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Biodiversity of arbuscular mycorrhizal fungi in plant roots and rhizosphere soil from different arid land environment of Qatar

Khazna Alrajhei | Iman Saleh [iD] | Mohammed H. Abu-Dieyeh [iD]

Biological Science Program, Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, Doha, Qatar

Correspondence
Mohammed H. Abu-Dieyeh, Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, P.O. Box 2713, Doha, Qatar.
Email: dandelion@qu.edu.qa

Abstract

Recently more attention has been observed toward the role of arbuscular mycorrhizal fungi (AMF) in plant growth. Qatar belongs to the Arabian Gulf region with hot and dry climatic conditions. The study aims to investigate the species composition and abundance of AMF in Qatar, rhizosphere soil samples, and roots of plants from 12 families and 8 different locations. The AMF were identified based on the sequencing of the polymerase chain reaction (PCR) product of the amplified conserved ITS region. The reported AMF infection rate was found to vary with location and plant species. *Tamarix aphylla* recorded the highest AMF infection rate (100%), followed by *Blepharis ciliaris* (98%) and *Sporobolus ioclados* (92%). AMF spore counts ranged from 29.3 spores in *Blepharis ciliaris* to 643 spores/100 g soil in *Fagonia indica*. No correlation was detected between colonization rate and spore counts. While all AMF identified at species levels were reported in other regions, new species are still expected since some were identified only at higher taxonomic levels. *Claroideoglomus drummondii* and *Rhizophagus irregularis* were the most widespread while *Claroideoglomus claroideum* and *Diversispora aurantia* were the least present. Our results fill the gap of knowledge of AMF in the region and opens new research toward its future applications for sustainable agriculture.

KEYWORDS
arbuscular mycorrhizal fungi, arid land, colonization, halophytes, Qatar

1 | INTRODUCTION

Mycorrhizal fungi are essential components of soil in many ecosystems. At least seven different types of mycorrhizal associations have been recognized. These are: ectomycorrhiza, endomycorrhiza particularly arbuscular mycorrhiza, ericoid mycorrhiza, arbutoid mycorrhiza, monotropoid mycorrhiza, ect-endomycorrhiza, and orchidaceous mycorrhiza (Varma & Kharkwal, 2009). Survey study done by (Wang & Qiu, 2006) found that mycorrhizal plants account for between 80 and 92% of all land plant species and families studied. In addition, they found among land plants, arbuscular mycorrhizal fungi (AMF) is the most common and ancestral mycorrhiza type. AMF establish symbiotic association with plants. They are a general constituent of rhizosphere microflora and AMF is associated with over 70% of the world's vascular plant species, and the symbiosis can be found in nearly all terrestrial habitats (Brundrett, 2009). The morphological and anatomical characteristics of their spores, as well as other current approaches like molecular tools, have been used to classify AMF.

Recently, molecular methods, like ribosomal DNA sequencing of chosen species, have revealed an entirely new picture of AMF

systematics at the genus, family, and higher taxonomic levels. The molecular phylogenetic analysis based on the SSU rRNA sequences carried out by (Schüβler et al., 2001) resulted in considerable alterations in the classification of AMF. As a result, AMF were removed from the polyphyletic Zygomycota and assigned to the Glomeromycota, a newly formed monophyletic group (Stürmer & Siqueira, 2006) which has been published in sections for many years (Schüßler & Walker, 2010). Currently, 342 AMF species have been identified in the phylum Glomeromycota.

The presence of fungi that form mycorrhizae in desert plants has been reported in a number of studies, and their activities are critical to these plants (Al-whaibi, 2009). AM fungus associated with most plants are prevalent in the arid soil of India's Thar Desert, according to (Tarafdar et al., 1999). The geographic distribution of AMF species, which is impacted by edaphic variables, is critical for understanding fungus dynamics, quantification, and identification, as well as predicting levels of indigenous AMF populations.

Factors such as soil temperature, soil nutrient, and composition, root exudations, habitat, and plant community type, rainfall; pH and the competition associated with other microorganisms that have interactions with them have been suggested to contribute to patterns of AMF distribution (Chaudhary et al., 2014; Gong et al., 2012; Jefwa et al., 2012; Kivlin et al., 2011; Liu et al., 2009; Mosbah et al., 2018; Opik et al., 2006). Although all these factors have been confirmed as essential factors determining AMF community, there is no one major factor affecting the community of mycorrhiza fungi (Chaudhary et al., 2014; Gong et al., 2012; Melo et al., 2017).

Diversity and distribution of AMF have remained an area of active interest across agriculturists, botanists, and environmental scientists. The interests in the mycorrhizal relationship are attributed to the benefits extended by the fungal partner in enriching the hosts with minerals and waters. In return, AMF derives their carbon skeleton from the hosts. It was revealed that AMF enters plant root cortical cells, which enables the root of morphology to alter and develop its tolerance against weeds, pests and diseases (Siddiqui & Futai, 2008). AMF also enhances the plants capability to cope with salt pressure by developing the absorption of mineral nutrients, keeping ion, preserving the acts of enzyme, and facilitating water uptake, while the rise of salinity resistance in many plants including tomato and maize (Al-Karaki et al., 2001). The plant-symbiosis with AMF can significantly improve the overall growth as the lengths of the roots are considerably enhanced. The relationship is also known to increase leaf area, plant biomass as well as nutrient uptake in the plants under dry conditions (Latef et al., 2016; Mohammadi et al., 2011).

Due to the importance and usefulness of these fungi, in certain countries the research is advanced to commercialize products of different species of AMF mainly produced for agricultural use. Currently products of Mycorrhizae fungi are produced by 28 factories around the world (Basiru et al., 2021). The Glomeraceae account for 100% of the items, with three species dominating the Family: *Rhizophagus irregularis* (39%), *Funneliformis mosseae* (21%), *Claroideoglomus etunicatum* (21%) and *Rhizophagus clarus* is the least popular (16%) (Basiru et al., 2021). Despite the importance of arbuscular mycorrhizae fungi

in improving plant growth and increasing yield, structural colonization studies and spore populations in various countries with desert plants, particularly the Arabian Gulf region, have very few studies about occurrence and diversity of AMF (Al-Qarawi et al., 2012). We hypothesized that arid environments are rich sources of potential arbuscular mycorrhizal associations that should be explored for uses in agriculture and conservation applications. Hence the importance of this research was to discover the types of arbuscular mycorrhizae fungi in the rhizosphere of several plant species in Qatar as an arid environment. Results from the current proposed research will be important to provide vital data about Qatari mycorrhizae and their future applications and management options. Considering that there are not many studies on mycorrhizal fungi in Qatar, the current study is conducted to further establish the trending facts for a better understanding of the importance of AMF in agriculture. Thus, embarking on the screening for AMF from the rhizosphere of Qatari flora and collecting information about the prevalence of AMF in plant roots and the surrounding soil with a major goal to establish future research ideas about agricultural sustainability in the arid land.

2 | MATERIAL AND METHODS

2.1 | Study sites

Qatar (25°35′48″N and 51°18′39″E) is a small peninsula that is located in the Arabian Gulf with an approximate area of 11,000 km² (Ben Hassen et al., 2020). It lies in the northern hemisphere desert. It can be described as a warm and semi-desert ecosystem. Rainfall in this region is highly unpredictable and erratic both in space and time with an annual average of about 80 mm. Moreover, because of its variability and low intensity, it is characterized by a high temperature of more than 40°C in the summer with high rates of evaporation (Frenken, 2009).

During this study, plant roots and rhizosphere soil were collected from eight different locations in Qatar and 16 different plant species. Figure 1 shows the distribution of the collection sites in Qatar.

2.2 | Samples collection

Using a metal core borer of 10 × 20 cm, soil and plant roots samples were collected from rhizosphere soil of eight different locations as listed in Table 1. Soil sub-samples from various sites within each uniform sampling region were combined to generate a composite sample (a total of 16 samples per representative plant species). All samples were collected in March 2020. Ten field trips were conducted to collect random samples of soil with consideration to obtaining representative samples from different plant families. The dominant species in each location was chosen. The samples were transferred into labeled polythene bags and kept at a temperature below 10°C in an icebox until arrival at the lab, then transferred to a refrigerator (4°C) for later determination of the number of spores and respective
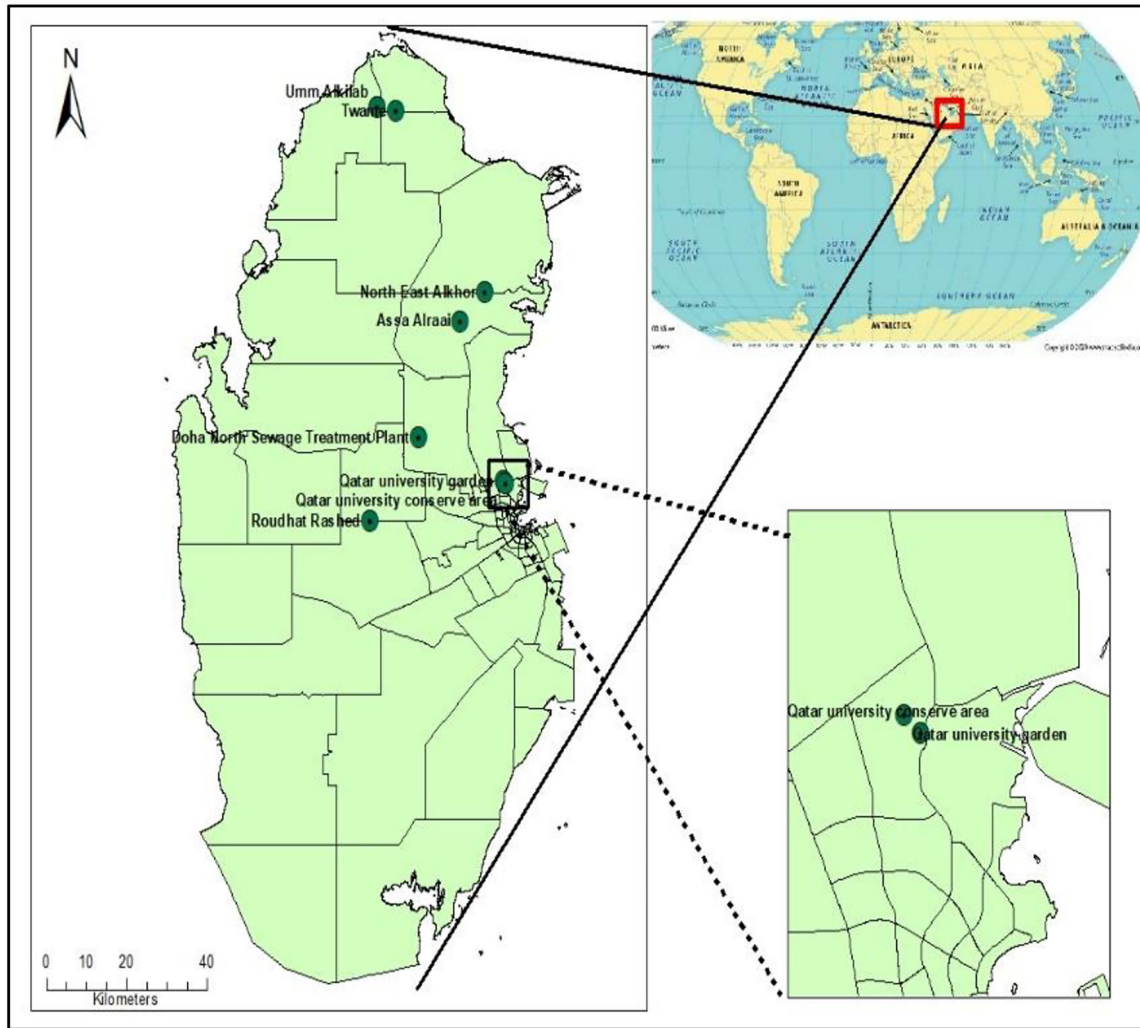
FIGURE 1 A map of State of Qatar showing the samples collection sites

identification for future analysis. All plant samples were collected after proper permissions and all methods were carried out in accordance with relevant guidelines and regulations.

Until processing, the root samples were stored in 95% ethyl alcohol. Soil samples were divided into two halves, the first part was used to determine the total number of spores in 100 g of dry soil, while the second part was used to analyze the physical and chemical properties of the soil.

2.3 | Physical and chemical properties of soil

Soil samples were tested for their pH (using ASTM 9045D method), salinity, total dissolved solids (TDS) (YSI EC 300–conductivity meter), total organic matters (TOM), and granules' size by finding the percentages of clay, sand and silt, as well as texture. They were also tested for the available amounts of minerals including calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), chloride (Cl), and calcium carbonate ($CaCO_3$). Physicochemical characteristics

and texture quality were examined using acid digestion method of soils by flame atomic absorption spectrometry (FLAA) for inorganic parameters and Manual of Oceanographic Observations and Pollutant Analyses Methods (MOOPAM) for organic parameters (Edgell, 1988; Kimbrough & Wakakuwa, 1989).

2.4 | Extraction arbuscular mycorrhizal fungi spores from soil

One hundred grams of soil were kept in a 1-L bucket and subsequently filled up to three quarters of tap water to complete wetting of the soil. The suspension was sieved by wet sieving and decanting method (Walker et al., 1982). Three sieves of 500, 250, and 32 μm were used respectively for sequential soil sieving. Post sieving, the solution with spores were distributed equally into two tubes and centrifuged at 2000 rpm for 5 minutes. The supernatant was discarded, and the tubes were filled with 50% sucrose. The spores are then mixed with sucrose solution and centrifuged at 2000 rpm for 1 min.

TABLE 1 Studied plants species and their families and location

Location	Host plant	Family
Doha North Sewage Treatment Plant	*Zygophyllum qatarense*	Zygophyllaceae
	Tamarix aphylla	Tamaricaceae
	Launaea nudicaulis	Asteraceae
Assa Alraai	*Sclerocephalus arabicus*	Caryophyllaceae
	Fagonia indica	Zygophyllaceae
Umm Alkilab	*Spergula fallax*	Caryophyllaceae
	Cynodon sp.	Poaceae
Twame	*Plantago ovata*	Plantaginaceae
North east Alkhor	*Salvia aegyptiaca*	Lamiaceae
	Lycium shawii	Solanaceae
Roudhat Rashed	*Aizoon canariense*	Aizoaceae
	Pulicaria undulata	Compositae
Qatar university campus	*Malva parviflora*	Malvaceae
	Paronychia arabica	Caryophyllaceae
Qatar university protected field	*Blepharis ciliaris*	Acanthaceae
	Sporobolus ioclados	Poaceae

The supernatant was sieved through 38-μm mesh and washed rapidly with water to remove residual sucrose adhering to the spores. The spores were then transferred from the sieve to the Petri dish. For each rhizosphere sample, spores were counted using a stereoscopic (LEICA 10450028) at 2X to 10X magnifications and the total number of spores in a 100-g soil sample was determined. There were three replicates for each soil sample (Abrol et al., 1988; Gerdemann & Nicolson, 1963; Parial et al., 2014).

2.5 | Assessment of arbuscular mycorrhizal fungi root colonization

Fresh plant roots from different Qatari native plants were separated from the soil and washed with water. The roots were cleaned with 10% KOH to remove root colors and cellular components. Roots were stained with .05% trypan blue (a chitin specific stain that stains the cell wall of AM fungi) and stored in 50% glycerol to remove the excess stain. Roots were then examined using a compound light microscope (YMPUS BX43) at different magnifications. Percent root colonization was counted according to the following equation (Brundrett et al., 1984).

$$\text{Percent Root colonization} = \frac{\text{Number of Root Segments Colonized}}{\text{Number of Root segments observed}} \times 100$$

2.6 | DNA extraction of rhizosphere soil samples

Soil samples (2 g/sample) were properly prepared by drying them at room temperature for a week and sifting them with a 500-μm sieve. The dried samples were packaged and sent to SYMPLANTA laboratory in Germany (the end of March 2020). DNA used in the metagenomic analysis was extracted from the 16 soil samples using a modified protocol of the FastDNA Spin Kit for soil (MP Biomedicals, Heidelberg, Germany) and an MP FastPrep-24 5G machine. For each sample, 500 mg of fine homogenous soil was transferred and repeated bead-beating was performed using ¼ inch ceramic beads in lysing matrix A to insure a better DNA yield. Samples were then treated as per the manufacturer's instructions except that 2X40s disruption was used instead of 1X40s (Senés-Guerrero et al., 2014). Extracted DNA was then stored at −20°C for further analysis.

2.7 | Polymerase chain reaction

2.7.1 | Nested polymerase chain reaction

Four sets of primers were used for a nested polymerase chain reaction (PCR) that amplifies the large subunit (LSU) of the rDNA of AMF allowing species-specific identification. The PCR method used was described by Senés-Guerrero et al., 2020 and Kruger et al. 2009. DNA template used for the first PCR was between 1 and 5 μl, and the first set of primers were SSUmAf and LSUmAr. As for the nested PCR, .5 μl of the DNA template was used with the forward primer LSUD2Af and reverse primer (Table S1). The fragment amplified included a part of the small subunit of the rRNA gene (SSU), the ITS region (ITS1-5.8S-ITS2) and a part of the large subunit of the rRNA gene (SSU) (Krüger et al., 2009; Senés-Guerrero et al., 2020). Three PCR replicates were conducted for each of the extracted DNA sample of the 16 soil samples. The target specific nested PCR (TS-PCR) products were run on a 1% agarose gel (EvaGreen stained) to analyze the quality of the generated amplicons and to evaluate their sizes. The TS-PCR nested PCR amplicons were separately purified using solid phase reversible immobilization (SPRI) paramagnetic bead-based technology (AMPure XP beads, Beckman Coulter) with a Bead to DNA (PCR-product) ratio of .8:1 (v/v).

2.7.2 | Index polymerase chain reaction

The purpose of the index PCR is to tag the amplicon generated by the nested PCR to prepare for Illumina sequencing. Figure S1 shows the details of the index PCR, the inner target-specific (TS) primer pairs used in the nested PCR already had a universal tag. Index PCR was performed directly on the tagged TS-PCR LSU amplicons to barcode all samples with different indices for pooling, using 5 μl of each TS-PCR product as template with the index primer pair

comprising a complementary tag, indices and sequencing adapters. By incorporating sample-specific indices (Table S2), all PCR products generated can be pooled together to run in a single sequencing experiment.

The 50-µl index PCR reactions contained: 5-µl DNA, 5-µl Nextera XT Index Primer 1, 5-µl Nextera XT Index Primer 2, 25-µl Phusion Polymerase MasterMix, 10-µl PCR Grade water. PCR conditions were adopted to the Phusion® polymerase as follows: 98°C for 3 min, 8 cycles of (98°C for 30 s, 57°C for 30 s, 72°C for 20 s), final elongation 72°C for 5 min.

The index PCR amplicons were separately purified using solid phase reversible immobilization (SPRI) paramagnetic bead-based technology (AMPure XP beads, Beckman Coulter) with a Bead:DNA (PCR-product) ratio of 1:1 (v/v).

2.8 | Pool preparation and library qualification

For the purified index-PCR products, one library pool was generated from all samples by mixing 5 µl of each of the purified amplicon samples. The library was stored at −20°C until sequencing. The High sensitivity DNA LabChip Kit (Agilent Technologies) was used on the 2100 Bioanalyzer (Agilent Technologies) to analyze the integrity and peak distribution of the library pool of the purified and pooled index-PCR amplicons. The library pool was quantified using the highly sensitive fluorescent dye-based Qubit® dsDNA HS Assay Kit (Invitrogen).

The pool comprised a main amplicon peak at 587 bp and a second one with 661 bp, corresponding with the expected amplicon size and no signs of contamination with sequence-compromising primer dimers in lower molecular ranges. Some weak higher molecular weight background signals were detected; however, this is of no concern as cluster generation highly favors lower molecular weight fragments.

The pool library was quantified using the highly sensitive fluorescent dye-based Qubit® dsDNA HS Assay Kit (Invitrogen) and it showed a final DNA concentration of 219 ng/µl and a molar concentration 48 nM (calculated for a length of 600 bp).

2.9 | Sequencing

Library pool was subjected to a denaturation step using NaOH, to ensure the presence of single stranded DNA (ssDNA) fragments for cluster generation. Thus, the library consisted of ssDNA fragments with sequencing adapters and indices. The library pool was diluted and used for loading on the MiSeq® system for cluster generation and sequencing. Sequencing was performed at a final concentration of 16 pM and with a 20% genomic DNA control library spike-in. Cluster generation and sequencing was done using Miseq Reagent kit v3 600 cycles. Bidirectional sequencing was performed producing a maximum of 600 bases of sequence information in 2 × 300 bp paired-end (PE) reads.

2.10 | Data processing

The Illumina software MiSeq® Reporter (MSR) on the MiSeq® system and the Illumina Sequence Analysis Viewer (SAV) were used for imaging, data processing and evaluation of the sequencing run. Raw sequence reads were analyzed using dada2 implemented in QIIME2 (Callahan et al., 2016). Data was then subjected to an evolutionary placement algorithm (EPA) analysis by RAxML EPA. The principle methods, including the MAFFT alignment of individual sequences to a reference alignment, were performed as per Senés-Guerrero et al. 2016 (Senés-Guerrero & Schüßler, 2016). Identified species were given EP numbers. Evolutionary placement numbers (EP) are species-identifier numbers based on numbers previously used by SYMPLANTA (Loján et al., 2017; Senés-Guerrero et al., 2014; Senés-Guerrero & Schüßler, 2016). EP numbers were used because they do not change through the process of taxonomic changes, and they allow comparison of species communities between studies.

2.11 | Biodiversity indices calculation

Shannon-Wiener (H) and Pielou's evenness (E) diversity indices were calculated to assess the diversity of samples collected from different locations. Indices were calculated using the following formulas (Suleiman et al., 2019).

$H = -\Sigma pi.log\ (pi)$, where pi is the proportion of the frequency of number of reads of a certain identified representative sequence over the total number of reads of all sequences identified in a location.

$E = H/log(S)$, where S is the total number of identified sequences in a location.

2.12 | Statistical analysis

The Spearman correlation test was conducted at a significance level of $P \leq .05$ to test for the correlation between the number of spores per 100 g of soil sample, each of the number of AMF identified per sample and the Shannon biodiversity index.

3 | RESULTS

3.1 | Soil analysis of the sampling sites

During the study of soil analysis from eight locations, the results showed that the texture classes of soil samples generally were silty sand with alkaline pH ranging between 7.56–9.10. The soil is generally calcareous with total calcium carbonate ($CaCO_3$) values ranging between 302518.16 and 455782.04 ppm. The (EC) electrical conductivity on the rhizosphere soil samples ranges between (.1–3.5 ppt), indicating that the soil is suitable for normal plant growth. This is with exception to the soil collected from Qatar University's protected field which is to be considered moderately saline. On the other hand, the

TABLE 2 Physical and chemical characteristics of soil samples in studied location

Locations	Doha north sewage treatment plant	Assa Alraai	Qatar university protected field (Sabkha)	Northeast Alkhor	Umm Alkilab	Tweem	Qatar university campus	Roudhat Rashed
pH	7.56	8.22	8.65	8.88	8.53	8.69	8.91	9.1
Salinity, ppt	1.2	.2	3.5	.1	.1	.1	.1	.1
TDS, g/L	1.816	.283	5.03	.1889	.1748	.1723	.1809	.1216
Ca (ppm)	131,414	168,704	168,308	221,277	112,275	171,339	144,637	116,951
K (ppm)	8654	11,015	7882	8281	16,547	9607	8188	8946
Mg (ppm)	17,549	37,696	23,779	24,755	48,255	44,078	10,383	15,891
Na (ppm)	9215	5772	10,504	5792	4866	5338	9209	7881
P (ppm)	147	476	172	316	1028	400	423	177
% TOM	NIL	NIL	NIL	NIL	1.63	NIL	NIL	NIL
CaCO3 (%)	36.65	41.71	45.58	43.55	30.56	38.51	31.90	3.25
Cl (ppm)	2667.65	.08	8111.41	999.83	1164.05	1493.65	2501.93	1820.60
Clay %	1.16	5.41	1.55	3.18	3.99	3.17	0	1.04
Silt %	15.61	47.29	21.42	36.64	34.36	37.71	7.46	1.58
Sand %	83.23	47.3	77.03	60.18	61.75	59.12	92.54	88.38
Texture	Silty sand	Silty sand	Silty sand	Silty sand	Silty sand	Silty sand	Sand	Silty sand

soil was very poor in organic matter and a negligible proportion of organic matter appeared in only one site (Table 2).

3.2 | Plant identification

The total number of collected plants from the eight studied areas was 16 plants. Plant samples collected from each area were identified at the herbarium of Qatar University. The collected plant samples belong to 12 different plant families (see Table 1).

3.3 | Arbuscular mycorrhizal fungi colonization in roots

All root segments examined were colonized by AMF with the occurrence of hyphae, vesicles and arbuscules, whether internally and/or in combination with the roots. The intensity of infection with arbuscules was less in comparison to mycelium and vesicles. Most of the samples were found to have no arbuscular presence in their root systems.

The percentages of infection with the mycorrhizal fungi in the roots are presented in Table 3. The colonization rate among plant species varied, the high percentage of AMF root colonization was found to be 100% in *Tamarix aphylla*, and the lowest value was 12% in *Zygophyllum qatarense* (Figure 2).

3.4 | Arbuscular mycorrhizal fungi spores in soil

Spore population varied from 29 to 643 spores/100 g, the highest spore number was 643 spores/100 g of dry soil accompanied with *Fagonia indica* at Assa Alraai location followed by *salvia aegyptiaca* 581 at North East Alkhor, while the lowest value was 29 spores/100 g accompanied with *Blepharis ciliaris* at Qatar University's protected field.

The average number of spores per sample collection was calculated as spore count per 100 g soil. The highest spore count was recorded in soil samples of the Twame area with 562.7, followed by Assa Alraai 515.2, and Northeast Alkhor 494 spore/100 g soil (Figure 3).

## 3.5 | DNA integrity and concentration of the library

The integrity and purity of the AMPure XP beads purified library pool was checked using the High Sensitivity DNA LabChip Kit on the Bioanalyzer. The electropherogram of the purified amplicon library quality check is shown in Figure S2.

3.6 | Sequencing results' quality

Quality criteria according to Illumina Inc. were evaluated and are summarized in Table S3.

TABLE 3 Means of root colonization (±SD, $n = 3$) (%) of AMF and spore density (number/100 g soil) in different plants at different site locations

Location	Geographic location latitude(°N) /longitude (°E)	Host plant	Spores/100 g soil	AMF root colonization %
Doha north sewage treatment plant	25.454167/51.300564	*Zygophyllum qatarense*	171.7 ± 6.11	12%
		Tamarix aphylla	150.7 ± 6.11	100%
		Launaea nudicaulis	443 ± 6	63%
Assa Alraai	25.662816/51.393684	*Sclerocephalus arabicus*	387.3 ± 252.42	32%
		Fagonia indica	643 ± 6.08	32%
Umm Alkilab	26.049472/51.208722	*Spergula fallax*	318.3 ± 23.63	10.9%
		Cynodon sp	570.6 ± 88.94	49%
Twame	26.041618/51.249680	*Plantago ovata*	562.6 ± 76.96	77.8%
North east Alkhor	25.715596/51.449514	*Salvia aegyptiaca*	581 ± 243.91	78%
		Lycium shawii	407 ± 151.92	50.8%
Roudhat Rashed	25.303106/51.192371	*Aizoon canariense*	66 ± 6.24	32%
		Pulicaria undulata	39.7 ± 4.93	76.6%
Qatar university campus	25.374962/51.490011	*Malva parviflora*	129 ± 36.43	33.9%
		Paronychia arabica	220.3 ± 14.29	22%
Qatar university protected field (Sabkha)	25.370381/51.495726	*Blepharis ciliaris*	29.3 ± 8.33	98.3%
		Sporobolus ioclados	86.3 ± 8.5	92%

FIGURE 2 Fungal structures of arbuscular mycorrhizae (AM): (a) *Launaea nudicaulis* roots (4X). (b) *Tamarix aphylla* roots (10X). (c and d) *Pulicaria undulata* roots (40X). V: vesicle. H: hypha. A: arbuscular
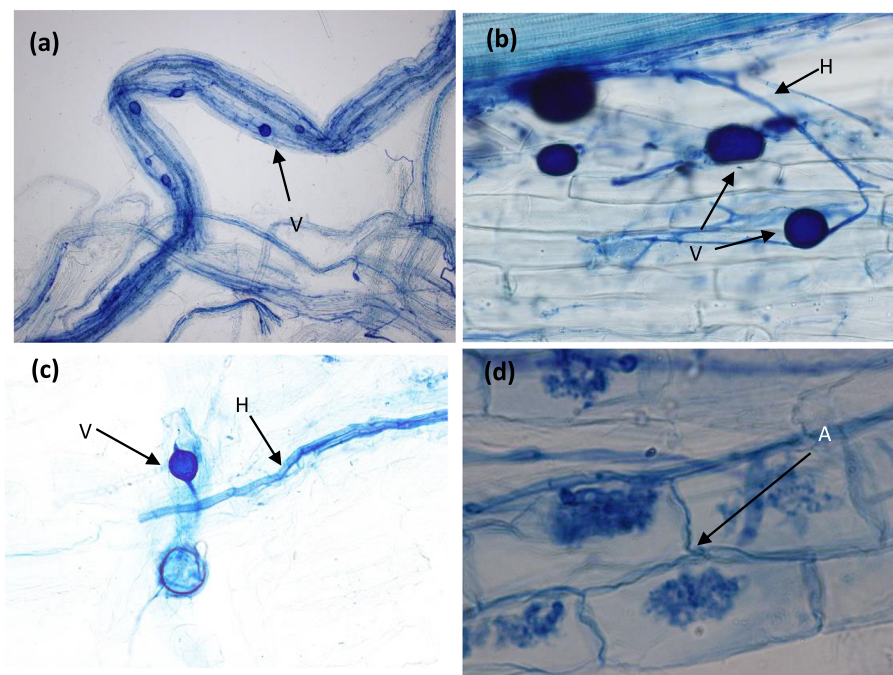
Read counts per sample showed high quality and enough read counts and thus are suitable for downstream in-depth data analysis. The filter-passed clusters led to an amount of up to 242,000 reads cumulated per custom sample, indicating the high variability of the samples. All samples reached the target number of approximately 10,000 reads per sample.

3.7 | Molecular characterization of the arbuscular mycorrhizal fungi communities in the soil samples

The total community DNA isolated from soil samples was subject to the evolutionary placement algorithm (EPA) analysis, MAFFT alignment permit the identification of 12 representative sequences at the
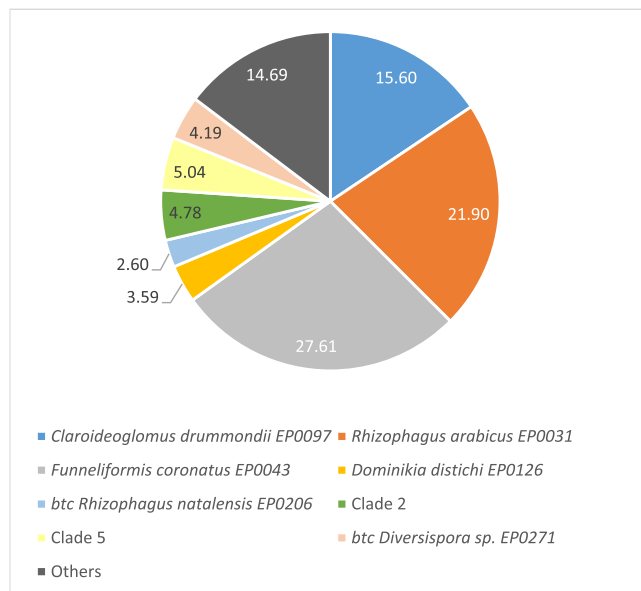
FIGURE 3 Identified arbuscular mycorrhizal fungi (AMF) species abundance (percentage) in respect to overall results. Clade 5: spores are affiliated to the node giving rise to *Diversispora varaderana* EP0222/insculpta EP0121/sp. EP0242/sp. env. EP0017. Clade 2: spores are affiliated to the node giving rise to Kamienskia divaricata EP0255/Kamienskia perpusilla EP0120/Mikrokamienskia peruviana EP0321

species level. The total abundance of each of the representative sequences in all samples was calculated, *Funneliformis coronatus* was found to be the most abundant and it dominated *Launaea nudicaulis*, *Zygophyllum qatarense*, and *T. aphylla*. *Rhizophagus arabicus* is the second most abundant species dominating samples *Malva parviflora*, *Paronychia Arabica*, *Aizoon canariense*, and *Pulicaria undulata*. Finally, *Claroideoglomus drummondii* is the third most abundant species dominating samples *Launaea nudicaulis* and *Blepharis ciliaris* (Figure 3).

Eleven representative sequences were identified to a basal to clade level (btc) which means that the sequences could belong to the species with the given EP number or they could belong to a sister species. Six representative sequences are originated from the node that is basal to the three or four species which EP numbers are listed. The remaining nine representative sequences were identified at a broader level with seven of them affiliated to the large clade giving rise to the species listed in the table and the last two affiliated to the basal node of the family listed in Table 5 and Figure 5.

Claroideoglomus drummondii is the most widespread (the only species detected in all samples) and the second most abundant after that was *Funneliformis coronatus* in terms of read numbers. *Pulicaria undulata* showed the highest number of identified representative sequences with 26 AMF species detected, followed by *Malva parviflora* and *Paronychia arabica* with 23 AMF species and *Salvia aegyptiaca* and *Plantago ovata* with 22 and 21 AMF species respectively. According to Shannon diversity index, *Salvia aegyptiaca* and *P. ovata* are the most diverse (Table 4). *Corymbiglomus pacificum* or a sister species was found with seven reads only in only one of the

samples (*Sporobolus ioclados*), this is extremely low, but cannot be explained with any artifact, thus is interpreted as a real, but low abundance occurrence. *Blepharis ciliaris* showed the lowest diversity, with three species, all in significant abundance (*Claroideoglomus drummondii* EP0097 66%, btc *Rhizophagus natalensis* EP0206 10%, btc *Rhizophagus silesianum* EP0318 24%).

Figure 4 shows the correlation between the total number of spores isolated per 100 g of a soil sample and the total number of identified AMF per sample in part A, and the total number of spores isolated per 100 g of a soil samples and the Shannon biodiversity index in part B. As we can see, there is more harmony between trend-lines of part B of the figure, with the exception of the first sample compared to part A. When observing the number of spores identified per 100 g of soil in comparison with the total number of identified AMF, the trend-lines did not show harmony in the first samples and in the last four samples. If we connect those results to the percentages of identified AMF in each soil sample represented in Figure 5, we notice that those samples have a dominant species, which occupies around 80% of the sample. This is in harmony with their low evenness in those samples (Table 4). Spearman correlation analysis support the results by showing a significant correlation between the number of spores per 100 g of soil and the Shannon diversity index ($P = .017 < .05$). Note that correlation between the number of spores per 100 g of soil and the number of identified AMF per sample was not significant.

Sample 1 is dominated by *Claroidoglomus drummodii* and *Funneliformis coronatus*, while the last four samples are dominated by a species related to *Septoglomus titan*. Considering that metagenomic analysis was used for AMF identification, the number of identified AMF does not necessarily reflect active germinated spores, it could be that the soil in locations 1, 13, 14, 15, and 16 are rich with dormant spores while the actual number of active mycorrhiza is low, which explains the low spore count per 100 g of soil. Figure 5 also shows that the samples with the most varied sequences are samples 8, 10, and 11. This is in great harmony with the Shannon indices calculated where sample 8 showed the highest diversity index (2.4) followed by samples 10 and 11 with diversity index of 2.3 and 2.0 respectively.

Table 5 shows the distribution of the identified AMF according to plant species. The species *Claroidoglomus drummodii* was found in all studied host plants. Other species occurred in 15 (out of 16) host plant species and few species were found only in one host plant (Table 5).

4 | DISCUSSION

Several factors can affect the life cycle of AMF, among those being temperature, anthropogenic disturbances, plant species dynamisms, light availability, rainfall, soil nutrient, and composition, root exudations, as well as competition associated with other microorganisms that have interactions with them (Chaudhary et al., 2014; Gong et al., 2012; Jefwa et al., 2012; Liu et al., 2009). Although significant correlation has been identified between the diversity index of each

TABLE 4 Frequency and diversity indices of samples from different origins

	QAT01	QAT02	QAT03	QAT04	QAT05	QAT06	QAT07	QAT08	QAT09	QAT10	QAT11	QAT12	QAT13	QAT14	QAT15	QAT16
Total number of species	10	6	8	19	14	3	10	22	14	21	10	18	23	23	18	26
Shannon–Wiener index H	.76	.23	1.21	1.30	1.57	.86	1.22	2.43	1.91	2.34	1.98	1.44	.96	.87	.71	.90
Pielou's eveness index E	.33	.13	.58	.44	.59	.78	.53	.79	.72	.77	.86	.50	.31	.28	.25	.28
e.H.	2.14	1.26	3.34	3.66	4.8	2.35	3.39	11.3	6.77	10.4	7.21	4.21	2.61	2.38	2.03	2.47

sample and the total number of spores per 100 g of sample, in other words indicating that soils with convenient conditions are likely to be diverse with different AMF species, however when the Spearman test was conducted between the number of spores and the raw data of total number of identified AMF per soil sample, correlation was far from significant, further look at the data shows that many samples rich with spores had one or two dominant species, which lower their diversity. As a conclusion, convenient soil conditions might lead to the growth of various types of AMF, while specific conditions might also lead to the domination of particular species that leave the soil rich with a specific type of AMF and eliminate others per competition.

Although properties of soil have been confirmed as essential factors determining the AMF community, there is no one major factor affecting the community of mycorrhiza fungi (Chaudhary et al., 2014; Gong et al., 2012; Melo et al., 2017). In our study, no specific correlation was found to be significant between each of the soil parameters and the number of spores, and neither with the percentage of root colonization. These results agree with the study by Oehl et al. (2010).

The pH range among our study sites were very narrow, from 7.56–9.10, so no significant correlation was found with soil spore count nor root colonization. Similarly a study conducted by Bainard et al. (2014) reached the same conclusion. This non-significant correlation with soil pH has been explained by the fact that optimum pH is variable among mycorrhizae species. The effect of pH may vary from one species to another. A study by Melo et al. (2017) found that members belonging to *Acaulosporaceae* expressed a negative correlation with pH, contrarily, the members belonging to Glomoid undetermined group expressed a positive correlation with pH. Other studies indicated that there is a positive relationship between the population of mycorrhizae and the pH, as the percentage of infection increases when pH rises (Gai & Liu, 2003; Gunasekaran et al., 1987; Postma et al., 2007)., while others found some species are strictly reported in acidic soil (Bainard et al., 2014).

The pH of the soil was also found to significantly affect the diversity of AMF populations (Mosbah et al., 2018), however this is not the case in our study which might be due to the narrow differences in pH among soil samples.

It can be noticed in the chemical analysis of the soil that the phosphorus amount has changed from one area to another. The highest amount of phosphorus encountered was in Umm Alkalib, which is linked to low roots colonization (10.9% and 49%). This makes sense as colonizing spores provide plants with the phosphorus from the soil and lack of colonization of AMF would lead to phosphorus accumulation in the soil.

In our study, the only soil sample that showed the highest salinity (3.5 ppt) was collected from a protected field at QU Campus where the number of fungal spores is the lowest compared to other studied sites. Salinity may negatively affect AM fungal development and hyphal augmentation (Gabchenko, 2008). Studies done by different scientists have different views on the effect of salinity on mycorrhizae development. Barrow et al. (1977) reported that sporulation and colonization of fungus are inversely related to the salinity of the soil. The decreased colonization is mainly associated with high concentrations
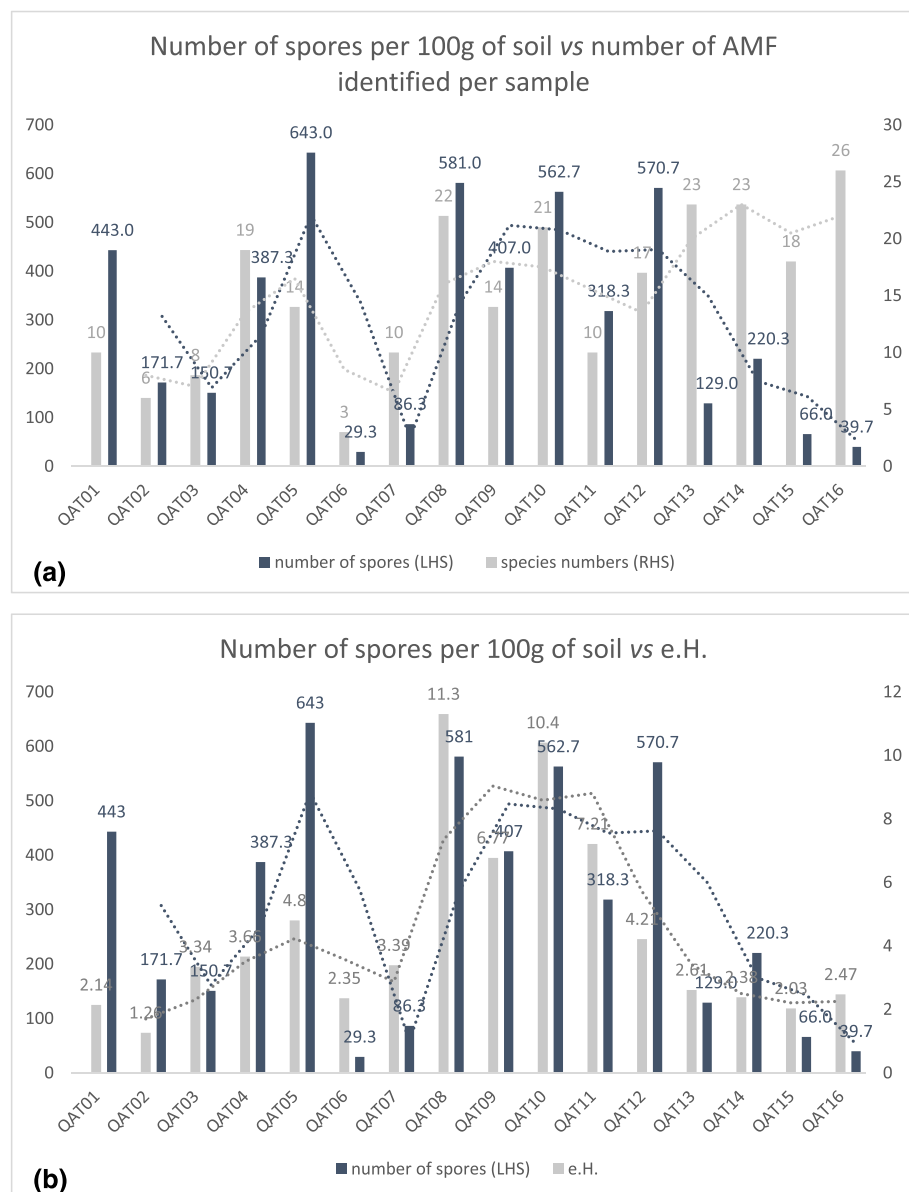
FIGURE 4 Correlation between the spores count per 100 g of soil sample and the number of AMF identified per sample (a) compared to the correlation between the spores count per 100 g of soil sample and exponential of Shannon diversity index (e.H.) (b) in the 16 studied samples

of sodium chloride (Barrow et al., 1997). Saint-Etienne et al. (2006) reported a negative correlation between salinity and mycorrhizal infectivity. An increase in salinity from 5% to 22% results in decrease in infectivity from 100% to 6% respectively. Thus, overall there are conflicting reports on the effects of high saline concentrations on fungus growth and germination (Saint-Etienne et al., 2006). The impacts of soil salinity on spore germination of AMF growths, and consequently hyphal generation, is a standout among the most critical adverse effects of salinity on mycorrhizal colonization (Juniper & Abbott, 2006). Therefore, the presence of AMF in salt land may depend on the type of host plant and may not on the environmental stresses (Nurbaity, 2014).

The amount of AMF spores found in rhizosphere soils are different from one plant species to another despite the fact they are from the same habitat. This implies that the distribution of AMF is not influenced by the zonation arrangement of vegetation, but instead is more linked with the plant's species. Therefore, differences in spore counts may be attributed to the variation in the behavior associated with each AMF species and its host, even in the same ecosystem (Klironomos et al., 2011).

The mycorrhizal colonization for selected plant species growing in Qatar was not studied before for their structural colonization with AMF. All the plants studied were infected with AMF but in different proportions. Biotic and abiotic factors affect the number and type of AMF, so the percentage of infection varies from plant to plant and from one region to another (Escudero & Mendoza, 2005; Moreira et al., 2006). For example, our findings of the Zygophyllaceae family are against the findings of Varma (1999) who mentioned that this family is mycorrhizae free. We found that the percentage of AMF infection of two plant species belonged to this

FIGURE 5 Percentages of identified AMF in each soil sample out of the total identified representative sequences per sample. Clade 1: spores are affiliated to the node giving rise to Septoglomus turnaue EP0113/jasnowskae EP0132/xanthium EP0263/fuscum EP0139. Clade 2: spores are affiliated to the node giving rise to Kamienskia divaricata EP0255/Kamienskia perpusilla EP0120/Mikrokamienskia peruviana EP0321. Clade 3: spores are affiliated to the node giving rise to Dominikia duoreactiva EP0260/D. achra EP0116/D. lithuanica EP0259. Clade 4: spores are affiliated to the node giving rise to Mikrodominikia litorea EP0304/Orientoglomus emiratum EP0267/Dominikia sp. EP0202. Clade 5: spores are affiliated to the node giving rise to Diversispora varaderana EP0222/insculpta EP0121/sp. EP0242/sp. env. EP0017. Clade 6: spores are affiliated to the node giving rise to Diversispora jakucsiae EP0122/arenaria EP0143/slowinskiensis EP0217
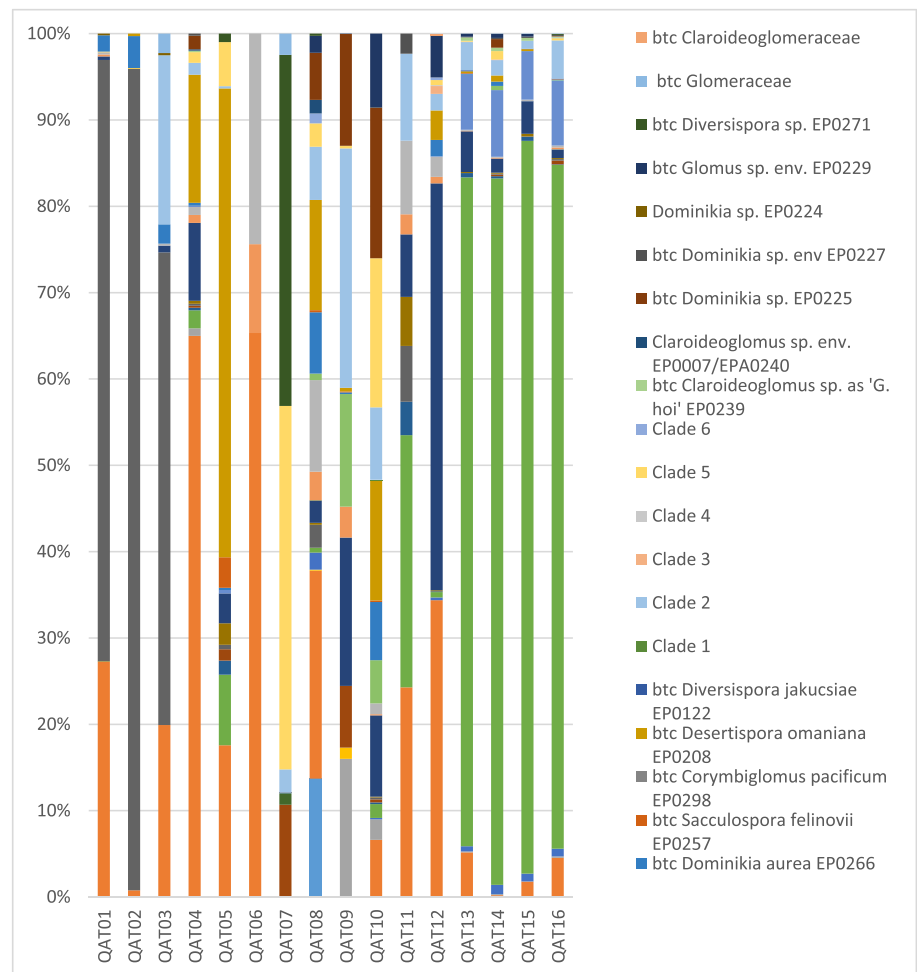
family, *Zygophyllum qatarense* and *Fagonia indica* was 14% and 32%, respectively.

Also a study by Chaudhry et al. (2013) showed that the status of root colonization of arbuscular mycorrhiza with *T. aphylla* was very variable even in the same rhizosphere, ranging from 46% -72% and the spore counts ranged between 60 to 246 spores/100 g soil, while in our study the percentage of roots infection for the same species was 100% and the average spore count was 150.7 spores/100 g soil. This discrepancy in the results confirms that the rate of the infection of the root with the number of AMF is affected by many interrelated factors such as salinity, temperature, and season among others.

In previous literature, authors have reported positive correlations between soil spore counts and AMF root colonization rates (Błaszkowski, 1994; Khakpour & Khara, 2012; Sivakumar, 2013), in contrary, some reported negative correlations (Louis & Lim, 1987), while others found both a positive and negative association between AMF colonization and spore population (Khanam et al., 2006). Our results imply no correlations between spore counts and root colonization, similarly as found by Diaz and Honrubia (1994). According to Hetrick and Bloom (1986), the number of AMF fungal spores in natural habitats is not always related to the amount of AMF colonization and this was explained by the fact that AMF spores of certain species

sometimes take a long time to germinate, in addition to the importance of seasonality and variations in environmental factors among different seasons (McGee, 1989).

Annotations that are said to originate from a node basal to species or a large clade giving rise to some species or even a basal node of a family could be new discovered species. However, they could be described species which are not defined by DNA sequences yet. This is not unexpected, as for fungi in general and AMF in particular, only 5%–10% of species are known, and even less are defined by DNA data. Therefore, for many AMF occurring in nature, no sequence data is yet available. The EPA methods allow to recognize such species, not represented in the databases, to a certain level, whereas an EPA affiliation at a "basal" node (e.g. basal to a family) might be composed by more than one species, as branching at deep nodes are unknown.

According to Schüßler and Walker (2010), *Claroideoglomus drummondii*, *Rhizophagus irregularis* is the most widespread species. Our results indicated that *Claroideoglomus drummondii* was the only species detected in all study site samples, which indicates the ability of this species to withstand high temperatures and alkaline soils in addition to its efficiency in establishing symbiosis with roots of different host plants. Similarly the species *R. irregularis*, was found in all our soil samples except one, however many studies mentioned that this

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TABLE 5   AMF distribution in various host plant collected from 16 study sites

No.	I-no.	Annotation	QA T01 Launaea nudicaulis	QA T02 Zygophyllum aduense	QA T03 Tamarix aphylla	QA T04 Sclerocephalus arabicus	QA T05 Fagonia indica	QA T06 Blepharis ciliaris	QA T07 Sporobolus ioclados	QA T08 salvia aegyptiaca	QA T09 lycium shawii	QA T10 Plantago ovata	QA T11 Spergula fallax	QA T12 Cynodon sp	QA T13 Malva parviflora	QA T14 Paronychia arabica	QA T15 Aizoon canariense	QA T16 Pulicaria undulata
1	425	Claroideoglomus claroideum EP0039												X				
2	462	Claroideoglomus drummondii EP0097	X					X			X							
3	530	Rhizophagus invermaius EP 0035				X					X							
4	584	Rhizophagus intraradices EP0032								X								
5	674	Rhizophagus irregularis EP0009	X					X			X		X					
6	776	Rhizophagus arabicus EP0031	X					X			X	X	X					
7	840	Sclerocystis sinuosa EP0204																
8	851	Kamienskia bistrata EP0119	X					X			X					X		
9	1150	Funneliformis coronatus EP0043	X					X			X							
10	1245	Nanoglomus plukenetiae EP0323								X								
11	1262	Dominikia distichi EP0126	X															
12	1979	Diversispora peloponnesiaca EP0322									X							
13	736	btc Rhiziphagus clarus EP0076													X		X	
14	2719	btc Praglomus laccatum EP0100																
15	705	btc Rhizophagus natalensis EP0206	X			X	X											
16	716	btc Rhizophagus silesianum EP0318	X		X													
17	761	btc Rhizophagus fasciculatus EP0030																
18	1032	btc Septoglomus titan EP0278												X				
19	1345	btc Dominikia aurea EP0266								X								
20	1918	btc Sacculospora felinovii EP0257	X					X										
21	2197	btc Corymbiglomus pacificum EP0298																
22	2228	btc Desertispora omaniana EP0208		X														
23	2102	btc Diversispora jakucsiae EP0122																X
24	928	btc Kamienskia divaricata EP0255 / Kamienskia perpusilla EP0120 / Mikrokamienskia peruviana EP0321			X				X	X	X						X	X
25	981	btc Septoglomus turnaue EP0113 / jasnowskae EP0132 / xanthium EP0263 / fuscum EP0139										X						
26	1304	btc Dominikia duoreactiva EP0260 / D. achra EP0116 / D. lithuanica EP0259												X				
27	1438	btc Mikrodominikia litorea EP0304 / Orientoglomus emiratum EP0267 / Dominikia sp. EP0202														X		
28	1940	btc Diversispora varaderana EP0222 / insculpta EP0121 / sp. EP0242 / sp. env. EP0017						X		X								X
29	2099	btc Diversispora jakucsiae EP0122 / arenaria EP0143 / slowinskiensis EP0217								X								
30	385	btc Claroideoglomus sp. as 'G. hoi' EP0239																X
31	355	Claroideoglomus sp. env. EP0007/EPA0240																
32	1338	btc Dominikia sp. EP0225				X												
33	1373	btc Dominikia sp. env EP0227				X						X						
34	1388	Dominikia sp. EP0224	X		X													
35	1504	btc Glomus sp. env. EP0229																
36	2055	btc Diversispora sp. EP0271					X											
37	360	btc Claroideoglomeraceae													X			
38	485	btc Glomeraceae			X			X										X

	Spores' representative sequences allowed identification at the species level with matching between our DNA samples and previously sequenced environmental samples (EP number)
	Spores' representative sequences allowed to relate the spores to the listed species in the table keeping a margin that it could also be a closely relate sister species (basal to clade)
	Spores' representative sequences indicates that the spores are affiliated to the node giving rise to the 3 or 4 species listed in the table[a]
	Spores' representative sequences idicates that the spores are affiliated to the large clade giving rise to the species listed in the table[a]
	Spores' representative sequences idicates that the spores are affiliated to the basal node of the family listed in the table[b]

[a]The EPA makes a maximum likelihood based computation of the representative sequence under consideration into the reference tree. It gives the likelihood value to where the sequence evolutionary belongs; it is based on real phylogenetic computation.

[b]It is usual that the representative sequence itself is unknown. In AMF maybe only 5%–10% of species are known and even less are defined by DNA data. Therefore, unknown sequences are either new discovered species or just previously described species which have their DNA not sequenced yet.

species is the same as *Glomus intraradices* (Beltrano et al., 2013; Berruti et al., 2016; Fileccia et al., 2017; Hashem et al., 2018). However a study carried out by Stockinger et al. (2009) genetically separated the two types. During our personal communication with Professor Arthur Schüßler (Head of R&D Wilhelms GmbH, Managing Director & Owner Symplanta GmbH & Co. KG) about the two species, he mentioned to us "what formally was named 'intraradices' is *irregularis*, in at least 99% of the cases." Numerous studies have proven the effectiveness and usefulness of *G. intraradices* in agricultural fields, from the aspects of promoting plant growth as well as enhancing soil potassium content (Zhang et al., 2017); increasing crop yields (Ceballos et al., 2013); inducing growth promotion of cucumber (Ravnskov & Larsen, 2016) and alleviating the damage caused by salt stress (Beltrano et al., 2013).

Diversispora aurantia EP0074 was found in only one sample. This species has never been found in its northern portion, according to the 6000 rhizosphere soils surveyed from around the world (Blanke et al., 2004; Błaszkowski, 2012), possibly due to the inability of this species to withstand extreme temperatures.

In this research, some of the recorded fungal species were already investigated by other authors to improve plant growth and help plants to overcome stress. For instance, *rhizophagus irregularis* (previously known as *Glomus irregulare*) which is one of the most widespread species in our study, was subjected as an inoculum in many studies to investigate plant growth promotion (Zhang et al., 2017), not only that but it was also formulated to become a commercial product with 39% production rate compared to other AMF. The results of the abovementioned product were positive in several research applications. For example: increased plant growth as well as potassium content (Zhang et al., 2017), increase cassava yields (Ceballos et al., 2013), caused growth promotion of cucumber (Ravnskov & Larsen, 2016) and alleviating the damage caused by salt stress (Beltrano et al., 2013). In addition to that *Rhizophagus intraradices* (previously known as *Glomus intraradices* and *Rhizoglomus intraradices*) experiments have also proven their positive effect on enhancing plant growth such as increasing dry matter biomass and chlorophyll content (Hajiboland et al., 2010) and increase phosphate content (Bayani et al., 2015).

The discovery and identification of these fungi that help plants to overcome various environmental conditions and bear to improve plant growth in the arid Qatari environment is essential for paving the way for future potential research into environmental protection and sustainable agriculture applications.

5 | CONCLUSION

Rhizosphere soil samples and roots of 16 plant species from eight locations were investigated. Twelve representative sequences have been identified at the species levels based on DNA molecular markers, while 2 representative sequences were identified at the family level which indicates that these two sequences are either newly discovered species or just previously described species which do not have their DNA sequenced yet. A part of these species was already investigated and proved to be effective in sustainable agriculture applications. However, strains that have been discovered to be effective in one environment might be with no effect in another environment, particularly in arid lands. Thus, relying on native AMF species is a better choice. Results from such a study will open research for agricultural applications in greenhouse and field crops.

For the selected plant species growing in Qatar, mycorrhizal colonization with AMF was not studied previously. The AMF species described in this study are the first to be discovered throughout the Qatar state which contributes to the literature about the number of species known to exist in this arid region. Because all our isolates came from arid environments, these AMF could help in conserving the biodiversity of desert ecosystems via biological mechanisms to mitigate the negative effects of abiotic stress. In addition, it may have beneficial applications, mainly in agriculture and food security.

CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

KA and MHA designed the study, KA performed the experiments, KA, IS, and MHA analyzed the results, KA wrote the manuscript. IS and MHA reviewed and edited the manuscript and MHA supervised the work.

ORCID

Iman Saleh https://orcid.org/0000-0003-3025-1401

Mohammed H. Abu-Dieyeh https://orcid.org/0000-0002-0417-3414

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