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Diversity of Arbuscular Mycorrhizal fungi in two perturbed ecosystems (dune and saline soil) in west Algeria

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ABSTRACT: AMF (Arbuscular Mycorrhizal fungi) diversity and population level was investigated in dune and saline regions in west Algeria, in association with different plants species. Sampling was conducted in four localizations (two for saline soil and dunes respectively). All studied plant species formed endomycorrhizal symbiosis but no ectomycorrhizal fungi were detected. In dune plant species, mycorrhizal colonization level was more than 80%, comparatively to halophyte plants where it was low. AMF spores densities average recorded in studied soils was ranged from 41 to ≥300 spores/100g of dried soil. Saline soils were the richest in AMF spores with ≥300 spores/100 g of dried soil. The morphological characters indicated that the spore populations consisted of 9–11 morphotypes. These spores belonged to *Glomineae* order and were grouped into three families of *Gigasporaceae, Glomaceae,* and *Acaulosporaceae.*
Keywords: Degraded sites; diversity; endomycorrhizal fungi; salinity; sand pit.

INTRODUCTION

Ecosystems disruption in Algeria as in Mediterranean countries was largely encountered due to natural and anthropological constraints, especially in coastal dunes and saline soils (Gaucher and Burdin, 1974; Ghodbani, 2008). Predominant form of salt in saline soils was NaCl, leading to increased sodium content and then affecting other minerals absorption (Greenway and Munns, 1980). On another hand, dune soils were generally poor in nutrients and water (Fisher et al., 1978; Hatimi, 1995), and the mobility of elements in particular phosphorus was reduced by low humidity level (Olson et al., 1961). Despite adverse conditions, these soils can harbor fungal microflora (Nicolson et Johnston, 1979; Hatimi, 1995) such as vesicular and arbuscular mycorrhizal fungi, which are ubiquitous microorganisms acting as an important factor in nutrient cycling regulation. These fungi were associated with many plant species on dunes and saline soils (Aliasgharzadeh et al., 2001; Hatimi and Tahrouche 2007; Karaarslan and Uyanöz, 2011). This symbiosis was one of the tolerance strategies developed by plants under stress conditions (Entry et al., 2002), contributing indirectly to dune fixation by sand grains aggregates formation (Koske et al., 1984), and playing an important role in improving growth and tolerance of plants in saline soils (Cantrell and Linderman, 2001; Zuccarini and Okurowska, 2008).

Vegetation degradation increases the disruption of these affected areas by erosion occurrence with the subsequent loss of soil and organic matter (Duponnois et al., 2001). To restore this vegetation; edaphic, botanical and microbiological analyzes of degraded soils are needed. Indeed, microbiological analyzes allow to understand the distribution of microbial flora, especially AMF fungi. Among these fungi present in disturbed areas, we could select effective indigenous isolates for later use in revegetation programs.

MATERIALS AND METHODS

Sites of study localization and samples collection

Four degraded sites were selected on the basis of salinity and dune nature (Figure 1). The first site was a sand pit situated in Terga which is a coastal region (Ain Témouchent); soils were collected from the rhizosphere of *Acacia saligna* and *Lotus creticus,* named respectively AT and LT. The second site was the sand pit of Ouled Boudjemaa in Ain Témouchent; soil was collected from the rhizosphere of *Lotus creticus* and named LB. The third and fourth sites were saline sites in Terga (Ain Témouchent) (SST) and Ain Skhouna (Saida) (3S); soils were collected for both from the rhizosphere of *Arthrocnemum macrostachyum.*

A bulk of soil from each site of sampling was obtained from five sub-samples point collected at 50 cm around plants chosen randomly and at a depth of 0-20 cm. Similarly, samples of fine roots were collected from specified plants.

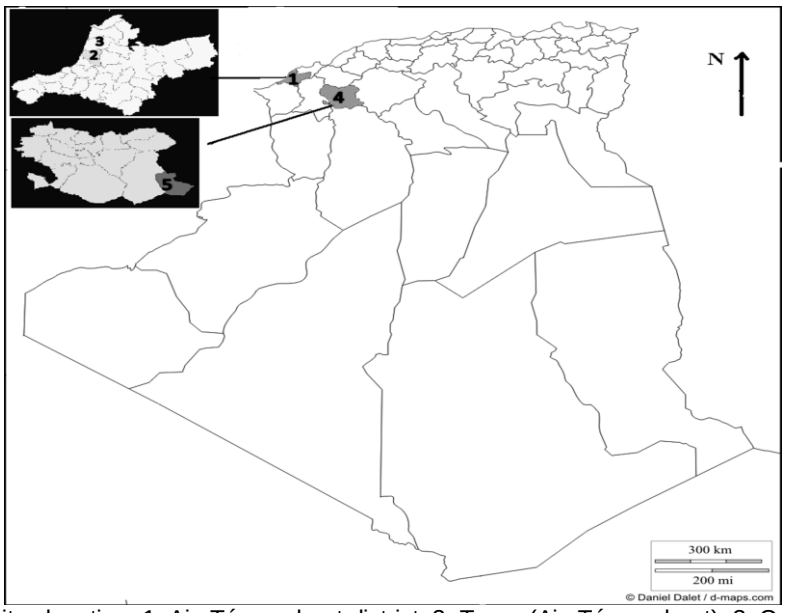

Figure 1. Sampling sites location. 1: Ain Témouchent district, 2: Terga (Ain Témouchent), 3: Ouled Boudjemaa (Ain Témouchent), 4: Saida district, 5: Ain Sekhouna (Saida).

Root clearing and staining

To reveal fungal structures, root samples were prepared following Philips and Hayman (1970) protocol. Treated roots were cut into 1 cm pieces. Twenty to thirty fragments randomly selected were crushed on slides in glycerol at the rate of 10 fragments per slide with three replicates. Fragments were observed under a photonic microscope (Grx40) to estimate AMF colonization parameters as described by Trouvelot et al., (1986): F: Mycorrhizal frequency in the root system, M: Mycorrhizal intensity in the root system.

Trapping culture

For each site, inocula consisted in rhizospheric soil containing plants root of interest, cut into small pieces and mixed thoroughly with the soil. The used substrate was sand which was autoclaved three times at 120 °C for one hour. The Morton and Walker (1992) trapping protocol was followed. Two replicates and one non AMF pots serving as control were grown under similar conditions. HYBRIDE PICO variety of maize was used as trapping plant. Seeds were disinfected in 3.8% sodium hypochlorite for 15 minutes and thoroughly rinsed with sterile distilled water. The pots were closed with perforated lid to allowed seedling emergence. Irrigation with sterile distilled water was applied daily; Hoagland's solution (Hoagland and Arnon 1938) was used when nutritional deficiencies were observed. The experiment was conducted in a growth chamber with controlled conditions (temperature 25°C and photoperiod of 16 h). After 42 days of culture, maize plants were harvested and the substrate of each pot was collected and dried at laboratory temperature.

Extraction, counting and morphological characterization of AMF spores

Spores extraction was realized on the basis of Gerdemann and Nicolson (1963) method. Soils collected from trapping culture were used for spore's morphological characterization, while spores' counting was performed on *in situ* sampled soils. Number, richness and relative abundance of fungal spores were estimated per 100 g of dry soil under binocular microscope. Morphological characterization of AMF spores was performed under photonic microscope, MOTIC version 2.1 connected to a computer. Spore identification was based on morphological features (color, shape, size, wall structure, hyphal attachment…etc). Species distinction and description were referred to the identification key of International Culture Collection of Arbuscular and Vesicular Arbuscular Mycorrhizal Fungi (INVAM) (http//www.invam.caf.wvu.edu/).

Statistical Analysis

Results were subjected to variance analysis with one factor (ANOVA) using SPSS 8.0 software; the averages were compared by multiple comparisons according to Tukey test.

Diversity of AMF in studied sites was estimated with diversity indices of Shannon and Weiner (1949) cited by Sokpon (1995); by Holou (2002); and Evenness indices of Piélou (1966).

H= - ∑ Ni / N log2 (Ni/ N)

H: Shannon diversity index; Ni: number of type; i individuals in a given category; N: total number of individuals of all types in a given category; Log2: logarithm base 2.

E= H / log2 S

E: Evenness index of Piélou; H: Shannon diversity index; S: numbers of species encountered; Log2: logarithm base 2.

RESULTS AND DISCUSSION

Physical and chemical analyzes of soil

Physical and chemical analysis (Table 1) showed that the three dunes soils of Ain Témouchent site were sandy or clay-loam sandy non saline with alkaline pH, high rate of nitrogen and low to moderate rate of phosphorus. 3S (Saida soil) and SST (Terga soil) soils were saline soils with EC (electrical conductivity) of 9.45 mS and 28.4 mS respectively. They were rich in nitrogen and poor in phosphorus, with alkaline pH and were characterized by respectively very fine silty and silty clay fine texture.

Table 1. Geographical, climatic and pedological soil characteristics of five studied soils.

	Localization	Climate	Clay	Silt	Sand	pH	EC (mS)	Total N %	Assimilable P (ppm)	Organic Matter %
AT	35° 25' 07" Nord 1° 10' 39" West	Semi-arid	1.58	13.74	84.68	8.95	0.0893	0.28	50	0.86
LT	35° 25' 07" Nord 1° 10' 39" West	Semi-arid	1.04	9.31	89.58	9.07	0.0893	0.39	20	2.13
LB	35° 28' 23" Nord 1° 11' 33" West	Semi-arid	24	12	64	8.26	0.0317	0.4	17.5	2.49
3S	34° 30' 20" Nord 0° 50' 59" East	Semi-arid	6.65	86.31	7.04	8.42	9.45	0.45	20	5.17
SST	35° 25' 07" Nord 1° 10' 39" West	Semi-arid	37.29	61.83	0.88	8.12	28.4	0.36	15	3.65

EC: electrical conductivity; mS: milli-Siemens.; N: Nitrogen; P: Phosphorus; ppm: parts per million.
AT: rhizosphere of Acacia saligna in Terga sand pit (Ain Témouchent); LT: rhizosphere of Lotus creticus in Terga sand pit (Ain Témouchent); LB: rhizospheres of Lotus creticus in Ouled Boudjemaa sand pit (Ain Témouchent); 3S: rhizosphere of Arthrocnemum macrostachyum in Ain Skhouna (Saida); SST: rhizosphere of Arthrocnemum macrostachyum in Terga (Ain Témouchent).

Sandy soils are generally poor in phosphorus (Ranwell, 1972; Koske and Halvorson 1981) and nitrogen (Fisher and Turner, 1978; Hatimi and Tahrouche, 2007). However, the high rate of nitrogen observed in studied dune soils was probably due to the beneficial effect of nitrogen fixation by various legumes species present in the site in association with soil bacteria, named rhizobia. For example, it is well known that Acacia species have an important role in improving soil nitrogen status (Dommergues 1994). This improvement resulted from the contribution of organic matter rich in nitrogen by root and shoots renewal and mainly by the fallout of litter (Bernhard-Reversat et al., 1998).

A difference in microbial activity between the studied soils was indicated by the difference in organic matter percentage which was low in AT (***A**cacia saligna* rhizosphere of **T**erga), moderate in LT (***L**otus creticus* rhizosphere of **T**erga), LB (***L**otus creticus* rhizosphere of Ouled **B**oudjemaa) and SST and high in 3S. Indeed, the region of Ain Sekhouna (3S) is a pastoral area which explains the large amount of organic matter of animal origin.

Natural root AMF colonization

Microscopic analysis of stained roots showed the presence of different endomycorrhizal structures except arbuscules in *Arthrocnemum macrostachyum,* halophytes living in saline soils. A difference in root colonization rate was noted between dunes and saline sites plant species (Table 2).

Root colonization rate was significantly lower in *Arthrocnemum macrostachyum* halophyte of Terga saline site (SST) compared to the same species sampled from Saida saline site (3S) ($p \leq 0.05$), this was negatively correlated with high salinity level of Terga saline soil (SST). *Arthrocnemum macrostachyum* species belongs to the so called non-mycorrhizal family of *Chenopodiaceae*, but it was screened for AM fungal association.

Low rate of mycorrhizal colonization observed in halophytes root was explained by the negative effect of salinity on plants and their endomycorrhizal symbiosis (Hirrel, 1981; McMillen et al., 1998; Jahromi et al., 2008; Evelin et al., 2009). It affects negatively the colonization ability; spore germination and hyphae fungal growth (Hirrel and Gerdemann, 1980; Menconi et al., 1995; Sheng et al., 2008), probably due to the direct effect of NaCl on AMF (Juniper and Abbott, 2006). Indeed, Tian et al., (2004); Sheng et al., (2008) reported that salinity reduced or eliminated the AMF formation.

Absence of arbuscules (which are the main site of nutrient transfer) in halophyte species living in saline conditions indicates the establishment of a non functional symbiosis (Hirrel et al., 1978; Malloch and Malloch, 1981). Plenchette and Duponnois (2005) hypothesized that a third type of mycorrhizae, without arbuscules, could exist when *Chenopodiaceae* were associated with AMF.

Table 2. Mycorrhizal frequency and intensity parameters (F: Mycorrhizal frequency. M: Mycorrhizal intensity) of different AMF species issued from studied soils.

	LB	LT	AT	3S	SST
F%	92.31[b]	89.70[b]	88.88[b]	78.30[b]	25.71[a]
M%	40.08[a]	24.46[a]	38[a]	28.26[a]	1.91[a]

Values followed by the same letter on the same line are not significantly different at P ≤ 0.05.
AT: rhizosphere of *Acacia saligna* in Terga sand pit (Ain Témouchent) ; LT: rhizosphere of *Lotus creticus* in Terga sand pit (Ain Témouchent) ; LB: rhizosphere of *Lotus creticus* in Ouled Boudjemaa sand pit (Ain Témouchent) ; 3S: rhizosphere of *Arthrocnemum macrostachyum* in Ain Skhouna (Saida); SST: rhizosphere of *Arthrocnemum macrostachyum* in Terga (Ain Témouchent).

Occurrence of mycorrhizal associations with sand dune plant roots was observed by several authors (Stahl, 1900; Asai, 1934; Jehne and Thompson, 1981; Read 1989), indicating that dominant plants and pioneer grasses were normally associated with AMF. However, the mycotrophic nature of studied dune species may explain the high mycorrhizal frequency levels (F %) (Table 2). Karaarslan and Uyanöz (2011) reported that plants growth was more dependent on endomycorrhizal symbiosis in arid and semi-arid areas. Indeed, factors affecting distribution and plants AMF colonization in dunes were: plant species, dunes stability level, seasons, climate, organic matter amount and microbial activity (Nicolson, 1960; Giovannetti and Nicolson, 1983; Giovannetti, 1985).

Sand dunes exhibit favorable conditions for the association and development of AMF with plants since they are deficient in phosphorus (Ranwell, 1972; Koske and Halvorson, 1981). The importance of AMF for the growth and succession of plant species in dunes was first recognized by Nicolson, (1959). Aggregation of sand grains and colonization of AMF with dune plants significantly stabilize the sand dunes (Sutton and Sheppard, 1976; Koske and Polson, 1984). Several temperate dunes: northeastern United States (Koske and Halvorson, 1981; Koske, 1987), Italy (Giovannetti and Nicolson, 1983; Giovannetti, 1985), Poland (Blaszkowski, 1997), Scotland (Nicolson 1960; Nicolson and Johnston 1979) and Morocco (Hatimi and Tahrouch, 2007) have been surveyed for the occurrence of AMF.

From these results, it appears that mycorrhizal colonization showed differences depending on the site of sampling and no on plant species.

Despite soils poverty of studied soils in phosphorus, there were major pioneer vegetations, du probably to endomycorrhizal microflora. These microsymbionts generally stimulate the growth of host plants, especially in soils deficient in phosphorus (Mousain et al., 1997), thanks to their benefit effect in the phosphate nutrition of host plants (Hatch, 1937; Landeweert et al., 2001). In fact, Phosphate transfer by AMF was demonstrated by Jakobsen et al., (1992). Harrison and van Buuren, (1995) identified phosphate transporters (GVPT, GIPT and GmosPT) which are involved in the fungus phosphate absorption from the soil. Solaiman et al., (1999) showed a phosphate accumulation in the vacuole, a polyphosphate form, at the extramatrical hyphae.

Richness of AMF spores in studied sites

Spores average number, relative abundance and morphotype were consigned in table 3. The average number of AMF spore was significantly high in saline soils of Saida and Ain Témouchent (3S and SST), with more than 300 spores per 100g of dried soil, while it was low in dune soils with 73s/100 gss at LB, 41s/100 gss at LT and 37s/100gss at AT ($p \leq 0.05$) (Table 3).

Aliasgharzadeh et al., 2001 reported that AMF may produce spores at low root colonization levels in severe saline conditions. AMF spores richness of saline soils has been reported by many authors (Aliasgharzadeh et al., 2001; Landwehr et al., 2002). Authors reported a relatively high spore number that did not significantly decreased with soil salinity. It could be resulted from stimulated sporulation under salt stress (Tressner and Hayes, 1971); or it could be due to the fact that salt inhibit spore germination and AMF hyphae growth (McMillen et al., 1998).

AMF spore density in saline soils of Ain Témouchent (SST) and Saida (3S) was higher than those obtained by Carvalho et al., (2001) in salt marsh in Portugal.

In dune soils, reduced number of AMF spores was related significantly ($p \leq 0.05$) to the low amount of organic matter, and probably to the degraded state of these soils. In fact, a reduced density of indigenous mycorrhizal propagules had occurred in degraded region (Sieverding, 1991) caused by soil texture (Brundrett, 1991). According to this author, sandy soils were not favorable to AMF formation, while high colloidal soils favor it; wich was the case of saline soils of Saida and Ain Témouchent.

Li et al., (2007) also showed that endomycorrhizal colonization and spore density varied greatly between plant species and different types of substrate. Indeed, spores density was lower in *Acacia saligna* (AT)

and *Lotus creticus* (LT) of Terga compared to *Lotus creticus* of Ouled Boudjemaa (LB). This result was positively and significantly correlated with root mycorrhizal colonization of these species ($p \leq 0.01$).
According to Cordoba et al., (2001), structural complexity level, vegetation diversity and dune stabilization level influence AMF spores abundance. These parameters were higher in old dunes than mobile and young coastal dunes (Koske, 1975).

Table 3. Average number, diversity and relative abundance of AMF spores morphotypes isolated from studied soils in west Algeria.

	LB		LT		AT		3S		SST	
	Spores number	Spores relative abundance	Spores number	Spores relative abundance	Spores number	Spores relative abundance	Spores number	Spores relative abundance	Spores number	Spores relative abundance
Black	5.6137	7.69%	3.9442	9.62%	3.441	9.30%	0	0	20.55	6.85%
Red	12.3589	16.93%	10.25	25%	6.882	18.60%	27.9	9.30%	0	0
Dark brown	20.2137	27.69%	10.25	25%	12.047	32.56%	52.32	17.44%	24.66	8.22%
Light brown	13.4758	18.46%	0	0	0	0	83.73	27.91%	49.32	16.44%
Yellow	16.8484	23.08%	5.5186	13.46%	4.3031	11.63%	136.05	45.35%	168.48	56.16%
Beige	0	0	7.093	17.30%	4.3031	11.63%	0	0	0	0
Orange	4.4895	6.15%	3.9442	9.62%	6.0236	16.28%	0	0	36.99	12.33%
Total N	73[b]		41[a]		37[a]		≥ 300[c]		≥ 300[c]	
Morphotype	11 [a]		10 [b]		9 [c]		9 [c]		6 [d]	

Values followed by the same letter on the same line are not significantly different at $P \leq 0.05$.
N: total number of individuals of all types in a given category.
AT: rhizosphere of *Acacia saligna* in Terga sand pit (Ain Témouchent); LT: rhizosphere of *Lotus creticus* in Terga sand pit (Ain Témouchent); LB: rhizospheres of *Lotus creticus* in Ouled Boudjemaa sand pit (Ain Témouchent); 3S: rhizosphere of *Arthrocnemum macrostachyum* in Ain Skhouna (Saida); SST: rhizosphere of *Arthrocnemum macrostachyum* in Terga (Ain Témouchent).

Repartition and specific diversity of Glomale in studied sites

According to Morton and Benny, (1990) classification, 45 morphotypes were detected in the five studied soils, in Terga (AT, LT, SST); Ouled Boudjemaa (LB) and Saida (3S), ranged from 6 to11. Most of the morphotypes were common to all sites, and few were specific (table 3, fig 2). All these morphotypes belonged to the *Glomineae* order represented by three families (*Gigasporaceae*, *Glomaceae* and *Acaulosporaceae*).

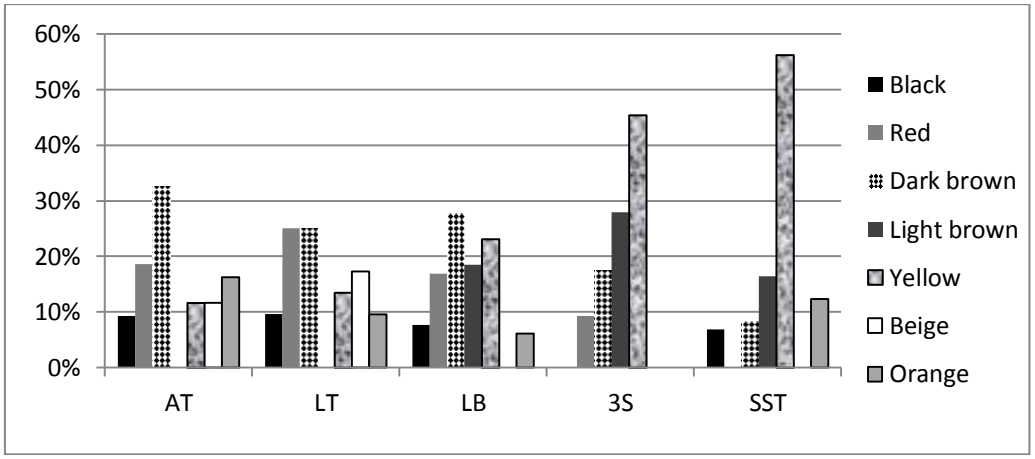

Figure 2. AMF species distribution in five disturbed soils localized in west Algeria.
AT: rhizosphere of Acacia saligna in Terga sand pit (Ain Témouchent); LT: rhizosphere of Lotus creticus in Terga sand pit (Ain Témouchent); LB: rhizospheres of Lotus creticus in Ouled Boudjemaa sand pit (Ain Témouchent); 3S: rhizosphere of Arthrocnemum macrostachyum in Ain Skhouna (Saida); SST: rhizosphere of Arthrocnemum macrostachyum in Terga (Ain Témouchent).

In Terga soils (LT and AT), dominance of dark brown and red morphotypes was observed (fig 2). This dominance was reported by several authors (Bergen and Koske, 1984; Beena et al., 2000; Hatimi and Tahrouch, 2007); such soils conditions were conducive to brown and red morphotypes development in Terga site. However, in Ouled Boudjemaa (LB) the dominant morphotypes were dark brown and yellow morphotypes and the absence of beige morphotype was signaled. Brown morphotype abundance in dune soils, indicated that this species sporulated abundantly in sandy soils and it was a major component of mycorrhizal flora of these soils.

AMF spores diversity in saline soils (3S and SST) was less important compared to dune soils. This diversity was dominated by yellow morphotype (table 3, fig 2), indicating that this species was adapted to salinity. This dominance was observed by Karaarslan and Uyanöz (2011).

Glomus genus was dominating in arid and semi-arid regions (Tarafdar and Kumar, 1996; Mathur et al., 2007) because of its resistance to soil high temperature (Al-Raddad, 1993). It has been reported that *Glomus* sp. was the most observed in saline soils (Allen and Cunningam, 1983; Ho, 1987; Wang et al., 2004).

Morphological characteristic variation of AMF spore was depending of plant species that affected physicochemical characteristics and fertility of soils, and consequently affected AMF community structure (Abbas et al., 2006). Gianinazzi-Pearson et al., (1989) showed that host plants root exudates influence some spore species germination. Gavin (2005) reported that host plants productivity and structure affected mycorrhizal diversity. In this regard, Burrows and Pfleger (2002) found that the richest plant community was richer in AMF.

Edaphic and anthropogenic factors, floristic and microbiological composition were very important to colonization, growth and distribution of AMF (Johnson et al., 1991; Xavier and Germida, 1999; Dahlberg, 2002; Jones et al., 2003; Treseder, 2004). Results showed that soil texture, EC and organic matter affected positively and/or negatively spores relative abundance.

Shannon and Weiner diversity index differed between dunes and saline sites (table 4). Values of this index were higher in dune sites that appeared to be favorable to all morphotypes and offered them the same chance of survival. Indeed, low values obtained in saline sites were sign of a specific morphotype specialization, indicating presence of large numbers of individuals at the expense of others (yellow morphotype).

Table 4. Shannon and Weiner diversity index and evenness index of Piélou in five disturbed soils of west Algeria basing on spores counting.

	H (SHANNON)	E (PIELOU)
LB	2.41	0.86
LT	2.47	0.88
AT	2.44	0.87
3S	1.78	0.63
SST	1.82	0.65

AT: rhizosphere of *Acacia saligna* in Terga sand pit (Ain Témouchent); LT: rhizosphere of *Lotus creticus* in Terga sand pit (Ain Témouchent); LB: rhizospheres of *Lotus creticus* in Ouled Boudjemaa sand pit (Ain Témouchent); 3S: rhizosphere of *Arthrocnemum macrostachyum* in Ain Skhouna (Saida); SST: rhizosphere of *Arthrocnemum macrostachyum* in Terga (Ain Témouchent).

Evenness index of Piélou values varied between 0 and 1. Values of this index obtained in saline sites indicated the presence of rare species which were disproportionately distributed**.** In contrast, high values obtained in dune sites (close to 1) testified to an equitable distribution of species**.**

CONCLUSION

Our results confirmed that AMF were omnipresent in dunes and saline areas. Despite stressful conditions of these ecosystems, recorded AMF show a significant microbiological activity. These densities exhibit a significant difference between dune and saline soils, indicating that spore germination and host plants root colonization were affected by salinity, leading to spore accumulation in soils.

According to morphological characters, a variety of Glomales associated with different plants species in two different ecosystems was highlighted. These Glomales were divided into three families of *Gigasporaceae, Glomaceae* and *Acaulosporaceae* in all studied soils. However, dominance of brown and yellow morphotypes in dune and saline soils respectively, suggested that edaphic and pedological conditions affect AMF fungi distribution. Our results allowed the establishment of AMF collection native of disturbed areas. That must be preserved and used in such ecosystems by including them in rehabilitation programs.

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