Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Metagenomic Study on the Variable Influence of Soil Microbiomes on *Oryza sativa* Seedling Microbiome Across Different Cultivated Soils

REMYA UNNIKRISHNAN

remyarose15@gmail.com

Mahatma Gandhi University https://orcid.org/0000-0003-2688-701X

Priya V.K

Mahatma Gandhi University

Padinjarakavil Soumya

Mahatma Gandhi University

Jayachandran K

Mahatma Gandhi University

Radhakrishnan E.K

radhakrishnanek@mgu.ac.in

Mahatma Gandhi University

Research Article

Keywords: Diversity analysis, Plant-microbe interaction, Rice, Metagenome, Soil microbiome

Posted Date: August 28th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10836456/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: The authors declare no competing interests.

Abstract

Understanding the role of soil microbiome in shaping plant associated microbial communities is essential for enhancing plant health and productivity. The current study has been designed to investigate bacterial and fungal composition of root and leaf of rice plant grown under different soil types. By using the 16S and 18S rRNA amplicon sequencing, the study could identify distinct microbial profiles at the same time diversity analysis has revealed significant shifts in the root and leaf microbiomes, with root-associated communities displaying greater diversity in response to the soil variations. Notably, *Xanthomonas* was observed to be predominant in root samples grown in different soil conditions when compared to the controls, while the soil was observed to have a more diverse bacterial population. Fungal analysis has showed a high abundance of *Aspergillus* in both the soil and leaf samples, whereas *Curvularia, Bipolaris*, and *Starmarella* were abundant in root samples. These findings underscore the critical role of soil type in shaping the plant microbiome, influencing nutrient cycling, plant microbe interactions, and disease suppression. The result of the study provide insight into this phenomenon in rice by revealing the microbial differential distribution across the plant compartments and thereby highlighting the impact of soil on microbial assembly.

Introduction

Plants coexist in harmony with their surrounding environment, thriving in balance with both the biotic and abiotic factors. Microorganisms inhabiting within the plant organs as endophytes play a pivotal role in its functional biology (Potts et al., 2023; Sharma et al., 2022). The structure and diversity of plant microbial community has already been reported to modulate the plant growth, yield, stress tolerance and disease resistance (Porter et al., 2020). Even though most of the studies have utilized the role or function of microorganism in plant, the major drivers affecting the microbial assembly is still lacking (Perreault et al., 2021). The early stages of plant growth such as seed germination, seedling development, and root initiation are critical and highly susceptible phases where microorganisms can have determining impact. By understanding the microorganisms involved in these processes and the major factors influencing these early interactions, novel insights could be generated on microbial associations that promote early-stage plant health (Olanrewaju et al., 2024). This could even open up opportunities to manage the microbiome and there by to contribute to sustainable alternative to synthetic chemicals to enhance the plant resilience, growth, and yield (Muhammad et al., 2024). At the same time, microorganisms possessing anti-phytopathogenic properties provide an effective alternative for managing plant disease. This disease suppression could work through the antibiosis, competition, parasitism, or predation (Arseneault and Filion, 2017). The presence of antibiotic producing microorganisms have also been shown to have the potential to reduce the disease severity caused by plant pathogens (Kinsella et al.,2009; Raaijmakers et al.,2009). In theory, the disease-suppressing abilities of these microorganisms could be harnessed within the plant systems to biologically control the pathogens (Roca and Matilla, 2023)

Niches play a crucial role in shaping the structure and assembly of microbial communities, as environmental factors favour the ecological traits and competitive dynamics within a given locality. Each organism within these communities exhibits distinct competitive abilities for the biotic and abiotic resources available, resulting in a coexistence through niche partitioning (Gustavo Santoyo 2022). A deeper understanding of these factors, both within and with plant hosts can enhance our understanding on the microbiome assembly patterns. This is important as the knowledge on microbial community composition in specific niches can provide insights into their plant beneficial properties. By detailed scientific insights into these native communities, it becomes possible to monitor them or even introduce new, beneficial communities tailored to support plant health, counteract soil borne pathogens, and also to enhance the crop resilience (Harman et al., 2021; Kumawat et al., 2023). For the same, identification and expansion of ecological niches of beneficial microorganisms is essential irrespective of their natural or introduced origin. However, not all the soil microorganisms have the ability to colonize or associate with the plant, as the environment and plant species can have determining effects on the associated microbial community (Liu et al., 2020; Walsh et al., 2021)

Although the influence of soil on plant microbiome is well documented across various plant species and geographic regions, still there is a lack of comprehensive understanding on how the soil microbiome composition varies across different soil types (Dastogeer et al., 2020; Walsh et al., 2021). This is especially important with respect to crop plants like rice where the soil microbiome influence on rice microbiome and there by plant productivity can open up novel opportunities to improve its yield. This knowledge gap is largely due to the variability in soil factors across different geographic regions, as well as fluctuations in biotic and abiotic factors. Given the complexity of studying the entire soil profile and microbial communities, a practical approach could be the monitoring of cultivated areas of rice across Kerala. This targeted monitoring can yield valuable insights into the plant-soil-microbe interactions in diverse environmental contexts and which thereby be helpful for predicting and to potentially manage the beneficial microbial dynamics. Influence of its soil microorganisms are the immediate source of plant microbial communities, a better understanding on this scenario will enable to explore the plant microbiome management and modulation (Trivedi et al., 2022).

Understanding the extent to which seed-associated microorganisms influence host resistance and productivity is crucial for enhancing plant growth and yield. Seeds harbor a diverse community of microorganisms that can colonize emerging seedlings, directly or indirectly impacting plant health and development. (Rodriguez et al., 2009; Malfanova et al., 2013; War et al., 2023). While the horizontal transfer of microorganisms from soil to plants has been widely studied, vertical transmission from seed to plant remains less understood, as the seed microbiome is often not directly characterized before planting (Abdelfattah et al., 2023;War et al., 2023). Seed surface sterilization, a common practice used to minimize the pathogens, can obscure the true contribution by the seed-associated microorganisms to the overall microbiome of germinating seedlings (Walsh et al., 2021). Exploring how the root microbiome influences the seedlings across diverse soil microbiome context can provide crucial insights into the relative role of seed and soil derived microbes in shaping plant functions (Ofek et al., 2011). This

knowledge could lead to more precise strategies for fostering the beneficial microbial interactions in plants and thereby to enhance the productivity through microbiome management.

To understand the influence of the soil microbiome on plant, it requires sampling studies by exposing seedlings to diverse cultivated soil types. To achieve this, current study has been designed to collect various soil samples from various paddy fields of Kerala, India, to examine the impact of soil microbiome on microbial distribution of growing seedlings.

Materials and methods

Soil collection

For this study, soil samples were collected from Uma rice (MO16) from cultivated fields across five districts in Kerala, India (Supplementary Table 1). To ensure the comprehensive representation, composite soil sampling was conducted during October 2024 which is the second season for Uma cultivation (Mundakan- winter season). From each site, approximately 30 volumetric ounces of soil were collected from a depth of 10 cm, after removing any litter layer if present. The collected samples were transported to the laboratory at ambient temperature and stored at 4°C until further processing. Each soil sample was sieved to 2 mm, homogenized, and used for DNA extraction, while the remaining soil was stored at 4°C.

Seed germination study

To assess the influence of distinct soil microbial communities on the rice seedling, Uma rice variety were collected from the Rice Research Station Pattambi, Kerala. Seeds were placed in rooting trays containing soils collected from five districts of Kerala. The rooting trays were incubated at room temperature for seven days. Each soil sample was tested with 5–8 seeds per slot, with two replicates per soil type (N = 5 × 2 = 10) (Supplementary Table 2). Uma control samples were cultivated in a sterilized agar medium to exclude any influence of soil microbiome.

After one week of growth, seedlings exhibited varying sizes, with roots averaging 7.0 cm and shoots averaging 9.0 cm in length. The germination rate of the Uma cultivar was same for all the five soil types/soil treatments. For the DNA extraction, seedling tissues were processed by carefully removing the seeds and seed casings by using sterile forceps. The remaining plant tissue, consisting of roots and leaves, were collected from all germinated seedlings, placed on parchment paper, and stored at -20°C until further processing.

Sequencing of 16S rRNA and 18S rRNA

The V4 region of the 16S rDNA gene was amplified by using the primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACNVGGGTWTCTAAT-3'), while the 18S rDNA gene

was amplified with SSUF04 (5'-GCTTGTCTCAAAGATTAAGCC-3') and SSUR22 (5'-GCCTGCTGCCTTCCTTGGA-3') (Walsh et al., 2021). PCR was performed under the conditions of initial denaturation at 95°C for 15 s, annealing at 60°C for 15 s, extension at 72°C for 2 min, followed by a final extension at 72°C for 10 min and cooling at 4°C. Amplification was conducted in duplicate for 10 root samples, 10 leaf samples, 5 composite soil samples, and 2 control samples each for root and leaf. The samples were sequenced at the School of Biosciences, Mahatma Gandhi University (Kottayam, India), using the Oxford Nanopore Technologies (ONT) GridION platform. The amplicons were pooled, tagged with barcode sequences, and processed for sequencing.

Sequence processing and data cleaning

The reads generated were analyzed by using the metagenomics workflow of the EPI2ME software. This workflow utilizes Minimap2 (version 2.26-r1175) to align sequences against the NCBI 16S and 18S databases, as well as the SILVA database. Kraken (version 2.1.3) was used to assign taxonomic labels to the metagenomic sequences. The study included five leaf samples, five root samples, and five composite soil samples from rice cultivated areas in Kerala along with the root and leaf control samples. Both the 16S rDNA and ITS analyses were conducted to identify the bacterial and fungal populations present in the samples. The abundance table generated for bacterial and fungal populations contained entries belonging to groups, which were manually removed. The abundance tables and taxonomy files were subsequently used for further analysis in R (R Core Team 2021). To normalize the sequencing depth when necessary, samples were rarefied before downstream analyses (Yinglin and Xia, 2023). The R packages employed for data analysis included phyloseq (McMurdie and Holmes 2013) vegan (Oksanen et al., 2024), DESeq2 (Love et al., 2014), and ampvis2 (Andersen et al., 2018), while plots were created using ggplot2 and UpSetR. Statistical significance was assessed by using the Wilcoxon test.

Tree construction

The top 500 organisms in the abundance table were selected for the phylogenetic analysis. Among these, several organisms did not found to have a corresponding 16S sequence annotated in the SILVA database and were therefore removed from the analysis. Additionally, organisms with sequences shorter than 1200 nucleotides in the SILVA database were excluded. As a result, only 417 sequences were included for the bacterial study, and only 251 were selected for the fungal study in the phylogenetic analysis. Phylogenetic analysis was performed using PhyML (http://www.atgc- montpellier.fr/phyml/) and the substitution models were estimated by using the Bayesian Information Criterion with random starting trees, and bootstrapping was conducted 100 times and the final tree was visualized using the ggtree, ape, and ggplot2 packages.

Results

To investigate the influence of the soil microbiome on seedling microbiome development, rice seeds were cultivated in five distinct soil types, each representing a separate treatment. To evaluate the contribution of seed-derived microorganisms in shaping the seedling microbiome, the control seeds were not surface-sterilized, allowing for the characterization of the associated bacterial and fungal taxa. In this study, bacterial and fungal communities in the root, leaf, and soil were analysed by using 16S rDNA and 18S rDNA sequencing.

Microbial richness comparison across sample groups

The richness analysis, represented by Operational Taxonomic Units (OTUs), highlights significant variations in bacterial and fungal diversity across soil, root, and leaf samples, emphasizing the impact of environmental conditions and treatment effects (Fig. 1). In bacterial communities, soil composite exhibited the highest microbial richness (901.4 ± 239.5 OTUs). Leaf control maintained relatively low bacterial richness (190 OTUs), while leaf treatment showed a reduction in diversity (172.8 ± 63.1 OTUs), suggesting the treatment caused a shift in microbial composition. Root treatment samples exhibited moderate bacterial richness (435 ± 181.8 OTUs), reinforcing their role as a stable microbial reservoir when compared to the root control 540 (Fig. 1).

Similarly, for the fungal communities, the leaf treatment was found to have the highest richness (878.8 ± 234.93 OTUs), indicating an increase in microbial diversity when compared to the leaf control (709 OTUs), which showed the low richness. Root treatment samples were also found to display higher (613.2 ± 146.02 OTU) than the root control (261), which further supported the idea that applied treatment might have microbial recruitment of root associated microbial communities. Soil composite samples were found to have moderate fungal richness, reinforcing soil as a major reservoir for the plant associated microbiomes. The lower microbial richness in root control compared to the treated root samples suggests a less diverse microbial community in the untreated roots (Fig. 1).

Overall, these findings suggest that the soil treatment used in the study enhanced the microbial diversity in both leaf and root samples, while the soil was found to have a balanced and diverse microbial community. The observed variations in microbial richness underscore the critical role of environmental conditions and plant-microbe interactions in shaping the microbial communities across plant compartments

Rank abundance analysis of microbial communities

The rank abundance analysis illustrated distinct microbial distribution patterns across the soil, root, and leaf samples, highlighting differences in diversity and evenness among the sample types (Fig. 1). Soil samples exhibit the highest microbial evenness, with a gradual slope indicating a well-balanced community where no single taxon dominates, reinforcing its role as a microbial reservoir that contributes to microbial recruitment in root and leaf compartments. In contrast, the root and leaf control samples

displayed a steeper curve, suggesting that a few dominant taxa contribute significantly to the microbial abundance, leading to lower overall evenness due to strong plant selection pressures. Treatment effects are particularly evident in root samples, where the microbial distribution became more evident when compared to the control, indicating increased microbial richness and diversity. In leaf treatment samples, microbial dominance remained relatively unchanged, suggesting that plant specific selection pressures continue to shape the microbiome despite the treatment. The observed variations in rank abundance across the used conditions suggest that the treatment used could enhance the microbial diversity and evenness, particularly in roots, leading to potential microbial shifts that could influence the plant health and productivity. These findings emphasize the selective nature of plant microbe interactions, where the specific microbial taxa become dominant depending on the environmental conditions and treatment effects, shaping the overall microbial community structure in agricultural systems.

Microbial diversity assessment using Shannon Index

The Shannon Diversity Index (H') analysis has highlighted distinct bacterial and fungal diversity patterns across the leaf, root, and soil samples, with higher values indicating greater species diversity and evenness. In bacterial communities, soil composite samples exhibited the highest Shannon index (3.3 ± 0.3), suggesting a highly diverse and well-balanced microbial community, significantly different from root treatment ($p = 0.019$) and other sample types ($p = 0.28$). Root treatment samples here displayed moderate bacterial diversity (2.8 ± 0.28), which is significantly higher than root control ($p = 1.1e-05$), indicating the soil treatment could enhance the microbial colonization in the rhizosphere (Fig. 2). In leaf treatment, the bacterial diversity is the lowest (1.6 ± 0.06). In fungal communities, soil also maintained the highest diversity (3.9 ± 0.5), reinforcing its role as a microbial reservoir. Root treatment samples showed a higher fungal diversity (3.6 ± 0.24) when compared to the root control (2.8, $p = 0.27$), suggesting that treatment influences fungal recruitment. Leaf samples exhibited the lowest microbial diversity, with leaf treatment showing a slight increase (2.8 ± 1.03) compared to the leaf control (2.4, $p = 0.075$), indicating that treatment positively impacts fungal and bacterial diversity but remains limited by plant specific selection pressures in the phyllosphere. Statistical comparisons further confirm significant differences between root and soil samples ($p = 0.00067$ for bacteria), reinforcing the trend of increasing microbial diversity from leaves to roots to soil (Fig. 2). These findings emphasize the impact of environmental conditions and treatment on microbial community structure, with soil treatment significantly enhancing root associated bacterial and fungal diversity, offering potential applications for the soil health, plant microbe interactions, and sustainable agriculture.

Beta diversity analysis (Bray-Curtis Dissimilarity)

The beta diversity analysis, based on Bray-Curtis dissimilarity and Principal Coordinates Analysis (PCoA) has revealed distinct bacterial community composition across the soil, root, and leaf samples, with the first two principal coordinates (PCo1 and PCo2) explaining 50.3 and 16.6% of the variation, respectively. Soil composite samples form a well separated cluster, which indicated a unique microbial community distinct from the plant associated compartments. Root associated samples (root control and treatment)

clustered separately, with root treatment forming a more compact group, suggesting a moderate shift in microbial composition due to the treatment, while root control exhibited greater variation. Similarly, leaf associated samples (leaf control and treatment) displayed distinct clustering, with leaf treatment forming a tighter group, due to the treatment induced microbial shift, whereas the leaf control samples were observed to be more scattered.

For fungal community, the clustering patterns along PCo1 (33.5%) and PCo2 (5.5%) highlighted the fungal communities to be primarily influenced by both plant compartment (leaf, root and soil) and treatment conditions (Fig. 3). These findings suggest that treatment applied might have resulted in a more consistent and distinct fungal composition in both root and leaf samples, while the soil maintains a stable but separate microbial profile.

Differential abundance of microbial communities at the Phylum level

The differential abundance analysis at the Phylum level has revealed distinct bacterial and fungal community compositions across the leaf, root, and soil samples, emphasizing the influence of plant compartments and environmental conditions on microbial diversity. Leaf samples exhibited a relatively stable microbiome, with bacteria dominated by *Proteobacteria* (*Pseudomonadota*) and *Verrucomicrobiota* and fungi primarily consisting of *Ascomycota*, with lower but consistent abundances of *Basidiomycota* and *Zoopagomycota*. The conserved nature of the leaf microbiome has suggested strong plant specific selection pressures that limit the microbial colonization. Root samples displayed greater microbial diversity, with bacterial communities enriched with *Bacillota, Bacteroidota*, and *Actinomycetota*, while the fungal communities included *Ascomycota, Basidiomycota*, and *Mucoromycota* (Fig. 4). The increased microbial richness in root samples highlights the rhizosphere as a microbial hotspot, whereas the beneficial groups such as *Bacillota* contribute to plant growth and pathogen suppression, and fungal taxa such as *Curvularia, Bipolaris*, and *Starmarella* in root treatment has suggested treatment induced fungal recruitment influencing the plant health. Soil samples harbor the highest microbial diversity, with bacteria including *Acidobacteriota*, *Chloroflexota*, *Planctomycetota*, *Cyanobacteria*, and *Fusobacteria*, and fungi dominated by *Ascomycota, Basidiomycota, Mucoromycota*, and *Cryptomycota*, reinforcing the role of soil as a microbial reservoir supporting the plant associated microbial colonization. The presence of unknown fungal taxa, particularly in leaf treatment and soil samples, suggests the potential for novel or understudied microbial groups with ecological significance. The observed differences highlight the selective nature of plant microbe interactions, with the soil acting as the most diverse microbial environment, roots serving as an intermediate microbial exchange zone, and leaves maintaining a more selective microbiome. Treatment induced shifts in microbial communities further emphasize the potential for microbiome based interventions in sustainable agriculture, where the targeted manipulation of bacterial and fungal populations could enhance plant growth, stress tolerance, and nutrient dynamics.

Upset plot analysis of microbial community overlaps

The upset plot analysis highlights the distribution of bacterial and fungal taxa across the soil, root, and leaf microbiome, emphasizing the shared and unique microbial features influenced by environmental conditions and treatment effects. In bacterial communities, intersection of all treatment groups (274 OTUs) represents a core microbiome common across all the multiple environments. Soil samples exhibited the most number of unique OTUs (795), functioning as a reservoir for microbial recruitment into the plant associated compartments. Root (199) and leaf (184) microbiome displayed distinct bacterial compositions, with treatment induced shifts evident in both the compartments, suggesting treatment enriched beneficial microorganisms or suppresses specific taxa (Fig. 4).

Diversity and community composition of soil, root and leaf

The soil samples exhibited a distinct microbial composition and abundance compared to the root and leaf samples, reflecting the diverse environmental conditions from which they were collected. These soils were sourced from the highest paddy-cultivated fields of Kerala, each characterized by unique terrain and soil properties. The microbial composition varied significantly among different sample types, including soil composite, root treatment, root control, leaf treatment, and leaf control reflecting the influence of soil environment and plant-microbe interactions on microbial diversity.

The unknown category exhibited the highest relative abundance, particularly in soil composite, root treatment, and leaf control samples, suggesting the presence of unclassified or less studied microbial taxa. *Xanthomonas*, a plant associated bacterial genus, was notably more prevalent in leaf treatment samples, indicating its potential role in the rice phyllosphere microbiome. *Escherichia* and *Salmonella* also appeared across multiple sample types, implying environmental transmission and possible seed or soil origins (Supplementary Fig. 1).

Comparing the microbial compositions, soil composite samples exhibited the highest diversity, reflecting the complexity of the soil microbiome. Root associated samples showed an enrichment of *Burkholderia* and *Bacillus*, indicating microbial selection at the root level, while leaf-associated samples displayed distinct microbial compositions, with increased abundance of *Brucella* and *Limnospira*, suggesting the microbial colonization from environmental sources. Differences between treatment and control samples highlighted the impact of soil inoculation on microbial recruitment, as root and leaf treatment samples displayed distinct microbiomes compared to their respective controls. Control samples exhibited lower diversity and microbial abundance, suggesting a more limited microbial community. Overall, the results suggest that soil inoculation significantly influences the seedling microbiome composition, with distinct microbial recruitment patterns in root and leaf compartments. The presence of both beneficial and potentially pathogenic bacteria, such as *Bacillus* and *Xanthomonas*, highlights the potential role of microbial communities in plant health and development.

The relative abundance analysis at the genus level further revealed distinct fungal community structures across the soil, root, and leaf samples under different conditions. *Aspergillus* was highly abundant in soil and leaf treatment, suggesting its dominance in soil and treated leaf environments. The presence of

unknown fungal taxa in high proportions, particularly in leaf treatment was also observed. *Penicillium, Malassezia*, and *Sarocladium* were present at lower but consistent abundances across all the sample types, while *Curvularia*, *Bipolaris*, and *Starmarella* were enriched in the root treatment, suggesting that treatment influenced root-associated fungal communities (Supplementary Fig. 1). Soil samples exhibited the highest fungal diversity, reinforcing their role as microbial reservoirs, whereas both the root and leaf communities showed treatment induced shifts. These findings highlight the impact of environmental and treatment factors on the fungal composition and suggest potential microbial interactions influencing the plant health.

Keystone species analysis

The analysis of bacterial and fungal keystone species across the leaf treatment, root treatment, and soil composite revealed distinct community structures, with minimal overlap between environments. The leaf treatment exhibited the highest bacterial diversity, spanning multiple phyla and forming a highly interconnected network, indicative of dynamic microbial interactions (Fig. 6). In contrast, the root treatment harbored a more selective microbial community with fewer but stronger interactions, likely reflecting functional specialization. The soil composite displayed a broader yet balanced microbial network, with *Candidatus saccharibacteria* uniquely present, suggesting its role in soil stability. *Pseudomonadota* was a dominant keystone group across all treatments, underscoring its ecological significance in plant-associated microbiomes. While bacterial keystone species were distributed across 5 to 10 different phyla, fungal keystone species were primarily restricted to *Basidiomycota* and *Ascomycota* across treatments and the soil composite. Notably, *Talaromyces viridis* was the only fungal keystone species common to both the leaf treatment and soil composite. Supplementary Tables (3 and 4) provide a detailed list of the identified bacterial and fungal keystone species. This variation could be influenced by the environmental factors, metabolite availability, and the specific ecological functions of these organisms. Interestingly, despite these differences, the keystone species predominantly belong to a limited number of phyla, which may reflect their specialization and interdependent interactions within their respective ecosystems.

Phylogenetic tree

A total of 417 bacterial taxa and 251 fungal taxa were retained for the phylogenetic analysis, ensuring high-quality sequence data for the accurate evolutionary assessments. The phylogenetic trees for bacteria and fungi were provided as Supplementary Figures (2 and 3).

Discussion

Understanding the influence of the soil microbiome on plant-associated microbiomes is complex, as it is difficult to isolate this relationship from the effects of climatic and soil edaphic factors. Many studies have emphasized the critical role of the soil microbiome in shaping the plant health, particularly through its influence on nutrient cycling, disease suppression, and plant growth promotion (Mendes et al., 2013; van der Heijden et al., 2008). In our study, we also identified the presence of beneficial microorganisms

that could contribute to plant growth and resilience. However, while the role of the soil microbiome in nutrient cycling and plant health has been extensively studied, the vertical transmission of microbes from soil to seeds and leaves remains a relatively underexplored area.

Microbial transmission from soil to plants occurs through multiple pathways, including root colonization, phyllosphere interactions, and potential seed endophyte recruitment (Compant et al., 2010). While some studies suggest that certain microorganisms may be vertically transmitted through seeds (Shade et al., 2017), the mechanisms governing the microbial movement from soil to aboveground plant tissues, including leaves, require further investigation. Our findings align with the previous studies by identifying microbial taxa associated with different plant compartments, emphasizing the need for further research on how soil-derived microbes colonize and persist within plant tissues over time. Understanding these dynamics could have significant implications for sustainable agriculture, as harnessing beneficial microbes for plant health and productivity may provide an alternative to chemical fertilizers and pesticides.

In our study, soil samples exhibited the highest microbial diversity, with *Desulfuromonas, Bacillus, Burkholderia*, and *Brucella* being the most abundant, highlighting their roles in sulfur cycling, plant growth promotion, and soil fertility maintenance. Root samples, particularly in the treated group, show an increased relative abundance of *Xanthomonas, Limnospira*, and *Escherichia*, whereas the control root samples displayed lower microbial diversity, indicating that the soil treatment significantly influences root-associated microbial communities. The dominance of *Xanthomonas* in root samples suggests the presence of plant-associated pathogens, necessitating further investigation. Leaf samples were found to follow a similar microbial pattern as that of the root samples, with *Brucella* and *Limnospira* being predominant in the treated group, while control leaves exhibited the lowest microbial diversity among all samples, reinforcing the idea that plant compartments selectively recruit specific microorganisms. Notably, the microbial composition at the phylum level remains stable in leaf samples, while root and soil communities share greater similarities. The observed microbial diversity trends suggest that soil serves as a microbial reservoir, influencing the root and leaf-associated microbiomes. The differential colonization of beneficial microorganisms like *Bacillus* and *Limnospira* alongside potential pathogens like *Xanthomonas* and *Escherichia* highlights complex plant-microbe interactions that could impact the plant health and productivity. These findings emphasize the need for further functional studies to understand the implications of these microbial communities on the plant growth and crop yield.

Also the relative abundance analysis at the genus level revealed distinct fungal community structures across the soil, root, and leaf samples, indicating that environmental conditions and treatments significantly influence the fungal composition. The dominance of *Aspergillus* in both soil and leaf treatment suggests its adaptability and potential functional significance in soil and plant environments. *Aspergillus* species are well documented for their roles in organic matter decomposition, soil nutrient cycling, and plant-pathogen interactions (Perrone & Susca, 2017; Niji etal., 2023). Some *Aspergillus* species have been reported as beneficial endophytes, while others can act as opportunistic pathogens, emphasizing the need for further characterization on its functional role in the plant microbiome. Several

fungal genera, including *Penicillium, Malassezia*, and *Sarocladium*, were consistently present across all sample types. *Penicillium* species are widely recognized for their ability to produce secondary metabolites with antimicrobial properties, making them important contributors to plant disease suppression (Visagie et al., 2014). *Malassezia*, a genus typically associated with human and animal skin, has recently been detected in plant microbiomes, raising questions about its ecological role in plants (Lorch et al., 2018). Meanwhile, *Sarocladium* includes both plant-beneficial and pathogenic species, with some strains known to enhance plant growth or contribute to disease resistance (López-Fernández et al., 2018). The enrichment of *Curvularia, Bipolaris*, and *Starmarella* in root treatment samples suggests that the treatment exerts a strong influence on root-associated fungal communities. *Curvularia* and *Bipolaris* are commonly reported in plant-microbe interaction studies, with some species acting as pathogens while others function as plant-growth-promoting fungi (Phoulivong et al., 2012). *Bipolaris* species, for instance, have been associated with disease resistance mechanisms in certain crops, as well as with endophytic benefits under specific conditions (Manamgoda et al., 2014). The presence of *Starmarella*, a lesser known fungal genus, may indicate a niche specific adaptation in response to the treatment, warranting further functional studies. Soil samples exhibited the highest fungal diversity, reinforcing their role as microbial reservoirs that contribute to the recruitment of plant-associated microbes. This aligns with previous findings showing that soil harbors a vast and functionally diverse fungal community that can influence plant health through mechanisms such as nutrient solubilization, pathogen suppression, and mycorrhizal associations (van der Heijden et al., 2015; Bahram et al., 2018).

The beta diversity analysis, based on Bray-Curtis dissimilarity and Principal Coordinates Analysis (PCoA), highlights distinct microbial community compositions across the soil, root, and leaf samples, indicating that both plant compartment and treatment conditions significantly shape microbiome structure. The first two principal coordinates (PCo1 and PCo2) have revealed a clear separation between soil and plant-associated microbial communities. These findings align with previous studies that emphasize the compartmentalization of microbial communities within plant-associated niches due to the selective pressures exerted by the host plant (Bulgarelli et al., 2013; Edwards et al., 2015).

In our study, soil composite samples formed a distinct cluster, reinforcing the concept that soil harbors a unique and relatively stable microbial community. This observation is consistent with prior research, which has demonstrated that soil microbiomes are primarily shaped by edaphic factors such as pH, organic matter content, and nutrient availability (Fierer, 2017; Mendes et al., 2013). However, while soil serves as the primary microbial reservoir, only a subset of microorganisms successfully colonizes the plant roots and leaves, highlighting microbial selection and niche differentiation. This selective recruitment has been well documented, particularly in rhizosphere studies showing that plants actively shape their microbiomes through root exudates and immune responses (Berendsen et al., 2012; Sasse et al., 2018).

The differentiation between root control and root treatment samples in our study further supports the idea that external treatments (different soils) can influence the microbial selection. The more compact clustering of root treatment samples suggests that the applied treatment led to a structured and stable

microbial community, whereas the greater variation in root control samples indicates a more heterogeneous root microbiome. Similar findings have been reported in studies where biofertilizers and organic amendments altered the root-associated microbial communities, leading to increased recruitment of beneficial microbes involved in nutrient acquisition and plant growth promotion (Hartman et al., 2018; Lareen et al., 2016).

A comparable pattern was observed in leaf associated microbiomes, where the leaf treatment samples exhibited more compact clustering, whereas leaf control samples showed greater variability. This suggests that treatment conditions play a role in shaping the phyllosphere microbiome, potentially through systemic plant responses or microbial migration from the rhizosphere. Previous studies have reported similar results, indicating that phyllosphere microbial communities are highly dynamic and influenced by environmental factors such as humidity, UV exposure, and microbial dispersal from soil or airborne sources (Vorholt, 2012; Meisner et al., 2018). Our results support the hypothesis that root to shoot microbial migration may contribute to leaf microbial assembly, a phenomenon that has been increasingly recognized in plant-microbe interaction studies (Compant et al., 2010; Hardoim et al., 2015). Our findings align with prior studies that have demonstrated how plant microbiomes differ significantly across root, leaf, and soil compartments, driven by plant-specific selection pressures (Bulgarelli et al., 2013; Edwards et al., 2015). However, the previous studies have extensively characterized the rhizosphere microbiome and its role in plant health, the vertical transmission of microbes from soil to seed and leaves remains less explored. Our study contributes to this growing field by providing evidence on microbial differentiation across plant compartments and highlighting the role of treatment in shaping microbial assembly in *Oryza sativa*. Future research should employ whole genome, metagenome and transcriptomic approaches to further investigate the functional roles of these microbial shifts and their implications for plant growth, health, and resilience under varying environmental conditions.

Conclusion

This study underscores the crucial role of microbial communities in shaping the plant health and development, highlighting the influence of environmental conditions and the impact of different soil types on root and leaf microbiomes. Soil serves as a microbial reservoir, contributing to the recruitment and establishment of distinct microbial communities in plant compartments. Variations in soil composition could influence the microbial diversity, with different soils supporting unique bacterial and fungal taxa that shape the root and leaf microbiomes. The presence of beneficial and potentially pathogenic taxa suggests complex plant-microbe interactions that affect the plant resilience, nutrient cycling, and disease suppression. These findings reinforce the dynamic nature of plant-associated microbiomes and the potential for targeted microbial interventions to enhance the plant health and productivity. Further research is needed to explore the functional roles of these microbial communities and the long-term impact of soil driven microbial variations on plant ecosystems.

Declarations

Conflict of interests

The authors declare that they have no conflict of interests.

Clinical trial number

not applicable

Funding

This work was supported by Rashtriya Uchchatar Shiksha Abhiyan (RUSA 2.0), Government of India.

Author contributions.

RU contributed towards conceptualization, methodology, ONT Giridion sequencing, data interpretation, writing, editing and finalization of original manuscript. PVK conducted bioinformatics analysis, editing and finalization of manuscript. PS conducted manuscript preparation and sequencing, editing and finalization of manuscript. JK and REK contributed towards conceptualization, methodology, fund acquisition, editing and finalization of manuscript.

Acknowledgements

The authors acknowledge the lab facilities and instrumentation facilities utilized from: (a) RUSA 2.0 (b) KSCSTE-SRS (c) DBT-SURE (d) Kairali Gaveshana Puraskaram Research Grant- KSHEC (e) SAIF instrumentation facility, IIUCN, School of Chemical sciences, School of Environmental Sciences.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Abdelfattah A, Tack AJM, Lobato C, Wassermann B, Berg G (2023) From seed to seed: the role of microbial inheritance in the assembly of the plant microbiome. Trends Microbiol 31(4):346–355. 10.1016/j.tim.2022.10.009
2. Andersen KS, Kirkegaard RH, Karst SM, Albertsen M (2018) ampvis2: an R package to analyse and visualise 16S rRNA amplicon data. bioRxiv. https://doi.org/10.1101/299537

3. Arseneault T, Filion M (2017) Biocontrol through antibiosis: exploring the role played by subinhibitory concentrations of antibiotics in soil and their impact on plant pathogens. Can J Plant Pathol 39(3):267–274. 10.1080/07060661.2017.1354335
4. Bahram M, Hildebrand F, Forslund SK, Anderson JL, Soudzilovskaia NA, Bodegom PM, Bork P (2018) Structure and function of the global topsoil microbiome. Nature 560(7717):233–237
5. Berendsen RL, Pieterse CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17(8):478–486
6. Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–838
7. Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–838
8. Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved, and prospects for utilization. Appl Environ Microbiol 76(13):4945–4950
9. Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S, Sundaresan V (2015) Structure, variation, and assembly of the root-associated microbiomes of rice. Proceedings of the National Academy of Sciences, 112(8), E911-E920
10. Fierer N (2017) Embracing the unknown: Disentangling the complexities of the soil microbiome. Nat Rev Microbiol 15(10):579–590
11. Gustavo Santoyo (2022) How plants recruit their microbiome? New insights into beneficial interactions. J Adv Res 45–58. doi.org/10.1016/j.jare.2021.11.020
12. Hardoim PR, van Overbeek LS, van Elsas JD (2015) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 23(12):749–758
13. Harman G, Khadka R, Doni F, Uphoff N (2021) Benefits to Plant Health and Productivity From Enhancing Plant Microbial Symbionts. Front Plant Sci 11:610065. 10.3389/fpls.2020.610065
14. Hartman K, Tringe SG, Buckley DH (2018) Metagenomic insights into the impacts of organic amendments on soil health. Annu Rev Plant Biol 69:589–614
15. Kinsella K, Schulthess CP, Morris TF et al (2009) Rapid quantification of Bacillus subtilis antibiotics in the rhizosphere. Soil Biol Biochem. 2009;41(2):374–379
16. Kumawat KC, Sharma B, Nagpal S, Kumar A, Tiwari S, Nair RM (2023) Plant growth-promoting rhizobacteria: Salt stress alleviators to improve crop productivity for sustainable agriculture development. Front Plant Sci 13:1101862. 10.3389/fpls.2022.1101862
17. Lareen A, Burton F, Schäfer P (2016) Plant root-microbe communication in shaping root microbiomes. Plant Mol Biol 90(6):575–587
18. Liu Q, Cheng L, Nian H, Jin J, and Tengxiang Lian (2023) Linking Plant Functional Genes to Rhizosphere Microbes: A Review. Plant Biotechnol J 21:902–917. https://doi.org/10.1111/pbi.13950

19. López-Fernández S, Sampedro I, Sanz M, Dopazo A, Silva L (2018) Sarocladium species: Expanding their role in plant-microbe interactions. Mycologia 110(1):59–68
20. Lorch JM, Palmer JM, Lindner DL, Ballmann AE, George KG, Griffin KJ, Blehert DS (2018) Malassezia vespertilionis sp. nov.: a new cold-tolerant species of yeast from bats susceptible to white-nose syndrome. Mycologia 110(1):27–39
21. Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15(12):550
22. Malfanova N, Kamilova F, Validov S, Chebotar V, Lugtenberg B (2013) Is L-arabinose important for the endophytic lifestyle of Pseudomonas spp? Arch Microbiol 195:9–17
23. Manamgoda DS, Rossman AY, Castlebury LA, Crous PW, Madrid H, Chukeatirote E, Hyde KD (2014) The genus Bipolaris. Stud Mycol 79:221–255
24. McMurdie and Holmes (2013) phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE
25. Meisner A, Jacquiod S, Snoek BL, ten Hooven FC, van der Putten WH (2018) Drought legacy effects on the composition of soil fungal and prokaryotic communities. Front Microbiol 9:294
26. Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37(5):634–663
27. Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37(5):634–663. https://doi.org/10.1111/1574-6976.12028
28. Muhammad A, Kong X, Zheng S, Bai N, Li L, Khan MHU, Fiaz S, Zhang Z (2024) Exploring plant-microbe interactions in adapting to abiotic stress under climate change: a review. Front Plant Sci 15:1482739. 10.3389/fpls.2024.1482739
29. Nji QN, Babalola OO, Mwanza M (2023) Soil Aspergillus Species, Pathogenicity and Control Perspectives. J Fungi (Basel) 9(7):766. 10.3390/jof9070766
30. Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J (2024) vegan: Community Ecology Package. R package version 2.6–6.1
31. Ofek M, Hadar Y, Minz D (2011) Colonization of cucumber seeds by bacteria during germination. Environ Microbiol 13:2794
32. Olanrewaju OS, Glick BR, Babalola OO (2024) Metabolomics-guided utilization of beneficial microbes for climate-resilient crops. Curr Opin Chem Biol 79:102427. 10.1016/j.cbpa.2024.102427
33. Perreault R, Laforest-Lapointe I (2022) Plant-microbe interactions in the phyllosphere: facing challenges of the anthropocene. ISME J 16, 339–345 (2022). https://doi.org/10.1038/s41396-021-01109-3

34. Perrone G, Susca A (2017) Penicillium species and their associated mycotoxins. Methods Mol Biol 1542:107–119. 10.1007/978-1-4939-6707-0_5
35. Phoulivong S, Cai L, Chen H, McKenzie EH, Abd-Elsalam KA, Chukeatirote E, Hyde KD (2012) Colletotrichum: Species, ecology, and novel appressorial morphologies. Fungal Divers 54(1):107–125
36. Porter SS, Bantay R, Friel CA et al (2020) Beneficial microbes ameliorate abiotic and biotic sources of stress on plants. Funct Ecol. 2020;00:1–12. https://doi.org/10.1111/1365-2435.13499
37. Potts J, Brown RW, Jones DL, Cross P (2023) Seasonal variation is a bigger driver of soil faunal and microbial community composition than exposure to the neonicotinoid acetamiprid within Brassica napus production systems. Soil Biol. Biochem. 2023, 184, 109088
38. R Core Team (2021) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org
39. Raaijmakers JM, Paulitz TC, Steinberg C et al (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil 321:341–361. https://doi.org/10.1007/s11104-008-9568-6
40. Roca A, Matilla MA (2023) Microbial antibiotics take the lead in the fight against plant pathogens. Microb Biotechnol 16:28–33. doi.org/10.1111/1751-7915.14185
41. Rodriguez RJ, White JF, Arnold AE, Redman RS (2009) Fungal endophytes: Diversity and functional roles. New Phytol. 2009, 182, 314–330
42. Sasse J, Martinoia E, Northen T (2018) Feed your friends: Do plant exudates shape the root microbiome? Trends Plant Sci 23(1):25–41
43. Shade A, Jacques MA, Barret M (2017) Ecological patterns of seed microbiome diversity, transmission, and assembly. Curr Opin Microbiol 37:15–22. 10.1016/j.mib.2017.03.010
44. Sharma B, Singh BN, Dwivedi P, Rajawat MVS (2022) Interference of climate change on plant-microbe interaction: Present and future prospects. Front Agron 3:725804. 10.3389/fagro.2021.725804
45. Trivedi P, Batista BD, Bazany KE and B. K. Singh. (2022)Plant-Microbiome Interactions Under a Changing World: Responses, Consequences and Perspectives. New Phytol 234: 1951–1959. https://doi.org/10.1111/nph.18016
46. 47, van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11(3):296–310
47. 48, van der Heijden MGA, Martin FM, Selosse MA, Sanders IR (2015) Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytol 205(4):1406–1423. 10.1111/nph.13288
48. Visagie CM, Houbraken J, Dijksterhuis J, Seifert KA, Jacobs K, Samson RA, Kraak B (2014) Diversity and taxonomy of Penicillium in indoor environments. Stud Mycol 78:1–14
49. Vorholt JA (2012) Microbial life in the phyllosphere. Nat Rev Microbiol 10(12):828–840

50. Walsh CM, Becker-Uncapher I, Carlson M, Fierer N (2021) Variable influences of soil and seed-associated bacterial communities on the assembly of seedling microbiomes. ISME J 2748–2762. 10.1038/s41396-021-00967-1
51. War AF, Bashir IF, Zafar A, Kardol RP, Rashid I (2023) Insights into the seed microbiome and its ecological significance in plant life, Microbiological Research
52. Xia Y (2023) Jun Sun Bioinformatic and Statistical Analysis of Microbiome Data. Springer. https://doi.org/10.1007/978-3-031-21391-5

Figures

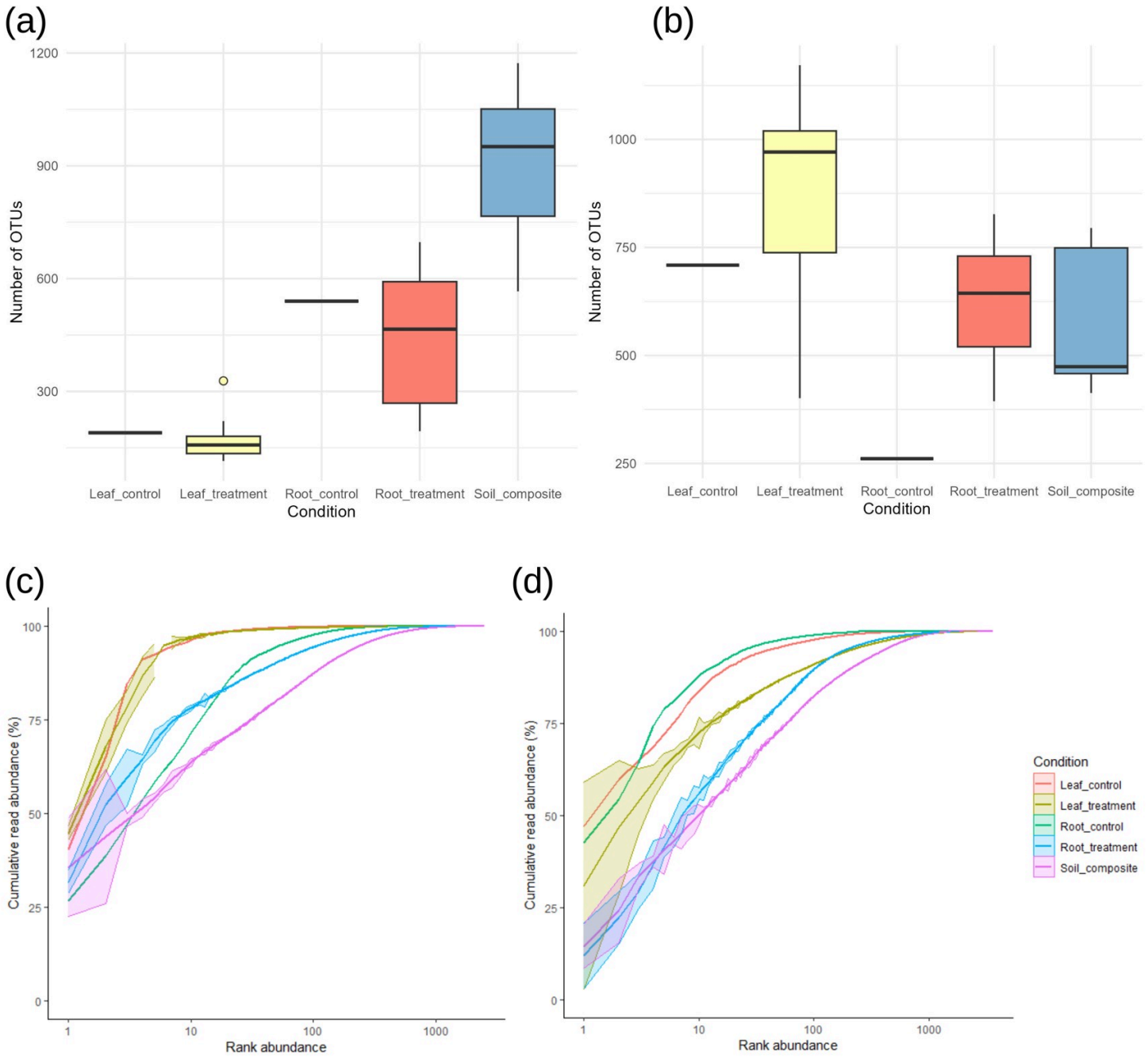


Figure 1

Boxplots comparing OTU richness across different study groups for showing the number of OTUs present in each group in (a) bacteria and (b) fungi. The rank abundance plots showing the species richness and evennes of the groups of (c) bacteria and (d) fungus

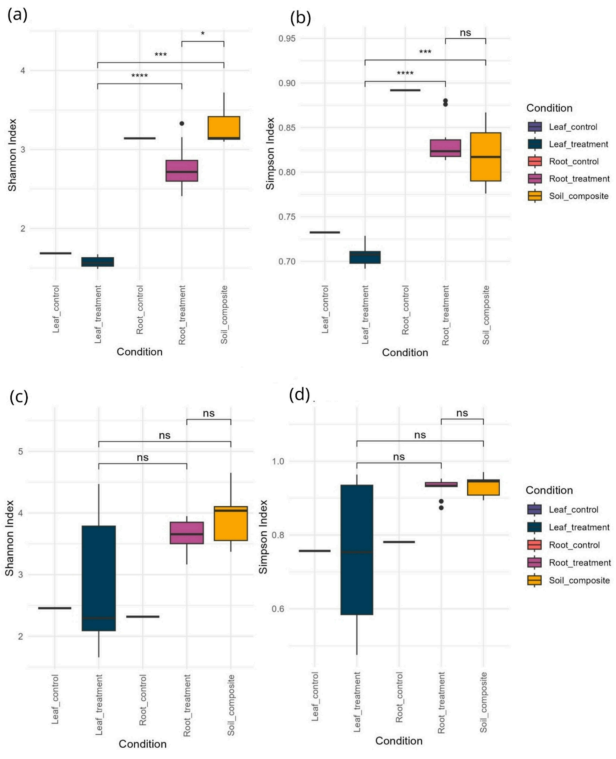


Figure 2. Shannon Diversity Index and Simpson Diversity Index values representing the bacterial and fungal diversity across different sample groups. The figure illustrates the variation in species richness and evenness, with higher index values indicating more diverse and evenly distributed microbial communities. Bacterial (a) Shannon and (b) Simpson and fungal (c) Shannon and (d) Simpson diversity index.

Figure 2

See image above for figure legend.

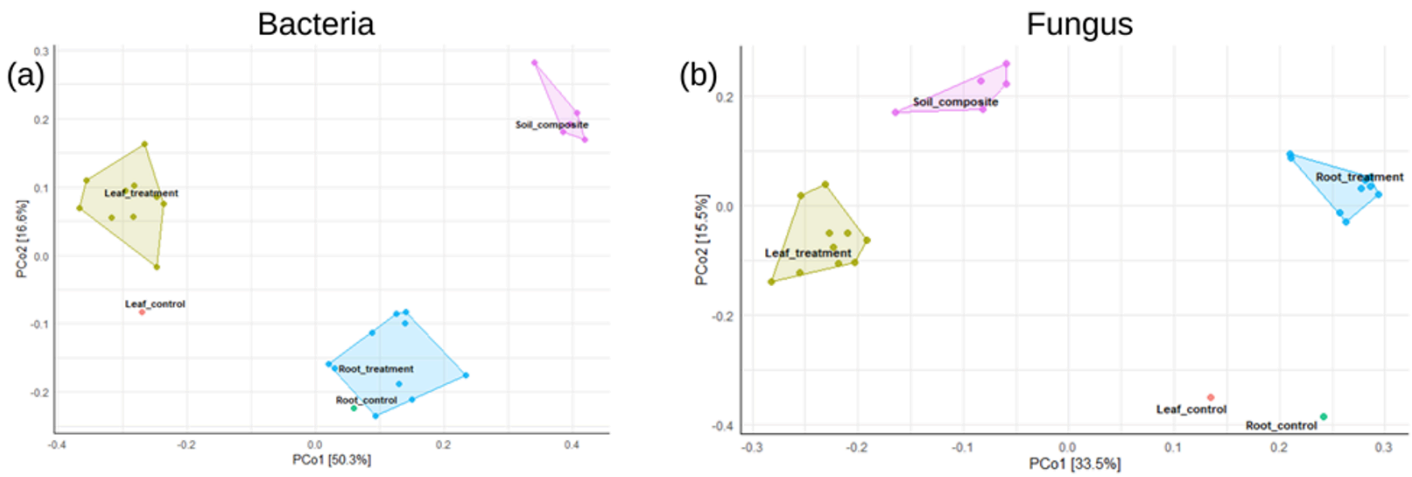


Figure 3

Beta diversity analysis of (a) Bacteria and (b) Fungus

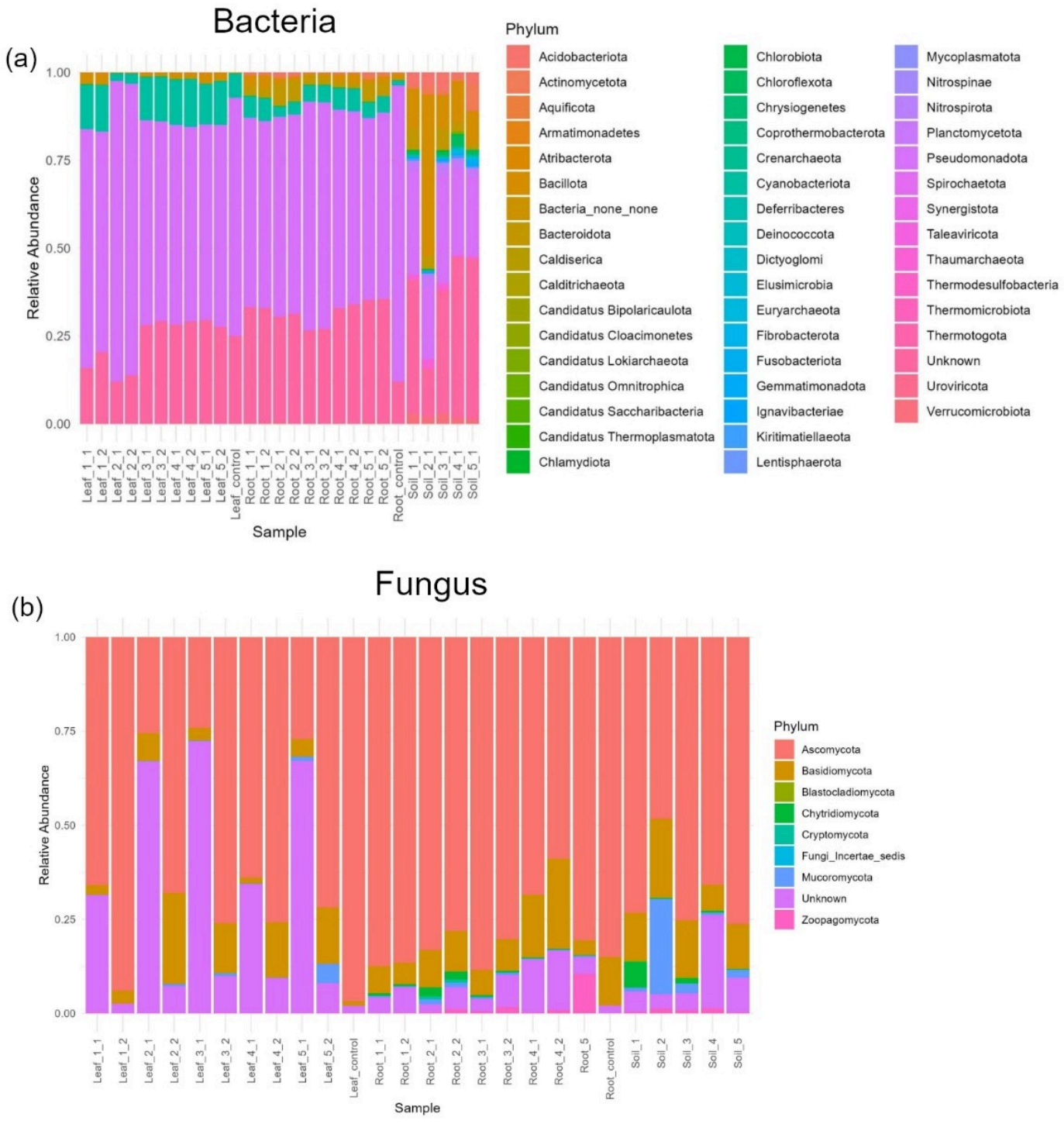


Figure 4

Differential abundance of various phyla in the samples in under the study (a) Bacteria and (b) Fungus.

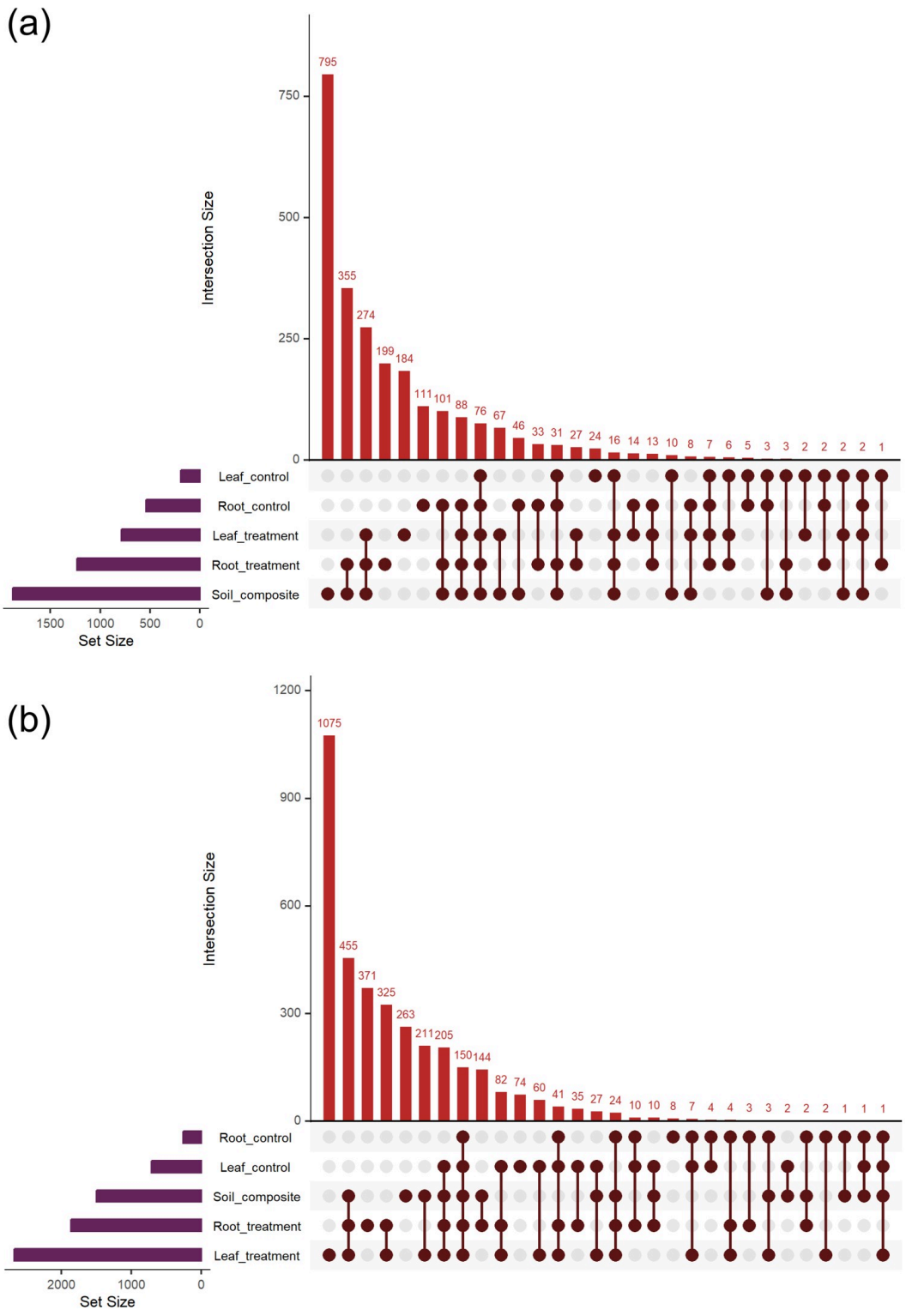


Figure 5

Upset plot illustrating the shared and unique bacterial taxa among different sample groups (a) Bacteria and (b) Fungus. The horizontal bars represent the total number of microbial taxa in each group, while the vertical bars indicate the number of taxa shared among specific sample combinations. The connected dots below the bars highlight the intersections between groups, providing insights into microbial community overlap and exclusivity across different environmental conditions or treatments.

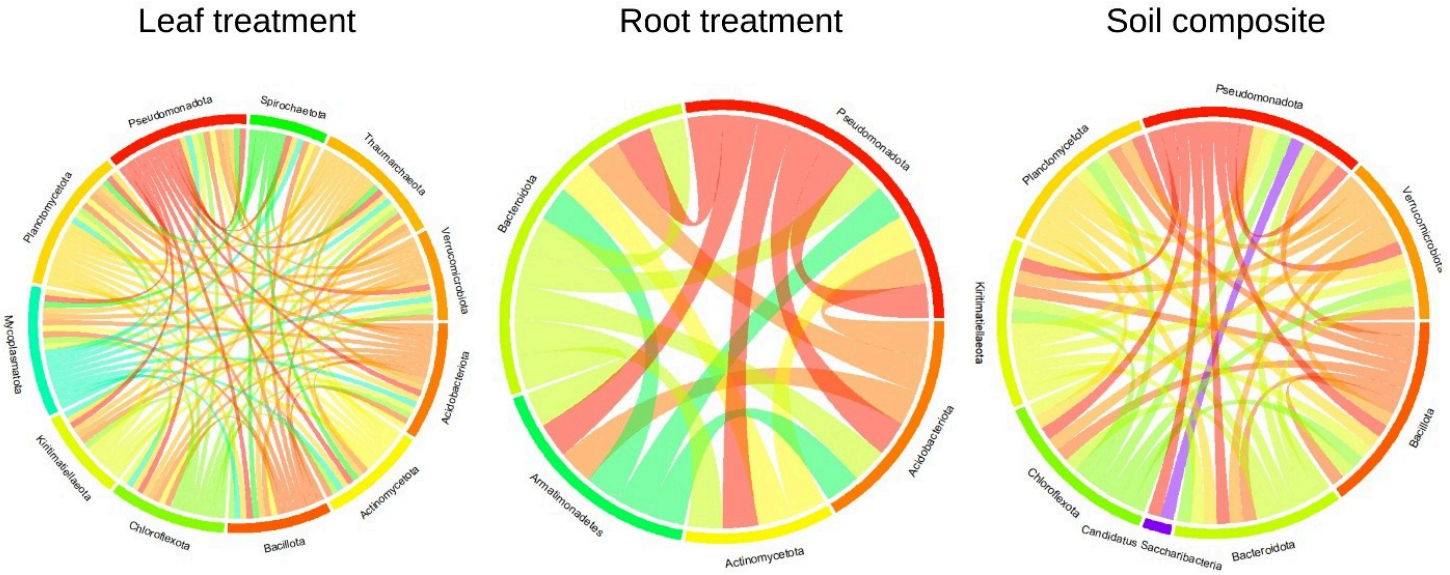


Figure 6

Chord diagram showing the Phyla of bacteria identified as keystone species