Absence of Gigasporales and rarity of spores in a hot desert revealed by a multimethod approach

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A multimethod approach to assess arbuscular mycorrhizal fungal diversity in a hot arid and hyperalkaline region

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Abstract

Hot deserts impose extreme conditions on plants growing in arid soils. Deserts are expanding due to climate change, thereby increasing the vulnerability of ecosystems and the need to preserve them. Arbuscular mycorrhizal fungi (AMF) improve plant fitness by enhancing plant water/nutrient uptake and stress tolerance. However, few studies have focused on AMF diversity and community composition in deserts, and the soil and land use parameters affecting them. This study aimed to comprehensively describe AMF ecological features in a 5,000 m^2 arid hyperalkaline region in AlUla, Saudi Arabia. We used a multimethod approach to analyse over 1,000 soil and 300 plant root samples of various species encompassing agricultural, old agricultural, urban and natural ecosystems. Our method involved metabarcoding using 18S and ITS2 markers, histological techniques for direct AMF colonization observation and soil spore extraction and observation. Our findings revealed a predominance of AMF taxa assigned to Glomeraceae, regardless of the local conditions, and an almost complete absence of Gigasporales taxa. Land use had little effect on the AMF richness, diversity and community composition, while soil texture, pH and substantial unexplained stochastic variance drove their structuring in AlUla soils. Mycorrhization was frequently observed in the studied plant species, even in usually non-mycorrhizal plant taxa. Date palms and *Citrus* trees, representing two major crops in the region, displayed however a very low mycorrhizal frequency and intensity. AlUla soils had a very low concentration of spores, which were mostly small. This study generated new insight on AMF and specific behavioral features of these fungi in arid environments.

Introduction

Hot arid and hyperarid environments encompass approximately 19% of the Earth's terrestrial surface (Laity 2009) and are currently expanding as a result of climate change (IPCC 2022). Within these regions, soils have low organic matter contents, neutral to alkaline pH, high salinity, carbonate accumulation, reduced biological activity and low humidity (De Deyn et al. 2008; Laity 2009). Furthermore, hot arid environments are characterized by high temperatures, intense ultraviolet radiation, infrequent and irregular rainfall (Almazroui 2013). Plants exposed to these harsh conditions have thus developed specific adaptations, such as symbiosis, that enable them to survive and develop in arid and hyperarid ecosystems.

Among these, arbuscular mycorrhizal fungi (AMF) are a group of obligate plant root symbiotic fungi belonging to the Glomeromycotina subphylum (Spatafora et al. 2017). This type of symbiosis is the most prevalent worldwide as it occurs among ~ 80% of vascular plant species (Brundrett and Tedersoo 2018). AMF develop a network of fine hyphae that increase the exchange surface with the soil, thereby improving the plant's assimilation of nutrients such as phosphorus, nitrogen, calcium, magnesium and iron (Apple 2010). Notably, these hyphae are able to extract water from small pores in soil that would typically remain inaccessible to plant roots (Dayana et al. 2020; Vasar et al. 2021). They can also stabilize soil aggregates, thus preventing erosion, through a combination of biophysical, biochemical and biological processes (Rillig and Mummey 2006). Finally, AMF hyphae can jointly improve plant tolerance

to biotic and abiotic stresses, including salinity, heavy metal exposure, heat stress and drought (Apple 2010; Duc et al. 2018), as well as pathogen tolerance (Jaiti et al. 2007). Thanks to these features, AMF is a crucial symbiont in desert soils with low nutrient and water availability, while enabling the plants to establish and survive (Al-Whaibi 2009; Albaqami et al. 2018). Overall, arbuscular mycorrhizae could help reduce irrigation and fertilization needs in agriculture, while reducing the impact of plant diseases (Tyagi et al. 2017; Vishwakarma et al. 2022). AMF could also help reduce crop water consumption in arid and hyperarid regions where water is a scarce and depleted resource (Bi et al. 2018; Koech and Langat 2018; Seraphin et al. 2022), while mitigating desertification, which is why these fungi are currently being studied and used in revegetation programs (He 2020; Silva et al. 2022).

Even with these potential benefits of AMF on desert plants, non-mycorrhizal plants are considered to be more common and diverse in arid lands (Soudzilovskaia et al. 2017; Brundrett and Tedersoo 2018). Desert plants also have amongst the lowest AMF root colonization and root AMF biomass levels as this colonization is dependent on the fine root number, which is low in deserts (Treseder and Cross 2006). Glomeraceae and Diversisporaceae are the most abundant AMF families, while *Glomus* is generally the most abundant genus detected in desert soils probably because of their capacity to thrive in a broad range of niches (Beena et al. 2000; Albaqami et al. 2018; Adenan et al. 2021). Yet few studies to date have focused on desert environments and soils (Harrison and Griffin 2020; Chu et al. 2020), so data on AMF are still lacking in fungal reference databases such as MaarjAM, GenBank and FungalRoot (Ryberg et al. 2009; Öpik et al. 2010; Soudzilovskaia et al. 2020). New studies are needed to enhance our knowledge and our databases on AMF spanning in arid lands and to further understand the impacts of the specific environmental conditions on arbuscular mycorrhizae.

The aim of the present study was to describe and understand the AMF communities found in the hyperarid region of AlUla, Saudi Arabia. We specifically focused on identifying the AMF taxa present in this hyperarid and hyperalkaline region, while determining the parameters that shaped the AMF communities in these conditions, the plants that formed mycorrhizae and the spore profiles in these soils. Understanding the nature and structure of AMF communities in deserts is essential for exploring applications of arbuscular mycorrhization in agriculture and land restoration programs.

Arbuscular mycorrhization can be studied by describing soil spores, observing AMF root colonization rates or environmental DNA sequencing, with each method having its advantages and pitfalls. Once extracted, AMF spores may be quantified and morphologically described. This can generate information on the mycorrhizal potential of the soils and the morphological traits of the spores. However, spore quantification has many shortcomings, as AMF sporulation conditions, ability and colonization rates are highly diverse (Sharmah et al. 2010). The spore number can thus provide information on the mycorrhizal potential but is insufficient to fully approach it (Gemma and Koske 1988). Spore extraction can be combined with visual taxonomic spore identification based on identification keys. This identification approach may, however, not be very efficient as it relies on distinguishing morphological variations among AMF spores. The morphology of spores of genetically distinct species can be identical, whereas high intraspecific morphological diversity can arise due to varying environmental conditions (Walker et

al. 2021). Metabarcoding offers the opportunity for more reliable taxonomic identification based on molecular markers and for relative quantification of AMF and their communities. This is, however, an indirect method that does not give direct information on the effective root colonization and metabolically active AMF. Otherwise, histological analysis and microscopic observation allows direct observation of AMF in plant roots so as to be able to measure the AMF root colonization frequency and intensity. Yet these three methods are seldom combined although it could generate more overall and precise insight into arbuscular mycorrhization processes in specific environments. Here we used these three combined methods to describe arbuscular mycorrhizae in soils and roots collected out of a representative variety of environments (e.g. geological formations, urbanization, soil types), land uses and plant species in a 5,000 km² hyperarid region.

Material and Methods

1.1. Area of study and land uses in the region of AlUla

AlUla region in northwestern Saudi Arabia (Fig. 1a) is characterized by hot arid climatic conditions, with maximum daily temperatures regularly exceeding 45°C from July to August, while rainfall is rare and irregular (30–170 mm/year) (Toumi et al. 2015). The studied area spans from 26°26'32.577" N 37°14'29.861" E to 26°54'54.34"N 38°15'5.93"E, covering > 5,000 km² (Fig. 1a). The city of AlUla found in this hyperarid desert is surrounded by natural ecosystems and farms that are irrigated with water from the Saq-RAM Aquifer (Seraphin et al. 2022). In our study, we identified four distinct land uses: natural ecosystems (e.g. in the Sharaan Nature Reserve), urban areas, and active or abandoned agrosystems. Date palm (*Phoenix dactylifera* L.), various C*itrus* species and alfalfa (*Medicago sativa* L.) are mostly cultivated in these agrosystems (Odnoletkova and Patzek 2023).

1.2. Workflow of approaches, datasets and investigated parameters

Several approaches and datasets were used in this study. The methodological workflow can be found in Fig. S1. This section outlines: i) the purpose of each approach, ii) how each dataset was generated, and iii) variables and parameters investigated in each dataset. Briefly, three approaches were used to characterize AMF in the AlUla region: i) AMF species identification using metabarcoding, ii) plant root mycorrhizal histological assessments, and iii) *in situ* status of AMF in the soils based on spore extraction. For metabarcoding, three sampling protocols were used: i) soil sampling along a kilometric grid, ii) collection of soil, rhizosphere, and roots of six plants species with contrasted symbiotic status in the Sharaan Nature Reserve, and iii) date palm root sampling in farms and natural ecosystems (Fig. 1 and S1).

1.3. Sampling protocols for molecular identification of AMF species

1. Kilometric grid sampling

The kilometric grid (hereafter called “Grid”) approach was used to investigate the diversity and composition of AMF genera at the regional scale, including environmental drivers such as soil geochemical properties (Fig. 1b, Fig. S1). The Grid shape is presented on Fig. 1b, its dimensions were about 55 km (North to South) by 30 km (East to West), with a sample collected every kilometer. Overall, during the 2019 and 2020 fall seasons, 593 soil samples were collected at 40 cm depth over a 1,039 km² area in the AlUla region. The Grid was not continuous due to the actual field conditions and discrepancies (absence of soil to collect or risky access conditions). Samples were classified according to the four land uses representative of the region: urban area, active agrosystems, abandoned agrosystems and natural ecosystems. All results generated via this approach were included in the Grid dataset.

2. Sampling protocol for the Sharaan plant species dataset

While the Grid dataset only included soils, we also investigated AMF species found on multiple plant species naturally occurring in desert ecosystems, notably in the Sharaan Nature Reserve. Details on the Sharaan plant species (hereafter called “SPS”) sampling protocol were previously described in Maurice et al. (2024b). A total of 689 root and soil rhizosphere samples were collected in natural ecosystems, from six plants species commonly found within and in the vicinity of the Shaaran Nature Reserve (Fig. 1b, Fig. S1). The collected plants were *Astragalus spinosus* (Forssk.) Muschl., *Haloxylon persicum* Bunge., *Haloxylon salicornicum* (Moq.) Bunge ex Boiss., *Helianthemum lippii* (L.) Dum.Cours., *Stipagrostis plumosa* L. Munro ex T. Anderson, and *Retama raetam* (Forssk.) Webb & Berthel. For each plant species, roots and soil rhizosphere were collected at 20–40 cm depth between two seasons, i.e. 2021 summer (August) and 2022 Spring (March). Additional bulk soil samples were collected as controls. All of these data were gathered for the SPS dataset.

3. Date palm sampling protocol

Apart from soils and natural ecosystems, we studied the most emblematic crop in the AlUla region: *Phoenix dactylifera* L., known as date palm (Gros-Balthazard et al. 2023). Roots were collected between October 2021 and October 2022 at least one meter away from the stipe (trunk) at 40 cm depth in different locations, including the AlUla urban area, the Madâin Sâlih agricultural area (north of AlUla), and in natural ecosystems of the Sharaan Nature Reserve (Fig. 1b, Fig. S1). A total of 21 date palms were sampled with: 14 individuals in farms (cv. Barni, the most common local cultivar in the region), and 7 individuals collected in natural ecosystems. Additional soil was collected in the vicinity of date palms for assessment of the pH_{H2O}, pH_{KCl} and acidification potential. Date palm data were called “Palm” dataset.

1.4. Metabarcoding datasets, library preparation and sequencing

Details on all of the molecular biological methods and pipelines used for metabarcoding analyses were described in Maurice et al. (2023). Detailed methodology can be found in Supp methodology. Briefly, DNA extraction was done on roots and soil samples. Their concentration was assessed and then samples were amplified with ITS86F (5' GTGAATCATCGAATCTTTGAA 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') primers for the fungal ITS2 region (Op de Beeck et al. 2014) for both the Grid and SPS dataset samples and with AMADf (5' GGGAGGTAGTGACAATAAATAAC 3') and AMADGr (5' CCCAACTATCCCTATTAATCAT 3') primers for the 18S rRNA gene (Berruti et al. 2017) in the SPS and Palm dataset samples. Samples from the SPS dataset amplified with the 18S and ITS2 markers are hereafter named SPS 18S and SPS ITS2, respectively. Biological material in the Grid dataset (593 samples) and the SPS dataset (689 samples) were sequenced in two independent Illumina NovaSeq runs by the Fasteris SA company (Switzerland). The 21 Palm dataset samples were sequenced in one Illumina MiSeq run (2 x 250 bp), by the Fasteris SA company (Switzerland).

Details on the data processing pipeline are available in Perez-Lamarque et al. (2022). Operational taxonomic units (OTUs) were formed after merging, cleaning and quality check of the sequences followed by a clustering with 97% similarity.

In the Grid and SPS datasets, OTUs with < 100 reads were removed. In the Palm dataset, OTUs with < 10 reads were removed because of the sequencing depth differences between NovaSeq and MiSeq. After decontamination and cleaning, the Illumina NovaSeq for SPS ITS2 yielded 101,508,231 sequence reads and 17,028,263 reads for SPS 18S; the NovaSeq for the Grid ITS2 yielded 212,457,568 sequence reads, while the Miseq for Palm 18S yielded 326,001 sequence reads. Taxonomic assignment of OTUs was performed using the UNITE V8.3 online database for ITS2 (Kõljalg et al. 2020) and the SILVA database for 18S (Quast et al. 2013). The UNITE database was used as a reference for AMF taxonomy because it included the greatest number of AMF genera, while all genera detected in SILVA were listed in UNITE.

All sequences generated in this study were deposited on the on the NCBI Sequence Read Archive (SRA) as follows: i) the Grid dataset sequences were deposited on the NCBI public repository (PRJNA1058874), ii) the Palm dataset sequences were deposited on NCBI (PRJNA1078326). SPS data sequences were retrieved from the NCBI public repository (PRJNA1061359).

1.5. Histology and AMF observation

The roots of 277 individual plants representative of the AlUla plant diversity were collected in agrosystems and natural ecosystems for microscopic observation of AMF structures (Fig. 1c, Fig. S1). These plants belonged to 25 families and 46 plant species. Regarding crops, the roots of 13 cultivated *Citrus* spp. and 127 cultivated date palms were collected in farms. Eight feral (i.e. spontaneous, not cultivated) date palms were collected in Sharaan Nature Reserve for comparison with those growing in agrosystems. The roots were stored in a glycerol, ethanol and water 1:1:1 (v:v:v) solution and stored at 4°C. Plant species were identified according to reference works (Migahid 1996; Chaudhary 1999–2001; Collenette 1999).

Detailed operation for root staining can be found in Supp methodology. Briefly, roots were stained with Trypan blue in Amman lactophenol after clearing in KOH solution following the protocol of Phillips and Hayman (1970). Five to 20 fragments of 1 cm were observed depending on the available material. When the AMF presence was confirmed and the root fragment number and quality were sufficient, the mycorrhizal colonization frequency and intensity were assessed according to the method of Trouvelot et al. (1986).

1.6. AMF spore contents in soils

A total of 12 soil samples were collected in natural ecosystems and agrosystems (Fig. 1d, Fig. S1). In natural ecosystems, two types of soils were sampled in relation to plant categories with: i) the presence of perennial plants such as date palm or *Vachellia* spp. (9 samples), and ii) only with annual plants, such as *Anthemis* or *Asphodelus* (3 samples). Preliminary tests revealed that there were no spores in 100 g of soil. As a result, the protocol was modified accordingly and 1 kg of soil was sampled per site for spore extraction.

Detailed method for AMF spore extraction can be found in Supplementary method. The method of Gerdemann and Nicolson (1963), followed by 60% sucrose/water centrifugation was used for the spore extraction. Briefly, soil was sieved sequentially through 3 sieves of 2, 1 mm and 40 µm Ø mesh. Spores were collected between the two sieves (< 1 mm, > 40 µm) and then a 60% sucrose/water solution was added before a centrifugation step. Spores were collected at the sucrose-water interface. Total spore counting was performed for each sample under a binocular magnifier to determine the number of spores/kg.

1.7. Soil chemical analysis

The pH_{H2O}, pH_{KCl} and acidification potentials were measured in all soils. All Grid dataset soils were also analyzed with X-ray fluorescence (XRF) for atomic abundance. The pH of soils was measured after mixing them at 1:5 (v:v) with H_2O or a KCl (1 $mol.L^{-1}$) solution, with a Knick 766 pH Meter (Knick International, Berlin, Germany). The acidification potential was measured as the difference between pH_{H2O} and pH_{KCl}. Grid dataset soils were prepared and analyzed using the XRF S1 Titan analyzer (Bruker, Billerica, Massachusetts, USA) to determine the atomic abundance of elements from magnesium to uranium. Triplicates of each soil were mixed with 1:3 (v:v) of SpectroBlend® (SCP Science, Baie-D'Urfé, Québec, Canada), placed in aluminum capsules, then pressed at 20 t for 2 min. Each capsule was measured three times using Geoexploration calibration. The resulting nine measures per soil sample were averaged and calibrated according to the limit of optical detection (LOD) of each analyzed element (provided by Brucker). For further analysis, the XRF values were centered log ratio (clr) because of the compositional nature of XRF data, as recommended by Reimann et al. (2012). Major elements in the soil composition that varied across samples were considered as variables: magnesium (Mg), aluminum (Al), silicium (Si), phosphorus (P), potassium (K), calcium (Ca), iron (Fe) and titanium (Ti).

1.8. Statistical analysis

All statistical analyses (graphs and calculations) were performed in R (version 4.3.0).

The soil coordinates and geochemical analyses (pH measures and clr-transformed XRF values) were visualized using a principal component analysis (PCA). Differences in soil properties between land uses in the Grid dataset were assessed using ANOVA, with clr transformation of the element atomic abundances.

For all metabarcoding data analyses, the Glomeromycotina subphylum was extracted from the total fungal diversity. Each AMF dataset was independently analyzed, mainly because the genetic markers, sample numbers, and taxonomic assignments of OTUs differed in the Grid, SPS, and Palm datasets.

Alpha diversity of AMF in each dataset was measured using Hill numbers at three diversity orders: 0 (species richness); 1 (exponential of Shannon index); and 2 (inverse of Simpson index), using the *hilldiv* package version 1.5.1 (Alberdi and Gilbert 2019). Differences in Hill numbers between groups (genetic markers or land uses) were assessed using a Wilcoxon test.

Before exploring the beta diversity of AMF communities, the Grid dataset was first rarefied to 36,947 (minimum) reads, and then only Glomeromycotina phylum reads were retained. The AMF communities were visualized using distance-based ReDundancy Analyses (dbRDA), based on Bray-Curtis distances, using the *microeco* package (Liu et al. 2021). Ellipses (95% confidence interval) were drawn based on land uses. The overall influence of land uses on AMF communities was first assessed through a permutational analysis of variance (PERMANOVA). If the influence was significant ($p < 0.05$), detailed two-by-two comparisons of land uses were obtained with multivariate analyses of variance (MANOVA). Both PERMANOVA and MANOVA were conducted with the adonis2 function in the *vegan* package (Oksanen et al. 2022). We further investigated how the environmental parameters could affect AMF communities by fitting the geochemical data and coordinate parameters into the dbRDA. Geochemical data and coordinate parameters were investigated one-by-one using PERMANOVA to test their influence on the AMF diversity. Shared taxa between land uses were identified via Venn diagrams (with the Venny 2.1 online software package; Oliveros, 2015).

Results

2.1. Overall identification of arbuscular mycorrhizal fungi genera using metabarcoding

The raw number and proportion of AMF reads in every dataset differed. Illumina sequencing allowed us to detect 49,552 AMF reads (representing 15.2% of the total fungal reads) in the Palm dataset, 3,507,822 AMF reads (20.6%) in the SPS 18S dataset, 1,725,640 AMF reads (1.7%) in the SPS ITS2 dataset and 2,337,033 AMF reads (1.1%) in the Grid dataset. Based on the UNITE database, the Glomeromycotina taxa found in our study covered all fungal classes (3/3), orders (6/6), 39% of families (7/18), and 52% of genera (24/46) (Fig. 2). The number and proportion of genera identified also differed between datasets:

Palm dataset (9 genera), SPS 18S dataset (10 genera), SPS ITS2 dataset (14 genera), and Grid dataset (19 genera).

Five genera were detected in all of the datasets: *Claroideoglomus*, *Funneliformis*, *Glomus*, *Rhizophagus* and *Septoglomus*. Glomeraceae was the most abundant AMF family (50.8% mean relative abundance) followed by Diversisporaceae (10.3%). A substantial number of AMF sequences were unassigned to the genus level (32.5% of the sequences) (Fig. S2). *Glomus* was the most abundant AMF genus (22.9%), followed by *Rhizophagus* (11.0%) and *Diversispora* (6.9%).

Regarding the Gigasporales order, only two OTUs were found in all of the datasets, i.e. one assigned to *Dentiscutata* that was found in both active and abandoned agrosystems in the Grid dataset, and another assigned to *Racocetra* in an active farm in the Palm dataset.

2.2. Gene marker affect identification of AMF communities but not diversity

The ITS2 marker had a deeper sequencing depth than 18S (101,508,231 vs. 17,028,263 reads) but the 18S marker was able to retrieve a higher proportion of AMF sequences (20.6% vs. 1.7% with ITS2). Regarding the alpha diversity, no significant differences in Hill numbers were found between the SPS ITS2 and SPS 18S datasets (Fig. S3). However, the AMF family proportions differed when comparing the SPS 18S and SPS ITS2 datasets. Indeed, the Glomeraceae family was highly dominant in the SPS 18S dataset (73.6%) compared to the SPS ITS2 dataset (34.7%). The SPS ITS2 dataset had a diverse AMF composition; with 15% Diversisporaceae (3.9% in the SPS 18S dataset) and 8% Claroideoglomeraceae (< 0.1% in the SPS 18S dataset). At the genus level, the Glomeraceae composition differed. The SPS 18S dataset was dominated by *Glomus* (56.4%) and *Rhizophagus* (14.0%), whereas *Dominikia* (15.2%) and *Kamienskia* (6.2%) predominated in the SPS ITS2 dataset. The proportion of unidentified Glomeromycotina in the SPS 18S and SPS ITS2 datasets represented 22.4% and 41.5% of the total AMF reads, respectively (Fig. S2).

2.3. Land use impacts the AMF community diversity and composition

After analyzing the overall AMF diversity in the datasets, we tested the potential impact of land uses on the AMF community structure and composition (Figs. 3 & 4). The AMF composition of the soils differed significantly ($p < 0.05$) across land uses (Fig. 3). R^2 values for the PERMANOVA and subsequent MANOVA tests were all < 0.018. The AMF composition of both active and abandoned agrosystems significantly differed from that of natural ecosystems and urban areas ($p < 0.05$). However, no differences were detected: i) between abandoned and active agrosystems, and ii) between natural ecosystems and urban areas (Fig. 3). The AMF alpha diversity was significantly higher in active agrosystems compared to natural and urban ecosystems (Fig. 4) for $q = 0$, 1 and 2. Hill numbers of abandoned agrosystems did not significantly differ from those of active agrosystems, natural ecosystems or urban areas, while Hill numbers of active agrosystems significantly differed from those of

natural and urban areas (Fig. 4). The proportion of AMF taxa in the Grid dataset shared among all land uses was of 63.2% (Fig. S4).

2.4. Effect of soil properties on AMF communities

Soil properties according to land uses

Geochemical properties in the Grid dataset were assessed as a complement to the metabarcoding analyses.

For all land uses together, the average pH_{H2O} and pH_{KCL} values ranged from 8.20 in active agrosystems to 8.96 in natural ecosystems. Si was the most abundant element, with average values > 89% in all land uses. Compared to other land uses, urban areas were found to have higher proportions of Al, Ti, P, F, and K. In contrast, natural ecosystems had significantly lower Ti, Ca, P, Fe, K, and Mg contents compared to urban areas and agrosystems. The statistical results revealed that the soil properties in active and abandoned agrosystems were not significantly different, except for the higher Al detected in abandoned agrosystems (Table. 1). Although significant differences in atomic elements were detected between land uses, the four ellipses on the PCA were not clearly separated (Fig. S5).

Table 1
Land uses differ lightly in coordinates, pHH2O and atomic abundances. Geochemical properties and coordinates of 593 soil samples in the AlUla region according to four land uses. The soil geographical and physicochemical property results are expressed as mean ± standard deviation. AP is the acidification potential.

Parameters	Active agrosystems n = 47	Abandoned agrosystems n = 67	Natural ecosystems n = 447	Urban area n = 32
Y longitude	26.74682 ± 0.14966 a	26.75629 ± 0.10245 a	26.72985 ± 0.13229 a	26.63633 ± 0.11450 b
X latitude	37.94533 ± 0.08535 ab	37.97742 ± 0.06682 a	37.96684 ± 0.08198 ab	37.94352 ± 0.04697 b
pH H_2O	8.72 ± 0.47 b	8.75 ± 0.58 b	8.96 ± 0.50 a	8.87 ± 0.49 ab
pH KCl	8.20 ± 0.35 a	8.30 ± 0.50 a	8.33 ± 0.48 a	8.27 ± 0.37 a
AP	0.52 ± 0.39 a	0.45 ± 0.36 a	0.63 ± 0.44 a	0.60 ± 0.42 a
Si (%)	92.9 ± 3.8 ab	89.1 ± 9.7 ab	92.0 ± 6.9 a	90.1 ± 5.3 b
Al (%)	0.683 ± 0.511 b	1.212 ± 1.176 a	1.058 ± 1.230 ab	1.439 ± 1.319 a
Ti (%)	0.495 ± 0.296 ab	0.470 ± 0.299 b	0.488 ± 0.332 b	0.614 ± 0.299 a
Ca (%)	2.099 ± 1.240 ab	4.052 ± 5.608 a	2.298 ± 2.712 b	2.853 ± 1.662 a
P (%)	0.064 ± 0.039 a	0.060 ± 0.044 ab	0.051 ± 0.038 b	0.062 ± 0.035 a
Fe (%)	1.218 ± 0.800 b	1.550 ± 1.415 b	1.495 ± 1.405 b	2.146 ± 1.561 a
K (%)	0.142 ± 0.103 b	0.312 ± 0.381 ab	0.242 ± 0.346 b	0.433 ± 0.465 a
Mg (%)	1.853 ± 0.750 ab	2.163 ± 1.482 a	1.727 ± 1.082 b	1.939 ± 0.690 ab

Soil properties and AMF communities in relation to land uses

The combination of AMF communities and soil properties (pH, atomic elements, and coordinates) were assessed by distanced-based redundancy analysis (db-RDA), as shown in Fig. 5, where the four land uses are represented with ellipses. The PERMANOVA results showed that all soil parameters on the db-RDA had an impact (at $p < 0.001$) on the repartition of AMF communities.

2.5. Plant root AMF observations in the AlUla region

Histological observations of the 277 root samples collected in the study area revealed that 29 out of the 46 plant species sampled (63%) were colonized by AMF (Table 2). The plant root AMF microscopic observations (hyphae, vesicles, and arbuscules) are available in Fig. S6. 102 individual plants were found to be colonized by AMF amongst the 277 samples (37%). Half of the root samples (49%) were from *P. dactylifera* date palm roots. Among these latter 135 samples, 23% were mycorrhized by AMF. Despite the

high AMF frequency (61%), the mycorrhizal intensity was quite low (average of 4%) in these mycorrhized date palms. Excluding *P. dactylifera*, 51% of the 142 remaining plant individuals were positive to AMF mycorrhization. The mycorrhizal frequency and intensity depended greatly on the plant species.

Table 2

Mycorrhizal status and observations of plant species in the AlUla region. The number of individuals (n) sampled is indicated for each plant species. The results are presented according to the percentage of mycorrhized individuals, the frequency and intensity of AMF root colonization. Non-determined (ND) means that AMF frequency and intensity measurements were unavailable due to low root fragment quality.

Plant taxonomy		**Mycorrhizal observations**			
Plant family	**Plant species**	**n**	**Mycorrhized individuals (%)**	**Frequency (%)**	**Intensity (%)**
Amaranthaceae	*Aerva javanica* (Burm.f.) Juss. ex Schult.	1	0	-	-
Amaranthaceae	*Chenopodiastrum murale* (L.) S. Fuentes, Uotila & Borsch	2	50	100	4
Amaranthaceae	*Haloxylon persicum* Bunge	13	38	19	17
Amaranthaceae	*Haloxylon salicornicum* (Moq.) Bunge ex Boiss.	20	31	34	14
Amaranthaceae	*Caroxylon imbricatum* var. *imbricatum*	1	0	-	-
Amaranthaceae	*Soda rosmarinus* (Bunge ex Boiss.) Akhani	1	0	-	-
Amaranthaceae	*Suaeda vermiculata* Forssk. ex J.F.Gmel.	1	0	-	-
Anacardiaceae	*Searsia tripartita* (Ucria) Moffett	2	100	ND	ND
Apocynaceae	*Rhazya stricta* Decne.	1	0	-	-
Arecaceae	*Phoenix dactylifera* L.	135	23	61	4
Asclepiadaceae	*Pergularia tomentosa* L.	1	100	ND	ND
Asteraceae	*Artemisia* sp.	1	100	ND	ND
Asteraceae	*Picris cyanocarpa* Boiss.	1	100	100	93
Asteraceae	*Sonchus oleraceus* L.	4	100	83	17
Capparaceae	*Capparis spinosa* L.	1	0	-	-
Cistaceae	*Helianthemum lippii* (L.) Dum.Cours.	9	100	82	5
Convolvulaceae	*Convolvulus spicatus* Peter ex Hallier f.	1	100	ND	ND
Cyperaceae	*Cyperus conglomeratus*	1	100	20	1

Plant taxonomy		Mycorrhizal observations			
Plant family	**Plant species**	**n**	**Mycorrhized individuals (%)**	**Frequency (%)**	**Intensity (%)**
	Rottb.				
Fabaceae	*Astragalus sieberi* DC.	8	100	82	37
Fabaceae	*Astragalus spinosus* (Forssk.) Muschl.	10	70	47.13	6
Fabaceae	*Retama raetam* (Forssk.) Webb & Berthel.	13	60	30	11
Fabaceae	*Senna italica* Mill.	3	66	ND	ND
Fabaceae	*Vachellia gerrardii* (Benth.) P.J.H.Hurter	2	100	ND	ND
Fabaceae	*Vachellia tortilis* subsp. *raddiana* (Savi) Kyal. & Boatwr.	2	100	ND	ND
Lamiaceae	*Lavandula coronopifolia* Poir.	1	0	-	-
Lamiaceae	*Teucrium leucocladum* Boiss.	1	0	-	-
Malvaceae	*Malva parviflora* L.	1	100	67	12
Moraceae	*Ficus palmata* Forssk.	2	50	ND	ND
Moraceae	*Ficus salicifolia* Vahl	1	100	ND	ND
Plantaginaceae	*Plantago lanceolata* L.	1	0	-	-
Poaceae	*Chloris barbata* Sw.	1	100	83	4
Poaceae	*Polypogon monspeliensis* (L.) Desf.	1	100	33	1
Poaceae	*Stipagrostis ciliata* (Desf.) De Winter	1	0	-	-
Poaceae	*Stipagrostis plumosa* (L.) Munro ex T.Anderson	2	0	-	-
Polygonaceae	*Calligonum comosum* L'Hér.	1	0	-	-
Polygonaceae	*Rumex vesicarius* L.	1	100	100	25
Resedaceae	*Ochradenus baccatus* Delile	1	100	100	20
Rhamnaceae	*Ziziphus nummularia* (Burm.f.) Wight & Arn.	2	50	ND	ND

Plant taxonomy		Mycorrhizal observations			
Plant family	**Plant species**	**n**	**Mycorrhized individuals (%)**	**Frequency (%)**	**Intensity (%)**
Rutaceae	*Citrus* spp.	13	0	-	-
Sapindaceae	*Dodonaea viscosa* Jacq.	1	100	ND	ND
Solanaceae	*Lycium shawii* Roem. & Schult.	4	0	-	-
Solanaceae	*Withania somnifera* (L.) Dun.	3	33	100	55
Solanaceae	*Solanum villosum* Mill.	1	0	-	-
Tamaricaceae	*Tamarix nilotica* (Ehrenb.) Bunge	1	0	-	-
Urticaceae	*Forsskaolea tenacissima* L.	1	100	50	1
Zygophyllaceae	*Zygophyllum bruguieri* (DC.) Christenh. & Byng	2	0	-	-

2.6. Number of AMF spores in soils in the AlUla region

Spores from 1 kg samples of 12 soils from the AlUla region were extracted and numbered (Table 3). Overall, there were < 150 spores/kg of soil, with an average of 58 ± 42 spores/kg of soil. No significant differences in spore number were found regarding the type of aboveground vegetation (annual or perennial plants), nor the different land uses (active agrosystems or natural ecosystems). Detected spores were mostly small (≈ 50 µm) with thick walls (Fig. S7).

Table 3
Number of AMF spores extracted from AlUla soils in different conditions. Plant Type corresponds to the type of vegetation observed in the vicinity of the soil sample (perennial or annual). The species observed at sampling time are given in the “Plant observed” column. The land use is indicated.

ID	Number of spores/kg	Plant Type	Plant observed	Land use
SP01	61	Annual	*Anthemis*/Poaceae/Diverse annuals	Natural ecosystem
SP02	38	Perennial	*Vachellia gerrardii*	Natural ecosystem
SP03	75	Perennial	Date palm	Active agrosystem
SP04	86	Perennial	*Haloxylon salicornicum*	Natural ecosystem
SP05	59	Perennial	*Vachellia tortilis*	Natural ecosystem
SP06	32	Annual	*Anthemis*/Poaceae/*Asphodelus*	Natural ecosystem
SP07	11	Perennial	Diversity	Natural ecosystem
SP08	144	Perennial	Date palm	Active agrosystem
SP09	34	Annual	*Anthemis*/Poaceae/*Asphodelus*	Natural ecosystem
SP10	30	Perennial	*Lycium shawii, Vachellia tortilis*	Natural ecosystem
SP11	118	Perennial	Date palm	Active agrosystem
SP12	8	Perennial	Date palm (+ *Citrus*)	Active agrosystem

Discussion

Our large-scale investigation of the AMF communities of the plants and soils in the region of AlUla revealed several interesting findings discussed below: i) reports of AMF in the roots of multiple plant species known as non-mycorrhized, ii) low mycorrhization rates of major crops (date palms and *Citrus* spp.), iii) very low numbers of AMF spores in the soils, iv) absence of several AMF families (e.g. Gigasporaceae) usually commonly found in farms and natural ecosystems, v) the slight impact of land uses on AMF diversity and soil parameters, and vi) the importance of using several approaches (metabarcoding, histology, and spore counting) on a large scale to improve our knowledge of AMF found in hyper-arid environments. With an average pH of 8.86 ± 0.53, up to 10.40, the hyper-arid region of AlUla

is also characterized by hyper-alkaline soils (Table 1). We highlight the small effect on AMF community composition of land uses compared to passive dispersal and soil physical and chemical parameters in this desert region. Finally, we discuss the importance of a vast sampling analysis dispositive with a variety of methods to study the AMF at a large scale. Arid and hyper-arid lands are sensible and rather unknown ecosystems, understanding further these ecosystems is crucial for both agriculture and land restoration in the context of climate change and increase in desertification.

3.1. Mycorrhizal status of hyper-arid ecosystems plants

In the present study we collected plant roots from 46 plant species (belonging to 25 families) and reported their mycorrhizal status, with mutualistic symbiosis with AMF found in at least one individual plant in 29 species (63%). We queried the scientific literature to determine if these results were in line with those of other previous studies (Table S1). Three main findings, as discussed below, emerged from this comparison: i) we filled a knowledge gap by clarifying the previously unknown mycorrhizal status of some plant species, ii) we challenged this status when the plant was previously described as being non-mycorrhizal, and iii) we did not observe AMF in some plant species previously described as being compatible with arbuscular mycorrhization.

To the best of our knowledge, this is one of the first studies to report the presence of arbuscular mycorrhizae in the roots of the 10 following plant species: *Astragalus sieberi* (Fabaceae); *Astragalus spinosus* (Fabaceae); *Convolvulus spicatus* (Convolvulaceae); *Ficus cordata* (Moraceae); *Ficus palmata* (Moraceae); *Forsskaoleae tenacissima* (Urticaceae); *Ochradenus baccatus* (Resedaceae); *Pergularia tomentosa* (Asclepiadaceae); *Picris cyanocarpa* (Asteraceae) and *Searsia tripartita* (Anacardiaceae). Some species of the *Astragalus*, *Convolvulus*, *Ficus*, *Pergularia*, *Picris* and *Searsia* genera are already known to be compatible with AMF (Table S1). We identified arbuscular in eight plant species from these genera, potentially filling a knowledge gap regarding their unknown mycorrhizal status. Moreover, this is one of the first reports of mycorrhization detection in *Forsskaolea* and *Ochradenus* plant species. We have also reported the presence of AMF in *Chenopodiastrum* (ex: *Chenopodium*) and *Rumex* genera, which are known for their variable mycorrhizal status (Fontenla et al. 1998; Karanika et al. 2008; Daisog et al. 2012; Safari Sinegani and Elyasi Yeganeh 2017; Kellogg et al. 2021). Some plant families are commonly known to be mycorrhizal, e.g. Asteraceae, Fabaceae, Poaceae and Solanaceae (Wang and Qiu 2006). All of the Asteraceae and Fabaceae plant species investigated here were mycorrhized, thereby confirming previous findings (Table S1). However, our data regarding Solanaceae and Poaceae species revealed poor mycorrhization rates (1/3 species mycorrhized, 1/8 individuals and 2/4 species, 2/5 individuals, respectively), which was much lower compared to the findings of other studies (Table S1). Amaranthaceae plant species are known to be compatible with AMF (Chaudhry et al. 2005; Wang and Qiu 2006; Santhoshkumar, et al. 2019), yet their mycorrhization rates are considered to be low to null (Tester et al. 1987). Here we reported much higher rates, with evidence of AMF root colonization in 3/7 Amaranthaceae species and in 12/39 individuals.

These differences in plant mycorrhizal status when comparing our data with previous reports could be explained by multiple factors, such as: i) driven by environmental conditions, ii) outdated, incomplete data, or iii) simply a lack of knowledge and investigations. Non-mycorrhizal plants are more common and diverse in arid lands (Soudzilovskaia et al. 2017; Brundrett and Tedersoo 2018). However, we could expect that plants growing in extreme desert conditions (e.g. extreme drought, low soil nutrients, discontinuous vegetation cover, high heat and soil alkalinity in the AlUla region) could greatly benefit from this mycorrhizal interaction feature which can help plants mobilize water and nutrients and survive heat stress (Duc et al. 2018; Harkousse et al. 2021; Madouh and Quoreshi 2023). Another example of such plant adaptations in extreme environments was reported in the highly polymetallic soils in New Caledonia, where Cyperaceae plants (known as non-mycorrhizal) were found to unexpectedly develop mycorrhization (Lagrange et al. 2011), which is in line with our observations in AlUla, where AMF was detected in *Cyperus conglomeratus*. Primary descriptions of the plant mycorrhizal status often come from temperate climatic conditions and unconstrained environments, while our results, along with those of other studies (O'Connor et al. 2001; Agwa and Al-Sodany 2003; Chaudhry et al. 2005; Lagrange 2009), were obtained in arid environments under harsh conditions (aridity, nutrient shortage, extreme pH, etc.). Environmental conditions, e.g. biotic stress, climate and soil nutrient content, are important factors underlying the establishment of AMF mutualistic symbiosis (Muthukumar et al. 2004; Lagrange 2009), plant taxonomy alone may not be discriminant enough to know the true mycorrhization potential of a plant species. For example, salinity, heat and drought can affect plant mycorrhization (Diatta et al. 2014; Duc et al. 2018). Land use, pH and geological parent material may also explain the mycorrhizal distribution in plants (Menzel et al. 2016).

Another interesting point concerns the energy cost of plant-AMF symbiosis in arid environments, where more energy is required to develop a functional mutualistic relationship (Koltai and Kapulnik 2010). Plants must feed their AMF symbionts with sugars produced by photosynthesis (which involves atmospheric carbon dioxide and water consumption). Overuse of this trade-off may be deleterious to plants in arid environments where water is already a scarce resource (Chaves et al. 2002). To conclude this section, our study challenged current views on the mycorrhizal status of desert plants, as we noted that the hyperarid and hyperalkaline environmental conditions in AlUla may be conducive to the development of plant-AMF mutualistic symbiosis.

3.2. Unexpected low AMF levels in crops

In this study, we observed mycorrhizal colonization of two major crops, i.e. date palm (*P. dactylifera*; Arecaceae), and *Citrus* spp. (Rutaceae). Date palm is: i) the main crop in the AlUla region, ii) adapted to desert conditions (e.g. drought, pH, heat and salinity), and iii) characterized by high mycorrhizal rates in other locations (Ramoliya and Pandey 2003; Abohatem et al. 2011; Bouamri et al. 2014; Arab et al. 2016; Hazzouri et al. 2020). *Citrus* spp. plants are often jointly cropped with date palms in AlUla orchards (Gros-Balthazard et al. 2023), and are highly dependent on mycorrhizal colonization (Wu and Xia 2006; Song et al. 2015). In our study, no AMF structures were found in any of the collected roots of 13 individual *Citrus* plants. Regarding date palm, only 23% of the 135 individuals were colonized, and none

of the 8 individuals collected in natural ecosystems displayed any root AMF. In comparison, Bouamri et al. (2014) found mycorrhized roots in all of the 10 date palms sampled in farms at Tafilalet (Morocco). Environmental DNA revealed high variability in AMF proportions across all date palm samples (ranging from 0 to 95% of the total reads issued from 18S sequencing). AMF reads were detected inside and on the surface of date palms roots, except in two individuals growing in natural conditions. These AMF mainly concerned the Glomeraceae family, i.e. mostly *Glomus*, *Rhizophagus* and *Sclerocystis* genera.

Drought stress alleviation is one of the main potential roles of AMF to benefit plant crops. Many studies have obtained evidence on the effectiveness of AMF in enhancing date palm growth and improving the tolerance of these trees to abiotic and biotic stress in date palm farms (Jaiti et al. 2007; Evelin et al. 2009; Abohatem et al. 2011; Al-Karaki 2013; Ait-El-Mokhtar et al. 2019). Water management is crucial in these desert regions, yet water supplies have been jeopardized. Over time, the use of non-renewable water resources from aquifers gradually increased the soil salinity, so farmers had to find alternatives, such as sea water desalinization (Shahin and Salem 2014; Zemni et al. 2022; Seraphin et al. 2022; Odnoletkova and Patzek 2023).

Arbuscular mycorrhizae in cultivated date palms was found to be reduced when water access is scarce (Baslam et al. 2014) which could explain the absence of mycorrhizae in natural conditions. However, date palm mycorrhization is generally low in the region, even on intensely irrigated farms. This observation is specific to date palms, because the mycorrhization rate was checked on plant species growing in the vicinity of date palms (i.e. *Chenopodiastrum murale, Sonchus oleraceus* and *Plantago lanceolata*). These adventitious plants were found to have an average of 71% arbuscular mycorrhizal structures, with a mean frequency of 81%, thereby indicating that the water scarcity and environmental conditions did not hamper the mycorrhization of these species. The absence of mycorrhizae noted in *Citrus* spp. trees and the low AMF colonization rate of date palms, i.e. two crops that are usually colonized by AMF, could have been the result of the extreme soil conditions. Indeed, even though water stress may be alleviated by irrigation, the soils remain nutrient-poor and alkaline, which could specifically affect interactions between AMF and these plants, while having less of an impact on other species (Oehl et al. 2017). However, poor soils (mainly lacking phosphorus) are commonly reported to favor plant-AMF interactions (George et al. 1995; Ma et al. 2023). Regarding water scarcity, it is unclear if this factor enhances or reduces AMF colonization, and this may vary according to the plant species, fungal species, environmental conditions, and water stress intensity (López-Ráez 2016).

3.3. Environmental selection of spores and alternative AMF dispersal strategies in arid environments

AlUla soils displayed an extremely low quantity of spores, regardless of the sampling location and conditions, with only 58 spores/kg of soil detected. Similar studies on arid or semi-arid desert soils (rocky to sandy soils) in natural ecosystems or farms, reported much higher spore numbers in soils, ranging from 200 spores/kg of soil in a semi-arid ecosystem (Requena et al. 1996) to up to 18,400 spores/kg of soils under date palms in farms (Bouamri et al. 2006). Despite this considerable variability

in spore quantities in desert soils, to our knowledge this is the first time that such a low number of AMF spores has been reported in these soils.

The low quantity of spores in AlUla soils could be attributed to two non-mutually exclusive processes: i) high spore degradation in these soils due to environmental factors (e.g. heat, UV radiation, sand abrasion), or ii) the natural selection in the region in favour of AMF species producing low spore numbers. AMF fitness may be linked to the spore morphology and characteristics. The first hypothesis that could be put forward is that hyperarid environments are naturally conducive to the selection of small thick-walled spores (e.g. *Archaeospora*, *Rhizophagus*), while other spores that are unfit for survival in desert ecosystems are quickly disappearing and/or just absent. Requena et al. (1996), for instance, reported that many spores they observed in a Spanish semi-arid region were degenerated. Regarding AMF natural selection, AMF spores and cells harbor many nuclei (Hijri and Sanders 2005; Kokkoris et al. 2020) that may be genetically distinct and could be selected and transferred (or not) to the offspring enabling them to rapidly select traits like production of spore (Verbruggen and Kiers 2010).

Despite the low spore abundance in AlUla soils we monitored, our results showed that plants were still able to establish plant-AMF interactions (especially in herbaceous species). In Namibia, the spore content in soils could not be correlated with the arbuscular colonization of plants (Uhlmann et al. 2004), and it is unlikely that spores are important for inducing AMF colonization of plant roots (López-Sánchez and Honrubia 1992). These considerations suggest that spores may not be the main AMF propagation pathway in hyperarid regions. There are other means of AMF survival and propagation, such as via hyphae or root fragments colonized by AMF, through the so-called propagules (Salomon et al. 2022). In our case, these propagules might be the most probable way of AMF dispersion, with colonized root fragments offering shelter and nutrients for the fungi (Müller et al. 2017), whereas the low extent of plant cover on the soils may indicate that there was a low mycelium hyphae density in the soils.

3.4. Predominance of Glomeraceae and absence of Gigasporaceae in the hyperarid AlUla region

Overall, AMF detected in AlUla soils and plants showed high dominance of Glomeraceae species, mostly with 18S, in addition to Diversisporaceae and Claroideoglomeraceae, which were observed throughout the ITS2 datasets (Fig. 3; 4). Datasets sequenced with 18S markers were almost exclusively composed of Glomeraceae taxa, with the main genera being *Glomus* and *Rhizophagus*, in addition to *Sclerocystis* in date palm roots. Glomeraceae, mostly *Glomus*, is often predominant in soils exposed to abiotic stress as they tend to be opportunistic while bearing features that are advantageous in adverse environments (e.g. ability to rapidly colonize plant roots). This predominance of Glomeraceae has been reported in numerous studies in different arid regions worldwide using 18S sequencing and spore identification (Symanczik et al. 2014; Qiang et al. 2019; Harrower and Gilbert 2021; Adenan et al. 2021; Zhao et al. 2022). Diversisporaceae are also regularly identified in deserts (Qiang et al. 2019; Vasar et al. 2021; Harrower and Gilbert 2021; Zhao et al. 2022). Other AMF families detected in these arid environments include Paraglomeraceae (Zhao et al. 2022), Claroideoglomeraceae (Symanczik et al. 2014; Zhao et al.

2022), Acaulosporaceae (Zhao et al. 2022), and Scutellosporaceae (Gigasporales) (Harrower and Gilbert 2021).

AMF diversity in the date palm rhizosphere has until now mostly been described on the basis of spore morphotypes. Our study is one of the first in which AMF communities in date palm roots were investigated using a metabarcoding method based on the 18S. Several studies on the date palm rhizosphere reported a dominance of Glomeraceae taxa, mostly *Glomus* spores along with recurrent Gigasporales (Gigasporaceae, Scutellosporaceae and Racocetraceae) (Al-Yahya'ei et al. 2011; Khirani et al. 2020; Dalal and Solanki 2021). The dominance of Glomeraceae, especially *Glomus* detected via both 18S sequencing and spore observation, is consistent with our results. However, authors of other studies have often reported detecting spores from the Gigasporales order in date palm orchards, which is inconsistent with our metabarcoding results (only two OTUs on soils of farms in the Grid dataset, representing 0.015% of the AMF reads; and in date palm roots in the Palm dataset, representing 0.8% of the AMF reads), and is also out of line with our soil spore extractions which mostly revealed small sized spores. Gigasporales spore sizes reportedly range from 120 to 640 µm (Souza 2015) yet we extracted spores between sieves with meshes of 40 µm and 1 mm. Our spore extraction method is thus suitable for trapping Gigasporales spores. In cases where no spores of this order were found, it could be hypothesized that this order was either absent, or highly preyed upon by other organisms. This scant presence of Gigasporales taxa in dryland habitats has already been described (Adenan et al. 2021), with only 1 OTU found to belong to *Scutellospora*. Gigasporales were reported to be more preyed on and degraded in dunes than in temperate environments (Lee and Koske 1994). Taxa of other orders such as Archaeosporales, Entrophosporales and Paraglomerales were also seldom found in AlUla, in line with the findings of other previous studies conducted in desert environments (Adenan et al. 2021).

AMF identification via metabarcoding also depends on the database used for the taxonomic assignment (SILVA for 18S and UNITE for ITS2). This difference of database may play a role in the differences in community composition observed between markers. Datasets sequenced with ITS2 showed more Unidentified Glomeromycotina than those sequenced with 18S, but in both datasets those sequenced mostly belonged to the Glomerales and Diversisporales orders. The high proportion of unidentified AMF obtained reflected an overall knowledge gap that exists with regard to desert ecosystems, particularly in reference databases. Studies like ours, involving high sequencing depth and the combined use of ITS2 and 18S, could enhance the overall understanding through the detection of a broader range of AMF taxa.

3.5. Soil parameters and location rather than land use shape AMF communities in AlUla

Despite the fact that there were no significant differences in soil characteristics, shifts in AMF communities were identified between land uses. Although the alpha-diversity (Hill numbers) was mostly similar under all conditions, the beta diversity (PERMANOVA on Bray-Curtis distances) differed between agrosystems, natural ecosystems and urban areas. These results are consistent with those reported by Yu et al. (2022), where the microbial composition was more sensible than the diversity index. Our R^2

PERMANOVA values explained < 0.01% of the community differences, but even the slightest difference can be significant when the sample number is sufficient. Overall, our results suggest that the fungal community composition obtained for abandoned agrosystems was an intermediate pattern between the active agrosystem and the natural ecosystem land uses, thereby suggesting that agriculture had a legacy effect on the microbial communities, as described in (Maurice et al. 2024a). Moreover, our definition of abandoned agrosystems included farms that had been abandoned recently or longer ago, while having different extents of past anthropic pressure. Compared to natural ecosystems, these past anthropic disturbances potentially modified the soils: i) in terms of hygrometry, ii) lowering the pH, and iii) fertilizer and pesticide use, patterns also described by Tchabi et al. (2008). In the light of all of these elements, we expected that farming and past anthropic disturbances would lead to marked disparities in AMF communities between the four land uses. The small differences in AMF communities between land use is inconsistent with the findings of other studies where AMF were highly affected by land uses and agricultural practices, mainly tillage (Thiele-Bruhn et al. 2012). However, tillage and other mechanical treatments are not required in date palm cultivation, so there should be very little alteration in the soil structure. There might be a link between the lack of differences in soil parameters between land uses (Fig. S5) and the small differences between land uses for AMF community composition. This lack of a marked effect of land uses on AMF communities has also been reported by Jansa et al. (2014).

The main drivers of AMF community structure in AlUla were generally the longitudinal sample distribution, along with the proportion of silicon detected as compared to other measured elements and pH, i.e. mostly pH_{KCl}. The high effect of the geographical distribution on the map i.e longitude, latitude (Fig. 5) unveils that soils collected at close distances tend to show more similar AMF community. Microbial dispersal is considered to be mostly passive, which implies that geographically close points would tend to be affected by the surrounding microbial communities due to the limited dispersal. In our results, the extent of the distance effect on the AMF community structure could thus be an indicator of the importance of dispersal limitation (Zhou and Ning 2017).

The soil pH is a very common AMF community structuring driver (Tedersoo et al. 2014, 2022; Davison et al. 2015, 2021). The high Si concentration, as opposed to that of other elements, is an indication of the sandy texture of soils. Compared to other soil types, sandy soils are characterized by high porosity, warmer temperatures, high dryness and low nutrient contents (De Deyn et al. 2008; Laity 2009). In the study of Al-Ghamdi and Jais (2013), the AMF colonization rate increased along with the soil sand content. The effect of the soil texture, notably the sand content, is considered to be an important factor governing the AMF community composition (de Oliveira Freitas et al. 2014). Overall, the desert microbial community composition differs when comparing biomes in temperate or tropical regions, and the communities display a lower phylogenetic diversity (Murgia et al. 2019). Davison et al. (2015) highlighted low AMF richness in semi-desert areas in comparison with other environments, such as in forests, grasslands and shrublands. Other key parameters are known to shape AMF communities, including temperature, precipitation and organic carbon levels (Davison et al. 2015). There were no noteworthy temperature and precipitation differences in our study because all samples were collected in the same region (AlUla). However, the temperature was often lower and the humidity higher in active farms

because of irrigation and the date palm vegetation cover (de Grenade 2013). Organic carbon was very low in all of the analyzed soils sampled in farms and natural ecosystems (data not shown). In these environments, however, changes at the microenvironment scale can have a marked impact on plant and microbial communities by creating fertility islands, which could partly explain the variability in composition and diversity of AMF communities that we noted at the regional scale (Maurice et al. 2023).

3.6 Combined use of AMF metabarcoding, root histology and spore extraction in a desert region with diverse landscapes and land uses

Based on molecular data and direct observations, we conducted a large-scale study to characterize AMF in the AlUla region (Saudi Arabia) and took into account how the different land uses (natural ecosystems, active/abandoned agrosystems, and urban areas) and soil parameters shaped the fungal microbiome and environment. The combined use of different methods of analysis allowed us to describe directly and indirectly the arbuscular mycorrhization in both soils and roots in a variety of conditions and plant species. Note that in our study—spanning an arid 5,000 km² area—we used a total of 7,620,047 AMF reads, characterized mycorrhization in 46 different plant species with 277 root samples, while extracting spores from 12 soils. By comparison, other large-scale studies that have been carried out to describe microbial communities often involved distant sampling areas, while focusing on specific environments and extrapolating findings to infer intermediary conditions (O'Connor et al. 2001; Davison et al. 2015; Tedersoo et al. 2022). Multiple methods of analysis mixing high throughput analysis and plant and soil characterization Other large-scale kilometric grid sampling has also been carried out, e.g. in the studies of Karimi et al. (2019) and Djemiel et al. (2023), who mapped bacteria and fungi throughout France, with samples collected 16 km apart. Menzel et al. (2016) sampled AMF across Germany based on 130 km² sampling cells. Our kilometric grid had a more accurate 1 km² mesh, covering an area of 1,039 km². Although challenging, this region-wide sampling effort generated useful information regarding AMF variations at regional and local scales. We highly recommend using this method in future studies in arid deserts to gain greater insight into these specific ecosystems and to gather data that could be of use in further research carried out the current climate change setting.

Conclusion

In the present large-scale investigation on AMF communities hosted in plants and soils in the hyperalkaline and hyperarid AlUla region, we highlighted: i) the importance of conducting field surveys to assess and eventually challenge the mycorrhizal status of commonly known non-mycorrhized plant species, and ii) the potential impact of local agricultural practices on AMF species and mycorrhizal mutualistic symbiosis. Moreover, we found that the environmental drivers (e.g. hyperalkalinity, hyperaridity, soil parameters, and geographical coordinates) may: i) favor alternative AMF dispersal modes in the AlUla region, ii) be incompatible with ecological niches of commonly occurring AMF families (e.g. Gigasporaceae), iii) have more of an impact than land use on the composition, structure, and shifts of local AMF communities. Finally, our use of several approaches (metabarcoding, histology and spore counting) on a large scale enhanced our knowledge on AMF communities in hyperarid

environments. These overall findings should pave the way for a better understanding of the current status of AMF in hyperalkaline and hyperarid regions, which is essential for developing suitable renewable agriculture practices in the current climate change setting.

Declarations

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Author Contribution

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Figures

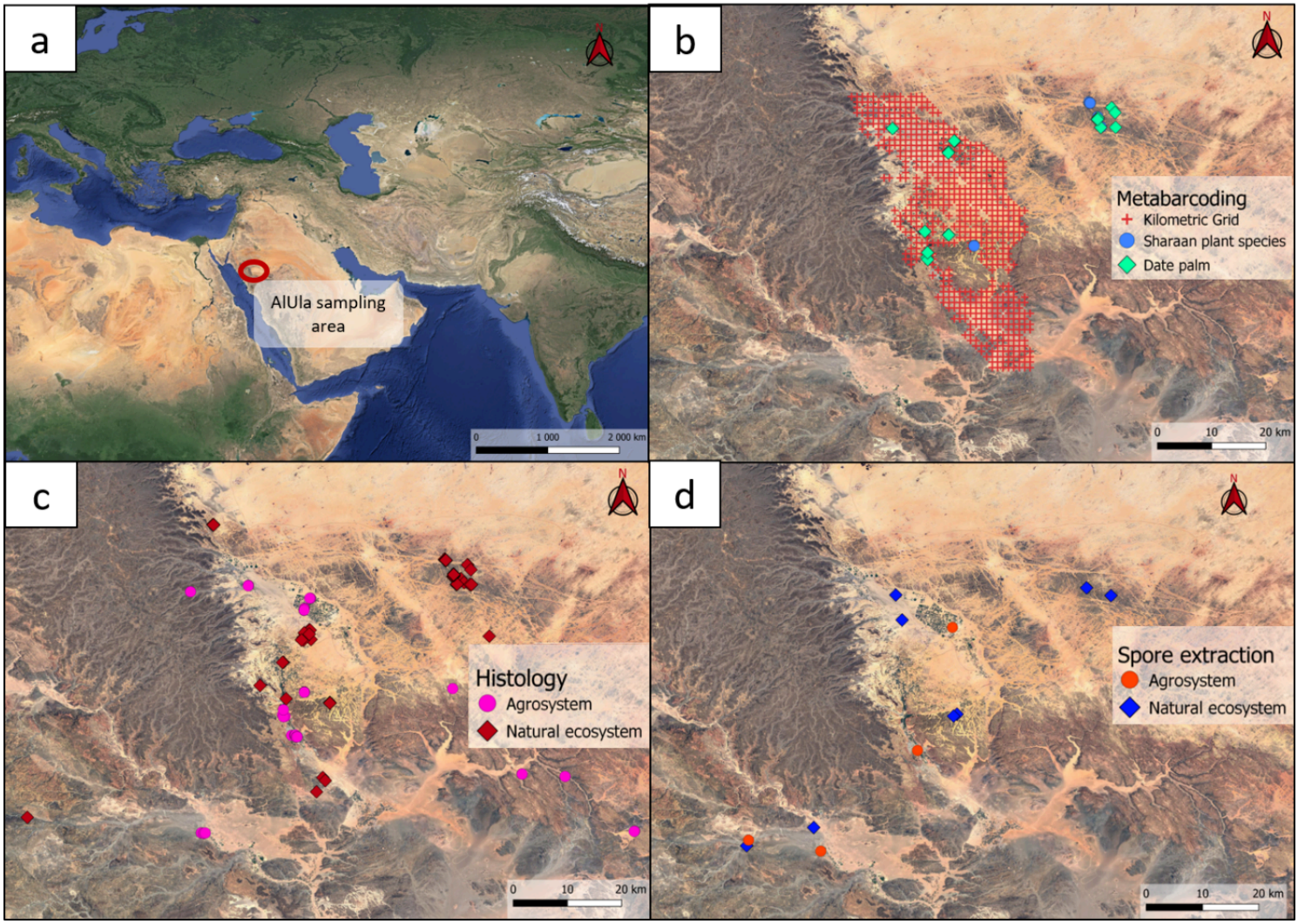

Figure 1

Maps and locations of the samples collected for metabarcoding, histological analysis and spore extraction. **a**: Overview of the sampling area and location of the AlUla region; **b**: Metabarcoding Glomeromycota samples collected from: i) soils sampled along a kilometric grid (red crosses), ii) date palm roots (green diamonds), and iii) soils, rhizospheres, and roots associated with Sharaan plant species (blue dots); **c**: Root sampling to check plant arbuscular mycorrhization in agrosystems (pink dots) and natural ecosystems (cyan diamonds); **d**: Spore samples extracted from soils collected in agrosystems (red dots) and natural ecosystems (blue diamonds).

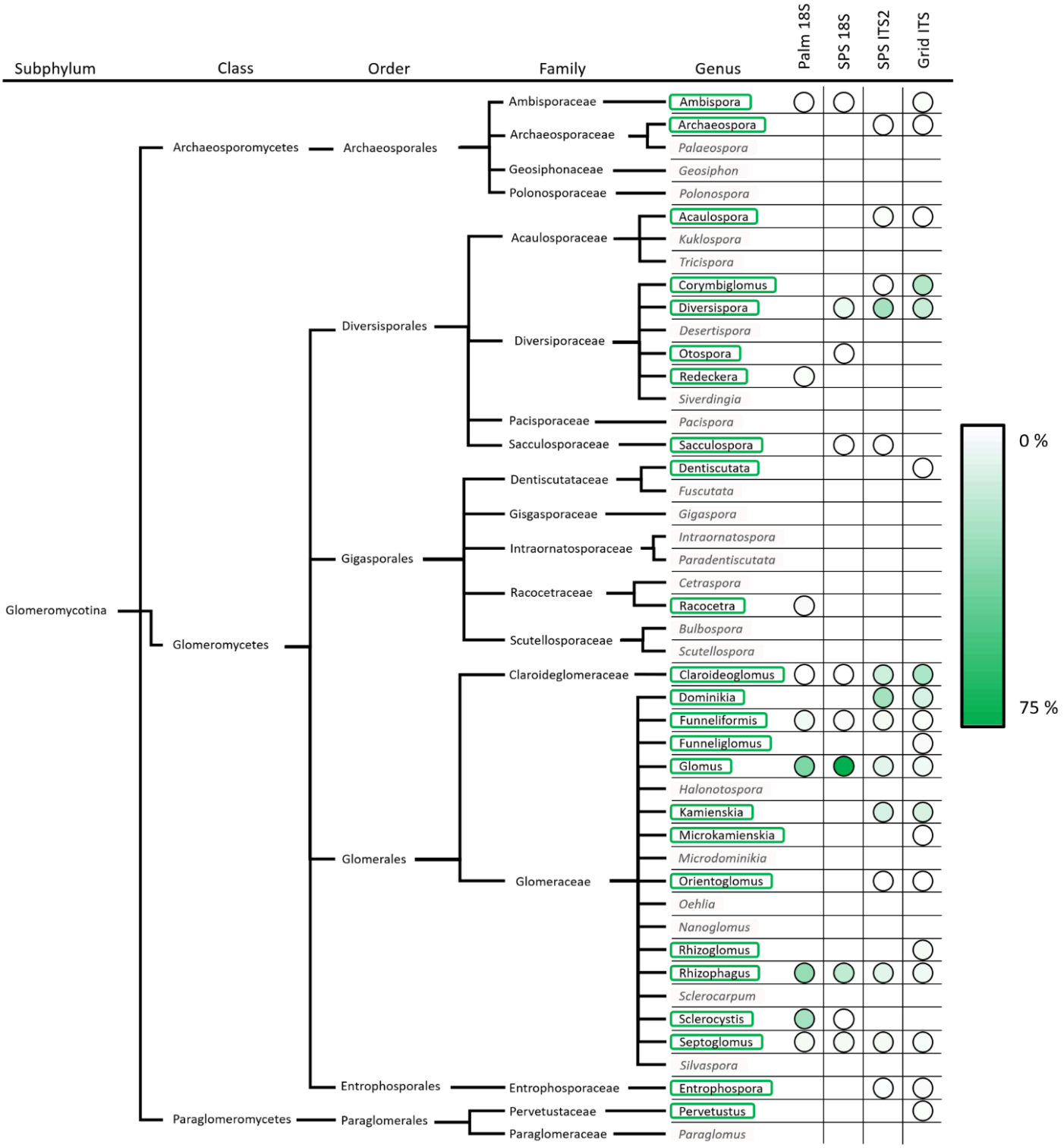

Figure 2

A large number of the AMF genera identified worldwide were detected in the AlUla region using metabarcoding analysis. Detected genera are outlined in green. The relative abundance of each AMF genus among AMF taxa detected in the metabarcoding datasets (in columns) is shown with a green color gradient. Unidentified genera of OTUs are not shown here. The cladogram of taxonomic relationships includes all AMF genera listed in the UNITE database.

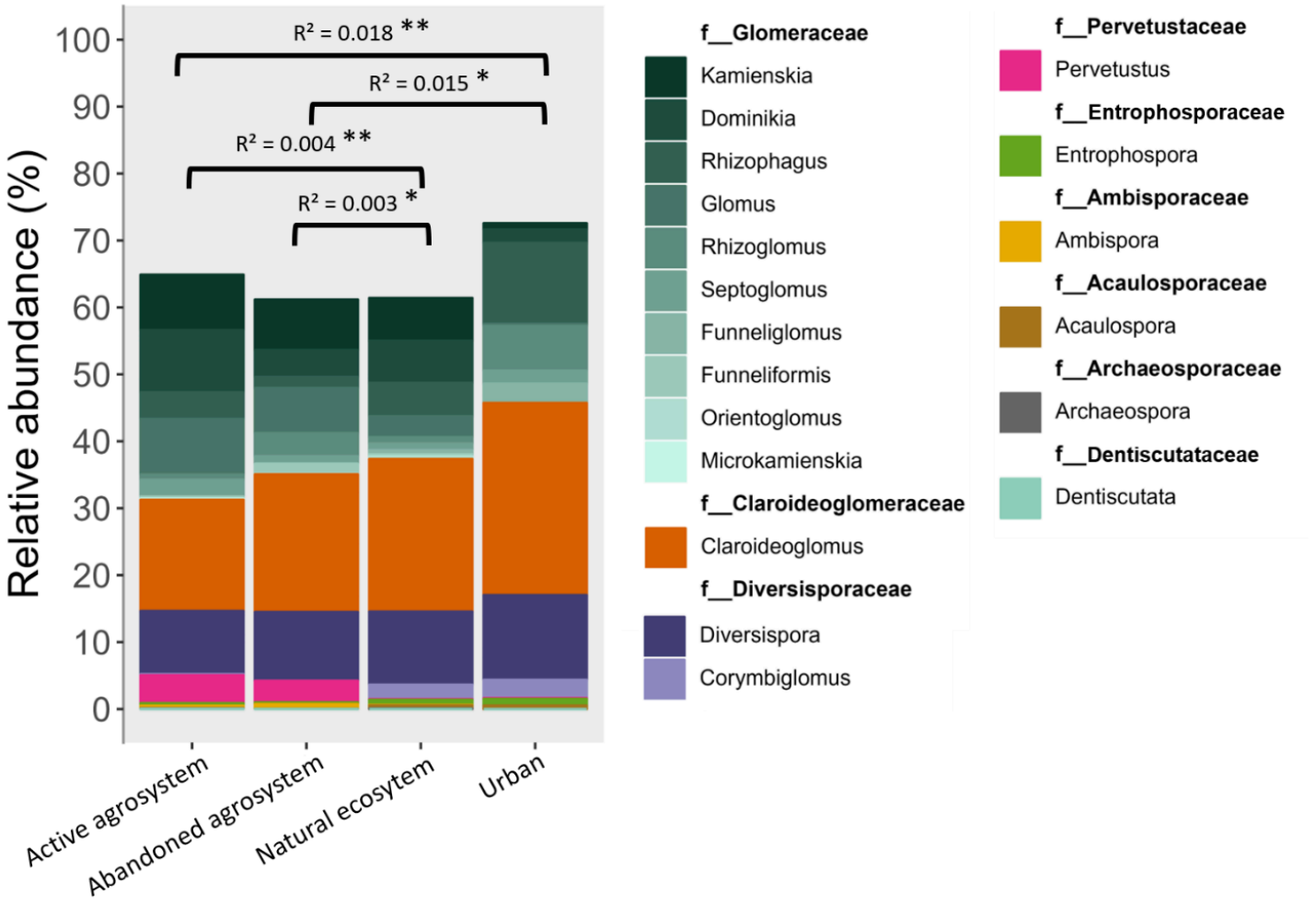

Figure 3

Land use affects the relative abundances of AMF**.** Mean relative abundances of rarefied AMF for the different land uses in the Grid dataset. Colors represent the genera with the corresponding families (in bold). Unidentified Glomeromycotina genera are not colored on the plot. AMF communities were compared two-by-two for the different land uses using MANOVA tests. The statistical results are expressed as R^2 values followed by the corresponding p-values (** at $p < 0.01$; * at $p < 0.05$).

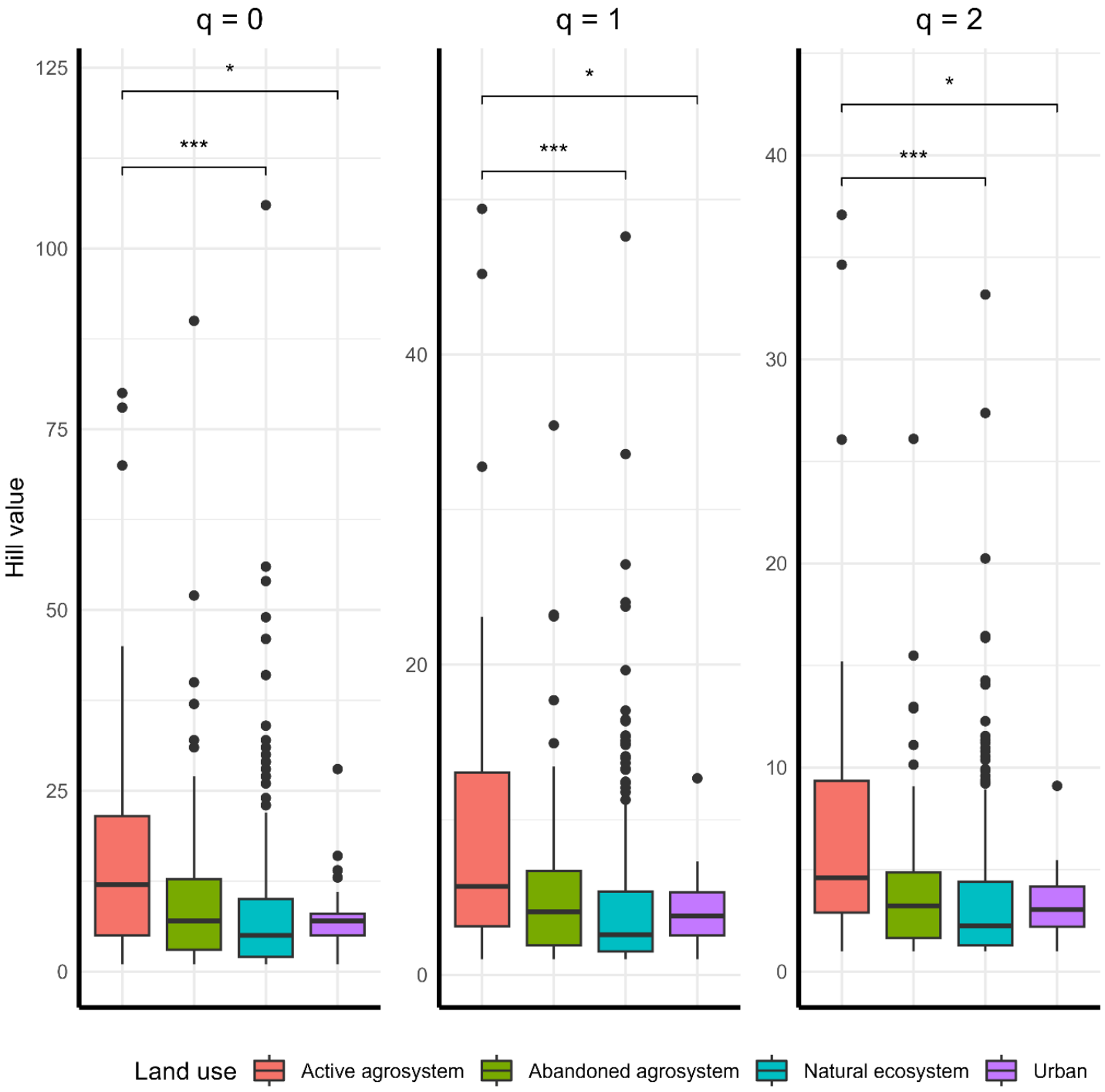

Figure 4

Active agrosystems have the highest AMF diversity. Hill diversity across three diversity orders (q = 0, 1, 2) for AMF OTUs detected in the Grid dataset according to the different land uses. Significance levels were calculated with a Wilcoxon test (* at $p < 0.05$, *** at $p < 0.001$).

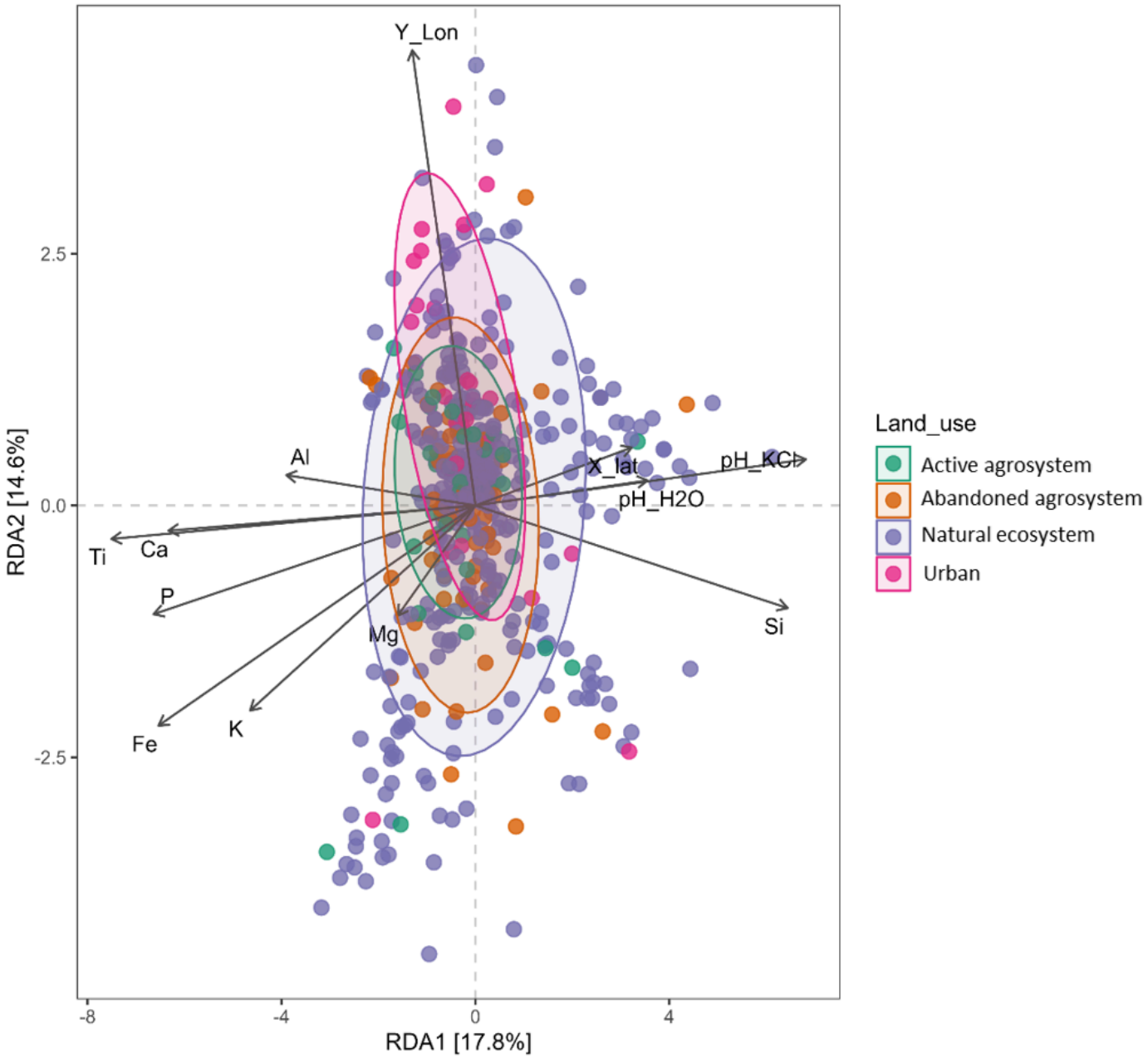

Figure 5

Soil AMF communities are mainly driven by their longitudinal repartition, soil pH, silicon, calcium and titanium. Distance-based redundancy analysis (db-RDA) visually representing the effects of soil and geographical variables on AMF communities based on the Bray-Curtis distance. Contributions of the XRF atomic composition, pH and XY coordinates to the first two dimensions are represented by vectors. Samples are colored according to their land uses.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfigures.pptx
- FigureS1analysisworkflow.png
- FigureS2relativeabundancedatasets.png
- FigureS3Hillnumbersmarkergene.png
- FigureS4Venn.png
- FigureS5PCA.png
- FigureS6AMFobservations.png
- FigureS7Spores.png
- TableS1.xlsx
- Suppmethodology.docx
- floatimage1.jpeg