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Revealing the microbial composition changes and relationship with *Fusarium* caused by rot disease in the *Crocus sativus* L.

Jia Song[1], Xiaoyuan Xi[2], Xiaodong Qian[2], Jing Li[2], Yuanyuan Tao[2], Liqin Li[2*] and Guifen Zhou[1*]

Abstract

Purpose Rot disease caused by *Fusarium* poses a formidable threat to the growth of saffron (*Crocus sativus* L.), resulting in substantial damage to both yield and quality. It is paramount to delve into the root causes of rot disease in saffron to optimize both yield and quality. Existing preventive and treatment modalities have exerted deleterious effects on corms and the natural environment. Consequently, the quest for efficacious and eco-friendly methods such as biological control agents has become an urgent imperative.

Methods The disparate distribution of microbial communities between rhizospheric microorganisms and saffron serves as the foundational exploration for uncovering the underlying causes of rot disease. Samples from various saffron organs and rhizosphere soil were gathered, and the sequencing data from the microbial communities were interpreted using 16S rRNA and ITS gene sequencing methods. This facilitated an in-depth examination of the composition and changes of microorganisms in both healthy and diseased saffron plants.

Results The findings indicated rot disease reduced the abundance and diversity of microorganisms in saffron, and the fungal co-occurrence networks were less stable and their communities were more sensitive to rot disease than the bacterial community. *Fusarium* was the predominant genus in diseased samples, accounting for 99.19% and 89.77% of the communities in diseased leaves and corms. With corms and leaves displaying heightened susceptibility to infection compared to other plant organs. Some of the beneficial bacterial taxa enriched in the diseased plants were also identified in networks, they showed an antagonistic relationship with *Fusarium*, suggesting a potential for these bacteria to be used in biologically based control strategies against rot disease. These insights could prove invaluable for the development of biocontrol agents aimed at combating this plant ailment.

Conclusion These findings significantly advance our understanding of saffron-microbiome interactions and could provide fundamental and important data for improving saffron yield and quality in the process of sustainable development.

Keywords Saffron, Bacterium, Fungus, Microorganisms diversity, Biological control agent

*Correspondence:
Liqin Li
liliqin@hzhospital.com
Guifen Zhou
609493235@qq.com
Full list of author information is available at the end of the article

Introduction

Crocus sativus L., commonly known as saffron, is a perennial herbaceous plant renowned for its red stigma, which serves as a valuable raw material in the production of spices, dyes, and beauty cosmetics. Beyond its commercial applications, saffron exhibits a variety of pharmacological activities, including neuroprotection (Hosseini et al. 2018), treatment of Alzheimer's disease (D'Onofrio et al. 2021), antidepressant effects (Akhondzadeh et al. 2020), antileukemic properties (Moradzadeh et al. 2019), and anti-arteriosclerotic effects (Kakisis 2018). However, saffron's reproductive mechanism poses a unique challenge. Being triploid, saffron can only propagate asexually through corms. The yield and quality of saffron are intricately linked to the growth of these corms. Nevertheless, the vulnerability of corms to microbial invasion during their growth phase can lead to basal stem rot, yellowing, branch withering and corm decay (Di Primo and Cappelli 2000), ultimately severely affecting the yield and quality of both corms and stigma.

Fungal pathogens such as *Fusarium oxysporum*, *Fusarium solani*, *Penicillium citrosulfuratum*, *Penicillium citrinum*, and *Stromatonia gladioli* are responsible for causing corm rot diseases in saffron (Hu et al. 2021; Tian et al. 2022). These infections have been reported to result in a mortality rate exceeding 50% (Zhang et al. 2022). Notably, *Fusarium oxysporum*, a prevalent plant pathogen found in soil, poses a particular threat by producing toxic secondary metabolites known as mycotoxins (González-Jartín et al. 2019). Excitingly, the utilization of biological control agents offers a promising solution to combat these fungal threats. Biological control agents can be by studying the relationship between saffron and microorganisms is a common method used to identify beneficial microorganisms that can help plants resist pathogens and safeguard them from harm (Ambardar and Vakhlu 2013; Mansotra et al 2023). For instance, the bacterial endophyte BG-E39 has demonstrated efficacy in conferring resistance against *F. oxysporum* corm rot in saffron (Ahmad et al. 2022).

The microorganisms inhabiting the rhizosphere soil, the region closest to plant roots, represent a focal point of extensive research due to their intricate interactions and mutualistic relationships. When plants face pathogenic microorganisms, the rhizosphere microorganisms orchestrate adaptive responses, primarily through two pivotal mechanisms. Firstly, plants can actively shape the composition of rhizosphere microbial communities to deter pathogen intrusion (Berendsen et al. 2012; Li et al. 2021). Secondly, beneficial rhizosphere microorganisms play a crucial role in modulating the host plant's immune system; for instance, certain *Bacillus* strains facilitate nutrient uptake, stimulate growth via plant hormone synthesis, and shield plants from pathogens and abiotic stressors (Saxena et al. 2020). Conversely, the proliferation of pathogenic bacteria can disrupt the delicate equilibrium between plants and microorganisms, leading to competitive interactions in ecological niches (Chapelle et al. 2016). This dynamic interplay is subject to a multitude of influencing factors, and any perturbation that tilts the balance between the host microbiome and the plant can significantly elevate the risk of pathogen invasion (Dastogeer et al. 2022).

Establishing a stable barrier between plants and microorganisms is crucial for safeguarding plant health by promoting antagonistic interactions among microorganisms. Using 16S and ITS rRNA sequencing methods to obtain the data, researchers analyzed and identified *Fusarium* as a common pathogen causing rot disease in various plant species, including saffron (Ren et al. 2023). Further investigation into potential symbiotic or antagonistic relationships between *Fusarium* and other microorganisms in the context of rot disease development in saffron is warranted. Are there any microorganisms that collaborate with *Fusarium* to induce or exacerbate rot disease in saffron, or conversely, exhibit resistance against *Fusarium* to enhance saffron survival rates and yields?

The samples of different organs and rhizosphere soil from diseased and healthy saffron were collected and sequencing data of microbial communities using 16S rRNA and ITS gene sequencing methods. By analyzing the impact of rot disease on the abundance and structure of microbial diversity in saffron organs and rhizosphere soil, further find microorganisms that are synergistic or antagonistic to *Fusarium*. These antagonistic microorganisms might be used in biologically based control strategies against rot disease. This provides theoretical guidance for improving saffron yield and quality.

Materials and methods

Experimental design and sampling

In this study, after flowering saffron samples were collected in Huzhou City, Zhejiang Province, China (longitude 120.6°E, latitude 30.52°N). The incidence rate about 30% of the total number of corms showed disease symptoms. The disease indexes were recorded using the following scale: 0 = no symptoms of corm rot, 1 = rotting outside the corm, 2 = rotting inside the corm, 3 = rotting both inside and outside the corm, and 4 = whole corm rot (Zhou et al. 2021; Mansotra et al. 2023). Plants displaying evident signs of decay in corms (disease index ≥ 2) and robust health were collected but not in the decaying areas, separately, across various plant tissues (Fig. S1). Three replicates of each healthy and diseased sample were collected. Following the complete excavation of the plant, the process of shaking it to gather the soil dislodged

from the roots was conducted to obtain rhizosphere soil. The specimens were subdivided into eight parts: Healthy Leaves (HL), Healthy Corms (HQ), Healthy Roots (HR), Healthy Rhizosphere Soil (HS), Diseased Leaves (DL), Diseased Corms (DQ), Diseased Roots (DR) and Diseased Rhizosphere Soil (DS). Each replicate consisted of a composite sample obtained by mixing three individual samples, resulting in a total of 24 samples. All samples were stored at -80 °C until further experimental procedures were initiated.

DNA extraction and PCR amplification

DNA was extracted using the E.Z.N.A® soil DNA Kit (Omega Bio tek, Norcross, GA, U.S.) assessed DNA quality via gel electrophoresis, and subsequently determined DNA concentration and purity. The hypervariable region of the bacterial 16S rRNA gene was amplified employing 799F (5'- ACTCCTACGGGGGGCAG-3') and 1193R (5'- GACTACHVGGGTWTCTAAT-3') primers (Zhang et al. 2021), while the fungal ITS fragment was amplified using ITS1F (5 '- CTTGCGCATTTAGAGGAGAGT AAA-3') and ITS2R (5 '- GCTGCGTTCTTCATCGAT GC-3') primers (Huang et al. 2023). PCR amplification was conducted using a thermal cycler. The amplification protocol for the 16S rRNA and ITS genes involved initial heating of the reaction mixture to 95 °C for 3 min, denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. A final extension step was performed at 72 °C for 5 min, followed by cooling to 12 °C. The reaction mixture was prepared following the kit's instructions. After analyzing the PCR products via electrophoresis on a 2% agarose gel, purification of the products was carried out, followed by quantification.

Illumina MiSeq sequencing

Illumina MiSeq sequencing was performed using the same method reported (Huang et al. 2023) and the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw data has been uploaded to the NCBI database and Figshare (Accession Number: SRP468807; 10.6084/m9.figshare.24557236).

Processing of sequencing data

The sequencing data were processed starting with demultiplexing, then subjected to quality filtering using fastp version 0.20.0 (Chen et al. 2018), and paired-end reads were merged with FLASH version 1.2.7 (Magoč and Salzberg 2011). The specific criteria for processing were as follows: i the average quality score dipped below 20 of 300 bp reads was truncated across a 50 bp sliding window; any trimmed reads shorter than 50 bp or those containing ambiguous nucleotides were eliminated from the dataset; ii only sequences with an overlap of over 10 bp were assembled, with a maximum permissible mismatch ratio of 0.2 in the overlapping region and unassembled reads were discarded; iii identification of individual samples was based on barcode and primer recognition, with adjustments made for sequencer read direction—barcodes had to match exactly and below 2 nucleotide mismatch in primer matching. Operational taxonomic units (OTUs) with 97% similarity cutoff (Stackebrandt and Goebel 1994; Edgar 2013) were clustered using UPARSE version 7.1 (Edgar 2013), and the chimeric sequences were detected and subsequently discarded. The taxonomic classification for each representative OTU sequence was executed by the RDP Classifier version 2.2 (Wang et al. 2007) against a 16S rRNA database (eg. Silva v138) applying a 0.7 confidence threshold for taxonomic assignment.

Diversity and Statistical Analysis

The alpha diversity was assessed using Kruskal–Wallis rank sum test and False Discovery Rate (FDR) corrections for multiple tests. Non-metric multidimensional scaling (NMDS) analysis at the genus level for bacterial and fungal communities was conducted utilizing the Bray–Curtis distance algorithm. To analyze the relative abundance and the function prediction of microorganisms, online tools provided by the Majorbio cloud platform were employed. Furthermore, the platform includes features for data visualization and plotting (https://cloud.majorbio.com/page/tools/). Student's t-test and Kruskal–Wallis rank sum test were used to compare two and multiple groups. The association between core microorganisms and *Fusarium* was visualized using Cytoscape.

Co-occurrence network analysis

The co-occurrence pattern was reconstructed by calculating multiple abundance correlations utilizing the genus-level matrix and the CoNet application within Cytoscape (Gao et al. 2021). A robust co-occurrence was defined by a Spearman correlation coefficient exceeding 0.70 and a P-value below 0.05. To minimize the P-value, the Benjamini–Hochberg procedure was employed, tailored to reduce the likelihood of false positive signals. Subsequently, Gephi was utilized for network visualization, with each microbial genus depicted as a node and edges symbolizing interactions between distinct microbial genera (Gao et al. 2021).

The calculation of topological features encompassed various metrics, including the number of positive co-occurring correlations, negative mutually exclusive correlations, average path length, network diameter, average clustering coefficient, average connectivity, and modularity within both bacterial and fungal networks. The functional roles of individual nodes were elucidated through

an evaluation of their degree and closeness centrality metrics. Notably, the top 10 nodes exhibiting the highest degree and closeness centrality were identified as hub species within each network. Network stability was gauged through the ratio of negative to positive correlations and the degree of modularity, providing insights into the robustness and structure of the networks under scrutiny.

Results

Corm rot disease affected the microbial diversity in saffron

A total of 3856 OTUs were obtained, resulting in the identification of 3,141 bacterial OTUs and 715 fungal OTUs in the healthy and severe corm rot-diseased saffron (Fig. S1). NMDS analysis at the genus level revealed significant differences in microbial distribution between the healthy and disease groups (Fig. 1). The analysis based on the Shannon index, which represented community diversity, showed that each organ sample within the diseased groups exhibited decreased diversity compared to the healthy group (Fig. S2A, B; $P<0.05$). Alpha diversity analysis based on the Chao index, which assesses community richness, showed that bacterial communities across the diseased samples had reduced richness in contrast to the healthy group (Fig. S2C; $P<0.05$). In the bacterial community, *Ralstonia* was highest in the healthy leaves and lower levels in diseased leaves, while *Enterobacter* showed the opposite trend in leaves and corms. *Burkholderia-Caballeronia-Paraburkholderia* was more prevalent in diseased corms and roots, but less in healthy samples. In the fungal community, *Fusarium* was more abundant in diseased samples and less prevalent in healthy samples. The dominant bacterial composition exhibited a significant contrast between diseased and healthy samples, illustrating a competitive trend of "As one falls, another rises" (Fig. S3; $P<0.05$). In summary, all results indicated that rot disease has had a discernible impact on the microbial diversity of saffron.

Corm rot disease altered the microbial composition across various organs of saffron

Compared to healthy leaves, a notable decline in fungal community diversity was evident in diseased leaves (Fig. 2B). Intriguingly, compared with the healthy samples, the diversity of bacterial communities in the diseased corm significantly changed and the corm rot disease reduced the diversity of microbial community within the rhizosphere soil (Fig. 2).

Core microbial taxa were identified in both healthy and diseased plants, revealing 310 core bacterial taxa in healthy plants and 220 in diseased plants. Additionally, 14 core fungal taxa were present in healthy plants and 17 in diseased plants. Notably, 196 bacterial taxa and 7 fungal taxa were consistent across all samples (Table S1). Analysis using Venn diagram illustrated a reduction in the core microbial community in diseased leaves compared with the healthy leaves. Moreover, a significant decrease in the core bacterial community was observed in the corm, while the fungal community in the rhizosphere also exhibited a notable decline (Fig. 3; Table S2, S3). These findings substantiate the decline in core microbial taxa attributed to rot diseases.

Remarkably, 11 bacterial genera displayed relative abundance, with *Burkholderia-Caballeronia-Paraburkholderia* representing 55.75% of the bacterial community in diseased corms. Among the 20 fungal genera showing

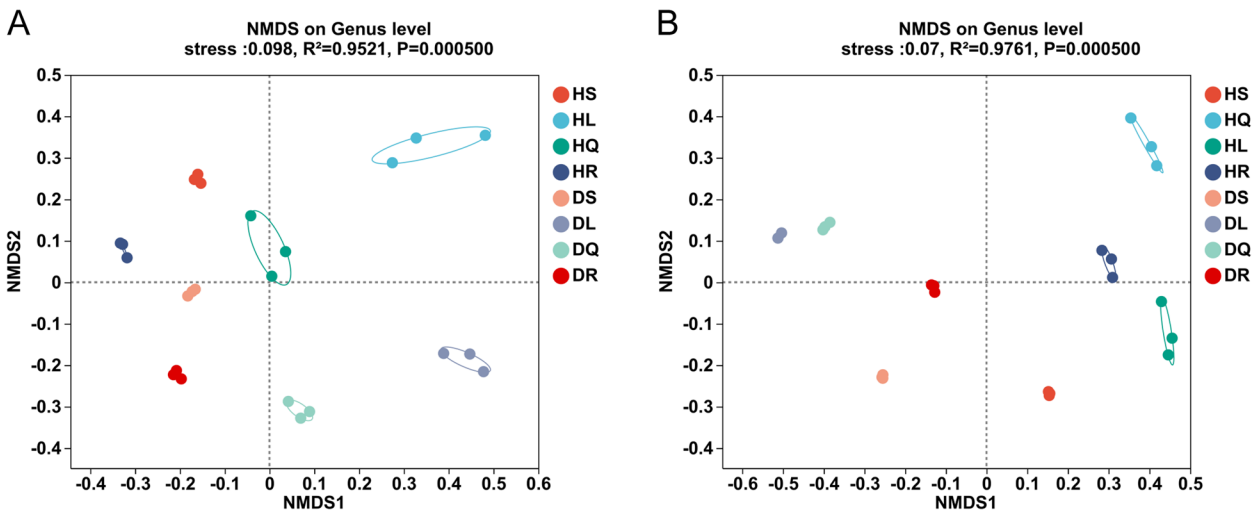

Fig. 1 NMDS analysis of fungal and bacterial communities in four different organ samples of saffron. **A** Fungal community. **B** Bacterial community. "H*" represents the healthy group, while "D*" represents the diseased group. "*S", "*R", "*Q", and "*L" stand for rhizosphere soil, root, corm, and leaf

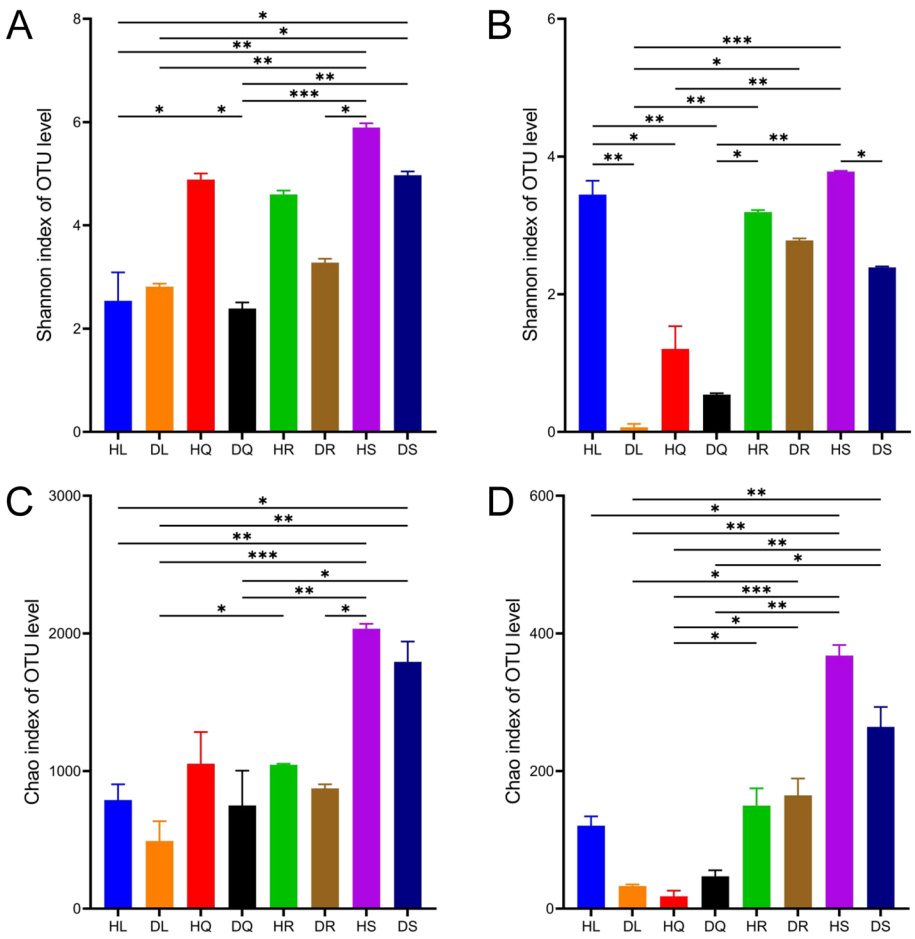

Fig. 2 Alpha diversity analysis of fungal and bacterial communities in four different organ samples of saffron. Shannon diversity index of (**A**) bacterial and (**B**) fungal communities in the different organs of healthy and diseased samples. Chao diversity index of (**C**) bacterial and (**D**) fungal communities in the different organs of healthy and diseased samples. ***$P < 0.001$. **$P < 0.01$. *$P < 0.05$

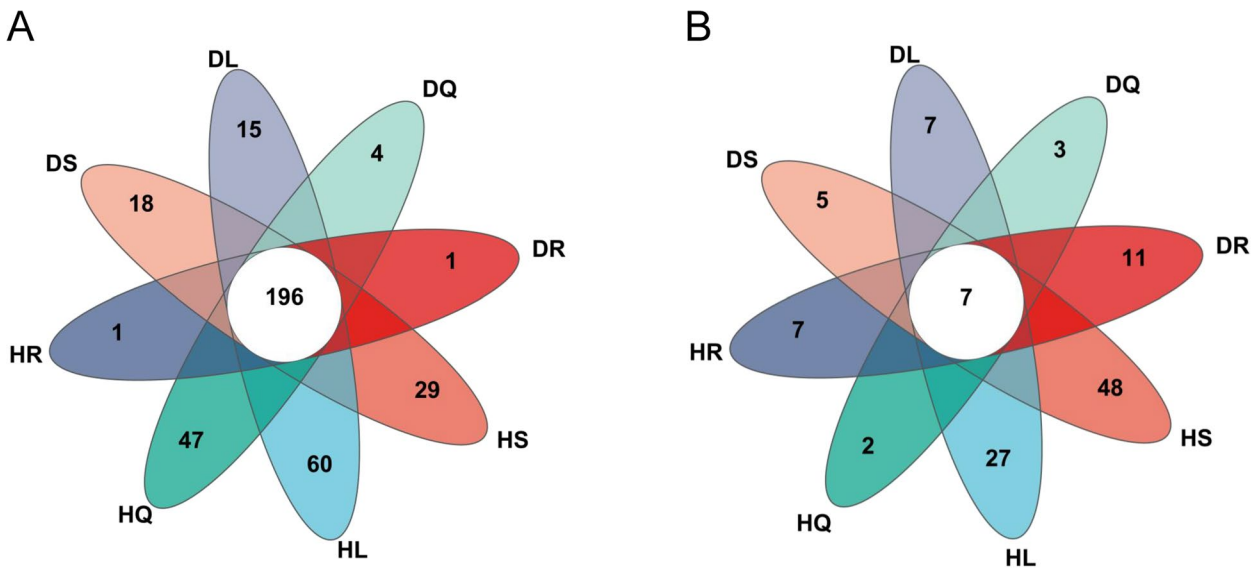

Fig. 3 Venn diagram of fungal and bacterial communities in four different organ samples of saffron. **A** Bacterial community. **B** Fungal communitiy

relative abundance, *Fusarium* dominated with 99.19% and 89.77% prevalence in diseased leaves and corms, respectively, surpassing its occurrence in diseased rhizosphere soil (23.17%) and roots (23.26%) (Fig. 4; Tables S4, S5). In summary, the invasion of pathogens resulted in varying impacts on each plant organ, with leaves experiencing the most severe effects, followed by the corm. This observation may suggest a contrasting scenario to bottom-up host resistance.

Fungi responded more sensitive to corm rot disease

The primary factor influencing changes in fungal community composition was identified as the rot disease through NMDS analysis. To unravel the intricate interplay among the microbiota present in saffron, a co-occurrence network analysis was conducted using the Spearman correlation coefficient of OTUs. The topological characteristics of the resulting networks were further explored to investigate microbial communities (Table 1).

The number of nodes and edges of fungal and bacterial taxa was higher in the healthy network than in the diseased network and the proportion of negative edges and modularity were higher in diseased networks than in the healthy networks (Fig. 5; Table 1). Those results indicated that rot diseases have altered the microbial co-occurrence network. In analyzing fungal communities, it was observed that the average clustering coefficient of healthy samples was lower than that of diseased samples (FH: 0.361, FD: 0.404). Conversely, the average path length (health: 2.795; disease: 2.285) and diameter (health: 9; disease: 6) exhibited opposite trends. For bacterial communities, the average clustering coefficient and average path length were lower in healthy samples, while the diameter was higher. Mutually negative interactions, indicating ecological competition, can improve microbiome stability by dampening the destabilizing effects of cooperation (Gao et al. 2021). The proportion of negative edges and modularity in bacterial networks (healthy negative edges/modularity ratio: 10.70%/0.238; diseased

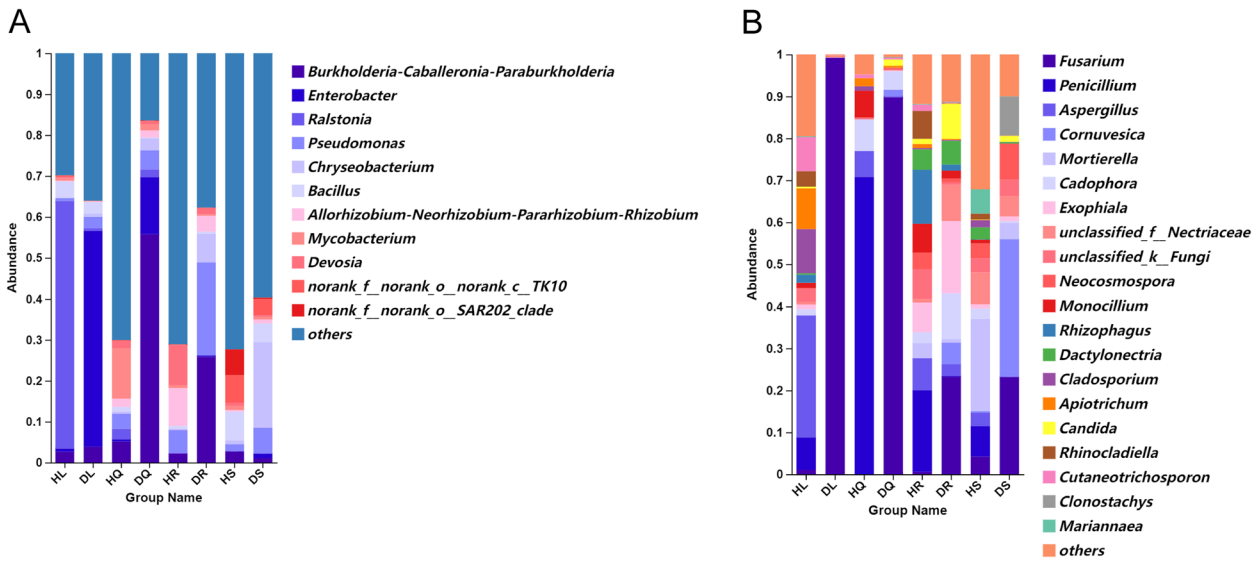

Fig. 4 Relative abundance of microorganisms in all samples at genera levels. **A** Bacterial community. **B** Fungal community

Table 1 Summary of topology attributes in groups

Sample	Nodes	Edges	Average degree	Avg.Weighted Degree	Network Diameter	Modularity	Avg.Clustering Cofficient	Avg.Path Length
FH	233	3398	14.584	14.854	9	0.361	0.396	2.795
FD	162	2023	12.488	12.488	6	0.404	0.410	2.285
BH	724	39717	54.858	54.858	10	0.238	0.348	2.466
BD	900	34295	38.106	38.106	10	0.391	0.312	2.897

FH, FD, BH, BD: Fungal community in healthy samples, Fungal community in diseased samples, Bacterial community in healthy samples, Bacterial community in diseased samples. The number of nodes and edges represented the total number of OTUs in the dataset and their interactions, the average degree was the average number of connections per node in the network, and the average path length referred to the average network distance between all pairs of nodes. The average clustering coefficient was the ratio of the actual number of edges to the maximum number of edges in a node that was fully connected

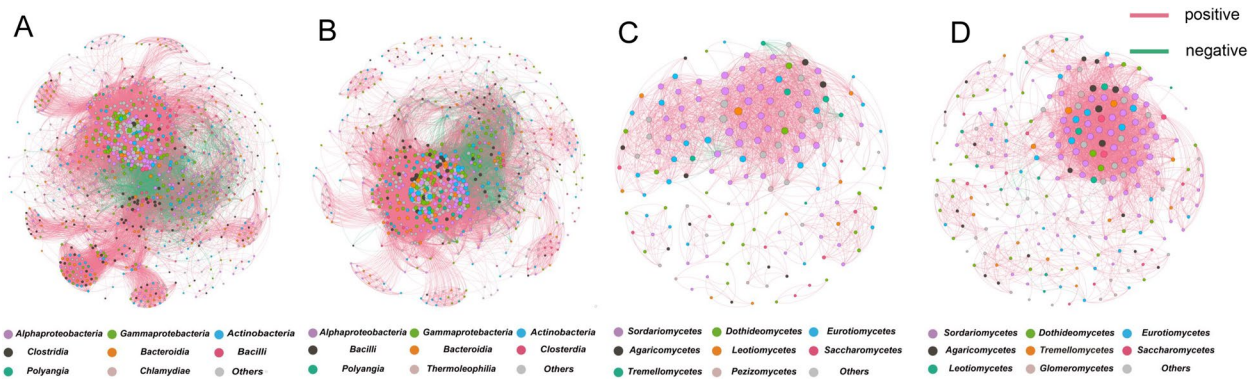

Fig. 5 Co-occurrence networks of bacterial and fungal communities in healthy and diseased samples. Bacterial community in (**A**) diseased and (**B**) healthy samples. Fungal communities in (**C**) diseased and (**D**) healthy samples

negative edges/modularity ratio: 13.42%/0.391) surpassed that in fungal networks (healthy negative edges/modularity ratio: 0.74%/0.361; diseased negative edges/modularity ratio: 4.65%/0.404). Bacterial networks recorded a higher number of nodes and edges (Table 1). Further, the edges of the top 10 hub nodes with high degree and closeness centrality values in the bacterial networks were higher in the diseased network compared with the healthy networks, while the same pattern was observed among the fungal taxa (Fig. 5; Table S6). However, the edges of the top 10 hub nodes in the healthy fungal networks had no negative correlation (Table S6). Notably, the network structure of fungal communities appeared to be simpler, suggesting a greater susceptibility of fungi to the effects of rot disease.

Rot disease altered the composition of the microbial community structure

The composition of the bacterial community and fungal community at the genus level is shown in Fig. 5. Within the bacterial community, *Burkholderia-Caballeronia-Paraburkholderia* and *Pseudomonas* were consistently present across all samples. However, the abundance of *Burkholderia-Caballeronia-Paraburkholderia* was notably higher in diseased corms and roots compared to other samples, constituting 55.75% and 25.85% of the respective communities. *Enterobacter* was predominantly found in diseased leaves and in smaller quantities in other samples. *Ralstonia* emerged as the predominant bacterial genus in healthy leaves, representing a substantial 60.43% of the community, while its presence was relatively diminished in other samples. Interestingly, in the fungal community, *Fusarium* and *Penicillium* were prevalent in both healthy and diseased samples. *Fusarium* was the predominant genus in diseased samples, accounting for 99.19%, 89.77%, 23.26%, and 23.17% of the communities in diseased leaves, corms, roots, and rhizosphere soil, respectively. *Fusarium* played a dominant role in reshaping the microbial structure in diseased leaves and corms. *Penicillium*, on the other hand, accounted for 7.80%, 70.73%, 19.45%, and 7.28% of healthy leaves, corms, roots, and rhizosphere soil, respectively (Table S4 and S5). A comparison with healthy organs revealed a shift in the dominant microorganisms present in diseased organs, accompanied by varying increases or decreases in other microorganisms. This shift underscores the significant impact of rot disease on altering the microbial structure within the affected plant organs.

The interaction between core microorganism and *Fusarium*

With its widespread presence and elevated concentrations in afflicted samples, efforts have been directed toward exploring the microbial communities that interact closely with *Fusarium* as core taxa. This pursuit aims to discern microorganisms that exhibit antagonistic behavior towards *Fusarium*, thereby laying a foundational framework for the advancement of biological control agents.

To understand the relationship between core microorganisms and pathogenic microorganisms, the quest extends to identifying beneficial microorganisms that demonstrate synergistic effects, as well as those that counteract pathogenic microorganisms. The key core microorganisms have been identified and delineated: *Enterobacter*, *Chryseobacterium*, *Ochrobactrum*, and *Sphingobacterium* have shown a positive correlation with *Fusarium* (Fig. 6), suggesting a potential facilitative role in *Fusarium*'s invasion of host plants and the subsequent onset of rot disease. Conversely, instances of negative correlations between microorganisms signify mutual inhibition. *Microbulbifer*, *norank_f__BIrii41*, *Tepidamorphus*, *Lysinimonas*, *Iamia*, *Nocardia*, *Tumebacillus*, *Bauldia* and others have exhibited an inverse relationship with *Fusarium*, indicating a protective function during

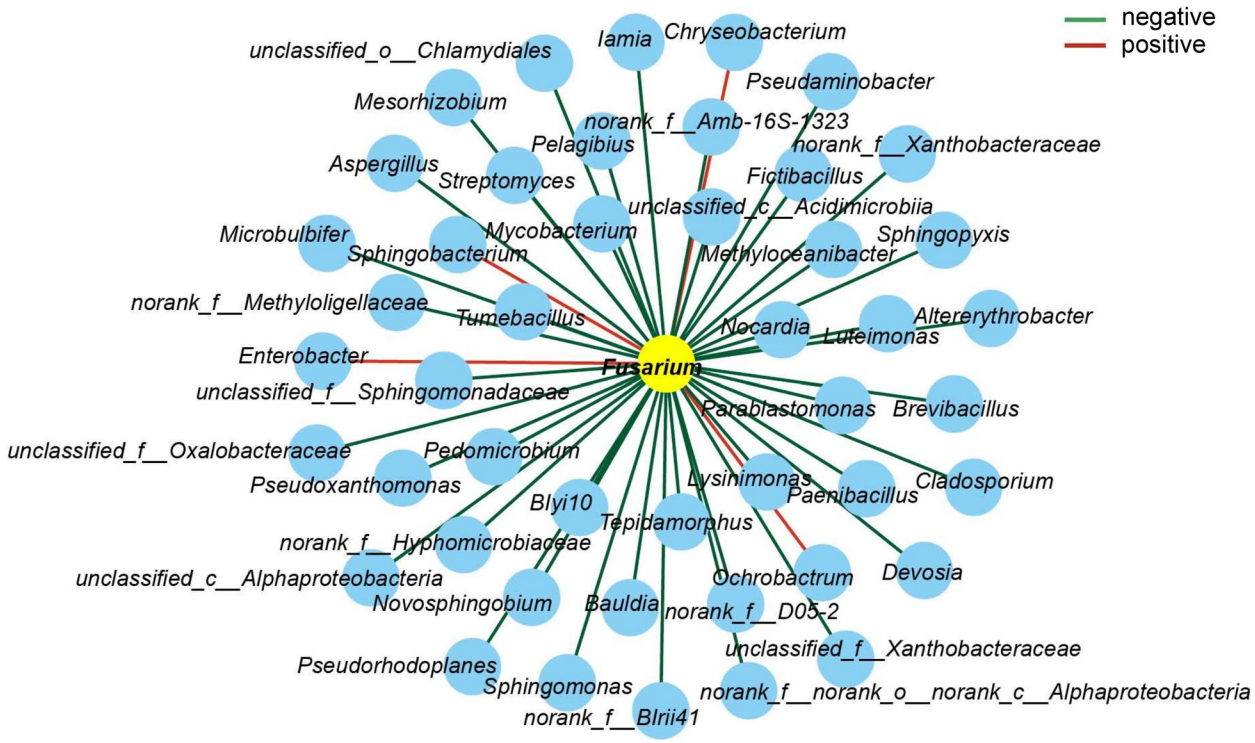

Fig. 6 Relationship between the core microbial communities and *Fusarium*

the process of *Fusarium* invasion and proliferation. These findings underscore the potential of these microorganisms in serving as candidates for further exploration in the realm of biological control agents.

Function prediction of microorganisms

The KEGG function annotation in bacterial communities based on Tax4Fun could obtain OTU annotation information for each functional level of KEGG, as well as the abundance information of each function in different samples. The functional predictions derived from all samples showed their involvement in crucial pathways including carbohydrate metabolism, cofactor and vitamin metabolism, energy metabolism, amino acid metabolism, membrane transport, signal transduction, and other essential biological processes. Notably, carbohydrate metabolism emerged as the most abundant function within both the healthy and diseased groups. While the disparity between the healthy and diseased cohorts was not statistically significant, subtle variations were observed in select pathways like membrane transport and signal transduction (Table S7). These findings suggest relative stability in the microbial functional profiles within the bacterial community, with limited alterations discernible between the healthy and diseased states.

According to FUNGuild (Fig. 7), the functional categorization of fungi present in the sample, along with the abundance data for each functional category across various samples were acquired. The analysis identified 16 distinct fungal functional groups (excluding unassigned taxa), including categories such as Undefined Saprotroph, Endophyte-Litter Saprotroph-Soil Saprotroph-Undefined Saprotroph, and Animal Pathogen-Endophyte-Lichen Parasite-Plant Pathogen-Soil Saprotroph-Wood Saprotroph. The prevalence of undefined saprophytes was notably higher in the healthy group compared to the diseased group, ranging from 43 to 89%, with the highest occurrence observed in healthy corms. In contrast, the Endophyte-Litter Saprotroph-Soil Saprotroph-Undefined Saprotroph category exhibited the highest abundance in the healthy rhizosphere soil samples. Conversely, the diseased group exhibited a relatively elevated presence of the Animal Pathogen-Endophyte-Lichen Parasite-Plant Pathogen-Soil Saprotroph-Wood Saprotroph category, particularly in diseased leaves and corms, ranging from 89 to 99%. Furthermore, the abundance of plant pathogens in the diseased rhizosphere soil samples surpassed that of other samples, constituting 42% of the total composition. These findings strongly suggest that the proliferation of harmful fungi within the diseased samples

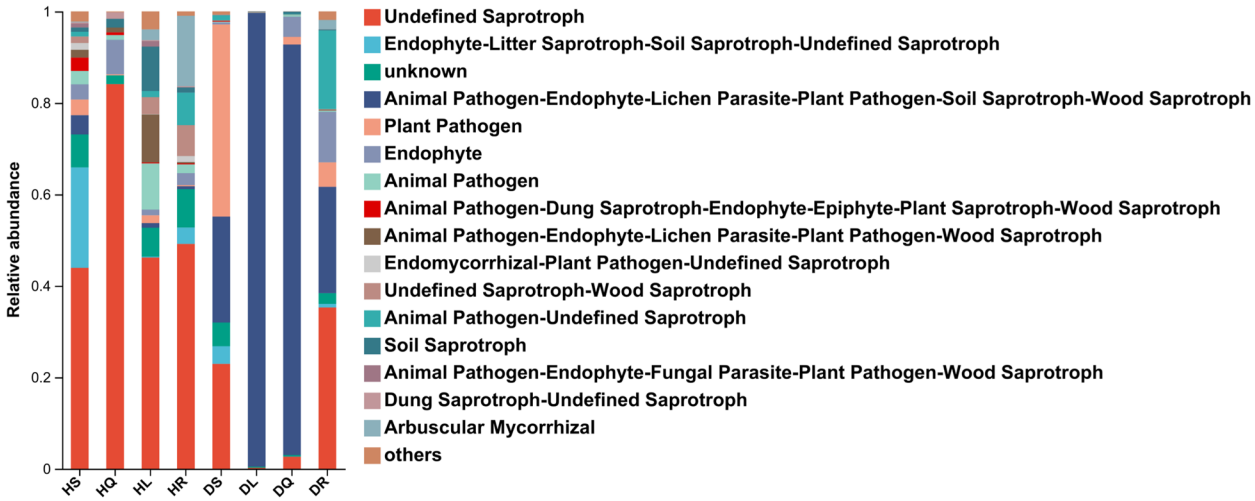

Fig. 7 Functional changes in the composition of fungal groups inferred by FUNGuild

significantly contributed to the onset of saffron rot disease.

Discussion

Corm rot disease changed the microbial diversity in saffron

Microorganisms play a crucial role in the growth and development of plants (Yan et al. 2019; Gao et al. 2021; Boro et al. 2022), and the alterations in microbial communities are intimately associated with their health. Our study reveals that microorganisms are more abundant and diverse in healthy saffron plants compared to diseased ones, aligning with microbial variations observed in prior research on tomato and *Polygonati Rhizoma* diseases (Trivedi et al. 2020; Yin et al. 2020; Pang et al. 2022). When plants become diseased, the pathogen proliferates in large quantities, which can further impact the diversity of rhizosphere microorganisms (Zhang et al. 2023), so we found a notable decrease in rhizosphere microorganism counts in disease saffron plants. Our study suggests the pathogenic microorganisms and beneficial microorganisms' competitive relationship in saffron and rhizosphere soil. These beneficial microorganisms aid plants in acclimating to their surroundings, promoting growth and development, boosting stress resistance, and restricting the proliferating of pathogenic microorganisms (Zipfel and Oldroyd 2017; Li et al. 2022). Plant recognizes the presence of microorganisms by detecting their signals through their inherent defense and adaptation mechanisms, and allow beneficial microorganisms to colonize and establish symbiotic relationships (Zipfel and Oldroyd 2017). Instead, when faced with pathogenic microorganisms, plants may trigger immune responses to constrain invaders and safeguard their health (Zipfel and Oldroyd 2017).

Corm rot disease affected the microbiome of roots and rhizosphere soil less than leaves and corms

Understanding plant susceptibility during pathogen invasion provides crucial insights for safeguarding saffron crops and enhancing yield (Zhu et al. 2023). Analysis of bacterial and fungal communities sequencing data revealed that leaves were the most susceptible organ to disease, followed by corms (Fig. 3; Table S2, S3), while the root microbiome exhibited heightened resistance owing to its rich species diversity, intricate relationships, and abundance of beneficial microorganisms (Liu et al. 2020). Differences in the organizational structure and environment of plant tissues give rise to significant variations in community structure and functional traits (Liu et al. 2020). Compared to other organs, roots employ a range of robust defense mechanisms (such as monitoring and defense systems) to fend off pathogenic microorganisms more effectively than leaves and corms (De Coninck et al. 2015). Moreover, roots possess structural advantages over corms and leaves, enabling them to swiftly recognize and respond to pathogen invasion, thereby impeding the proliferation of *Fusarium oxysporum* (Gordon 2017; Carbajal-Valenzuela et al. 2022). In instances of *Fusarium* infection, fusaric acid-induced damage to the plant leaves results in rapid water loss (Dong et al. 2012; Wang et al. 2012), potentially accounting for the observed reduction in leaf-associated microorganisms.

Increasing the diversity of fungal community helped to resist corm rot disease

Corm rot disease caused by *Fusarium* and *Penicillium* has become a serious threat to saffron production with an average incidence of 30 to 40% (Mirghasempour et al 2022; Hu et al. 2021; Tian et al. 2022). When plants face

pathogenic invasions, the microbial community within the plant changes (Trivedi et al. 2020). The researchers observed that the fungal community, as opposed to the bacterial community, exhibits significant correlations with plant health and disease, with a notably reduced diversity in tissues affected by rot disease (Gao et al. 2021). Research indicates that the diversity of fungal communities is closely linked to their capacity to withstand pathogen attacks and enhanced fungal community diversity has been shown to bolster resistance against pathogen intrusion (Penton et al. 2014; Tang et al. 2020). The augmentation in both the quantity and diversity of microorganisms can foster more intricate network relationships in future studies, thereby enhancing their ability to fend off pathogen invasions, ultimately improving plant survival rates and yield (Gao et al. 2021).

Screening beneficial microbial community helped in the development of biological control agents

Plants can recruit a diverse array of beneficial microbiota, forming intricate co-occurrence microbial network structures (Cha et al. 2016; Wu et al. 2021). Understanding these intricate plant-microorganism relationships is crucial for harnessing the potential of beneficial microorganisms in combating pathogen invasion, safeguarding plant growth, development, and overall health, as well as laying the groundwork for developing effective biological control agents in saffron (Trivedi et al. 2020). The utilization of biological control agents has emerged as a prominent area of research due to their environmentally friendly characteristics in the prevention and management of plant diseases. Investigating the interactions among highly antagonistic strains in biocontrol strategies is essential for designing effective microbial consortia (Li et al. 2021). Many studies reported that *Fusarium* was the main fungus causing many plant diseases and had high pathogenicity (Michielse and Rep 2009; Ma et al. 2016; Geng et al. 2014). Our study has found that the negative correlation between certain microbial communities like *Mycobacterium*, *Microbulbifer, Tepidamorphus, Lysinimonas, Iamia, Nocardia, Tumebacillus* and *Bauldia* with *Fusarium* presents a promising avenue for the development of novel biological control agents (Fig. 6). Among these microorganisms, *Mycobacterium* is consistently detected across all samples. Although its abundance diminishes in diseased samples but remains higher than that of certain other bacterial genera (Fig. 4; Tables S1). Bouam et al. found that some of *Mycobacterium* can colonize plant roots and promote its growth (Bouam et al. 2018). Moreover, other genera have been shown to offer protection to plants and the environment. For example, *Microbulbifer* demonstrates the most significant negative correlation with *Fusarium*. Zhu et al. (2022) demonstrated that *Microbulbifer* sp. YX04 exhibits its potent algicidal activity against *P. globosa* through the production and secretion of metabolites. However, they are infrequently associated with *Fusarium*. Although many antagonistic microorganisms have been found, the antagonistic effects of these genera on *Fusarium* still need further verification.

Conclusions

These investigations have established that corm rot disease diminished both the number and variety of microorganisms in saffron crops. Fungi community demonstrated a higher susceptibility to rot disease compared to bacterial populations, with the corms and leaves being more easily influenced by infections than other parts of the plant. Additionally, diseased saffron can recruit beneficial microbial community to protect plants, those beneficial microorganisms could be used for the research on biological control agents. The current research has deepened the understanding of the impact of corm rot disease on the microorganisms in saffron and its rhizosphere soil, providing basic data for the sustainable development of saffron.

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

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Not applicable

Authors' contributions
Jia Song was a major contributor to writing the manuscript, Xiaoyuan Xi and Xiaodong Qian analyzed the study data and drew the figures and tables, Jing Li and Yuanyuan Tao collected samples, Liqin Li and Guifen Zhou proposed ideas and revised the articles.

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Availability of data and materials
The raw data has been uploaded to the NCBI database and Figshare (Accession Number: SRP468807; 10.6084/m9.figshare.24557236).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou 310053, Zhejiang, China. [2]TCM Key Laboratory Cultivation Base of Zhejiang Province for the Development and Clinical Transformation of Immunomodulatory Drugs, Huzhou Central Hospital, Affiliated Central Hospital of Huzhou University, Huzhou, Zhejiang, China.

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