Full Length Article

Soil Properties Shape Species Diversity and Community Composition of Native Arbuscular Mycorrhizal Fungi in *Retama raetam* Roots Growing on Arid Ecosystems of Tunisia

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Abstract

The aim of this study was to identify the major environmental factors affecting the Arbuscular Mycorrhizal Fungi (AMF) diversity and community composition in the roots of *Retama raetam* growing on arid ecosystems of Tunisia. AM Fungi were assessed by cloning and sequencing of the AMF SSU rRNA gene and therefore checked against NCBI GenBank and Maarj*AM* database. Thirteen operational taxonomic units (OTUs) were identified in *R. raetam* roots and AMF community was dominated by *Glomeraceae*. Of the totality of OTUs, ten matched previously known virtual taxa (VTX) and 3 OTUs were treated as novel VT and grouped into 3 putative new AMF taxa (pNTX). Correlation analysis reported that high available phosphorus content and high electrical conductivity in the soil decrease AMF richness and diversity. Interestingly, Principal Component Analysis (PCA) and Monte Carlo permutation tests on Canonical Correspondence Analysis (CCA) indicated that soil physic-chemical properties such as soil total nitrogen (TN), soil-available P, EC, and soil texture affected the AMF community composition. No significant relation was found between AMF distribution and soil organic carbon (OC) and soil pH. Our findings provide insights into the nature of AMF communities colonizing *R. raetam* roots and help to link fungal distribution to specific soil properties, which could be exploited in the identification of a wider variety of fungal strains as potential inoculants for rehabilitation and restoration program. © 2020 Friends Science Publishers

Keywords: Arbuscular mycorrhizal fungi; Diversity; Soil properties; distribution; *Retama raetam*; Arid areas

Introduction

Arbuscular mycorrhizal fungi are major components of rhizosphere soils, globally distributed (Munkvold *et al.* 2004) and form symbiosis with the fine roots of terrestrial plants (He *et al.* 2016). This association is strongly implied in plant nutrient uptake and productivity (Smith and Read 2008), host protection against pathogens (Sikes *et al.* 2009) and tolerance to adverse environmental stresses (Zhu *et al.* 2016; Yang *et al.* 2018). In this symbiosis, the AMF acquire carbon compounds from the host plant and in return enhances the uptake of nutrient elements and water in plants (He *et al.* 2016).

An increasing number of reports have highlighted the effect of environmental variables on AMF communities. Soil texture and soil pH are found to be essential factor shaping the AMF community composition (Bainard *et al.* 2014; Xiang *et al.* 2014; Alguacil *et al.* 2016). Furthermore, soil salinity is known to be crucial factors in determining mycorrhizal communities (Wild *et al.* 2009; Guo and Gang 2013). Interestingly, soil available phosphorus was found to have a deep influence on both AMF abundance and diversity (Geel *et al.* 2014; Beenhouwer *et al.* 2015; Liu *et al.* 2016). However, other reports revealed that roots AMF communities are considerably influenced by the host species (Hazard *et al.* 2012; Gosling *et al.* 2013). Liang *et al.* (2015) suggested that AMF composition and diversity are determined by both soil properties and plant communities. Researches describing the environmental drivers of AMF communities in Mediterranean arid area are lacking until now.

Degradation processes and desertification in Mediterranean arid ecosystems is the combined result of anthropogenic factors related to human activities and natural factors such as scarce, irregular rainfall, dry and hot summer (Requenaf *et al.* 1997). These conditions disturb biodiversity (Djellouli 1996), soil properties and major plant nutrients especially P and N (Requenaf *et al.* 1997).

The rehabilitation of degraded landscapes is thus a major preoccupation in Mediterranean arid area of South Tunisia. Among plant species adapted to xeric conditions, *Retama raetam,* is a desert shrub, common to arid ecosystems around the Mediterranean basin (Pnueli *et al.* 2002). It revealed a great ecological importance and is widely used for dune stabilization and soil protection from wind erosion thanks to its very developed root system (Ferchich 1996). This makes *R .raetam* a legume of choice for use in sustainable production systems. Previous reports mainly focus on the capacity of this specie to establish symbiotic associations with nitrogen-fixing bacteria (Mahdhi *et al.* 2018) and arbuscular mycorhizal fungi (Mosbah *et al.* 2018). However, the knowledge about the relation between soil properties, AMF diversity and structure of communities in *R. raetam* roots is not available in this region. Therefore, understanding the nature of AMF communities colonizing *R. raetam* roots and their correlation to soil properties under harsh conditions is of interest in order to identify a wider variety of fungal strains as potential inoculants for rehabilitation and restoration programs. The main objectives of the current study were to (i) characterize AMF communities in the roots of *R. raetam* across four different sites located in arid area of South Tunisia. (ii) Detect specific AMF taxa for *R. raetam* roots. (iii) Explore, for the first time, the major environmental factors affecting the diversity and communities' composition of AMF in the roots of this legume under harsh conditions.

Materials and Methods

Study sites and sampling

The four study sites are located in South Tunisia in arid Mediterranean climate (Fig. 1). These areas are marked by a mean annual temperature above 20°C (mean temperature is 12°C in December and 30°C in July) and annual mean rainfalls vary between 200 and less than 100 mm. Sites are located in (1) BouHedma National Parc (SidiBouzid coordinates, 34°29'N–9°29'E), (2) Institute of Arid Regions-Medenine (Medenine coordinates, 33°29'N–10°38'E), (3) Institute of Arid Regions-Chenchou (Gabès coordinates, 33°53'N–9°53'E) and (4) Oued Dkouk Natural Reserve (Tataouine coordinates, 32°37'N–10°18'E) (Fig. 1). Fine roots of the plant species *R. raetam* were carefully collected. Rhizosphere soil was collected from soil surrounding the roots of the studied plant.

Soil analysis

Rhizosphere soil samples were subjected to the following analyses. Soil texture was measured according to Robinson's pipette method (Naanaa and Susini 1988). Soil pH was determined in soil water suspensions by pH meter (Matest, Italia). Electrical conductivity was measured using

Fig. 1: Map of Tunisia indicating the location of the four studied sites in the arid area of southern Tunisia

a conductivity meter (Bibby Scientific, UK). Total soil nitrogen (TN) and organic carbon (OC) contents were measured using an elemental analyzer (commercial service). The soil available phosphorus that is sodium bicarbonate-extractable phosphorus (Olsen *et al.*1954) was determined by the colorimetric method.

Estimation of AM root colonization

The roots were washed with water and clarified with a potassium hydroxide (KOH) solution (10%) in the water bath at 90°C for 45 min. Root segments were then rinsed with distilled water and stained with Trypan blue 0.05% (w/v). They were finally placed on slides, mounted in a glycerol solution and observed with a bright-field microscope (×400) (Trouvelo *et al.* 1985). The frequency (F%) and intensity of mycorhization (%M) were estimated using Mycocalc program (http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download.html).

Isolation and enumeration of AM fungal spores

Spores were isolated from the rhizosphere soil samples through the wet-sieving and decanting method described by Gerdemann and Nicolson (1963). One hundred grams for each soil sample was independently suspended in 1 L water and then stirred for 10 min. We used 3 sieves to retain the spores (25, 100 and 500 μm). The spores retained on the 25 and 100 *μ*m sieves were independently recovered by sucrose gradient centrifugation and suspended in water for checking under a binocular stereomicroscope (×45). Two different size spore classes (25–100 *μ*m) and (100–200 *μ*m) were counted.

Root DNA extraction and nested PCR

Dried roots (50°C) were crushed in liquid nitrogen with mortar and pestle. DNA extraction from 50 mg crushed roots was performed using DNeasy® Plant Mini Kit (Qiagen). Root DNA quantification was determined by fluorescence with the Quant-iT™ PicoGreen®dsDNA Assay Kit (Invitrogen) on a microplate reader (Tecan) (Infinite M200). PCR was carried out in an Applied BiosystemsVeriti 96 well thermocycler and the GoTaq® DNA Polymerase (Promega) in a volume of 25 μL. Root DNA extracts served as template DNA in a first PCR amplification step using the universal eukaryote primers NS1(5'-GTAGTCATATGCTTGTCTC-3') and NS4(5'-CTTCCGTCAATTCCTTTAAG-3') to amplify 1.2 kb fragments of the small subunit (SSU) rRNA gene (White *et al.* 1990). PCR was performed using 2 μL of composite root DNA (10 ng), 200 μM dNTPs, 0.5 μM of each primer, 200 ng μL^{-1} BSA, 1 U Taq DNA polymerase and the reaction buffer. The thermic profile consisted in an initial denaturation at 94°C for 3 min, 30 cycles at 94°C for 30 s, 40°C for 1 min, 72°C for 1 min, followed by a final elongation step at 72°C for 10 min. Undiluted amplicons were directly used as target DNA (2 μL) in a second PCR step using the universal eukaryote primer NS31 (5'-TTGGAGGGCAAGTCTGGTGCC-3') (Simon *et al.* 1992) and the AMF-specific primer AM1(5'-GTTTCCCGTAAGGCGCCGAA -3') (Helgason *et al.,* 1998) to amplify ca. 550 bp fragments. Reagents concentrations in the reaction mix were the same as in the PCR step 1 but the annealing temperature was set to 60°C.

Cloning and sequencing

The PCR products were cooled in gel, purified with the Pure Link Quick Gel Extraction Kit (Invitrogen), eluted in 30 μL buffer, then quantified with a Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific) at 260 nm and further cloned with the pGEM-T Easy vector (Promega) in XL2-Blue ultracompetent cells (Agilent Technologies). For each library, 96 white clones were randomly selected, and DNA was extracted from cell aliquots by 7 heat shocks (96°C – 2 min, 4°C – 10 sec) in a volume of 30 μL sterile water. Cell debris were then pelleted at 3,000 $\times$ *g*. The supernatants were used as template DNAs in an amplification (PCR) using the vector primers 17 merpUC/M13 (Promega). PCR products having the expected size were sequenced by Genoscreen (Sanger Technology, http://www.genoscreen.fr).

Glomeromycota identification and phylogenetic analysis

Sequences were blasted using the BLASTn algorithm and compared with sequences found in the GenBank database (Altschul *et al.* 1997). Non-AMF sequences were excluded. Representative sequences were aligned with a selection of AMF reference sequences using the ClustalX algorithm

(Thompson *et al.* 1997) then grouped into operational taxonomic units (OTUs) based on 97–100 % sequence identities using the Mothur program (Schloss *et al.* 2009) and compared to the virtual taxa (VTX) listed in the Maarj *AM* database (http://maarjam.botany.ut.ee/) (Öpik *et al.* 2010), VTX are sequence groups based on bootstrap support and identity value (ID) ≥97% sequence similarity. Sequences not assigned to existing VTX (ID<97%) were considered as putative new taxa (pNTX). Matrices of pairwise distances were produced with the Maximum Composite Likelihood (MCL) approach (based on a 277 nt alignment) using Mega Xsoftware (Kumar *et al.* 2018). A specific pNTX includes sequences with a distance greater than 3%. A selection of AMF ribosomal reference sequences published in GenBank were included in the phylogenetic analysis, while *Archaeospora trappei* and *A. schenckii* were used as outgroups. Maximum-likelihood (ML) phylogenetic analyses (Kimura 2-parameters, 1000 replications) were produced using Molecular Evolutionary Genetics Analysis Mega-X software (Kumar *et al.* 2018).

Nucleotide sequence accession numbers

A total of 194 Glomeromycota of 18S rDNA sequences were obtained. Twenty-eight representative sequences have been deposited at the National Centre for Biotechnology Information (NCBI) GenBank under the accession numbers KU707432-KU707459.

Statistical analyses

All the statistical analyses were performed using XLSTAT (v2010.5.04) software. Significant differences between quantitative values (spore numbers, mycorrhization scores, soil properties) were determined by the one-way analysis of variance ANOVA and Fisher's least significant difference (LSD) at a significance level of $P < 0.05$. The Richness (S) indicates the number of different AMF OTUs in the sampled community. The Shannon-Weaver (H') index was calculated as a measure of AMF diversity from the equation H' = - $\sum pi$ (lnpi) where pi is the proportion of individuals found in the ith species (pi is estimated as ni/N, [here and throughout, ni is the number of individuals in the each species]). Pearson's correlation coefficients were calculated to evaluate relations among the soil properties, root mycorrhizal colonization (F% and M%), spore density, Richness (S)and the Shannon–Wienerdiversity index (H') at a significance of $P<0.05$. A Venn diagram showing the number of site-specific and shared AMF OTUs was shown using a 4-way Venn diagram created in Venny (Oliveros 2007–2015). In order to examine the multivariate relations between the soil characteristics and AMF OTUs distribution, principal component analysis (PCA) was performed. To evaluate the effects of soil properties, Monte Carlo permutation test on canonical correspondence analysis (CCA) was then applied and was conducted using 1000 random permutations. The forward-

selection procedure arranged the variable according to their importance and significance for the distribution of the OTUs.

Results

Soil analysis

The soil physicochemical parameters are summarized in Table 1. The soil texture was sandy loam in soils from BouHedma, Medenine and Oued Dkouk and sandy clay loam in Chenchou's soil. pH ranged from 8.34 to 8.6. Organic carbon (OC) was significantly higher in BouHedma soils than other sites. Total nitrogen (TN) values ranged from 0.08% in Oued Dkouk's soil to 0.35% in Chenchou's soil. Both Electric conductivity and available P contents showed the highest values in Oued Dkouk's soils whereas the lowest values were from Medenine's soils.

Root colonization

All root samples observed for AMF colonization contained AMF. The percentage of mycorhizal colonization was high for all sites and ranged from 94.64% to 97.78%. The intensity of mycorhization was moderate to high and ranged from 36.02 to 76.26%, respectively in *R. raetam* roots from Chenchou and BouHedma soils (Fig. 2).

AMF spores number

High density of AMF spores was generally observed in field soils. The highest average density of the smallest size spores (25–100 μm) was evidenced in *R. raetam* soils from Medenine (4748 spores per 100 g soil) (Fig. 3) and the lowest average density was in rhizosphere from BouHedma (2057 spores per 100 g soil). Moreover, the density of spores of the largest class (100–200 μm) was far below that of smaller spores and reached at best ca. 655 spores per 100 g soil rhizosphere from Medenine.

AMF community composition

OTUs abundance and diversity: Partial SSU rDNA sequences from roots were successfully amplified by nested PCR (550 bp). A total of 194 sequences belonging to AMF were obtained from the 4 clone libraries. Sequences not affiliated to AMF were eliminated from sequence analysis and community structure analysis. After BLASTn and
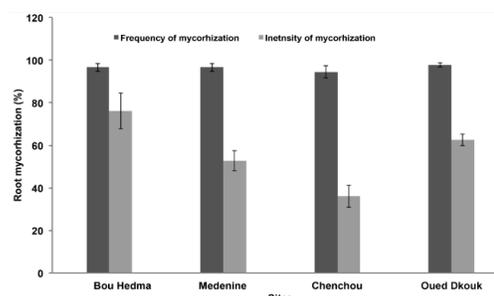

Fig. 2: Root mycorhization of *R. raetam*: mycorhizal frenquency (F) (dark grey bars) and mycorhizal intensity (M) (pale grey bars). Data are reported as (mean ± SE) for three replicate per samples. Significant differences between the samples are indicated by different letters above the bars and were determined by using Fisher's least significant difference (LSD) at the 0.05 level after one-way ANOVA

Fig. 3: AMF spore number in rhizosphere soil of *R. raetam* in four different sites: spores of the smallest class (25-100 μm) (dark grey bars), spores of the largest class (100-200 μm) (pale grey bars). Data are reported as (mean ± SE) for three replicate per samples. Significant differences between the samples are indicated by different letters above the bars and were determined by using Fisher's least significant difference (LSD) at the 0.05 level after one-way ANOVA
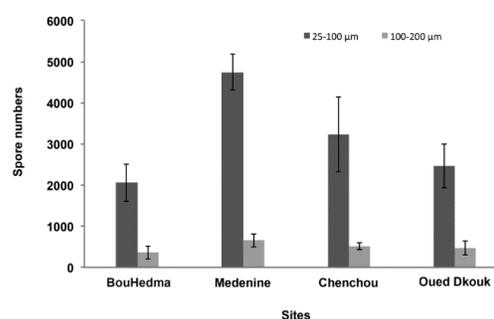
phylogenetic analyses the sequences were clustered into 13 OTUs supported by a bootstrap value > 97% (Fig. 4, Table 2). Among the 13 OTUs, 12 OTUs (182 sequences) belonged to the Glomeraceae family, while only 1 OUT (12 sequences) represented Diversisporaceae family. The genera of Glomeraceae belonging to *Glomus, Funneliformis, Rhizoglomus, Septoglomus* were found. The most abundant genara were represented by *Funilloformis*, accounting for 40.72% of the sequences, followed by *Rhizophagus*

Table 1: Physical and chemical properties of the studied soils

	Bou Hedma	Medenine	Chenchou	Oued Dkouk
Soil texture	Sandy loam	Sandy loam	Sandy clay loam	Sandy loam
Sands (%)	68	69	66	70
Silt (%) 24	21	17	20	
Clay (%)	6	8	15	7
pH	8.3	8.35	8.7	8.32
Electrical Conductivity EC (μS/cm)	2.52	1.55	1.83	3.46
Organic Carbon (%)	1.27	0.73	0.85	0.45
Total Nitrogen (%)	0.1	0.2	0.35	0.08
Available Phosphorus (mg/Kg soil)	15.59	9.58	12.12	23.5

Different letters indicate significant differences at *P* < 0.05 (n=3)

Table 2: Distribution of 13 AMF OTUs taxa (10 VTX and 3pNTX) detected in the root samples of *R. raetam* from the four studied sites

| OTU name	Taxonomic affiliation	Maarjam database virtual taxa	BouHedma	Medenine	Chenchou	Oued Dkouk
	Funilliformis mossea	VTX00105	■		■	■
Glo1	*Uncultured glomus*	VTX00092			■	■
Rhi1	*Rhizophagus intraradices*	VTX00113		■	■	
Glo2	*Uncultured glomus*	VTX00114	■		■	
Glo3	*Uncultured glomus*	VTX00115		■		
Glo4	-	pNTX C	■			
Glo5	-	pNTX A	■	■		
Glo6	-	pNTX B	■			
Rhi2	*Rhizophagus iranicus*	VTX00155			■	
Glo7	*Uncultured glomus*	VTX00342	■	■		
Div	*Diversispora spurca*	VTX00263		■		
Sept	*Septoglomus constrictum*	VTX00064		■		
Glo8	*Uncultured glomus*	VTX00063		■		
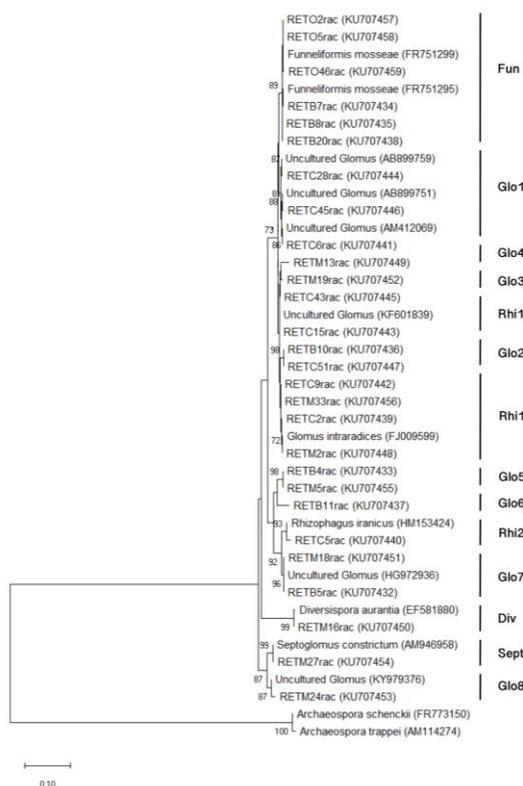
Fig. 4: Maximum likelihood tree based on partial 18S rDNA gene sequences amplified from root DNA from *V. tortilis*. Bootstrap values calculated for 1000 replications are indicated. Scale bar = 10% nucleotide divergence. Accession numbers from GenBank are given in brackets. Sequences are labeled from the left according to the name of legume specie (RET: *Retama raetam*), the site identity (*e.g.*, M, with M for Medenine, B for BouHedma, C for Chenchou, O for Oued Dkouk), No. attributed to the sequence (*e.g.*, 16)

(26.3%), *Glomus* (18.56%), *Diversispora* (6.19%). *Septoglomus* corresponded to less than 1% of total sequences. Twelve OTUs were ascribed to Glomeracea family, within the genus Gloms, were retrieved 8 OTUs, of which 5 OTUs (Go1, Glo2, Glo3, Glo7 and Glo8) represented sequences of uncultured *Glomus* species and 3 OTUs (Glo4, Glo5 and Glo6) didn't matched to any Known Glomycomycota sequence. In the genera *Funneliformis* and *Septoglomus*, OTUs named Fun and Sept were identified as *Funneliformis mossea* and *Septoglomus constructum* respectively. OTUs Rhi1 and Rhi2 assigned to Rhizophagus matched to sequences of Known species (*Rhizophagus intraradices* and *Rhizophagus iranicus*). While, OTUs belonging to Diversispora (Div) matched only to sequences of known species (*Diversispora spurca*).

OTUs identification based on the Maarj *AM* database: The MaarjAM database of published Glomeromycotina SSU rRNA gene sequences was used to confirm the assignment of our OTUs to sequences of Glomeromycota. Ten OTUs (>97% similarity) matched previously-known virtual taxa (VT; *i.e.*, a molecular operational taxonomic unit) and 3 OTUs (<97% similarity to previously known VT belonging to Glomeraceae) were considered as novel VT and grouped into 3 putative new taxa (pNTX) (Table 3).

OTUs distribution: *Funniliformis mossea* (Fun) representing the most abundant OTU was present in all sites except those of Medenine, *Rhizophagus intraradices* (Rhi1) occurred in two sites (Medenine and Chenchou). Two OTUs, Glo5 and Glo7 were found in both BouHedma and Medenine's sites. In the same way, Glo3, Glo4, Glo8, Div and Sept were found exclusively in *R. raetam* roots from Medenine location. Glo6 was only present in the roots from BouHedma. *R. iranicum* (Rhi2) and Uncultured Glomus (Glo1) were found exclusively in *Retama* roots from Chenchou site.

The shared and site-specific AMF OTUs for sites were illustrated in a Venn diagram (Fig. 5). We found that five OTUs (Fun, Rhi1, Glo2, Glo5 and Glo7) accrued in more than one site, Glo5 and Glo7 were found only shared by BouHedma and Medenine's sites, Rhi1was found shared by Medenine and Chenchou's sites. *F. mossea* was found shared by BouHedma and Chenchou's sites. The remaining eight OTUs (Glo1, Glo3, Glo4, Glo6, Glo8, Div, Sept and Rhi2) were specific to one site. No site-specific AMF OUT was determined in Oued Dkouk. In return one (Glo6), two (Glo1, Rhi2) and five AMF OUTs (Glo3, Glo4, Glo8, Sept and Div) were specific to BouHedma, Chenchou and Medenine sites, respectively.

Table 3: Putative new taxa (pNTX) generated by Pairwise Distances Matrix Sequences sharing distances less than 3% where considered as belonging to the same pNTX and specified by an alphabetic letter (A, B, C)

pNTX	Representative sequence	RETB4rac (KU707433)	RETB11rac (KU707437)	RETM13rac (KU707449)	RETM5rac (KU707455)
pNTXA	RETB4rac (KU707433)	1			
pNTX B	RETB11rac (KU707437)	0.0399	1		
pNTX C	RETM13rac (KU707449)	0.068	0.076	1	
pNTX A	RETM5rac (KU707455)	0.0001	0.0399	0.0068	1

Sequences sharing distances less than 3% where considered as belonging to the same pNTX and specified by an alphabetic letter (A, B, C)
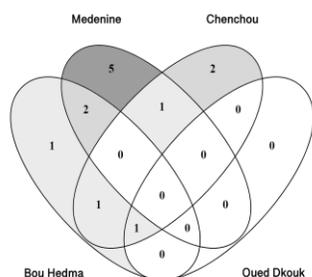
Fig. 5: Venn diagram reporting the number of unique and shared OTUs detected in Retama roots among studied sites

Effect of soil properties on phylogenetic diversity and AMF abundance

R. raetam from Medenine soil showed the highest OTU Richness (8 OTUs) while only one OTU was detected in *R. raetam* from Oued Dkouk's soil. According to the OTU diversity estimated by Shannon's index, the greatest AMF diversity was found in *R. raetam* roots from Medenine soil (1.68), followed by Chenchou soil (1.51) and BouHedma soil (1.45). A null AMF diversity was found in Oued Dkouk soil. The Pearson's correlation coefficients between soil chemical properties, Shannon diversity index (H') and Richness (R) are presented in Table 4. There was a significant and negative relationship between avail P, EC and S and H', while there was no correlation between other soil variables (TN, soil pH, OC), soil texture (clay, sand and silt contents) and AMF richness and diversity.

Effect of soil physicochemical properties on AMF community composition

PCA (principal component analysis) was performed to infer the relationship between AMF distribution and the soil variables. The resulting ordination is presented in Fig. 6. The cumulative percentage variance of species data showed that the first two PCA axes explain 77.62% of the variability in species data. PCA on AMF distribution and soil variables showed that AMF communities in *R. raetam* roots differed significantly between the studied sites and suggested that many AMF (Rhi1, Glo3, Glo4, Glo5, Glo8, div, Sept) prefer soil conditions with lower soil salinity (EC) and available P. The relative preference for higher salinity and available P in Fun, Glo1, Glo2, Glo6, Glo7 and Rhi2 AMF was also

suggested. PCA showed also that Glo1, Glo2, Rhi1 and Rhi2 prefer soil with high nitrogen. The results of Monte Carlo permutation tests on canonical correspondence analysis indicated that environmental variables, such as soil TN, soil-available P, EC, and soil texture affected the AMF distribution significantly ($P<0.05$), in which the effect of soil available P, TN and EC was stronger than that of other variables ($P < 0.0001$) (Table 5). No significant relation was found between AMF community composition and soil organic carbon (OC) and pH.

Discussion

Our data revealed that, in Arid area of Tunisia soil properties shaped the community composition of arbuscular mycorrhizal fungi in *R. raetam* root, which nevertheless always maintained high spore density and high mycorhization levels. Here, we identified AMF species colonizing *Retama* roots and described their diversity, distribution and community composition usingnested PCR, cloning and sequencing of the chromosomal small subunit ribosomal RNA (SSU) gene marker.

The occurrence of indigenous mycorrhizal infection and spore density varied among rhizospheres (Fig. 2 and Fig. 3). The spore density is known to vary considerably among different ecosystems. Values range from a few dozen to 10,000 spores per 100 g soil (Johnson and Wedin 1997; Tao and Zhiwei 2005; Bouazza *et al.* 2015), although spore density in rhizosphere of *R. raetam* was relatively high. Significant differences were found in spore density of small (25–100 μm) and large (100–200 μm) spores in all rhizospheres, and more AMF small sized spores were found. The dominance of such small sized spores was also reported in arid and semiarid regions (Stutz *et al.* 2000; Tao and Zhiwei 2005; Wu *et al.* 2014). In our study, R. *raetam* growing in arid area characterized by high temperature was typically AM plants with high frequency of mycorhization (F> 90%) showing the capacity of this legume to enrich the soil with "infective" mycorrhizal propagules. This finding was consistent with other reports on drought ecosystems (Stutz *et al.* 2000; Tao *et al.* 2003).

Pearson's correlation analysis demonstrated that soil properties have no influence on AMF colonization or spore density (Table 4). Halder *et al.* (2016) showed that correlation between edaphic factors and AMF colonization of six plant species was not significant. Aliasgharzadeh *et al.* (2001) found that spore number was not correlated to soil

Table 4: Pearson correlation coefficients between soil properties, OTU Richness and Shannon Wiener diversity index, root mycorhization and spore number among study sites

	OC	Clay	Silt	Sand	pH	EC	Avail P	TN	S	H'	F	M	Sp (25-100 μm)	Sp (100-200 μm)
OC	1	-0.081	0.546	-0.513	-0.012	-0.462	-0,411	0,034	0,414	0,656	-0,331	0,366	-0,312	-0,519
Clay		1	-0.877	-0.813	**0.996**	-0.411	-0,422	**0,953**	0,142	0,316	-0,908	-0,919	0,246	0,291
Silt			1	0.439	-0.844	0.070	0,103	-0,763	0,141	0,094	0,596	0,927	-0,291	-0,434
Sand				1	-0.853	0,588	0,567	-0,827	-0,323	-0,625	**0,969**	0,565	0,012	0,090
pH					1	0,604	0,635	-0,124	-0,779	-0,481	-0,097	0,179	-0,917	-0,842
EC						1	**0,998**	-0,663	**-0,953**	**-0,972**	0,652	0,434	-0,662	-0,497
Avail P							1	-0,675	**-0,955**	**-0,957**	0,644	0,465	-0,702	-0,546
TN								1	0,435	0,563	-0,939	-0,918	0,463	0,452
S									1	0,924	-0,392	-0,241	0,735	0,559
H'										1	-0,631	-0,264	0,484	0,289
F											1	0,743	-0,198	-0,145
M												1	-0,555	-0,628
Sp (25-100 μm)													1	**0,973**
Sp (100-200 μm)														1

OC, organic carbon; EC, electric conductivity; Avail P, avaiable phosphorus; TN, total nitrogen; S, OTU Richness; H'; Shannon–Wiener diversity index; F, Frequency of mycorhization; M, Intensity of mycorhization, Sp, Spores number. The values highlighted in bold are statistically significant ($P < 0.05$). Correlations above 0.75 are strong, 0.35-0.75 moderate and less than 0.35 weak

Table 5: Results of Monte Carlo permutation tests (1000 permutations) of the Canonical Correspondence Analysis CCA for the relationships between soil properties and AMF community

	F value	*P* value
Sand	4.146	**0.006**
Silt	4.876	**0.002**
Clay	5.299	**0.002**
pH	2.688	0.056
EC	6.751	**<0.0001**
OC	2.526	0.06
T N	6.140	**<0.0001**
Avail P	7.019	**<0.0001**

EC Electric conductivity; OC, Organic carbon; TN, total nitrogen; OC, Organic carbon; Avail P, Available Phosphorus. The values highlighted in bold are statistically significant ($P < 0.05$)
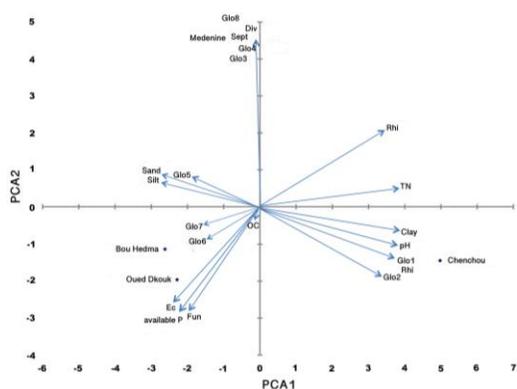

Fig. 6: Principal-component analysis (PCA) of the AMF community composition in the roots of *Retama raetam* in four different soil types. The model explained 77.62% of the whole variance. OC, organic carbon; EC, electric conductivity; Avail P, available phosphorus; TN, total nitrogen

factors like EC, pH, and sodium absorption ratio, but correlated with soil texture.

A total of 194 symbiotic AMF clones associated to native *R. raetam* roots growing on arid Tunisian soils were analysed using the nested PCR, cloning, sequencing of the chromosomal small subunit ribosomal RNA (SSU) gene marker. Results showed that diverse AMF fungal communities are present in *R. raetam* roots (13OTUs or VTXs) (Table 2 and Fig. 4). Ten of the 13OTUs detected in this study clustered with virtual taxa from the MaarjAM database and were clearly grouped with sequences of known taxa (Fun, Rhi1, Rhi2, Sept and Div) or also with sequences of uncultured *Glomus* spp (Glo1, Glo2, Glo3, Glo7 and Glo8). These uncultured *Glomus* are originating from different host plants distributed in different geographical areas, even including different continents as was shown by Alguacil *et al.* (2008). The three other OTUs(Glo4, Glo5 and Glo6) were separate from any AMF reference sequence and treated as 3 putative new taxa (pNTX) (MaarjAM database) and they may constitute new species within the Glomeraceae species suggesting that there is a substantially novel diversity of AMF taxa in arid area of South Tunisia, which was in agreement with other diversity studies in gypsum soils under semiarid area of southeastern Spain (Alguacil *et al.* 2008) and salinized south coastal plain of Laizhou Bay in China (Guo and Gong 2013). In our study, *Glomeraceae* species was the dominant family in all studied sites which is consistent with previous studies in arid and semi-arid areas (Mathur *et al.* 2007; Bai *et al.* 2013); this could be explained by the fact that this family tolerates high soil temperature (Al-Raddad 1993).

The richness and diversity of AMF in the roots of *R. reatam* were found to be different between studied soils. Significant negative correlations were found between the soil salinity (EC) and available P and AMF richness and diversity (Table 4). Cherif *el al.* (2015) explained that salinity alters propagules hydratation and unable their germination; which maintain spores under long term dormancy stage. Consequently, roots infection by spores decreased (Saint-Etienne *et al.* 2006). Similarly, negative effect of high available P on AMF richness and diversity (Bainard *et al.* 2014; Geel *et al.* 2014; Beenhouwer *et al.*

2015) could be explained by the fact that AMF taxa differ in their tolerance to high P availability (Verbruggen *et al.* 2012), and that rising P levels diminish the provision of plant assimilated carbohydrates towards the mycorrhizal symbionts as supported by Liu *et al.* (2012) leading to a competition for carbohydrates between AMF and can consequently reduce AMF diversity on the host plant root. According to PCA biplot, the determinal effect of soil avail P and Electric conductivity was not limited on AMF richness and diversity, but also on AMF community distribution. Privious AMF studies mention that soil available P (Liu *et al.* 2016) likewise soil salinity (Wild *et al.* 2009; Guo and Gang 2013) is crucial drivers of mycorrhizal communities. Some AMF were found to be dominant under high soil available P and electric conductivity. *F. mosseae* is one such AMF OUT, this AMF specie is commonly found in soils and have a worldwide distribution. Sahodaran *et al.* (2019) testing the effect of root inoculation on plant and soil showed that inoculation with this specie increase the soil available P. *F. mosseae* was also observed in soil with high salinity levels (Aliasgharzadeh *et al.* 2001) and is known by its capacity in protecting plants from determinal effects of salt stress (Bharti *et al.* 2013).

Other soil physicochemical properties (nitrogen content, soil Clay, Sit, and Sand contents) appears also to be strong factors driving the distribution of AM fungal communities. PCA and Monte Carlo Permutation test on CCA analysis showed a positive significant relationship between *Glo1, Glo2, Rhi1, Rhi2* and soil total nitrogen (TN). It seems that total N can be a determinant in improving AM hyphal length and mycorrhizal biomass as previously reported by Treseder and Allen (2002). Furthermore, soil silt, sand and clay contents affected AMF distribution. The involvement of soil texture in the structuring of AM fungal communities has often been reported (Herrmann *et al.* 2016). Interestingly, soil pH and soil OC were not significantly related to AMF community distribution.

Conclusion

In our study, a high spore density and high mycorrhization levels were revealed across the different sites. We identified the *R. raetam* roots AMF communities at the species and genus level and described new AMF lineages within the Glomeraceae species. High available Phosphorus content and electric conductivity in the soil decrease AMF richness and diversity. Abiotic factors, in particular soil properties (soil total nitrogen, available P, electric conductivity and soil texture) shape AMF communities on *Retama* roots. For the first time, our findings provide insights into factors affecting AMF diversity and communities' distribution of AMF colonizing *Retama* roots, which could be exploited in the identification of a wider variety of fungal strains as potential inoculants for rehabilitation and restoration program.

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