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Metagenomic Analysis of Microbial Community Dynamics in Konjac Rhizosphere During Soft Rot Disease Progression

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Abstract

Amorphophallus konjac, the sole glucomannan-rich species in the family Araceae, faces significant yield and quality losses due to soft rot disease. Understanding the relationship between soil microbial communities and soft rot incidence is critical for sustainable konjac production. Metagenomic analysis was employed to systematically investigate rhizosphere microbial dynamics associated with soft rot progression. Microbial community richness peaked at the mature stage of diseased plants compared to healthy and latently infected counterparts. Principal coordinate analysis revealed greater variability in rhizosphere communities of diseased plants, with distinct separation from healthy/latently infected samples. At the phylum level, *Chloroflexi* and *Acidobacteria* abundances in healthy mature plants exceeded those in diseased plants by 11.54% and 4.6%, respectively, while pathogenic *Rhizopus arrhizus* and *Rhizopus microsporus* were significantly enriched in diseased mature plants. Correlation analyses demonstrated predominantly negative associations between bacterial species and soil factors, contrasting with positive fungal correlations. KEGG pathway annotation identified carbohydrate metabolism and amino acid synthesis as core microbial functions in the konjac rhizosphere. These findings suggest that *Chloroflexi* and *Acidobacteria* enhance disease resistance, whereas Rhizopus species promote soft rot development. Our results provide

actionable insights for designing microbiome-based strategies to mitigate konjac soft rot, reducing reliance on conventional agrochemicals.

Key points

• Diseased mature plants show highest rhizosphere microbial richness; *Chloroflexi/Acidobacteria* enrich in healthy ones, aiding resistance.

• Pathogenic *Rhizopus* spp. drive soft rot; bacteria negatively, fungi positively correlate with soil factors, revealing divergent responses.

• Lays groundwork for microbiome-based strategies to cut agrochemicals, fostering sustainable konjac soft rot control.

Keywords Amorphophallus konjac • rhizosphere microbiome • soft rot disease • metagenomics • biological control

Introduction

Amorphophallus konjac, a unique glucomannan-rich species in the Araceae family, has gained significant attention for its versatile applications in food technology, biomedicine, and material sciences due to the exceptional gelation and film-forming properties of konjac glucomannan (Devaraj et al., 2019; Tan et al., 2021). However, a major constraint in konjac cultivation is its high susceptibility to soft rot disease caused by *Pectobacterium* spp. This soil-borne pathogen induces rapid tissue maceration in corms and petioles, with infection rates reaching 30-50% in typical cases and exceeding 80% in severe outbreaks, often resulting in total yield loss (He et al., 2022). Current management strategies, including chemical controls and disease-resistant cultivars, remain largely ineffective due to the pathogen's soil persistence and complex host-microbe interactions.

The rhizosphere microbiome plays a critical role in plant-pathogen dynamics, functioning as a frontline defense against soil-borne diseases (Wogene et al., 2024). Comparative studies in other crops demonstrate that pathogens must overcome resident microbial communities to establish infections, as evidenced in tomato bacterial wilt (Wei et al., 2019), tobacco root rot (Gao et al., 2019), and banana Fusarium wilt (Shen et al., 2015). Disease progression significantly alters microbial

composition, with pathobiome signatures including increased *Deinococcus-Thermus* and *Firmicutes* abundance in cotton verticillium wilt (Zhang et al., 2010) and reduced *Proteobacteria* populations in citrus Huanglongbing disease (Trivedi et al., 2011). Although preliminary investigations by Wu et al. (2017) indicate that the microbial diversity in the rhizospheres of healthy konjac plants is lower than that in diseased ones, several crucial knowledge gaps remain. Firstly, we lack a clear understanding of the temporal assembly patterns of microbial communities during the latent infection stages. Secondly, the functional shifts in microbiome dynamics as the disease progresses are still not well - characterized. Finally, there is a need to identify early biomarkers that can predict disease outcomes accurately.

We propose that the binary disease outcomes (healthy vs. severely infected) observed in konjac under homogeneous conditions may stem from early divergence in rhizosphere microbiome assembly. This hypothesis aligns with recent findings demonstrating microbiome-mediated disease suppression in other plant-pathogen systems (Gu et al., 2022). Through the analysis of the development paths of the microbial community in konjac plants at various health stages, this research study has the following specific aims: Firstly, to precisely describe and define the functional changes and succession processes within the rhizosphere microbiomes during the infection by *Pectobacterium*. Secondly, to determine and recognize the key microbial taxa that are closely associated with the suppression of the disease. The insights gained from this study will significantly enhance our comprehension of the complex interactions among plants, the microbiome, and pathogens. Moreover, these findings will provide valuable guidance for the development of innovative biocontrol strategies, which are essential for the sustainable cultivation of konjac.

Materials and methods

Rhizosphere soil collection system and sampling design

The experimental trials were conducted at the Agricultural High Technology Zone (30.3172°N, 109.4772°E; 421.5 m a.s.l.) in Enshi Tujia and Miao Autonomous Prefecture, Hubei Province, China. A specialized “rootbox” system (Wei et al., 2019) was employed to non-destructively collect rhizosphere soil from individual

Amorphophallus konjac plants across distinct developmental stages. The system comprised nine detachable nylon mesh compartments (dimensions: height = 136 mm, width = 18-21 mm, thickness = 1-2 mm; pore size = 150 μm), enabling stage-specific soil sampling. After removing plant debris, the soil was sieved (< 2 mm), homogenized, and divided into the nylon bags, with each bag filled with 4 g of soil. The soil used in the "rootbox" was field soil from a plot of konjac under continuous cropping for five years, with severe soft rot disease.

Field experimental design and retrospective sampling strategy

The tissue-cultured seedlings of *Amorphophallus konjac* K. Koch cv. Qingjiang were obtained from the Konjac Research Institute, Enshi Academy of Agricultural Sciences (Enshi, China). On April 10, 2023, a total of 200 seedlings were transplanted into pre-prepared rhizobox systems and subsequently cultivated under controlled greenhouse conditions.

Monitoring the progression of the disease, we found that the incidence of soft rot quantified at three phenological stages was as follows. At the initial stage (July 10th), the infection rate was 25.0%. By the vegetative stage (August 10th), it had reached 46.0%. And at the maturity stage (September 10th), the infection rate was 65.0%. At each monitoring timepoint, three randomly selected nylon mesh compartments were harvested per rhizobox. Samples from identical plants across stages were pooled, homogenized, and cryopreserved at -80°C for downstream analyses. This describes a rhizosphere sampling protocol where the selected compartments are processed in a specific way to prepare the samples for further analysis.

Rhizosphere soil samples and konjac plants were collected at the maturity stage. Quantitative PCR was used on September 10th to quantify the number of soft rot bacteria in konjac plants. Individual plants that showed no soft rot symptoms and had a concentration of pathogenic bacteria in the stem tissue $<10^2$ CFU·g^{-1}, were classified as healthy plants. Plants without visible soft rot symptoms but with a concentration of pathogenic bacteria between10^2 CFU·g^{-1}and 10^6 CFU·g^{-1}in the stem tissue were classified as latently infected. Plants exhibiting soft rot symptoms with bacterial counts exceeding 10^6 CFU·g^{-1}were classified as diseased. No plants displaying soft rot

symptoms without detectable pathogenic bacteria in their stems were observed in this study. The repeated sampling strategy allowed us to trace back the health condition (diseased, latently infected, or healthy) of individual plants over time. For instance, if a plant was identified as diseased in the final sampling period, soil samples from earlier periods associated with the same plant were also classified as related to a diseased plant. Specifically, soil samples collected during the initial stage (July 10^{th}) were labeled as DIS, those from the vegetative stage (August 10^{th}) as DVS, and those from the maturity stage (September 10^{th}) as DMS. Similarly, if a plant was classified as latently infected in the final sampling period, soil samples collected during the initial, vegetative, and maturity stages were labeled as LIS, LVS, and LMS, respectively. For plants identified as healthy in the final sampling period, the corresponding soil samples were marked as HIS, HVS, and HMS for the initial, vegetative, and maturity stages, respectively. Six biological replicates per health category were randomly selected for micro-biome profiling across all developmental stages.

Soil physicochemical analysis

Physicochemical analysis was conducted to assess the soil's physicochemical characteristics under different plant health statuses and growth stages. Triplicate measurements were carried out to evaluate the field soil conditions. Soil pH was measured by preparing a mixture of soil and CO_2-free deionized water at a 1:2.5 (w/v) ratio. Organic matter (OM), available nitrogen (AN), available phosphorus (AP), and available potassium (AK) were determined following established protocols (Wu et al., 2020).

DNA extraction, quantitative PCR, and metagenomic sequencing

Total genomic DNA was extracted from konjac rhizosphere soil samples using the Mag-Bind® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer’s instructions. The concentration and purity of the extracted DNA were determined using TBS-380 and NanoDrop2000, respectively. One aliquot of the DNA extract was used for quantitative polymerase chain reaction (qPCR) analyses, while another aliquot was fragmented to an average size of ~400 bp using Covaris M220

(Gene Company Limited, China) for paired-end library construction. The paired-end library was prepared using NEXTFLEX Rapid DNA-Seq (Bio Scientific, Austin, TX, USA). The raw data have been submitted to a public repository (NCBI) and the accession number was SUB15290670. The *Pectobacterium chrysanthemi* (*P.ch*) pathogen abundance was determined following previous methods (Wu et al., 2011).

High-throughput sequencing and data processing analysis

High-throughput sequencing of the microbial metagenomic DNA library was performed using the Illumina NovaSeq 6000 sequencing platform (Shanghai Majorbio Bio-pharm Technology Co., Ltd.). Data analysis was conducted on the Majorbio Cloud Platform (www.majorbio.com). Sequencing data quality was assessed using FastQC, while Trimmomatic, IDBA-UD, Prodigal, and CD-HIT software were used to purify, assemble, predict the open reading frame (ORF), and remove redundancy, respectively. The gene abundance was analyzed using Bowtie 2 and SAM tools. DIAMOND software was then employed to compare homology between the non-redundant protein sequences in the gene set and microbial protein sequences in the NCBI database. Finally, annotated species classification and relative abundance information were obtained.

Results

Disease status classification in konjac plants

Through systematic evaluation of symptomatic manifestations and quantitative PCR (qPCR) detection of *Pectobacterium chrysanthemi* in stem tissues, the experimental population (n=200) exhibited distinct health stratification. Among them, 65.0% (n = 130) were classified as diseased, showing characteristic soft rot symptoms and pathogen loads exceeding 10^6 CFU·g^{-1}. Meanwhile, 21.5% (n = 43) were latently infected, remaining asymptomatic yet harboring pathogens at levels ranging from 10^2 to 10^6 CFU·g^{-1}. Additionally, 13.5% (n = 27) were considered healthy, displaying neither symptoms nor detectable pathogens (<10^2CFU·g^{-1}). This categorical distribution demonstrated significant association between symptom development and pathogen proliferation (χ^2=167.3, df=2, p<0.0001), confirming the biological relevance of the classification criteria.

Metagenomic profiling of konjac rhizosphere microbiota

Taxonomic composition based on NR database annotation

Gene sequences of microorganisms in konjac rhizosphere soil were annotated by species through comparison with the NR database (Table 1). As shown in the table, bacteria were the predominant microorganisms in each sample, accounting for 99.04% of the total species, followed by *Heunggongvirae* (0.48%) and *Archaea* (0.18%), while fungi were relatively rare, comprising only 0.09%. In this study, bacteria and fungi with potential functional significance were selected for subsequent analysis.

Table 1 Taxonomic distribution of microbial communities in konjac rhizosphere soil

Domain	Kingdom/Phylum	Read Counts	Relative Abundance (%)
Bacteria	*All phyla*	431,501,482	99.04
Viruses	*Heunggongvirae*	2,080,076	0.48
	Bamfordvirae	163,354	0.04
	Other viral taxa	91,796	0.02
Archaea	*All phyla*	795,568	0.18
Eukaryota	*Viridiplantae*	436,704	0.10
	Fungi	392,790	0.09
	Metazoa	115,064	0.03
unclassified	-	85,498	0.02

Notes: Data filtered at≥0.01% relative abundance threshold. Viral subcategories consolidated under "Viruses" domain for hierarchical clarity.

Alpha diversity index of microbial communities in rhizosphere of konjac

The α-diversity indices of bacterial and fungal communities in the konjac rhizosphere, across different health statuses and growth stages, are presented in Tables 2 and 3. For both healthy and diseased plants, the Chao1 indices (reflecting community richness) for bacteria and fungi were higher during the mature stage compared to the initial stage, while the Simpson indices (reflecting community diversity) decreased correspondingly. The highest Chao1 values and the lowest Simpson indices were observed in the diseased plants at the mature stage (DMS). These results suggest that microbial community richness and diversity in the konjac

rhizosphere vary with growth stage and health status, peaking during the mature stage of diseased plants.

Table 2 Alpha diversity indices of bacterial communities in the rhizosphere of konjac under different health statuses and growth stages

Sample	Chao 1	Shannon	Simpson
DIS	366.67±10.975ab	4.0990±0.0126a	0.0572±0.0013b
DVS	372.33±51.541ab	4.1972±0.3863a	0.0599±0.0261b
DMS	413.00±6.429a	2.3849±0.1781b	0.2996±0.0549a
HIS	348.33±12.811ab	4.2582±0.1576a	0.0590±0.0105b
HVS	338.33±11.893b	3.8288±0.0262a	0.0855±0.0017b
HMS	351.67±20.078ab	4.1484±0.0097a	0.0685±0.0060b
LIS	325.33±12.441b	3.9642±0.0585a	0.0682±0.0061b
LVS	397.33±10.682ab	4.1046±0.0036a	0.0607±0.0015b
LMS	374.33±20.019ab	3.9380±0.1047a	0.0699±0.0075b

Notes: 1. Different lowercase letters indicate significant differences between treatments ($P < 0.05$). 2. Abbreviations: DIS, diseased plants at initial stage; DVS, diseased plants at vegetative stage; DMS, diseased plants at mature stage; HIS, healthy plants at initial stage; HVS, healthy plants at vegetative stage; HMS, healthy plants at mature stage; LIS, latently infected plants at initial stage; LVS, latently infected plants at vegetative stage; LMS, latently infected plants at mature stage.

Table 3 Alpha diversity indices of fungal communities in the rhizosphere of konjac under different health statuses and growth stages.

Sample	Chao 1	Shannon	Simpson
DIS	21340.67± 147.931d	5.3896±0.0633b	0.0228±0.0012b
DVS	21417.33±189.711d	5.4278±0.1716b	0.0252±0.0036ab
DMS	22876.33±39.048a	6.0730±0.0091a	0.0109±0.0002c
HIS	20792.67±223.570e	5.2049±0.1631b	0.0317±0.0046a
HVS	21437.00±144.133d	5.1836±0.0556b	0.0313±0.0020a
HMS	22436.33±185.869ab	5.3875±0.0557b	0.0259±0.0012ab
LIS	20652.33±298.241e	5.3089±0.1003b	0.0267±0.0025ab
LVS	21810.33±104.260cd	5.2339±0.0356b	0.0293±0.0011ab

LMS	22183.00±125.012bc	5.2924±0.0281b	0.0279±0.0010ab

Notes: Same as Table 2.

The similarity in bacterial and fungal species composition across health statuses and growth stages was visualized using Venn diagrams (Fig. 1). Notably, 6.39% of fungal species and 1.52% of bacterial species were unique to the diseased mature stage (DMS), compared to 2.95% and 0.15%, respectively, in the healthy mature stage (HMS). This highlights a greater abundance of unique rhizosphere microbial species during the mature stage of konjac soft rot disease (DMS).

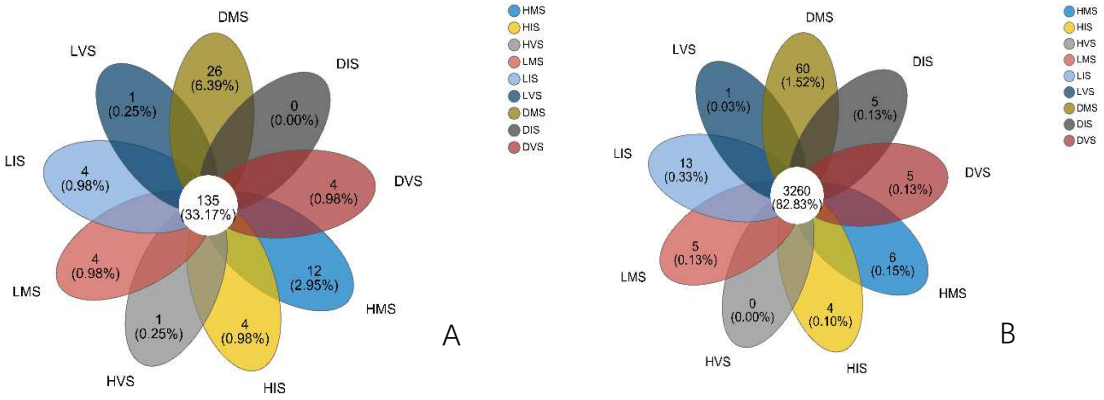


Fig.1 Venn diagram of fungal (A) and bacterial (B)communities in the rhizosphere of konjac under different health statuses and growth stages.

Beta diversity of microbial communities in the rhizosphere of konjac

Principal component analysis (PCA) was used to evaluate differences and similarities in bacterial and fungal communities within the konjac rhizosphere across varying health statuses and growth stages (Fig. 2). The results showed clear separation of sample points based on health status for both bacterial and fungal communities, with most points distributed along the horizontal axis PC1 (explaining 39.18% and 71.57% of variance for bacteria and fungi, respectively). This demonstrates significant divergence in rhizosphere microbial community composition among different health statuses. Variations related to growth stages were primarily distributed along PC2 (explaining 19.29% and 28.38% of variance for bacteria and fungi, respectively), indicating that health status was the dominant factor shaping bacterial and fungal community structure in the konjac rhizosphere, while growth stage played a secondary role. Furthermore, diseased sample points exhibited greater

dispersion compared to healthy and latently infected samples, suggesting higher variability in microbial diversity within the rhizosphere soil of konjac plants affected by soft rot disease.

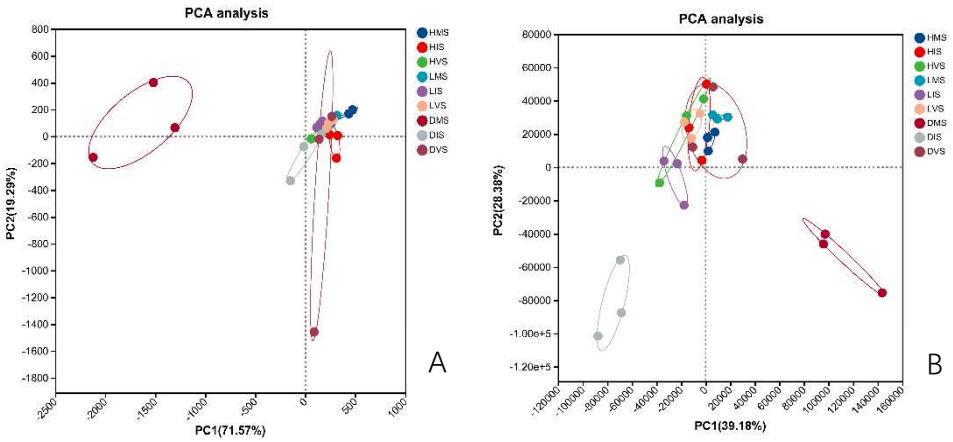


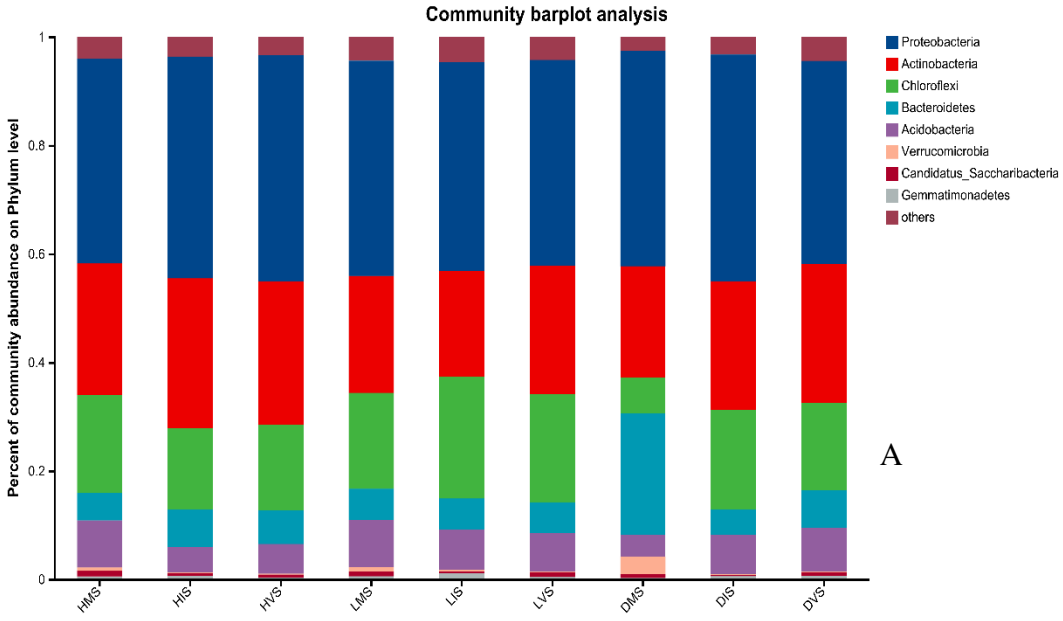
Fig. 2. PCA of fungal (A) and bacterial (B) communities in the rhizosphere of konjac under different health statuses and growth stages.

Composition of microbial communities in the rhizosphere of konjac

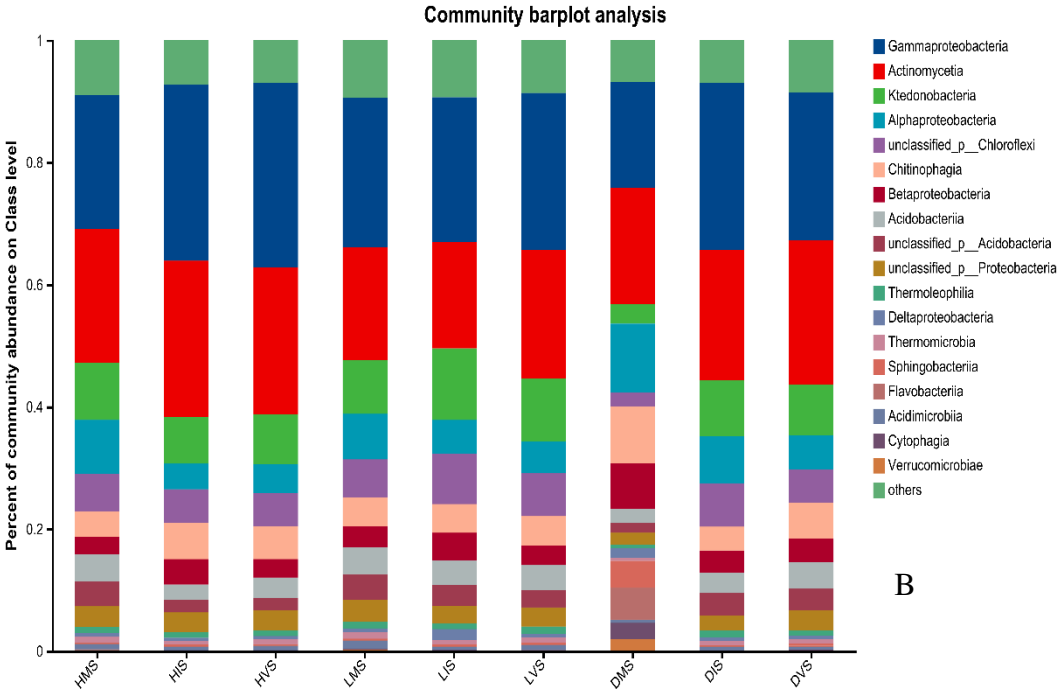
Across all samples, bacterial communities were annotated into 160 phyla, 268 classes, 478 orders, 989 families, 3,936 genera, and 29,372 species, while fungal communities comprised 9 phyla, 36 classes, 99 orders, 250 families, 407 genera, and 828 species. The relative abundances of dominant bacterial and fungal taxa at each taxonomic level were analyzed (Fig. 3).

The dominant bacterial phyla in the konjac rhizosphere were Proteobacteria and Actinobacteria, collectively accounting for 62.25% of the community, followed by *Chloroflexi, Bacteroidetes, Acidobacteria, Verrucomicrobia, Candidatus_Sacchari bacteria,* and *Gemmatimonadetes* (Fig. 3A). Both growth stage and health status influenced the relative abundance of dominant taxa. In diseased plants at the mature stage, the abundance of *Chloroflexi* (6.55%) and *Acidobacteria* (4.03%) was significantly lower compared to healthy plants at the same stage (18.09% and 8.69%, respectively). Conversely, Bacteroidetes (22.35%) and *Verrucomicrobia* (3.27%) were enriched in diseased plants relative to healthy plants (5.05% and 0.63%, respectively), suggesting their potential role in disease response. At the class level, dominant taxa such as *Ktedonobacteria*, unclassified *Chloroflexi,* unclassified *Acidobacteria*, and

unclassified *Proteobacteria* were reduced in diseased mature plants. In contrast, *Alphaproteobacteria, Chitinophagia, Betaproteobacteria, Sphingobacteriia, Flavobacteriia, Cytophagia,* and *Verrucomicrobiae* were more abundant in diseased plants (Fig. 3B).



A



B

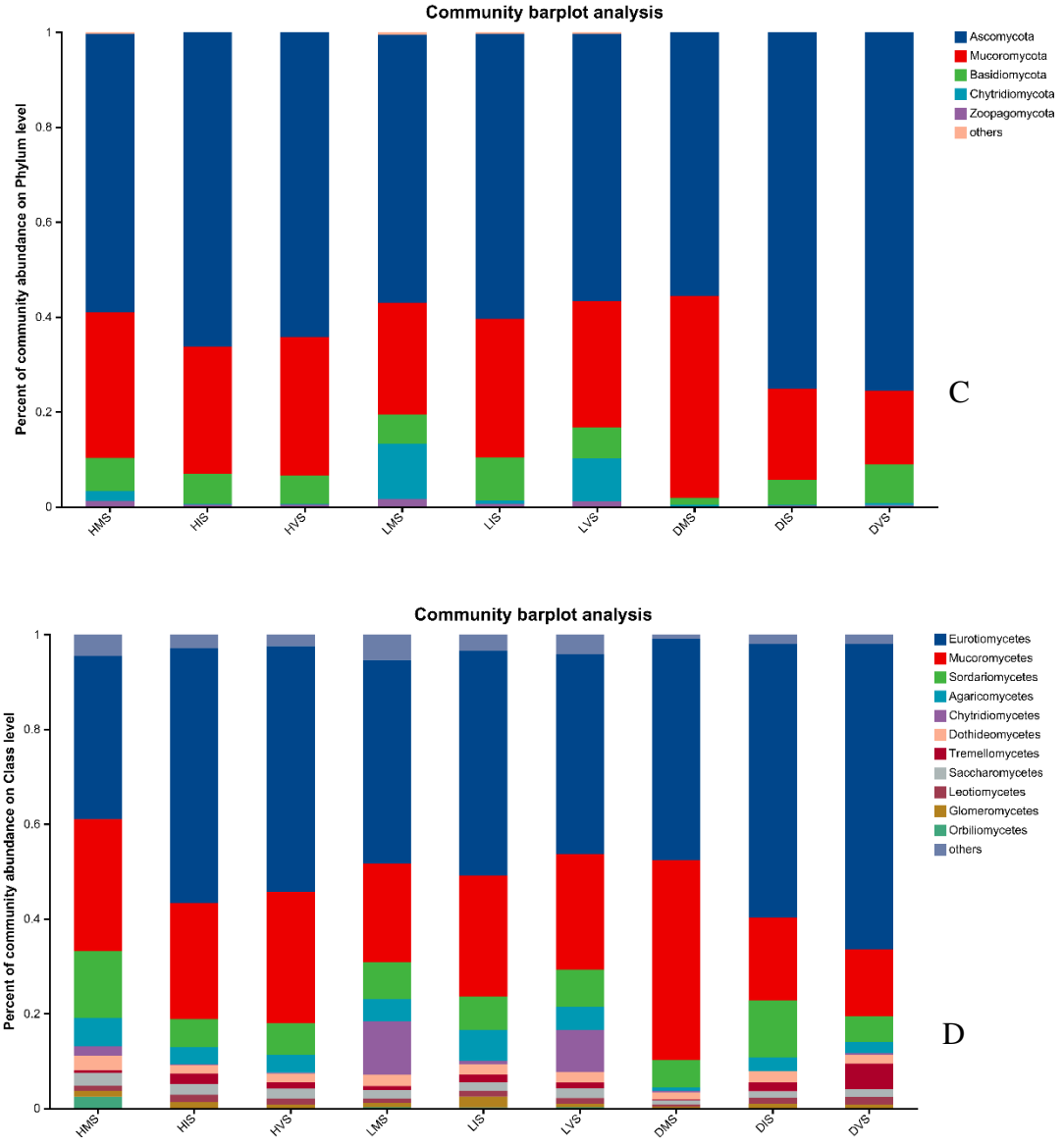
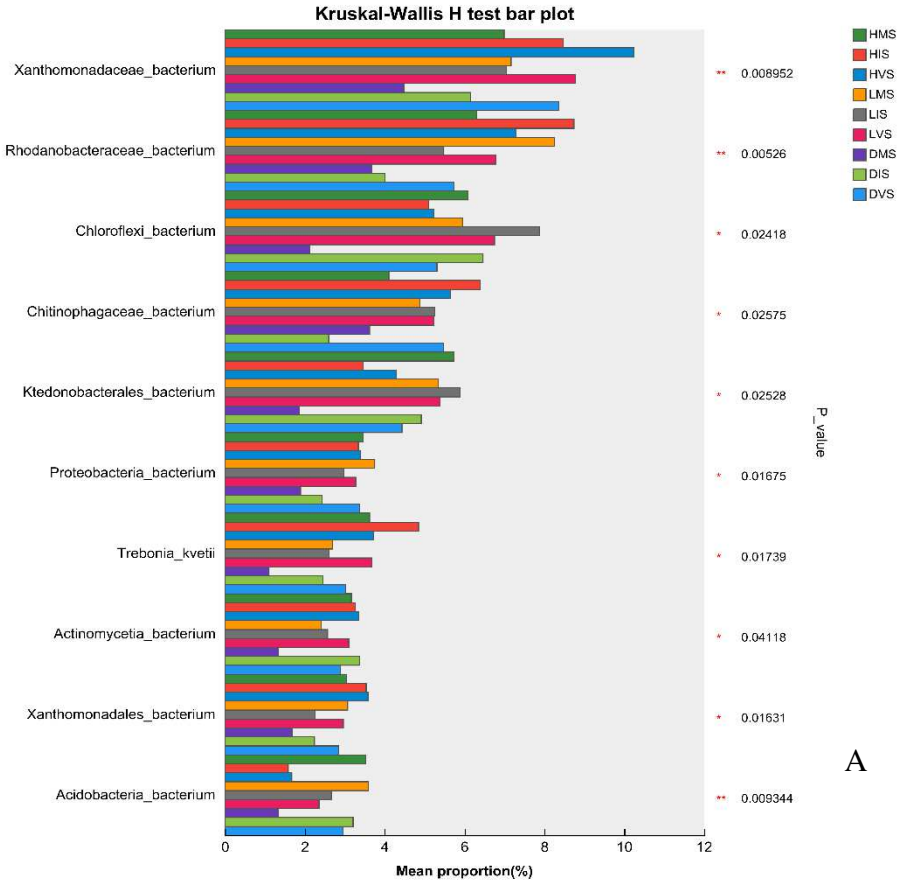


Fig. 3. Relative abundance of dominant bacterial (A: phylum, B: class) and fungal (C: phylum, D: class) taxa in the konjac rhizosphere under different health statuses and growth stages.

Dominant fungal phyla included *Ascomycota, Mucoromycota,* and *Basidiomycota* (Fig. 3C). At the mature stage, diseased konjac exhibited lower abundances of *Chytridiomycota* and *Zoopagomycota* compared to healthy and latently infected plants (Fig. 3D). During the initial growth stage, fungal abundances across health states were similar. In the vegetative stage, latently infected plants showed higher *Chytridiomycetes* abundance than healthy or diseased plants. By the mature

stage, *Chytridiomycetes* abundance in diseased plants was significantly lower than in healthy and latently infected plants. Healthy plants displayed higher *Chytridiomycetes* abundance at maturity compared to earlier stages, while latently infected plants showed increased *Chytridiomycetes* abundance at later stages. Disease plant exhibited elevated *Mucoromycota* abundance at the mature stage relative to earlier stages.

The Kruskal-Wallis H test identified significant differences in bacterial and fungal species abundance under varying health conditions and growth stages (Fig. 4). Among the top 10 bacterial species, *Xanthomonadaceae_bacterium*, *Rhodanobacteraceae_bacterium*, and *Acidobacteria_bacterium* showed highly significant differences, with their abundances lowest under disease conditions. *Xanthomonadaceae_bacterium* peaked during the vegetative stage, following the trend: healthy>latently infected>diseased (Fig. 4A). For fungi, *Rhizopus arrhizus* and *Rhizopus microsporus* were significantly enriched in diseased mature plants, whereas *Blyttiomyces helicus* was abundant in latently infected mature plants (Fig. 4B).



A

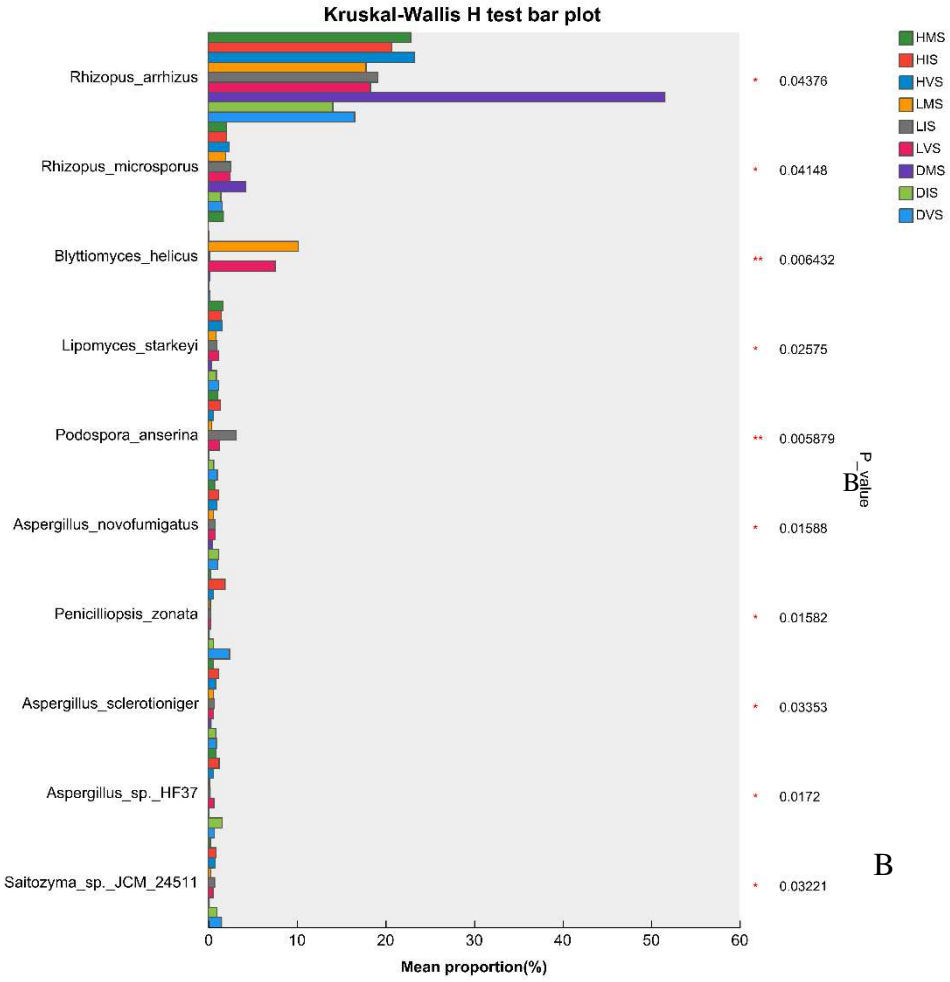


Fig. 4. Differential species analysis of (A) bacterial and (B) fungal communities in the konjac rhizosphere.

A Spearman correlation heatmap revealed relationships between microbial species and soil factors (Fig. 5). Most bacterial species showed significant negative correlations with soil properties. For example, *Alphaproteobacteria_bacterium* and *Rhodanobacter* sp. *C06* were positively correlated with pH, while *Rhodanobacter glycinis*, *Rhodanobacter sp.*, and *Acidobacteria_bacterium* correlated positively with available nitrogen (AN) and potassium (AK). In contrast, *Ktedonobacteraceae_bacterium* was negatively correlated with organic matter (OM). *Acidobacteriaceae_bacterium*, *Alphaproteobacteria_bacterium*, and *Acidobacteria_bacterium* correlated positively with available phosphorus (AP) but negatively with AN and AK (Fig. 5A). Most fungal species exhibited positive correlations with soil factors. *Podospora anserina* was negatively correlated with pH,

while other fungi showed positive correlations. AN and AK negatively correlated with *Blyttiomyces helicus* and *Hirsutella minnesotensis* but positively with *Saitozyma sp. JCM 24511* and *Penicilliopsis zonata* (Fig. 5B).

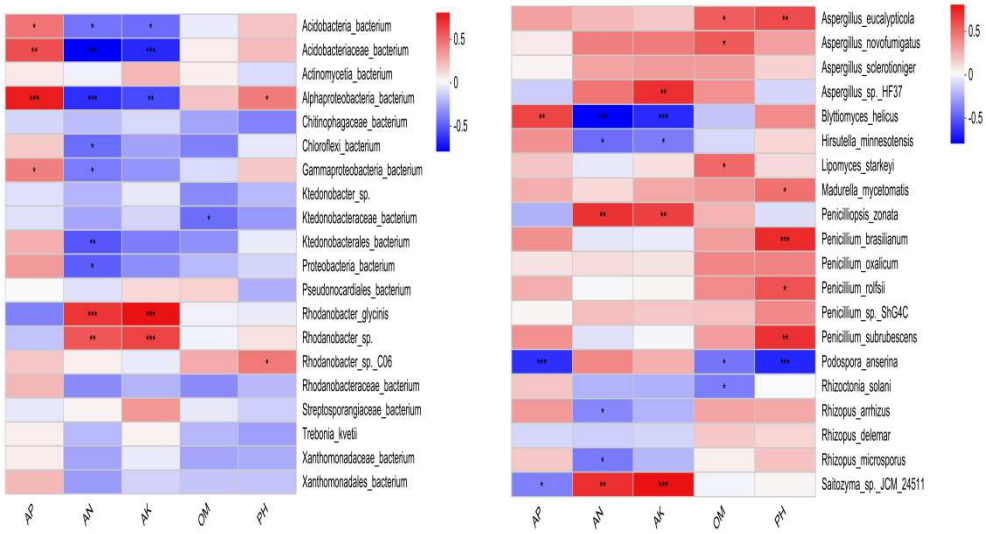


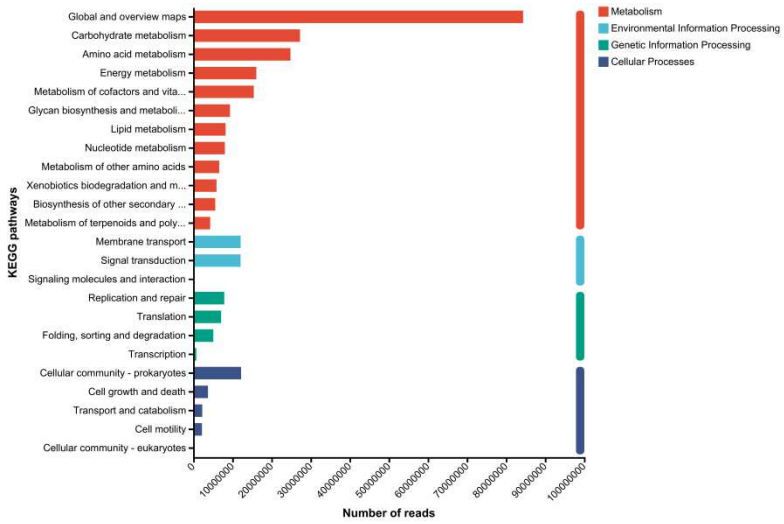
Fig. 5. Spearman correlation heatmap between soil factors and microbial species (A) bacterial and (B) fungal.

Notes: OM, organic matter; AN, available nitrogen; AP, available phosphorus; AK, available potassium.

KEGG functional profilingof konjac rhizosphere soil based on metagenomic analysis

KEGG functional annotation

Functional annotation of microbial genes in the rhizosphere soil was performed using the KEGG database (Fig. 6). At **Level 1**, "Metabolism" exhibited the highest abundance, representing the primary functional role of rhizosphere soil microorganisms, followed by "Environmental Information Processing" and "Genetic Information Processing." At **Level 2**, the most abundant functional categories included "Global and Overview Maps," "Carbohydrate Metabolism," "Amino Acid Metabolism," and "Energy Metabolism."

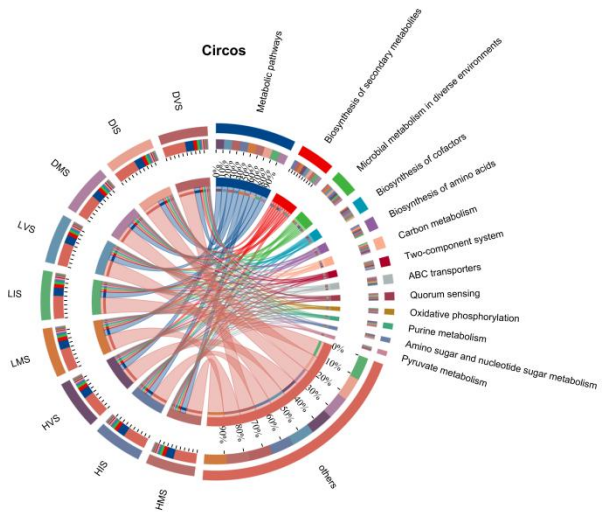


316

317 Fig. 6. Pathway statistics of konjac rhizosphere genes annotated in the KEGG
318 database.

319 **KEGG functional composition and differential analysis**

320 Dominant KEGG functional pathways (Level 3) with higher abundances in
321 rhizosphere microorganisms under varying health statuses and growth stages were
322 visualized using a chord diagram (Fig. 7). Key pathways included "Metabolic
323 Pathways," "Biosynthesis of Secondary Metabolites," "Microbial Metabolism in
324 Diverse Environments," "Biosynthesis of Cofactors," "Biosynthesis of Amino Acids,"
325 "Carbon Metabolism," "Two-Component Systems," and "ABC Transporters," with
326 average proportions of 17.6%, 7.80%, 5.23%, 3.15%, 2.76%, 2.61%, 2.08%, and
327 2.02%, respectively.



328 Fig. 7. Relative abundance of KEGG-annotated pathways in the konjac rhizosphere

under different health statuses and growth stages

Health status and growth stage have notable effects on the functional pathways of rhizosphere microorganisms. Regarding health status, diseased plants exhibited a diminished functional abundance in "Metabolic Pathways" and "Amino Sugar and Nucleotide Sugar Metabolism" when contrasted with healthy and latently infected plants. On the contrary, "Microbial Metabolism in Diverse Environments," "Biosynthesis of Cofactors," and "Amino Sugar and Nucleotide Sugar Metabolism" were more prevalent in healthy plants. Additionally, "Oxidative Phosphorylation" was more enriched in latently infected plants as opposed to diseased and healthy plants. In terms of the growth stage, the "Biosynthesis of Cofactors" showed lower abundance at the initial stage compared to the vegetative and mature stages. The abundance of "Oxidative Phosphorylation" and "Purine Metabolism" declined at the mature stage, whereas "Two-Component Systems," "ABC Transporters," and "Quorum Sensing" showed an increase. Moreover, "Metabolic Pathways" and "Amino Sugar and Nucleotide Sugar Metabolism" were more abundant during the vegetative stage than at other growth stages.

Discussion

Changes in the Rhizosphere Microbial Community

The diversity and composition of rhizosphere microbial communities are closely linked to the occurrence of soil-borne diseases (Li et al., 2015). This study revealed that microbial community richness and diversity in the konjac rhizosphere peaked during the mature stage of diseased plants (Tables 2 and 3). The Chao1 indices for bacterial and fungal communities in diseased konjac rhizosphere were 413.00 and 22,876.33, respectively, indicating higher microbial diversity in diseased rhizosphere soil compared to healthy plants. This aligns with findings in pepper plants affected by bacterial wilt, where diseased rhizosphere soil exhibited greater microbial diversity than healthy soil (Liu et al., 2021). Principal coordinate analysis (PCoA) demonstrated significant differences in bacterial and fungal community structures across varying plant health conditions and growth stages, with diseased samples showing higher dispersion (Fig. 2). Similarly, Zhang et al. (2023) reported distinct

rhizosphere microbial community structures between healthy Chinese cabbage and clubroot-diseased plants.

At the phylum level, the relative abundances of *Chloroflexi* and *Acidobacteria* in healthy plants at the mature stage were 11.54% and 4.66% higher, respectively, than in diseased plants (Fig. 3). These taxa are recognized as key biological indicators of soil disease suppression (Wang et al., 2019; Wang et al., 2020). *Acidobacteria* actively interact with plants as growth-promoting bacteria (Kielak et al., 2016), while both *Chloroflexi* and *Acidobacteria* contribute to konjac's resistance against soft rot infection.

In diseased plants, the abundance of *Chytridiomycota* was significantly reduced compared to healthy plants (Liao et al., 2021), whereas *Mucoromycota* was enriched in soils of root-diseased plants such as ginseng with rusty root syndrome (Wei et al., 2020). *Chytridiomycota* enhances crop resistance, while *Mucoromycota* is linked to reduced disease resistance. In this study, the relative abundances of *Chytridiomycota* in DMS (diseased mature stage), LMS (latently infected mature stage), and HMS (healthy mature stage) were 0.28%, 11.69%, and 2.11%, respectively, while *Mucoromycota* abundances were 42.45%, 23.60%, and 30.74% (Fig. 3C).

The fungal species *Rhizopus arrhizus* and *Rhizopus microsporus* were significantly enriched in diseased mature plants. *R. arrhizus* is a known pathogen of sunflower rot (Abeywickrama et al., 2020) and postharvest bulb rot (Gao et al., 2024), while *R. microsporus* induces water-soaked, puffy, and dark-gray lesions in leaf mustard (Wang et al., 2020). *R. microsporus* releases toxins that kill host cells, facilitating necrotrophic growth for both the fungus and associated bacteria (Partida-Martinez & Hertweck, 2005). These observations suggest that *R. arrhizus* and *R. microsporus* may promote the proliferation of konjac soft rot pathogens, exacerbating disease severity.

Impact of the Rhizosphere Microbial Functions

The metagenome represents the collective genomic content of environmental microorganisms. Compared to other omics approaches, metagenomics enables more efficient and accurate predictions of soil microbial functions. In this study,

metabolism was identified as the dominant functional category of the konjac rhizosphere microbial community, with the hierarchical order at Level 1 being: Metabolism > Environmental Information Processing > Genetic Information Processing > Cellular Processes (Fig. 6). This highlights that microbial activity in the konjac rhizosphere is predominantly metabolic. Enhanced microbial metabolic capacity improves plant nutrient absorption and compound synthesis, which are critical for rapid plant growth (Prabha et al., 2019). Annotation of secondary metabolic pathways via the KEGG database further revealed that carbohydrate metabolism and amino acid synthesis are central functions of the konjac rhizosphere microbial community. These processes underscore the role of rhizosphere microorganisms in ensuring nutrient supply for plant growth (Wei et al., 2019).

Conclusions

Microbiome composition diverges early from an initially homogeneous state, and its variability is strongly associated with disease outcomes. Since pathogen density alone often cannot predict disease onset in a timely manner for effective intervention (such as the case of *Ralstonia solanacearum* surge coinciding with tomato wilt symptoms), early identification of key taxa involved in pathogen suppression is crucial. Through combining fluorescence quantitative PCR detection of soft rot pathogens with plant symptom analysis, konjac plants were classified into diseased, latently infected, and healthy states. Dynamic rhizosphere microbiome analysis showed that microbial shifts occur before observable changes in pathogen density, thus enabling earlier disease prediction. Key findings include that rhizosphere bacterial and fungal communities vary significantly among different health statuses and growth stages, with the highest diversity at the diseased mature stage. *Rhizopus arrhizus* and *Rhizopus microsporus* (known rot pathogens) are enriched in diseased plants, while *Chytridiomycota* (which promotes crop resistance) decreases and *Mucoromycota* (which reduces resistance) increases. Early microbiome divergence patterns imply that intrinsic microbial dynamics drive plant health outcomes. These insights contribute to the development of biological control strategies for konjac soft rot and emphasize microbiome diagnostics as a tool for pre-symptomatic disease management. Targeted manipulation of key microbial taxa can reduce the reliance on

chemical pesticides and enhance disease resilience. However, further research is required to clarify the mechanistic roles of specific taxa in pathogen suppression.

Declarations

Ethical Approval

This study did not involve human or animal subjects; thus, ethical approval from an ethical committee or internal review board was not applicable. No procedures related to human or animal research were conducted, and therefore, statements regarding consent to participate and consent to publish in the context of ethical approval for such studies are not relevant to this research.

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Availability of data and materials

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

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