Arbuscular mycorrhizal fungal species in saline environments of Central Argentina: seasonal variation and distribution of spores at different soil depths

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Natural saline soils of Central Argentina have rarely been the focus of arbuscular mycorrhizal fungi (AMF) which are Glomeromycota. We explored the vertical distribution of spores of different AMF species in the rhizosphere of *Atriplex lampa* in two saline environments (Salinas Grandes and Salinas de Ambargasta) during wet and dry seasons. 18 AMF species were identified. Spore numbers were highest at Salinas Grandes during the wet season. Soil depth showed an influence on spore abundance of some specific species. Our results highlight the effect of soil depth, seasons and soil characteristics on sporulation of AMF species under saline conditions.

Keywords: saline soils; soil profile, *Atriplex lampa*, mycorrhizal species, Glomeromycota.

In Central Argentina natural saline soils cover 14 % of the area (Cabido & Zak 1999, Augé *et al.* 2006). The vegetation shows a zonal species distribution dependent on salt concentration, with halophytes mainly of the Chenopodiaceae, representing 9 % of total vegetation cover. The genus *Atriplex* of this family has received great attention (Mulgura de Romero 1981) because it is often used for restoration of saline sites (Salem *et al.* 2010). *Atriplex lampa* (Moq.) D. Dietr. is a valuable fodder shrub due to its high mineral and protein content available even during drought periods (Passera & Borsetto 1989).

Halophytes have effective mechanisms of nutrient uptake (Khan & Duke 2001). In particular, their association with arbuscular mycorrhizal fungi (AMF) may improve plant tolerance to drought and salt (Smith & Read 2008). Although the Chenopodiaceae family has been traditionally regarded as non-mycorrhizal (Hirrel *et al.* 1978), some *Salicornia* spp., *Suaeda* spp., *Atriplex* spp. and *Salsola* spp. were found to be colonized by AMF (Allen 1983,

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Allen & Allen 1990, Sengupta & Chaudhuri 1990, Aguilera *et al.* 1998, Hildebrandt *et al.* 2001, Plenchette & Duponnois 2005). In Argentina *Atriplex lampa* was found to be mycorrhizal (Fontenla *et al.* 2001). Although a high salt content is unfavorable for AMF spores germination and hyphae growth (Juniper & Abbot 1993, 2004), AMF communities occur naturally in saline environments (Hildebrandt *et al.* 2001, Landwehr *et al.* 2002, Wang *et al.* 2004). Ecological studies on the community structure of AMF are often restricted to the top 20 cm of soil, where most of the root biomass is concentrated (Brundrett 1991). Few studies have shown AMF diversity changes among soil profile (Oehl *et al.* 2005, Cuenca & Lovera 2010) and seasonal dynamics (Carvalho *et al.* 2001, Lugo & Cabello 2002, Füzy *et al.* 2008). There are no studies of AMF communities in natural saline soils of Central Argentina, and the present work reports vertical distribution and seasonal variation of spore abundance of the members of the AMF community in the rhizosphere of *A. lampa* in two saline environments of Central Argentina.

Materials and methods

The study was conducted in the Córdoba Province, Central Argentina: "Salinas de Ambargasta" (64° 18` W, 29° 27` S) and "Salinas Grandes" (64° 31` W, 29° 44` S) (Fig. 1). Together both sites occupy approximately 600000 ha along the northwestern limit of the Córdoba Province. The climate is dry and warm, with a mean annual precipitation below 500 mm and mean temperature of 19.9 °C. Wet season is between September and February (spring and summer) and dry season between March and August (autumn and winter). The vegetation shows a zonal species distribution dependent on salt concentration. The most elevated areas (with lowest salt concentration) are occupied by a xerophytic forest and the understory vegetation is represented by halophytes. On the edge of the saline depression, where this study was carried out, halophytes mainly belonging to the Chenopodiaceae are dominant (Cabido & Zak 1999).

Sampling was conducted in both sites during wet season (March 2007) and dry season (August 2008). At each site, five *A. lampa* individuals, situated 50 m apart from each other, were randomly chosen. Five soil samples were carefully taken at different soil depths (0–10, 10–20, 20–30, 30–40, and 40–50 cm) with a corer (3 cm diameter) from under the canopy of each of the five plants. The samples were placed in plastic bags and stored at 4 °C.

From soil samples 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 following Nelson & Sommers (1982), carbon : nitrogen ratio and soil texture. Total nitrogen was determined using the micro-Kjeldhal method (Bremner & Mulvaney 1982).

AMF spores were extracted from each soil sample by wet sieving and decanting (Gerdemann & Nicolson 1963), followed by centrifugation in sucrose gradient (Walker *et al.* 1982). A fine sieve (38 µm) was used to collect

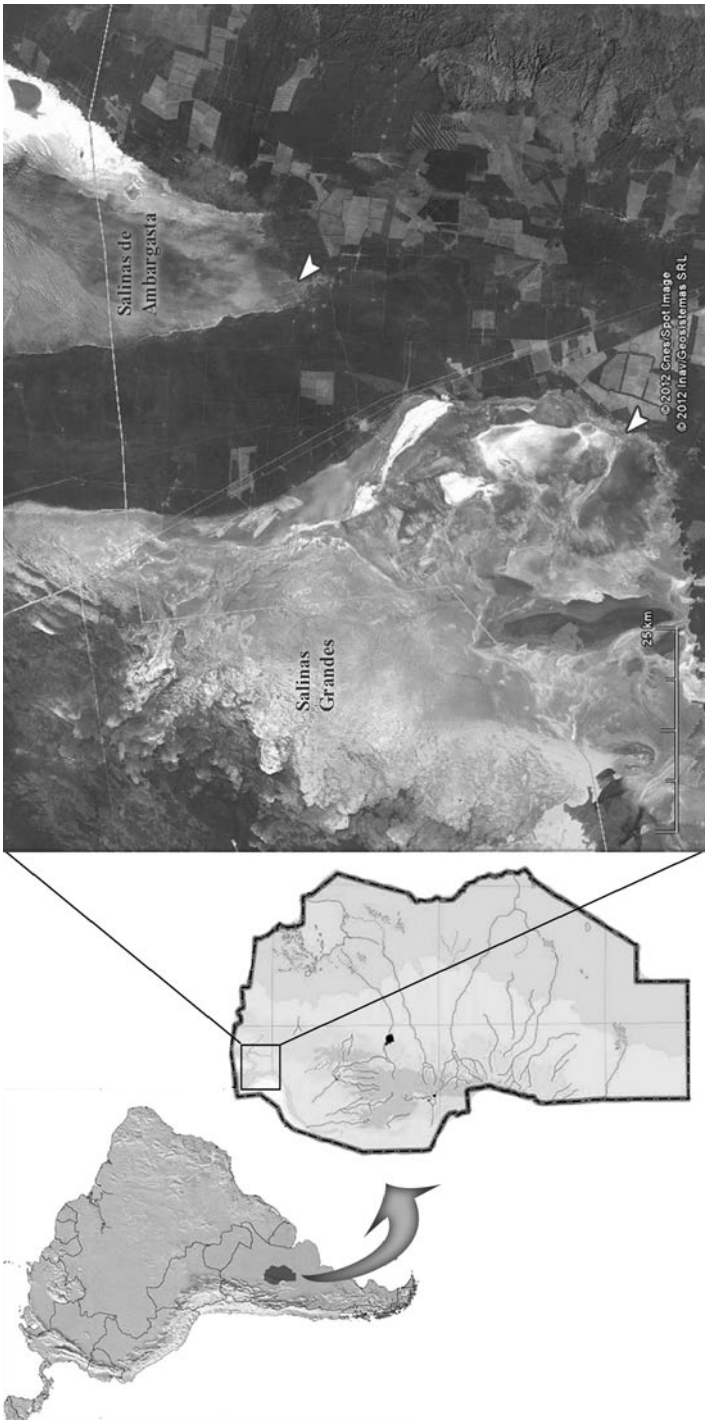

Fig. 1. Study sites (arrowheads) in the two saline environments (Salinas Grandes and Salinas de Ambargasta) of the northwestern Córdoba Province, Central Argentina.

small spores, and the material remaining on the top sieve (125 µm) was also checked for sporocarps and larger spores. Only apparently healthy spores of individual, morphologically different spores were counted under stereomicroscope, and recorded as number of spores per 100 g dry soil.

For the taxonomic identification, fungal spores and sporocarps were mounted onto slides using PVA with and without Melzer´s reagent (Omar *et al.* 1979) and examined with a compound microscope. AMF species identification was based on current species descriptions and identification manuals of Schenk & Perez (1990), INVAM (http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm) and Oehl *et al.* (2011).

To relate soil depth, season and location with soil parameter, AMF spore numbers, species richness, Shannon-evenness and Shannon-diversity index, a repeated measures analysis of variance (ANOVA) followed by a Tukey post-hoc test with a significance level of 0.05 was performed. When measures were not independent (tested with Mauchly's sphericity test) a multivariate analysis of variance (MANOVA) was performed. All residuals were tested for normality and homocedasticity with Shapiro-Wilks and Levene´s tests, respectively. All statistics were performed using STATISTICA program of statsoft (http://www.statsoft.com/).

Results and discussion

Soils of Salinas de Ambargasta (SA) and Salinas Grandes (SG) were Aridisol-Orthid typic Salorthids (INTA 2003). Both sites showed a sandy clay loam texture, high pH and electrical conductivity, and slightly organic matter content (Fig. 2). Soils of SA showed higher electrical conductivity and phosphorous levels (15.7 ± 4.8 and 9.8 ± 6.1, respectively) than SG soils (12.6 ± 4.9 and 7.4 ± 3.6, respectively). Soil depth had little influence on soil parameters, only organic matter content decreased significantly in SA soil while C/N relation increased significantly in this soil with depth (Fig. 2).

The 18 AMF species identified belonged to eight genera: *Acaulospora*, *Ambispora, Claroideoglomus*, *Diversispora*, *Funneliformis*, *Glomus*, *Scutellospora* and *Septoglomus*. Thirteen spore types could be identified to species level: *Acaulospora bireticulata* Rothwell & Trappe*, A. scrobiculata* Trappe, *A.* aff. *undulata* Sieverd., *Ambispora leptoticha* (N. C. Schenck & G. S. Sm.) C. Walker, Vestberg & A. Schüßler, *Claroideoglomus etunicatum* (Becker & Gerd.) C. Walker & A. Schüßler, *C. luteum* (L. J. Kenn., J. C. Stutz & J. B. Morton) C. Walker & A. Schüßler, *Diversispora spurca* (C. M. Pfeiffer, C. Walker & Bloss) C. Walker & A. Schüßler, *Funneliformis geosporum* (T. H Nicolson & Gerd.) C. Walker & A. Schüßler, *F. mosseae* (T. H Nicolson & Gerd.) C. Walker & A. Schüßler, *Glomus brohultti* Sieverd. & Herrera, *G. clarum* T. H. Nicolson & N. C. Schenck, *G. magnicaule* I. R. Hall and *Septoglomus* aff. *constrictum* (Trappe) Sieverd., G. A. Silva & Oehl. *Acaulospora scrobiculata* was present only in SG soils. *Ambispora leptoticha* and *Glomus* sp. 2 were only found during the dry season. For morphological characteristics of unidentified species found in the soil samples of *A. lampa* see Tab. 1.

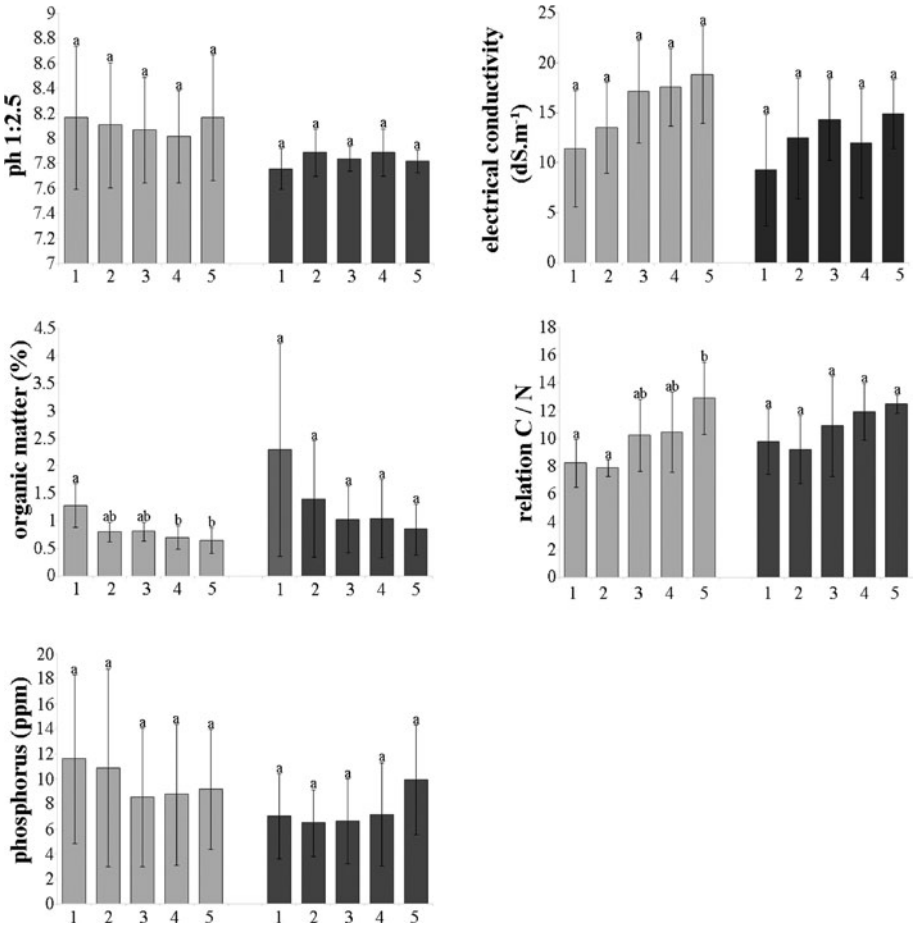

Fig. 2. Soil properties of the two study sites, Salinas de Ambargasta (SA, gray) and Salinas Grandes (SG, black), at five depth levels: 1 (0–10 cm), 2 (10–20 cm), 3 (20–30 cm), 4 (30–40 cm), and 5 (40–50 cm). Different letters indicate significant differences among soil depths according to Tukey HSD test at $P = 0.05$ (n = 5 replicates).

The AMF biodiversity found in Argentina 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 *Funelliformis geosporum* and *F. mosseae* have 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 etunicatum* 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.

Tab. 1. Morphological characteristics of unidentified species found in rhizospheric soil samples of *Atriplex lampa* in Salinas Grandes and Salinas de Ambargasta.

	Color and shape	Size Distribution (µm)	Subtending hyphae	Pore closure	Other characteristics
Funneliformis sp. 1	Orange to reddish brown; globose	94–150 × 96–150	Funnel shaped; wall continuous with spore wall and slightly lighter in color than spore wall	Septum under spore base	
Glomus sp. 1	Reddish brown; globose to subglobose	72–130 × 82–130	Straight to curved; wall continuous with spore wall and slightly lighter in color than spore wall	Septum under spore base	Irregular globular projections (3–5) × (5–6) µm slightly lighter in color than spore wall
Glomus sp. 2	Orange; globose to subglobose	60–112 × 60–112	Straight; wall continuous with spore wall and slightly lighter in color than spore wall	Constricted at spore base	Irregular globular projections slightly lighter in color than spore wall. Usually very long subtending hyphae
Glomus sp. 3	Yellow; globose to subglobose	34–45 × 34–49	Straight; wall continuous and concolorous with spore wall	Open	Always found forming compact sporocarps

Altogether the number of AMF spores ranged between 5 and 1418 per 100 g dry soil. It varied from 5 to 350 per 100 g dry soil in SA (51 ± 13 and 101 ± 15 in wet and dry seasons, respectively) and from 17 to 1418 per 100 g dry soil in SG (601 ± 74 and 122 ± 29 in wet and dry seasons). For both sites and seasons, AMF spore numbers were comparable to other saline environments as reported by Aliasgharzadeh *et al.* (2001) for the Tabriz Plain of Iran, Hildebrandt *et al.* (2001) for Central Europe, and Landwehr *et al.* (2002) for the Hungarian steppe, suggesting that salt concentrations do not negatively affect AMF sporulation. A significant interaction among season x site ($F = 7.87$, $P < 0.01$) was observed, with the significant highest spore number in SG during the wet season. Differences in soil characteristics between the sites may have affected AMF spore formation, as was observed in other *Atriplex* species (Aguilera *et al.* 1998). Soil depth had no significant influence on spore numbers ($F = 0.39$, $P = 0.808$ and $F = 3.70$, $P = 0.092$ for SA and SG, respectively).

Tab. 2. Relative spore abundance [a] (in percentage) and total spore number per 100 g dry soil (± standard error) of AMF species found in Salinas Grandes (SG) and Salinas de Ambargasta (SA) during (a) wet and (b) dry seasons across different soil depths[b].

a)

	SA					SG				
	1	2	3	4	5	1	2	3	4	5
Acaulospora bireticulata	0	0	0	0	0	I	I	I	I	I
A. scrobiculata	0	0	0	0	0	I	0	I	0	0
A. aff. *undulata*	0	I	0	0	0	I	0	0	0	0
Ambispora leptoticha	0	0	0	0	0	0	0	0	0	0
Claroideoglomus etunicatum	I	I	I	I	I	I	I	I	I	I
C. luteum	0	0	0	0	0	0	I	0	I	0
Diversispora spurca	I	0	0	I	I	0	I	I	I	I
Funneliformis geosporum	I	I	I	I	I	II	II	II	II	II
F. mosseae	II	II	I	I	I	I	I	I	I	I
Funneliformis sp. 1	0	I	0	I	I	I	I	I	I	0
Glomus brohultti	II	I	I	I	I	IV	III	III	III	II
Glomus clarum	I	II	II	III	III	I	I	II	I	I
G. magnicaule	0	0	0	0	0	I	I	I	0	I
Glomus sp. 1	I	I	I	I	I	I	I	I	I	I
Glomus sp. 2	0	0	0	0	0	0	0	0	0	0
Glomus sp. 3	0	0	0	I	I	I	I	I	I	I
Scutellospora sp.	II	II	II	IV	I	I	II	I	I	II
Septoglomus aff. *constrictum*	V	IV	IV	II	III	III	III	III	III	III
Total spore number	86 (57)	52 (24)	32 (14)	47 (24)	37 (15)	636 (233)	418 (114)	847 (138)	665 (188)	437 (104)

[a] Relative spore abundance are symbolized as follows: 0 = absent; I 1–10 %; II 11–20 %; III 21–30 %; IV 31–40 %; V 41–50 %; VI 51–60 % of total spore numbers.

[b] 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, 40–50 cm).

b)

	SA					SG				
	1	2	3	4	5	1	2	3	4	5
Acaulospora bireticulata	0	0	0	0	I	0	0	0	0	0
A. scrobiculata	0	0	0	0	0	0	0	0	0	0
A. aff. *undulata*	0	0	0	0	0	0	0	0	0	0
Ambispora leptotichia	0	I	0	0	I	0	0	0	0	0
Claroideoglomus etunicatum	I	I	I	I	I	I	I	I	I	I
C. luteum	I	I	I	I	0	I	I	I	II	I
Diversispora spurca	0	I	III	IV	I	VI	V	VI	VI	V
Funneliformis geosporum	I	I	II	I	II	I	I	I	I	I
F. mosseae	I	I	I	I	I	I	I	I	I	I
Funneliformis sp. 1	0	I	I	I	0	I	I	I	0	I
Glomus brohultti	I	II	II	II	II	I	I	I	I	I
G. clarum	I	I	0	I	I	I	I	I	I	I
G. magnicaule	0	0	0	0	I	0	I	0	0	0
Glomus sp. 1	I	I	I	I	I	I	0	0	0	0
Glomus sp. 2	0	I	0	0	0	0	II	0	0	0
Glomus sp. 3	I	I	I	I	I	II	I	I	I	I
Scutellospora sp.	II	I	I	II	II	0	0	0	0	0
Septoglomus aff. *constrictum*	V	IV	III	I	III	I	I	II	III	IV
Total spore number	118 (42)	126 (57)	98 (23)	78 (17)	84 (27)	147 (68)	210 (112)	155 (45)	65 (21)	33 (6)

[a] Relative spore abundance are symbolized as follows: 0 = absent; I 1–10 %; II 11–20 %; III 21–30 %; IV 31–40 %; V 41–50 %; VI 51–60 % of total spore numbers.

[b] 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, 40–50 cm).

The same as soil parameters, AMF species were almost not influenced by soil depth. Species richness, Shannon-evenness and Shannon-diversity index did not show significant differences between the variables studied (depth, season and site). Nevertheless, the relative spore abundance in relation to total AMF spore numbers, showed differences with soil depth for some species, *Glomus clarum* during wet season in SA and *Septoglomus* aff. *constrictum* during dry season in SG were increasingly found with increasing soil depth, while *Glomus brohultti* and *Glomus* sp. 3 in SG showed a decreasing relative spore abundance with increasing soil depth during wet and dry season, respectively (Tab. 2). Variation of spore abundance of different AMF species with soil depth was also reported by Oehl *et al.* (2005). Similar as observed by Aliasgharzadeh *et al.* (2001), Wang *et al.* (2004) and Wilde (2009) in other saline soils, also here the AMF most commonly found were *Glomus* spp. It was speculated that *Glomus* spp. are adapted to stressful conditions (Carvalho *et al.* 2001, Landwehr *et al.* 2002).

As far as we know this is the first study of AMF species of saline soils of Argentina. Since natural saline soils of Central Argentina cover 14 % of its surface, saline ecosystems are important for agriculture and environment. Our study shows that AMF species are involved in the plant-soil interactions, and if adapted plants in such ecosystems systems shall be used more intensively, the soil genetic AMF resource should be identified in broader future research. This can be done by including classical methods like using trap plants for the establishment of AMF collections (Brundrett 1991, Bellgard 1993) from which AMF species can be better identified by using molecular biological tools (Oehl *et al.* 2005, Wilde *et al.* 2009).

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