



Diversity of arbuscular mycorrhizal fungi in the rhizosphere of *Asteriscus maritimus* (L.) Less., a representative plant species in arid and saline Mediterranean ecosystems



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ABSTRACT

Native arbuscular mycorrhizal fungi (AMF) from saline areas could be a critical issue for success in recovering areas affected by salinity, either in natural environments or in agricultural lands. Despite its important role, there is little information on the distribution and abundance of the different mycorrhizal associations in saline environments in European Mediterranean areas. In the present study the community of AMF is investigated in the rhizosphere of a representative plant species adapted to saline Mediterranean areas, *Asteriscus maritimus*. Samples of the rhizosphere of twenty plants were taken from the most arid region in Europe, Cabo de Gata Natural Park, in two different areas: a dune and a salt marsh. A total of 30 AMF species belonging to three classes, five orders, nine families and 13 genera within the phylum Glomeromycota were found from both sites. In the rhizosphere of the salt marsh spore densities were six times higher than in the dunes, although the diversity was similar in both systems. One new AMF species has been described in the dune and four species could not be unequivocally identified, suggesting possible new species.

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1. Introduction

Seasonal aridity characterises Mediterranean environments. Damage to soil and plants in arid and semiarid areas is not easily repaired due to the fragility of these ecosystems (Pascual et al., 2000). The latter is inducing desertification of great areas in Southeast Spain which leads to increases in salinity (Albaladejo et al., 1996). Salinity is recognized as a severe global ecological problem being one of the most influential abiotic factors limiting plant growth and yield (Porcel et al., 2012).

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil inhabitants belonging to the phylum Glomeromycota which establish mutualistic symbiotic associations with most land plants (Smith and Read, 2008). Symbiosis between plants and AMF is one of the

plant strategies for growing under a variety of stress conditions (Entry et al., 2002). The ecological impact of AMF is particularly relevant for arid and semi-arid ecosystems where they would enable greater plant tolerance of environmental stresses characteristic of these ecosystems (Allen, 2007) and it is well documented that AMF improve plant growth and soil structure (van der Heijden et al., 2006). Due to the key ecological functions played in soils and plants by AM symbiosis, a diverse community of AMF is necessary for the development and maintenance of plant diversity (van der Heijden et al., 1998). Assessment of the native species composition is an important issue as AMF play important roles in the vigour of plant communities and the restoration of disturbed ecosystems (Renker et al., 2004). Moreover, the use of AMF adapted to salinity could be a critical issue for success in recovering saline areas either in natural environments or in agricultural lands that became salinised due to inappropriate land use (Estrada et al., 2013).

Despite its important role, there is little information on the distribution and abundance of the different mycorrhizal associations in saline environments in European Mediterranean areas. The aim of the present work was to analyse the diversity of AMF species

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in sensitive ecosystems affected by desertification and salinity in Southeast Spain, selecting two ecosystems: salt marshes and dunes. Cabo de Gata Natural Park is the most arid ecosystem in Europe (Geiger, 1973) and was selected as the target area. AMF were screened in the rhizosphere of *Asteriscus maritimus* (L.) Less., a halophyte member of the Asteraceae family, highly mycotrophic (Mason, 1928), and native of lands surrounding the Mediterranean Sea, especially Spain (Lendínez et al., 2011). It is found both in salt marshes and dunes and has a high ecological value in Mediterranean ecosystems (Rodríguez et al., 2005). However, differences in the AMF communities associated with this plant can be assumed dependent on the ecosystems. For this purpose AMF were studied either directly in samples from the target soil or from one year bait plant cultures established to increase AMF spore population, to reveal their maximum complement possible.

To identify the different AMF species in natural saline sites, morphological and developmental criteria were applied. The morphological identification of AMF can accurately be done with spores (Oehl et al., 2011), while the fungal structures within the roots (intraradical hyphae, arbuscules, vesicles) may vary only within the family or order but not at the species level (Dodd et al., 2000). Our primary purpose was to understand the species composition of AMF in the rhizosphere of *A. maritimus* in two arid and saline habitats.

2. Material and methods

2.1. Site description

The study was carried out in a representative saline Mediterranean ecosystem Cabo de Gata Natural Park in Almería (Andalucía, Spain). The climate is dry and hot, with an average annual temperature of 18.5 °C, irregular rainfall occurring mostly in autumn with a mean annual rainfall less than 200 mm and annual solar hours of 2.960. Two habitats were selected: a natural sand dune (36°44'41"N 02°07'26"W) and a salt marsh (36°45'24"N 02°13'17"W) (Fig. 1).

2.2. Plant and soil sampling

Most of the halophytes in saline sites belong to the *Chenopodiaceae*, *Junceaceae*, *Cyperaceae* or *Brassicaceae* families which are non- or weakly mycorrhizal. For the present study the halophyte

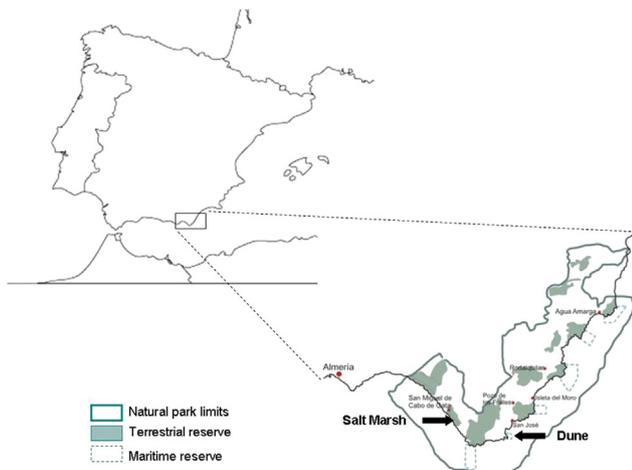


Fig. 1. Sampling sites location, salt marsh and dune, at Cabo de Gata Natural Park, Southeast Spain.

A. maritimus, belonging to the Asteraceae family was selected as the target plant, as it is well known to be colonized by AMF (Mason, 1928).

Soil samples were taken from the rhizosphere of twenty *A. maritimus* plants, ten of them growing in the sand dune system and the other ten plants in the salt marsh, in February 2010 to analyse spore populations. Plants were randomly collected in an area of 500 m² at each site with a drill with their intact root systems up to 40 cm soil depth taking up to 1 L of soil per sample. Root fragments in these samples were rinsed cleansed with tap water and then cleared in 10% KOH and stained with 0.05% trypan blue to confirm AM colonization (Phillips and Hayman, 1970).

The analysis of selected soil parameters was carried out by the Analytical Service of the Centro de Edafología y Biología Aplicada del Segura, CSIC, Murcia, Spain. pH and electrical conductivity were measured in the 1.5 aqueous extract (CRISON), organic carbon and total nitrogen were measured in a C/N analyser (Flash EA 112 Series-Leco TRUSPEC). Available P was estimated by the Olsen et al. (1954) method.

2.3. AMF bait cultures

AMF bait cultures were established and maintained by transplanting each *A. maritimus* plants with their surrounding rhizosphere soils into 1 L pots and transferring them to the greenhouse at EEZ in Granada immediately after sampling. The pots were irrigated three times per week and fertilized every four weeks with Long-Aston nutrient solution (Hewitt, 1952). The bait cultures were used for propagation of indigenous AMF communities to be characterised after one year, in March 2011.

2.4. AMF spore isolation and identification

For the analyses of AMF spores, 50 g of each rhizospheric soil were taken in March 2010 and 25 g in March 2011 from each of the twenty bait cultures due to the increase in spore numbers. AMF spores, both from the field collected soil and from the bait cultures, were isolated, identified and counted separately for each location. They were separated from the soil samples by a wet sieving and decanting method, followed by sucrose centrifugation process (Sieverding, 1991). After centrifugation, the supernatant was poured through a 50 µm mesh and quickly rinsed with tap water. Turgid spores (suggesting viability) were counted under a dissecting microscope using up to 90-fold magnification. For identification of AMF species, spores (about 50–70% of the total numbers) were picked under the dissecting microscope and mounted in polyvinyl alcohol-lactic acid-glycerine (PVLG) (Koske and Tessier, 1983) or PVLG mixed 1:1 (v/v) with Melzer's reagent (Brundrett et al., 1994) for permanent slides. The spores were then examined using a compound microscope at up to 400× magnification. The AMF species identification was based on current species descriptions and identification manuals (e.g. Schenck and Pérez (1990) and the webpage "Arbuscular mycorrhizal fungi (Glomeromycota), Endogone, and Complexipes species" deposited in the Department of Plant Pathology, University of Agriculture in Szczecin (Poland): <http://www.zor.zut.edu.pl/Glomeromycota/>), and on own type specimen analyses of almost all AMF species described. In this study, an AMF "species" is either a clearly identified species based on its spore morphology, or a species as yet unknown to us. We follow the glomeromycotean classification of Oehl et al. (2011).

2.5. Species richness and specific density

In the present work species richness and specific density have been evaluated at the dune and salt marsh. The species richness is

defined as the total number of species found in a community. The specific density (D_i) is the proportion of individuals of one particular species in the sample (i) relative to the total number of individuals (N):

$$D_i = n_i/N$$

3. Results

3.1. Root colonization by AMF

All surveyed samples were colonized by AMF and formed typical arbuscular mycorrhizal structures. Intra- and intercellular hyphae, vesicles and arbuscules were abundant in the root tissues.

3.2. Soil analysis

At the dune site, the soil was alkaline with pH 8.2, organic carbon 15.3 g kg⁻¹, total nitrogen 1.9 g kg⁻¹, available P 27.0 mg kg⁻¹, and soil electrical conductivity 0.5 dS m⁻¹. At the salt marsh, the soil was alkaline with pH 8.7, organic carbon 2.6 g kg⁻¹, total nitrogen 0.3 g kg⁻¹, available P 47.0 mg kg⁻¹, and soil electrical conductivity ranging from 3.95 dS m⁻¹ at the surface to 7 dS m⁻¹ at the deeper soil layer of the *A. maritimus* rhizospheric soil.

3.3. AMF spore densities at different ecosystems

The AMF spore densities differed among the sites. Samples from the salt marsh had higher spore densities than the dune, both in the rhizosphere soil of the field samples, and in the bait cultures. In March 2010, the spore densities were 1 and 7 g⁻¹ soil at the dune and the salt marsh, respectively. After one year in the bait cultures, the spores densities were more than doubled, with 3 and 18 g⁻¹ soil at the dune and the salt marsh. The relation between dune and salt marsh remained the same, with the spore density around six times higher at the salt marsh than at the dune.

3.4. AMF species richness

In total 30 AMF species could be distinguished from the field samples across all sites and both years of survey. They belong to all three current classes, to all five current orders, to nine families and thirteen genera currently known in the Glomeromycota (Table 1). The majority of the isolated species (16) were of the order Glomerales (3 *Funneliformis* species, 9 *Glomus* species and one *Septoglomus* species of the Glomeraceae and two species of *Claroideoglossum* and one *Entrophospora* species of the Entrophosporaceae). Of the Diversisporales, four *Diversispora*, two *Pacispora* and one *Acaulospora* were detected. Of the Gigasporales, two *Racocetra* and one *Scutellospora* were found. Finally, two *Archaeospora* and one *Ambispora* species of the Ambisporales and one *Paraglossum* species of the Paraglomerales were detected. Four species (*Glomus* sp. BEV1, *Glomus* sp. BEV2, *Entrophospora* sp. BEV3 and *Diversispora* sp. BEV4) could not be unequivocally identified as a known species and might represent new species.

3.5. AMF species richness in sand dune

In the field samples of March 2010, 16 AMF species belonging to 9 genera were found in the sand dune. In March 2011, after one year of bait culturing, 15 AMF species reproduced spores in the cultures belonging to 10 genera. From field and bait cultures, a total of 23 AMF species were detected belonging to 11 AMF genera were identified. Of these species, 7 were found recovered only from the field samples and 8 only from the bait cultures, while 9 species

Table 1

Presence and species richness of AMF at dune and salt marsh determined directly from field-collected soil samples in 2010 and from bait cultures in 2011.

AMF species	Sand dune		Salt marsh	
	In field sample 2010	Trap culture harvest 2011	In field sample 2010	Trap culture harvest 2011
Glomerales				
Glomeraceae				
<i>Funneliformis coronatus</i>	x	x	x	x
<i>Fu. geosporus</i>			x	x
<i>Fu. mosseae</i>		x	x	x
<i>Glomus badium</i>	x		x	
<i>Gl. intraradices</i>	x	x	x	x
<i>Gl. macrocarpum</i>	x		x	
<i>Gl. sp. BEV1</i>			x	
<i>Gl. magnicaule</i>	x			
<i>Gl. sp. BEV2</i>	x		x	
<i>Gl. microaggregatum</i>	x	x	x	x
<i>Gl. microcarpum</i>			x	
<i>Gl. rubiforme</i>	x			
<i>Septoglomus constrictum</i>	x	x	x	x
Entrophosporaceae				
<i>Claroideoglossum claroideum</i>	x	x	x	x
<i>Cl. etunicatum</i>		x	x	x
<i>Entrophospora</i> sp. BEV3			x	
Diversisporales				
Diversisporaceae				
<i>Diversispora aurantia</i>		x		x
<i>Di. clara</i>		x		
<i>Di. versiformis</i>		x		x
<i>Di. sp. BEV4</i>	x		x	
Acaulosporaceae				
<i>Acaulospora scrobiculata</i>	x	x		
Pacisporaceae				
<i>Pacispora dominiki</i>	x	x		
<i>Pa. franciscana</i>	x	x		
Gigasporales				
Racocetraceae				
<i>Racocetra persica</i>	x		x	
<i>Ra. verrucosa</i>			x	
Scutellosporaceae				
<i>Scutellospora calospora</i>	x	x	x	
Archaeosporales				
Archaeosporaceae				
<i>Ambispora gerdemannii</i>			x	
<i>Archaeospora myriocarpa</i>			x	x
<i>Ar. trappei</i>		x		x
Paraglomerales				
Paraglomeraceae				
<i>Paraglossum occultum</i>		x		x
AMF species richness site ⁻¹ year ⁻¹	16	16	20	13
Total AMF species richness site ⁻¹	23		24	
AMF species richness of study	30			

were detected both in the field samples and the bait cultures (Table 1).

In 2010, the species detected (in order of decreasing specific density), were: *Septoglomus constrictum*, *Diversispora* sp. BEV4, *Claroideoglossum claroideum*, *Funneliformis coronatus*, *Glomus badium*, *Glomus intraradices*, *Glomus macrocarpum*, *Glomus* sp. BEV2,

Scutellospora calospora, *Glomus rubiforme*, *Racocetra persica*, *Acaulospora scrobiculata*. In 2011, the species recovered from the sand dune (in order of decreasing specific density, see Fig. 2) were: *Se. constrictum*, *Fu. coronatus*, *Cl. claroideum*, *Pacispora dominikii*, *Gl. intraradices*, *Claroideoglomus etunicatum*, *Diversispora clara*, *Diversispora versiformis*, *Funneliformis mosseae*, *Glomus microaggregatum*, *Sc. calospora*, *Diversispora aurantia*, *Paraglomus occultum*, *Archaeospora trappei*, *Pacispora franciscana*, *Ac. scrobiculata*. In the field (2010) and in the bait cultures (2011), *Se. constrictum* was the most abundant species.

3.6. AMF species richness in salt marsh

In the salt marsh system, a total of 24 AMF species, also belonging to 11 AMF genera, were detected (Table 1). In the field samples from the salt marsh, 20 species were identified in 2010 belonging to 10 AMF genera. After one year of bait culturing, in 2011, 12 AMF species had reproduced spores belonging to 7 genera. Of these species, 11 were only found in the field samples (2010) and 4 only in the bait cultures (2011), while 9 species were recovered from both the field samples and the bait cultures.

In 2010, the species detected (in order of decreasing specific density, see Fig. 2), were: *Se. constrictum*, *Diversispora* sp. BEV4, *Glomus* sp. BEV2, *Entrophospora* sp. BEV3, *Gl. intraradices*, *Cl. claroideum*, *Fu. coronatus*, *Sc. calospora*, *Gl. macrocarpum*, *Fu. mosseae*, *Funneliformis geosporus*, *Gl. badium*, *Cl. etunicatum*, *Ra. persica*. In 2011, the species recovered from the sand dune (in order of decreasing specific density, see Fig. 2) were: *Pa. occultum*, *Gl. intraradices*, *Fu. coronatus*, *Cl. claroideum*, *Cl. etunicatum*, *Se. constrictum*, *Fu. mosseae*, *Di. versiformis*, *Gl. microaggregatum*, *Di. aurantia*, *Fu. geosporus*, *Archaeospora myriocarpa*, *Ar. trappei*. In the field samples, *Se. constrictum* was the most abundant species, but not in the bait cultures where *Pa. occultum* had the highest specific density.

3.7. AMF differences in both ecosystems

AMF species and genus richness was similar in both ecosystems (Table 1) with 23 respective 24 out of 30 AMF species and each 11

out of 13 AMF genera detected, respectively. *Ambispora* and *Entrophospora* genera were only found in the salt marsh but not in the dune. Other remarkable difference was the presence of *Fu. geosporus*, *Entrophospora* sp. BEV3, *Gl. microcarpum*, *Glomus* sp. BEV1, *Racocetra verrucosa*, *Ambispora gerdemanni* and *Ar. myriocarpa* only in the salt marsh. On the other hand, *Acaulospora* and *Pacispora* genera were only found in the dune (Table 1) besides the species of *Ac. scrobiculata*, *Pa. dominikii*, *Pa. franciscana*, *Gl. magnicaule* and *Gl. rubiforme*.

4. Discussion

The data presented in this paper show the AMF species found in the rhizosphere of *A. maritimus* from Cabo de Gata Natural Park, Southeast Spain, which it is a typical Mediterranean ecosystem affected by salinisation and desertification. The two sampling areas under study, dune and salt marsh, have in common high levels of salinity and the ability to accommodate common species of plants, as the target species of this study *A. maritimus*, although they differ in many physical, chemical and biological characteristics. Thus, it is not rare to detect common AMF species at both zones. Spores were isolated directly from the field samples and from one year old bait cultures, since it is well known that the use of successive and extended bait cultures detects higher number of species than extraction of spores directly from field soil (e.g. Ferrol et al., 2004). Actually, low AMF species richness was often reported for arid and semiarid ecosystems by extractions of spores from field soil, reflecting limitations in the sporulation patterns under field conditions (e.g. Stutz et al., 2000), but this might depend also on the sampling and isolation effort (Bashan et al., 2007) and we found high AMF species richness at both sites. Since the plant species is not an experimental variable, the reasons for similarities and differences of spore densities and species richness should be based on the differential characteristics between both areas (García and Mendoza, 2008). For instance, it is noteworthy that AMF belonging to the family Glomeraceae were the predominant sporulators in field and bait conditions and also presented the highest number of species diversity both in dune and salt marsh. These

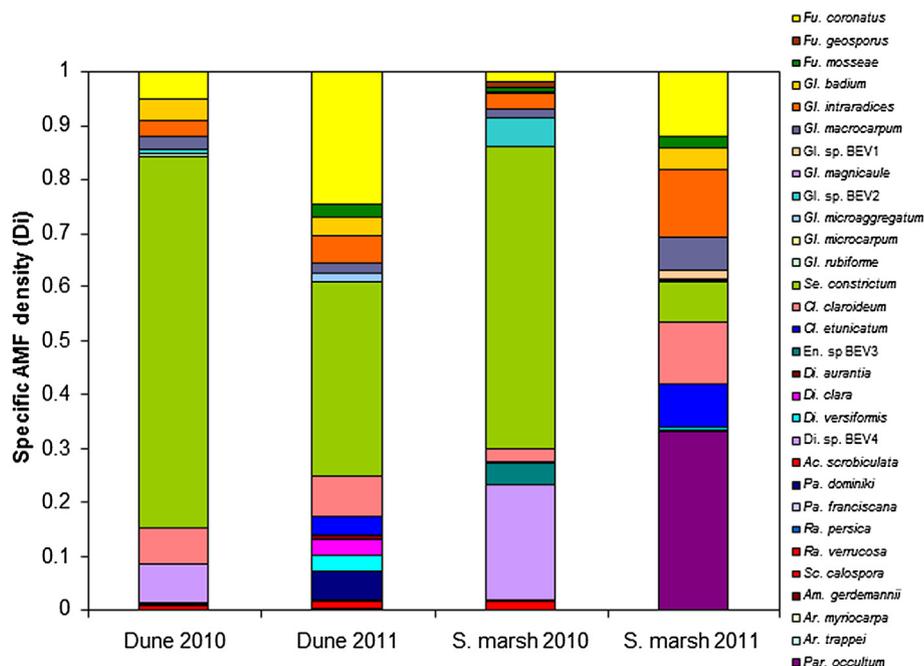


Fig. 2. Specific density (D_i), described as the proportion of individuals of one particular species in the sample relative to the total number of individuals, of AMF found in dune and salt marsh at two consecutive years. 2010 and 2011 refers to field-collected soil and to bait cultures respectively.

species may be more adapted in adjusting patterns of sporulation to environmental stress conditions (Jacobson, 1997). A similar restriction in species composition to mostly fungi belonging to *Glomus* spp. was previously reported in arid and semiarid ecosystems (Jacobson, 1997; Stutz et al., 2000).

Previous work indicated that saline soils contain up to 80% of all spores belonging to one single AMF species, *Fu. geosporus* (Landwehr et al., 2002; Sonjak et al., 2009). In agreement with previous studies, *Fu. geosporus* has been found in the salt marsh, both at field site and bait culture. However, we found higher AMF species diversity than those published, and *Fu. geosporus* was not the most abundant species. In the field samples, *Se. constrictum* was the most abundant species in both habitats while in the bait cultures it only remained predominant in the dune but in the salt marsh it was replaced by *Pa. occultum*. *Se. constrictum* and the genus *Paraglomus* have been widely found in arid and saline environments (Ferrol et al., 2004; Rodríguez-Echeverría and Freitas, 2006; Hammer et al., 2011; Wilde et al., 2009). Some other Glomeraceae species, *Gl. intraradices*, *Fu. mosseae* and *Cl. etunicatum* for instance, have been also found in saline environments (Camprubí et al., 2010; Hammer et al., 2011; Landwehr et al., 2002; Sonjak et al., 2009; Wilde et al., 2009). In the Glomerales order, three species could not unequivocally be identified, suggesting possible new species; two of them belonging to the Glomeraceae family, *Glomus* sp. BEV1 and *Glomus* sp. BEV2, and the other to the Entrophosporaceae family, *Entrophospora* sp. BEV3. The other unidentified species belong to the order Diversisporales, *Diversispora* sp. BEV4, and a new species that has been described, *Di. clara* (Estrada et al., 2011). For further identification studies, bait cultures have been established to propagate the unknown fungi from this study.

In this study, only one Acaulosporaceae, *Ac. scrobiculata*, could be found in the dune, where the genus *Pacispora* was very common and widely represented but not in the salt marsh. Controversially, Wilde et al. (2009) found a species of the genus *Pacispora*, *Pa. scintillans*, in the rhizosphere of *Puccinellia maritima* in Terschelling salt marsh. Our results also report the occurrence of *Ra. persica* among other Gigasporales species. This species has been recovered in the Northwestern coast of Italy (Turrini et al., 2008) and had previously been described from other sand dunes (Blazkowski and Tadych, 1997; Rodríguez-Echeverría and Freitas, 2006; Camprubí et al., 2010). Contrary to some results, we did not find any *Gigaspora* species in our samples while other authors found several species (Selvaraj and Kim, 2004; Rodríguez-Echeverría and Freitas, 2006). The latter may be explained according to Turrini et al. (2008) who pointed out that *Gigaspora* species are rare in ecosystems with anthropogenic disturbance.

This study confirms the existence of a rich diversity of AMF in the rhizospheric soils of Spanish saline Mediterranean ecosystems.

5. Conclusions

The study reveals a high taxonomic diversity of AMF in the rhizosphere of *A. maritimus* in Spanish saline Mediterranean ecosystems, although environments affected by salinity are dominated for non-AMF plants. The Glomerales order were the predominant sporulators under field and bait conditions, both in salt marsh and dune, and also presented the highest number of diversity of species. Common AMF species both in dunes and salt marshes were found, suggesting the importance not only of soil characteristics but the host plant. The genus *Pacispora* was very common and widely represented in dunes but not in salt marshes and we could not identify any spore belonging to *Gigaspora* genus. One new species, *Di. clara*, has been recently described and published and four other species are still under study.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jaridenv.2013.05.019>.

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