

Article

Exploring Protist Communities in the Rhizosphere of Cultivated and Wild Date Palms

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Abstract

Protists represent a major component of eukaryotic diversity within the soil microbiome, playing critical roles in mediating carbon and nitrogen cycling and influencing nutrient availability and soil health. Their diversity is shaped by multiple factors, including temperature, pH, organic matter content, and land use. In this study, we investigated the protist diversity in rhizosphere soils from both wild and cultivated date palm varieties. Our results identified nitrate, nitrite, calcium, and carbon content as key soil factors significantly correlated with protist diversity. Only 9.2% (42) of operational taxonomic units (OTUs) were shared across all soil samples, suggesting that these taxa possess traits enabling adaptation to extreme environmental conditions. The dominant protist families belonged to Rhizaria, Alveolata, Amoebozoa, and Archaeplastida, primarily comprising bacterial consumers, alongside taxa from Stramenopiles, Opisthokonta, Hacrobia, and Excavata. At the class level, Filosa-Sarcomonadea, Colpodea, Variosea, Tubulinea, and Chlorophyceae were the most abundant. Filosa-Sarcomonadea and Colpodea were positively correlated with bacterial and fungal genera, suggesting their role as consumers, while Variosea showed a negative correlation with bacteria, reflecting predator-prey dynamics. Notably, the protist community composition in wild date palm rhizosphere soils was distinct from that in cultivated soils, with Opisthokonta being particularly abundant, likely reflecting adaptation to drought conditions. Overall, this study highlights the significant differences in protist diversity and community structure between wild and cultivated date palm ecosystems.

Keywords: dryland microbiome; *Phoenix dactelifera*; protist diversity; soil biodiversity; soil microbiome



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1. Introduction

Protists are all eukaryotes except fungi, animals and green plants such as *Amoeba* and *Amoeboflagellate* [1,2]. They play a crucial role in structuring soil microbial communities through their predation on various microorganisms, including bacteria, fungi, and other protists from diverse taxonomic groups [1]. They dominate the eukaryotic kingdom and

have key functions such as cycling of carbon and nutrient in all ecosystems [3]. Previous study showed soil protists drive ~40% of terrestrial nitrogen mineralization, critically supporting soil fertility [3]. Furthermore, protists are essential in increasing carbon and nitrogen content through symbiotic relationships with bacteria as well as stimulating plant growth promoting bacteria and affecting plant biomass through protist predation on bacteria and releasing nutrients and make them available for the plants [2,4]. Protist can enhance functions of plant and suppression of disease through direct consumption of pathogens making them less abundant in the soil community [4]. Other protist species are considered pathogenic to human such as *Plasmodium falciparum* that is a causing agent in malaria and *Giardia duodenalis* and *Entamoeba histolytica* which considered a intestinal parasite. Also, some protist can be pathogenic to plant such as plasmodiophorids from Rhizaria group [5]. Previous studies on protist were exploring different soil and water samples and investigated a number of protist groups including *Ciliophora*, *Cercozoa*, *Acanthamoeba*, *Kinetoplastea*, and *Apusomonads* [6–8]. Protists were identified mainly by using microscope and DNA sequencing techniques [9,10]. Previous and recent advancements in environmental DNA isolation and ultra-deep high-throughput sequencing have facilitated the discovery of previously undetectable protist diversity [5,11,12].

Different abiotic factors such as temperature, heavy metals and organic matter as well as biotic factors such as soil pathogens, and land use have been demonstrated to influence protist diversity in soil ecosystems [10,13]. Climate change exacerbates these effects, with drought and extreme rainfall events shown to alter protist community structure within single growing seasons [3,14]. Under drought condition, protist can inhabit tiny water films and may form resistant drought cysts in which it can maintain its existence [2]. The impact of land use on belowground biodiversity, particularly in protist communities, remains insufficiently studied. Land use plays a critical role in shaping the stability and composition of protist communities [13–15]. Research indicates that protist community variability is lower in grassland ecosystems than in arable land [13]. This could result from the high disturbance and environmental pressure in arable lands compared to grasslands [13]. Other studies focusing on alkaline paddy soils revealed a significant abundance of *Amoebozoa* (29.5%), *Stramenopiles* (23.7%), *Rhizaria* (19.5%), and *Alveolata* (12.6%) [16]. Protist diversity was assessed across different vegetation types, including woodlands, shrublands, and forests [17]. Although the alpha diversity was significantly different across these vegetation types, the diversity of protist based on the trophic level was similar. Different protist were identified in these vegetation type including *Alveolata*, *Rhizaria*, and *Archaeplastida* [17].

Additionally, another factor that influences the diversity of soil protists is soil management practices. Soil management practice includes the use of fertilizers [18]. The application of fertilizers is recognized as a key factor in shaping protist diversity in soils [19–22]. A study analyzing various soil types, including black, yellow, and red, highlighted nitrogen fertilizers' impact on protist diversity. It was noted that phagotrophic protists were particularly affected, with a significant reduction in their relative abundance across all soil types. These protists exhibited a negative relationship with precipitation and nitrate [23]. In contrast, phototrophic protist abundance was positively correlated with other factors such as solar radiation, precipitation, soil moisture, pH, and organic carbon [23]. These pH and nutrient alterations may create unfavorable conditions for certain protists while favoring others, leading to a reorganization of the entire soil food web [23]. The results demonstrated that nitrogen fertilizer application decreased the soil pH and increased nitrate, explaining the observed reduction in phagotrophic protist abundance [23].

Biotic factors such as plant pathogens can also affects the trophic level diversity of protist [16]. Notably, both phagotrophic and phototrophic protists have been observed to exhibit a negative correlation with plant pathogens [16]. This suggests that the environmen-

tal factors and agriculture practices influence the protist communities. Their ecological role as microbial predators makes them important indicator to the health of soil and its nutrient cycle efficiency. Further research is needed to understand the long-term implications of protist dynamics for the health of soil and the agricultural sustainability.

Differences in the soil microbiome between wild and cultivated plants have been extensively studied for bacteria [24] and fungi [25], but remain largely unexplored for protists. Plant domestication has been shown to influence the microbiome, with wild plants typically harboring a greater abundance of symbiotic microorganisms and a lower presence of plant pathogens compared to their cultivated counterparts [25]. Research on the soil microbial communities associated with date palms has primarily focused on bacteria and fungi [26,27], while the diversity of protists in date palm rhizosphere soils remains poorly characterized [5,7].

Date palm (*Phoenix dactylifera*) is one of the 14 species in the *Phoenix* genus, each with multiple cultivar, and it is mainly distributed in the Asia, Africa and South Europe [15,28]. Date palm fruits are valued in local communities as a cost-effective source of carbohydrates, amino acids, and fibers and are rich in phenolics, polyphenols, flavonoids, carotenoids, tannins, antioxidants and anti-cancer compounds [29]. As date palm is very important plant and the protist plays an important role in soil; the influence of protist on date palm soil should be investigated.

The present study examines protist community structure, diversity, and richness associated with the rhizosphere of wild and cultivated date palms. Firstly, we hypothesize that diversity and richness will be relatively consistent and higher in commercial date palm cultivars across farms compared to the coastal wild date palm population. This expectation is based on the more stable environmental conditions provided by farms, including regular irrigation and nutrient supplementation. Also, we hypothesize that the protist community associated with wild date palms are likely to be shaped by frequent drought stress and nutrient limitations, due to sporadic rainfall and nutrient-poor soils that are not supplemented with fertilizers [19]. Thus, we hypothesize that protist community structure in the rhizosphere of wild date palms will be differentiated from the protist communities associated with the commercial cultivars in the farm. Furthermore, given previous findings that bacterial diversity does not differ significantly among date palm cultivars within the same location [19], we predict that protist diversity and richness within the rhizosphere will be similar across cultivars growing at the same site, with differences primarily reflected in the relative abundance of specific taxa.

2. Materials and Methods

2.1. Study Sites and Soil Sampling

Rhizospheric soil samples were collected from both cultivated and wild date palm with a total of 55 soil samples. Five date palm cultivars were selected: Barhi, Shishi, Nabot Saif, Khalas, and Khenazi and their soil were sampled across two farms in Qatar: Qatar University Farm (25°48'29.8" N, 51°20'47.0" E) and Rawdat Al-Faras Farm (25°49'22.3" N, 51°19'58.1" E). Using a sterilized small shovel, 10 to 15 cm of soil were removed before collecting the soil sample from the rhizosphere near the roots. Samples were collected from 5 date palm trees randomly selected for each of the five cultivar type avoiding borders trees having 25 samples from each farm. Similarly, five wild date palms trees' rhizosphere samples were collected from Umm Bab coastal region (25°13'07.8" N, 50°46'04.5" E). Approximately 50 g of soil was collected using a sterile trowel and placed in sterile paper bag. The collected samples were left to air dry for 4 days and were stored at −20 °C to evaluate the microbial communities in the Environmental Science Center (ESC) laboratory in Qatar University.

2.2. Soil Physiochemical Characteristics

Pre-treatment of soil was done before chemical analysis by drying the soil in glass petri-dish in oven at 60–62 °C for 48 h. The dried soil samples were then crushed to a fine powder in a rotary ball mill (Retch Mill, Haan, Germany) at 250 rpm for 40 min and passed through a 2 mm mesh of a standard sieve. pH, salinity, conductivity and TDS were measured with YSI prop by preparing 1:2 soil to distilled water ratio. Chemical analysis of total carbon (TC), total nitrogen (TN) was done using CHN analyzer. Nitrate and nitrite were measured using UV spectrophotometer. For nitrate, two grams of soil were mixed with 50 mL of KCl solution and shaken for one hour. The mixture was then centrifuged at 5000 rpm for 15 min. Ten milliliters of the supernatant were collected, and 0.5 mL of nitrate reagent then the absorbance was measured. For nitrite, 10 mL of soil sample was taken into new tube and diluted to 50 mL with distilled water. The sample was passed through cadmium column after adding 1 mL of ammonium chloride then 20 mL was collected, and 0.5 mL of nitrate reagent was added then the absorbance was measured. The concentration of key elements (Ca, K, Mg, Cd, and Pb) was analyzed with ICP-OES after digestion. In Teflon tube, 0.25 g of soil was weighed and mixed with 9 mL of nitric acid. The tube set for 30 min under 95 °C hotblock then add 9 mL HF and set for another 30 min. Further, the temperature was increased to 135 °C for one hour then increased for 150 °C for one more hour. This was done using previously published ESC protocols adapted from EPA method #207 in the ESC ISO 17025-2017 accredited facilities [26].

2.3. DNA Extraction, Library Preparation and Illumina Sequencing

Before DNA extraction, composite samples was prepared from equal weight of the five tree samples collected for each cultivar. Environmental DNA was extracted from composite soil samples using a DNeasy PowerSoil Pro kit (Qiagen, Germany) following the manufacturer's instructions. A 30-ng qualified DNA template and 18S rRNA gene fusion primers: TAREuk454FWD1 (5-CCAGCASCYGC GGTAATTCC-3) and TAREukREV3 (5-ACTTTCGTTCTTGATYRA-3) were added to the polymerase chain reaction (PCR). The condition of the PCR cycle was as following; 95 °C for 10 min then 50 °C for 30 s finally 72 °C for 1 min and then 10 min. All PCR products were purified by Agencourt AMPure XP beads, dissolved in an elution buffer, and eventually labeled to finish library construction. The DNA library size and concentration were determined by Agilent 2100 Bioanalyzer (Shenzhen, Guangdong, China). Qualified libraries were sequenced at Begin Genomics Institute (Shenzhen, Guangdong, China) using the HiSeq 2500 platform with the sequencing strategy MiSeq-PE300 (MiSeq Reagent Kit) [30].

2.4. Bioinformatics and Statistical Analysis

The raw data were filtered to obtain high-quality clean data by removing adaptors and low-quality ambiguous bases. 18S microbial analysis was performed using MOTHUR (v1.39.5) [31] in the galaxy.org service (<https://usegalaxy.org/>, accessed on 24 February 2024). These paired-end reads were added to tags using the Fast Length Adjustment of Short reads program (FLASH, v1.2.11) [32]. These tags overlap with each other and form clusters as OTU with a 97% cutoff value using UPARSE software (v7 0.0.1090) [33]. The Ribosomal Database Project database was used to do taxonomic classifications to the OTU using the Protist Ribosomal Reference database (PR2) [34]. The OTU-abundance statistics table for each sample was constructed by comparing all tags back to OTU using the USEARCH_global [35]. Protist families pie chart was constructed for all samples. Functional classification of protist (Producer, consumer, parasite) [36] was performed based on the OTUs and taxonomic annotation [19]. In order to estimate alpha diversity of the samples, the rarefaction curves was first generated. Alpha diversity at the OTU level was

analyzed using MOTHUR (v1.39.5) [31]. The core pan flower chart was done by using SRplot (<https://www.bioinformatics.com.cn/en>, accessed on 1 October 2024) [37]. ANOVA analysis was done for Shannon index representing diversity and for (sobs) representing richness of cultivars type as well as samples' locations to observe statistically significant differences (p -value = 0.05). PCA for OTU and chemical components was done by R version (v3.4.1) software using package gplot, performance, stats, FactoMineR and factoextra.

3. Results

Sequence analysis of the protist communities associated with the sampled date palm cultivars and wild populations resulted in the identification of 456 distinct operational taxonomic units (OTUs). Of these, 42 OTUs were consistently detected across all samples, representing a putative core microbiome common to both cultivated and wild date palms (Figure 1). The Berhi cultivar, sampled from two different farms, exhibited the highest number of unique OTUs, 20 and 14 respectively, indicating a notably diverse and distinct microbial assemblage. In comparison, the wild date palm sample from Umm Bab harbored 10 unique OTUs, underscoring the differences in protist community composition between wild and cultivated hosts. The remaining samples displayed between 4 and 9 unique OTUs each, reflecting variable levels of taxonomic uniqueness among the different groups.

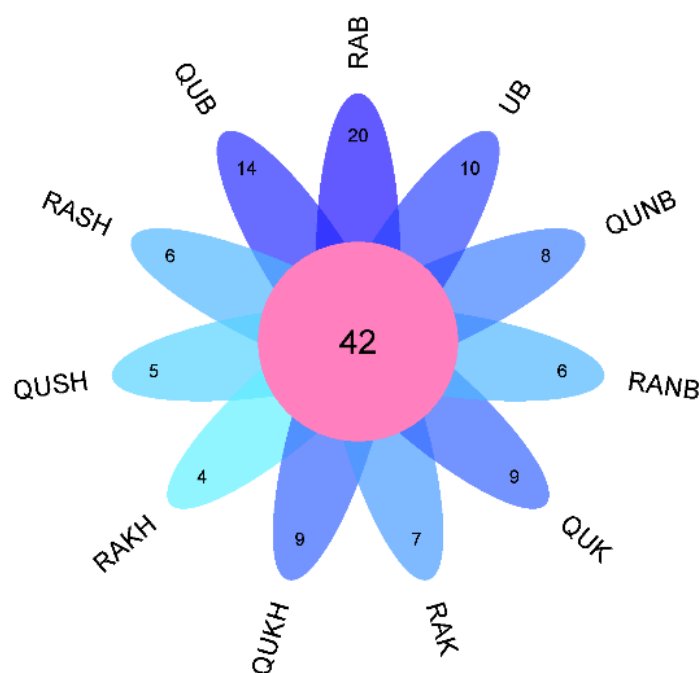


Figure 1. Core–Pan flower chart illustrating the distribution of operational taxonomic units (OTUs) across all samples. The central circle indicates the number of OTUs shared among all samples (core microbiome), while each petal represents the number of unique OTUs associated with individual cultivars or the wild date palm population. Sample abbreviations are as follows: RAB (Rawdat Al-Faras Berhi), UB (Umm Bab, wild date palm), QUNB (Qatar University Nabot Saif), RANB (Rawdat Al-Faras Nabot Saif), QUK (Qatar University Khalas), RAK (Rawdat Al-Faras Khalas), QUKH (Qatar University Khenazi), RAKH (Rawdat Al-Faras Khenazi), QUSH (Qatar University Shishi), RASH (Rawdat Al-Faras Shishi), and QUB (Qatar University Berhi).

Analysis of protist family-level composition across all samples revealed distinct patterns in relative abundance. The most dominant taxonomic groups were Rhizaria (18%), Alveolata (15%), Amoebozoa (12%), and Archaeplastida (11%), which together accounted for a substantial portion of the total protist community (Figure 2A). These four groups comprised the majority of the observed protist diversity. Additional, less abundant groups

included Stramenopiles, Opisthokonta, Hacrobia, and Excavata, each contributing smaller proportions to the overall community composition. Functional classification of the protist communities by trophic strategy revealed that heterotrophic protists—classified as energy consumers—were the most prevalent functional group, representing 16.85% of the total protist population (Figure 2B). Parasitic protists were present at much lower levels, comprising only 0.54% of the total composition. Phototrophic protists were the least represented, with minimal presence in the soil samples. Notably, the majority of detected OTUs could not be confidently assigned to a specific trophic category, indicating the presence of a substantial proportion of functionally uncharacterized protists.

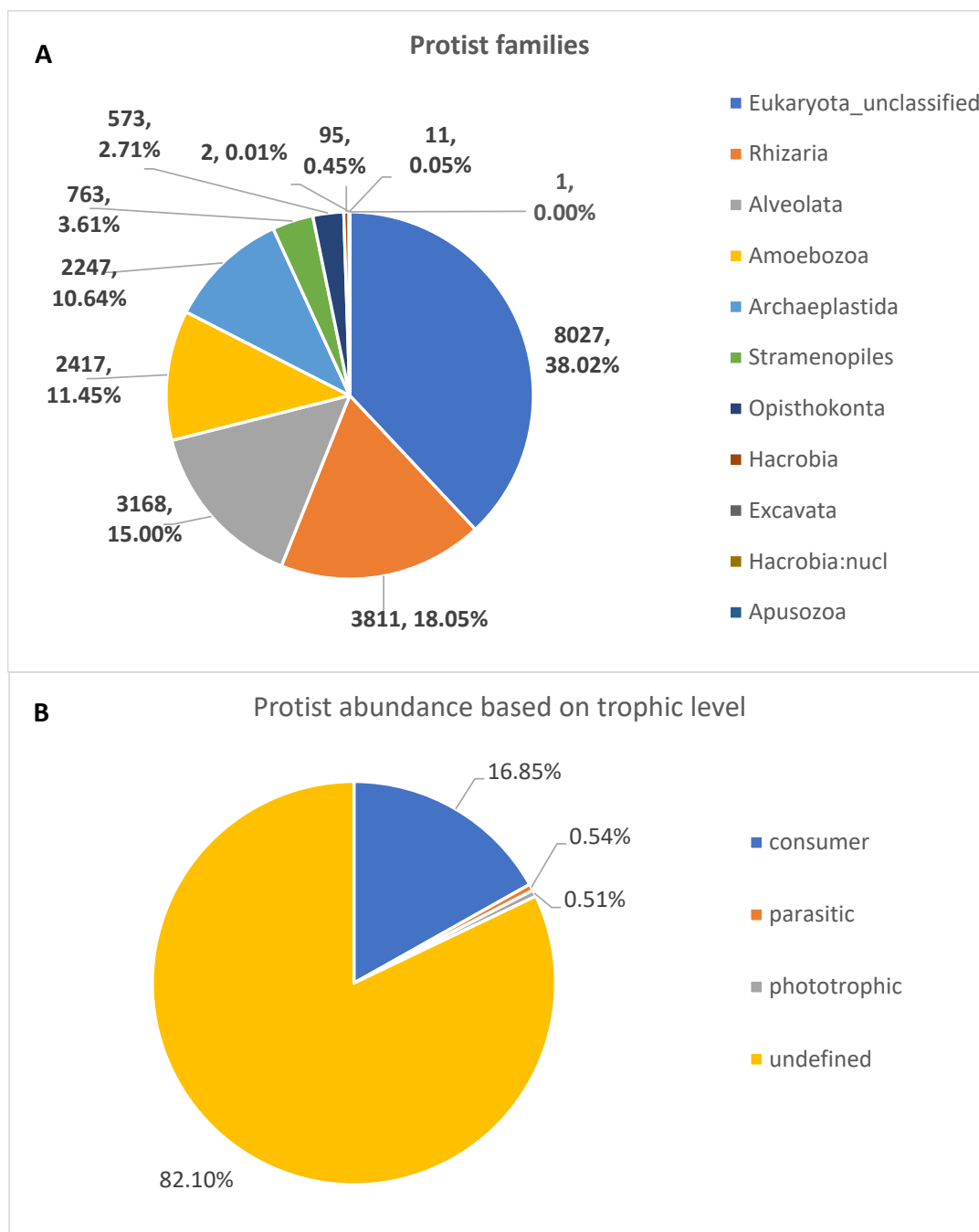


Figure 2. (A) Percentage of protist families in the rhizosphere of date palms across all sampled groups (%). (B) Distribution of protist orders categorized by trophic functional groups.

The distribution of protist classes across the sampled groups exhibited distinct patterns in relative abundance. The most prevalent classes included Filosa-Sarcomonadea, Colpodea, Variosea, Tubulinea, and Chlorophyceae, which together accounted for a substantial proportion of the overall protist community (Figure 3). These dominant taxa were consistently detected across nearly all samples, showing a similar trend in relative abundance profiles. Despite the prominence of these groups, a considerable fraction of the protist diversity remained taxonomically unresolved. Specifically, approximately 45% of the total relative abundance was attributed to unclassified protist taxa, indicating a large proportion of the community remains unidentified at the class level. Additionally, around 25% of the relative abundance was distributed among numerous low-abundance classes, highlighting the presence of diverse but less dominant lineages within the samples. A notable deviation was observed in the wild date palm sample, which exhibited a comparatively higher abundance of Opisthokonta relative to the cultivated groups, suggesting possible ecological or environmental influences on community composition.

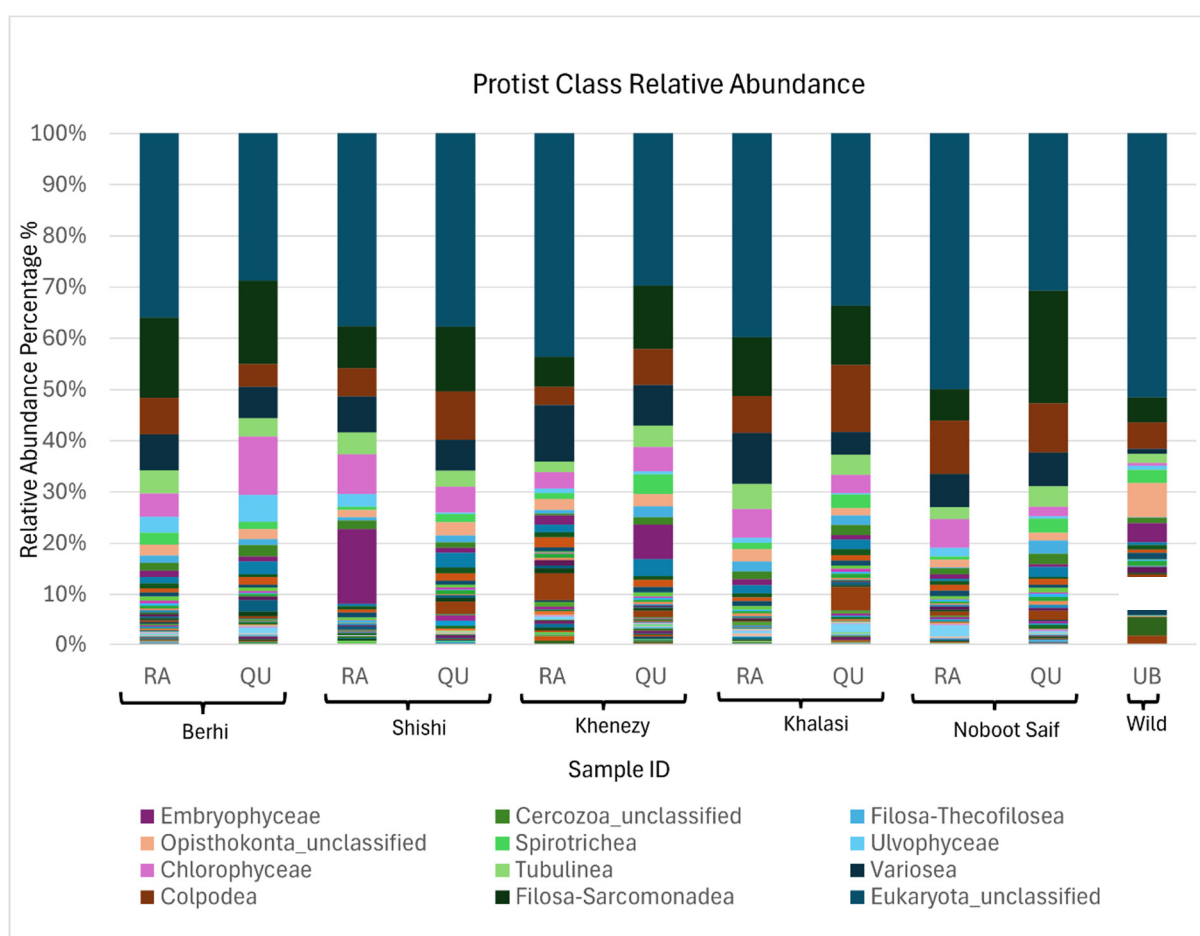


Figure 3. Relative abundance of protist classes in the rhizosphere across all date palm cultivars and the wild population. Sample groups include RA (Rawdat Al-Faras Farm), QU (Qatar University Farm), and UB (wild date palm population from Umm Bab).

Comparative analysis of protist diversity across sampling locations indicated no significant differences in either protist diversity ($p = 0.85$) or species richness ($p = 1.00$) among Qatar University Farm, Rawdat Al-Faras Farm, and the wild date palm population at Umm Bab (Figure 4A). However, when diversity was assessed at the cultivar level, some variability was observed. Although the differences among cultivars were not statistically significant ($p = 0.46$), the Berhi cultivar exhibited the highest protist diversity, followed by

Shishi and Nabot Saif cultivars (Figure 5A). Similarly, species richness was greatest in Berhi and Nabot Saif (Figure 5B). The rhizosphere of the wild date palm displayed moderate protist diversity and low species richness, comparable to that observed in the cultivated varieties. Consistent with diversity patterns, differences in species richness among cultivars were also not statistically significant ($p = 0.47$).

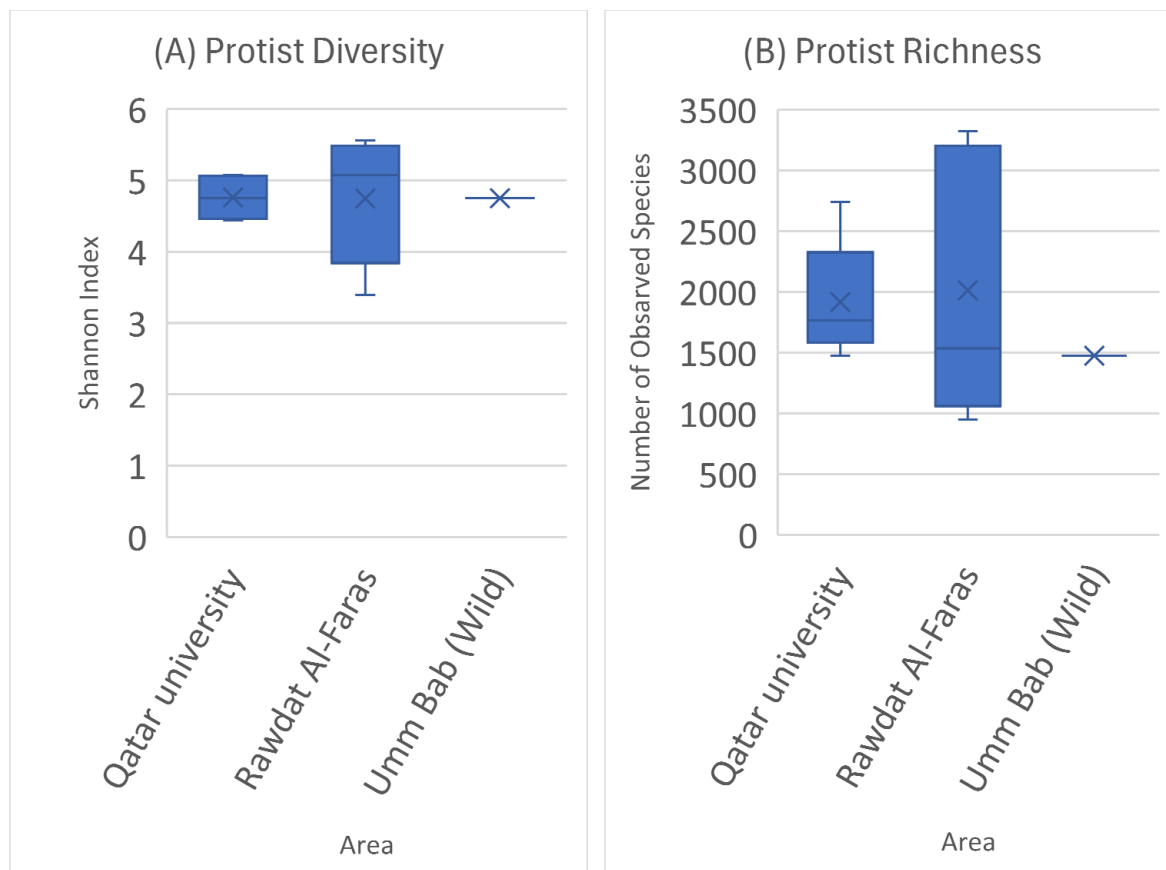


Figure 4. (A) Protist diversity in the rhizosphere across farmed areas (Qatar University Farm, Rawdat Al-Faras Farm) and the wild date palm population (Umm Bab). (B) Protist species richness in the rhizosphere across cultivated and wild date palms. Statistical analysis indicated no significant differences in diversity (Shannon index, $p = 0.85$) or species richness ($p = 1.00$) among locations.

Soil physiochemical analysis point out the difference between cultivated date palm and wild date palm total carbon, total nitrogen and trace metal concentrations (Figure 6). In the wild date palm, carbon percentage and calcium concentration were higher compared to the cultivated date palm with total nitrogen and the rest of trace metals being the lowest. Among different cultivars, khenezy from Rawdat Al-Faras farm showed the lowest concentrations of salinity and conductivity and nitrate concentration and the highest concentrations of cadmium and phosphate. Depending on the samples location, it is noticed that carbon and nitrogen percentage in both farm were similar compared to the wild date palm location. Similarly, this difference in concentration between wild and cultivated date palm for calcium, cadmium and magnesium was noticed.

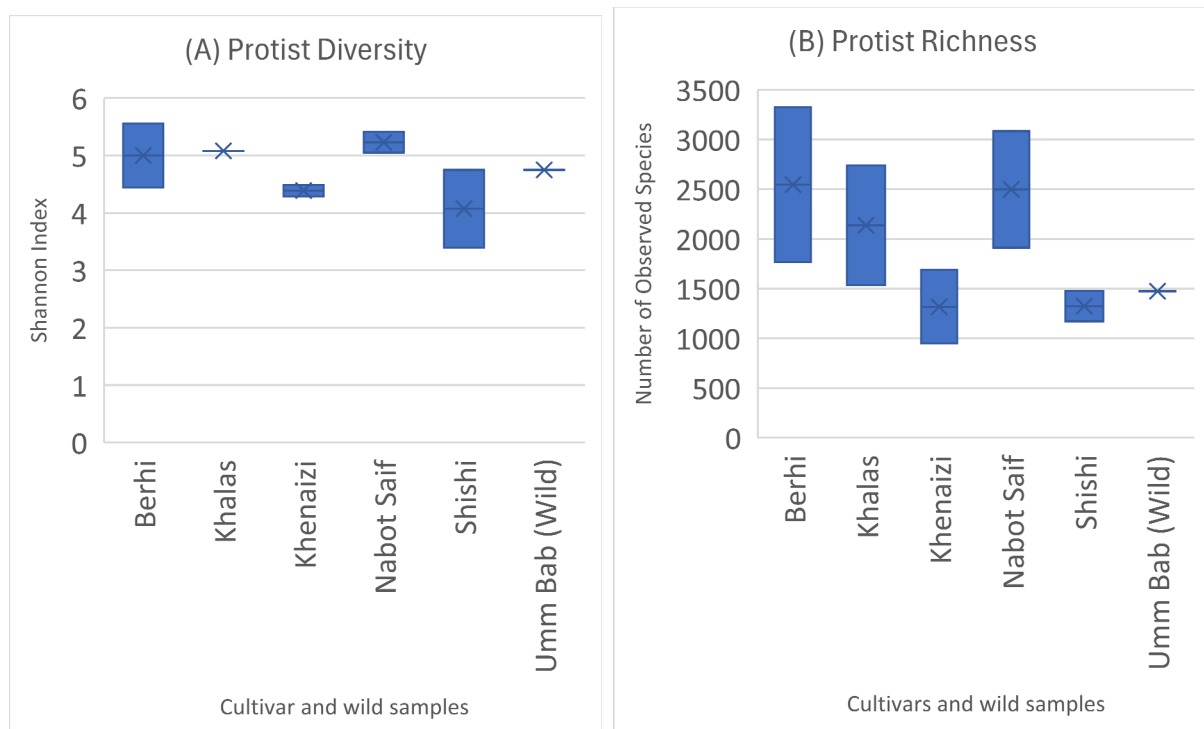


Figure 5. (A) Protist diversity in the rhizosphere of different date palm cultivars and the wild population. (B) Protist species richness in the rhizosphere across wild and cultivated date palms. Statistical comparisons showed no significant differences in diversity ($p = 0.46$) or richness ($p = 0.47$) among the groups.

Principal component analysis (PCA) (Figure 7) revealed distinct associations between soil chemical properties and protist community composition across different growing environments. The first principal component (Dim1) accounted for 38.7% of the total variance and primarily distinguished samples based on nitrate/nitrite concentrations versus calcium content and pH. Protist OTUs exhibited strong positive correlations with nitrate and nitrite levels, indicating that these nitrogen species are key drivers of community structure. In contrast, negative correlations with calcium and pH suggest that these factors may constrain the presence or abundance of certain protist taxa. Phosphorus and total carbon content appeared to have minimal influence on protist community distribution.

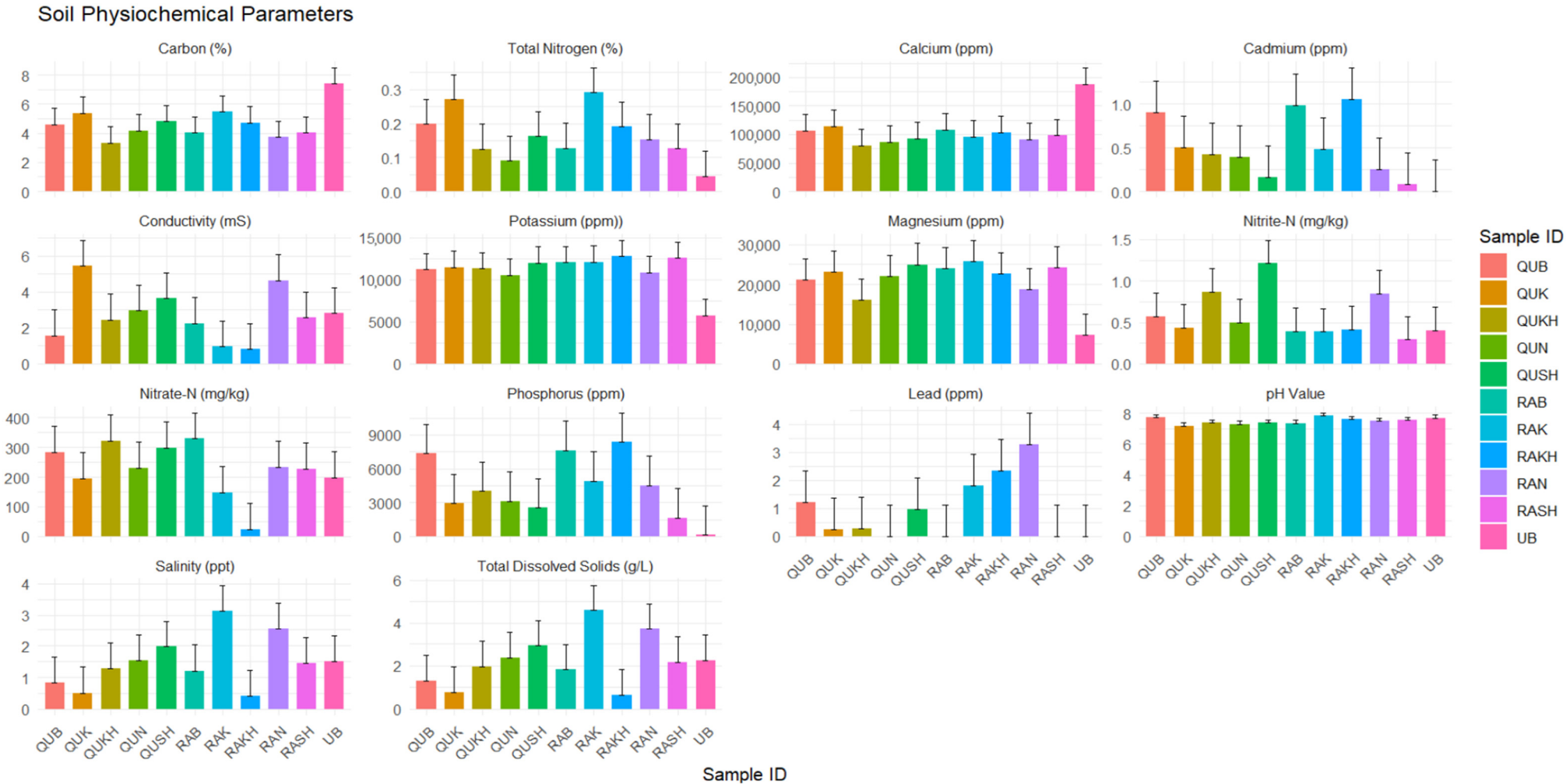


Figure 6. Soil Physiochemical Parameters for each cultivar and wild date palm.

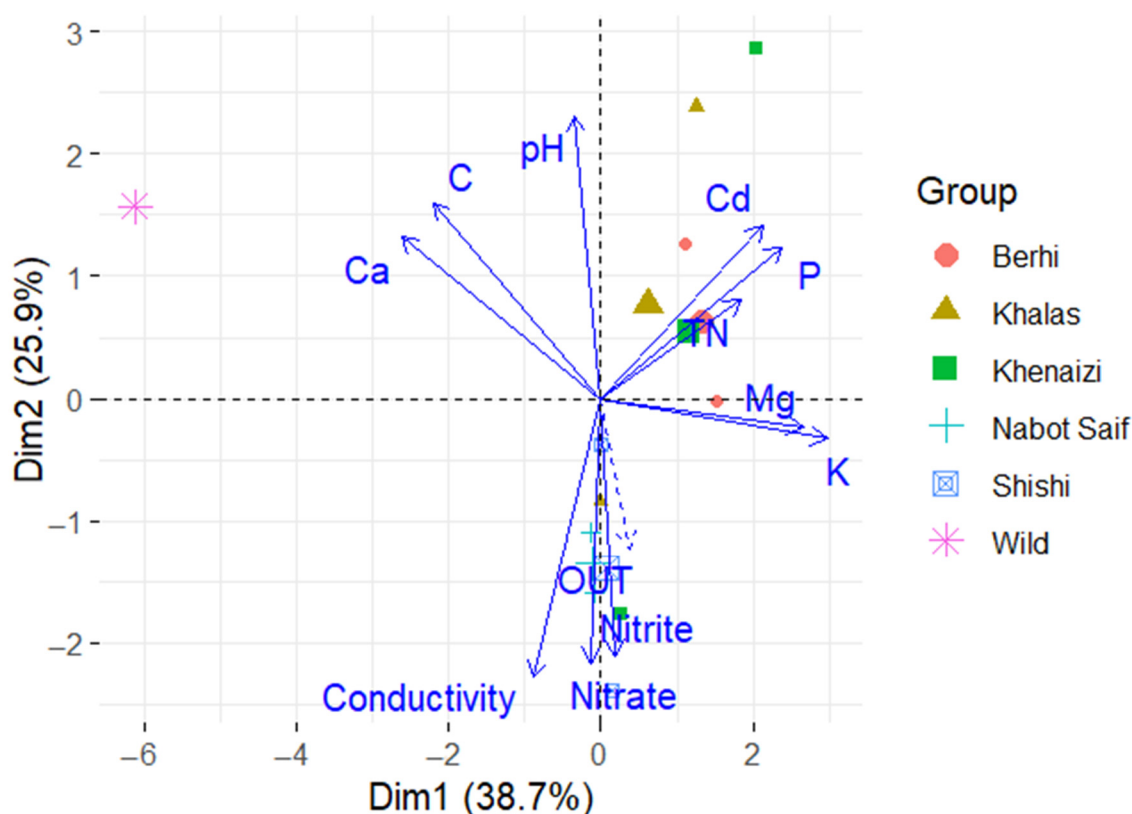


Figure 7. PCA of samples OTU and chemical component (p -value = 0.005).

4. Discussion

Metagenomic analysis identified 42 operational taxonomic units (OTUs) that were shared across all samples, indicating that the species corresponding to these OTUs are broadly adapted to the diverse environmental conditions present at all sampling sites, including both saline and agricultural areas. Notably, the Berhi cultivar at both farm locations exhibited the highest number of unique OTUs, highlighting the influence of cultivar genotype on the species richness of protists. Genotype-specific selection observed in our study is consistent with findings from a previous investigation of protist diversity in paddy soils, which reported similar genera—including Amoebozoa, Stramenopiles, Rhizaria, and Alveolata—but with differing relative abundances [16]. Amoebozoa and Rhizaria are common bacterial consumers and play a critical role in soil food webs by promoting the release of ammonium, a form of nitrogen readily available to plants [38,39]. Overall, the relative abundance of major protist classes was comparable across samples, with the exception of certain classes, suggesting that not all protists are equally influenced by the genotype or location of the date palm cultivars. The wild date palm population exhibited a distinct protist community characterized by a higher abundance of *Opisthokonta*, which we suggest is associated with drought and stress conditions, similar to observations in urban soils [40]. In contrast, previous studies in forest soils have linked higher *Opisthokonta* abundance to environments with greater cation exchange capacity and higher organic matter content [41]. The divergence in protist community structure observed in the wild samples relative to cultivated ones is expected, given the harsher environmental conditions and limited nutrient availability. In contrast, farm management practices and stabilized soil conditions in cultivated areas likely contribute to more consistent protist community composition. These findings support our hypothesis that the relative abundance of protist taxa differs significantly between wild and cultivated date palm environments.

Our hypothesis that protist diversity would be higher in farmed areas compared to wild habitats was not supported, as no significant differences were detected between farms and wild date palms. Previous studies examining land-use impacts on soil microbiota have shown that agricultural soils, benefiting from stable environmental conditions and consistent inputs of fertilizers and water, tend to exhibit higher protist diversity compared to stressed environments such as saline or polluted soils [41]. Further research is needed to assess whether reductions in protist diversity are linked to functional trade-offs affecting plant health or agricultural sustainability.

Across different soil types, similar protist taxa, including Chlorophyceae, Sarcomonadea, Variosea, and MAST, have been detected, although their relative abundances vary [36]. Dominant protist classes such as Filosa-Sarcomonadea and Colpodea have been found to correlate positively with bacterial and fungal genera, suggesting their key roles in structuring soil microbial niches [17]. A comparative study across vegetation types reported that protist diversity was highest within the Rhizaria supergroup, followed by Alveolata, Archaeplastida, and Amoebozoa, with minimal variation between vegetation types [17]. In line with previous studies [36], our results showed that consumer protists were the most abundant functional group, followed by phototrophs and parasites at similar proportions. However, other environments display different dominance patterns; for example, wetlands tend to support a higher diversity of pathogenic protists compared to drier habitats [42]. In the tomato rhizosphere, seven OTUs were identified as phagotrophic protists, including one amoebozoan and six cercozoans, which primarily feed on bacteria, alongside two phototrophic protist OTUs [18]. Similarly, in paddy soils, functional group analysis showed that 67.7% of protists were decomposers and predators, followed by autotrophs (21.0%) and pathogens (9.2%) [16].

In our study, neither cultivar type nor sampling area significantly influenced protist richness or diversity. However, the protist community associated with the wild date palm population was distinct from those at the two farm sites, likely reflecting the effects of harsher environmental conditions, including nutrient limitation and water scarcity. Similarly, a study across shrublands, woodlands, and native forests reported variation in phototrophic protist alpha diversity and shifts in the relative abundance of the Archaeplastida supergroup [17]. High soil salinity reduces plant water availability, disrupts cytosolic ion homeostasis, and enhances the production of reactive oxygen species (ROS), all of which can impact protist communities via common downstream signaling pathways and stress response mechanisms [43–45]. In most stress responses, calcium signaling and ROS act as second messengers, indicating that these signaling networks could influence protist community dynamics under environmental stress [46].

Although multiple protist classes were identified, more than 25% of sequences were assigned to unclassified eukaryotes. This is likely due to the limited study of protists across spatial and temporal scales [47] as well as the incompleteness of reference databases, the presence of novel or rare taxa, high genetic diversity, or biases in existing reference sequences [34].

As hypothesized, protist communities in the rhizosphere were distinctly separated from the cultivars on the farms. Unlike findings from paddy field soils, where pH was a primary driver of protist diversity [16], pH did not significantly influence protist communities in date palm soils. Instead, nitrate and nitrite levels exhibited strong positive correlations with protist OTU abundance. Similarly, prior studies have shown that nitrogen fertilizer applications, which increase nitrate concentrations, can affect soil pH and lead to a reduction in phagotrophic protist abundance [48]. In agreement with our findings, nitrate content was negatively correlated with salinity levels.

Conversely, studies in agricultural soils have reported that nitrogen fertilization can negatively impact protist diversity, with effects varying depending on the specific protist taxa involved [49]. Unexpectedly, in our study, total nitrogen content showed only a weak correlation with protist diversity, contrasting with previous reports suggesting that nitrogen enrichment promotes protist proliferation [23,50]. A study in paddy soils similarly found no relationship between nitrogen content and protist diversity, likely due to uniform nitrogen distribution across samples [16]. Other soil elements, including potassium and magnesium, also exhibited low correlations with protist OTU abundance. In addition, soil water content, heavily influenced by climate conditions and management practices, has been shown to affect protist diversity [16], potentially explaining the divergent patterns observed under the extreme environmental conditions of Qatar.

Collectively, these findings offer valuable insights into how fundamental soil properties shape protist life in both managed and natural ecosystems. To deepen our understanding of these complex communities, future studies should integrate functional metagenomics approaches to characterize unclassified taxa and elucidate how microbial interactions contribute to ecosystem functioning in arid agricultural systems.

5. Conclusions

This study provides a comprehensive characterization of rhizospheric protist communities associated with cultivated and wild date palms in arid environments. Despite considerable taxonomic variation, 42 OTUs were shared across all samples, indicating a core microbiome likely adapted to diverse soil conditions. The Berhi cultivar exhibited the highest number of unique OTUs, suggesting host genotype influences certain microbial associations. However, neither cultivar type nor location significantly affected overall diversity or richness, highlighting the dominant role of environmental conditions in structuring these communities. Dominant groups included Rhizaria, Alveolata, Amoebozoa, and Archaeplastida, with heterotrophic protists being functionally most abundant. A large proportion of unclassified taxa underscores the need for expanded reference databases and functional annotation tools. The distinct community composition in wild palms, particularly the higher abundance of Opisthokonta, suggests a response to abiotic stressors such as drought and salinity. Soil nitrate and nitrite levels were key environmental drivers, while pH and other nutrients showed weaker correlations. These findings underscore the complexity of protist-environment interactions and the resilience of protist communities across managed and natural arid ecosystems. Future work integrating functional metagenomics and long-term monitoring will be essential to better understand the ecological roles of protists in soil health and sustainable agriculture under extreme environmental conditions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/soilsystems9030079/s1>.

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