applied sciences

MDPI

Article

Characterization of Core Microbiomes of Olive Tree Rhizospheres Under Drought Stress Conditions

Andrea Visca [1,*], Lorenzo Nolfi [1,2], Luciana Di Gregorio [1], Manuela Costanzo [1], Elisa Clagnan [1,3], Filippo Sevi [1], Federico Sbarra [1,4], Roberta Bernini [2], Maria Cristina Valeri [5], Edoardo Franco [5], Ornella Calderini [5], Luciana Baldoni [5], Gaetano Perrotta [1,*] and Annamaria Bevivino [1]

1 Department for Sustainability, ENEA, Italian National Agency for New Technologies, Energy and Sustainable Economic Development, Casaccia Research Center, Via Anguillarese 301, 00123 Rome, Italy; lorenzo.nolfi@unitus.it (L.N.); luciana.digregorio@enea.it (L.D.G.); manuela.costanzo@enea.it (M.C.); elisa.clagnan@unimi.it (E.C.); filippo.sevi@enea.it (F.S.); federico.sbarra@unito.it (F.S.); annamaria.bevivino@enea.it (A.B.)
2 Department of Agriculture and Forest Sciences, University of Tuscia, 01100 Viterbo, Italy; roberta.bernini@unitus.it
3 Gruppo Ricicla Labs., Dipartimento di Scienze Agrarie e Ambientali-Produzione, Territorio, Agroenergia (DiSAA), Università degli Studi di Milano, Via Celoria 2, 20133 Milan, Italy
4 Department of Life Sciences and Systems Biology (DBIOS), University of Turin, Via Verdi 8, 10124 Turin, Italy
5 Institute of Biosciences and Bioresources, CNR, Via Madonna Alta, 130, 06128 Perugia, Italy; mariacristina.valeri@ibbr.cnr.it (M.C.V.); edoardo.franco@ibbr.cnr.it (E.F.); ornella.calderini@cnr.it (O.C.); luciana.baldoni@cnr.it (L.B.)
* Correspondence: andrea.visca@enea.it (A.V.); gaetano.perrotta@enea.it (G.P.)

Featured Application

The findings of this study can be applied to develop microbiome-based strategies for enhancing drought resilience in olive trees and other Mediterranean crops. By identifying core microbial communities associated with plant roots and rhizospheres under water-limited conditions, this work lays the foundation for sustainable agricultural practices aimed at mitigating the impacts of climate change on traditional cultivars.

Abstract

Drought stress poses a significant threat to olive cultivation in Mediterranean regions. This study investigated the resilience and functional adaptation of root-associated and rhizosphere soil microorganisms of four olive cultivars under contrasting water regimes (irrigated vs. drought) across seasons. Using a combination of amplicon-targeted metagenomics, phylogenetic analysis, and text mining of the scientific literature, we identified a conserved core microbiome and revealed that drought stress significantly alters the structure of root-associated—but not rhizosphere soil—bacterial communities. Potential functional profiling indicated that drought conditions enriched for genes involved in stress response pathways, including branched-chain amino acid transport, glutathione S-transferase activity, thioredoxin reductase, and chemotaxis. Text mining co-occurrence networks highlighted strong associations between some key bacterial genera and plant growth-promoting functions like phytohormone production and biocontrol. Furthermore, we identified *Solirubrobacter*, *Microvirga*, and *Pseudonocardia* as the primary contributors to these drought-resilience functions. The stability of the soil microbiome suggests functional redundancy, whereas the restructuring of the root endophytic compartment indicates active plant selection for beneficial microbes. Our findings provide a foundation for developing tailored microbial consortia (SynComs) to enhance drought tolerance in olive trees and support sustainable agriculture in water-limited environments.

check for updates

Academic Editors: Pantelis E. Barouchas and Flynn Trevan

Received: 29 July 2025
Revised: 29 August 2025
Accepted: 30 August 2025
Published: 2 September 2025

Citation: Visca, A.; Nolfi, L.; Di Gregorio, L.; Costanzo, M.; Clagnan, E.; Sevi, F.; Sbarra, F.; Bernini, R.; Valeri, M.C.; Franco, E.; et al. Characterization of Core Microbiomes of Olive Tree Rhizospheres Under Drought Stress Conditions. *Appl. Sci.* **2025**, *15*, 9667. https://doi.org/10.3390/app15179667

Keywords: bacteria; roots; SynComs; functional redundancy; long reads; text mining

1. Introduction

The root–rhizosphere interface represents a crucial zone of interaction between plants and microorganisms, where many processes essential for plant health and development take place [1]. This interface plays a pivotal role in enhancing the uptake of nutrients and water by plants and is increasingly recognized as a strategic target for microbiome-based interventions in agriculture [2]. By modulating the microbial communities associated with plant roots, it is possible to improve nutrient acquisition and bolster resistance to both biotic and abiotic stressors [3]. As a result, understanding the dynamics of these interactions is becoming a priority for developing sustainable agricultural practices.

In recent years, growing attention has been placed on identifying specific microbial taxa or functional groups within the rhizosphere that can be leveraged to enhance crop performance [4]. However, the complexity and diversity of microbial communities, along with the variability between different plant species and environmental conditions, present significant challenges [5]. One approach to narrowing the search for beneficial microorganisms is to define and characterize the so-called "core microbiome", the subset of microbial taxa that are consistently found across different samples within a given habitat [6]. These core members are thought to play a more central role in influencing soil processes and in shaping the growth, health, and resilience of their host plants [7].

Nonetheless, identifying the core microbiome solely on the basis of taxonomic presence may offer only a partial picture [4]. Rhizosphere microbial diversity is known to vary widely in response to soil properties (e.g., pH, texture, and organic matter content), plant genotype, and broader environmental factors such as temperature, moisture, and nutrient availability [8]. Increasingly, research suggests that it is the functional traits of microorganisms that primarily determine their recruitment and persistence in the rhizosphere, whereas taxonomic classification provides a descriptive framework rather than a causal explanation of microbial assembly [9].

In terrestrial environments, microbial and plant life must frequently adapt to fluctuations in water availability, which occur daily and seasonally [10,11]. Because plants are sessile during their lifecycle, they have evolved multiple physiological and morphological strategies to cope with water limitation, including alterations in root system architecture and stomatal closure [12]; at the same time, dispersal via seeds or other diaspores represents an additional adaptive mechanism to overcome spatial and temporal environmental variability. Recent findings also highlight the importance of microbial partners in helping plants manage drought conditions [13]. Drought stress is known to significantly reshape the composition and functional potential of the root-associated microbiome, and these changes can, in turn, influence plant responses to stress [14–16].

With the frequency and severity of drought events expected to increase in the future, it is essential to develop innovative, microbiome-based solutions that can be quickly applied in agricultural settings [17]. A deeper understanding of the interactions between plants and their microbiomes during drought episodes will be fundamental to enhancing crop resilience.

In this context, this study aimed to explore how drought stress affects the composition and structure of the rhizosphere bacterial community. Specifically, the objectives were to (i) compare the microbial communities associated with different olive tree cultivars across contrasting water regimes (drought vs. irrigation); (ii) identify the bacterial taxa consistently associated with olive roots and rhizospheres (core microbiome) and distinguish

those taxa that are specifically enriched under drought conditions (responsive taxa); (iii) investigate the functional attributes of the core and responsive microbes, with a focus on traits potentially linked to drought adaptation; and (iv) perform a literature-based co-occurrence analysis to contextualize the ecological functions of core microbial taxa. The insights gained from this research offer a valuable basis for developing microbial applications that can support plant adaptation to drought and promote more sustainable agricultural practices.

2. Materials and Methods

2.1. Seasonal Study

This study was carried out in olive groves located in Central Italy near Perugia (Boneggio site: 43°03′26.0″ N 12°21′54.4″ E; Lugnano site: 42°32′24.1″ N 12°19′01.1″ E). Four different olive cultivars were included in the sampling: Arbequina, Koroneiki, Chemlal de Kabilye (Chemlal in the text), and Shengeh, originating from Spain, Greece, Algeria, and Iran, respectively. The first two cultivars were reported as more drought-susceptible than the latter two [18,19]. Plants were eight years old at the sampling dates and were raised without any pruning in order to avoid trauma to branches and root systems. Average air temperatures at 2 m above ground level, measured by the regional agrometeorological network and expressed as monthly means, ranged from 3.23 °C (minimum) to 10.55 °C (maximum) in January, up to 20.81 °C (minimum) to 33.90 °C (maximum) in July. Two contrasting water regimes were adopted: dry, with only the contribution of natural rainfall, where an average annual rainfall of 692.2 mm over the last five years was reported—with 56.6 mm, 50 mm, 38.1 mm, and 75 mm as monthly precipitation along the winter, spring, summer, and autumn seasons, respectively (Figure S1); and irrigated (wet), supplying approximately 3.6 m^3 of water per plant by subsurface drip irrigation during the summer dry season, equivalent to ~180 mm of water depth when normalized to the average root zone area (20 m^2 per plant), which allows for direct comparison with the precipitation data. Soil was managed with natural vegetation cover, periodically cut during the dry season. Plant volume, canopy density, and production, which were evaluated separately, displayed similar results for both dry and irrigated treatments, with a slight alternating production in previous years in the case of dry plants. Soils at both orchards were classified using the WRB (IUSS Working Group WRB, 2022) and USDA Soil Taxonomy based on EU-scale soil maps (SoilGrids v2.0; ESDAC). At the Boneggio site (43°03′26.0″ N, 12°21′54.4″ E), soils are dominated by Cambisols (WRB; commonly calcaric) corresponding to Inceptisols (USDA). At the Lugnano site (42°32′24.1″ N, 12°19′01.1″ E), soils are predominantly Luvisols/Cambisols (WRB; often chromic/calcaric), corresponding to Alfisols/Inceptisols (USDA). These assignments reflect regional mapping and are consistent with the hilly calcareous landscapes of central Umbria; site-specific horizon descriptions were not collected. Their main physico-chemical properties are reported in Table S1. Parameters included texture (sand, silt, clay percentages), pH, organic matter, electrical conductivity, total nitrogen, assimilable phosphorus, cation exchange capacity (CEC), and exchangeable cations (Ca, Mg, K).

2.2. Sampling and DNA Extraction from Soil- and Root-Associated Microorganisms

The sampling of rhizospheric soil and roots was performed both in summer and winter seasons, under both dry and irrigated conditions. For each olive cultivar × treatment × season combination, 3 independent trees were sampled, resulting in a total of 24 trees (4 cultivars × 2 treatments × 2 seasons × 3 replicates). Each root and rhizospheric soil sample was obtained from four collection points per plant, taken from four opposite sides, approximately 50–70 cm from the trunk, exploring the layer at 20–30 cm depth

after removing the top soil. This design ensured biological replication and accounted for within-orchard variability. No a priori power analysis was performed; replication levels were chosen to match standard practices in rhizosphere microbial ecology, where three biological replicates per factor combination are considered sufficient to capture community-level trends.

Samples for DNA analysis were frozen in liquid nitrogen, then shipped to the ENEA laboratories in dry ice, where they were stored at −80 °C until analysis. Rhizosphere samples were first homogenized and then 0.25 g of soil was taken for DNA extraction using the DNAeasy PowerSoil Pro kit (Qiagen Inc., Venlo, The Netherlands). Root samples were first washed three times with sterile H_2O, lyophilized, homogenized, and finally processed for DNA extraction, following the same procedure described for rhizosphere samples. Extracted DNA was quantified with a Nanodrop spectrophotometer and Qubit (Thermo Fisher Scientific ©, Waltham, MA, USA), to assess DNA quantity (ng/mL) and quality (260/280 and 260/230 ratios). Moreover, DNA samples were also run on 1% agarose gel to assess fragmentation. DNA extractions were carried out in triplicate.

2.3. Library Construction and Sequencing

Library construction was carried out using PCR amplification performed on 25 ng of DNA from each sample. The broad-spectrum profiling of the bacterial community was achieved using universal primers 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-TACGGYTACCTTGTTACGACTT-3′). Amplification was conducted with Taq polymerase (Bioline, Meridian Bioscience, London, UK), and the PCR products were verified by gel electrophoresis on a 1% agarose gel. Each assay was run in triplicate, including the no-template controls. Any possible PCR inhibition was assessed by conducting an inhibition test using samples diluted from 10- to 100-fold, and no inhibition was observed. The PCR amplicons for each sample were purified using AMPure beads, and approximately 45 ng of purified product was utilized for library preparation. Libraries were constructed using the Rapid Barcoding Kit 24 (SQK-RBK 114.24), according to the manufacturer's instructions (Oxford Nanopore Technologies, Oxford, UK). Sequencing was performed on a Flongle Flow Cell (R10.4.1) using the Mk1B device (Oxford Nanopore Technologies, UK) overnight. An overall sequencing depth of 50× was achieved for each sample. Basecalling and demultiplexing were carried out using MinKNOW software (v23.08.9) integrated with Dorado software (v0.5.2). The raw sequences are deposited in the repository PRJNA1194004. Detailed protocols for sequencing and library preparation are available through the official channels of the supplier (Oxford Nanopore).

2.4. Bioinformatic Analysis for Taxonomy Classification and Functional Annotation

The raw reads obtained from the sequencing were basecalled with Dorado v0.5.2. The raw fastq files were filtered using "cutadapt" v4.4 and classified with Emu (https://gitlab.com/treangenlab/emu; accessed on 1 August 2025) using the SILVA database (version 138.1). A custom workflow was used to format the Emu output for the functional annotation. Briefly, the R library "tidyverse" was used to manipulate the Emu output and create an OTU-like table. A consensus sequence was created by aligning all the sequences with the same tax_id with Clustal Omega v1.2.4 and then creating a consensus sequence from the multiple alignments using EMBOSS v6.6.0.0. Both the OTU-like table and the consensus sequences were used for functional prediction. The software Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2, Version 2.4.2; https://github.com/picrust/picrust2; accessed on 1 August 2025)—which can predict the function of bacterial communities according to the proportion of marker gene sequences in samples—was used to infer approximate functional potential of microbial communities

using default parameters, including a standard NSTI (Nearest Sequenced Taxon Index) cutoff of 2.0.

2.5. Data Mining

To study the interactions between selected bacterial genera and functions associated with drought resistance and plant growth promotion (PGP), a text mining analysis of the scientific literature was conducted. A Python script in the Jupiter Lab environment (version 4.0.11) was developed using specialized libraries to perform a systematic search through the Scopus database using Unpaywall APIs [20] for the download of research papers, following predefined guidelines and the APIs' limitations. Two separate searches were performed on soil and endophytes, respectively. The search queries used were as follows:

1. "TITLE-ABS-KEY (microbiome or microbes or bacteria) AND (drought or "water stress") AND (soil or rhizosphere) AND olive";
2. "TITLE-ABS-KEY (microbiome or microbes or bacteria) AND (drought or "water stress") AND (endophyte*) AND olive".

Two manually curated keyword lists were prepared: the first containing the bacterial genera of interest [21,22], and the second including terms related to key ecological functions [23]. The script analyzed the full texts of the resulting articles and calculated the co-occurrence frequencies between terms in the two lists. The data obtained were structured into a co-occurrence matrix, where for each pair of terms, a value representing the number of times they appeared together in the scientific literature was assigned. This matrix was imported into Gephi software (version 0.10.1) for visualization and analysis of co-occurrence networks [24,25].

2.6. Statistical Analysis

Statistical analyses of the microbial community structure and function data were performed in the R environment (v 4.3.3). Specifically, the plots were created using the *ggplot2* package (https://ggplot2.tidyverse.org/; accessed on 1 August 2025), while PERMANOVA (Permutational Multivariate Analysis of Variance) on community structure data was conducted using the adonis() function in the vegan package (https://vegandevs.github.io/vegan/; accessed on 1 August 2025), based on Bray–Curtis dissimilarities. Non-metric Multidimensional Scaling (NMDS) was performed using the metaMDS() function in vegan to ordinate samples in a reduced dimensional space based on Bray–Curtis dissimilarities. A single NMDS ordination including all samples was calculated to ensure comparability, with treatments (season, irrigation, cultivar) distinguished by graphical coding (colors, shapes). Stress values were reported to evaluate the goodness-of-fit. Functional analysis, to assess the differential expression of microbial community functions under varying environmental conditions—with a specific focus on identifying functions potentially linked to drought resistance—was carried out using the *Deseq2* package [26]. For clarity in visualization, functional categories in heatmaps were abbreviated (F01–F30), with the corresponding full descriptions provided in Table S2. The overlap of microbial taxa among treatments was illustrated using Venn diagrams generated with the Venndiagram package [27]. These diagrams are used solely for visualization and were based on results from variance partitioning performed with the varpart() function in vegan, which decomposes community variation attributable to season, irrigation, and cultivar effects. To identify the most relevant functional contributors (KOs), first, the mean abundance of each KO across water-level conditions has been calculated. Statistical testing was then performed for each KO to assess differences between water conditions. Specifically, a two-sided *t*-test (for two water levels; in the case of more conditions, an ANOVA could be used) was applied. KOs with a *p*-value < 0.05 were considered statistically significant. From this subset of significant KOs, which

were ranked by mean abundance across samples, the top 30 were selected (Table 1) to highlight those contributing most strongly to the functional profile. This approach ensured that only taxa showing both statistical significance and ecological relevance (in terms of relative abundance) were reported as "top contributors."

Table 1. Top contributors to functions related to drought resistance.

KO	Genus	Description
K00384	*Microvirga*	trxB, TRR; thioredoxin reductase (NADPH) [EC:1.8.1.9]
K00799	*Microvirga*	GST, gst; glutathione S-transferase [EC:2.5.1.18]
K01897	*Pseudonocardia*	ACSL, fadD; long-chain acyl-CoA synthetase [EC:6.2.1.3]
K01997	*Microvirga*	livH; branched-chain amino acid transport system permease protein
K01998	*Solirubrobacter*	livM; branched-chain amino acid transport system permease protein
K01999	*Solirubrobacter*	livK; branched-chain amino acid transport system substrate-binding protein
K03406	*Microvirga*	mcp; methyl-accepting chemotaxis protein
K03654	*Solirubrobacter*	recQ; ATP-dependent DNA helicase RecQ [EC:3.6.4.12]
K03704	*Pseudonocardia*	cspA; cold shock protein (beta-ribbon, CspA family)

3. Results

3.1. The Literature-Based Co-Occurrence Analysis of Drought-Related Microbes and Functions

The literature search retrieved 689 soil-related articles and 349 endophyte-related articles. These corpora were processed using co-occurrence text-mining analysis to identify statistically supported associations between bacterial genera and functional terms related to drought tolerance and plant growth promotion (PGP). Associations were considered significant when their co-occurrence frequency exceeded the 95th percentile of randomized permutations ($p < 0.05$).

The soil-derived network (Figure 1) comprised 59 nodes (bacterial genera and functions) and 1214 edges, resulting in a network density of 0.512. The average degree, defined as the mean number of edges per node, was 29.695. The weighted average degree, which reflects the cumulative strength of all connections by incorporating co-occurrence frequencies, was much higher (251.729). This disproportionate value highlights that certain genera are not only broadly connected but also strongly supported by frequent functional associations in the literature, amplifying their relative importance in the network. The clustering coefficient averaged 0.77, with 6582 closed triangles, indicating a highly cohesive structure. Within this network, *Rhizobium* emerged as one of the most connected genera, showing strong associations with key functional terms such as "microbiome," "phytohormones," "biocontrol," and "siderophores." *Streptomyces* was also highly connected, linking to "microbiome," "biocontrol," and "secondary metabolites." *Paenibacillus* exhibited significant associations with "biocontrol," "microbiome," and "phytohormones." Finally, *Bradyrhizobium* showed strong connections with *Rhizobium*, "microbiome," and "phytohormones," underscoring its role in nitrogen fixation and the promotion of soil health.

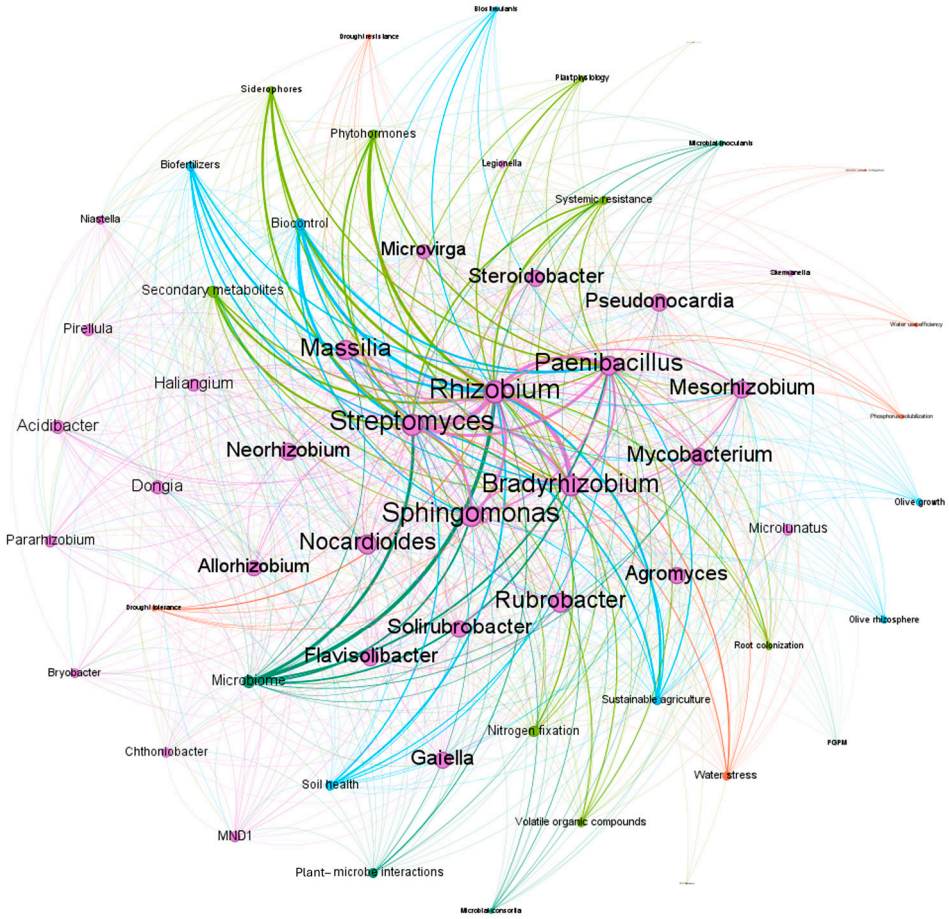

Figure 1. Network referring to documents related to soils. Data mining analysis revealed the co-occurrence patterns among microbial genera from "core microbiome" text mined from the literature and the top-occurring keywords in the documents.

The network of root-associated microorganisms (Figure 2) also contained 59 nodes but with fewer edges (775), corresponding to a network density of 0.453. The average degree was 26.271, while the weighted average degree reached 185.864, again reflecting the strength of recurrent associations. The clustering coefficient remained high (0.771), with 5165 closed triangles, indicating that this network—although less connected than the soil network—retained strong internal cohesion. In the root-associated network, *Rhizobium* again stood out as one of the most connected genera, with significant associations with "microbiome," "phytohormones," "biocontrol," and "siderophores." *Streptomyces* displayed strong links with "microbiome," "biocontrol," and "siderophores," suggesting possible synergistic interactions with *Rhizobium*. *Paenibacillus* also showed relevant associations with "biocontrol," "microbiome," and "phytohormones," while *Bradyrhizobium* maintained a central role through connections with *Rhizobium*, "microbiome," and "phytohormones."

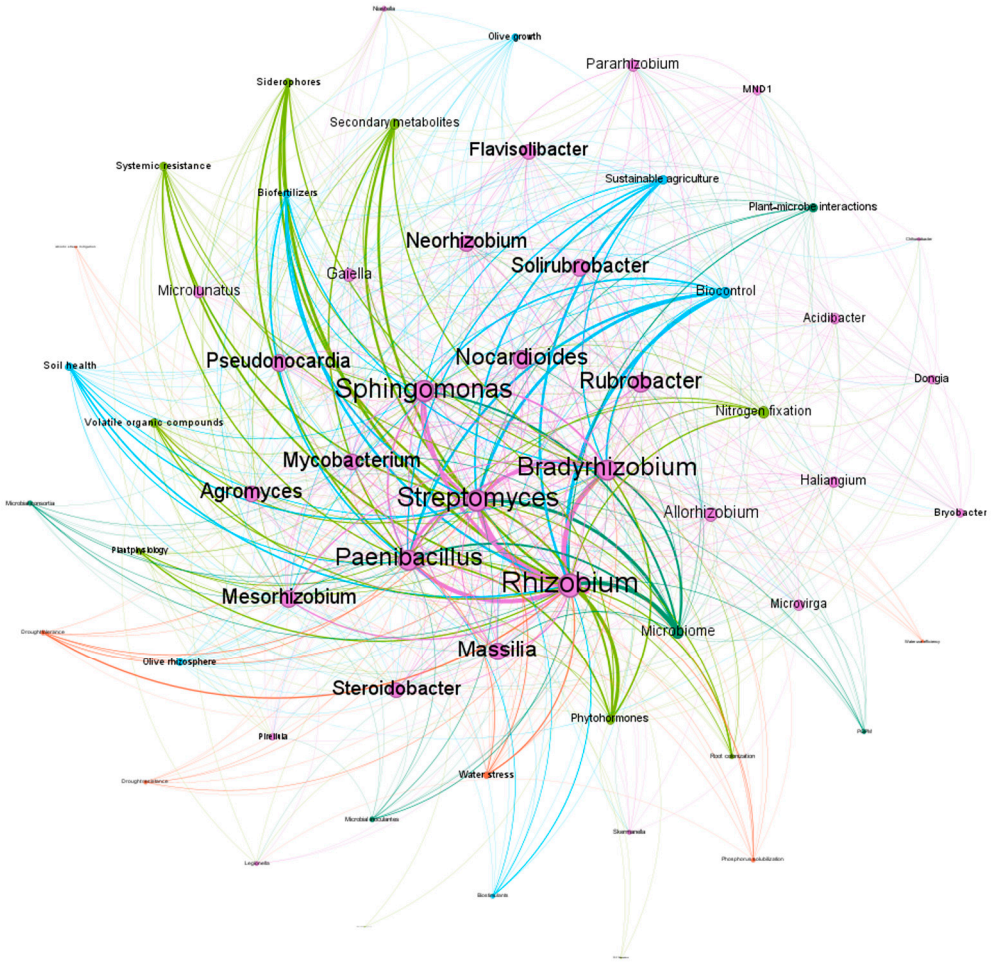

Figure 2. Network referring to documents related to root-associated microorganisms. The data mining analysis revealed the co-occurrence patterns among microbial genera from "core microbiome" text mined from the literature and the top-occurring keywords in the documents.

3.2. Taxonomic and Functional Core of the Bacterial Communities

The results shown in Figure 3 illustrate the differences in microbial community structure in rhizospheric soils and root-associated samples. Although NMDS ordination revealed visually distinct clustering of samples according to water regime, especially for root-associated samples, PERMANOVA did not detect statistically significant differences (Table S3, $p > 0.05$). This apparent discrepancy likely reflects high intra-group variability and limited sample size, which reduce statistical power. Nevertheless, the low stress value (0.078) indicates that the ordination faithfully represents community dissimilarities, suggesting that water regime exerts a structuring effect that, while evident at the visual level, does not reach formal significance in multivariate testing.

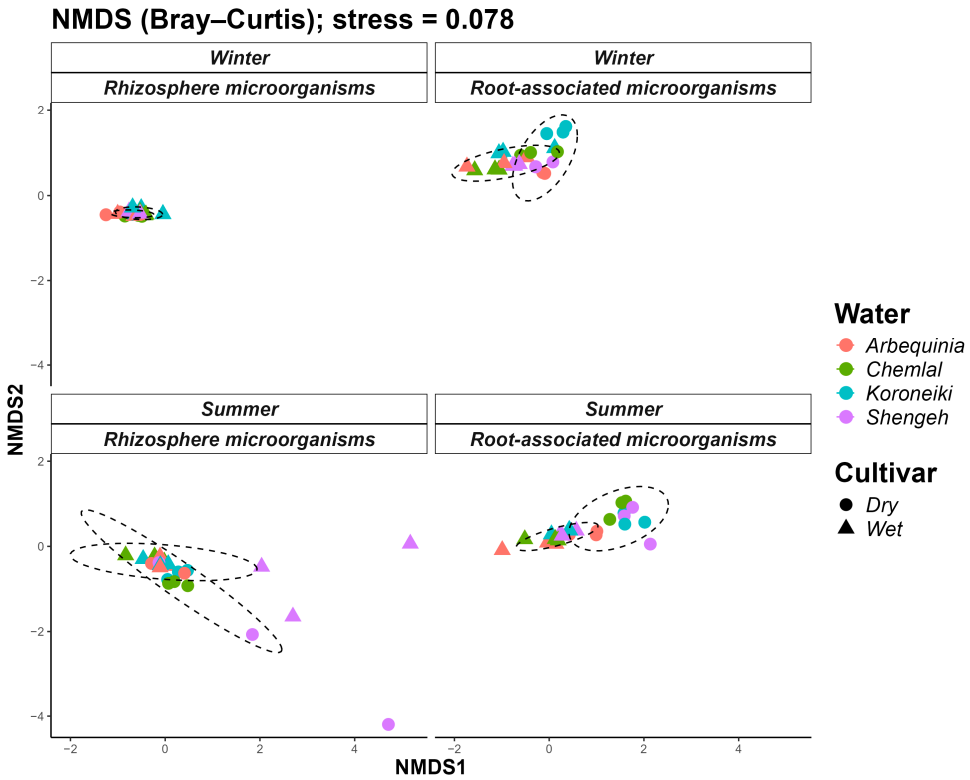

Figure 3. Soil rhizosphere and root-associated microorganisms' structure distribution related to water regime (dry, wet) and crop (Arbequinia, Chemlal, Koroneiki, and Shengeh), split by season (winter, summer). Dotted ellipses indicate the 95% confidence intervals around the centroids of water regime groups.

The functional analysis performed using DESeq2 revealed an almost complete absence of significant differences in the rhizospheric soil samples (Figure 4).

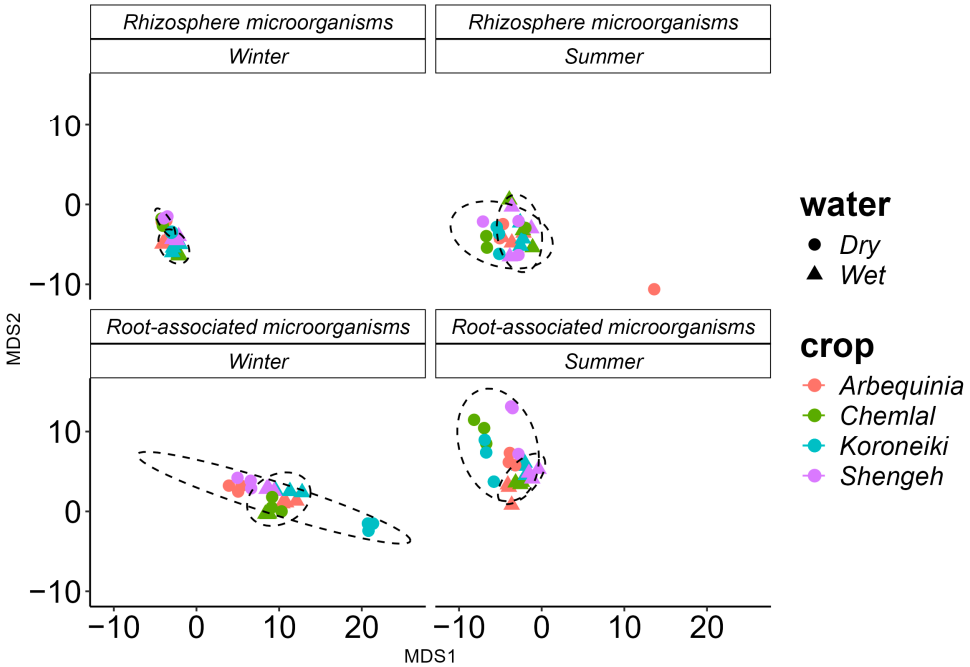

Figure 4. Soil rhizosphere and root-associated microorganisms' potential function distribution related to water regime (dry, wet) and crop (Arbequinia, Chemlal, Koroneiki, and Shengeh), split by season (winter, summer). Dotted ellipses indicate the 95% confidence intervals around the centroids of water regime groups.

On the other hand, functions associated with root-associated microorganisms showed differences related to water regime mainly at the cultivar level (Figure 4). In the winter samples, a significant difference in functional profiles between drought-stressed and well-watered conditions was observed primarily in the cultivar *Koroneiki*, and to a lesser extent in *Arbequina* and *Shengeh*.

During the summer season, the most notable functional differences were found in the samples of the cultivars *Chemlal* and *Koroneiki*.

3.3. Core Microbiomes

The Venn diagram (Figure 5) illustrates the overlap of taxa identified across treatments. It is based on inertia partitioning analysis (variance explained by each factor), and is provided as a visual summary rather than an independent statistical method. The root-associated wet samples show the highest number of unique genera (58), while the soil rhizosphere dry samples exhibit fewer unique taxa (13). A total of 66 genera are shared among all four conditions, suggesting the presence of a conserved core microbiome. The overlaps among groups indicate that both water availability and microbial niche influence community composition, with a higher variability observed in the root-associated microbiomes compared to the rhizosphere soils.

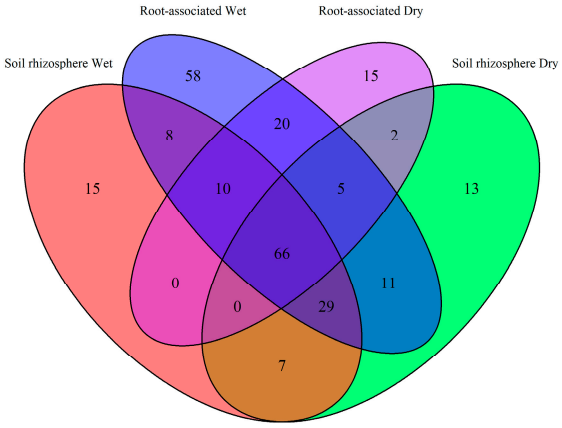

Figure 5. Venn diagram showing the unique and shared microbial genera among the four different conditions: soil rhizosphere, wet (red); root-associated, wet (blue); root-associated, dry (violet); and soil rhizosphere, dry (green).

3.4. Functions Related to Stress Response and Enrichment

The functions of the 30 most abundant and significantly different ($p < 0.05$) rhizosphere and root-associated microorganisms, predicted using the PICRUSt2 tool, are reported in Figures 6A and 6B, respectively. Interestingly, the functions related to branched-chain amino acid (BCAA) transfer permease were significantly higher under drought in both rhizosphere and root-associated microorganisms. The function related to glutathione S-transferase (GSTs) was also found to be significantly higher in rhizosphere drought conditions, as well as the function associated with thioredoxin reductase and those associated with chemotaxis; interestingly, the latter function was also different when combining the sampling time and cultivar effect (Tables S6 and S7) together with water level.

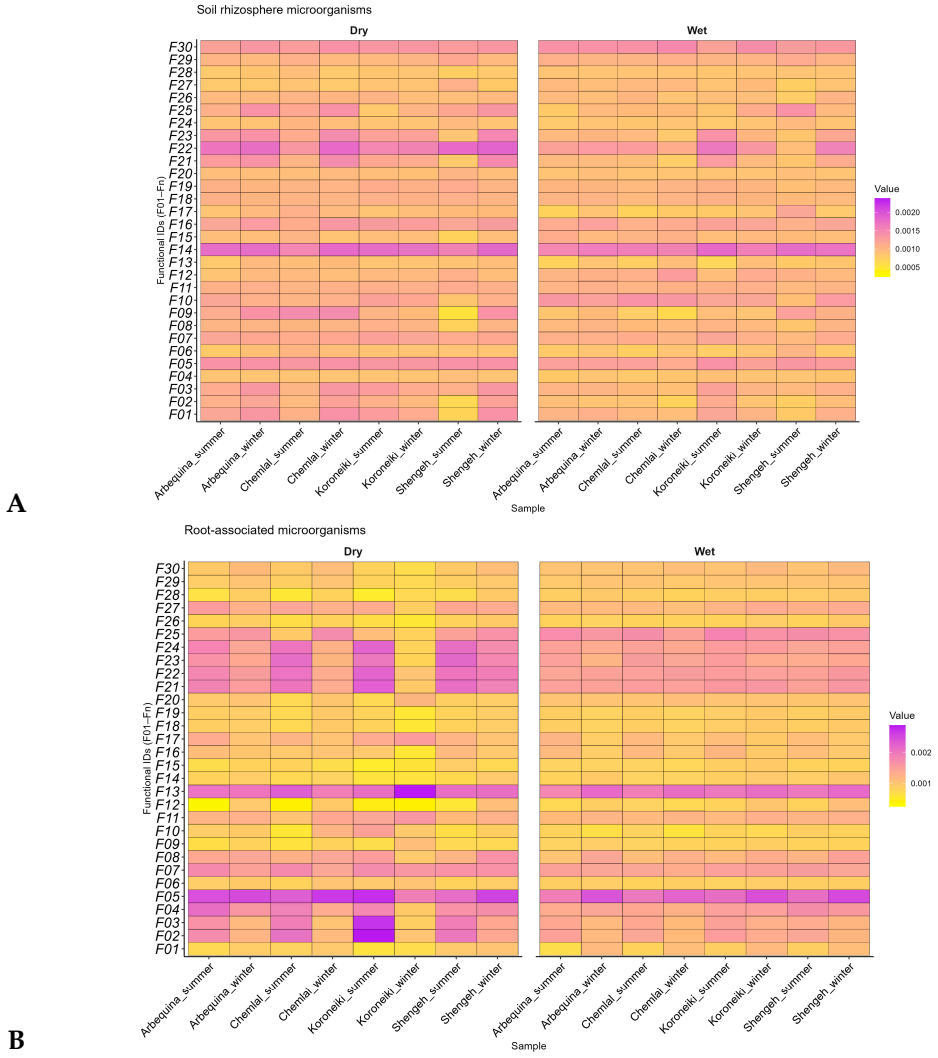

Figure 6. The 30 most abundant and significantly different ($p < 0.05$) functions of (**A**) rhizosphere and (**B**) root-associated microorganism samples split by treatment (dry; wet) and related to each olive variety (Arbequina; Chemlal; Koroneiki; Shengeh) in the two seasons (winter; summer). The values range from low (yellow) to high (violet). Abbreviated functional codes (F01–F30) are displayed in the heatmap for readability; full names are listed in Table S2.

Regarding the root-associated functions (Figure 6B), it is worth noting that, in addition to a difference between dry and wet conditions, a significant seasonal difference was also found in 23 of the 30 functions considered (Table S7). Moreover, a significantly higher abundance in the function related to cold shock protein has been found in all drought samples, which was also found for the function associated with long-chain acyl-CoA synthetase. Interestingly, the function related to an ATP-dependent DNA helicase (RecQ) has been found to be significantly different in both the time of sampling and cultivar (Table S7), together with the water level.

Table 1 shows the main bacterial genera responsible for contributing to the functions identified as significantly different between dry and wet conditions, and associated with traits related to drought stress resistance and/or plant growth promotion (PGP). In particular, three bacterial genera were found to be the major contributors to the nine identified functions: *Solirubrobacter*, *Microvirga*, and *Pseudonocardia*.

4. Discussion

4.1. Rhizosphere Stability Under Drought

The resilience of soil microbial communities under drought conditions is a remarkable phenomenon, with direct implications for plant adaptability to environmental stressors. Our findings reveal that the composition of the olive rhizosphere microbiome remains largely unchanged between wet and dry conditions, highlighting the stability of these ecosystems. This resilience can be attributed to functional redundancy and robust ecological networks that allow soil microbes to maintain critical functions despite fluctuations in water availability [28–30]. Many soil bacteria establish complex mutualistic relationships with plant roots, promoting stress tolerance, nutrient acquisition, and water conservation [31]. One key mechanism underlying this stability is microbial dormancy. Many soil bacteria can enter a dormant state during periods of drought, reactivating their metabolism once conditions improve [32]. This ability to alternate between active and dormant states preserves the overall microbial structure and functionality, effectively buffering the community against water scarcity [33,34]. Additionally, the presence of drought-adapted taxa in the rhizosphere supports the concept of a core microbiome, a subset of microbial species that remain functionally stable despite environmental fluctuations [6,35].

Interestingly, our analyses identified functional markers in soil microbiomes strongly related to drought tolerance, including branched-chain amino acids (BCAAs), glutathione S-transferases (GSTs), thioredoxin pathways, and chemotaxis. BCAAs, which accumulate during drought, are typically derived from protein breakdown rather than new synthesis, underscoring their role in microbial stress response [36–39]. GSTs detoxify reactive compounds and peroxides, thereby protecting plant and microbial cells from oxidative damage [40–43]. Thiourea, another protective compound, has been shown to enhance drought tolerance by modulating aquaporin expression and improving cellular water balance [44–48]. Finally, chemotaxis—the ability of bacteria to migrate toward chemical signals—is a critical trait for plant growth-promoting bacteria (PGPB) [49,50]. In our study, the methyl-accepting chemotaxis protein (K03406) showed a very strong correlation with the water regime, olive cultivar, and sampling season, supporting its key role in regulating drought-stress responses in olive systems.

4.2. Root-Associated Microbial Shifts and Functional Significance

In contrast to rhizosphere stability, root-associated microbial communities exhibited significant compositional shifts between irrigated and dry olive groves. These results confirm that endophytic and root-associated bacteria are more sensitive to environmental changes [51,52]. Endophytes colonizing the internal root tissues play a central role in plant health, facilitating root development, enhancing mineral uptake, and providing disease resistance [53]. Under drought stress, root-associated communities reorganize, favoring taxa that confer greater stress tolerance [54,55]. Many of these taxa produce osmolytes and antioxidants that mitigate water stress [56,57] or modulate plant hormones such as abscisic acid (ABA) and ethylene to optimize drought responses [58–60].

Root exudates likely drive these shifts as plants selectively recruit beneficial microbes under stress [61,62]. Such recruitment enhances nutrient uptake when absorption is limited and strengthens root structures to improve water retention. Functional analyses further confirm this adaptation: root-associated microorganisms upregulated stress-related molecular responses, including heat shock proteins (HSPs) and long-chain acyl-CoA synthetases (LACSs) [63–65]. These mechanisms help maintain cellular homeostasis under drought stress, supporting the view that root-associated microbes actively contribute to plant resilience [66–69].

Three genera emerged as central members of the olive root "core microbiome": *Solirubrobacter*, *Microvirga*, and *Pseudonocardia*. *Solirubrobacter*, often associated with arid soils, contributes to resilience by degrading complex organic matter and maintaining nutrient cycling under low-moisture conditions [70]. *Microvirga*, a nitrogen-fixing genus, provides bioavailable nitrogen under drought, compensating for reduced nutrient uptake [71]. *Pseudonocardia* produces secondary metabolites such as osmoprotectants, antioxidants, and phytohormones (e.g., IAA), enhancing root growth and water uptake [72]. These genera have also been identified in the literature as key taxa in stress-resilient microbiomes [73–75]. Our findings also support the notion that the definition of a *core microbiome* may be more meaningful when based on functional traits rather than taxonomic composition. While the rhizosphere microbiome remained taxonomically stable, root-associated shifts revealed specific taxa linked to drought-related functions such as nitrogen fixation, chemotaxis, and phytohormone production. This functional definition of core taxa provides a more robust framework for understanding plant–microbe interactions under drought stress [6].

4.3. Applications for Drought-Resilient Farming

By integrating the functional and the literature-based analyses, this study highlights the translational potential of olive-associated microbiomes for agricultural applications. The identification of *Solirubrobacter*, *Microvirga*, and *Pseudonocardia* as core contributors to drought resilience suggests their utility as foundational members of synthetic microbial consortia (SynComs) designed for Mediterranean crops. Each genus contributes complementary functions—nutrient cycling, nitrogen fixation, and hormone modulation—that are highly desirable for promoting drought tolerance.

Moreover, functional markers such as GSTs, chemotaxis proteins, and thioredoxin pathways provide candidate traits that can guide the selection of strains for microbial inoculants or biostimulant formulations. These traits could be explicitly targeted in the development of next-generation microbial products tailored for climate-smart agriculture. Such products may support irrigation optimization (by enhancing plant water-use efficiency), cultivar selection (through improved compatibility with beneficial microbiomes), and microbiome manipulation (by fostering the establishment of beneficial taxa).

Altogether, our results provide both fundamental ecological insights and a roadmap for applied innovation. Functionally defined core microbiomes offer a promising avenue for the design of microbial-based solutions that enhance olive resilience to drought, while also contributing to broader strategies for sustainable farming in water-limited agroecosystems.

5. Conclusions

Our study demonstrates the resilience and adaptability of soil- and root-associated microbial communities under drought conditions. While root-associated communities undergo significant compositional shifts, favoring taxa linked to stress tolerance, soil microbiomes exhibit remarkable stability, likely reflecting functional redundancy and microbial dormancy strategies. These patterns highlight how microbial plasticity contributes to maintaining ecosystem processes under environmental fluctuations.

The observed shifts in root-associated microbiota suggest that olive trees may selectively recruit beneficial microbes via root exudates, thereby enriching taxa with stress-mitigating capabilities. Functional inference further supports this view by pointing to associations with processes such as osmolyte production, hormone modulation, and antioxidant activity. Although these functions were inferred rather than directly measured, they represent potential microbial contributions to plant drought resilience. In this sense, the identified functional markers serve as indicators of microbial processes potentially linked to adaptation, rather than as direct causal agents.

The identification of a root-associated core microbiome enriched under drought, including genera such as *Microvirga*, *Solirubrobacter*, and *Pseudonocardia*, provides insights into ecological strategies that may support plant performance through biocontrol, phytohormone production, nutrient mobilization, and possible pathogen defense. At the same time, it is important to acknowledge that drought tolerance in olive trees may also be strongly influenced by plant physiological traits, and that the relative contributions of plant and microbial responses remain to be disentangled.

From an applied perspective, our findings have several implications. First, they point to the potential of integrating microbiome-based approaches (e.g., synthetic microbial consortia and targeted microbial inoculants) with irrigation management to sustain productivity under water-limited conditions. Second, they highlight the value of considering cultivar-specific microbial associations when selecting olive varieties for drought-prone environments. Together, these insights advance the framework for leveraging plant–microbe interactions as nature-based solutions to improve the resilience of Mediterranean agroecosystems to climate change.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/app15179667/s1, Table S1: Physico-chemical parameters of soils; Table S2: Abbreviated functional codes (F01–F30); Table S3: Results of the PERMANOVA; Table S4. Results of the post-hoc Tukey-HSD rhizosphere; Table S5. Results of the post-hoc Tukey-HSD root-associated microorganisms; Table S6. Results of the post-hoc Tukey-HSD samples; Table S7. Results of the post-hoc Tukey-HSD test functions; Table S8. Core microbiome composition; Figure S1. Monthly rainfall; Figure S2. Enriched functions in the microbial community.

Author Contributions: A.V.: Writing—Original Draft, Visualization, Software, Methodology, Investigation, Formal Analysis, Data Curation, Conceptualization. L.N., L.D.G., M.C., and E.C.: Writing—Review and Editing, Software, Methodology. F.S. (Filippo Sevi) and F.S. (Federico Sbarra): Investigation, Methodology, Software. R.B.: Supervision, Investigation, Validation. M.C.V., E.F., O.C., and L.B.: Sample Collection, Review and Editing, Funding Acquisition. G.P. and A.B.: Writing—Review and Editing, Supervision, Resources, Project Administration, Funding Acquisition, Conceptualization. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the PRIMA Project "BIOMEnext—Modelling integrated biodiversity-based next-generation Mediterranean farming systems" (D.R., 0000672.22-03-2021).

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Orozco-Mosqueda, M.D.C.; Fadiji, A.E.; Babalola, O.O.; Glick, B.R.; Santoyo, G. Rhizobiome Engineering: Unveiling Complex Rhizosphere Interactions to Enhance Plant Growth and Health. *Microbiol. Res.* **2022**, *263*, 127137. [CrossRef]
2. Pandey, K.; Saharan, B.S. Soil Microbiomes: A Promising Strategy for Boosting Crop Yield and Advancing Sustainable Agriculture. *Discov. Agric.* **2025**, *3*, 54. [CrossRef]
3. Anas, M.; Khalid, A.; Saleem, M.H.; Ali Khan, K.; Ahmed Khattak, W.; Fahad, S. Symbiotic Synergy: Unveiling Plant-Microbe Interactions in Stress Adaptation. *J. Crop Health* **2025**, *77*, 18. [CrossRef]
4. Castellano-Hinojosa, A.; Strauss, S.L. Insights into the Taxonomic and Functional Characterization of Agricultural Crop Core Rhizobiomes and Their Potential Microbial Drivers. *Sci. Rep.* **2021**, *11*, 10068. [CrossRef] [PubMed]
5. Shu, W.-S.; Huang, L.-N. Microbial Diversity in Extreme Environments. *Nat. Rev. Microbiol.* **2022**, *20*, 219–235. [CrossRef]
6. Neu, A.T.; Allen, E.E.; Roy, K. Defining and Quantifying the Core Microbiome: Challenges and Prospects. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2104429118. [CrossRef] [PubMed]
7. Banerjee, S.; Van Der Heijden, M.G.A. Soil Microbiomes and One Health. *Nat. Rev. Microbiol.* **2023**, *21*, 6–20. [CrossRef] [PubMed]

8. Sneha, G.R.; Swarnalakshmi, K.; Sharma, M.; Reddy, K.; Bhoumik, A.; Suman, A.; Kannepalli, A. Soil Type Influence Nutrient Availability, Microbial Metabolic Diversity, Eubacterial and Diazotroph Abundance in Chickpea Rhizosphere. *World J. Microbiol. Biotechnol.* **2021**, *37*, 167. [CrossRef]
9. Ling, N.; Wang, T.; Kuzyakov, Y. Rhizosphere Bacteriome Structure and Functions. *Nat. Commun.* **2022**, *13*, 836. [CrossRef]
10. Aley, P.; Singh, J.; Kumar, P. Adapting the Changing Environment: Microbial Way of Life. In *Microbiome Under Changing Climate*; Elsevier: Amsterdam, The Netherlands, 2022; pp. 507–525, ISBN 978-0-323-90571-8.
11. Toor, M.D.; Ur Rehman, M.; Abid, J.; Nath, D.; Ullah, I.; Basit, A.; Ud Din, M.M.; Mohamed, H.I. Microbial Ecosystems as Guardians of Food Security and Water Resources in the Era of Climate Change. *Water Air Soil. Pollut.* **2024**, *235*, 741. [CrossRef]
12. Kalra, A.; Goel, S.; Elias, A.A. Understanding Role of Roots in Plant Response to Drought: Way Forward to Climate-resilient Crops. *Plant Genome* **2024**, *17*, e20395. [CrossRef]
13. Poudel, M.; Mendes, R.; Costa, L.A.S.; Bueno, C.G.; Meng, Y.; Folimonova, S.Y.; Garrett, K.A.; Martins, S.J. The Role of Plant-Associated Bacteria, Fungi, and Viruses in Drought Stress Mitigation. *Front. Microbiol.* **2021**, *12*, 743512. [CrossRef]
14. Dao, J.; Xing, Y.; Chen, C.; Chen, M.; Wang, Z. Adaptation of Rhizosphere Bacterial Communities of Drought-Resistant Sugarcane Varieties under Different Degrees of Drought Stress. *Microbiol. Spectr.* **2023**, *11*, e01184-23. [CrossRef]
15. Santos-Medellín, C.; Liechty, Z.; Edwards, J.; Nguyen, B.; Huang, B.; Weimer, B.C.; Sundaresan, V. Prolonged Drought Imparts Lasting Compositional Changes to the Rice Root Microbiome. *Nat. Plants* **2021**, *7*, 1065–1077. [CrossRef]
16. Wang, H.; Wang, Y.; Kang, C.; Wang, S.; Zhang, Y.; Yang, G.; Zhou, L.; Xiang, Z.; Huang, L.; Liu, D.; et al. Drought Stress Modifies the Community Structure of Root-Associated Microbes That Improve *Atractylodes lancea* Growth and Medicinal Compound Accumulation. *Front. Plant Sci.* **2022**, *13*, 1032480. [CrossRef]
17. Malhi, G.S.; Kaur, M.; Kaushik, P. Impact of Climate Change on Agriculture and Its Mitigation Strategies: A Review. *Sustainability* **2021**, *13*, 1318. [CrossRef]
18. Gholami, R.; Fahadi Hoveizeh, N.; Zahedi, S.M.; Gholami, H.; Carillo, P. Effect of Three Water-Regimes on Morpho-Physiological, Biochemical and Yield Responses of Local and Foreign Olive Cultivars under Field Conditions. *BMC Plant Biol.* **2022**, *22*, 477. [CrossRef] [PubMed]
19. Boussadia, O.; Zgallai, H.; Mzid, N.; Zaabar, R.; Braham, M.; Doupis, G.; Koubouris, G. Physiological Responses of Two Olive Cultivars to Salt Stress. *Plants* **2023**, *12*, 1926. [CrossRef] [PubMed]
20. Piwowar, H.; Priem, J.; Larivière, V.; Alperin, J.P.; Matthias, L.; Norlander, B.; Farley, A.; West, J.; Haustein, S. The State of OA: A Large-Scale Analysis of the Prevalence and Impact of Open Access Articles. *PeerJ* **2018**, *6*, e4375. [CrossRef]
21. Kuzyakov, Y.; Blagodatskaya, E. Microbial Hotspots and Hot Moments in Soil: Concept & Review. *Soil. Biol. Biochem.* **2015**, *83*, 184–199. [CrossRef]
22. Chen, L.; Liu, Y.; Xu, Z.; Song, Y. Editorial: Beneficial Microbe-Plant Interactions under Biotic/Abiotic Stress Conditions. *Front. Microbiol.* **2023**, *14*, 1294042. [CrossRef]
23. Rubin, R.L.; Van Groenigen, K.J.; Hungate, B.A. Plant Growth Promoting Rhizobacteria Are More Effective under Drought: A Meta-Analysis. *Plant Soil.* **2017**, *416*, 309–323. [CrossRef]
24. Pokorny, J.J.; Norman, A.; Zanesco, A.P.; Bauer-Wu, S.; Sahdra, B.K.; Saron, C.D. Network Analysis for the Visualization and Analysis of Qualitative Data. *Psychol. Methods* **2018**, *23*, 169–183. [CrossRef]
25. Jacomy, M.; Venturini, T.; Heymann, S.; Bastian, M. ForceAtlas2, a Continuous Graph Layout Algorithm for Handy Network Visualization Designed for the Gephi Software. *PLoS ONE* **2014**, *9*, e98679. [CrossRef]
26. Love, M.; Anders, S. DESeq2 2017. Available online: https://bioconductor.org/packages/release/bioc/html/DESeq2.html (accessed on 1 August 2025).
27. Chen, H.; Boutros, P.C. VennDiagram: A Package for the Generation of Highly-Customizable Venn and Euler Diagrams in R. *BMC Bioinform.* **2011**, *12*, 35. [CrossRef] [PubMed]
28. Fan, W.; Tang, F.; Wang, J.; Dong, J.; Xing, J.; Shi, F. Drought-Induced Recruitment of Specific Root-Associated Bacteria Enhances Adaptation of Alfalfa to Drought Stress. *Front. Microbiol.* **2023**, *14*, 1114400. [CrossRef] [PubMed]
29. Gao, Y.; Cui, J.; Ren, G.; Wei, S.; Yang, P.; Yin, C.; Liang, H.; Chang, J. Changes in the Root-Associated Bacteria of Sorghum Are Driven by the Combined Effects of Salt and Sorghum Development. *Environ. Microbiome* **2021**, *16*, 14. [CrossRef] [PubMed]
30. Verma, S.K.; Sahu, P.K.; Kumar, K.; Pal, G.; Gond, S.K.; Kharwar, R.N.; White, J.F. Endophyte Roles in Nutrient Acquisition, Root System Architecture Development and Oxidative Stress Tolerance. *J. Appl. Microbiol.* **2021**, *131*, 2161–2177. [CrossRef]
31. Ayangbenro, A.S.; Babalola, O.O. Reclamation of Arid and Semi-Arid Soils: The Role of Plant Growth-Promoting Archaea and Bacteria. *Curr. Plant Biol.* **2021**, *25*, 100173. [CrossRef]
32. Ben Gaied, R.; Brígido, C.; Sbissi, I.; Tarhouni, M. Sustainable Strategy to Boost Legumes Growth under Salinity and Drought Stress in Semi-Arid and Arid Regions. *Soil. Syst.* **2024**, *8*, 84. [CrossRef]
33. Kour, D.; Yadav, A.N. Bacterial Mitigation of Drought Stress in Plants: Current Perspectives and Future Challenges. *Curr. Microbiol.* **2022**, *79*, 248. [CrossRef]

34. Ma, Y.; Dias, M.C.; Freitas, H. Drought and Salinity Stress Responses and Microbe-Induced Tolerance in Plants. *Front. Plant Sci.* **2020**, *11*, 591911. [CrossRef]
35. Abideen, Z.; Cardinale, M.; Zulfiqar, F.; Koyro, H.-W.; Rasool, S.G.; Hessini, K.; Darbali, W.; Zhao, F.; Siddique, K.H.M. Seed Endophyte Bacteria Enhance Drought Stress Tolerance in Hordeum Vulgare by Regulating, Physiological Characteristics, Antioxidants and Minerals Uptake. *Front. Plant Sci.* **2022**, *13*, 980046. [CrossRef]
36. Cui, X.; He, W.; Christensen, M.J.; Yue, J.; Zeng, F.; Zhang, X.; Nan, Z.; Xia, C. Abscisic Acid May Play a Critical Role in the Moderating Effect of Epichloë Endophyte on *Achnatherum inebrians* under Drought Stress. *J. Fungi* **2022**, *8*, 1140. [CrossRef]
37. Muhammad, M.; Wahab, A.; Waheed, A.; Mohamed, H.I.; Hakeem, K.R.; Li, L.; Li, W.-J. Harnessing Bacterial Endophytes for Environmental Resilience and Agricultural Sustainability. *J. Environ. Manag.* **2024**, *368*, 122201. [CrossRef] [PubMed]
38. Ahlawat, O.P.; Yadav, D.; Walia, N.; Kashyap, P.L.; Sharma, P.; Tiwari, R. Root Exudates and Their Significance in Abiotic Stress Amelioration in Plants: A Review. *J. Plant Growth Regul.* **2024**, *43*, 1736–1761. [CrossRef]
39. Ansari, M.; Devi, B.M.; Sarkar, A.; Chattopadhyay, A.; Satnami, L.; Balu, P.; Choudhary, M.; Shahid, M.A.; Jailani, A.A.K. Microbial Exudates as Biostimulants: Role in Plant Growth Promotion and Stress Mitigation. *J. Xenobiotics* **2023**, *13*, 572–603. [CrossRef] [PubMed]
40. Zhao, H.; Kosma, D.K.; Lü, S. Functional Role of Long-Chain Acyl-CoA Synthetases in Plant Development and Stress Responses. *Front. Plant Sci.* **2021**, *12*, 640996. [CrossRef] [PubMed]
41. Yang, X.; Lu, M.; Wang, Y.; Wang, Y.; Liu, Z.; Chen, S. Response Mechanism of Plants to Drought Stress. *Horticulturae* **2021**, *7*, 50. [CrossRef]
42. Kumar, P.; Paul, D.; Jhajhriya, S.; Kumar, R.; Dutta, S.; Siwach, P.; Das, S. Understanding Heat-Shock Proteins' Abundance and Pivotal Function under Multiple Abiotic Stresses. *J. Plant Biochem. Biotechnol.* **2024**, *33*, 492–513. [CrossRef]
43. Aghaie, P.; Tafreshi, S.A.H. Central Role of 70-kDa Heat Shock Protein in Adaptation of Plants to Drought Stress. *Cell Stress. Chaperones* **2020**, *25*, 1071–1081. [CrossRef] [PubMed]
44. Hamid, R.; Ghorbanzadeh, Z.; Jacob, F.; Nekouei, M.K.; Zeinalabedini, M.; Mardi, M.; Sadeghi, A.; Ghaffari, M.R. Decoding Drought Resilience: A Comprehensive Exploration of the Cotton Eceriferum (CER) Gene Family and Its Role in Stress Adaptation. *BMC Plant Biol.* **2024**, *24*, 468. [CrossRef]
45. Srivastava, S.; Yadav, S. A Positive Role of Polyunsaturated Fatty Acids on Sustainable Crop Production against Salt Stress: An Overview. *Biologia* **2024**, *79*, 1599–1610. [CrossRef]
46. Wei, H.; Movahedi, A.; Zhang, Y.; Aghaei-Dargiri, S.; Liu, G.; Zhu, S.; Yu, C.; Chen, Y.; Zhong, F.; Zhang, J. Long-Chain Acyl-CoA Synthetases Promote Poplar Resistance to Abiotic Stress by Regulating Long-Chain Fatty Acid Biosynthesis. *IJMS* **2022**, *23*, 8401. [CrossRef] [PubMed]
47. Abdul Rahman, N.S.N.; Abdul Hamid, N.W.; Nadarajah, K. Effects of Abiotic Stress on Soil Microbiome. *IJMS* **2021**, *22*, 9036. [CrossRef]
48. Aqeel, M.; Ran, J.; Hu, W.; Irshad, M.K.; Dong, L.; Akram, M.A.; Eldesoky, G.E.; Aljuwayid, A.M.; Chuah, L.F.; Deng, J. Plant-Soil-Microbe Interactions in Maintaining Ecosystem Stability and Coordinated Turnover under Changing Environmental Conditions. *Chemosphere* **2023**, *318*, 137924. [CrossRef]
49. Philippot, L.; Griffiths, B.S.; Langenheder, S. Microbial Community Resilience across Ecosystems and Multiple Disturbances. *Microbiol. Mol. Biol. Rev.* **2021**, *85*, e00026-20. [CrossRef]
50. De Mandal, S.; Sonali; Singh, S.; Hussain, K.; Hussain, T. Plant–Microbe Association for Mutual Benefits for Plant Growth and Soil Health. In *Current Trends in Microbial Biotechnology for Sustainable Agriculture*; Yadav, A.N., Singh, J., Singh, C., Yadav, N., Eds.; Environmental and Microbial Biotechnology; Springer: Singapore, 2021; pp. 95–121, ISBN 978-981-15-6948-7.
51. Bandopadhyay, S.; Li, X.; Bowsher, A.W.; Last, R.L.; Shade, A. Disentangling Plant- and Environment-Mediated Drivers of Active Rhizosphere Bacterial Community Dynamics during Short-Term Drought. *Nat. Commun.* **2024**, *15*, 6347. [CrossRef]
52. Loiko, N.; Islam, M.N. Plant–Soil Microbial Interaction: Differential Adaptations of Beneficial vs. Pathogenic Bacterial and Fungal Communities to Climate-Induced Drought. *Agronomy* **2024**, *14*, 1949. [CrossRef]
53. Bundschuh, M.; Mesquita-Joanes, F.; Rico, A.; Camacho, A. Understanding Ecological Complexity in a Chemical Stress Context: A Reflection on Recolonization, Recovery, and Adaptation of Aquatic Populations and Communities. *Environ. Toxicol. Chem.* **2023**, *42*, 1857–1866. [CrossRef] [PubMed]
54. Sorensen, J.W.; Shade, A. Dormancy Dynamics and Dispersal Contribute to Soil Microbiome Resilience. *Phil. Trans. R. Soc. B* **2020**, *375*, 20190255. [CrossRef] [PubMed]
55. Zhou, Y.; Liu, D.; Li, F.; Dong, Y.; Jin, Z.; Liao, Y.; Li, X.; Peng, S.; Delgado-Baquerizo, M.; Li, X. Superiority of Native Soil Core Microbiomes in Supporting Plant Growth. *Nat. Commun.* **2024**, *15*, 6599. [CrossRef] [PubMed]
56. Maisnam, P.; Jeffries, T.C.; Szejgis, J.; Bristol, D.; Singh, B.K.; Eldridge, D.J.; Horn, S.; Chieppa, J.; Nielsen, U.N. Severe Prolonged Drought Favours Stress-Tolerant Microbes in Australian Drylands. *Microb. Ecol.* **2023**, *86*, 3097–3110. [CrossRef]
57. Heinemann, B.; Hildebrandt, T.M. The Role of Amino Acid Metabolism in Signaling and Metabolic Adaptation to Stress-Induced Energy Deficiency in Plants. *J. Exp. Bot.* **2021**, *72*, 4634–4645. [CrossRef]

58. Ingrisano, R.; Tosato, E.; Trost, P.; Gurrieri, L.; Sparla, F. Proline, Cysteine and Branched-Chain Amino Acids in Abiotic Stress Response of Land Plants and Microalgae. *Plants* **2023**, *12*, 3410. [CrossRef] [PubMed]
59. Le, X.H.; Millar, A.H. The Diversity of Substrates for Plant Respiration and How to Optimize Their Use. *Plant Physiol.* **2023**, *191*, 2133–2149. [CrossRef] [PubMed]
60. Shim, J.S.; Jeong, H.I.; Bang, S.W.; Jung, S.E.; Kim, G.; Kim, Y.S.; Redillas, M.C.F.R.; Oh, S.-J.; Seo, J.S.; Kim, J.-K. Drought-Induced Branched-Chain Amino Acid Aminotransferase Enhances Drought Tolerance in Rice. *Plant Physiol.* **2023**, *191*, 1435–1447. [CrossRef]
61. Stavridou, E.; Voulgari, G.; Michailidis, M.; Kostas, S.; Chronopoulou, E.G.; Labrou, N.E.; Madesis, P.; Nianiou-Obeidat, I. Overexpression of A Biotic Stress-Inducible Pvgstu Gene Activates Early Protective Responses in Tobacco under Combined Heat and Drought. *IJMS* **2021**, *22*, 2352. [CrossRef]
62. Xu, J.; Xing, X.-J.; Tian, Y.-S.; Peng, R.-H.; Xue, Y.; Zhao, W.; Yao, Q.-H. Transgenic Arabidopsis Plants Expressing Tomato Glutathione S-Transferase Showed Enhanced Resistance to Salt and Drought Stress. *PLoS ONE* **2015**, *10*, e0136960. [CrossRef]
63. Yang, G.; Wang, Y.; Xia, D.; Gao, C.; Wang, C.; Yang, C. Correction to: Overexpression of a GST Gene (ThGSTZ1) from *Tamarix hispida* Improves Drought and Salinity Tolerance by Enhancing the Ability to Scavenge Reactive Oxygen Species. *Plant Cell Tissue Organ Cult.* **2022**, *150*, 247–249. [CrossRef]
64. Zhang, Y.; He, J.; Xiao, Y.; Zhang, Y.; Liu, Y.; Wan, S.; Liu, L.; Dong, Y.; Liu, H.; Yu, Y. CsGSTU8, a Glutathione S-Transferase From Camellia Sinensis, Is Regulated by CsWRKY48 and Plays a Positive Role in Drought Tolerance. *Front. Plant Sci.* **2021**, *12*, 795919. [CrossRef]
65. Ahmed, N.; Rahman, K.; Rahman, M.; Sathi, K.S.; Alam, M.M.; Nahar, K.; Islam, M.S.; Hasanuzzaman, M. Insight into the Thiourea-Induced Drought Tolerance in Two Chickpea Varieties: Regulation of Osmoprotection, Reactive Oxygen Species Metabolism and Glyoxalase System. *Plant Physiol. Biochem.* **2021**, *167*, 449–458. [CrossRef]
66. Pandey, M. Thiourea and Hydrogen Peroxide Priming Improved K^+ Retention and Source-Sink Relationship for Mitigating Salt Stress in Rice. *Sci. Rep.* **2021**, *11*, 3000. [CrossRef]
67. Sahu, M.P. Role of Sulfhydryl Bioregulator Thiourea in Mitigating Drought Stress in Crops. In *Plant Stress Mitigators*; Elsevier: Amsterdam, The Netherlands, 2023; pp. 293–304, ISBN 978-0-323-89871-3.
68. Sehar, Z.; Gautam, H.; Iqbal, N.; Alvi, A.F.; Jahan, B.; Fatma, M.; Albaqami, M.; Khan, N.A. The Functional Interplay between Ethylene, Hydrogen Sulfide, and Sulfur in Plant Heat Stress Tolerance. *Biomolecules* **2022**, *12*, 678. [CrossRef]
69. Sun, Q.; Liu, X.; Kitagawa, Y.; Calamita, G.; Ding, X. Plant Aquaporins: Their Roles beyond Water Transport. *Crop J.* **2024**, *12*, 641–655. [CrossRef]
70. Matilla, M.A.; Krell, T. Bacterial Amino Acid Chemotaxis: A Widespread Strategy with Multiple Physiological and Ecological Roles. *J. Bacteriol.* **2024**, *206*, e00300-24. [CrossRef] [PubMed]
71. Xu, Q.; Ali, S.; Afzal, M.; Nizami, A.-S.; Han, S.; Dar, M.A.; Zhu, D. Advancements in Bacterial Chemotaxis: Utilizing the Navigational Intelligence of Bacteria and Its Practical Applications. *Sci. Total Environ.* **2024**, *931*, 172967. [CrossRef]
72. Xiong, Y.-W.; Li, X.-W.; Wang, T.-T.; Gong, Y.; Zhang, C.-M.; Xing, K.; Qin, S. Root Exudates-Driven Rhizosphere Recruitment of the Plant Growth-Promoting Rhizobacterium *Bacillus flexus* KLBMP 4941 and Its Growth-Promoting Effect on the Coastal Halophyte *Limonium sinense* under Salt Stress. *Ecotoxicol. Environ. Saf.* **2020**, *194*, 110374. [CrossRef] [PubMed]
73. Gupta, A.; Mishra, R.; Rai, S.; Bano, A.; Pathak, N.; Fujita, M.; Kumar, M.; Hasanuzzaman, M. Mechanistic Insights of Plant Growth Promoting Bacteria Mediated Drought and Salt Stress Tolerance in Plants for Sustainable Agriculture. *IJMS* **2022**, *23*, 3741. [CrossRef]
74. Mishra, P.; Mishra, J.; Arora, N.K. Plant Growth Promoting Bacteria for Combating Salinity Stress in Plants–Recent Developments and Prospects: A Review. *Microbiol. Res.* **2021**, *252*, 126861. [CrossRef]
75. Parasar, B.J.; Sharma, I.; Agarwala, N. Root Exudation Drives Abiotic Stress Tolerance in Plants by Recruiting Beneficial Microbes. *Appl. Soil. Ecol.* **2024**, *198*, 105351. [CrossRef]