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Impact of soil salinity on arbuscular mycorrhizal fungi biodiversity and microflora biomass associated with *Tamarix articulata*Vahll rhizosphere in arid and semi-arid Algerian areas

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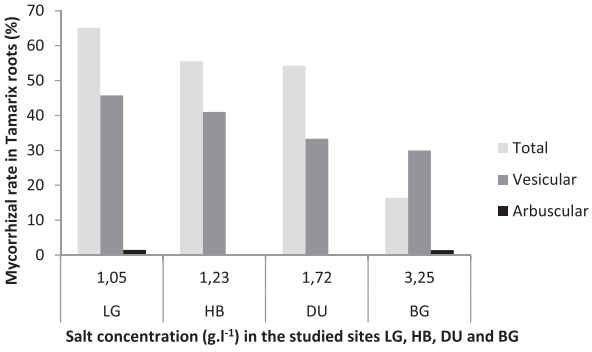
HIGHLIGHTS

- 7 arbuscular mycorrhizal fungi (AMF) species were isolated from Tamarix saline soils.
- Salinity level decreased *T. articulata* mycorrhizal rate but didn't inhibit it.
- Soil salinity increased AMF and bacteria but reduced saprotrophic fungal biomass.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil salinization is an increasingly important problem in many parts of the world, particularly under arid and semi-arid areas. Unfortunately, the knowledge about restoration of salt affected ecosystems using mycorrhizae is limited. The current study aims to investigate the impact of salinity on the microbial richness of the halophytic plant *Tamarix articulata* rhizosphere. Soil samples were collected from natural sites with increasing salinity (1.82–4.95 ds.m^{-1}). Six arbuscular mycorrhizal fungi (AMF) species were isolated from the different saline soils and identified as *Septoglomus constrictum*, *Funneliformis mosseae*, *Funneliformis geosporum*, *Funneliformis coronatum*, *Rhizophagus fasciculatus*, *and Gigaspora gigantea*. The number of AMF spores increased with soil salinity. Total root colonization rate decreased from 65 to 16% but remained possible with soil salinity. Microbial biomass in *T. articulata* rhizosphere was affected by salinity. The phospholipid fatty acids (PLFA) C16:1ω5 as well as i15:0, a15:0, i16:0, i17:0, a17:0, cy17:0, C18:1ω7 and cy19:0 increased in high saline soils suggesting that AMF and bacterial biomasses increased with salinity. In contrast, ergosterol amount was negatively correlated with soil salinity indicating that ectomycorrhizal and saprotrophic fungal biomasses were reduced with salinity. Our

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findings highlight the adaptation of arbuscular and bacterial communities to natural soil salinity and thus the potential use of mycorrhizal *T. articulata* trees as an approach to restore moderately saline disturbed arid lands.

1. Introduction

Saline soils occupy over 7% of the total earth's land surface and 70% of all agricultural soils worldwide. They have devastating and deleterious global effects and expected to be responsible of 30% land loss within the next 25 years, and up to 50% by the year 2050. Soil salinity represents an increasingly problem in many parts of the world, particularly in arid and semi- arid areas (Rengasamy, 2006). Dagar and Tomar (2002) indicate that saline soil affect plant growth adversely, by disrupting their physiological mechanisms due to toxicity of excessive Na^+ and Cl^- ions towards the cell. The toxic effects include disruption of enzyme structure and other macromolecules, disruption of photosynthetic efficiency, gas exchange, membrane organization and water status. Salinity may directly or indirectly inhibit cell division and enlargement and finally the growth, productivity and yield of the whole plant (Badda et al., 2014; Sinclair et al., 2014). In Algeria, about 2 991 013 ha lands exhibit low to high saline levels (DGF, 2008) mainly located under arid and semi-arid areas (Halitim, 1988).

Saline soils are composed of several types of salts, mainly sodium ions and calci-magnesic ions (Ramdan, 2001). To counteract this problem, many strategies have been proposed including new salt-tolerant plant (Badda et al., 2014). In fact, reforestation programs for saline soils require the proper selection of tree species and planting techniques (Dagar and Tomar, 2002). *Tamarix articulata* Vahll commonly known as *Tamarix*, could be ranked among the tolerant species for both saline soils and underground salt water (Chaudhry et al., 2013). The species was introduced in Algerian steppic areas around 1948 (DGF, 2000). Well adapted to the steppic areas, it became gradually naturalized over the years. *T. articulata* is an important halophytic evergreen tree species of *Tamaricaceae* family. It has some interesting medical value such: flower galls used as astringent and gargle, bark used for treating eczema and other skin diseases. It is used in apiculture producing a dark brown honey with minty aroma (Orwa et al., 2009). Wood is used as fiber, timber and fuel source, leaves as forage of livestock (Ozenda, 1983), galls and bark as mordant for dyeing and tree is highly valuable for stabilizing sand dunes and soil erosion (Orwa et al., 2009; Chaudhry et al., 2013). In arid and semi-arid areas like the steppic region in Algeria, harsh climatic conditions, high salt concentrations, low rainfall and poor physico-chemical soil composition are all deleterious growth environment to Tamaris. To overcome the detrimental effects and to improve plant tolerance to stresses, particularly salt stress, the plants adopt a wide variety of strategies including deep and branched root systems, scaly leaves with thick cuticle and waxy layer on the epidermis and most importantly the development of symbiosis with arbuscular mycorrhizal fungi (AMF). The AMF are obligate symbionts that colonize the roots of more than 80% of the terrestrial plants and benefit to plant growth, plant survival and soil quality (Smith and Read, 2008). The symbiotic association may also increase host plant resistance against biotic and abiotic stresses, including salinity, drought and pollution (Smith and Read, 2008). In this respect, biological processes such as AMF application to alleviate salt stress would be a potential beneficial option resulting in better increment, vigorous growth and consequently higher yield and reforestation. The use of arbuscular mycorrhizal biofertilizer technology could be an interesting way for the management of native flora and restoration of natural habitats with minimal chemical inputs. However the knowledge about restoration of salt affected ecosystems using mycorrhizae is limited. In order to evaluate the beneficial impact of AMF on *Tamarix* plant potential for saline site restoration, it is necessary to investigate the impact of salinity on the microbial richness of the soil and the ability of AMF to colonize the plant under such adverse conditions. The soil microorganisms behavior under saline growth conditions has been the subject of several studies, but the results remained controversial (Juniper and Abbott, 1993, 2006; Johromi et al., 2008; Barin et al., 2013).

Previous works on *T. articulata* mycotrophy focused on the AMF spatial distribution and root colonization (Chaudhry et al., 2013). Investigations were done on field and greenhouse grown plants, with added inoculants but never in relation with saline soils (Beauchamp et al., 2005; Kelly and Gehring, 2013). The use of native AMF inoculants isolated from indigenous soils could bring substantial benefits in restoration activities as the indigenous AMF species may demonstrate high adaptation to local condition together with low environmental risks.

The present study aimed to isolate and identify AMF spores species naturally associated with *T. articulata* rhizosphere from sites showing a gradient in their soil salinity and to evaluate AMF root colonization levels in the field. The impact of soil salinity on the viability of telluric microorganisms from *T. articulata* rhizosphere in different saline sites has been evaluated for different groups of microorganisms using specific lipid biomarkers. AMF were characterized by the presence of the phospholipids fatty acids (PLFA) and the neutral lipids (NLFA) C16:1ω5. Ergosterol was used for saprotrophic and ectomycorrhizal fungal biomass indicator and total specific PLFA were used as indicator of soil bacterial biomass.

2. Materials and methods

2.1. Experimental sites

Four different sites (LG, HB, DU and BG) located in arid and semi-arid Algerian areas were chosen in the present study according to their soil salinity concentrations (Table 1). Whereas LG and BG are sites where *T. articulata* was grown spontaneously, HB and DU are planted sites. HB was planted with *T. articulata* in 1952 in order to set ground sandy soil around train rails and DU is an urban site planted in 1985.

2.2. Soil and root sampling

Five mature *T. articulata* trees, at a minimum of 20 m apart, were selected from each site. Three roots and soil samples were collected from each tree at a depth of 40 cm and samples were stored at 4 °C until use. For each site, soil samples were collected in each season and pooled to obtain one sample/year.

Table 1
Description of studied sites.

Sites	LG	HB	DU	BG
Salinity ($g.l^{-1}$)	1.05	1.23	1.72	3.24
Site name/distance from Algiers	Laghouat 400 km southern	HassiBahbah 250 km southern	Djelfa 300 km southern	Boughzoul 185 km southern
Site localization	33°48′24″N 2°52′56″ East.	35°04′33″N 3°01′37″ East.	34°40′00″N 3°15′00″ East.	35°41′59″N 2°50′52″ East.
Altitude (m)	750	841	1131	635
Bioclimatic stage	Saharan	Semi arid	Semi arid	Arid
Vegetation	Natural vegetation	Planted in 1952	Planted urban site	Natural vegetation

2.3. Pedological parameters

The soil total nitrogen content was determined according to the Kjeldahl method (Petard, 1993). Available phosphorus was determined with Olsen et al. (1954) method. Walkley–Black method modified according to CEEAEQ (2003) was used to measure organic matter and organic carbon content. pH_{water} and Electrical Conductivity (EC) were determined according to1:5 (W/V) soil water suspension at 25 °C (AFNOR, 1995). Soil salinity was established according to (Szaboles, 1979) Calcareous was determined with Bernard Calcimeter classical method described by Petard (1993). Soil texture was determined with American method based on textural triangle (USDA, 2013).

2.4. Indigenous AMF spore isolation and identification

AMF spores were extracted from 100 g of soil for each soil sample using the wet-sieving method (Gerdemann and Nicolson, 1963). The spores were manually isolated from supernatant, classified under the dissecting microscope by spore morphotype (size, color, hyphae), and mounted in polyvinyl alcohol-lacto-glycerol (PVLG) with or without Melzer's reagent on microscopic slides. Observations were carried out under an optical microscope (x40, x60). Spore abundance was evaluated by counting the number of spores per identified or characterized species. Identification, based on morphological characters, was performed using original species descriptions using Blazkowski identification keys (Blaszkowski, 2012) and specialized websites (Blaszkowski *http://www.zor.zut.edu.pl/Glomeromycota*, and INVAM: *http://invam.wvu.edu*).

2.5. AMF root colonization

The measurement of AMF root colonization levels was performed on fresh roots. Roots were soaked during 5 min in H_2O_2 (30%) bath to remove pigments, cleared in KOH (10%) and stained with Trypan blue (0.05%) (Phillips and Hayman, 1970). Root mycorrhizal rates were calculated using magnified grid line intersect method (McGonigle et al., 1990). Two thousand root fragments from 20 plants sampled from the studied sites were observed under an optical microscope (×100), intersections were counted in the following categories: negative (no fungal material in root), arbuscules, vesicles and hyphae.

2.6. Fatty acid analyses

After removing plant debris from soil samples, four replicates of 3 g of freeze-dried soil (from each soil sample) were analyzed to measure the fatty acid content. Lipid extraction was performed according to Frostegård et al. (1991). The extracted lipids were fractionated into neutral lipids, glycolipids and polar lipids on a silica acid column by successively eluting with chloroform, acetone and methanol (1: 2: 1, V: V: V). The chloroform fraction (containing the neutral lipids) and the methanol fraction (containing the phospholipids) were subjected to a transesterification using a base solution (KOH 0.2 M) prepared in methanol to transform the PLFA and the NLFA into free fatty acid methyl esters. Final extracts were analyzed with the use of a PerkinElmer Autosystem gas chromatograph (GC) equipped with a flame-ionization detector (Norwalk, CT) and an EC-1000 (Alltech) capillary column (30 m × 0.53 mm i.d) with hydrogen as carrier gas (3.6 ml min^{-1}). The temperature program included a fast rise from 50 °C to 150 °C at 15 °C min^{-1} and then a rise from 150 to 220 °C at 5 °C min^{-1}. Fatty acid quantification was made by using nonadecanoic acid methyl ester (C19:0) as an internal standard. Their identification relied on the retention times of a wide range of standards (fatty acid methyl ester mixtures C4–C24:1, Sigma Aldrich) and mass analysis. The system was operated in the constant linear velocity (70 cm s^{-1}) using helium as a carrier gas. Fatty acids were identified by comparing their mass spectra with the standard mass spectra in the NIST MS library.

The amounts of the phospholipid fatty acids (PLFA) 16:1ω5 and the neutral lipid fatty acids (NLFA) 16:1ω5 in the soil of the different experimental plots were determined and used as indicators of the AMF biomass (Frostegård et al., 1991). The ratio between NLFA and PLFA 16:1ω5 is high in AMF (1–200), while it is low in bacteria (<1) (Olsson et al., 1997). Gram-positive bacteria biomasses were estimated by the quantification of the PLFA: i15:0, a15:0, i16:0, i17:0, a17:0 amounts and Gram-negative bacteria biomass by the quantification of the PLFA: cy17:0, C18:1ω7 and cy19:0 amounts in the soil.

2.7. Ergosterol analysis

Four replicates of 4 g freeze-dried soil from each studied site were analyzed for free ergosterol content, in order to estimate the level of living saprophytic fungi (Yuan et al., 2008). Ergosterol was extracted in 5 ml of methanol in the dark. The samples were mixed in a vortex apparatus for 1 min, extracted overnight and then refluxed at 70 °C for 90 min. After cooling, 1 ml H_2O and 2 ml cyclohexane were added. The samples were mixed in a vortex apparatus for 20 s, centrifuged for 5 min at 3000 rpm. After removal of the upper phase, ergosterol was extracted from the methanol fraction with further 1.5 ml cyclohexane. The cyclohexane was evaporated under N_2. Final extracts were analyzed with the use of a PerkinElmer Autosystem (GC) equipped with a flame-ionization detector (Norwalk, CT) and a HP5 capillary column. Ergosterol contents in the soil samples were measured using a standard curve of different ergosterol dilutions.

2.8. Statistical analysis

Multivariate analysis was elaborated with Statgraphics centerion 15.0, for analyzing soil parameters, mycorrhizal colonization, fungal and bacterial specific fatty acid content and ergosterol content, according to the least significant difference test (LSD < 5%). A correlation between soil salinity and different parameters: total spores number, mycorrhizal rate, vesicles, and total bacterial content, PLFA C16:1ω5, NLFA C16:1ω5, as well as ergosterol content was established according to Pearson test using Graphpad Prism 5.

3. Results

3.1. Soil characteristics

Physicochemical characteristics of soil samples are presented in Table 2. Soil salinity level varies from low (1.05 g l^{-1}) to high

Table 2
Physical and chemical characteristics of the soils associated with *T. articulata* rhizosphere in the four different studied sites.

	LG	HB	DU	BG
Salt ($g.l^{-1}$)	1.05^{c}	1.23^{bc}	1.72^{b}	3.25^{a}
EC ($ds.m^{-1}$)	1.82^{bc}	2.13^{b}	2.95^{b}	4.95^{a}
$pH_{(water)}$	7.99^{c}	7.78^{b}	7.73^{b}	7.48^{a}
OM (%)	1.32^{b}	0.87^{b}	1.34^{a}	1.43^{c}
$CaCO_3$ ($mg.g^{-1}$)	7.06^{b}	4.24^{a}	10.24^{c}	12.65^{d}
C org ($mg.g^{-1}$)	7.7^{b}	5.1^{a}	7.8^{b}	8.3^{c}
Available P ($mg.kg^{-1}$)	0.13^{ab}	0.14^{b}	0.1^{a}	0.21^{c}
Total N ($mg.g^{-1}$)	0.203^{a}	0.23^{b}	0.231^{b}	0.420^{c}
C : N	37.9^{d}	22.1^{b}	33.7^{c}	19.7^{a}
Moisture (%)	4.21^{a}	10.17^{d}	8.01^{c}	4.94^{b}
Sand (%)	16.03^{a}	88.91^{d}	50.24^{b}	70.50^{c}
Silt (%)	49.47^{d}	4.58^{a}	33.75^{c}	19.99^{b}
Clay (%)	34^{c}	6.5^{a}	12.00^{b}	9.5^{ab}
Texture	Loam–Clayy	Sandy	Loam–Sandy–Clayy	Sandy–Loamy

Data are presented as mean ± standard deviation. The means were obtained from four replicates (n = 4). For each parameter, different letters indicate significant difference between the sites according to the LSD test ($p < 0.05$). OM: Organic matter, EC: Electrical conductivity, $CaCO_3$: Total calcareous, C: Carbone, N: Nitrogen, C/N ratio carbon on Nitrogen.

(3.25 g l^{-1}) respectively in LG and BG sites. The high salinity soil of BG is also characterized by a high electrical conductivity (EC), 3 fold higher than the low one, and higher levels of Ca (12.6%), organic matter (1.44%), 0.42 mg.g^{-1} and slightly higher available phosphorous (0.21 mg kg^{-1}) . Soil textural analysis indicates that studied sites were mostly loam and sandy soil.

3.2. AMF diversity and root colonization

Seven AMF species were isolated from the studied sites (Table 3). According to their morphology, six species were identified: *Septoglomus constrictum*, *Funneliformis mosseae*, *F. geosporum*, *F. coronatum*, *Rhizophagus fasciculatus* and *Gigaspora gigantea*. *F. coronatum*, *S. constrictum*, *F. mosseae* and *F geosporum* species were isolated from the four locations but their abundance varied from site to site. The most abundant species was *S. constrictum* representing 34.7, 20.20, 41.8 and 43.44% of the AMF spore population respectively in LG, HB, DU and BG sites. *F. geosporum* was the most abundant in BG site making 38.5% of spore population. *F. mosseae* species population remained stable in the four sites sampled. Some isolated species were specific to one site such as *F. coronatum*, found only in the highest saline site BG and *Gi. gigantea* observed only in HB site. *S. constrictum* and *F. geosporum* spore populations tended to increase with salinity but remained quite high in all studied sites.

The AMF structures observed in stained roots were vesicles, intraradical hyphae and rarely arbuscules. Vesicles were the most frequent observed structures. Their percentages decreased with salinity level from 45.75% in the less saline site LG to 29.95% in the most saline site BG. Arbuscules were occasionally observed in BG, HB and LG sites. Total root colonization rates clearly decreased with soil salinity, being four times higher in roots from low saline than high saline soil (Table 4).

3.3. Salinity and soil microbial biomass

In order to determine the effect of salinity on microbial biomass, specific lipid biomarkers were assessed and compared between the different saline sites.

The amounts of PLFA C16:1ω5, a fatty acid used to quantify AMF in soils, were found to be 2-fold higher in the most saline sites (BG and DU) compared to the less saline sites (LG and HB) (Fig. 1). By contrast, the NLFA C16:1ω5 soil content, mainly reprsenting mainly the vesicle storage structures, was found inversely correlated to soil saline concentrations. It was 15 times higher in the less saline site (LG) than in the most saline one (BG) (Fig. 2). The NLFA /PLFA C16:1ω5 ratio was found much higher in the lowest saline soil LG (ratio = 10) than in the most saline site BG (ratio = 0.3) (Fig. 3).

Ergosterol content, used to quantify ectomycorrhizal and saprotrophic fungi, was less important in HB, DU, BG saline soils by comparison to LG site (Fig. 4).

Gram negative biomass (estimated by the sum of the PLFA cy17:0, C18:1ω7 and cy19:0) as well as Gram positive bacteria (quantified by the sum of the PLFA : i15:0, a15:0, i16:0, i17:0 and a17:0) increased by 2.3, 3.3 and 2 fold higer inHB, DU and BG saline sites respectively by comparison with LG site. However, ergosterol content was less higher in the most saline site BG compared to HB and DU (Fig. 5).

Table 3
Native AMF spore species isolated from the different soils associated with *T. articulata* rhizosphere in the different studied sites.

	LG	HB	DU	BG
Septoglomus constrictum	433	271	594	977
Funneliformis mosseae	393	370	341	390
Funneliformis geosporum	324	523	343	745
Funneliformis fasciculatum	nd	46	92	nd
Funneliformis coronatum	nd	nd	nd	64
Gigaspora gigantea	nd	131	nd	nd
Funneliformis sp1	97	nd	49	74
Total spore number /100 g of soil	1247	1341	1419	2248

nd : not detected.

Table 4
Arbuscular mycorrhizal fungal colonization rates of *T. articulata* roots in the studied sites.

Sites	Soil salinity (g l^{-1})	Total colonization rate (%)	Arbuscules (%)	Vesicles (%)
LG	1.05^{c}	65.12^{a}	1.50^{a}	45.75^{d}
HB	1.23^{bc}	54.02^{b}	0.10^{b}	41^{c}
DU	1.72^{b}	55.66^{a}	nd	33.33^{b}
BG	3.24^{a}	16.43^{b}	1.44^{a}	29.95^{a}

Data are presented as mean ± standard deviation. Means were obtained from four replicates (n = 4). For each parameter, different letters indicate significant difference between different sites according to the least significant difference LSD test ($P < 0.05$). nd: not detected.

3.4. Correlation analysis

Pearson's correlation coefficients for the different measured parametres in *T. articulata* rhizosphere versus soil salinity level are presented in Table 5. Correlation analysis showed that AMF root colonization, vesicles, NLFA C16:1ω5 and NLFA/PLFA C16:1ω5 ratio were negatively correlated with soil salinity levels. However, PLFA C16:1ω5 and total spore number were positively correlated with soil salinity concentration. No correlation was found between soil salinity level ergosterol and total bacterial amounts.

4. Discussion

The first objective of the present study was to investigate the biodiversity of indigenous AMF species associated with *T. articulata* rhizosphere in four sites characterized by a gradient of soil salinity. The morphospecies of the Glomeraceae family clearly dominated in these ecosystems with the exception of *Gi. gigantea* (Gigasporaceae) that occurred to be an auxiliary species found in a unique site of medium low salinity level. Glomoid spore species, that make the majority of AMF species, are worldwide distributed and recognized to be well adapted to environments subjected to disturbance and stresses (Daniel et al., 2001). Several studies have shown that *Glomus* species are typical of semi-arid Mediterranean ecosystems and can support high salinity soil conditions (Juniper and Abbott, 2006; Sànchez-Castro et al., 2012). The few studies dedicated to the AMF populations of Tamarix rhizosphere confirmed the abundance of Glomeraceae (Beauchamp et al., 2005; Kelly and Gehring, 2012; Chaudhry et al., 2013; Taniguchi et al., 2015). Among the species found in Tamarix rhisosphere, *F mosseae* is the one having a worldwide distribution (Öpik et al., 2009). It is

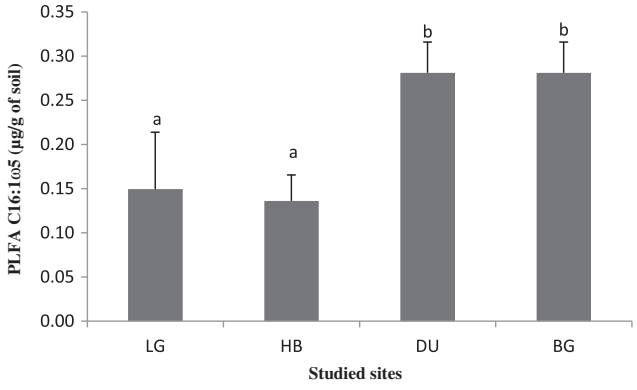

Fig. 1. Influence of soil salinity on PLFA C16 :1ω5 amount of the soils associated with *T.articulata* in the four studied sites. Data are presented as mean ± standard deviation. Means were obtained from four replicates (n = 4). Different letters indicate significant difference between the sites according to the LSD test ($p < 0.05$).

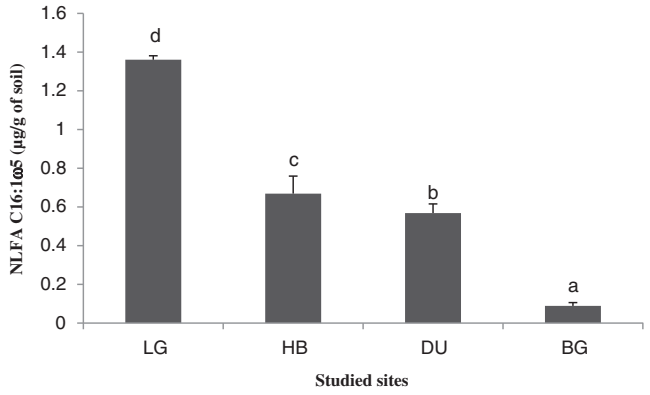

Fig. 2. Influence of soil salinity on NLFA C16:1ω5 amount of the soils associated with *T. articulate* in the four studied sites. Data are presented as mean ± standard deviation. Means were obtained from four replicates (n = 4). Different letters indicate significant difference between the sites according to the LSD test ($p < 0.05$).

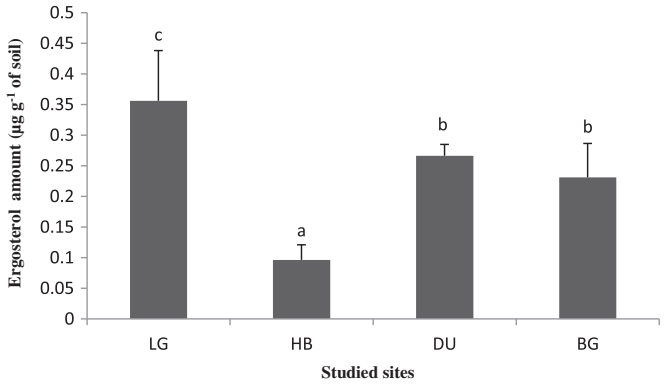

Fig. 4. Influence of salinity on ergosterol amounts of the soils associated with *T. articulata* in the four studied sites. Data are presented as mean ± standard deviation. Means were obtained from four replicates (n = 4). Different letters indicate significant difference between the sites according to the LSD test ($p < 0.05$).

considered highly tolerant to deleterious environmental conditions and capable of adaptation strategy to soil disturbances (Sýkorová et al., 2007), pollution (Hassan et al., 2011), cold (Gai et al., 2009), drought (Mohammad et al., 2003; Tian et al., 2009) and salinity (Krishnamoorthy et al., 2014). The predominant species in Tamarix rhisosphere, *F. geosporum* was often detected under saline environments. Landwehr et al. (2002) found that *F. geosporum* tolerated the European saline, sodic and gypsum soils and has been regularly identified from salt marshes ecosystems (Hildebrandt et al., 2001; Carvalho et al., 2003; Wilde et al., 2009). The species was recently found abundant in the rhizosphere of *T. ramosissima* growing under high salinity soil condition (Taniguchi et al., 2015).

Based on the present data, the number of AMF spores found in the rhizosphere of *T. articulata* increased with soil salinity level. Indeed, a positive Pearson coefficient correlation ($r = 0.7692$, $p < 0.001$) was found between the soil salinity concentration and the spore number. This is in agreement with numerous studies that reported high AMF spore populations in saline soils associated with different halophytic plants (Aliasgharzadeh et al., 2001; Hildebrandt et al., 2001; Landwehr et al., 2002). This could be considered a survival strategy implemented by AMF species, sporulation being a resistance behavior to help surviving adverse environmental conditions. In fact, AMF sporulation is recognized to be stimulated under salt-stress conditions (Hirrel, 1981; Aliasgharzadeh et al., 2001). McMillen et al. (1998) reported that the inhibition of AMF spore germination and of hyphal growth by NaCl resulted in an increase of spore populations in saline soils.

Tamarix AMF root colonization clearly decreased with soil salinity, showed by a significant negative correlation between mycorrhizal rate and soil salinity ($r = -0.7988$, $p < 0.001$). These results corroborate several studies that reported a reduction in root mycorrhizal rates at high salinity level (Giri et al., 2007; Asghari et al., 2008; Badda et al., 2014; Taniguchi et al., 2015). Even though most of those investigations were done under controlled conditions, their similarity with the present data obtained from natural environments confirmed the general behavior of AMF under saline growing conditions. Moreover, the vesicle percentages decreased with salinity level. This result was consistent with the amounts of NLFA C16:1ω5 which were found to be negatively impacted by salinity. Indeed, the neutral lipids mainly represent vesicle storage products (Olsson et al., 1995). By contrast, whatever the site, arbuscules were scarce. Allexander et al. (1988) suggested that soil salinity level may alter life duration of arbuscules. However, one cannot exclude that the low percent of arbuscules in *Tamarix* roots may be due to the field sampling. *Tamarix* root systems from natural environments required delicate manipulation to be extracted properly from compacted steppic soils. As a result, several of the fragile root apex may be destroyed at harvesting, lowering proportionally the level of arbuscules detected after root staining. Indeed, it is well known that arbuscules inhabit preferentially the first cm of fine root apex (Smith and Read, 2008). Contrary to herbaceous plants, woody plants such as the *T. articulata*, have woody roots rich in tannins and a root morphology which is not conducive to colonization by hyphae. As a consequence, the only non-pigmented root tips developing close to the soil surface served to evaluate root colonization levels.

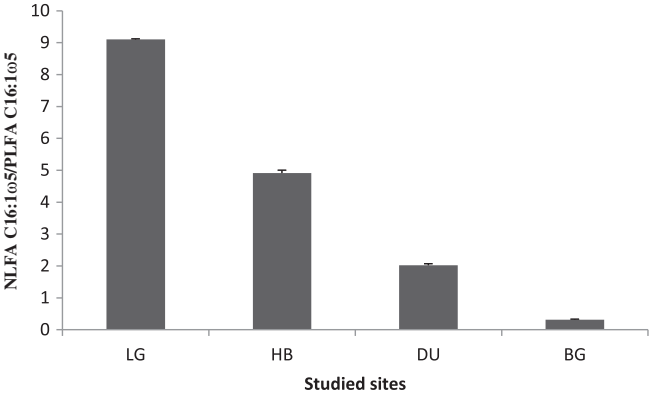

Fig. 3. Influence of salinity on NLFA/PLFA ratio C16: 1ω5 amount of the soils associated with *T. articulata* in the four studied sites. Data are presented as mean ± standard deviation. Means were obtained from four replicates (n = 4). Different letters indicate significant difference between the sites according to the LSD test ($p < 0.05$).

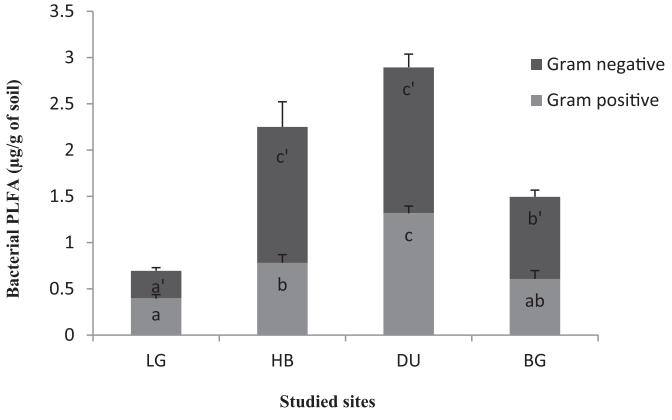

Fig. 5. Influence of soil salinity on bacterial PLFA amounts of the soils associated with *T. articulata* in the four studied sites. Data are presented as mean ± standard deviation. Means were obtained from four replicates (n = 4). Different letters indicate significant difference between the sites according to the LSD test ($p < 0.05$).

Table 5
Pearson's correlation coefficients between the differents AMF parametres and soil salinity level.

Parameter	Pearson r	P value summary
Spore number	0.7692	***
Mycorhizal rate	−0.7988	***
vesicles	−0.8297	****
Total bacterial PLFA	0.01541	ns
NLFA/PLFA	−0.6232	**
NLFA C16:1w5	−0.8329	****
PLFA C16:1 W5	0.728	**
Ergosterol	0,0684	ns

Pearson r: Pearson coefficient of parametric correlation sampled from Gaussian population. R square: coefficient of determination. ns: No significant correlation. **correlation is significant at $p < 0.01$, *** correlation is significant at $p < 0.001$, ****correlation is significant at $p < 0.0001$.

Several authors explained that salinity can affect directly the fungal development, reducing spore germination, hyphal formation and consequently host root colonization. Indeed, salinity could reduce AMF colonization directly by affecting spore germination (Porcel et al., 2012; Evelin et al., 2009; Juniper and Abbott, 2006). In saline site, the colonization process may be stopped because of dry and environmentally unwelcoming soil conditions. The propagule cannot be properly hydrated and unable to germinate with as a result, spore accumulation maintained under long term dormancy stage. Johromi et al. (2008) explained that salinity induced hyphae morphological changes especially in their elongation and ramification behavior, thus affecting their symbiotic capacity. Juniper and Abbott (2006) reported that under *in vitro* growth conditions, the germination rates and timing in presence of NaCl differed greatly between the AMF strains and for all strains, the hyphal length produced per germinated spore at a given time was reduced in the presence of NaCl. For some fungi, the reduction in the amount of hyphae in the presence of increasing concentrations of NaCl was primarily associated with a delay in spore germination, while for other fungi the specific rate of hyphal production was also substantially reduced in the presence of NaCl. Otherwise, it cannot be totally excluded that low mycorrhizal rate in saline soil can be explained by spore dormancy and subsequent activation in response to relatively specific signals that allow fungi to survive in soil when conditions are unfavorable (Sussman, 1976). The decrease in AMF colonization with increasing soil salinity may be due to plant physiological changes directly affecting their symbionts (Juniper and Abbott, 1993).

The impact of salinity on the microbial biomass was investigated through the quantification of specific lipid biomarkers for AMF, saprotrophic and ectomycorrhizal fungi as well as bacteria. All the specific lipid biomarkers tested allowed to detect strong response of microbial soil organisms to salinity. The ratio NLFA/PLFA has been shown to decrease with salinity. These results are consistent with those of Barin et al. (2013), which reported a negative correlation between the ratio NLFA/PLFA C16:1ω5 and soil salinity in the rhizosphere of *Medicago* and *Allium*.

The increase of PLFA 16:1ω5 and the decrease of the NLFA/PLFA 16:1ω5, until reaching a value <1 in the most saline soil, could be due to an increase of the bacterial community. In fact, the bacterial specific biomarkers indicate that the total bacterial biomass increase with soil salinity. Likewise, Frey-Klett et al. (2007) explained that under soil stress, microbial communities were dominated mostly by aerobic bacteria. Mendpara et al. (2013) reported that the majority of bacterial species that can tolerate saline soil belong to Gram negative bacteria especially to *Pseudomonas spp*. The association AMF/*Pseudomonas spp*. could serve as the ideal bioinoculant to promote growth for crops in saline soils (Nakbanpote et al., 2014). To support Gram negative bacteria potential in mycorrhizal plant interaction, most bacteria found enkysted in the mucilaginous outside AMF spore layer and soil hyphae belonged to Gram negative bacteria (Roesti et al., 2005). Some bacterial species are responsible for multiple helper effects because they influence both plants and associated mycorrhizal fungi (Bonfante and Anca, 2009).

Furthermore, our findings pointed out that ectomycorrhizal and saprotrophic fungal biomasses decreased when soil salinity increased, as measured by ergosterol content, the specific lipid biomarker of those fungi categories (Wichern et al., 2006; Ben-David et al., 2011). Ishida et al. (2009) and Hrynkiewicz et al. (2015) recorded relatively low frequency of ectomycorrhizal fungi in saline soils. Likewise, for saprotrophic fungi, Zizzo (2009) observed a decrease in growth and in hyphal density of four studied saprotrophic fungi in saline soil (*Laetiporus sulphureus*, *Ganoderma lucidum*, *Trametes versicolor* and *Pleurotus ostreatus*). Hyperosmotic stress in fungi is associated with inhibition of cell wall extension and cellular expansion, leading to a growth reduction. Moreover, an excess of Na^+ and/or Cl^- ions in fungal cells may alter enzymatic activity and nucleic acid structure decreasing the potential of ectomycorrhizal fungi for successful colonization of the plant roots (Bois et al., 2006; Hrynkiewicz et al., 2015).

The lowest ergosterol amount found in HB site may be due to the less organic carbon content in this soil (Table 2). Indeed, Montgomery et al. (2000) explained that when the organic carbon level in soils is moderate, the fungal ergosterol content is lower.

5. Conclusion

This first investigation on the microbiome associated with *T. articulata* saline rhizosphere in Algerian steppic arid and semi-arid areas revealed interesting AMF diversity and *T. articulata* root colonization under adverse saline environmental conditions. Moreover, our findings showed that whereas salinity has slight detrimental effects on soil saprotrophic biomass in *T. articulata* rhizosphere, bacterial biomass increased with salinity level. This study highlighted the ability of microbial community to adapt to the increased natural soil salinity and thus the potential use of mycorrhizal *T. articulata* trees as an approach to restore the microbiome in saline disturbed arid lands.

In the future, it will be interesting to use the indigenous AMF isolated from the naturally saline soils to produce a mycorrhizal inoculants tolerant to salinity in order to use it in saline soil restoration programs.

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