



Taxonomic and functional differentiation of soil and thallus microbiomes in *Riccia sorocarpa*

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Abstract

Liverworts, as early-diverging land plants, host unique and ecologically important microbiomes, yet remain largely understudied in microbial ecology. In this study, we investigated the taxonomic and functional composition of microbial communities associated with *Riccia sorocarpa*, focusing on the thallus and adjacent rhizosphere soil. Using nanopore sequencing and metagenomic analysis, we reveal a significantly higher microbial diversity and functional specialization in the thallus microbiome compared to soil. Despite a shared core microbiota, specific bacterial and fungal taxa are selectively enriched in the thallus, likely driven by host-derived factors. Functional profiling highlights an overrepresentation of nitrogen and sulfur cycling, phototrophy, and plant-associated pathways in the thallus, whereas soil communities are dominated by heterotrophic decomposition processes. These findings support the concept of *R. sorocarpa* as a holobiont and emphasize the ecological and biotechnological potential of liverwort-associated microbiomes. Our results provide new insights into plant-microbe interactions in early land plants and their roles in ecosystem function and resilience.

Keywords Bryophytes · Functional diversity · Metagenomics · Microbiome · *Riccia sorocarpa* · rhizosphere

Introduction

In recent years, scientific advancements have greatly expanded our understanding of microorganisms living in symbiosis with plants, opening new perspectives in plant microbiome research – a key factor influencing plant health and adaptability to changing environments (Compant et al. 2019; Trivedi et al. 2020; Trivedi et al. 2022). The plant microbiome comprises microorganisms such as bacteria, fungi, and viruses that live in close association with plants, supporting their growth, disease resistance, and nutrient uptake (Vandenkoornhuyse et al. 2015; Poudel et al. 2021). This microbiome includes distinct groups such as endophytes, epiphytes, and rhizosphere organisms inhabiting the root zone (Harman and Uphoff 2019).

Each of these groups plays a unique and critical role in plant health and development (Igíehon and Babalola 2018).

Endophytes colonize the internal tissues of plants (Weber et al. 2022), protecting them from pathogens and enhancing growth by improving nutrient uptake, particularly nitrogen (Fadiji and Babalola 2020). Epiphytes, which live on the plant's outer surfaces, also contribute protective functions, such as shielding plants from harmful UV radiation and aiding adaptation to abiotic stresses like drought, flooding, and temperature fluctuations (Zotz and Hietz 2001; Flores-Núñez et al. 2020). Moreover, the plant microbiome plays an essential role in mediating interactions between plants and other organisms, including pollinating insects, underscoring its ecological significance (de Vega et al. 2021).

While the plant microbiome is vital, other less-studied but equally crucial ecosystems, such as the soil microbiome, also profoundly influence terrestrial ecosystems (Alcaraz et al. 2018). Soil microorganisms contribute by cooperating with plants in key processes like nitrogen fixation and soil purification (Compant et al. 2019; Trivedi et al. 2020; Trivedi et al. 2022). Among pioneer organisms, liverworts (bryophytes) are some of the first to colonize new environments. As important agents of ecological succession, they accelerate processes such as rock weathering and water retention in forest ecosystems, which

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is particularly valuable during droughts (Wietrzyk-Pełka et al., 2020). Additionally, liverworts form biological soil crusts – complex communities comprising microorganisms such as cyanobacteria, green algae, other bryophytes, and lichens – that interact with soil particles and affect key ecosystem processes (Bowker et al. 2018; Weber et al. 2022). These functions support plant adaptation to changing environmental conditions and establish the foundation for vegetation development in challenging habitats (Trivedi et al. 2022).

Technologies such as nanopore sequencing, developed by Oxford Nanopore Technologies, have substantially advanced our capacity to analyze microbiomes with unprecedented resolution and speed (Branton et al. 2008; Deamer et al. 2016; Sun et al. 2022). These innovations have facilitated uncovering complex plant-microorganism interactions. For example, studies on moss microbiomes have shown they can form symbioses with mycorrhizal fungi, a relationship that was previously debated (Satjarak et al. 2022). In particular, the moss *Takakia lepidozoides* S. Hatt. & Inoue hosts the fungus *Leotia lubrica* (Scop.) Pers., identified as forming ericoid mycorrhiza. Such findings provide new insights into plant symbioses and reveal how diverse microorganisms support plant growth and adaptation to specific environmental conditions, such as nutrient-poor soils or extreme temperatures (Satjarak et al. 2022).

Despite their small size, liverworts play significant ecological roles. They inhabit various environments, including soil, tree and rock surfaces, and flood zones (Alcaraz et al. 2018; Krawczyk et al. 2025). As early colonizers, they contribute importantly to nutrient cycling (Wikström et al. 2009; Bowker et al. 2010). Their rhizoids and protonema help retain water within forest ecosystems, supporting vegetation during drought periods (Söderström et al. 2016; Ortiz-Rivero et al. 2023; Wicaksono et al. 2023). Furthermore, research has shown that the *Riccia* microbiome includes specific bacterial communities, such as methylobacteria, which play crucial roles in elemental cycling – including carbon and nitrogen – thereby influencing long-term ecosystem balance (Wicaksono et al. 2023).

The aim of this study is to comprehensively characterize the biodiversity of microorganisms symbiotic with the liverwort *Riccia sorocarpa* Bisch. We analyze the potential ecological functions of these microorganisms and explore reasons why liverworts and mosses are often considered pioneer species. Additionally, by integrating literature review with microbial analyses, we seek to identify novel microorganisms with potential biotechnological applications, such as in biopreparation development. Although this hypothesis requires further investigation, it may open new avenues for sustainable environmental management (Vandenkoornhuysen et al. 2015; Mesny et al. 2023).

Materials and methods

Plant and material

The study was conducted in an agricultural field located in Butryny, Warmian-Masurian Voivodeship, Poland (53.603N, 20.595E). This region is characterized by a temperate climate with an average annual temperature of approximately 8.2 °C and mean annual precipitation of 600–700 mm. The soils in the Purda commune, which includes the village of Butryny, are classified as “Brunatne właściwe” (type B) in the Polish soil classification system (Chief Inspectorate of Environmental Protection 2025). These correspond to Haplic Cambisols in the WRB classification. The field was selected due to the confirmed presence of *Riccia sorocarpa*, a liverwort species commonly found in disturbed habitats, including agricultural fields, gardens, fallow lands, and lakeshores (Krawczyk et al. 2025). *R. sorocarpa* forms small rosettes up to 2 cm in diameter, with a characteristic gray-green thallus featuring a deep, V-shaped notch at the apex. The distribution of this species within the study site was assessed through a preliminary survey conducted in early spring.

A total of five composite soil samples and five thallus samples of *R. sorocarpa* were collected. The soil samples were taken from the top 5 cm of the substrate using a sterile stainless-steel soil auger. Each composite soil sample consisted of three subsamples collected within a 1 m² plot to account for microhabitat variability. The thallus samples were carefully detached using sterile tools, ensuring minimal disturbance to the surrounding soil. To avoid cross-contamination, gloves and sterile tools were used during collection, and samples were immediately transferred to sterile polypropylene tubes. The samples were then stored in a portable cooler at approximately 4 °C and transported to the laboratory for further processing. The thallus samples were rinsed with sterile distilled water to remove surface contaminants.

DNA extraction, library preparation and sequencing

DNA was isolated from each sample. For soil samples, the PureLink™ Microbiome DNA Purification Kit (Invitrogen) was used. A soil DNA extraction protocol was performed by adding Lysis Buffer to a Bead Tube with soil, followed by Lysis Enhancer and vortexing. The next steps were performed in accordance with the manufacturer's protocol. DNA from thallus samples was isolated using the DNeasy® Plant Mini Kit (Qiagen). Samples were first disrupted with a mortar and pestle, then mixed with Buffer AP1 and RNase A. The mixture was vortexed and

incubated at 65 °C for 10 min with occasional inversion. Further steps were carried out according to the instruction. The elution step was performed twice to ensure maximum DNA yield. The DNA sample purity was determined spectrophotometrically with a Cary 60 spectrophotometer (Agilent). DNA concentration was measured using a Qubit fluorometer along with the Qubit™ dsDNA BR Assay Kit (Invitrogen, Carlsbad, NM, USA). If necessary, the extracted DNA was further purified and concentrated using the Genomic Purification Kit (New England Biolabs, NEB), following the manufacturer's instructions.

The long-read libraries were constructed using the Ligation Sequencing gDNA – Native Barcoding Kit 24 V14 (Oxford Nanopore Technologies). The libraries were sequenced using MinION Mk1C sequencer and MinION R10.4.1 flow cell. Basecalling was performed using dorado v0.9.0 (<https://github.com/nanoporetech/dorado>).

Identification of organisms in soil and thallus samples

The resulting bam files were converted to fastq format using samtools v1.21 (Danecek et al. 2021) with the bam2fq option. Analysis of organism identification in soil and thallus samples was performed using the commercial BugSeq software, which enables a highly accurate metagenomic classifier for nanopore reads 10.1186/s12859-021-04089-5.

Bioinformatics analysis

The previously generated Kraken reports produced by BugSeq were converted into BIOM table format using kraken-biom v1.0.1 (<https://github.com/smdabdoub/kraken-biom>) to enable subsequent analyses. Alpha diversity (including observed species, Shannon, and Simpson indices) analysis,

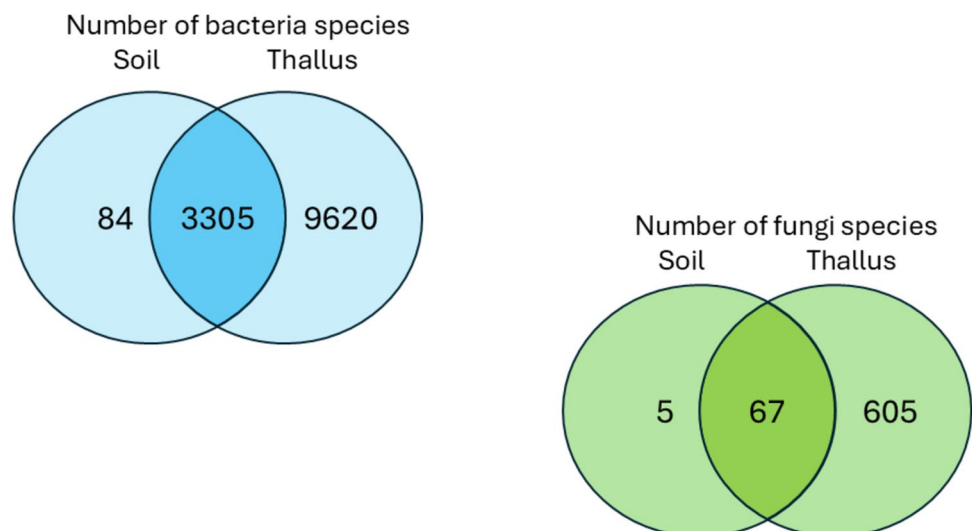
principal coordinate analysis (PCoA) to visualize microbial community patterns, and differential analysis to identify statistically significant taxonomic features were subsequently conducted using MicrobiotaProcess v1.16.1 (Xu et al. 2023). A p-value of less than 0.05 was adopted for statistically significant results in the discriminant analysis between soil and thallus samples.

The FAPROTAX database was used to establish metabolic or other ecologically relevant functions. A previously generated biom file and the collapse_table.py function with the following flags: –output_format_collapsed classical –n'columns_before_collapsing_excluding_unassigned' –collapse_by_metadata'taxonomy' were utilized for the analysis. Subsequently, the processes were subjected to a Wilcoxon statistical test, which helped to determine differences between soil and thallus samples (Louca et al. 2016). Additionally, FDR (padj) correction of p-values was performed. Processes with an FDR < 0.05 were considered statistically significant. Furthermore, Log2FoldChange was determined to observe the level and shift of change between soil and thallus.

Results

The data analysis encompassed a total of 658 411 reads for soil samples, of which 268 695 reads were taxonomically classified. For thallus samples, 13 834 586 reads were analyzed, with 4 936 261 reads assigned to specific taxa. In soil samples, 3 389 bacterial species and 72 fungal species were identified, while thallus samples contained 12 925 bacterial species and 672 fungal species (Fig. 1). The analysis revealed that 3 305 bacterial species and 67 fungal species were shared between soil and thallus communities (Fig. 1).

Fig. 1 The Venn diagram illustrating the number of identified bacterial and fungal species, as well as the number of species shared between them



Based on BugSeq analysis, the ten dominant bacterial orders identified in the samples were: Hyphomicrobiales, Burkholderiales, Kitasatosporales, Micrococcales, Mycobacteriales, Pseudomonadales, Micromonosporales, Nostocales, Propionibacteriales, and Sphingomonadales (Fig. 2).

Similarly, ten dominant fungal orders were detected: Chaetothyriales, Eurotiales, Pleosporales, Hypocreales, Xylariales, Mucorales, Glomerales, Sordariales, and Seriales (Fig. 3).

In the next phase of the study, the ten most frequently occurring and recurrent bacterial species present in both soil and thallus samples were identified as follows: *Nostoc punctiforme* Hariot, *Oscillatoria nigro-viridis* Thwaites ex Gomont, *Microcoleus asticus* Churro, *Bradyrhizobium algeriense* Ahnia, *Bradyrhizobium roseum* Zhang, *Mycolicibacterium hodleri* (Kleespies) Gupta, *Bradyrhizobium sediminis* Jin, *Variovorax ginsengisoli* Im, *Mycolicibacterium stelleriae* Nouiou, and *Tardiphaga robiniae* De Meyer (Supplementary 1).

An analogous analysis was performed for fungi, resulting in identification of the ten most frequently occurring and recurrent fungal species across both sample types: *Exophiala bonariae* Isola & Zucconi, *Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler, *Rhodotorula graminis* Di Menna, *Puccinia striiformis* Westend., *Pseudozyma flocculosa* (Traquair,

L.A. Shaw & Jarvis) Boekhout & Traquair, *Apiospora marii* (Larrondo & Calvo) Pintos & P. Alvarado, *Puccinia recondita* Roberge ex Desm., *Hirsutella rhossiliensis* Minter & B.L. Brady, *Durothea rogersii* (Y.M. Ju & H.M. Hsieh) Læssøe & Srikit., and *Tilletiopsis washingtonensis* Nyland (Supplementary 1).

Principal Coordinates Analysis (PCoA) revealed a clear separation between soil and thallus samples along the first principal coordinate (PCo1), which accounted for 71.08% of the total variability in microbial community composition (Fig. 4A). This distinct clustering indicates fundamental differences in taxonomic structure between microbiomes inhabiting these two environments. Moreover, thallus samples exhibited lower intra-group variability than soil samples, as reflected by the tighter clustering of blue points on the plot.

Alpha diversity analysis further supported these observations (Fig. 4B). Thallus microbiomes displayed significantly higher diversity than soil microbiomes in terms of both species richness and community evenness. This was evidenced by higher Shannon and Simpson index values for thallus samples. The observed differences were statistically significant ($p=0.0079$), indicating that microbial communities associated with the thallus are not only richer in taxa but also more evenly distributed, reflecting a more complex and balanced ecosystem.

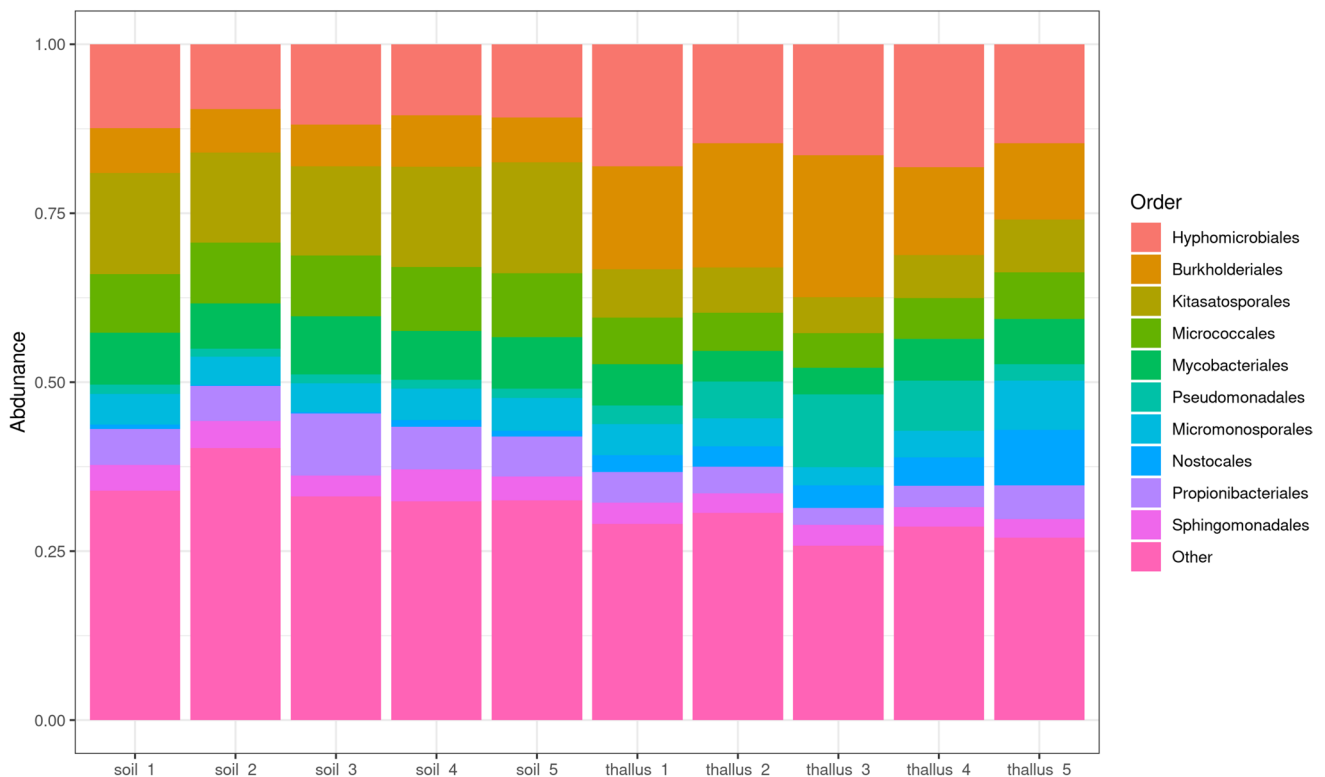


Fig. 2 Composition of microbial communities. The relative abundance of distinct taxa in bacterial communities was visualized at the order level

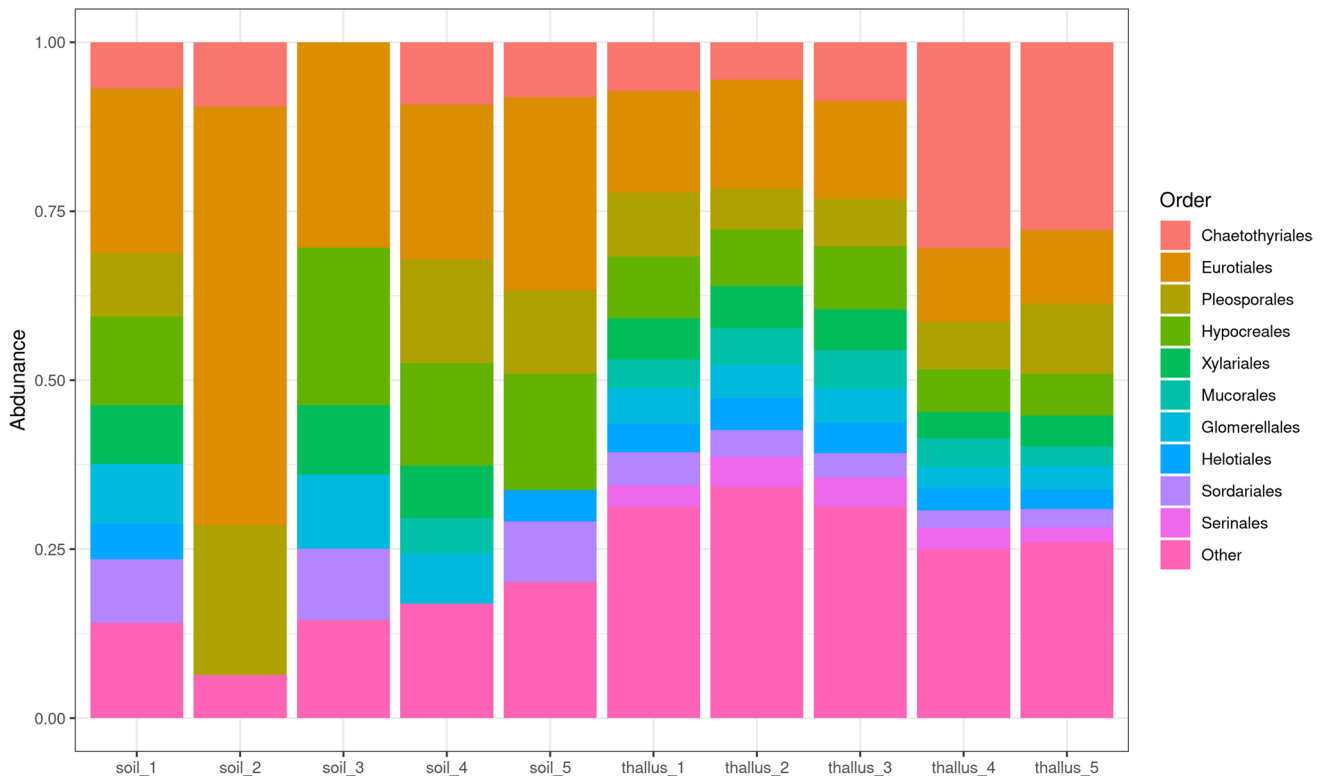


Fig. 3 Composition of microbial communities. The relative abundance of distinct taxa in fungal communities was visualized at the order level

Taxonomic analysis revealed specific microbial orders significantly associated with either soil or thallus environments. As shown in the Manhattan plot (Fig. 4C), seven microbial orders – Cytophagales, Eubacteriales, Hyphomicrobiales, Lactobacillales, Micrococcales, Mycobacteriales, and Sphingomonadales – were significantly enriched in thallus samples. These orders exhibited high $-\log_{10}(\text{p-value})$ scores, indicating strong and consistent association with the thallus microbiome. Conversely, the orders Eucoccidioida and Piroplasmida were significantly enriched in soil samples, suggesting adaptation or preference for the soil environment. Grouping of data points by phylum in the plot further emphasizes the taxonomic structure underlying these associations.

Functional profiling based on the FAPROTAX database (Fig. 5) revealed significant differences in microbial metabolic potential between soil and thallus samples. Processes such as phototrophy, ligninolysis, ureolysis, nitrogen and sulfur cycling, and plant pathogenicity were more abundant in the thallus microbiome, as indicated by positive Log_2 Fold Change values. In contrast, chemoheterotrophy and aromatic compound degradation were enriched in soil microbiomes. The adjusted p-values corroborated the statistical significance of these differences, with the most significant processes highlighted by darker-colored bubbles.

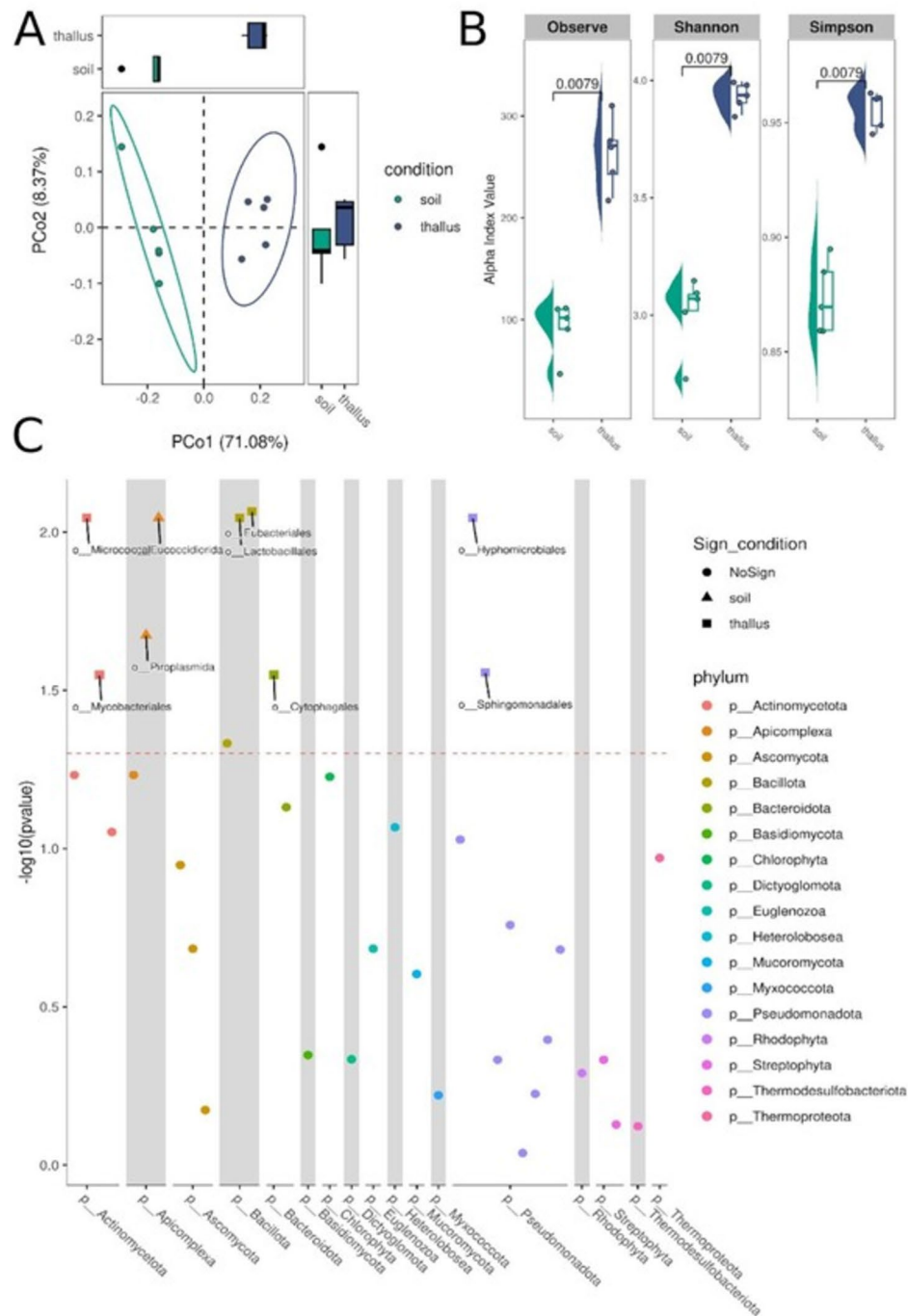
Discussion

Microbial richness and community overlap

Sequencing of microbial communities revealed a markedly higher number of reads and taxonomically classified sequences in thallus samples compared to soil. This difference likely reflects both deeper sequencing coverage and a more taxonomically diverse microbial community within the thallus. Although microbial biomass was not directly measured, the substantially greater number of taxa identified indicates that the thallus provides a microhabitat conducive to higher species richness. Notably, the thallus hosted nearly four times as many bacterial species and almost ten times as many fungal species compared to soil. These findings support previous observations that bryophytes, particularly thalloid liverworts, create microhabitats favorable for microbial colonization due to their poikilohydric hydration strategy, high surface-to-volume ratio, and production of selective secondary metabolites (Marks et al. 2018; Ortiz-Rivero et al. 2023).

Despite pronounced differences in species richness, a large number of microbial species (3 305 bacteria and 67 fungi) were common to both compartments. This overlap

Fig. 4 **A** Soil samples (green points) are clustered on the left side of the plot (negative PCo1 values), while thallus samples (blue points) are located on the right side (positive PCo1 values). The boxplots at the top and right of the figure show the distribution of values along the PCo1 and PCo2 axes. **B** Each panel presents data for a specific alpha diversity index. The distributions of values are shown as density plots (shapes on the sides) and boxplots, which visualize the median, quartiles, and data variability. Green points represent soil samples, while blue points represent thallus samples. Above the boxplots, the p-value ($p=0.0079$) indicates a significant difference between the groups for each alpha diversity index. **C** Manhattan plot visualizes the results of a statistical analysis for various organism orders in the context of their association with soil or thallus samples. The Y-axis represents the $-\log_{10}(p\text{-value})$, while the X-axis represents different phylum. The plot is grouped by phylum, and the points represent specific orders. Points above the dashed line are habitat-related groups



suggests ongoing microbial exchange or recruitment from surrounding soil into the thallus, potentially mediated by environmental filtering or host–microbe mutualistic compatibility (Rillig et al. 2015). However, the considerable number of species unique to the thallus implies selective enrichment, likely driven by host-derived factors such as nutrient exudation, antimicrobial compounds, or the physicochemical properties of the thallus surface (Wicaksono et al. 2023; Berdaguer et al. 2024). Such mechanisms probably parallel those shaping root microbiomes in

vascular plants, although bryophytes lack roots and instead develop simpler yet functionally relevant microbial interfaces (Wicaksono et al. 2021; Satjarak et al. 2022).

Taxonomic structure and ecological functions

The dominant bacterial orders – Hyphomicrobiales, Burkholderiales, Kitasatosporales, Micrococcales, Mycobacteriales, among others – encompass taxa with versatile metabolic capabilities. Many include nitrogen-fixing,

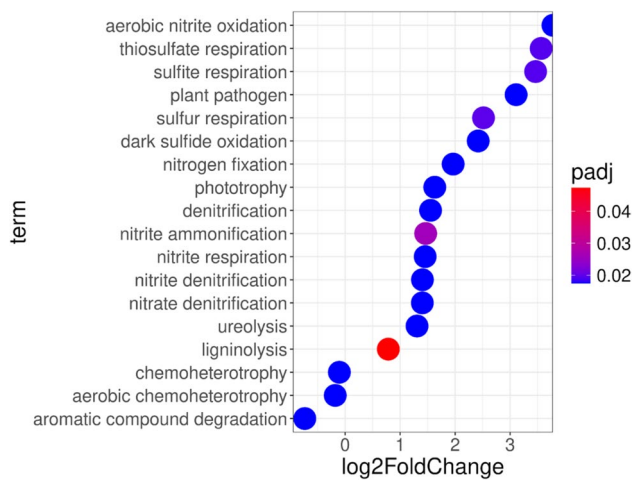


Fig. 5 Bubble chart of significant processes according to the FAPROTAX database. The Y-axis represents processes from the FAPROTAX database, and the X-axis represents Log2FoldChange. The color of the dots depends on the adjusted p-value (padj)

phosphate-solubilizing, and plant growth-promoting bacteria (PGPB) known to colonize plant surfaces and tissues as epiphytes or endophytes (Barka et al. 2016; Dohnalkova et al. 2017; Berkelmann et al. 2020). For example, Hyphomicrobiales and Burkholderiales are frequently associated with diazotrophic lifestyles and phytohormone production, such as indole-3-acetic acid (IAA), which may enhance liverwort growth and stress tolerance (Wang et al. 2017; Palberg et al. 2022). The detection of Nostocales, including *Nostoc punctiforme* and *Microcoleus asticus*, highlights the potential for nitrogen fixation and phototrophy within the thallus microbiome, as cyanobacteria are known symbionts of hornworts and some liverworts, contributing notably to nitrogen budgets especially in nutrient-poor substrates (Garcia-Pichel and Belnap 2021; Mishra et al. 2021; Álvarez et al. 2023).

The fungal community was similarly enriched in ecologically important taxa. Dominant orders such as Chaetothyriales, Eurotiales, Pleosporales, Hypocreales, and Glomerales encompass functionally diverse groups including saprotrophs, endophytes, and mycorrhizal fungi (Doilom et al. 2020; Deb et al. 2020; Santos et al. 2021). The presence of *Rhizophagus irregularis* (Glomeromycota) is notable, suggesting potential analogues of mycorrhizal associations in these non-vascular plants (Begum et al. 2019; Le Pioufle et al. 2019). Although liverworts lack true mycorrhizae, several species have been shown to engage with Glomeromycota in symbioses reminiscent of early land plants, reflecting a deep evolutionary origin of such associations (Rimington et al. 2018). Other detected fungi, like *Exophiala bonariae* and *Pseudozyma flocculosa*, are recognized for antifungal activity, environmental stress tolerance, or endophytic lifestyles, potentially contributing to a protective

microbial barrier around the host liverwort (Begum et al. 2019; Le Pioufle et al. 2019; Santhanam et al. 2023).

Core microbiota and host-microbe specificity

A key component of this study was the identification of ten recurrent and abundant bacterial and fungal species shared across both soil and thallus samples. The presence of taxa such as *Bradyrhizobium*, *Mycolicibacterium*, and *Variovorax ginsengisoli* within the core bacterial group suggests functional roles including nitrogen fixation, oxidative stress mitigation, and modulation of plant signaling pathways (Sindhu et al. 2019; Nouiouei et al. 2019; Zhang et al. 2020). This functional redundancy, commonly observed in association with higher plants, implies ecological plasticity and possible adaptation to early land plant hosts in *R. sorocarpa* (Sindhu et al. 2019; Nouiouei et al. 2019; Zhang et al. 2020).

On the fungal side, the detection of phytopathogens such as *Puccinia striiformis* and *Puccinia recondita* raises intriguing ecological and agricultural questions. Liverworts may serve as reservoirs or intermediate hosts, facilitating pathogen persistence in the environment (Bolton et al. 2008; Chen et al. 2014; Wicaksono et al. 2023). Conversely, the co-occurrence of protective fungi like *Pseudozyma flocculosa* and *Hirsutella rhossiliensis* highlights a complex microbial balance, where antagonistic interactions may regulate pathogen establishment (Chen and Liu 2005; Firrincieli et al. 2015; Sun et al. 2024).

Structural differentiation of microbial communities

The pronounced separation of microbial composition along the first principal coordinate (PCo1), with thallus and soil samples clustering distinctly, provides strong evidence for differential habitat filtering. The thallus represents a biologically active microhabitat with unique physicochemical conditions – including consistent hydration via capillary water retention, elevated organic exudates, and secondary metabolites – that selectively promote or inhibit colonization by specific microbial taxa (Carella and Schornack 2018; Ortiz-Rivero et al. 2023; Wicaksono et al. 2023). In contrast, soil, although rich in organic matter and diverse niches, is less influenced by host-derived biochemical cues, supporting a broader array of generalist microbes (Cline and Zak 2015; Deltedesco et al. 2020). Additionally, the lower intra-group variability among thallus-associated communities suggests a conserved core microbiome, possibly stabilized by host factors such as surface morphology, metabolite secretion, or antimicrobials (Stelmasiewicz et al. 2023). This pattern aligns with previous bryophyte-associated microbiome studies demonstrating selective pressures leading to reproducible, host-specific microbial assemblages (Carella and Schornack 2018; Holland-Moritz et al. 2021).

Alpha diversity and ecological implications

The significantly higher alpha diversity indices (Shannon, Simpson) observed in thallus microbiomes compared to soil ($p=0.0079$) indicate a microbial community that is both more species-rich and taxonomically balanced. This suggests liverwort microhabitats – especially in early successional or extreme environments – function as microbial biodiversity hotspots (Ortiz-Rivero et al. 2023; Wicaksono et al. 2023). Such heightened diversity likely reflects a finely tuned ecological balance orchestrated by the host. Evenly distributed microbial communities often correspond to increased functional redundancy and ecological stability, enhancing resilience of the plant–microbe holobiont to environmental fluctuations (Allison and Martiny 2008; Wittebolle et al. 2009). Moreover, diverse, even communities facilitate cooperative interactions like syntrophy, mutualism, and cross-feeding, broadening the functional capacity of the host-microbiome system (Miki et al. 2010; García-García et al. 2019).

The distinct taxonomic profiles in soil and thallus samples reflect strong environmental filtering and niche specialization. Enrichment of bacterial orders such as Cytophagales, Eubacteriales, Hyphomicrobiales, and Sphingomonadales in the thallus suggests adaptation to host association with possible roles in nutrient cycling, stress alleviation, or signaling. The presence of Sphingomonadales and Cytophagales on vascular plant leaves supports conserved ecological functions across host types (Angel et al. 2016; Pan et al. 2023; Dao et al. 2023). Hyphomicrobiales and Eubacteriales, however, appear uncharacteristic of above-ground vascular parts, revealing potentially unique liverwort-associated taxa. Conversely, dominance of Eucoccidiorida and Piroplasmida in soil likely reflects adaptation to free-living or specific edaphic niches. Manhattan plot clustering underscores the fundamental role of habitat in microbial community structure.

Functional profiling of microbial communities

Functional profiling of the microbiomes associated with *Riccia sorocarpa* thallus and soil, based on the FAPROTAX database, revealed significant differences in the metabolic potentials of these microbial communities. These differences reflect ecological adaptations of the microbiomes to their unique environmental conditions and the roles they play within their respective microhabitats.

The thallus microbiome was clearly enriched in functions related to nitrogen and sulfur cycling, including processes such as aerobic nitrite oxidation, thiosulfate respiration, and denitrification. This indicates active microbial participation in the transformation of inorganic compounds, often under microaerophilic or anoxic conditions that may develop

within thallus microenvironments. The presence of these processes suggests that the structure and properties of the thallus create a microhabitat where microbes use compounds like nitrites, sulfites, and thiosulfates as electron acceptors, increasing the efficiency of biogeochemical transformations.

Additionally, phototrophic functions, particularly associated with cyanobacteria such as *Nostoc punctiforme*, emphasize the autotrophic potential of the thallus microbiome. *Nostoc punctiforme* is a well-known diazotroph and phototroph, suggesting a symbiotic relationship that supports *R. sorocarpa* in nitrogen acquisition – a critical nutrient, especially in nitrogen-poor environments (Warshan et al. 2018). This symbiosis likely contributes to the survival and growth of the liverwort in challenging ecological conditions with limited nitrogen availability.

Furthermore, the functional profile of the thallus microbiome highlights its role as a specialized microhabitat supporting both autotrophic and anaerobic metabolic processes, indicating a complex biogeochemical cycling system primarily driven by microbial interactions. These findings align with prior studies on other liverworts, which have been shown to host diverse microorganisms participating in nutrient cycling, particularly of nitrogen and sulfur (Song et al. 2016; Field et al. 2019).

In contrast, the soil microbiome was predominantly enriched in heterotrophic functions. The prevalence of aerobic chemoheterotrophy and aromatic compound degradation suggests that soil microorganisms are adapted to decompose organic matter such as plant residues and lignin. This is essential for the carbon cycle and maintenance of soil fertility (Kroeger et al. 2021). The degradation of recalcitrant aromatic compounds, including lignin, highlights the capacity of soil microbes to mineralize complex organic substances, thereby promoting carbon recycling and enhancing soil health (Fuchs et al. 2011).

The described functions of the soil microbiome underscore its fundamental ecological role in organic matter decomposition and soil formation processes, contrasting with the more specialized and integrated role of the thallus-associated microbiome. It is also noteworthy that these functional differences underscore how distinct environments shape community composition and metabolic capabilities, highlighting microbial adaptations to specific ecological niches, such as plant structures (thallus) versus soil.

Holobiont perspective and evolutionary context

Collectively, the data suggest that *R. sorocarpa* does not simply harbor random microbes, but forms a functionally integrated holobiont with a selected microbiome contributing to host fitness via metabolic complementation, stress mitigation, and nutrient supply (Wicaksono et al. 2023). Such integration likely reflects ancient co-evolutionary trajectories

since liverworts are among the most basal land plants. Their capacity for symbioses with fungi (e.g., Glomeromycota) and cyanobacteria has been proposed as a key innovation facilitating plant terrestrialization (Ligrone et al. 2007; Carella and Schornack 2018). Our results provide contemporary support for this, showing *R. sorocarpa* maintains a structurally distinct, functionally enriched microbiome supporting nitrogen acquisition, carbon cycling, and microbial homeostasis.

Ecological insights

This study extends our understanding of liverwort microbiomes and their functional relevance in early land plants. The observed microbial diversity and specialization mark *R. sorocarpa* as a promising model for investigating host–microbe interactions. Future work should include functional assays via metatranscriptomics or metabolomics to elucidate roles of specific taxa in nutrient acquisition, host defense, and stress responses. Manipulative experiments, such as gnotobiotic liverwort systems, could clarify causal links between host traits and microbiome composition. Additionally, seasonal, spatial, and microclimatic variations in microbial assemblages warrant investigation, given bryophytes' sensitivity to environmental fluctuations (Marks et al. 2018).

Our findings reveal a profound and statistically significant divergence between microbial communities inhabiting the *R. sorocarpa* thallus and adjacent soil. This divergence, mainly captured by PCo1 explaining 71.08% of variance, indicates that the thallus provides a highly selective, functionally distinct ecological niche influencing both taxonomic assembly and microbial function. This corroborates previous findings reporting that *Riccia* liverwort thalli harbor microbial communities distinct from surrounding soil (Wicaksono et al. 2023).

Conclusions

This study provides comprehensive insights into the microbial diversity and functional specialization associated with the thallus of the liverwort *Riccia sorocarpa* compared to the surrounding agricultural soil. We found that the thallus hosts a richer and more taxonomically balanced microbial community, distinguished by a distinct core microbiota, structural specificity, and significant functional enrichment in nitrogen and sulfur cycling, phototrophy, and plant-associated processes. In contrast, soil microbiomes mainly engage in heterotrophic organic matter decomposition and aromatic compound degradation.

These results suggest that *R. sorocarpa* actively shapes its unique microbiome through selective pressures, fostering

microbial processes essential for survival in nutrient-poor and stressful environments. The presence of beneficial microbes with plant growth-promoting traits and potential biotechnological applications further highlights the ecological and applied significance of liverwort-associated microbiomes.

Our findings reinforce the concept of liverworts as holobionts and emphasize their value as model systems for investigating early land plant–microbe coevolution. Future studies employing functional genomics and synthetic microbiome approaches will be crucial to unravel the mechanistic bases of these interactions and explore their potential in sustainable ecosystem management.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11756-025-02025-z>.

Author contributions PW performed laboratory steps of the study, analysed data, carried out bioinformatic analyses, wrote the manuscript, MM carried out bioinformatic analyses, wrote the manuscript, JS collected samples, obtained funding and revised the manuscript, KK wrote and revised the manuscript.

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Data availability Not Applicable.

Declarations

Ethical approval Not Applicable.

Informed consent Not Applicable.

Conflict of interest The authors declare no conflict of interest.

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