Metagenome reveal the relationship between rhizosphere microorganisms and changes in soil environment of *Cunninghamia lanceolata* root rot

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Abstract

Cunninghamia lanceolata (*C. lanceolata*) is a crucial species in China, valued for its significance in timber production, afforestation, and commercial purposes due to its economic and ecological importance. However, since the 1980s, young *C. lanceolata* plantations in various regions of Sichuan, China, have been plagued by root rot. While previous studies have primarily focused on isolating and identifying pathogens responsible for root rot in *C. lanceolata*, little attention has been paid to the rhizosphere microbial community. This study represents the first attempt to investigate the impact of seasonal variation on the rhizosphere microbial community of *C. lanceolata* affected by root rot, utilizing high-throughput sequencing technology. The findings revealed that seasonal changes significantly influenced the physicochemical properties and microbial composition in the rhizosphere soil of *C. lanceolata* affected by root rot. Root rot notably led to a decrease in soil pH, an increase in total organic carbon (TOC) and total nitrogen (TN) contents, a reduction in the abundance of *Rhizophagus,* an increase in the abundance of certain bacteria in the *Acidobacteriota* phylum, and an enhancement of soil carbon and nitrogen metabolism throughout the year. These effects were particularly pronounced during the summer, coinciding with the peak severity of root rot. This study offers a valuable microbial resource for future investigations, aiming to enhance soil quality and facilitate ecological management strategies for root rot in *C. lanceolata*.

Keywords Root rot of *C. lanceolata*, Seasonal changes, Rhizosphere microbes, Environmental factors, Metagenomic sequencing

1. Introduction

Cunninghamia lanceolata (C. lanceolata) stands as a pivotal indigenous tree species in southern China (Guangyi et al., 2017). Its significance in forestry is underscored by the vast stock volume, which has reached 620.3645 million m3 in China, constituting a third of the nation's main tree species plantation. Moreover, *C. lanceolata'*s carbon storage, totaling 63.69 Tg, represents 1.71% of the country's forest carbon reservoir (Wang et al., 2001). Given its substantial contribution to plantation management and development, addressing challenges such as root rot is imperative.

Root rot poses a significant threat to *C. lanceolata*, particularly in young plantations, jeopardizing the ecological balance necessary for its growth. This disease, caused by microbial infection of plant roots, manifests as a gradual weakening of water and nutrient absorption functions, ultimately leading to plant demise. Symptoms include the yellowing and wilting of plant leaves (Ma et al., 2020; Pang et al., 2022; Zhao et al., 2018). However, research on root rot disease in *C. lanceolata* has been limited due to geographical constraints.

The plant rhizosphere serves as the primary defense mechanism for plant roots against soil-borne pathogens, hosting a diverse array of microorganisms (Cook et al., 1995; Mendes et al., 2013). These microorganisms play pivotal roles in enhancing plant resilience to stressors or pathogens and promoting overall plant health and growth by facilitating nutrient acquisition and producing plant hormones (Mishra et al., 2022). Additionally, members of the rhizosphere microbiome can combat soil-borne pathogens through various mechanisms, including antibiotic production, competition for nutrients, and parasitism (Druzhinina et al., 2011; Duffy et al., 2003; Haas and Défago, 2005; Lugtenberg and Kamilova, 2009). Moreover, they regulate plant immune responses by inducing systemic resistance (Conrath, 2006; Pieterse, 2012; Yang et al., 2009).

However, alterations in the rhizosphere environment, including changes in abiotic factors such as soil pH, moisture content, and root exudates, can disrupt the balance of the rhizosphere microbiome, leading to pathogen proliferation and disease onset (Lauber et al., 2009; Lebeis et al., 2012; Philippot et al., 2013). Seasonal variations also exert significant direct and indirect

effects on rhizosphere microbial communities. Studies have demonstrated that root rot severity can intensify over time, with the second year often marked by critical transformations in rhizosphere microbial communities (Bi et al., 2023; Yang et al., 2023a). Furthermore, the presence of pathogens has been linked to seasonal fluctuations in bamboo root microbiomes, resulting in reduced bacterial richness (Li et al., 2023) c

Our investigation at the Hongya County Forest Farm in Sichuan Province revealed varying degrees of *C. lanceolata* root rot severity across different seasons, suggesting a correlation between environmental changes and rhizosphere microbial composition. To test this hypothesis, we employed high-throughput sequencing to assess the structure and function of rhizosphere microbial communities in *C. lanceolata* root rot during all four seasons. Additionally, we measured changes in rhizosphere soil physicochemical properties to elucidate their influence on *C. lanceolata* root rot and identify healthy rhizosphere microorganisms to enhance *C. lanceolata* 's resistance to pathogens. This study aims to provide a theoretical foundation for the biological control of *C. lanceolata* root rot.

2. Material and methods

2.1 Soil sample collection

Samples were collected from the State-owned Forest Farm in Hongya County, Meishan City, Sichuan Province (102°49'-103°32' E, 29°24'-30°00' N). Rhizosphere soils from both healthy (CK) and root rot diseased (RR) C. lanceolata were collected in mid-April (SP) for spring, mid-July (SU) for summer, mid-October (F) for fall, and mid-January (W) for winter. Sampling was conducted in the morning to minimize the effects of rainfall and sunlight. Four biological replicates were collected for each group (SPCK, SPRR, SUCK, SURR, FCK, FRR, WCK, and WRR). Soil samples were collected without roots and gently shaken. Root-adhered soils were stored in sterile bags and snap-frozen at -80°C for subsequent DNA extraction. Shaken soil samples were air-dried for the determination of soil physicochemical properties.

2.2 Metagenome DNA extraction and shotgun sequencing

Total microbial genomic DNA was extracted using the OMEGA Mag-Bind Soil DNA Kit (M5635-02, Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions.

DNA quality and quantity were assessed using a Qubit 4.0 fluorometer (Invitrogen, USA) and Qubit™ 1X dsDNA HS Assay Kit (Invitrogen, USA), respectively. Shotgun sequencing was performed on the Illumina NovaSeq platform, where total metagenomic DNA was randomly fragmented into short fragments, and insert fragment libraries of approximately 400bp were constructed for paired-end sequencing.

2.3 Data processing and bioinformatics analysis

Raw sequencing reads were processed to obtain quality-filtered reads for further analysis. First, sequencing adapters were removed from sequencing reads using Cutadapt (v1.2.1) (Martin, 2011). Secondly, low quality reads were trimmed using a sliding-window algorithm in FASTP (Chen et al., 2018). Then, the reads were aligned to the genome of the *C. lanceolata* using BMTagger (Rotmistrovsky and Agarwala, 2011) to remove host contamination. The obtained reads after quality control were classified using Kraken2 (Wood et al., 2019) for the metagenomic sequencing reads from each sample from the RefSeq-derived database, which includes genomes from archaea, bacteria, viruses, and fungi.

The meta-large parameter of Megahit (v1.1.2) (Dinghua et al., 2015) was used to assemble each sample. The generated contigs (longer than 300bp) were then clustered together and clustered in "easiy-linclust" mode using mmseqs2 (Steinegger and Söding, 2017)， with the sequence identification threshold set to 0.95 and shorter contigs covered to 90%. Non-redundant contigs were aligned to the NCBI-nt database in "taxonomic" mode via mmseqs2 (Steinegger and Söding, 2017) to obtain the lowest common ancestor classification, and contigs assigned to Viridiplantae or Metazoa were removed in subsequent analyses. Genes in the group were predicted using MetaGeneMark (Zhu et al., 2010). The cluster module of the software MMseqs2 was used to obtain the non-redundant protein sequence set according to the similarity of 95% and the coverage of alignment region of 90% (the proportion of short sequences).To assess the abundances of these genes, the high-quality reads from each sample were mapped onto the predicted gene sequences using salmon (Rob et al., 2015) in the quasi-mapping-based mode with “-meta -minScoreFraction=0.55 ”, and the CPM (copy per kilobase per million mapped reads) was used to normalize abundance values in metagenomes. The functionality of

the non-redundant genes were obtained by annotated using mmseqs2 (Steinegger and Söding, 2017) with the "search" mode against the protein databases of KEGG databases,

The Kruskal-Wallis test method was used to compare the difference between the two groups, and the Benjamini-Hochberg method was used to control the False discovery rate (FDR). At the same time, the fold difference relationship of each taxon between each sample (group) was obtained, which was expressed as Log10 (Fold_change_value). By default, taxa with adj.P < 0.05 were selected by default as species with significant abundance differences. Based on the taxonomic and functional profiles of non-redundant genes, LEfSe (Linear discriminant analysis effect size) was performed to detect differentially abundant taxa and functions across groups using the default parameters (Segata et al., 2011). Beta diversity analysis was performed using Bray-Curtis distance metrics (Bray and Curtis, 1957), and visual analysis was performed using principal coordinate analysis (PCoA) (Ramette, 2007) to investigate the composition and functional changes of microbial communities in the samples.

2.4 Determination of soil physicochemical properties

Soil pH, total organic carbon (TOC), total nitrogen (TN) and total phosphorus (TP) contents were measured according to Chinese forestry standards(Li et al., 2020). The contents of ammonium nitrogen (AMN) and nitrate nitrogen (NN) in soil were determined by indigo colorimetry and ultraviolet spectrophotometer. 10 ± 0.05g of fresh soil was extracted with 50 mL 2 mol/L potassium chloride, and the content of NN was determined by ultraviolet spectrophotometry correction factor method (the determination wavelength is: 220 nm and 275 nm), and AMN content was determined by an indigophenol blue-colorimetric method (wavelength: 625 nm).

2.5 Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 26.0 (IBMCorp., Armonk, N.Y., USA). One-way ANOVA was used for group comparisons, with data presented as mean ± SD. Correlation analysis between soil physicochemical properties and microbial community was conducted using the genescloud tools platform

(https://www.genescloud.cn).

3. Results

3.1 Library sequencing data and gene prediction

Sequencing yielded an average of 92,171,463 raw reads, with 89,939,222 clean reads obtained after quality control, representing 97.58% of raw reads. The average base number per sample was 13,917,890,998 bp before quality control, and 13,572,686,193 bp after. Following BBCMS correction, read sequences were assembled into contigs, averaging 448,359 (Table S1).

3.2 Microbial diversity and community structure in the rhizosphere

According to Chao1 and Shannon index, the diversity of bacterial and fungal communities in rhizosphere soil was affected by seasons and diseases. We found that the richness of bacterial and fungal communities in healthy rhizosphere soil was lower in summer than in other seasons, and the Shannon index showed that SPCK was higher than SPRR, and CK in other seasons. This indicated that rhizosphere soil contained rich and diverse microflora in spring (Fig 1A, B). PCoA of beta diversity analysis was used to explore the differences and similarities among samples. The results showed that bacterial communities of healthy rhizosphere and diseased roots were far apart in different seasons (Fig 1C), indicating that root rot had a great influence on rhizosphere bacterial community composition in different seasons, while the healthy rhizosphere and root rot rhizosphere fungal communities were only far apart in fall. In spring, summer, and winter, the fungal communities of healthy rhizosphere and root rot were close to each other, indicating that the season had little effect on the fungal community (Fig 1D).

To characterize the distribution of bacteria and fungi in rhizosphere microbes, we performed an analysis of microbial composition. For bacterial composition, *Acidoferrum* and *Sulfotelmatobacter* were the dominant genera. *Acidoferrum* in FCK (10.84%) and *Sulfotelmatobacter* in WCK (7.07%) were the most abundant species. Notably, in SUCK, *Terracidiphilus* had a higher abundance than any other group, about twice as much (5.54%) (Fig 1E). As for the composition of fungi, *Rhizophagus* and *Ambispora* were the dominant genera. Among them, SPCK had the highest abundance of *Rhizophagus* (29.47%) and SPRR

had the highest abundance of *Ambispora* (7.36%) (Fig 1F).

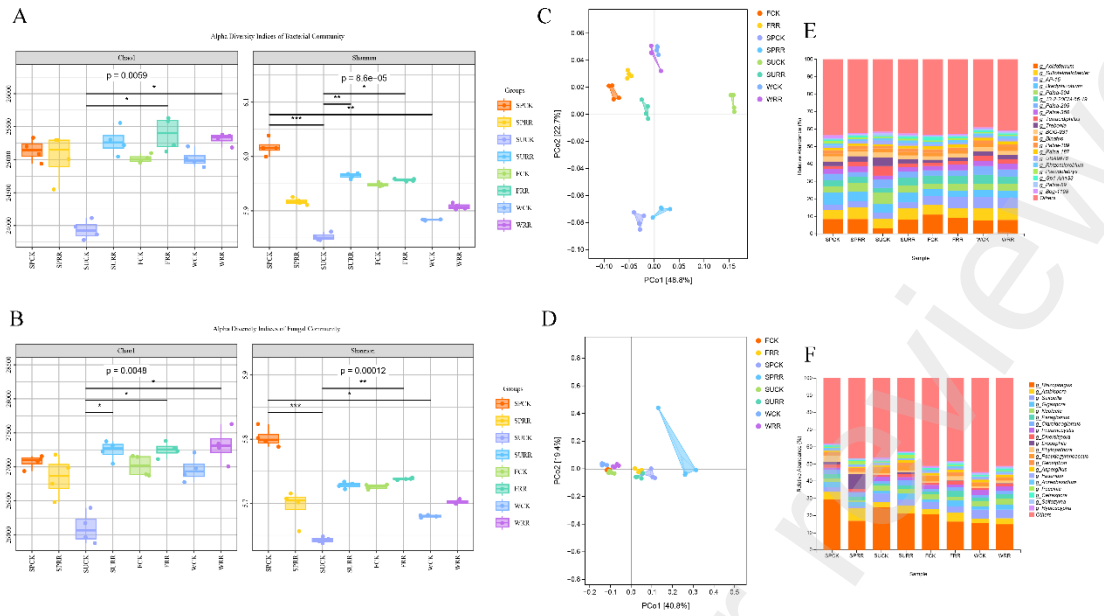

Fig 1. Diversity and composition of bacterial and fungal communities in the rhizosphere of root rot and healthy *C. lanceolata* during different seasons. (A). Chao1 and Shannon indices of rhizosphere bacterial community. (B). Chao1 and Shannon indices of fungal communities in the rhizosphere. (C). PCoA analysis of rhizosphere bacterial communities. (D) PCoA analysis of rhizosphere fungal communities. (E) Relative abundance of rhizosphere bacterial genera (Top20). (F) Relative abundance of rhizosphere fungal genera (Top20).

3.3 Differences in rhizosphere microbes

The Kruskal-Wallis test method was used to compare each group to obtain the statistical analysis results of difference significance, and Top10 was used for mapping. The results showed that the top 10 differential genera in the samples were *Acidoferrum, Sulfotelmatobacter, Bradyrhizobium,* and *Terracidiphilus* as top differential bacterial genera, with *Acidoferrum* notably higher in fall and *Terracidiphilus* lower in spring (Fig 2A). The top 10 differential fungal genera have *Rhizophagus, Ambispora, Claroideoglomus,* and *Paraglomus* these arbuscular mycorrhizal fungi (AMF), *Rhizophagus* in the healthy rhizosphere in summer has high abundance, is significantly higher than the healthy rhizosphere in fall; The abundance of *Ambispora* was significantly lower in the healthy rhizosphere in winter than that in summer. *Paraglomus* had similar differences to *Ambispora*, with a lower abundance of health in winter

and also significantly lower than in the summer rhizosphere (Fig 2B).

A LEfSe analysis was used to compare the different species in each group and identify the signature species. Rhizosphere biomarkers were also significantly affected by seasonal variation, as observed in microbial diversity and composition. Figure S1 shows that rhizosphere bacteria are abundant throughout the year, and there are many bacterial markers of rhizosphere soil for root rot. However, fungal biomarkers were scarce and were not detected in rhizosphere soils with root rot in summer and autumn (Fig S2).

Throughout the year, rhizosphere bacterial markers of root rot were much higher than those of healthy rhizosphere, whereas rhizosphere fungal markers of root rot were not detected. Rhizosphere bacterial markers of root rot included *Planctomycetota*, *Burkholderiaceae*, *Polyangiales*, *Bryobacterales*, *Pedosphaerales*, *Ktedonobacteraceae* and others (Fig 2C). Markers of healthy rhizosphere fungi included *Agaricomycetes*, *Pezizomycetes*, *Helotiales* and *Rhizophagus* (Fig 2D).

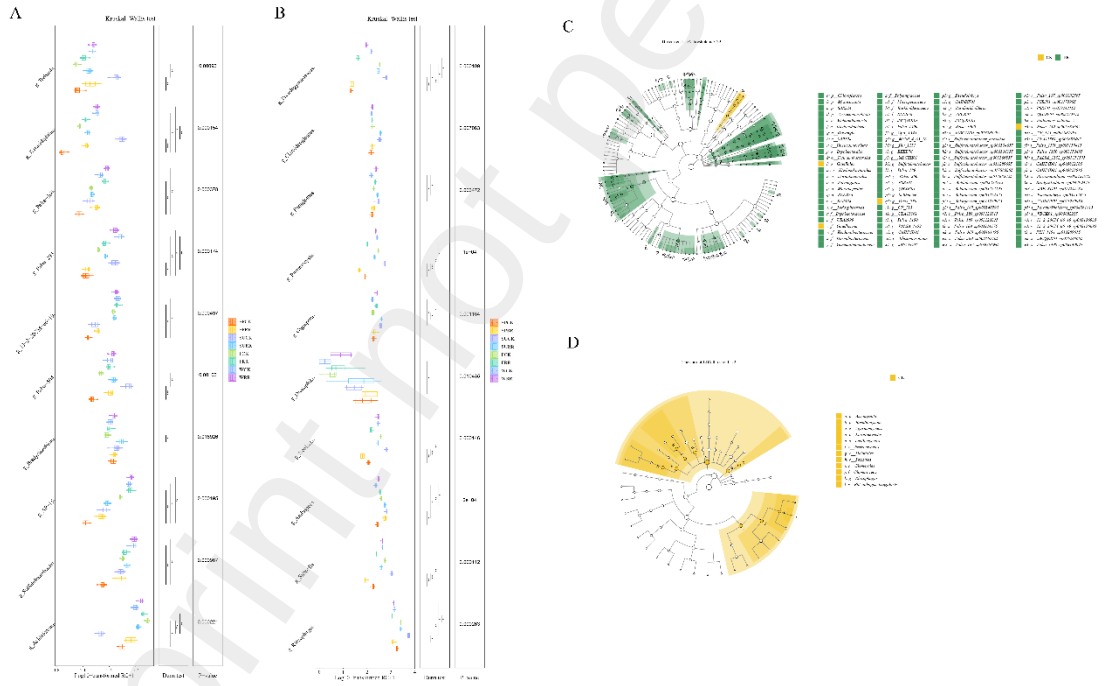

Fig 2. Analysis of species differences. (A) Top 10 bacterial genera with differences. (B) Top 10 fungal genera with differences. (C) Branching plots generated by LEfSe analysis show bacterial taxa with the highest differential abundance enriched in the rhizosphere (LDA value ≥ 2) (D) Branching plots generated by LEfSe analysis show fungal taxa with the highest differential abundance enriched in the rhizosphere (LDA value ≥ 2).

3.4 Metagenomic analysis of rhizosphere microbial functions

Based on KEGG, the functions of bacteria and fungi in the rhizosphere soil of *C. lanceolata* were defined. Lipid metabolism, Amino acid metabolism, Carbohydrate metabolism, Metabolism of terpenoid and polyketide compounds of bacteria in the healthy rhizosphere soil in winter was significantly higher than that in the healthy rhizosphere in spring (Fig 3A). The functional changes in Carbohydrate metabolism, Lipid metabolism, Amino acid metabolism, Energy metabolism, and Signal transduction of fungi in rhizosphere soil in the four seasons were almost the same, and the levels of these metabolism in healthy rhizosphere soil in summer were significantly higher than those in healthy rhizosphere soil in spring (Fig 3B). LEfSe analysis showed that root rot caused changes in Biotin metabolism, Thiamine metabolism, Glycosaminoglycan degradation, and Folate biosynthesis in rhizosphere soil bacteria (Fig 3C). The Hippo signaling pathway, Rap1 signaling pathway, Sulfur metabolism and Glucosinolate biosynthesis in rhizosphere soil fungi were also significantly changed (Fig 3D). In addition, we found that the function of rhizosphere microbes was also affected by season. In spring, root rot mainly affected rhizosphere functions such as Transport and catabolism, Carbohydrate metabolism, Biosynthesis of some secondary metabolites, Amino acid metabolism, Plant hormone signal transduction, and Plant pathogen interaction. In summer, root rot mainly affected Cell growth and death, Signal transduction, RNA degradation, Cysteine and methionine metabolism, and Porphyrin metabolism in the rhizosphere. In fall, root rot affected Membrane transport, Polysaccharide biosynthesis, Energy metabolism, and Plant pathogen interaction in rhizosphere. Finally, root rot affected Geraniol degradation, Ribosome biosynthesis, and Galactose metabolism in the rhizosphere during winter (Fig S3 and S4).

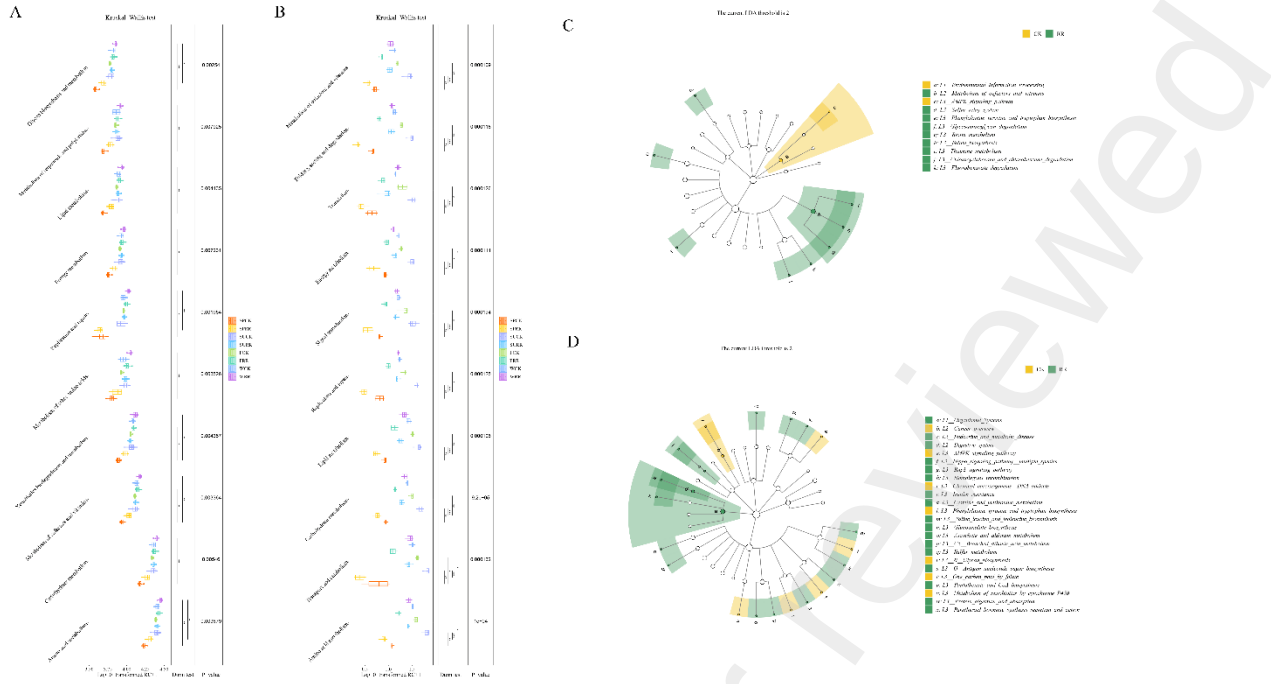

Fig 3. Analysis of KEGG functional differences. (A) prediction based on bacterial KEGG relative abundance (Top 10). (B) prediction based on fungal KEGG relative abundance (Top 10). (C) Branching plots generated by LEfSe analysis show KEGG functional groups enriched with the highest differential abundance for rhizosphere bacteria under LDA score (LDA value ≥ 2) (D) Branching plots generated by LEfSe analysis show KEGG functional groups enriched with the highest differential abundance for rhizosphere fungi under LDA score (LDA value ≥ 2).

3.5 Root rot affects soil physicochemical properties

Table 1 shows that season and root rot significantly affected soil physicochemical properties. Rhizosphere soil of *C. lanceolata* was acidic throughout the year, and root rot would further reduce soil pH. Root rot increased soil TOC and TN values, which were significantly higher than those in the healthy rhizosphere soil. The contents of TOC, TN and TP were higher in summer and fall. Root rot significantly increased the contents of AMN and NN in winter, but decreased in summer. The content of NN in summer was significantly different from that in the healthy rhizosphere soil.

Table 1. Soil physicochemical properties

Sample	pH	TOC (g/kg)	TN(g/kg)	TP(g/kg)	AMN(mg/kg)	NN(mg/kg)
SPCK	4.0825±0.0877 b	33.5900±0.6364 d	2.7550±0.0968 e	0.2725±0.0320 e	4.2550±0.7683 c	15.1950±1.6032 c
SPRR	3.8675±0.0842 c	37.6900±0.3685 c	3.2425±0.0650 c	0.2875±0.0222 de	5.2300±0.7671 bc	18.3975±0.5944 b
SUCK	3.8850±0.0823 c	37.3000±1.0451 c	3.0425±0.0655 d	0.3400±0.0216 c	4.1275±0.4282 c	22.9200±1.3456 a
SURR	3.3175±0.1056 d	44.6325±1.6702 a	3.8350±0.1085 a	0.4125±0.0171 a	4.1125±0.2266 c	18.1325±0.7568 b
FCK	4.2300±0.0600 a	36.7950±1.8716 c	3.2700±0.0852 c	0.3700±0.0141 b	7.0500±1.0075 a	22.1425±1.3861 a
FRR	3.9175±0.0499 c	42.0125±1.5714 b	3.5900±0.1068 b	0.3125±0.0126 cd	7.9525±0.9726 a	22.8900±1.2087 a
WCK	4.2425±0.0150 a	31.4050±1.4062 e	2.6175±0.1804 e	0.3325±0.0171 c	5.6600±0.7332 b	18.4250±0.4017 b
WRR	4.0650±0.0557 b	36.3625±0.5932 c	3.2075±0.0670 c	0.2825±0.0171 de	6.9800±0.4474 a	22.2975±2.7972 a

± before is mean (n = 4), ± after is standard deviation. Lowercase letters following numbers indicate significant differences.

3.6 Correlation between microbial communities and soil physicochemical properties

To observe the interactions between environmental factors and microbial communities, the top 10 species with genus level differences were selected for correlation analysis with environmental factors. As shown in Figure 4A, AMN was significantly correlated with most bacteria in the rhizosphere, and was significantly negatively correlated with *Bradyrhizobium*, while significantly positively correlated with *Acidoferrum* and *Sulfotelmatobacter. Bradyrhizobium* was negatively correlated with pH. There was no significant correlation between TOC and the top 10 bacterial genera. TN was significantly positively correlated with *Acidoferrum*, and NN was significantly positively correlated with most bacteria. In addition, pH was negatively correlated with the genera *Paraglomus*, *Gigaspora*, *Claroideoglomus* and *Ambispora*. TOC and TN were significantly positively correlated with the four fungal genera. TP and NN showed significant positive correlations with most fungi, while AMN showed significant negative correlations with *Drosophila*, *Rhizophagus*, *Claroideoglomus* and *Gigaspora* (Fig 4B).

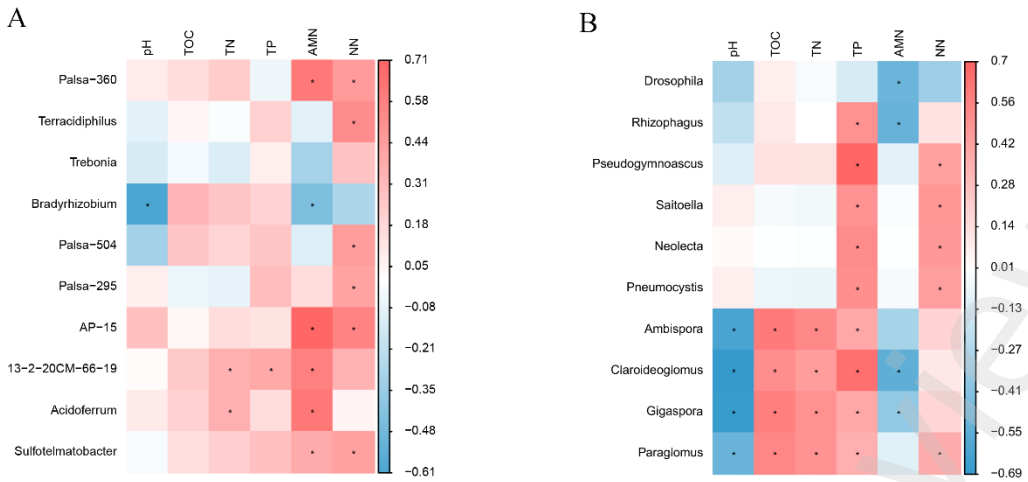

Fig 4. Correlation between environmental factors and microbial communities. (A) Correlation heatmap between environmental factors and top 10 differential bacterial genera. (B) Correlation heatmap between environmental factors and top 10 differential fungal genera. Red squares indicate positive correlations. Blue squares indicate negative correlations. "*" indicates a significant correlation at the 0.05 level.

4. Discussion

The interaction between host, pathogen and environment is known as the disease triangle of plants. The main factors related to plant pathogens include climate, landform, soil, plant community, etc. These factors can directly or indirectly affect plant pathogens (Makiola et al., 2021; Scholthof, 2007). A large number of studies have shown that seasonal variation has a strong regulatory effect on soil physicochemical properties and microorganisms (Li et al., 2020; Li et al., 2019; Onwuka, 2018; Upton et al., 2019). However, no studies have evaluated the seasonal changes in soil physicochemical properties and microbial communities in the rhizosphere of root rot of *C. lanceolata*. We found that rhizosphere soil pH was significantly lower and TOC and TN contents were significantly higher in diseased plants than in healthy rhizosphere soils. Previous study showed that soil pH decreased and TOC increased in *Psammosilene tunicoides* with root rot, and it was speculated that root rot may be related to reduced plant disease resistance due to lower pH in the soil, while excess TOC may lead to root rot (Yang et al., 2023b). Interestingly, Liu et al.(Liu et al., 2023) found that higher pH could improve the N uptake of *Aconitum carmichaeli* and the abundance of potentially beneficial

microorganisms in the soil, reducing the incidence of disease. However, in-depth investigations of the relationship between N nutrients and host nutrition and disease severity have shown that TN is associated with plant disease severity (Huber and Watson, 1974). Smiley et al. (Smiley et al., 2016) reported that wheat infected with *Fusarium pseudograminearum* had lower levels of root rot under low N conditions. These differences may be due to the differences in environmental factors induced by root rot in different plant species. In this study, season was an important factor affecting the physicochemical properties of the soil with root rot of *C. lanceolata*, the pH value was lowest in summer and highest in winter, and the contents of C, N and P were highest in summer, a result consistent with previous studies (Zhang et al., 2020). The early summer is rainy, the environment in the soil is acidic, and the ventilation is poor, which makes the roots suffocate, and therefore increases the incidence of root rot.

It has been reported that root rot changes the composition of plant rhizosphere microbial communities (Guo et al., 2022; Liao et al., 2022; Tang et al., 2023a; Tang et al., 2023b; Wang et al., 2024; Yang et al., 2023b). Here, we found that there was significant differences in rhizosphere microbiota between root rot and healthy *C. lanceolata*, with root rot reducing the abundance of *Rhizophagus* and increasing the abundance of *Acidoferrum* and *Sulfotelmatobacter*. As an AM fungus, *Rhizophagus* can promote the resistance of host plants to adverse environments (Oliveira et al., 2022; Romero-Munar et al., 2023). *Acidoferrum* belongs to *Acidobacteriota*, and the fungi in *Acidobacteriota* usually have carbon and nitrogen metabolism genes, so they play a full role in the cycling of organic matter and nitrogen in soil (Kalam et al., 2020), *Sulfotelmatobacter* is a candidate genus Acidobacteriota proposed by Hausmann et al.(Hausmann et al., 2018), which has a putative isosulfur metabolism. *Acidoferrum* and *Sulfotelmatobacter* were more abundant in the root rot samples, suggesting that plants may increase their carbon and nitrogen metabolism to maintain normal physiological activities under the condition of root rot. *Acidoferrum* showed a significant positive correlation with soil TN and AMN contents, and *Sulfotelmatobacter* was significantly positively with soil AMN and NN contents, confirming this statement. In the present study, five AM fungi, *Rhizophagus*, *Paraglomus*, *Gigaspora*, *Claroideoglomus* and *Ambispora* were significantly and

positively correlated with phosphorus content, this is in contrast to previous studies that reported that P is a key limiting factor for plants to establish a symbiotic relationship with mycorrhizal fungi (Breuillin et al., 2010; Nouri et al., 2014). Previous studies on the negative effects of P on mycorrhizal fungi were conducted at high P content; however, due to the low TP content in this study, it was not sufficient to inhibit mycorrhizal fungi. In addition, the differences in rhizosphere microbes vary with season (Upton et al., 2019). In summer, *Acidoferrum* was significantly increased in root rot samples compared to healthy samples. The abundance of *Rhizophagus* and *Claroideoglomus* was higher in summer, and *Rhizophagus* did not differ significantly between healthy and diseased samples in winter. However, in spring and winter, disease increases the relative abundance of *Ambispora*, which is also one of the AMF. When attacked by pathogens or insects, plants are able to recruit some AM fungi and enhance microbial activity, thereby inhibiting rhizosphere pathogens(Mendes et al., 2013). The different changes of *Ambispora* and *Rhizophagus* may be due to the inconsistent roles of different types of AMF when plants are under biotic stress.

Furthermore, KEGG enrichment analysis showed that root rot affected rhizosphere microbial community function and significantly increased the level of Biotin metabolism in rhizosphere. Biotin (vitamin H or biotin) belongs to a class of sulfur-containing fatty acid derivatives with a seven-carbon atom skeleton. In organisms, biotin mostly exists in the form of protein modification and participates in a series of important metabolic processes including sugars, proteins and fatty acids. From the perspective of energy consumption, biotin is also a kind of small biomolecules with high energy consumption, so its synthesis pathway is usually carefully regulated (Zhang et al., 2021). In recent years, biotin has been identified as a limiting/trophic virulence factor (Feng et al., 2015; Feng et al., 2014), therefore, the biotin metabolism of pathogenic bacteria is particularly important for their pathogenic processes. It is worth mentioning that root rot enhanced plant-pathogen interactions in spring and fall. In the co-evolution of host-microbe interactions, pathogens acquired the ability to deliver effector proteins to plant cells to suppress the primary immune response (PTI), allowing pathogen growth. In response to the delivery of pathogen effector proteins, plant-acquired surveillance

proteins (R proteins) are used to directly or indirectly monitor the presence of pathogen effector proteins (Chisholm et al., 2006). During these two seasons, *C. lanceolata* was able to increase its resistance to pathogens through the regulation of rhizosphere microorganisms, resulting in a low level of root rot.

In summary, we analyzed the changes of rhizosphere microbial community and soil physicochemical properties under the four seasons of *C. lanceolata* root rot. Root rot decreased the relative abundance of beneficial microorganisms such as *Rhizophagus*, increased the relative abundance of some bacterial genera in *Acidobacteriota*, and made the soil environment acidic. The increases of TOC and TN contents enhanced carbon and nitrogen metabolism and increased the level of disease, especially in summer. These findings provide new ideas for the effective use of plant microecological control measures to control root rot of *C. lanceolata*.

Acknowledgements

We thank Shanghai Personalbio Technology Co., Ltd for providing the sequencing services in the research.

Funding

This study was supported by the Natural Science Foundation of Sichuan Province (2024NSFSC1193). JP was supported by the Spanish Government grant TED2021-132627 B–I00 funded by MCIN and the China Scholarship Council (CSC) Grant (202206910028).

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data statement

All raw sequences were deposited in the NCBI Sequence Read Archive under accession

number PRJNA1104786.

Author contributions

Xiaokang Dai: Conceptualization, Methodology, Software, Funding acquisition, Writing - Original Draft. **Yuqing Dong:** Data curation, Formal analysis, Writing- Original draft**.** **Zebang Chen:** Resources, Visualization**. Shuying Li:** Supervision, Funding acquisition. **Josep Penuelas:** Funding acquisition, Writing - Review & Editing. **Tianhui Zhu:** Data curation, Writing - Review & Editing.

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