SHIFT IN SOIL MICROBIAL COMMUNITY OF COWPEA DUE TO ROOT ROT INFECTION

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Abstract. The impact of plant health status change, specifically the effect of root rot infection, on rhizosphere microbial structure remains unclear. To address this knowledge gap, we investigated the dynamic variations in soil nutrient and rhizosphere microbial community during cowpea root rot infection. Samples of healthy and moderately to severely diseased cowpea rhizosphere soil were collected from a field in Xiangyang, Hubei, China, and high-throughput sequencing was used to detect changes in soil microbial communities. Our results showed that cowpea biomass decreased significantly with increased disease severity, and differences in soil pH and nutrients (NO_3^--N, NH_4^+-N, and available phosphorus) were observed between healthy and diseased rhizosphere, particularly under moderate diseased conditions. The process of root rot infection affected the bacterial and fungal community structure in the rhizosphere, but with inconsistent responses. Specifically, bacterial richness decreased with increased disease severity, while minor differences were observed in fungal alpha diversity. Principal coordinate analysis revealed significant disparities in rhizosphere bacteria and fungi under healthy and diseased conditions (moderate and severe). Moreover, while bacterial communities underwent notable alterations, fungal communities exhibited no significant changes between moderate and severe diseased conditions. In addition, Redundancy analysis showed that bacteria beta diversity was determined by available potassium and pH in the rhizosphere, whereas fungal beta diversity was determined by soil organic matter and pH. Overall, with root rot infection, both the soil nutrients and microbial community structure of cowpea significant changed. As a result, our study provides insights into the molecular mechanism underlying cowpea root rot and other plant rhizosphere microbial communities.
Keywords: *bacterial, fungal, root exudates, microbial community, soil-borne disease*

Introduction

Cowpea (*Vigna unguiculata* L.) is an important food legume providing plant proteins and nutrients for humans (Oyeyinka et al., 2021; Wang et al., 2021), but it is susceptible

to soil-borne pathogens that can cause severe yield and quality losses due to perennial continuous cropping (Priyanka et al., 2022). Cowpea root rot is a serious disease that starts with individual branch and fibrous roots and spreads to the taproot, leading to weakened nutrient absorption (Priyanka et al., 2022). Traditional methods of managing the disease involve using fumigants and pesticides, but environmentally friendly strategies like soil biological control are needed for sustainable production (Jee et al., 2003; Priyanka et al., 2022). To develop alternative management strategies, we need to investigate the links between soil microbial communities and the onset of cowpea root rot disease.

Soils host diverse microbial communities that are vital for soil functionality and ecosystem stability (Bardgett et al., 2014; Vries et al., 2017), with the rhizosphere being the interface between plants and microbes that directly (nutrients cycle) affects plant growth and health (Qu et al., 2020). Pathogens in the rhizosphere interact with other microbes, which can determine whether plants become infected (Chaparro et al., 2012). When plants are infected, soil microbial communities can undergo changes due to root exudate profiles altered by stress signals or nutrient leakage through pathogen-inflicted wounds (Guo et al., 2021; Wen et al., 2021). Such changes may suppress pathogens and prevent soil-borne diseases by stimulating plant hormone production, competing for soil nutrients (Gu et al., 2020; Deng et al., 2022), and producing antibacterial/antifungal compounds (Pieterse et al., 2014; Syed-Ab-Rahman et al., 2019; Liu et al., 2021). For example, plant root exudates contain phenolic acids, including salicylic acid, vanillic acid, and cinnamic acid, which play a crucial role in regulating soil microbiota (Badri et al., 2013; Chaparro et al., 2013). In addition, recent studies have shown that phenolic acids can disrupt the balance of soil microbiota by promoting soil-borne pathogens and inhibiting beneficial microbes (Wu et al., 2016, 2017; Zhou et al., 2018), leading to the development of soil-borne diseases (Wang et al., 2017). Thus, studying changes in microbial communities during cowpea root rot could provide insights into rhizosphere engineering for disease prevention. However, there is a lack of comprehensive and dynamic comparisons during the infection process, and the key factors determining soil bacteria and fungi remain unclear.

This research investigated the differences in microbial communities and soil nutrient levels between healthy and diseased cowpea plants' rhizospheres (moderate and severe), as well as the relationships between these factors and cowpea root rot incidence. A total of 12 rhizosphere soil samples were collected from a cowpea field in Xiangyang, Hubei, China. The findings provide valuable insights into the vital function of rhizosphere microbial communities in preserving soil ecology and preventing soil-borne diseases, offering a theoretical foundation for promoting sustainable agriculture and maintaining soil health.

Materials and methods

Field experiment and sampling

Soil samples for this study were collected from a field plot at Tuanshan experimental station in Xiangyang City, Hubei Province (31.54 ^{0}N, 112.11 ^{0}E), where cowpea has been grown continuously for several years. The area has a subtropical monsoon climate, with an average temperature of 18.68 ^{0}C and annual precipitation of 1653.74 mm. The soil was classified as Red soil, and the initial characteristics of the 0-20 cm soil layer were recorded in *Table S1*. On April 9, 2022, 60,000 plants ha^{-1} of cowpea (cv.

Ejiangdou 8) provided by Xiangyang Academy of Agricultural Sciences, China, were sowed.

Sampling was carried out on May 26, 2022, during the cowpea branching phase. Before sampling, the field was divided into twelve plots (2×4 m) based on the health status of the plants. According to previous literature (Zhang et al., 2018; Chen et al., 2021), the morphology of cowpea disease is described as follows: in the early stage (moderate disease), the lower leaves of the infected plant became yellow, and withered from the edge of the leaves, but did not fall off. When the infected plant was pulled out, the underground part of the taproot and stem became dark brown. Later stage (severe disease), the diseased part is slightly sunk, sometimes cracking, and penetrating into the cortex. The vascular bundle turned brown when the stem was dissected. The lateral root of the diseased plant rots and dies, and when the taproot all rots, the diseased plant shrivels and dies. There were four healthy plots, four moderately diseased plots, and four severely diseased plots where more than half of the plants showed typical symptoms of root rot. Three cowpea plants were randomly selected from each plot as replicates, resulting in 12 samples each of healthy (H), moderate (M), and severe (S) categories. Rhizosphere soil samples were collected by shaking off the loosely adhered soil after excavating the plants.

Soil and plant analysis

To obtain the biomass, cowpea was dried (65 ^{0}C) and weighed. Soil samples were divided into three parts, sieved through a 2 mm filter, and stored at -80 ^{0}C in sterilized zip lock bags until DNA extraction. Approximately 300 g of fresh soil was used to determine the soil nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) while the remaining soil was air-dried for the determination of other soil properties. All soil chemical properties determination methods were consistent with those reported by Chen et al. (2022).

Soil quality index (SQI) was evaluated based on linear score equations converting soil factor to a value of 0-1 using either "more is better" or "less is better" scoring curves, depending on the sensitivity of the soil parameter. SQI was calculated using an SQI-area approach by comparing the area on a radar graph comprising of all soil parameters (Kuzyakov et al., 2020).

DNA extraction, PCR amplification, and high-throughput sequencing of soil samples

To extract DNA, 0.5 g of rhizosphere soil was used with the MO BIO PowerSoil DNA Isolation Kit (San Diego, USA). The concentration of extracted DNA was measured with a NanoDrop spectrophotometer (USA). The bacterial 16S V4 region and the fungal ITS1 region were amplified with appropriate primers (Wales et al., 2008; Jiao et al., 2018). Libraries were verified using a Fluorometer and samples were sequenced with the Illumina MiSeq PE250 platform. Sequences were processed using the QIIME 2 pipeline and analyzed using the SILVA v132 and UNITE databases for bacterial and fungal identification, respectively (Caporaso et al., 2010; Callahan et al., 2016). High-quality sequences were obtained for further analysis. Samples in the ASV tables were rarefied according to the lowest reads (Zhang et al., 2021).

Statistical analysis

In order to distinguish between healthy and diseased plants, rhizosphere soil chemical properties and biomass were analyzed using one-way ANOVA. Multiple comparisons were conducted using Duncan's test with a p-value of less than 0.05 in IBM SPSS Statistics 22. Microbial alpha and beta diversity were analyzed using the "vegan" package in R 4.1.0 (Oksanen et al., 2015), with richness and Shannon indices used to indicate bacterial and fungal diversity (Wu et al., 2021). Beta diversity of microbial communities between groups was determined using principal coordinates analysis (PCoA), and significant differences were tested using analysis of similarities (ANOSIM) (Sun et al., 2021). The correlation between microbial communities and physicochemical variables was tested using Spearman's test. Linear discriminant analysis effect size (LefSe) was employed to identify bacterial and fungal biomarkers from the genus to phylum level in the rhizosphere soil of healthy and diseased plants (Segata et al., 2011).

Results

Cowpea biomass and soil physicochemical properties

Increasing levels of root rot infection caused a significant decrease in cowpea aboveground biomass (*Figure 1*). This decrease was more severe in moderate and severe diseased cowpea plants, with reductions of 52.7% and 77.3%, respectively, compared to healthy plant. In terms of rhizosphere soil, NH_4^+-N and NO_3^--N content was higher in the rhizosphere soils of moderate diseased cowpea plants than in healthy and severe diseased cowpea plants, whereas EC, pH and available phosphorus (AP) content were lower (*Figure 2a; Table S1*). No significant differences were observed in other elements between healthy and diseased plants. Furthermore, soil quality index (SQI) showed no difference in the rhizosphere soil between healthy and diseased cowpea plants (*Figure 2b*). Overall, Cowpea root rot infection changed plant growth and the physicochemical properties of rhizosphere soil, especially for soil active nutrient.
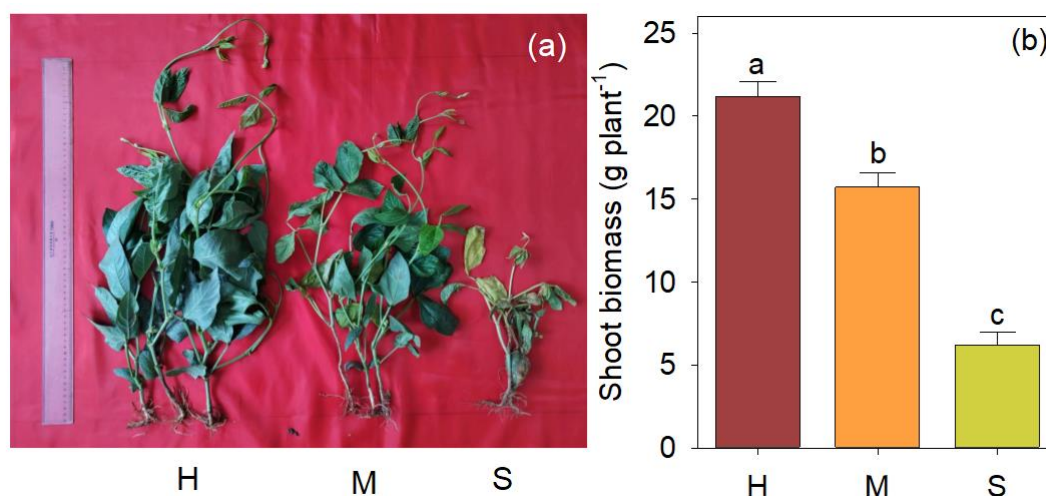

Figure 1. *The growth (a) and shoot biomass (b) of cowpea plant under healthy (H), moderate (M) diseased, and severe (S) diseased symptoms (n=4). Different lowercase indicates significant differences between healthy and diseased cowpea plants at a 1% level*
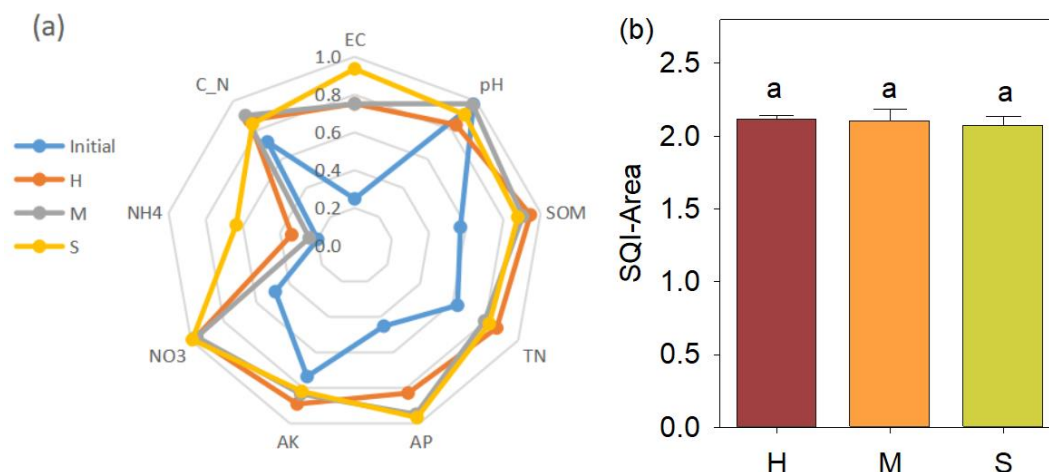
***Figure 2.** Physical and chemical properties (a) and soil quality index (SQI, b) in healthy (H), moderate (M), and severe (S) diseased cowpea rhizosphere soils (n=4). Different lowercase indicates significant differences between healthy and diseased cowpea plants at a 1% level*

Alpha and beta diversities of microbial community

The rhizosphere soil of healthy cowpea plants had significantly higher bacterial diversity than severe plants (Shannon and Simpson index, *Figure 3a,b*), while the fungal community diversity showed no significant difference (*Figure 3a-d*). Both bacterial and fungal communities in the rhizosphere soil formed distinct clusters, and ANOSIM indicated that the bacterial and fungal community structure were significantly affected by plant health (*Figure 4a,b*). In addition, the rhizosphere microbial community structure difference between healthy and diseased plants may be associated with variations in soil chemical properties (available potassium and pH contents for bacteria; SOM and pH for fungi, RDA, *Figure 5*). Overall, the dynamic changes of bacteria and fungi community were not consistent under different root rot infection degree.

Microbial community composition

At the phylum and class level (> 0.5%), we analyzed the bacterial and fungal community composition (*Figure 6a,b*). The predominant bacteria were Actinobacteriota, Proteobacteria, Chloroflexi, Firmicutes, Acidobacteriota, Gemmatimonadota, Bacteroidota, Patescibacteria, Myxococcota, Nitrospirota, Verrucomicrobiota, Planctomycetota. In contrast, the predominant fungi were Sordariomycetes, Mortierellomycetes, Tremellomycetes, Pezizomycetes, Eurotiomycetes, Dothideomycetes, unclassified_p__Ascomycota, Agaricomycetes, unclassified_k__Fungi, Rhizophlyctidomycetes, Saccharomycetes. Diseased rhizosphere soils showed lower abundance of Actinobacteriota, Nitrospirota, Cyanobacteria, and Fibrobacterota and higher abundance of Proteobacteria than healthy plant rhizosphere soil (*Table S2*).
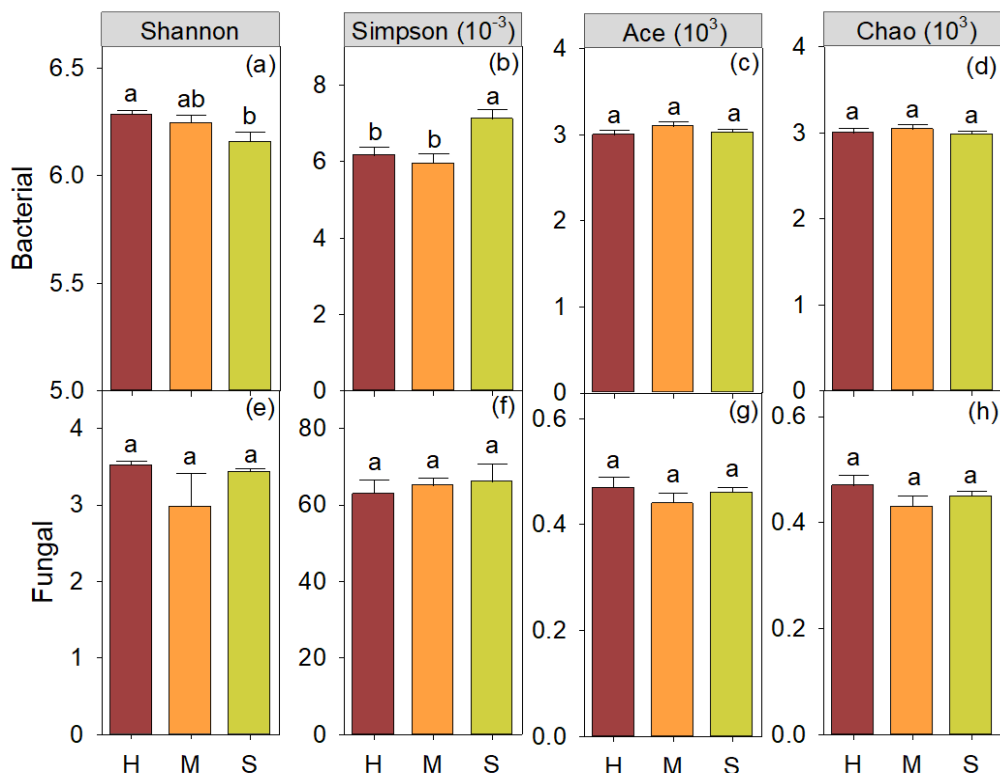
Figure 3. *Alpha diversity of the microbial communities in healthy (H), moderate (M), and severe (S) diseased cowpea rhizosphere soils. (a, b, c, and d) Shannon, Simpson, Ace, and Chao indices of bacterial community; (e, f, g, and h) Shannon, Simpson, Ace, and Chao indices of fungal community. Different lowercase indicates significant differences between healthy and diseased cowpea plants at a 1% level*
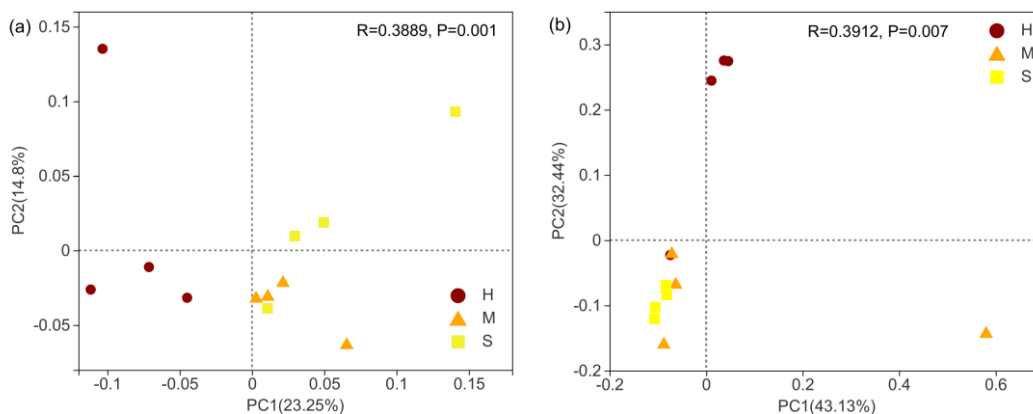

Figure 4. *Principal coordinates analysis (PCoA) of bacterial (a) and fungal (b) communities in healthy (H), moderate (M), and severe (S) diseased cowpea rhizosphere soils*

LEfSe identified biomarkers that were significantly different among different soil samples. Proteobacteria, Patescibacteria, Myxococcota, Chloroflexi, and Bacteroidota were the primary bacteria that changed significantly in the diseased conditions, while Zoopagomycota and Ascomycota were the primary fungi that changed significantly in the diseased conditions (*Figure 7*).
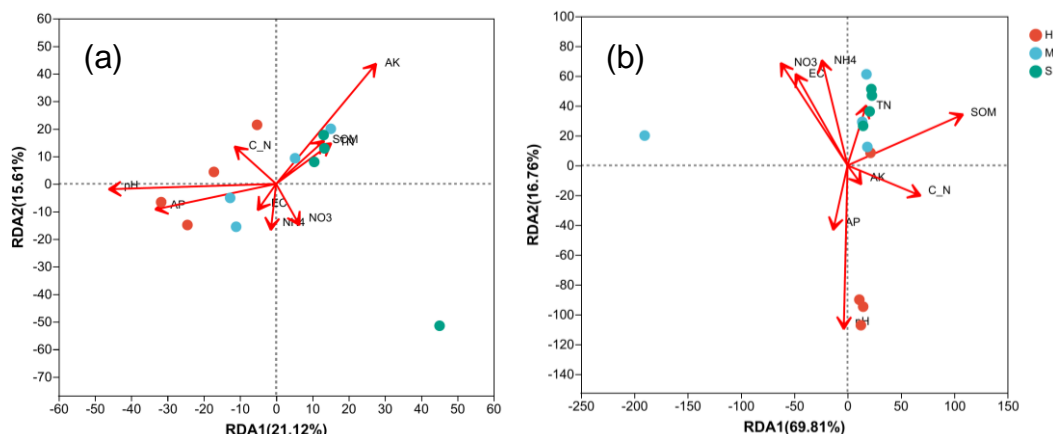
Figure 5. Redundancy analysis (RDA) between soil properties and soil microbial community with healthy (H), moderate (M), and severe (S) diseased cowpea rhizosphere soils
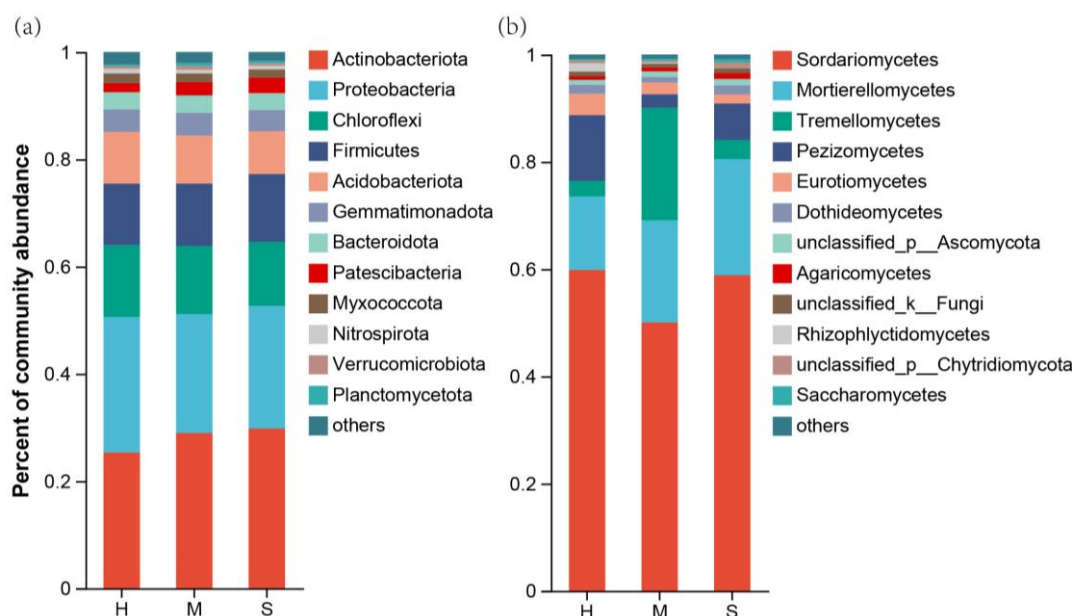

Figure 6. Microbial community composition in healthy (H), moderate (M), and severe (S) diseased cowpea rhizosphere soils. (a) Relative abundance of the major phyla in the bacterial community (>0.5%); (b) relative abundance of the major class in the fungal community (>0.5%)

Links among soil physicochemical properties, SQI, cowpea biomass, and microbial community

We utilized Spearman correlation analysis to examine the connections between soil physicochemical properties and different parameters, such as SQI, plant biomass, bacterial and fungal alpha diversity (*Figure 8*). Our findings revealed that cowpea biomass was positively linked to soil pH and available P, while SQI was significantly and positively associated with soil available K. Bacterial richness was positively correlated with soil EC, whereas fungal diversity was positively correlated with soil SOM.
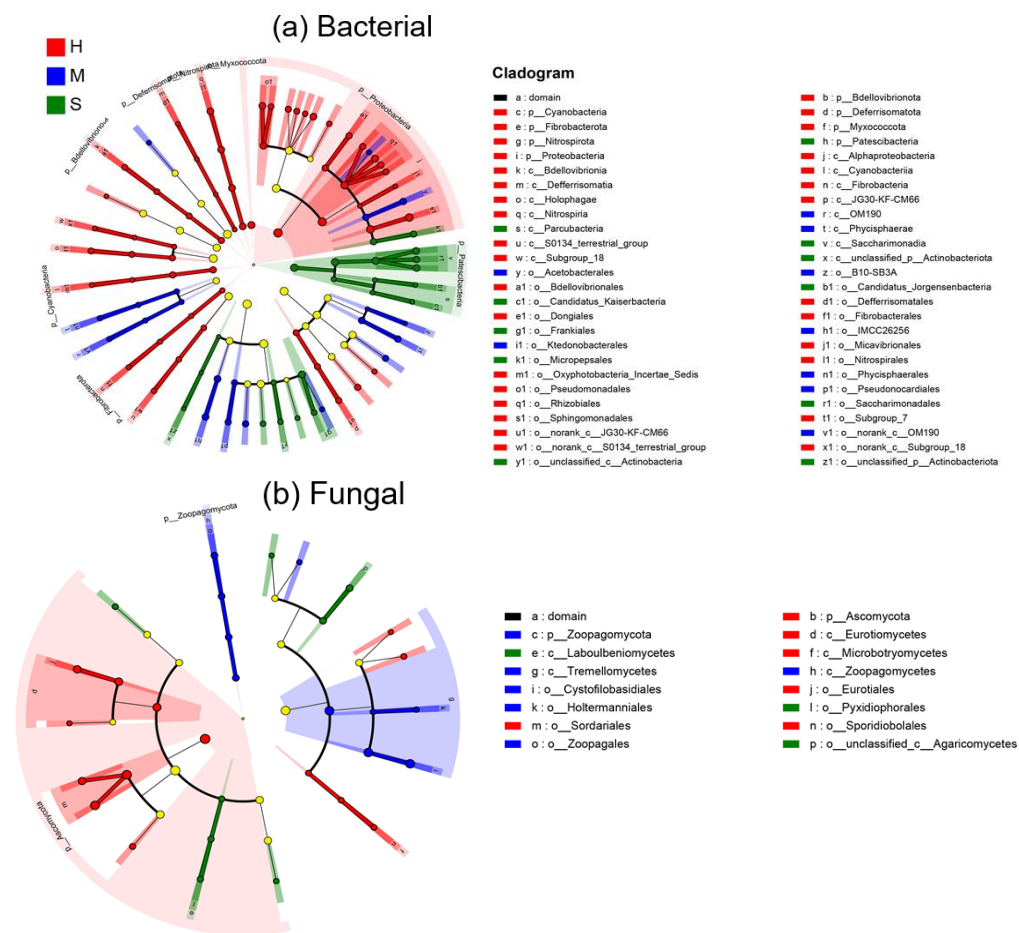
Figure 7. Composition of bacterial (a) and fungal (b) communities in three root-associated compartments and microbial biomarkers of healthy (H), moderate (M), and severe (S) diseased cowpea rhizosphere soils revealed by LEfSe analysis

*Figure 8. Spearman correlation coefficients between soil physical and chemical properties and alpha diversity of bacterial and fungal, soil quality index (SQI), and shoot biomass. * and ** indicate significant relationship differences between two indicators at 5% and 1% level, respectively*

Additionally, we conducted correlation analysis to investigate the relationships between soil characteristics, plant associations with bacterial and fungal communities in the rhizosphere soil of cowpea plants (*Figure 9*). Our findings suggested that there were potential beneficial and pathogenic microorganisms linked to the relationship between bacterial and fungal communities and plant biomass. At the genus level, the community components of bacteria and fungi were significantly correlated with soil physicochemical properties. Specifically, soil pH was significantly and negatively correlated with most genera, but only positively correlated with a few, while soil AK and SOM were positively correlated with most genera.
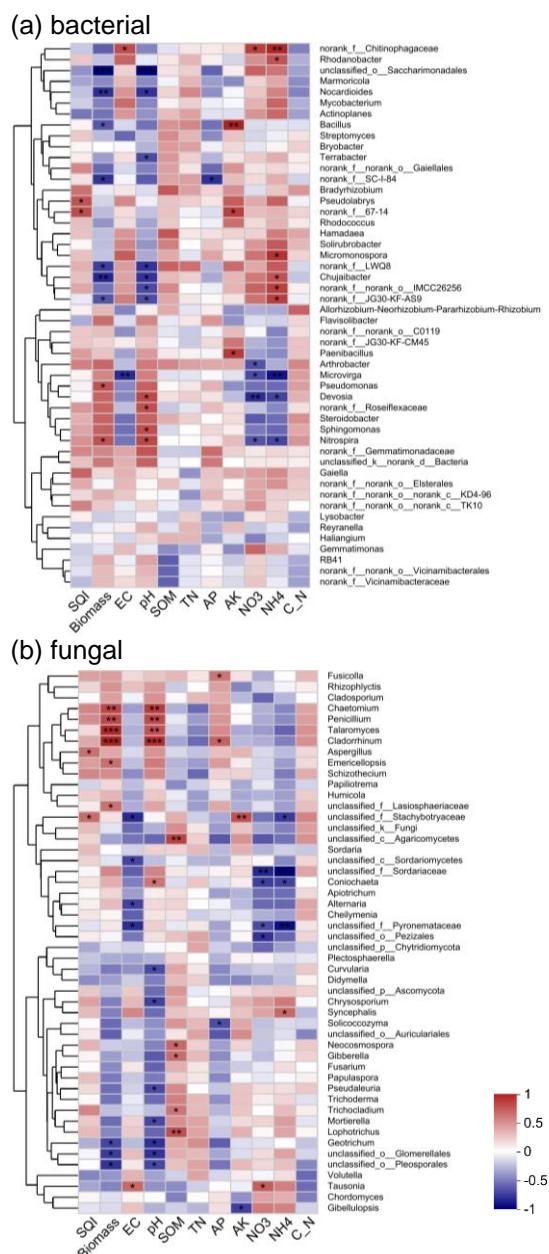

Figure 9. *Spearman correlation coefficients between relative abundance of predominant bacterial (a) and fungal (b) genera and shoot biomass, soil physical and chemical properties. * and ** indicate significant relationship differences between two indicators at 5% and 1% level, respectively*

Discussions

Response of cowpea plant and soil nutrients to root rot infection

Plant root metabolites can be classified into primary and secondary metabolites. Primary metabolites, such as carbohydrates, lipids, amino acids, nucleic acids, and other macromolecular compounds, are essential for plant growth, metabolism, and life maintenance activities (Kessler et al., 2018; Williams et al., 2020). Our findings suggest that the lower rhizosphere soil pH under diseased conditions in this study may be due to the organic acid substances secreted by root exudation. We also observed that soil NH_4^+-N and NO_3^--N content was significantly higher, while soil AP content was lower under moderate diseased conditions. The presence of high levels of NH_4^+-N in the soil, especially in the plant rhizosphere, has been proven to have a suppressive effect on fungal diseases like Fusarium wilt (Cao et al., 2016; Duan et al., 2016). Likewise, the addition of phosphorus to the soil is advantageous in managing fungal diseases because it promotes robust root growth in P-enriched soils, enabling plants to avoid pathogens and diseases (Dordas et al., 2008). However, most change of physical and chemical properties were only observed under moderate disease conditions, possibly due to the impact of extreme disease on plant growth and the subsequent decrease or insufficiency of plant root secretion. Overall, root exudation from diseased plants may be the main reason for the changes in soil NH_4^+-N, NO_3^--N, AP, and cowpea yield.

Response of cowpea rhizosphere microbial communities to root rot infection

Our study revealed that the bacterial richness index was significantly higher in the rhizosphere soil of healthy plants compared to diseased ones, which is consistent with many previous studies (Fu et al., 2017; Wu et al., 2017). However, contrasting results have also been reported due to confounding factors such as soil type, nutrient content, crop rotation, climate, host plant, and nosogenesis (Yuan et al., 2020). We observed distinct structures and compositions of bacterial and fungal communities between healthy and diseased rhizosphere soils. The variation of bacterial community showed a decreasing trend of most community composition, which may contain both beneficial nutrient circulation and pathogenic bacteria (*Table S2*). However, previous studies reported that most varied taxa both associated with promoting nutrients circulation and causing plant disease at the same time. For example, the Proteobacteria play a key role in the C, N, and S cycles in soil (Wang et al., 2020), but also contain some pathogenic bacteria (Zhang et al., 2010). By contrast, Patescibacteria was enriched in the rhizosphere soil of diseased cowpea plants in the present study. In a previous study, the Patescibacteria phyla was also observed significant difference between healthy and diseased conditions (Zhou et al., 2021).

As for the fungal community, less taxa changed induced by root rot infection. We found that the phyla Zoopagomycota was enriched in the rhizosphere soil of diseased cowpea plants. Pezizomycetes includes plant pathogens and saprobes (Marek et al., 2009) and can degrade dead organic matter (Hobbie et al., 2001). On the other hand, the Ascomycota is a phylum with the most species in the fungal kingdom, our study showed that the relative abundance of Ascomycota showed significant decline under diseased conditions, especially under moderate disease conditions. Therefore, imbalances in both bacterial and fungal community structures induced plant diseases and the degradation of dead plant material. Additionally, we found significant differences in bacterial communities between moderate and severe diseased conditions, whereas fungi did not

show such differences, indicating that bacteria may be more sensitivity to root rot disease in plants.

Relationship among microbial communities, soil nutrient, and cowpea biomass

This study found significant correlations between the genera level communities of bacteria and fungi and soil available nutrients, pH, organic carbon, phosphorus, and biomass. These correlations may be related to plant root exudates, which can affect rhizosphere microbial composition (Ulbrich et al., 2022). Soil pH and organic carbon were also found to be crucial factors contributing to the imbalance of soil microbial communities (Ao et al., 2022). Furthermore, rhizosphere secretions may provide a significant energy source for microorganisms, which is why soil organic carbon and phosphorus were closely related to bacteria and fungi (Ao et al., 2022). Certain taxa, such as Chaetomium, Penicillium, Talaromyces, and Nitrospira, were significantly related to biomass, indicating their potential importance for maintaining plant health (Haney et al., 2015; Berendsen et al., 2018). Therefore, our study suggests that rhizosphere pH and AP could serve as essential indicators of rhizosphere microecology.

Conclusion

Our study found that root rot infection had significant impacts on cowpea biomass, soil available nutrients, and soil microbial communities in the rhizosphere. Diseased conditions led to a reduction in cowpea biomass and changes in soil pH and nutrient levels (lower available phosphorus, higher NH_4^+-N and NO_3^--N). Bacterial and fungal communities responded differently to root rot infection, with bacterial alpha diversity showing significant changes between healthy and diseased conditions. Additionally, there were distinct differences in bacterial beta diversity between moderate and severe rhizosphere conditions, whereas fungal community structure showed the opposite trend. Soil beta diversity of bacteria was correlated with soil AK and pH in the rhizosphere, while fungal beta diversity was negatively correlated with SOM and pH. These findings highlight the complex interactions among soil nutrients, soil microbial communities, and cowpea root rot and have important implications for the development of control strategies against soil-borne diseases in the future.

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

Statements and Declarations. All the authors have approved the manuscript and agree with submission to the journal. There are no conflicts of interest to declare. All the authors ensure the accuracy or integrity of all aspects of the manuscript. Jie Zhu: Conceptualization, Methodology, Resources, Investigation, Writing-Review and Editing. Shunlin Tan: Validation, Writing-Reviewing and Editing. Yuqi Li: Writing-Reviewing and Editing. Lei Yang: Writing- Reviewing and Editing. HaixiaWen: Writing- Reviewing and Editing. Xiuli Su: Investigation. Jun Zhang: Writing- Reviewing and Editing. Peng Feng: Investigation. Wenjian Wang: Investigation. Bowei Cai: Writing- Reviewing and Editing. Zhiguo Fang: Supervision. Ruiqi Li: Writing-Review and Editing. Jie Zhang: Writing-Review and Editing, Supervision, Project Administration, Funding Acquisition. All authors have read and agreed to the published version of the manuscript.

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APPENDIX

Table S1. *Physical and chemical properties of surface (0-20 cm) soil and rhizosphere soil of cowpea under different diseased conditions (n=4)*

Treatment	EC (us)	pH (1:2.5 W/V)	SOM (g/kg)	TN (%)	Available P (mg/kg)	Available K (mg/kg)	NO_3^--N (mg/kg)	NH_4^+-N (mg/kg)	C/N ratio
0-20 cm	97.5±7.8	6.4±0.2	18.7±1.3	0.20±0	81.4±10.1	336.3±24.3	2.2±0.5	4.9±0.7	6.8±0.1
H	294.5±5.1b	6.4±0.1a	29.7±0.6a	0.22±0.01a	170.8±3.4a	381.2±17.3a	4.35±0.01b	6.03±0.26b	8.6±0.2a
M	294.8±12.8b	5.5±0.1c	31.1±0.7a	0.20±0.01a	149.3±3.6b	406.9±18.1a	4.47±0.02a	15.74±2.82a	8.3±0.4a
S	367.0±8.1a	5.9±0b	28.8±1.1a	0.21±0.01a	174.1±2.4a	374.4±31.5a	4.38±0.02b	8.37±0.70b	8.0±0.3a

Table S2. *Change in phyla composition of bacterial and fungal in the rhizosphere soil of cowpea associated with healthy (H) and moderate (M) and severe (S) infected with root rot*

	Species name	H	M	S	P-value
Bacterial	p__Proteobacteria	25.26±0.77	22.17±1.65	22.86±1.95	**0.03078**
	p__Patescibacteria	1.68±0.08	2.5±0.26	2.89±0.27	**0.01247**
	p__Myxococcota	1.81±0.15	1.59±0.13	1.52±0.11	**0.03618**
	p__Nitrospirota	0.85±0.09	0.68±0.08	0.69±0.05	**0.02444**
	p__Cyanobacteria	0.46±0.13	0.23±0.05	0.23±0.08	**0.02444**
	p__Bdellovibrionota	0.35±0.04	0.3±0.06	0.24±0.03	**0.02962**
	p__Fibrobacterota	0.05±0.02	0.01±0	0.01±0.01	**0.03294**
	p__Deferrisomatota	0.02±0	0.01±0	0.01±0	**0.01456**
Fungal	p__Ascomycota	79.20±1.50	57.42±23.88	71.25±3.16	**0.01832**
	p__Zoopagomycota	0.10±0.06	0.35±0.16	0.21±0.02	**0.04979**