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Unraveling the spatio-temporal dynamics of soil and root-associated microbiomes in Texas olive orchards

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Understanding the structure and diversity of microbiomes is critical to establishing olives in non-traditional production areas. Limited studies have investigated soil and root-associated microbiota dynamics in olives across seasons or locations in the United States. We explored the composition and spatiotemporal patterns of the olive-associated microbial communities and specificity in two niches (rhizosphere and root endosphere), seasons (spring, summer, and fall), and domains (bacteria and fungi) in the microbiome of the olive cultivar Arbequina across three olive orchards in Texas. Phylum Proteobacteria, followed by *Actinobacteriota*, dominated the bacterial populations in the rhizosphere and endosphere. *Rubrobacter* and *Actinophytocola* were dominant taxa in the rhizosphere and root endosphere at the genus level. Among fungal communities, phylum *Ascomycota* was prevalent in the rhizosphere and endosphere, while members of the *Chaetomiaceae* family outnumbered other taxa in the root endosphere. As per the alpha diversity indices, the rhizosphere at Moulton showed much higher richness and diversity than other places, which predicted a significant difference in rhizosphere between locations for bacterial diversity and richness. There was no significant variation in the bacterial diversity in the niches and the fungal diversity within the root endosphere between locations. Beta diversity analysis confirmed the effect of compartments—in influencing community differences. Microbial diversity was apparent within the endosphere and rhizosphere. The seasons influenced only the rhizosphere fungal diversity, contrasting the bacterial diversity in either niche. The research provided a comprehensive overview of the microbial diversity in olive trees' rhizosphere and root endosphere. The abundance and composition of OTUs associated with the rhizosphere soil of Arbequina suggest its role as a source reservoir in defining the potential endophytes.

Olive (*Olea europaea* L.) is cultivated commercially for the quality of its oil worldwide. Although early domestication of olive trees began in the Mediterranean region[1], in recent decades, commercial olive production expanded to non-traditional areas, such as Australia and North and South America[2], significantly varying in the agro-climatic conditions[3–5]. Olives were introduced into the United States in the late eighteenth century[6]. The United States only represents less than 5% of the global olive production[7]. While California is the primary center for olive cultivation, the industry spans other states, including Texas, Arizona, Georgia, Florida, Oregon, and Hawaii. In Texas, beginning in the mid-1990s[8], the high-density olive planting for oil production has been spread across approximately 1400 ha[9]. The productivity of Texas olives is primarily affected by stress from cold or heat and diseases, prominently the cotton root rot (*Phymatotricopsis omnivora*), which is prevalent in the high-pH soils of southwest Texas. Even if olive trees are adapted to drought and maintain an ability to produce fruit in extreme climates[10], the changing climate conditions have shown varied impacts on olive fruit maturation and oil composition in different cultivars, locations, and water availability[11–13].

Soil and plant-associated microbial communities are critical to plant productivity. Plant species, selection pressure, and environmental conditions define diversity within such communities. Soil microbes regulate the mineralization and competition of nutrients that sustain plant productivity[14]. At the same time, plant-associated

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microbiomes confer fitness advantages to the plant host, including growth promotion, nutrient uptake, stress tolerance, and resistance to pathogens[15]. In return, plants can affect soil microbial communities via host preference and changes in plant-derived inputs, such as litter, rhizodeposits, and root exudates[16].

Several reviews have highlighted the significance of soil and olive tree-associated microbiomes in defining olive tree productivity[17,18]. However, since the Mediterranean basin has been the world's production center for olives, research on the interactions with soil microbiota and/or olive tree microbiomes outside these non-traditional production areas is lacking. Microbiota, particularly at the root level, are critical in modifying plant physiology and metabolism under various climatic conditions[19]. The role of plant genotype in shaping the composition of its root-associated microbiome has been highlighted based on the differences and similarities between the microbial communities in different soils[20]. Healthy and highly stable root microbiota are critical in helping olive trees thrive in new environments and climatic conditions[21]. A study on the microbiomes (communities of bacteria and fungi) of the endo- and rhizosphere of various olive cultivars from the World Olive Germplasm Collection (WOGC) at the Institute of Agricultural and Fisheries Research and Training (IFAPA, Córdoba, Spain) revealed a robust genotypic influence and lower diversity in the endosphere than in the rhizosphere[20]. The changing dynamics of global climate (i.e., reduced rainfall, increasing drought and temperature) will likely expand the arid and semiarid environments characterized by low soil nutrients and organic matter, like the Texas climate, impacting olive productivity, soil microbiomes and ecosystem functioning. A study evaluating the impact of aridity on the bulk soil and the olive root-associated bacterial communities indicated that with the increment of aridity, distinct bacterial communities dominated by aridity-winner and aridity-loser bacteria negatively and positively correlated with increasing annual rainfall[22]. Likewise, the impact of variables such as plant age, organ type, altitudinal gradient, geographic location, and season, but not the cultivar, on the structure of microbial communities in commercial olive plants has been demonstrated[23,24].

On the contrary, studies have also shown the effect of the olive cultivar on the distinct differences in the endophytic and epiphytic microbial communities[25,26].

A deeper understanding of the structure and diversity of the soil and root microbiome in olive production would enable its utilization for abiotic or biotic stress alleviation, especially in non-traditional production areas. To our knowledge, no systematic investigation has been performed on this cultivar's soil/root-associated microbiota dynamics across seasons or locations in the United States or Texas. Here, we characterize the composition and spatiotemporal patterns in two niches (rhizosphere and root endosphere), seasons (Spring, summer, and fall), and domains (bacteria and fungi) in the microbiome of the olive cultivar 'Arbequina,' the primary cultivar used for commercial production in the United States planted at three locations (Carrizo Springs, Moulton, and Berclair) across Texas. We hypothesized that (a) the sampling location and seasons will strongly structure the olive-associated microbial communities over broadscale changes in climate and soil features across Texas, (b) the root microbial community (endosphere) structure would be more responsive than rhizosphere communities over the seasons or locations and (c) a conserved core microbial communities are associated with root endosphere and rhizosphere zones. The Illumina-based amplicon sequencing helped us characterize and compare the size and structure of olive tree-associated bacterial and fungal communities, providing comprehensive insights into their relevance in non-traditional production areas.

Results

The trees of the cultivar Arbequina grown at the three geographically distinct locations (Carrizo Springs, Moulton, Berclair) were selected to analyze the microbiome profile of olive rhizosphere soil and endosphere of roots over three seasons (Spring, summer, and fall). Amplicon sequencing of 16S rRNA and ITS regions on the Illumina Miseq platform generated 8,971,015 bacterial and 8,049,726 fungal raw sequence reads. After filtering, 8,879,079 bacterial and 6,868,796 fungal high-quality sequence reads were obtained.

Effects of location and seasons on OTUs

The number of OTUs was used to provide a comprehensive overview of the microbial structure and distribution, where 14,190 bacterial OTUs were identified in the rhizosphere (RS) and root endosphere (RE) samples. A Venn diagram representing OTU distribution in the rhizosphere and root endosphere of all samples revealed that 73.57% (10,441 OTUs) were only found in the rhizosphere soil as opposed to the roots (26.12%, 3707 OTUs), and both niche samples shared 0.03% (5 OTUs) of the total bacterial OTUs. Of all locations, the most unique bacterial OTUs were found in rhizosphere soils (14.13%, 1476 OTUs) in the Carrizo Springs and Moulton endosphere roots (17.56%, 651 OTUs). Across seasons, 1176 OTUs in spring (11.26%) and 639 OTUs in fall (17.23%) exhibited the unique rhizosphere and root endosphere OTUs (Fig. 1; Supplementary Fig. S1). In the case of fungi, 11,068 OTUs were generated. While both niches shared 7.17% (794 OTUs) of the total fungal OTUs, 94.71% (10,483 OTUs) were exclusively detected in rhizosphere soil compared to roots (5.29%, 585 OTUs). In Berclair, rhizosphere soils (30.5%, 2817 OTUs) and endosphere (22.57%, 195 OTUs) exhibited the most unique fungal OTUs. For seasons, spring (27.81%, 2304 OTUs) and summer (29.37%, 220 OTUs) displayed the unique OTUs for rhizosphere soils and root endosphere, respectively. (Supplementary Fig. S2).

Location and seasons structure the microbial community composition

The relative abundance (R.A) of dominant bacterial phyla varied across all locations and seasons for both sample categories. The analysis included the following acronyms: RS for rhizosphere, RE for root endosphere, CS for Carrizo Springs, M for Moulton, B for Berclair, Sp for spring, Su for summer, and F for fall. Phylum *Proteobacteria* (RS, 16.42%; RE, 15.50%) and *Actinobacteria* (RS, 12.63%; RE, 16.47%) dominated the bacterial communities in both niches (12–16%). The archaeal phylum *Crenarcheota* was found only in the rhizosphere (4.92%), while it showed zero abundance in the endosphere. At the genus level, *Bacillus* was identified as the dominant taxon

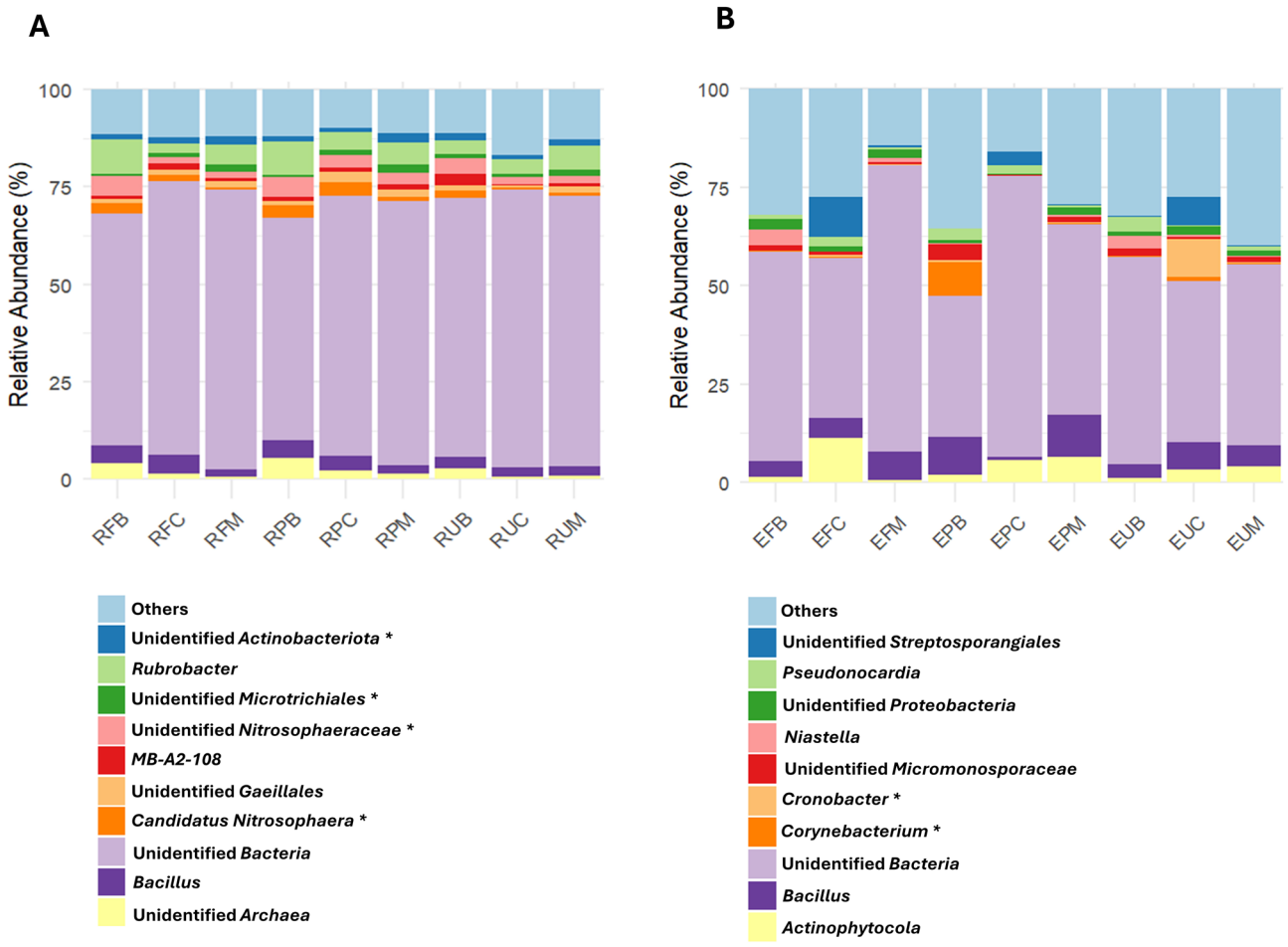

Figure 1. Relative abundances of the dominant bacterial genera across Rhizosphere (**A**) and Endosphere (**B**). The asterisks denote genera that were significantly different ($p < 0.05$), as shown by Linear discriminant analysis Effect Size (LEfSe). The stacked bar plots were based on the top 10 genera, while all remaining taxa (< 1%) were included in "Others". *RFB* Rhizosphere soil from Berclair in the Fall, *RFC* Rhizosphere soil from Carrizo Springs in Fall, *RFM* Rhizosphere soil from Moulton in Fall, *RPB* Rhizosphere soil from Berclair in Spring, *RPC* Rhizosphere soil from Carrizo Springs in Spring, *RPM* Rhizosphere soil from Moulton in Spring, *RUB* Rhizosphere soil from Berclair in Summer, *RUC* Rhizosphere soil from Carrizo Springs in Summer, *RUM* Rhizosphere soil from Moulton in Summer, *EFB* Rhizosphere soil from Berclair in Fall, *EFC* Root endosphere from Carrizo Springs in Fall, *EFM* Root endosphere from Moulton in Fall, *EPB* Root endosphere from Berclair in Spring, *EPC* Root endosphere from Carrizo Springs in Spring, *EPM* Root endosphere from Moulton in Spring, *EUB* Root endosphere from Berclair in Summer, *EUC* Root endosphere from Carrizo Springs in Summer, *EUM* Root endosphere from Moulton in Summer.

across niches, while *Actinophytocola* (3.49%) and Unidentified *Streptosporangiales* (2.38%) were identified only in the root endosphere. In addition, both niches were associated with a higher relative abundance of "Others" under phyla and genera, indicating the possibility of a diverse bacterial composition (Supplementary Fig. S3A,D).

Considering the influence of locations within seasons for given rhizosphere soil niches for taxonomic composition, the predominant bacterial phyla were *Actinobacteriota* in Moulton in all seasons, *Proteobacteria* in Carrizo Springs, and archaeal phyla *Crenarcheota* in Berclair location. In the root endosphere, there was an abundance of *Actinobacteriota* in Berclair during spring, Proteobacteria in Carrizo Springs, and Firmicutes relatively abundant in Moulton (Supplementary Fig. S3B,C, Supplementary Table S1). At the genus level, *Rubrobacter* was dominant in the rhizosphere of Berclair in the spring and fall seasons, followed by *Bacillus* in the spring and fall of Carrizo Springs. In contrast, the root endosphere of Carrizo Springs during Fall was enriched with *Actinophytocola* (11.19%), while the Berclair endosphere was abundant in *Corynebacterium* in spring (8.39%) (Fig. 1B,C, Supplementary Table S1). In addition, both niches were associated with higher relative abundance of "Others" under phyla and genus, indicating the possibility of a diverse bacterial composition.

For Fungi, Phylum *Ascomycota* dominated the fungi communities, the most abundant phylum in both niches (R.A > 40%). While *Mortierellomycota* was exclusively found in rhizosphere soil (3.60%), *Glomeromycota* was found in higher abundance in the root endosphere (7.72%). At the family level, members of *Chaetomiaceae* were identified as the dominant taxon across the rhizosphere in all seasons, while unidentified *Agaricales* and *Xylariales* were abundant in the root endosphere (R.A > 10%). Furthermore, much like the bacterial community, a higher percentage of "Others" under phyla and family was found in both niches, suggesting a potential for a diverse

fungal composition (Fig. 2A, Supplementary Fig. S4A,C). The top abundant fungal phyla in all rhizosphere niches of locations within seasons was *Ascomycota* (R.A > 75%), followed by *Basidiomycota*. *Mortierellomycota* was more prevalent in Berclair during spring and summer (7%). Phylum *Ascomycota* dominated the root endosphere and was particularly abundant in all locations in spring, while *Glomeromycota* showed the highest abundance in summer of all locations (Supplementary Fig. S4C,D, Supplementary Table S2).

At the family level, members of *Chaetomiaceae* were observed in higher abundance in all seasons of Berclair. Hypocrealeas in the rhizosphere of Moulton (24.32%) and *Cucurbitariaceae* (21.34%) in Carrizo Springs were abundant during summer. In contrast, in the root endosphere, in spring, members of unidentified *Xylariales* dominated Berclair (49.85%) and Moulton (48.21%). In summer, the Moulton root endosphere was abundant in *Herpotrichilleaceae,* and *Glomeraceae* was abundant in Carrizo Springs roots (Su, 25.77%) (Fig. 2B, Supplementary Table S2).

Using linear discriminant analysis of effect size (LEfSe), we aimed to identify distinct taxa as biomarkers that most likely explained the differential relative abundance between locations or seasons. The linear discriminant analysis effect size (LEfSe) with logarithmic LDA > 2 was used to identify markers. Overall, rhizosphere soil contained a more considerable number of bacterial markers than endosphere soil (Supplementary Fig. S5A–D). Between locations, members of *Nitrosophaeraceae, Microtrichiales,* and *Sphingomonas* were the predominant rhizosphere soil genera in Berclair, Moulton, and Carrizo Springs, respectively. The root endosphere revealed the presence of many distinct genera in Berclair (*Corynebacterium*, *Niastella, Rhizobium*, and *Promicromonospora)* (Fig. 3A,C).

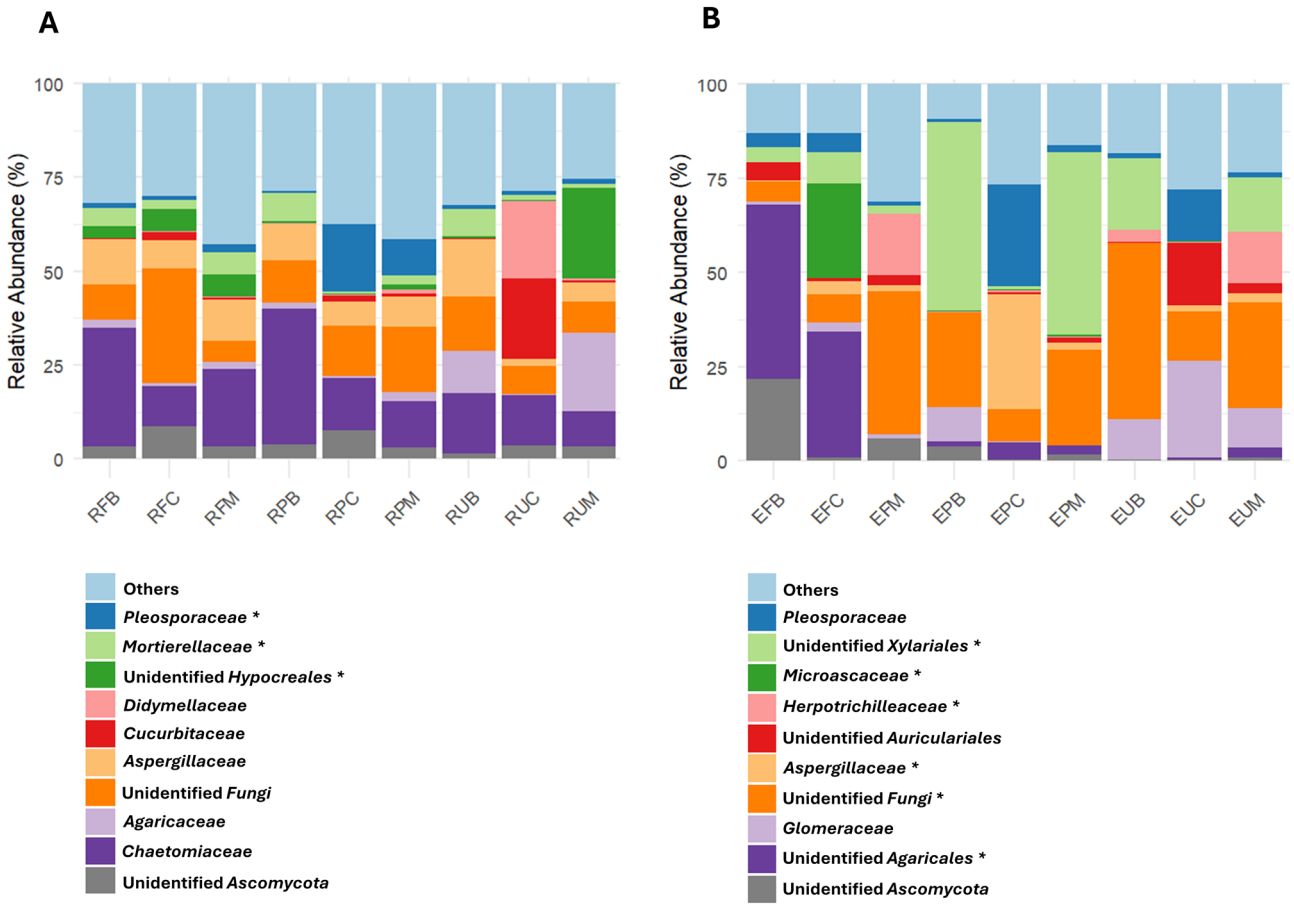

Figure 2. Relative abundances of the dominant fungi family across Rhizosphere (**A**) and Endosphere (**B**). The asterisks denote families that were significantly different ($p < 0.05$), as shown by Linear discriminant analysis Effect Size (LEfSe). The stacked bar plots were based on the top 10 genera, while all remaining taxa (< 1%) were included in "Others". *RFB* Rhizosphere soil from Berclair in Fall, *RFC* Rhizosphere soil from Carrizo Springs in Fall, *RFM* Rhizosphere soil from Moulton in Fall, *RPB* Rhizosphere soil from Berclair in Spring, *RPC* Rhizosphere soil from Carrizo Springs in Spring, *RPM* Rhizosphere soil from Moulton in Spring, *RUB* Rhizosphere soil from Berclair in Summer, *RUC* Rhizosphere soil from Carrizo Springs in Summer, *RUM* Rhizosphere soil from Moulton in Summer, *EFB* Rhizosphere soil from Berclair in Fall, *EFC* Root endosphere from Carrizo Springs in Fall, *EFM* Root endosphere from Moulton in Fall, *EPB* Root endosphere from Berclair in Spring, *EPC* Root endosphere from Carrizo Springs in Spring, *EPM* Root endosphere from Moulton in Spring, *EUB* Root endosphere from Berclair in Summer, *EUC* Root endosphere from Carrizo Springs in Summer, *EUM* Root endosphere from Moulton in Summer.

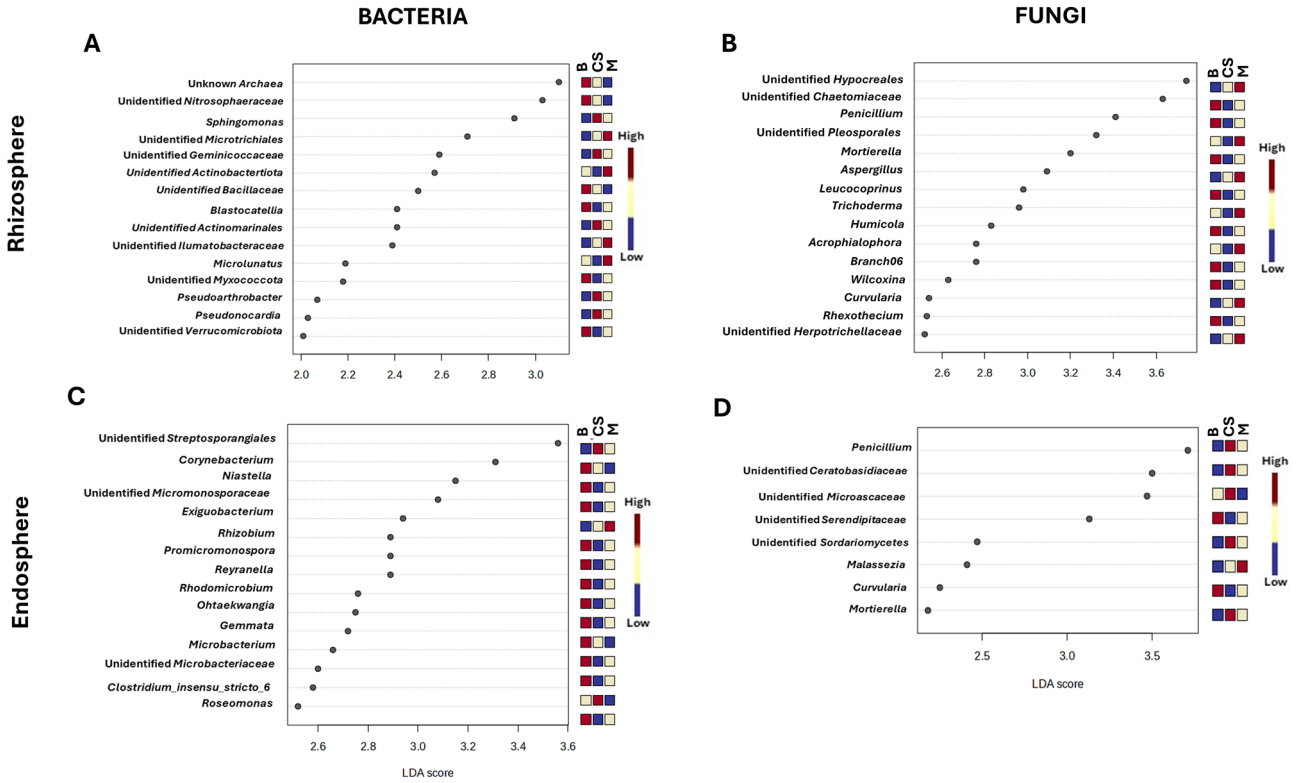

Figure 3. Dot plot illustrating the variation in the abundance of bacterial genera in the rhizosphere (**A**) and endosphere (**C**) and fungal genera in the rhizosphere (**B**) and endosphere (**D**) across different locations. The differentially abundant genera had LDA scores between 2 and 4 and were based on the FDR-adjusted $p < 0.05$. The colored bar represents the relative abundances of the differentially abundant genera, with the lowest indicated as blue and the highest as red. *B* Berclair, *CS* Carrizo Springs, *M* Moulton.

Concerning fungal biomarkers, members of *Hypocreales* and *Cheatomiaceae* were found in rhizosphere soils in Moulton and Berclair. While no unique distinguishable biomarker fungi existed in Carrizo Springs, they contained moderate numbers of *Hypocreales*. In contrast, members of families such as *Ceratobasidiaceae* and *Microascaceae* were enriched in Carrizo Springs more than in other locations in the root endosphere. (Fig. 3B,D).

We also examined the existence of distinct biomarkers between niches for each season. All seasons showed a higher abundance of *Rubrobacter* in rhizosphere soil. The root endosphere contained the genus *Actinophytocola*. Seasonally, spring and fall demonstrated greater enriched fungal biomarkers in the rhizosphere soil than in summer. Meanwhile, *Mortierella* was found in the rhizosphere throughout all seasons. *Wilcoxina* was exclusively seen in the fall, and *Pyrenocheta* was only detected in the summer. The predominant biomarkers in the root endosphere genera include *Malassezia* in the spring, respectively, whereas *Xylariales* were found across all seasons. (Supplementary Fig. S6A–D).

Bacterial and fungal taxonomic richness and diversity

Alpha diversity

Rarefaction curves indicated that the sequencing effort was sufficient to capture the total alpha diversity within the sample. With the increase in sample size, the Specaccum (species cumulative curve) showed the rate of increase of new species of bacteria and fungi (Supplementary Fig. S7A–C).

The alpha diversity metrics, Shannon (H′) and Inverse Simpson (1/D) indices, were used to assess the diversity of bacterial and fungal communities within samples across niches, locations, and seasons. Bacterial communities differed significantly (Kruskal–Wallis, chi-squared, $P < 0.001$) in diversity and species richness among overall sample categories, with rhizosphere soil exhibiting the highest diversity based on all metrics ($H' = 6.27$; $1/D = 162.61$) (Fig. 4A, Supplementary Fig. S8A). Season and site significantly affected the diversity of bacterial communities. For example, the site and the season explained 83.91% and 78.45% of the significant variation in bacterial diversity in the rhizosphere and root endosphere, respectively. Among locations, Moulton rhizosphere soil exhibited much higher richness and diversity than other locations ($H' = 6.45$). None of the used metrics revealed a significant difference in the α-diversity of the root endosphere niche in locations (Supplementary Table S3; Fig. 4C). For rhizosphere soils, seasonal differences in bacterial diversity and richness were not statistically significant. However, in the root endosphere, seasonal differences showed a significant variation in observed richness, while the evenness index comparison (InvSimpson) was not statistically significant (Kruskal–Wallis, chi-squared, $P > 0.05$). In particular, the root endosphere had a higher bacterial diversity in the fall season ($H' = 6.27$) than in the other seasons (Fig. 4C; Supplementary Table S3; Supplementary Fig. S8A,C,E). Fungi

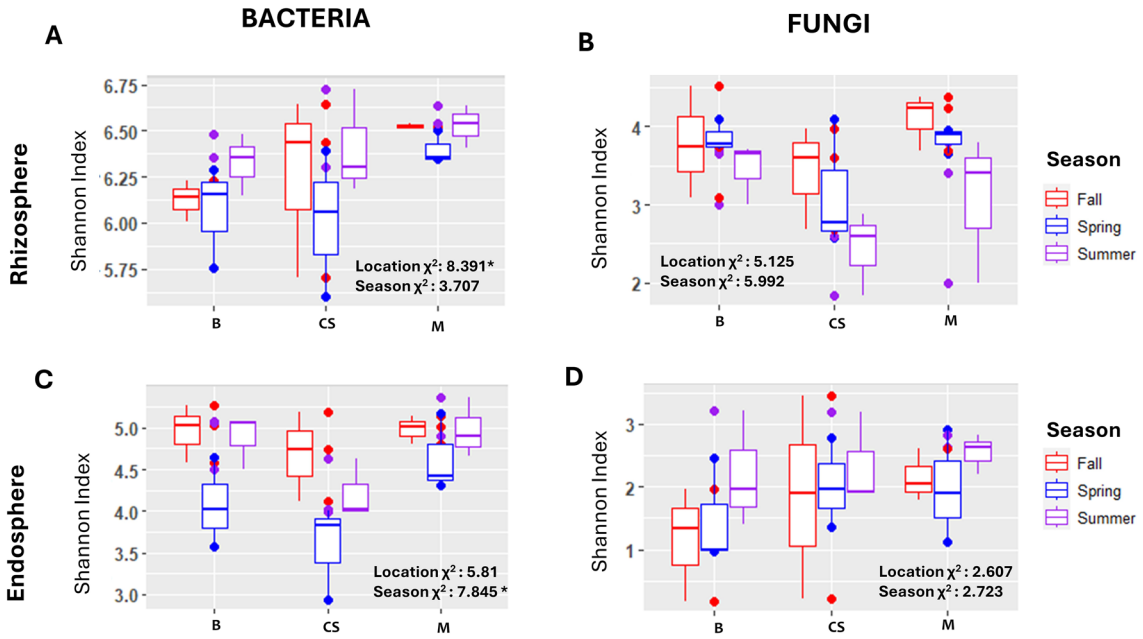

Figure 4. Box-and-Whiskers-plots visualize the Shannon diversity index across locations and seasons for bacteria (**A**–**C**) and fungi (**B**,**D**). Significant differences ($p \leq 0.05$)—are indicated by asterisks (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$). *B* Berclair, *CS* Carrizo Springs, *M* Moulton.

communities differed considerably (Kruskal–Wallis, chi-squared, $P < 0.001$) in diversity and species richness between overall niches, with rhizosphere soil showing the highest diversity across all locations and seasons ($H' = 3.44$; $1/D = 13.06$) (Supplementary Table S4; Fig. 4B; Supplementary Fig. S8B). However, for location and season, no statistically significant variation in fungal richness and evenness in the rhizosphere and root endosphere was observed according to the metrics used (Kruskal–Wallis, chi-squared, $P > 0.05$) (Fig. 4D,F; Supplementary Fig. S8D,F).

Beta diversity

Permutational multivariate analyses of variance (PERMANOVA) of the Bray–Curtis distance matrix showed that compartments (R^2; Bacteria: 0.451; Fungi: 0.123) significantly influenced microbial community differences ($P = 0.001$). Furthermore, locations in the rhizosphere (R^2; Bacteria:0.305, Fungi:0.216) and endosphere (R^2; Bacteria:0.146, Fungi:0.156) influenced microbial diversity. Seasons did not affect bacterial diversity in either niche; only fungal diversity was found in the rhizosphere ($R^2 = 0.084$, $P = 0.001$). The principal coordinate analysis (PCoA) based on Bray–Curtis's distance revealed that the 54 samples of the rhizosphere and endosphere bacterial communities from the same plant species exhibited a clear tendency to group based on sample type, with two principal component scores accounting for 45.7% and 4.3% of the total variations for bacteria and 14.8% and 7.0% of the total variations for fungi, respectively (Fig. 5). Rhizosphere bacterial samples from Berclair and Moulton tend to have separate clusters and Carrizo Springs formed separate clusters from other sites. There was an overlapping clustering for the root endosphere of Berclair and Moulton, while samples from Carrizo Springs within seasons were scattered (Fig. 5C,E), for fungi rhizosphere soil samples from Carrizo Springs were dispersed for all seasons, while Moulton and Berclair samples overlapped. For root endosphere samples, samples did not cluster clearly between Berclair and Moulton locations, while Carrizo Springs clustered from other locations, although not season-wise because they overlapped (Fig. 5D,F). These differences were also shown by NMDS, in which clear separation of samples according to the locations was observed.

In contrast, samples based on seasons displayed higher overlapping for bacteria and fungi (Supplementary Fig. S9A–F). Comparable results were observed in the Bray–Curtis dissimilarity distance-based hierarchical cluster analysis, demonstrating that the samples clustered into distinct groups based on the species composition of each sample (Supplementary Fig. S10A,B).

Discussion

Plant-associated microbial communities are critical determinants of plant health and productivity, contributing to nutrient availability and enhancing tolerance to abiotic and biotic stress[27,28]. With changing dynamics of the climate-driven amplification of abiotic stresses such as drought and heat, global olive cultivation has become highly vulnerable to sudden outbreaks of new diseases or herbivorous insect pests[29]. Unlike seasonal crops, fruit or nut trees like olives have a fundamentally different relationship with the soil. The olive-associated microbiome has been identified as a rich source of microbes that exhibit promise as agents that promote plant growth and exert biocontrol effects[30]. This study aimed to characterize the bacterial community compositions of the rhizosphere and endosphere of the Arbequina olive cultivar grown in three geographical regions from spring to fall.

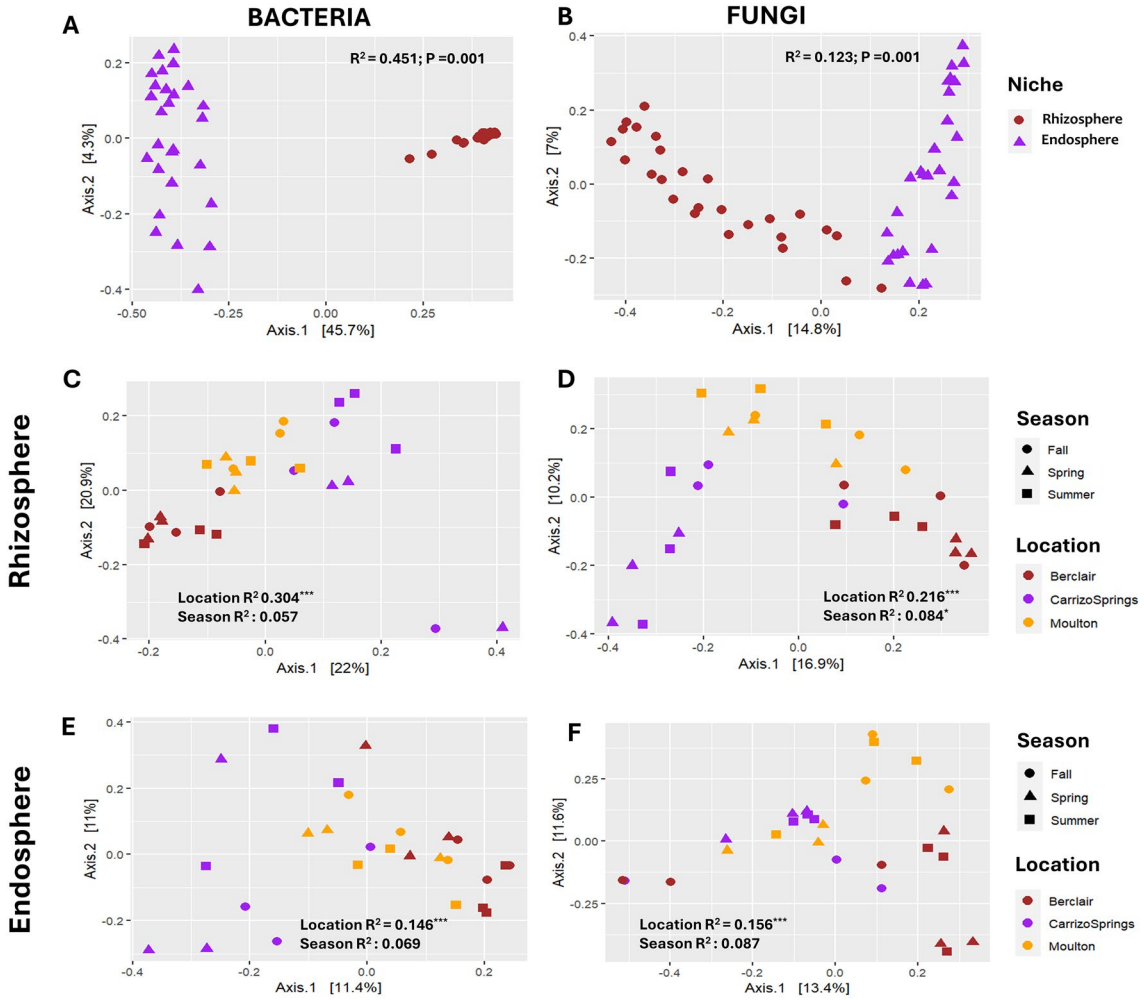

Figure 5. Beta diversity analyses for bacterial (**A**,**C**,**E**) and fungal (**B**,**D**,**F**) communities across locations and seasons. Beta diversity is shown as Principal coordinate analysis (PCoA) based on Bray-Curti's dissimilarity measures. Significance was tested using PERMANOVA. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

Numerous environmental and host-related factors, such as geographic location, plant genotype and phenotype, soil chemistry, and seasonal influences, will likely impact the microbial communities linked to plant hosts[31]. Our results showed a higher number of rhizospheres OTUs and, instead, a small number of roots endosphere OTUs for bacteria and mycobiota (Supplementary Fig. S4). This result aligns with other studies where the microbial communities from the olive root endosphere are less diverse than those from the rhizosphere[20]. The number of shared and unique OTUs suggests that rhizosphere samples contained most OTUs in bacterial and fungal datasets, confirming that rhizosphere soil is a primary reservoir for potential root endophytes[32].

The rhizosphere strongly influences the richness and diversity of microbial communitie

Overall, Alpha diversity analysis revealed that compared to roots, the rhizosphere soil had a significantly higher level of microbial diversity, presumably because the root endosphere tends to create an inner environment that is relatively stable, resulting in fewer changes in the microbial community within the plant. Locations specifically affected the rhizosphere bacterial abundance and fungal richness but did not impact community composition in the root endosphere. Moulton and Berclair demonstrated the highest diversity in community composition for bacterial evenness and fungal richness. Recent studies in several experimental systems have found that fungal communities are more spatially differentiated than prokaryotic communities[33–35], suggesting that fungal endemism may shape communities at multiple scales and habitats.

Contrary to other research findings, which indicated that the alpha diversity of the rhizo-biome increased between seasons[36], the seasons increased both richness and evenness for bacteria in the root endosphere. The greater alpha diversity in summer and community compositional differences between spring and summer suggest that root-associated microbiota alterations are linked to plant phenological processes. In addition to indirectly affecting microbes, climatic conditions also influence the rate of photosynthesis and, consequently, the rate of rhizodeposition, as demonstrated for trees and perennial plants[37]. Thus, seasonal changes in bacterial communities are most likely influenced by an enhanced carbon flux from increasing temperatures[38]. Together, these findings imply that the temporal dynamics in the root-associated microbiota are affected by plant phenology and

abiotic factors like weather, which may have an immediate impact on the microbiota or may have an indirect effect by altering plant physiological processes. However, seasons did not affect fungal alpha diversity in either compartment. Further research is needed to determine if these disparities are due to nutrient availability in different soils and sample types (rhizosphere vs. root endosphere) or the trophic characteristics of the bacterial and fungal species.

Geographic location influences the beta diversity of microbial communities

In the present study, the beta-diversity analysis revealed that rhizospheric compartments and geographical location were the primary drivers of the compositional variations of microbial communities. Comparing the rhizosphere to the endosphere, we observed a greater diversity of fungi in the rhizosphere. Variations in bacterial composition were nearly the same. Because of this, as previously described in[39], our observations further imply that endophytic root colonization is not a passive process and that olive plants can choose from soil microbial consortia. Mature olive trees, like the one studied in this study, may have established endophytic microbiomes with mutualistic links to their hosts, resulting in less diversity[40]. A distinct clustering was seen amongst sample niches due to their geographical locations, indicating that the microbial populations that occupy olive tree niches differ spatially. Intriguingly, the rhizosphere samples from Carrizo Springs are the most dispersed in the ordination plot, indicating that the rhizospheres of these plants exhibit extremely varied composition and structure, even if the root endosphere of this location formed a distinct cluster. The overlapping clusters of the microbial communities from Moulton and Berclair, which are geographically closer (135 km apart), were generally more similar than those from distantly located Carrizo Springs (which is 341 and 285 km from Moulton and Berclair, respectively). As seasons did not affect microbiome variation in our study, variations in soil attributes, such as physicochemical composition, soil conductivity, and pH, could likely have a prominent role[41,42].

Distinct microbial diversity associated with rhizocompartments across locations and seasons

Microbial populations respond differently at all taxonomic levels, forming distinct soil microbial communities in soils with varying physicochemical characteristics[43]. In our study, *Actinobacteriota* and *Proteobacteria* dominated the rhizospheric and root endosphere bacterial community, consistent with previous studies[20,40,44] across all locations. *Actinobacteriota*, the most abundant phylum in soil, produces extracellular enzymes, secondary metabolites (e.g., antimicrobial agents), and fast-degrading low-biodegradable organic compounds like hydrocarbons, lignin, and humus[45,46]. *Proteobacteria* live in nutrient-rich soils and mineralize many soil nutrients[47]. Our study observed *Proteobacteria* during summer, as in *Agave* species during the dry season[48].

Our data derived from rhizospheric soil and root microbiome are consistent with the study[20] that examined root endophytic core microbiome in olive varieties and found *Actinophytocola, Pseudonocardia, Bradyrhizobium* to be essential for plant fitness. An abundance of *Actinophytocola* could benefit olives, while *Rubrobacter* colonizes drought environments and metabolizes pesticides and pollutants. The prevalence of *Actinobacteriota* in our study (*Rubrobacter*, *Actinophytocola*, *Pseudonocardia*, unidentified *Micromonosopraceae*) in the root endophytic community suggests the possibility of isolating culturable representatives of these genera for their potential use as plant growth promotion (PGP) and biological control against olive tree pathogens. The rhizosphere of Berclair and Carrizo Springs and root endosphere of olives grown in Moulton were abundant in Phylum *Firmicutes*. Members of the genus *Bacillus*, well-known antagonists, and biocontrol agents are also the major components of the olive tree endosphere microbiota[49]. Synthesis of antimicrobial lipopeptide biosurfactants enabled this genus to be approved as a plant disease biocontrol agent[50]. No sequence reads from the kingdom *Archaea* were found in our investigation in the root endosphere, as observed in the other studies[20]. However, the rhizosphere soil was enriched with *Creanarcheota* (*Thermoproteota*). Members of this species are keystone members of agricultural soil communities due to their ability to promote the nitrogen cycle, fix carbon dioxide, and possess genes linked to plant growth promotion (PGP), suggesting their importance in these microbiomes[51]. The greater abundance of soil ammonia-oxidizing archaea *Candidatus_Nitrososphaeria* in the rhizospheric soil of Berclair in the spring and fall seasons was an intriguing finding. A thaumarchaeal candidate genus *Nitrososphaera* as a core microbiome member was also identified in the endosphere of olive varieties[49] and associated soil[52].

Regarding fungi composition, both rhizocompartments have *Ascomycota* and *Basidiomycota* as the major fungi, especially in Moulton and Berclair, which is consistent with earlier research on the endophytic and rhizospheric communities of olive trees[20,40]. Additionally, *Glomeromycota* was more prevalent in the root endosphere of Carrizo Springs. *Glomeromycota* is a monophyletic group of olive tree-dominant arbuscular mycorrhizal fungus (AMF)[53]. The AMF genera *Rhizophagus, Glomus, and Gigaspora* are known to improve host plant health by activating defense mechanisms against soilborne pathogens like *Phytophthora, Fusarium, and Verticillium*. Berclair soil was enriched with Members of phyla *Mortierellomycota*, known to solubilize soil phosphorus and enhance available phosphorus[54]. The rhizosphere soils of all locations were enriched with *Chaetomiaceae* members, while the root endospheres were abundant in *Glomeraceae*, unidentified members of *Agaricales* and *Xylariales*. While there were reports on bioactive metabolites produced by *Xylariales*[55], *Chaetomiaceae* members may play a part in defensive mutualism[56]. The presence of members of the *Agaricaceae* family, as well as unidentified *Agaricales* and *Auricualriales* that belong to both *Basidiomycota*, is in line with studies that have found numerous *Agaricomycetes* acting as saprophytes, mutualists, and plant endophytes[57].

This study also found seasonal variations in the rhizosphere and root endosphere fungal endophytes. Additionally, the fungal composition changed from late spring to fall. The most abundant endophytes, for example, were *Xylariales*, accounting for 33% of all isolates. Its relative abundance dropped to 11% by summer and 4% by fall. Many fungal species are predicted to colonize olive trees during the warm, humid spring and early summer months. Specific endophytes can eventually establish themselves, while others may decrease or disappear from the community, which could account for decreased endophytic fungal diversity from late spring to autumn[23].

However, throughout niches, the relative abundance of *Agaricaceae* and unidentified *Agaricales* members had significantly increased (up to 10% and 26%, respectively) in the summer and fall. The fungi community undergoes annual seasonal changes due to a successional process, which could be the cause of the observed variations[58]. In addition, it has been suggested that the recruitment of endophytic fungi may also occur due to interspecific competition among the fungi and alterations in the chemistry of plant tissues during the phenological growth stages of the tree[59].

Enrichment of specific microbial taxa in the rhizocompartments

Through LeFse analysis, we identified distinct microbial taxa, such as Genus *Sphingomonas*, which produces phytohormones and bioremediation, abundant in Carrizo Springs[60]. *Niastella* have been described as metal-tolerant and chitosan-hydrolyzing[61] in the root endosphere, whereas members of unidentified *Streptosporangiales* enriched in Carrizo Springs and during summer and fall seasons are known to produce antimicrobial compounds[27]. Regarding fungal biomarkers, rhizosphere-enriched ectomycorrhizal *Wilcoxina* and halotolerant *Humicola* species have demonstrated antifungal, antibacterial, and antiproliferative activities[62]. *Hypocreales* and *Acrophialophora* were unique biomarkers in Moulton and in Summer. Root endophytic *Hypocreales* decompose straw residue in arable soils and support plant growth[63], while *Acrophialophora* and *Malassezia* have been linked to alleviate drought stress and plant ectoparasitic defense[64,65], *Serendipitaceae* in Berclair, *spp*. can form a mutualistic symbiosis with crops and supply nutrients and water to the host crop[66].

The present investigation provides a detailed characterization of the microbiome composition in the rhizosphere and root endosphere of the olive cultivar Arbequina. Rhizosphere microbial communities, characterized by their substantial richness and diversity, are more strongly associated with specific locations than endosphere communities. Furthermore, the communities that exhibit higher abundance in the rhizosphere and endosphere may benefit plant growth and overall health. In conclusion, the presence of rhizocompartment and variations in geographic locations significantly impacted microbial populations across different geographical regions and seasons, with minimal influence observed from seasonal variations. Our findings highlight the need to consider the resident microbial population, soil environment, seasons, and plant genotypes in future microbiome research. However, studying the microbiome in all plant compartments is necessary to provide the complete context of the complexity of interactions between the host plant and microorganisms. Further, by employing shotgun metagenomics and microbiome-driven isolation techniques to identify members of persistent common taxa, one can elucidate the functional potential of the microbiome associated with olive trees. This fundamental knowledge establishes the basis for further investigation, which will utilize possible microbial consortia to conduct synthetic, in vitro community-based evaluation of this assembly process and the functional roles of the olive-associated microbes.

Materials and methods

Sample collection

Soil and root samples were collected from the olive groves located at three geographically distinct locations in Texas: Carrizo Springs/CZ (28° 31′ 27.5556” N, 99° 51′ 30.7836″ W), Moulton/M (29° 34′ 27.948″ N, 97° 8′ 48.444″ W), and Berclair/B (28° 31′ 31.404″ N, 97° 35′ 7.332″ W). ’Arbequina,’ the most grown variety in the United States, was selected for the study. The average age of trees was between 6 and 8 years old at each grove. The soil and root samples were collected in triplicates 4–7 inches deep within one meter from the trunk of the independent trees. The soil top layer (3 inches) was discarded for the rhizosphere soil sample collection. The soil rigidly attached to the roots was collected for rhizosphere analysis. Root samples were collected from the same samples to analyze root-associated microbial communities. The samples were collected during Spring/Sp (Mar–May), Summer/Su (June–Aug), and Fall/F (Sep–Nov) from each olive grove and transported to the Texas A&M AgriLife Research Center, Uvalde, Texas, in 2021 and stored at – 80 °C until processing (Table 1).

Total DNA extraction

DNA from each soil and root sample was extracted using the PowerSoil® DNA Isolation Kits (MO BIO Laboratories, Carlsbad, CA, USA) and ZymoBIOMICS DNA Kit (Zymo Research, Irvine, CA, USA), following the manufacturer’s recommendations. For the rhizosphere soil, samples were washed in phosphate-buffered saline (PBS) solution for 20 min, centrifuged at 2000 × g for 5 min, and the remaining soil pellet was frozen in liquid nitrogen and stored at – 80 °C. For the root endosphere DNA collection, after removal of adhering soil by shaking vigorously, roots were washed twice in PBS by shaking in 250 ml sterile flasks with 50 ml PBS for 20 min, sonicated (10 min of 25-s cycles at 3500 Hz), rinsed with sterile distilled water, flash-frozen in liquid nitrogen, and stored at – 80 °C until extraction.

Sequencing, filtering of reads, and assembly

The soil and root DNA samples were sequenced by the Novogene Corporation (USA) for microbial communities. In the case of rhizosphere soil for bacterial 16S analysis, the V3–V4 region was amplified using primers with barcodes 341F 5′-CCTACGGGAGGCAGCAG-3′ and 806 R 5′-GGACTACHVGGGTWTCTAAT-3′ and fungal ITS1 gene region was amplified using the ITS5-1737F (5′-GGAAGTAAAAGTCGTAACAAGG-3′) /ITS2-2043R (5′-GCTGCGTTCTTCATCGATGC-3′) primers. In the case of endosphere root samples, for bacterial 16S analysis, the V5-V7 region was amplified using barcoded primers 799F 5′-AACMGGATTAGATACCCKG-3′ and 1193R 5′-ACGTCATCCCCACCTTCC-3′ and fungal ITS1 gene region was amplified using the ITS1F-F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) /ITS1-1F-R (5′-GCTGCGTTCTTCATCGATGC-3′) primers. Unlike most studies, to minimize the plant-associated contamination of mitochondrial and chloroplast sequences, we have deliberately opted to use different pairs of primers based on the pre-validated analysis in olives that resulted in

Sample type	Season	Location	Abbreviated sample names	Description
Rhizosphere soil	Spring	Carrizo Springs	RPC1-1, RPC2-2, RPC3-3	Rhizosphere soil from Spring in Carrizo Springs
		Moulton	RPM1-19, RPM2-20, RPM3-21	Rhizosphere soil from Spring in Moulton
		Berclair	RPB1-37, RPB2-38, RPB3-39	Rhizosphere soil from Spring in Berclair
	Summer	Carrizo Springs	RUC1-4, RUC2-5, RUC3-6	Rhizosphere soil in Summer in Carrizo Springs
		Moulton	RUM1-22, RUM2-23, RUM3-24	Rhizosphere soil from Summer in Moulton
		Berclair	RUB1-40, RUB2-41, RUB3-42	Rhizosphere soil from Summer in Berclair
	Fall	Carrizo Springs	RFC1-7, RFC2-8, RFC3-9	Rhizosphere soil from Fall in Carrizo Springs
		Moulton	RFM1-25, RFM2-26, RFM3-27	Rhizosphere soil from Fall in Moulton
		Berclair	RFB1-43, RFB2-44, RFB3-45	Rhizosphere soil from Fall in Berclair
Root endosphere	Spring	Carrizo Springs	EPC1-10, RPC2-11, RPC3-12	Root endosphere from Spring in Carrizo Springs
		Moulton	EPM1-28, EPM2-29, EPM3-30	Root endosphere from Spring in Moulton
		Berclair	EPB1-46, EPB2-47, EPB3-48	Root endosphere from Spring in Berclair
	Summer	Carrizo Springs	EUC1-13, RPC2-14, RPC3-15	Root endosphere from Summer in Carrizo Springs
		Moulton	EUM1-31, EUM2-32, EUM3-33	Root endosphere from Summer in Moulton
		Berclair	EUB1-49, EUB2-50, EUB3-51	Root endosphere from Summer in Berclair
	Fall	Carrizo Springs	EFC1-16, RPC2-17, RPC3-18	Root endosphere from Fall in Carrizo Springs
		Moulton	EFM1-34, EFM2-35, EFM3-36	Root endosphere from Fall in Moulton
		Berclair	EFB1-52, EFB2-53, EFB3-54	Root endosphere from Fall in Berclair

Table 1. Sample designations for seasons and geographical locations.

higher capture of endospheric communities[67,68]. The primers 799F/1193R showed the lowest mitochondria 16S rRNA amplification, no chloroplast sequences, and the highest numbers of bacterial OTUs[68]. Even if different primer sets for plant vs. soil microbiomes have been shown to provide similar results for simultaneous studies on plant and soil microbiomes[69], authors comprehend the likely bias introduced while comparing the microbiomes across compartments due to the choice of different primers and suggest readers to restrict the taxonomic interpretations in the appropriate context and within specific rhizocompartment.

Despite the advantages, we agree that some differences in microbial communities could have resulted from the biased primer pairs selected. The PCR products from each sample were pooled, end-repaired, A-tailed, and further ligated with Illumina adapters. Libraries were sequenced on a paired-end Illumina platform to generate 250 bp paired-end raw reads. The library quality was checked with Qubit, real-time PCR for quantification, and bioanalyzer for size distribution detection. Quantified libraries were pooled and sequenced on Illumina platforms according to the required effective library concentration and data amount. Paired-end reads were merged using FLASH (V1.2.7)[70], and the splicing sequences were called raw tags. Quality filtering on the raw tags was performed to obtain the high-quality clean tags[71] according to the QIIME (V1.7.0) quality control process. The tags were compared with the reference database (SILVA138 and Unite V8.2 database) using the UCHIME algorithm[72]. Effective tags were obtained after the removal of chimeric sequences[73].

Operational taxonomy unit (OTU) cluster, taxonomic annotation, and diversity analysis

Sequence analyses were performed by Uparse software (Uparse v7.0.10)[72] using all the effective tags. Sequences with ≥ 97% similarity were assigned to the same OTUs, and a representative sequence for each OTU was used for further annotation. For each representative sequence, QIIME (Version 1.7.0) and the Mothur method were performed against the SSUrRNA database of SILVA 138 with a threshold set to 0.8–1[74], blast with BLASTALL (Version 2.2.25) and Unite V8.2 database[75] for species annotation at each taxonomic rank (kingdom, phylum, class, order, family, genus, and species). Statistical analysis and visualization of graphs were conducted in R studio v. 2023-06-16[76,77] unless stated otherwise. The microbial community analysis was carried out using the phyloseq R package, with the OTU tables and taxonomic classifications serving as the input dataset[78]. The dataset was rarefied by randomly selecting sequences with low read counts. Using "ggrare" from the "ranacapa" package, the rarefaction curves on species richness were computed[79]. The taxonomic composition was shown using plot bars. Using the rarefied dataset, the Kruskal Wallis chi-squared test was performed to assess changes in alpha diversity according to the Shannon diversity (H'), Inverse Simpson (1/D) metrics, followed by post-hoc Dunn's testing for multiple pairwise comparisons at $P < 0.05$. To check for variations in community structure between sample groups, Bray-Curti's dissimilarity-based permutational analysis of variance (PERMANOVA, 999 permutations) along with the Adonis test was employed to evaluate the effect of factors (niches, locations, and seasons) on microbial composition. Principal coordinate analysis (PCoA), non-metric multidimensional scaling (NMDS), and dendrograms were used to visualize and compare microbial community structure between sample groups based on the Bray–Curtis dissimilarity matrix. The linear discriminant analysis (LDA) effect size (LEfSe) method implemented in MicrobiomeAnalyst[80] was employed to discern distinct biomarkers of bacteria and fungi underlying the observed microbiome differences between the locations and seasons. A threshold LDA score of 2 and a significant α of 0.05 were applied to each feature to calculate its effect size.

Sequence accession numbers

The sequence data generated in this study are deposited in the National Center for Biotechnology Information (NCBI) under the BioProject PRJNA1032045 (root endospheric) and PRJNA1031998 (soil rhizosphere) for bacterial and PRJNA1032109 (soil rhizosphere), and PRJNA1032141(root endospheric) fungal microbiomes.

Data availability

The bacterial and fungal microbiome sequence data generated in this study are deposited in the National Center for Biotechnology Information (NCBI) under the BioProject numbers PRJNA1032045, PRJNA1031998, PRJNA1032109, and PRJNA1032141.

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Author contributions

V.J. conceived and designed the study. D.T., M.J., and A.M. conducted the experiments and collected the data. D.T. performed the statistical analysis. D.T. and V.J. drafted the manuscript. All authors approved the final version of the manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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