conditions and in Argentina ecosystems have not been previously examined for AMF presence.

Materials and Methods

Study area and sample collection

The study was conducted in two saline "Salinas de Ambargasta" -SA- (64º18' W, 29º27' S) and "Salinas Grandes" -SG- (64º31' W, 29º44' S), in the north of Córdoba Province, central Argentina. The climate in both sites is dry and warm, with a mean annual precipitation below 500 mm and mean temperature of 19.9 °C. The highest areas (170 masl, with low salt concentration) are occupied by a xerophytic forest of *Aspidosperma quebracho-blanco* Schltdl., *Prosopis flexuosa* DC., *Cercidium australe* Johnst., *Mimozyganthus carinatus* (Grisseb.) Bukart, *Ziziphus mistol* Griseb., *Prosopis torquata* (Cav. ex Lag.) DC., and *Stetsonia coryne* (Salm-Dyck) Britton & Rose;

the understory vegetation is represented by *Larrea divaricata* Cav. and some halophytes.

Our studied area, the edge of the salt flat, shows heavy constraints to the developing of any type of plant cover, being extremely open and scarce with the only presence of four species adapted to harsh environment: *Allenrolfea patagonica* (Moq.) Kuntze, *Atriplex argentina* Speg., *Heterostachys ritteriana* (Moq.) Moq. and *Suaeda divaricata* Moq. (Cabido and Zak, 1999; Cabido *et al.*, 2006). Sampling was made in an area of approximately 50 x 50 m in the two sites in end of the growing season (March, Summer) because during this period the plants present their full splendor (with flowers). Soil samples were randomly and carefully taken with a metal corer (3 cm of diameter) from under the canopy of five plants of each species to confirm connection between roots and shoots. The five soil samples per species were considered replicates.

Samples were collected from 0 to more than 40 cm in depth, at 10-cm intervals (at 0-10, 10-20, 20-30, 30-40, and 40-50 cm depths) in each site. Samples from each layer ($620 cm^3$ soil volume) of each replicate were placed in individual plastic bags and stored at 4 °C.

To characterize the soil from each site, four soil samples per depth level were taken and the following parameters were measured: electrical conductivity (mmhos/cm), extractable P determined with the method of Bray and Kurtz I (Jackson, 1964), pH in water (1:2.5), organic matter content (Nelson and Sommers, 1982), carbon: nitrogen ratio and soil texture. Total nitrogen was determined using the micro-Kjeldhal method (Bremner and Mulvaney, 1982).

AMF colonization and spores

Fresh roots were rinsed with water, cleared with 10% KOH (15 min at 90 °C) and bleached with 30% H_2O_2 (10 min, room temperature), acidified with 1% HCl (1 min, room temperature) and stained for 5 min in 0.05% trypan blue (Phillips and Hayman, 1970). To confirm mycorrhizal structure, in a second stage we then mounted the roots on glass slides for examination under a Kyowa 4-100x microscopic. The presence of arbuscules, vesicles, hyphal coils and intra- and intercellular hyphae without septa were used to designate AM associations. Quantification of AM root colonization was estimated visually as the proportion of the root which was colonized and characterized using five classes: very high (> 80%), high (60-79%), medium (40-59%), low (20-39%), and very low (1-19%), following Zangaro *et al.* (2002).

AMF spores were extracted from 100 g (dry weight) of each soil sample by wet sieving and decanting (Gerdemann and Nicolson, 1963), and the supernatant was centrifuged in a sucrose gradient (Walker *et al.*, 1982). The procedure included passage through 500-, 125-, and 38-μm sieves. The 500-μm sieve was checked for large spores, spore clusters, and sporocarps. The contents of the 125- and

38-µm sieves were layered onto a water-sucrose solution (70% [wt/vol]) gradient and centrifuged at 900 x *g* for 2 min. Only apparently healthy spores (those that contained cytoplasm, with no collapsed surface and no evidence of parasitism) were counted under stereomicroscope directly.

For taxonomic identification, fungal spores and sporocarps were mounted onto slides using PVA (polyvinyl alcohol) with and without Melzer reagent (Omar *et al.*, 1979) and examined with a compound microscope. AMF species were identified following original species descriptions and those presented by INVAM (International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi), Szcezecin University and Redecker *et al.* (2013). Vouchers were deposited in the Herbarium at the Spegazzini Institute (LPS), La Plata, Argentina.

Spore number and AMF species richness in different soil layers were expressed as follows: total number of spores and number of AMF species in 100 g dry soil. Soil moisture content was calculated for each soil sample as percent oven-dry weight of soil by drying at 80 °C for 48 h.

Species diversity was measured by the Shannon diversity index, which combines two components of diversity, species richness and evenness. It is calculated with the equation

$$H = \sum_{i=1}^{s} p_i (\log_2 p_i)$$

where p_i is the probability of finding each species i in one sample.

Statistical analysis

To evaluate the effect of depth level (within-subjects variables), sites and hosts species (between-subjects factors) on spore number, richness and diversity of AMF a repeated measures analysis of variance (ANOVA) followed by a Bonferroni multiple comparisons with a significance level of 0.05 was performed. All residuals were tested for normality and homocedasticity with Shapiro-Wilks and Levenes tests, respectively. AMF spore number was log transformed. All statistics were performed using STATISTICA program of statsoft (http://www.statsoft.com/) Version 8.

Results

Soils of Salinas de Ambargasta (SA) and Salinas Grandes (SG) were Aridisol-Orthid typic Salorthids (INTA, 2003). Both sites presented a sandy clay loam texture, high pH and electrical conductivity, and slightly CEC and organic matter content. Depth level influenced significantly some soil parameters (Table 1). A decrease in organic matter content, C, N, K and an increase in Na with increasing soil depth were observed (Table 1).

Roots from *A. patagonica*, *A. argentina*, *H. ritteriana* and *S. divaricata* were colonized and showed entry points, intraradical aseptate hyphae, intracellular hyphal coils and intracellular vesicles in both sites at all soil depths. Arbuscules were not detected. AM colonization in plant species was very low to low (5-31% and 2-37%) in *A. patagonica* and *S. divaricata*, respectively; in *H. ritteriana* and *A. argentina* AM colonization was very low to medium (0-45% and 4-50%), respectively.

A total of 19 morphologically distinctive AMF species were recovered, and 14 could be attributed to known species belonging to eigth genera (*Acaulospora*, *Ambispora*, *Claroideoglomus*, *Diversispora*, *Glomus*, *Funneliformis*, *Rhizophagus* and *Septoglomus*) (Table 2). The community of spores was dominated by *Glomus brohultti*, *Septoglomus* aff. *constrictum* and Funneliformis *geosporum*. In total four morphotypes belonging to *Glomus* were found (two glomoid morphotypes remain unidentified, Figure 1). Five morphotypes belongs to *Acaulospora* (two acaulosporoid morphotypes remain unidentified, Figure 1), three to *Claroideoglomus*, two to *Funneliformis* and *Rhizophagus*, one to *Ambispora, Diversispora* and *Septoglomus*.

Brief morphological characteristics of two glomoid and acaulosporoid unknown morphotypes are described below.

Glomoid morphotype sp. 1: Spores reddish brown; globlose to subglobose, 72-130 µm x 82-130 µm. Subtending hyphae: straight to curved; wall continuous with spore wall and slightly lighter in color than spore wall. Pore closure: septum under spore base. Other characteristics: irregular globular projections (3-5) x (5-6) µm slightly lighter in color than spore wall (Figure 1 a-b).

Glomoid morphotype sp. 2: Spores orange; globose to subglobose, 60-112 µm x 60-112 µm. Subtending hyphae: straight; wall continuous with spore wall and slightly lighter in color than spore wall. Pore closure: constricted at spore base. Other characteristics: irregular globular projections slightly lighter in color than spore wall. Usually, very long subtending hyphae (Figure 1 c-d).

Acaulosporoid morphotype sp. 1: Spores hyaline to orange, globose to subglobose, 75-150 µm x 60-180 µm. Spore wall with three layers. The outer hyaline, thin and flexible. Middle layer pale yellow, very fine, with numerous folds on the spore surface and appears "rugose". The third layer is a thin layer, less than 1 µm thick. It often is adherent to the spore wall in which case it cannot be detected (Figure 1 e).

Acaulosporoid morphotype sp. 2: Spores orange to red orange, globose to subglobose, 50-90 µm x 45-80 µm. The outer layer continuous with the wall of the sporiferous saccule. Spore wall with three layers, the outer layer degrading and sloughing. Middle layer orange, with circular to ovoid depressions (Figure 1 f). The third layer shows a positive Melzers reaction.

Table 1 - Soil properties of the two study sites, Salinas de Ambargasta (SA) and Salinas Grandes (SG), at five depth levels (0-10, 10-20, 20-30, 30-40, and 40-50 cm). [a]: Mean value of 4 samples. Values within a row followed by the same letter were not significantly different for each saline site among soil depth (p < 0.05). [b]: OM: organic matter content, C: carbon, N: total nitrogen; C/N: carbon/nitrogen ratio, P: available phosphorus, EC: electrical conductivity, Ca: calcium, Mg: magnesium, K: potassium, Na: sodium, CEC: cation exchange capacity.

Parameters		0-10 cm[a]	10-20 cm	20-30 cm	30-40 cm	40-50 cm
OM (%)[b]	SA	1.29 ± 0.40 a	0.81 ± 0.18 ab	0.82 ± 0.17 ab	0.71 ± 0.21 b	0.65 ± 0.23 b
	SG	2.30 ± 1.93 a	1.41 ± 1.06 a	1.04 ± 0.61 a	1.05 ± 0.71 a	0.86 ± 0.46 a
C (%)	SA	0.64 ± 0.20 a	0.41 ± 0.09 ab	0.41 ± 0.13 ab	0.36 ± 0.11 b	0.33 ± 0.11 b
	SG	1.15 ± 0.97 a	0.71 ± 0.53 a	0.52 ± 0.31 a	0.53 ± 0.36 a	0.43 ± 0.23 a
N (%)	SA	0.08 ± 0.03 a	0.05 ± 0.02 ab	0.04 ± 0.02 bc	0.04 ± 0.01 bc	0.03 ± 0.01 c
	SG	0.11 ± 0.07 a	0.08 ± 0.06 a	0.06 ± 0.04 a	0.04 ± 0.02 a	0.04 ± 0.02 a
C/N	SA	8.24 ± 1.72 a	7.90 ± 0.62 a	10.26 ± 2.57 ab	10.47 ± 2.88 ab	12.93 ± 2.59 b
	SG	9.79 ± 2.35 a	9.23 ± 2.48 a	10.91 ± 3.63 a	11.95 ± 2.04 a	12.52 ± 0.65 a
P (ppm)	SA	11.61 ± 6.74 a	10.89 ± 7.93 a	8.53 ± 5.55 a	8.76 ± 5.65 a	9.17 ± 4.81 a
	SG	7.04 ± 3.39 a	6.48 ± 2.68 a	6.60 ± 3.40 a	7.17 ± 4.15 a	9.93 ± 4.40 a
pH 1: 2.5	SA	8.17 ± 0.57 a	8.11 ± 0.50 a	8.07 ± 0.42 a	8.02 ± 0.37 a	8.17 ± 0.50 a
	SG	7.76 ± 0.16 a	7.89 ± 0.19 a	7.84 ± 0.10 a	7.89 ± 0.19 a	7.82 ± 0.09 a
EC (dS.m^{-1})	SA	11.45 ± 5.79 a	13.53 ± 4.53 a	17.19 ± 5.12 a	17.58 ± 3.90 a	18.85 ± 4.86 a
	SG	9.35 ± 5.56 a	12.48 ± 6.05 a	14.36 ± 4.04 a	11.98 ± 5.48 a	14.91 ± 3.42 a
Ca (Cmolc/kg)	SA	8.44 ± 4.94 a	8.21 ± 5.09 a	8.64 ± 5.20 a	8.98 ± 5.17 a	10.03 ± 5.98 a
	SG	8.94 ± 2.20 a	9.46 ± 2.73 a	9.85 ± 2.15 a	10.51 ± 1.51 a	11.11 ± 2.34 a
Mg (Cmolc/kg)	SA	1.19 ± 0.67 a	1.11 ± 0.62 a	1.13 ± 0.64 a	1.14 ± 0.64 a	1.19 ± 0.67 a
	SG	1.46 ± 0.07 a	1.39 ± 0.02 a	1.50 ± 0.07 a	1.45 ± 0.12 a	1.44 ± 0.12 a
K (Cmolc/kg)	SA	0.56 ± 0.31 a	0.45 ± 0.26 a	0.38 ± 0.22 a	0.37 ± 0.21 a	0.37 ± 0.23 a
	SG	0.82 ± 0.10 a	0.64 ± 0.14 ab	0.54 ± 0.03 bc	0.44 ± 0.07 c	0.43 ± 0.14 c
Na (Cmolc/kg)	SA	1.07 ± 0.58 a	1.12 ± 0.62 a	1.20 ± 0.67 a	1.22 ± 0.69 a	1.20 ± 0.70 a
	SG	1.25 ± 0.13 ab	1.21 ± 0.12 a	1.39 ± 0.17 ab	1.55 ± 0.23 b	1.56 ± 0.30 ab
CEC (Cmolc/kg)	SA	14.41 ± 2.23 a	13.50 ± 2.06 a	13.04 ± 2.57 a	14.10 ± 1.46 a	13.44 ± 6.11 a
	SG	11.31 ± 2.69 a	12.26 ± 3.07 a	12.48 ± 2.70 a	13.32 ± 2.36 a	13.59 ± 2.61 a

Table 2 lists the relative abundance of all AMF species collected ordered according to soil profile, sites and plant species. Clearly the majorities of AMF spore types were rare and apparently did not change along soil profile. The majority of AMF occurred in low densities (relative abundance < 1%). Depending on the plant species, some species were increasingly found with increasing soil depth, at least in relative terms. These were *Glomus magnicaule* in *Allenrolfea patagonica* and *Septoglomus* aff. *constrictum* in *Atriplex argentina*. Some species sporulated in the top layers; these were *G. brohultti* in *Atriplex argentina* and *Septoglomus* aff. *constrictum* in *Allenrolfea patagonica*.

The AMF spores number ranged between 3 and 1162 per 100 g dry soil (mean number:166 ± 75). Spore number varied from 7 to 591 per 100 g dry soil in Salinas de Ambargasta (mean number: 122 ± 37) and from 3 to 1162 per 100 g dry soil in Salinas Grandes (mean number: 210 ±

117). Spores number was high in the upper soil horizon (0-10 cm), decreasing with increasing soil depth. AMF spores differed among plant species throughout soil depths (Table 3). Significant differences were observed among rhizospheres of the different plant species and soil depths, and among sites and soil depths. A significant triple interaction among soil depth x plant species x site was observed, indicating that AMF spore number decreases as depth increases at both sites (Figure 2).

AMF species diversity and richness differed significantly among rhizospheres of the host species and sites (Tables 3, 4). Similarly to observed for spore number, a significant triple interaction among soil depth x host plant species x site were evidenced in AMF species diversity and richness, indicating that all ecological parameters decrease in plant species as depth increases at both sites. The highest value of species diversity and richness was recorded in *S. divaricata* (H = 2.3, richness 8 species).

Table 2 - Relative spore abundance [a] (in percentage) of arbuscular mycorrhizal fungi found in host plant species and sites [b] across different soil depths [c].

| Species	Allenrolfea pagatonica SA 1	2	3	4	5	SG 1	2	3	4	5	Atriplex argentina SA 1	2	3	4	5	SG 1	2	3	4	5	Heterostachys ritteriana SA 1	2	3	4	5	SG 1	2	3	4	5	Suaeda divaricata SA 1	2	3	4	5	SG 1	2	3	4	5
Acaulospora bireticulata																					I	I	I		I															
A. scrobiculata		II										I	I	I		I				I				IV	IV	II				I	I				I	I			I	I
A. aff. undulata	I							I	I	I		I	I	I										I							II									
Acaulospora sp. 1	I							I												I	I					I	I	I												
Acaulospora sp. 2	I										I										I																			
Ambispora leptoticha	I										I										I																			
Claroideoglomus claroideum																							I																	
Clar. etunicatum	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I					I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Clar. luteum	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		I			I	I	I	I	I	I		I			I	I	I	I	I	I	I	I	I	I
Diversispora spurca	I			I	I	I	I	I	I	I	I					I		I			I					I	II	II	II	II	I	I	I	I	II	I	I	I	I	II
Funneliformis geosporum	I	I	I	I	II	I	I	I	I	I	II	I	I	I	II	II	I	II	II	I	I			I	I	I	II	II	I	I	II	II	II	I	II	II	II	II	II	II
Fun. mosseae	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I					I	II				I	II			I	II	I	I	I	I	I	I	I	I	I
Glomus brohultii	I	II	I	I	I	II	I	I	I	I	II	II	I	I	II	II					I	I	II	I	I	I	I	I	II	I	II	II	II	I	II	II	II	II	III	II
G. magnicaule	I	I	I	I	I	I	I	II	II	II						I					I	I	I	I	I	I	II	II	I	I	I	I	I	I	I	I	I	I	I	I
Glomus sp. 1	I					I					I	I	I			I	I	I			I										I	I	I	I	I	I	I	I	I	I
Glomus sp. 2											I	I	I			I			I												I		I			I				
Rhizophagus clarus	I			I	I	I	I	I			I	I	I	I		I	I	I	I	I	I					I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Rhiz. intraradices												I	I	I								I							I				I							
Septoglomus aff. constrictum	V	V	IV	IV	IV	II	II	II	II	III	I	III	III	III	II	I	II	III	IV	III	IV	III	III		II	II	I	I	I	II	II	II	I	I	I	I	I	I	I	I

Relative spores abundance are symbolized as follows: I 0- ≤ 20%; II > 20- ≤ 40%; III > 40- ≤ 60%; IV > 60- ≤ 80%; V > 80-100%.
[b]Salinas de Ambargasta (SA) and Salinas Grandes (SG).
[c]Numbers 1-5 indicate the different soil depths (1, 0-10 cm; 2, 10-20 cm; 3, 20-30 cm; 4, 30-40 cm; 5, > 40 cm).
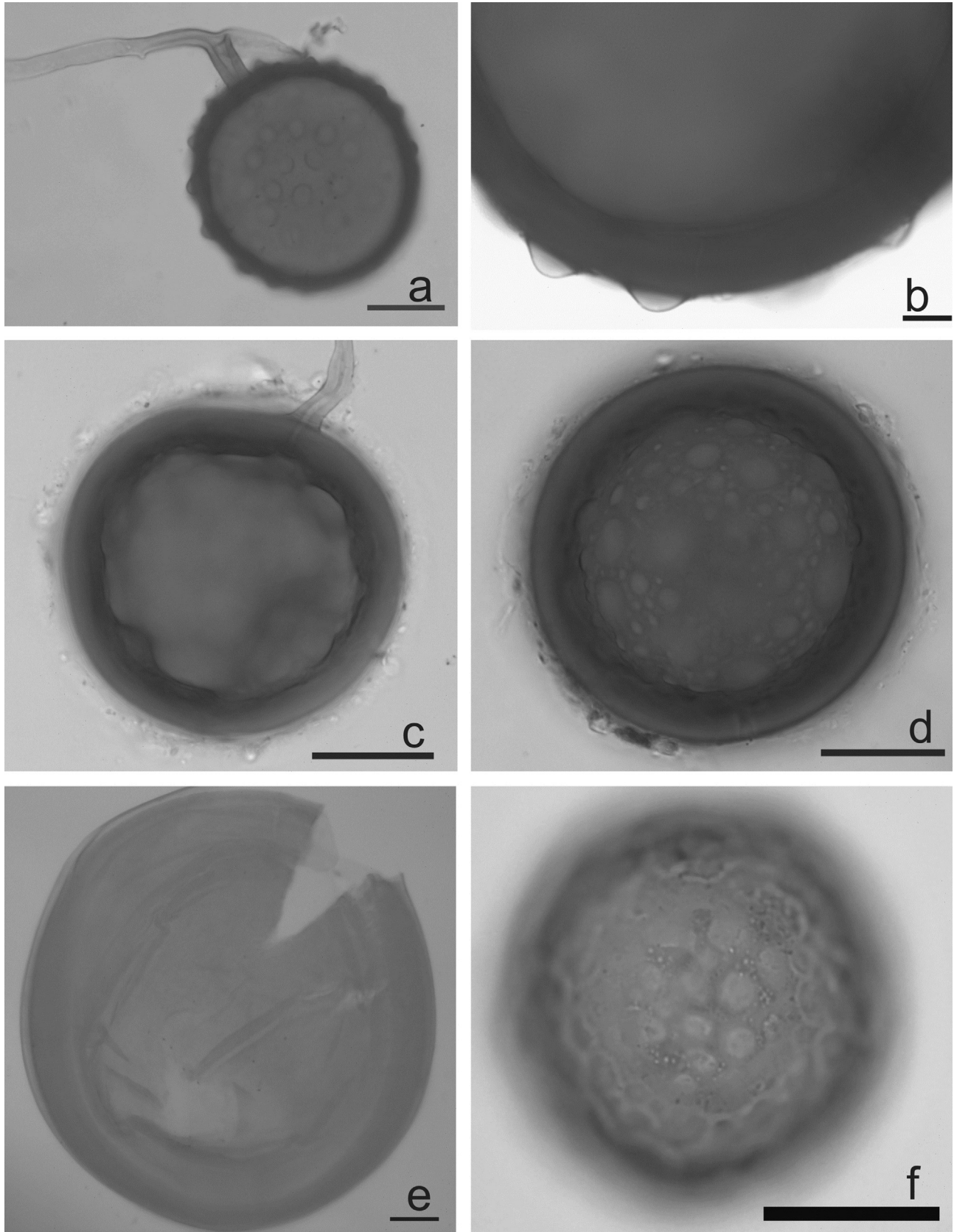
Figure 1 - Arbuscular mycorrhizal fungi found in Chenopodiaceae species from saline soils of Central Argentina. a-b: Glomoid morphotype sp. 1. c-d: Glomoid morphotype sp. 2 e: Acaulosporoid morphotype sp. 1. f: Acaulosporoid morphotype sp. 2. Scale bar a, c, d, e, f: 50 μm; b: 10 μm.
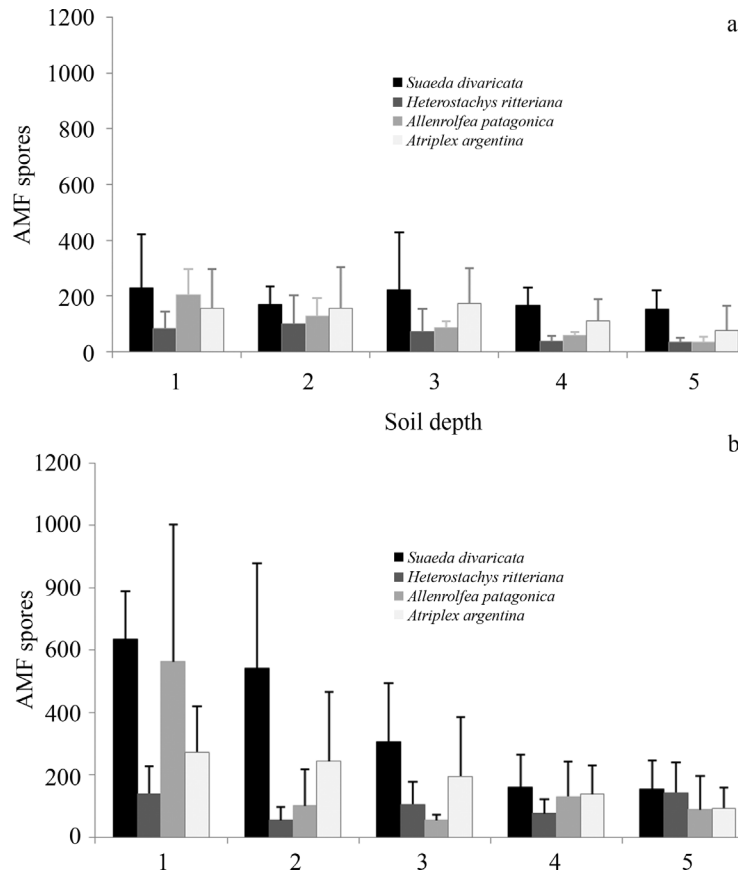
Figure 2 - Spore density of AMF (100 g dry soil) in four host plant species at different soil depths in both sites (a: Salinas de Ambargasta (SA), b: Salinas Grandes (SG)). Host plant species: *Suaeda divaricata*, *Heterostachys ritteriana*, *Allenrolfea patagonica* and *Atriplex argentina*. Soil depths 1 (0-10 cm), 2 (10-20 cm), 3 (20-30 cm), 4 (30-40 cm), 5 (> 40 cm); errors bars represent standard errors.

Table 3 - *F*-values from repeated measured ANOVA for Arbuscular Mycorrhizal Spores (AMS), Arbuscular Mycorrhizal Diversity (AMD) and Arbuscular Mycorrhizal Richness (AMR) in Host Plant Species (HPS), sites (S) (Salinas de Ambargasta and Salinas Grandes) and soil depth (SD).

	AMS	AMD	AMR
Within-subject effects			
Soil depth (SD)	17.407***	1.7680	1.8760
SD x HPS	2.456**	2.3100*	3.0614**
SD x S	2.460*	12.6139***	7.7972***
SD x HPS x S	2.265*	5.3024***	5.7144***
Between-subject effects			
Host plant species (HPS)	5.150**	16.5204***	16.5962***
Sites (S)	1.434	5.7848*	4.2074*
HPS x S	0.136	1.5919	0.3309

* Significant at the level 0.05. ** Significant at the 0.01 level. *** Significant at the 0.001 level.

Table 4 - Biodiversity index and species richness values for AMF morphotypes found in Salinas de Ambargasta and Salinas Grandes and hosts species. Data are means of one hundred replicates for each site and fifty replicates for each host. Values within a column followed by the same letter were not significantly different for sites ($p < 0.05$) for host species ($p < 0.0001$).

	Biodiversity index (H)	Richness (S)
Salinas de Ambargasta	1.72 b	5.47 b
Salinas Grandes	1.93 a	6.21 a
Allenrolfea pagatonica	1.5 c	5.04 a
Atriplex argentina	1.87 b	5.24 a
Heterostachys ritteriana	1.62 bc	5.04 a
Suaeda divaricata	2.30 a	8.04 b

Discussion

Mycorrhizal symbiosis is a key component in helping plants cope with adverse environmental conditions (Ruíz-Lozano and Azcón, 2000). In this study, AMF was found to

occur naturally in Chenopodiaceae plants across the soil profile in saline environments of Central Argentina.

The present study shows that the four species of Chenopodiaceae presented AM fungal structures in their roots. This finding is partly in agreement with the literature (Allen, 1983; Fontenla *et al.*, 2001; Plenchette and Duponnois, 2005), especially under drought and salt-stress conditions (Sengupta and Chaudhuri, 1990). AM colonization of the plants sampled in this study was significantly higher than values reported by Wang *et al.* (2004), but similar to

those of other plants species evaluated in saline soils (Hildebrandt *et al.*, 2001; Landwehr *et al.*, 2002).

The AMF diversity found in this study was higher than at two saline habitats in Netherlands and Northern Germany, where Wilde *et al.* (2009) found 14, 11 and 10 AMF species under *Aster tripolium*, *Puccinellia distans* and *Salicornia europaea*, respectively. In particular *Funneliformis geosporum* and *F. mosseae* has been widely reported for natural saline soils (Aliasgharzadeh *et al.*, 2001; Carvalho *et al.*, 2001; Hildebrandt *et al.*, 2001; Wilde *et al.*, 2009). Moreover, *Claroideoglomus etunicatus* was also found in saline soils of the Tabriz Plain of Iran (Aliasgharzadeh *et al.*, 2001) and *Ambispora leptoticha* in saline-alkaline soils of the Yellow River Delta of China (Wang *et al.*, 2004). As far as we know the other AMF species revealed here were not yet reported for saline soils.

AMF spore number in the rhizosphere of Chenopodiaceae plants, in deep soils, were similar to numbers in other saline soils (Aliasgharzadeh e*t al*., 2001; García and Mendoza, 2008; Hildebrandt *et al.*, 2001; Landwehr *et al.*, 2002). This suggests that AMF distribution is related to the physiological characteristics of the host and morphology and distribution of roots (Ingleby *et al.*, 1997; Wang *et al.*, 2004). Most spores were found in the surface soil layer (0-10 cm), decreasing in number with increasing soil depth. Spore production is concentrated near the soil surface (Abbott and Robson, 1991; Cuenca and Lovera, 2010; Ingleby *et al.*, 1997; Lovera and Cuenca, 2007; Oehl *et al.*, 2005) and could be associated with the greater presence of fine roots than in the deeper soil layers. Although Pearson's correlations between these variables have not been significant, we observed that root density was highest in the top soil and decreased with increasing depth (data not shown). AMF are fully dependent on host carbon; hence, the distribution of AMF spores associated with the fine root distribution across the soil profile was not surprising.

In the complex saline environments where soil physical and chemical properties, plant ecophysiological adaptation, and temperature-moisture characteristics are closely related, soil nutrient sources will certainly facilitate soil biota coexistence and activity (Barness *et al.*, 2009). The soils from central Argentina here analyzed were similarly to semi-arid and arid environments, where Chenopodiaceae are common (Aguilera *et al.*, 1998; Aliasgharzadeh *et al.*, 2001; Landwehr *et al.*, 2002; Wilde *et al.*, 2009). As increasing soil depth, differences in soil parameters (a reduction of OM, C and N in SA; a reduction of K and an increase of Na in SG) were found. These soil differences probably affect AMF spore number, as was observed in other Chenopodiaceae species (Aguilera *et al.*, 1998) and in other plant species (Verma *et al.*, 2010).

High levels of AMF diversity were observed in the saline environments of central Argentina. Diversity values recorded in the present study are similar to those found in saline-alkaline soils of the Yellow River Delta (Wang *et al.*,

2004). The AMF species richness differed among the host species investigated across the soil profile. The same effect was reported by Oehl *et al.* (2005) across soil profile in intensively cultivated soils in Central Europe. The highest number of species and the highest diversity was found in *Suaeda divaricata* in the first two soil depths (0-10, 10-20). As Bellgard (1993) and Lovera and Cuenca (2007) stated, AMF spores are concentrated in the first centimeters of soil and decrease significantly in the deepest layers of the soil profile.

The AMF community composition changed along soil depth. The community seemed to be dominated by *Glomus brohultti*, *Septoglomus* aff. *constrictum* and *Funneliformis geosporum*. Depending of the host plant, some species sporulated mainly, or exclusively, in the deeper soil layers (*Glomus magnicaule* in *Allenrolfea patagonica*, *Septoglomus* aff. *constrictum* in *Atriplex argentina*), others mainly in the top layers (*G. brohultti* in *Atriplex argentina* and *Septoglomus* aff. *constrictum* in *Allenrolfea patagonica*). As stated by Abe and Katsuya (1995), Ho (1987) and Wang *et al.* (2004). Glomeraceae species are the most commonly observed in stressful habitats.

AMF were described to protect plants against salinity (Ruíz-Lozano and Azcón, 2000) and AMF may have developed adaptive strategies to tolerate this stressful environment. In these saline environments, the interaction between host plant species and abiotic factors is so complex that AMF patterns are difficult to explain. The present study contributes to the knowledge of the vertical AMF distribution in extremely saline soils of two salines of Central Argentina and shows the high diversity of AMF in natural saline ecosystems. In addition, the results of our study demonstrate that these AMF species, belonging to different genera and families of Glomeromycota, are adapted to extreme environmental conditions and indicate the importance of conducting more exhaustive samplings (at different depths across the soil profile) to obtain a complete picture of AMF in the field.

Acknowledgments

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